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RESEARCH FOR RURAL Development 2010

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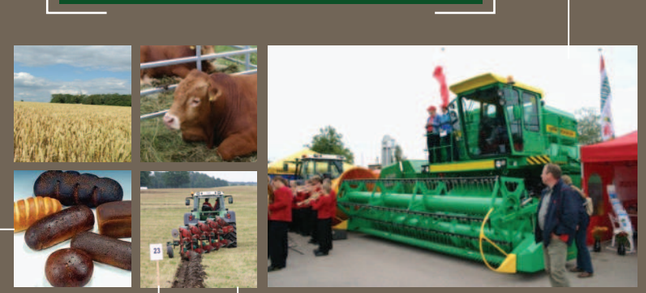

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EDITORIAL

With this issue of 2010, we bring 83 proceedings of the 108, which started life as presentations at the Annual 16th International Scientific Conference "Research for Rural Development 2010" held at the Latvia University of Agriculture, in Jelgava, on 19 to 21 May 2010.

In the retrospect of four months later, we can count the Conference as a great success. The theme – Research for Rural Development - attracted participation of 292 researchers with very different backgrounds. There were 11 presentations from Lithuania, 2 from Estonia, 1 from Romania and 107 from Latvia.

Four independent reviewers estimated each article.

The proceedings of the Annual 16th International Scientific Conference "Research for Rural Development 2010" is intended for academics, students and professionals researching in the area of crop production, animal breeding, agricultural engineering, agrarian and regional economics, food sciences, veterinary medicine, forestry, wood processing, water management, landscape architecture, rural engineering, information and communication technologies.

The proceedings will also be useful for researchers in educational sciences.

CONTENTS

TRANSGRESSIVE SEGREGATION FOR GRAIN QUALITY TRAITS IN THE CROSSINGS BETWEEN COVERED AND HULLESS BARLEY

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Abstract. The objectives of the investigation were to determine the transgressive lines of spring barley (*Hordeum vulgare* L.) for grain quality traits (1000 kernel weight, and content of crude protein, starch and β-glucans) in the six cross combinations between covered and hulless barley genotypes. The trial was carried out at the State Stende Cereal Breeding Institute during 2007 and 2008. Lines representing either positive or negative transgression were found in each cross combination if compared covered and hulless lines with their parent varieties. These lines during the two years of investigation exceeded both parents in positive or negative direction by one standard deviation according to any of the four analyzed grain quality traits. Seven positive transgressive lines were observed regarding 1000 kernel weight, and two transgressive genotypes regarding crude protein content within the population of covered barley lines. No line with positive transgression regarding starch and β-glucan content was found within the covered barley population. 27 hulless lines showed positive transgression regarding any of the four analyzed grain quality traits. The results show that it is possible to increase grain quality of spring barley grain by implementing selection practices.
Key words: covered and hulless barley, grain quality, transgression.

Introduction

Most of agronomic and grain quality traits show a continuous variation and are considered to be quantitatively inherited traits. An improvement of these traits is the main goal of breeding. The first step in the breeding process is choosing the most appropriate parent varieties for successful crossings. There is not found rapid and simple method for evaluation of parental genotypes in respect to predicting the progeny performance in a given cross combination still. The main reason is the nature of quantitative traits: they are determined by many genes with major or minor effects, which are influenced by environmental conditions. Therefore the results of quantitative studies are true only for the population on the basis of which these results were obtained (Falconer and Mackay, 1996).

The improvement of self-pollinated crops, including barley, is connected with production of homozygous selections superior to parental genotypes. The generation of these extreme phenotypes is referred to as transgressive segregation (Kuczynska et al., 2007a). According to L.H. Rieseberg et al. (1999), transgressive segregation in the production of F_2 or later-generation hybrid progeny with phenotypes that can fall outside the phenotypic range of the parental populations from which they were derived.

The frequency of transgression and symmetry/asymmetry in numbers of (+) and (-) segregants in a given population of homozygous lines depends both on trait and on cross combination (Rutger et al., 1966; Kuczynska et al., 2007b). In barley, transgressions in both directions have been observed for most of quantitative traits, e.g., for yield and its components, plant height, days to heading, and malting quality (Kuczynska et al., 2007a; Fregeau-Reid et al., 2001; Tinker et al, 1996; Thomas et al., 1995; Marquez-Cedillo et al., 2001). The results indicate that occurrence of transgressive segregants in a homozygous population should be considered as a phenomenon dependent simultaneously on several factors characterizing parental genotypes. The genetic distance and phenotypic differences between parental genotypes appeared to be significant for the frequency of transgressive lines (Kuczynska et al., 2007b). Greater numbers of transgressive progeny have been reported for offsprings between more distantly related species of barley (Vega, Frey, 1980). Usually transgressive individuals are most often found in early hybrid generations (F_2, F_3), which are often heterozygous. Later generations can not maintain transgressive properties (Kuczynska et al., 2007a).

Positive and negative transgressions occurred in populations derived from crosses between two-rowed (Thomas et al., 1995; Tinker et al., 1996), two-rowed × six-rowed (Marquez-Cedillo et al., 2001; Fregeau-Reid et al., 2001), and also between covered × hulless genotypes (Kuczynska et al., 2007a). Regarding the effect of hulless gene on different agronomic traits it was found that hullesness was associated with less plant height, lower seed weight, higher test weight, and yield reduction (Choo et al., 2001). The evaluation of barley lines derived from crosses made between covered and hulless varieties or outstanding lines is important to evaluate the hulless gene influence on grain quality.

The objectives of this investigation were to determine the transgressive lines of spring barley for traits of grain quality (1000 kernel weight, and content of crude protein, starch and β-glucans) in the cross combinations between covered and hulless barley genotypes.

Table 1

Cross combinations and number of lines used in the study

Cross No.	Parents (Origin)				Number of lines	
	Female parent	Type	Male parent	Type	Covered	Hulless
04-09	Justina (Germany)	covered	L 302 (Latvia)	hulless	5	8
04-10	Simba (Denmark)	covered	Wanubet (USA)	hulless	4	5
04-21	F_1 Austris/Danuta (Latvia/Germany)	covered	Freedom (Canada)	hulless	10	10
04-22	F_1 Austris/Danuta (Latvia/Germany)	covered	KM 2045 (the Czech Republic)	hulless	6	7
04-27	F_1 Linga/Kristaps (Latvia)	covered	SW 1291 (Sweden)	hulless	4	4
04-41	Gate (Latvia)	covered	Freedom (Canada)	hulless	5	6
				In total:	34	40

Materials and Methods

Thirty four covered lines and forty hulless F_4 and F_5 lines derived from F_2 covered and hulless populations from six cross combinations together with their parents were gown at the barley breeding nurseries of State Stende Cereal Breeding institute during the years 2007 and 2008 (Table 1). Covered and hulless genotypes were used as parents in the cross combinations.

The F_3 barley elite plants that derived from F_2 bulk populations were divided into covered and hulless plants. The structural analysis of F_3 elite plants regarding number of morphological and plant productivity traits was carried out and only the desirable material was selected. In 2007, 50 grains from each F_3 covered and hulless elite plants and their parents were planted in a one meter long row. Promising lines of F_4 generation and their parents were harvested. Grain quality traits for F_4 barley lines and their parents were evaluated. In 2008, F_4 covered and hulless barley lines and their parents chosen according to their superior agronomic performance were sown on 1 m^2 plot size. F_5 barley lines were harvested and the grain quality traits were evaluated.

The soil at the site was sod-podzolic sandy loam, humus content – 12-15 mg kg^{-1}, soil pH KCl – 6.0-6.7, pre-crop – potatoes, available for plants P – 88-94 mg kg^{-1} and K – 103-122 mg kg^{-1}. The plots were fertilized with N85 P43 K43 kg ha^{-1}.

The temperature and moisture conditions provided good barley field germination in 2007. In June the average temperature was by 2.2 °C higher than the long-term average, but the sum of precipitation was only 1.7 mm. July was cool and wet (average daily temperature 0.1 °C below long-term average, precipitation was 120% of long-term average). Mean temperature in August was by 2.2 °C higher than the long-term average. April of 2008 was comparatively warm with mean daily temperature by 2.2 °C higher than the long-term average. The moisture deficiency was observed in May (36% from long-term average) and also in the first decade of June. In June and July, the mean daily temperature was lower than the a norm (by 0.2 °C and 0.4 °C respectively). The 2nd decade of July was rich with precipitation (58.4 mm). Very high amount of precipitation was observed also in August (183% from long-term average).

1000 kernel weight (hereinafter/TKW, g) was evaluated according to the ISTA methodology. Grain quality chemical traits – content of crude protein, starch and β-glucans content - in the grain on dry matter (DM) basis were determined by automatic grain analyzer Infratec Analysis 1241. Fraction of barley grain over a 2.1 mm sieve was used for analysis.

Lines representing either positive or negative transgression were found in each cross combination comparing covered and hulless lines with their parent genotypes. These lines during the two years of investigation exceeded both parents in positive or negative direction by one standard deviation according to at least one of four analyzed grain quality traits.

Results and Discussion

One of the primary tasks of a breeder is, depending on the selection goal, to identify and select those plants or lines on the characteristics of interest that would be better than parent varieties to positive or negative direction. The probability to find transgressive homozygous lines is dependent on a combination, that is, the choice of parent varieties (Kuczynska et al., 2007a).

TKW is considered in experiments more frequently than other yield components for hybrids evaluation. In the breeding work this trait is used for both productivity and grain quality characterization. In four hybridization combinations (04-09, 04-21, 04-27, and 04-41) the stated difference between the

parent varieties was rather small (0.2-4.2 g), in two combinations (04-10 and 04-22) the difference was notably higher - from 5.8 to 18.3 g (Table 2).

There were found lines that also showed either positive or negative transgression if comparing with positive transgression was usually higher than that of lines with a negative effect. The most of positive transgressive lines belong to the cross combinations 04-21 (Austris/Danuta//Freedom) and 04-27 (Linga/Kristaps//SW1291). Hulless lines L-564, L-658 and

Table 2

Characterization of grain quality traits for covered and hulless parent varieties used in the cross combinations, 2007-2008

Cross combination	TKW, g		Crude protein, g kg^{-1}		Starch, g kg^{-1}		β-glucan, g kg^{-1}	
	♀ C[1]	♂ H	♀ C	♂ H	♀ C	♂ H	♀ C	♂ H
2007								
04-09	52.9	52.7	109.0	180.0	621.0	596.0	31.0	54.0
04-10	60.4	42.1	117.0	153.0	617.0	633.0	32.0	53.0
04-21	52.3	50.8	115.0	155.0	610.0	620.0	31.5	53.0
04-22	52.3	43.2	115.0	156.0	610.0	620.0	31.5	41.0
04-27	44.9	41.0	140.5	156.0	598.5	614.0	32.0	46.0
04-41	49.7	50.8	124.0	155.0	616.0	620.0	34.0	53.0
2008								
04-09	52.1	51.1	119.0	159.0	632.0	627.0	34.0	52.0
04-10	56.4	40.1	115.0	136.0	622.0	672.0	40.0	60.0
04-21	51.0	46.8	132.0	138.0	619.0	644.0	34.0	52.0
04-22	51.0	45.2	132.0	148.0	619.0	626.0	34.0	47.0
04-27	45.8	45.3	142.5	149.0	607.5	622.0	42.5	56.0
04-41	46.8	45.7	125.0	138.0	616.0	644.0	38.0	52.0

[1]C – covered; H – hulless.

TKW of covered and hulless lines with their parent varieties (Table 3). Within covered barley line population, the positive transgression was observed for seven lines regarding TKW. The difference between cross combinations was found in a number of transgressive lines too. Transgressive lines were detected in four (04-9, 04-21, 04-27, and 04-41) of the six cross combinations used in the study. These are the combinations, as already mentioned, where the difference between the parent varieties regarding 1000 kernel weight was low. It is also indicated in the literature that transgressive segregation more frequently is observed in those populations where the difference between the parent varieties is small and thus high additive (summarized) variability is noted (Surma et al., 1998; Surma et al., 2006). According to the results, eight transgressive lines for covered barley (21% from total number of lines), and nine lines for hulless barley (27%) were obtained. A relatively more lines were found with positive transgression than that with negative: seven covered barley lines and three hulless barley lines. Also A. Kucnzynska (2007a) pointed that transgressive segregants in both directions for TKW were observed in numerous studies and it was found that the frequency of lines L-715 should be especially marked due to their value of TKW which exceeded the covered parent used in the cross combinations. The above mentioned covered and hulless lines are considered as a promising material for future breeding work.

Average crude protein content of covered and hulless lines in the investigated cross combinations mostly falls in-between the two parent varieties (Bleidere and Belicka, 2009). There were also found covered and hulless lines characterized with transgressive properties according to the variability of crude protein content within the individual cross combinations (Table 3). In the population of covered barley, 6 such lines were found (15% from the total number of covered lines) - 4 lines characterized with negative transgression, and 2 lines with positive transgression. Seven lines (16% from the total number of hulless lines) only with a positive transgression according to the crude protein content were detected within hulless barley population. The results showed that barley lines characterized with positive transgression regarding crude protein content were observed more often than those with a negative effect. These lines could be used as a perspective breeding material in the breeding of high protein barley.

Table 3

Covered and hulless transgressive segregants for grain quality traits in the different cross combinations

Cross combination	Covered lines (n=40)		Hulless lines (n=34)	
	(-) transgression	(+) transgression	(-) transgression	(+) transgression
1000 kernel weight				
04-09	-	L-559	L-562, L569, L-571, L-555	L-564
04-10	-	-	-	-
04-21	-	L-657, L-671, L-672	L-648	L-658
04-22	-	-	-	-
04-27	-	L-713, L-723, L-728	-	L-715
04-41	L-836	-	L-824	-
Number of transgressive lines	1	7	6	3
% from total number of lines	3.0	18.0	18.0	9.0
Crude protein content				
04-09	-	-	-	-
04-10	-	-	-	L-587, L-590
04-21	L-655	L-669	-	L-646, L-647, L-650, L-662
04-22	-	-	-	-
04-27	-	L-713	-	L-720
04-41	L-830, L-834, L-835	-	-	-
Number of transgressive lines	4	2	-	7
% from total number of lines	10.0	5.0	0.0	18.0
Starch content				
04-09	L-559, L565, L568	-	L-564, L-570	L-555, L-556, L-557, L-562
04-10	L-581	-	-	-
04-21	L-657, L-665, L-671	-	-	L-648, L-649, L-652, L-661
04-22	L-686, L-692	-	-	L-698
04-27	-	-	-	-
04-41	-	-	-	L-822, L-824, L-828, L-829
Number of transgressive lines	9	-	2	13
% from total number of lines	23.0	0.0	0.0	38.0

Nine lines from four cross combinations, all with a negative transgression regarding starch content, were found within population of covered barley. Between hulless barley lines analyzed in the study, 15 lines from four cross combinations showed persistent transgessive properties to starch content - 2 lines with negative transgression, and 13 lines (38% from total number of hulless lines) with positive transgression (Table 3). Although no covered barley line characterized with positive transgression regarding starch content were found, there was a possibility to select 9 barley lines (from three cross combinations) which exceeded the covered parent according to the starch content in both years of investigation (data not shown).

A valuable feature for hulless barley, from the point of view of grain quality, is increased level of β-glucan content, especially if barley is used for food. As it is indicated in our previous studies, almost in all cross combinations the minimum and maximum value of β-glucan for hulless and covered lines were

between those values of parent varieties (Bleidere and Belicka, 2009). It is mentioned also in other studies (Li et al., 2008) that most of the barley lines showed β-glucan values which ranged from that of the low β-glucan parent to that of the high β-glucan parent. Transgressive line regarding β-glucan content was found only for hulless population, and only at one cross combination 04-22 (Austris/Danuta//KM 2045). Positive transgression was observed for lines L-679, L-682, L-694, and L-696.

It is advisable to cross covered and hulless genotypes in order to obtain covered and hulless barley lines with the consistent positive transgression from such cross combinations. The results show that it is possible to increase grain quality of spring barley grain by implementing selection practices.

Conclusions

1. Lines representing either positive or negative transgression were found in different cross combinations between covered and hulless parent varieties.
2. Seven positive transgressive segregants were observed regarding 1000 kernel weight, and two transgressive genotypes regarding crude protein content within the population of covered barley lines. No line with positive transgression regarding starch and β-glucan content was found within the covered barley population.
3. 27 hulless lines showed positive transgression regarding any of the four analyzed grain quality traits.
4. The covered and hulless lines with positive transgression are considered to be perspective material for the future breeding work.

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TRAITS INFLUENCING SPRING BARLEY COMPETITIVENESS AGAINST WEEDS UNDER ORGANIC AND CONVENTIONAL CONDITIONS

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Abstract. The aim of studies was to identify how different morphological and biological characteristics influence spring barley *(Hordeum vulgare* L.) competitiveness against weeds in various organic and conventional farming locations. Do those traits have an influence on the yield and in which growing conditions the selection of genotypes for organic farming should be done? The plant traits related to competitiveness against weeds at the beginning of the vegetation period are important in breeding for organic agriculture. Ten different varieties were included in trials for three years in two organic and two conventional environments. The included varieties were divided in four groups with respect to time of release and adaptation to growing environments: old extensive varieties and landraces for low input agriculture, medium old varieties with good adaptation to unfavorable conditions, modern varieties for low input, and modern varieties for high input conditions. Results showed that such traits as productive tillering ability, development speed and plant height at the beginning of stem elongation stage provide good soil shading. The most of traits were negatively related to the grain yield. Selection of genotypes for traits which influence spring barley competitiveness against weeds, such as a growth habit in the tillering stage, development speed in tillering stage, plant height at the beginning of stem elongation, length of flag leaf and the width of flag leaf, and the plant height before harvest, may take place in conventional conditions as well as in organic conditions.
Key words: conventional agriculture, organic agriculture, competitiveness against weeds, soil shading ability, selection.

Introduction

In organic agriculture weeds are considered as one of the main problems for farmers to deal with. Because in organic farming systems no herbicides are allowed, it is important to pay attention to prevention and control strategies which would have effect over a longer period of time. The aim of those activities is to restrict weed growth. These activities may be implemented in several levels: farm level (crop rotation, intercropping, distance between planting or sowing rows for adequate mechanical management), field crop level (optimizing growing conditions, thereby promoting crop competitiveness against weeds), and varieties level (plant architecture, rapid juvenile growth, deep rooting system). Not only crops differ in their competitiveness against weeds, but also within a crop species varieties have been found to differ in competitiveness (Lammerts van Bueren, 2002).

Species competitiveness against weeds is connected with a wide range of plant morphological and physiological traits and their interactions. This will include strengths in some characteristics compensating for weaknesses in others. Certain characteristics are indicated as desirable for organic varieties to improve weed suppression: good tillering ability, rapid early growth, taller plants. It is especially important under organic conditions, where the seed is not chemically treated and emergence is lower compared with conventional crops. Flag leaf attitude and high leaf area index, etc., are features that allow the plants to compete with weeds in the second half of the growing season (Hoad et al., 2005).

This study aims to identify how different morphological and biological characteristics influence competitiveness against weeds in organic and conventional farming locations. Do those traits have an influence on the yield and in which growing conditions the selection of genotypes for organic farming should be done?

Materials and Methods

A set of 10 contrasting two-row old and new spring barley cultivars was evaluated in detail during 3 growing seasons (2006-2008) in 4 replications of 12.6 m^2 plots in 4 growing environments. Varieties were divided in four intensity groups with respect to time of release and adaptation to growing conditions (Table 1).

The four locations include two organic sites: Organic 1 (O1) – situated at the Priekuli Plant Breeding Institute, and Organic 2 (O2) – at an organic farm which is within 5 km distance from the institute with similar soil and meteorological conditions. Both fields are certified for organic agriculture for more than 5 years. The other two locations are conventionally managed trial and seed production fields of the Priekuli Plant Breeding Institute: Conventional 1 (C1), and Conventional 2 (C2). The conventional fields were treated according to standard agricultural practices, including the use of herbicides, insecticides and synthetic fertilizers. As to the growing conditions, the soil type was sod-podzolic loamy sand, pH KCL 5.4-6.5. The humus content of the institute fields was low (17 – 27 g kg^{-1}), and the farmer's field O2 had a low to medium humus content (23 – 35 g kg^{-1}). The available P was high to very high - 150 – 242 mg kg^{-1}, and K was medium - 97- 157 mg kg^{-1}. Seed rate at all four locations was 400 seeds able to germinate per m^2. The conventional trial field C1 was fertilised with 80 kg ha^{-1} of N, 40 kg ha^{-1} of P, and

68 kg ha^{-1} of K. The C2 field received 100 kg ha^{-1} N before sowing and 20 kg ha^{-1} in tillering stage, 50 kg ha^{-1} P, and 83 kg ha^{-1} K. Weed control at conventional sites was accomplished with application of Granstar Premia 50 SX (tribenuron-methyl 50%) + Primus (florasulam) (2006-2007) or Secator 19 d.g. (NA-methil-iodosulfuron) (2008); and pests control - with the insecticide Fastak 50 (alfa-permethrin) (2006), or Sumi-alfa (esfenvalerate) (2007).

productive tillers per m^2 and the number of emerged plants per m^2.

Meteorological conditions in the years of study varied rather significantly. In 2006, the second half of vegetation period was extremely dry, thus the grain yield was low. In 2007, the growing conditions favoured the growth and development of barley. In 2008 there was not sufficient amount of rainfall in the first part of vegetation, and it was very rainy close to

Table 1

Description of the varieties included in trials 2006-2008

Variety	Intensity group		Year of registration or growing	Remarks
Primus	old, extensive	OE	1901	very tall plants, medium-prostrate, early plant growth, late maturing
Latvijas vietējie (Latvian local)			landrace ~1800	very tall plants, very late maturing
Dziugiai			1947	very rapid, early development, resistant to acid soil conditions
Idumeja	modern, low input	ML	2000	medium-tall plants, more erect, early plant growth, early maturing
Rubiola			2007	bred for organic farming
Inari	modern, high input	MH	1994	medium plant height, medium early maturing
Annabell			1999	currently the most popular variety in conventional farming
Ansis			1995	short plants, high-input type
Abava	medium old, with good adaptability	MA	1978	low-input type: good yield under poor growing conditions
Anni			1993	stable yield under low-input conditions, good stress resistance

At the O1 location, oil-seed rape was used as green manure with biomass yield approximately 20 tonnes per ha which provided of 26.5 kg ha^{-1} nitrogen. O2 (the organic farmer's field) was fertilised with manure at the rate of 20 tonnes per ha which provided of 41 kg nitrogen per ha. For weed control, in the O1 location harrowing was applied at tillering stage but no treatment was used in O2.

During the vegetation period, the following measurements and scorings were made: field emergence, plants per m^2; growth habit in tillering stage (according to Zadoks scale - 25 to 29), 1 - erect, 9 - planophile; development speed in tillering stage (GS 29-30), scores (1 - slow, 9 – rapid); plant height in the beginning of stem elongation (GS 30-31); soil shading (GS 30-31) (visually estimated plant covered area in the plot, %, - the more ground covered by the crop, the more shading); length of flag leaf (GS 47 – 50), cm; width of flag leaf (GS 47 – 50), cm; plant height before harvest (GS 90), cm; number of productive tillers per m^2 (GS 80-90). Approximate productive tillering capacity was calculated by dividing the number of maturity stage thus decreasing the grain quality.

The obtained results were statistically processed by SPSS 11.0. The obtained data were analysed using descriptive statistics and Pearsen correlation analysis. The significance of the differences between the samples was assessed using ANOVA.

Results and Discussion

Study results showed that the productive tillering capacity depends on both the growing conditions ($p<0.001$) and the variety ($p<0.001$). In organic farming conditions, tillering capacity was significantly lower ($p<0.001$) in comparison to conventional conditions. Although in O2 barley tillered more in comparison to O1, the number of productive tillers per unit of area during the harvest was smaller (Table 2), which was due to the low seedling emergence. Analyzing the correlation between the productive tillering capacity and emergence, it was found that on average there was a strong negative correlation ($r = - 0.629$, $p<0.01$); as a result the lower amount of seedlings was partially compensated by tillering. This could be explained

by both soil preparation quality before sowing and relatively later sowing dates which generally affected the growth of barley varieties and the development at O2 production site.

Comparing intensity groups of varieties, we found that the OE group varieties had the lowest productive tillering capacity in both organic and conventional locations.

with weeds compared with varieties which were with erect growth habit. More explicit erect growth habit in tillering stage was observed for OE group variety 'Dziugiai' and ML group varieties 'Idumeja' and 'Rubiola'. According to the opinion of Hoad et al. (2008), erectophile growth habit had advantages in sowings, where the weed risk was lower, especially at the first half of the growing season. There was a

Table 2

Mean values of traits associated with spring barley competitiveness against weeds at organic locations (O1 and O2) and conventional locations (C1 and C2) from 2006 to 2008

Traits		Environment			
		O1	O2	C1	C2
Emergence, plants per m^2	mean value	343.1	276.8	319.8	331.9
	differences **	O2	O1, C1, C2	O2	O2
Productive tillering capacity	mean value	1.3	1.6	1.8	1.7
	differences **	O2, C1, C2	O1, C1	O1	O1
Growth habit in tillering stage 1 - erect, 9 - plantophyle	mean value	4.4	4.3	4.6	4.5
	differences **	-	-	-	-
Development speed in tillering stage (1 - slow, 9 – rapid)	mean value	5.3	5.0	5.2	5.2
	differences **	-	-	-	-
Plant height at the beginning of stem elongation stage, cm	mean value	26.8	21.7	29.2	28.5
	differences **	O2	O1, C1, C2	O2	O2
Soil shading, %	mean value	65.1	60.0	62.2	57.3
	differences **	O2, C2	O1, C1	O2	O1
Length of flag leaf, cm	mean value	10.4	9.8	10.4	10.3
	differences **	-	-	-	-
Width of flag leaf, cm	mean value	0.75	0.75	0.79	0.78
	differences **	-	-	-	-
Number of productive tillers per m^2	mean value	440.6	365.1	587.9	549.8
	differences **	O2, C1, C2	O1, C1, C2	O1, O2	O1, O2
Plant height before harvest, cm	mean value	74.7	64.4	74.7	76.5
	differences **	O2	O1, C1, C2	O2	O2

** differences are significant at the 0.01 level

Early planophile growth habit in tillering was observed for MH group varieties 'Ansis' and 'Annabell'. The plant habit is highlighted as a significant feature that affects the competitiveness against the weeds (Hoad et al., 2008). Early planophile growth habit has several advantages in comparison with erect form. It has a higher light interception, and it shades weeds more effectively. The opinion of Hoad et al. (2008) is that, to a certain extent, it allows the plant to compensate for its length and therefore it is more suitable for short straw varieties at the first part of the vegetation period in the circumstances with a large proportion of weeds. In O2 conditions with poor emergence, the result was a large proportion of weeds. 'Ansis' and 'Annabell' competed less successfully

significant negative correlation ($r = -0.296$, $p<0.01$) between the growth habit in tillering stage and the soil shading ability (Table 3) indicating that erectophile growth habit was associated with better soil shading. It partly agrees with Hoad et al. (2005) acknowledgment that planophile or expanded habit during the beginning of the growth suppresses weeds very well, but the soil shading in this phase is relatively poor.

This could be explained by a different development speed in tillering stage of varieties. Correlation analysis showed that between the growth habit in tillering stage and the development speed in this time, there was a medium close negative correlation ($r = -0.682$, $p<0.01$).

Table 3

The correlation coefficients between the soil shading and traits associated with competitiveness against weeds in organic (O1 and O2) and conventional (C1 and C2) cultivation sites

Soil shading				
Trait	O1	O2	C1	C2
Emergence	0.003	-0.142	0.057	-0.081
Productive tillering capacity	-0.127	0.021	-0.139	-0.106
Growth habit in tillering stage	-0.359**	-0.241	-0.289	-0.320**
Development speed in tillering stage	0.667**	0.408**	0.545**	0.618**
Plant height at the beginning of stem elongation stage	0.602**	0.435**	0.577**	0.792**

** correlation is significant at the 0.01 level

The planophile grows habit in the tillering stage slowed was the development speed. In our experiment, 'Ansis' and 'Annabell' had a slower speed of development than other varieties.

But 'Dziugiai', 'Idumeja', and 'Abava ', which has more erect growth habit, developed faster under organic conditions. Between the growth habit in the tillering stage and the yield there was no significant correlation (Table 4), but there appeared a tendency for erect growth habit in the tillering stage under organic conditions to have advantages in competitiveness with weeds.

Comparison of correlation coefficients between the growing sites showed (Table 5) that growth habit in the tillering stage and development speed under both organic conditions closely correlated with the values in conventional sites. This suggests that it should be possible to make selection of genotypes for both these features within conventional conditions.

The faster the variety developed, reaching taller height in the elongation stage, the better soil shading ability it had. It was demonstrated by the significantly positive correlation between growth habit in the tillering stage, plant height at the beginning of stem elongation stage, and soil shading ability (Table 3). Plants which were grown organic conditions were shorter at the beginning of the stem elongation stage compared with those grown in conventional conditions. The shorter plant height at the beginning of stem elongation stage over an average of three years was in location O2 (Table 2). Tallest plants in this environment had the varieties with the fastest development speed: 'Dziugiai' from OE group, and 'Idumeja' and 'Rubiola', representing the ML intensity group. The shortest were MH group varieties 'Annabell' and 'Ansis' which also had the slowest development speed. Taking into account the low emergence in O2 conditions and the large proportion of weeds, the plant height in the beginning of stem elongation stage had a significant ($p<0.001$) effect on the soil shading ability (Table 3) and the yield (Table 4). Analyzing the correlative relationship between yield and plant height at the beginning of the stem elongation stage, in the O2, unlike other growing conditions, there was a significant positive correlation (Table 4). In the organic site O1, where agro technical management measures were done optimally, as well as

Table 4

Correlation between the barley yield and the traits which are associated with competitiveness against weeds in two organic (O1 and O2) and two conventional (C1 and C2) locations

Traits	Environment			
	O1	O2	C1	C2
Productive tillering capacity	0.406**	0.653**	-0.021	0.432**
Growth habit in tillering stage	-0.093	0.260	0.289	0.241
Development speed in tillering stage	-0.198*	-0.089	-0.077	-0.200
Plant height at the beginning of stem elongation stage	-0.381**	0.418**	-0.341**	0.164
Soil shading	0.113	0.086	0.259	0.201
Length of flag leaf	-0.463**	0.218*	-0.171	-0.224*
Width of flag leaf	-0.470**	0.362**	-0.005	-0.179
Plant height before harvest	-0.008	0.687**	-0.064	-0.306**
Number of productive tillers per m^2	0.428**	0.765**	0.147	0.453**

** correlation is significant at the 0.01 level

Table 5

Correlation coefficients between the traits which are associated with competitiveness against weeds expression in organic (O1, O2) and conventional (C1, C2) environments

Growing environment	O1			O2	
	O2	C1	C2	C1	C2
Tillering capacity	0.398**	0.219	0.09	0.237**	-0.001
Growth habit in tillering stage	0.825**	0.847**	0.815**	0.798**	0.883**
Development speed in tillering stage	0.821**	0.889**	0.908**	0.861**	0.812**
Plant height at the beginning of stem elongation	0.116	0.739**	0.574**	0.047	0.213
Length of flag leaf	0.304**	0.737**	0.451**	0.285**	0.377**
Width of flag leaf	0.089	0.665**	0.533**	0.131	0.251**
Soil shading	0.367**	0.660**	0.285	0.468**	0.236**
Plant height before harvest	0.614**	0.772**	0.892**	0.347**	0.581**
Number of productive tillers	0.348**	0.227*	0.327**	0.266**	0.359**

** correlation is significant at the 0.01 level

in the conventional sites, the plant height in the stage of elongation was higher in comparison with O2, but it had significantly negative effect on grain yield.

Assessing the correlation of plant height at the beginning of stem elongation stage between the environments, it was found that the values obtained in O1 and O2 did not significantly correlate between each other (Table 5). But a close positive correlation was observed between values in the O1 and conventional C1 locations. Therefore, in order to improve the barley's competitiveness against weeds at the beginning of the growing season genotypes may be selected for plant height at the beginning of stem elongation stage as well in the conventional conditions with medium input level of agrochemicals, except if breeding is done for low yielding organic environments.

Better soil shading at the beginning of vegetation period was observed for OE group varieties: 'Dzuigiai', 'Primus', and 'Latvijas vietējie'. ML and MA group varieties also better covered the soil in comparison to the new MH short stem varieties which at the beginning of stem elongation stage covered about half of the research plot. Soil shading had no significant correlation with the yield, and therefore it can not be used as an efficient valuation tool for yield assessment (Table 4), but it is necessary to assess this trait in the breeding for organic environments; a trait which ensures competitiveness against weeds.

ANOVA showed that the length of flag leaf and the width of flag leaf depend only on the genotype ($p<0.01$). Both of these characteristics positively correlated with each other ($r = 0.763$, $p<0.01$) and also with the height of the plant ($r = 0.370_{\text{Length of flag leaf}}$, $0.381_{\text{Width of flag leaf}}$, $p<0.01$). The highest and widest leaves were found for the OE group varieties. Also the plant height of the OE group varieties when they reached full maturity stage was the highest, which generally refers to good competitiveness against weeds throughout the vegetation season of those varieties. A significant positive correlation between both traits characterising leaf area and yield were found only in the location O2, whereas in O1 (Table 4) the correlation was both significant and negative. It indicates that selection for leaf parameters is more essential while breeding varieties for organic farms with lower yield level.

Length of flag leaf, width of flag leaf, and the plant height before harvest have a significant role of soil shading in the second half of growing season to increase the competitiveness of the varieties against late summer weeds. Late summer weeds may hinder the barley harvest. When parts of the weeds get into the crop harvest, they increase its total mass and moisture, thereby creating additional costs for drying; and during threshing, weed seeds which fall out in the field contaminate the soil. According to Hoad et al. (2005), recurved leaf inclination increases light interception and shading ability, especially under conditions of low soil fertility.

The plants under organic conditions in comparison with the conventional conditions were shorter, especially in location O2, which may create additional risks in this environment - that late summer weeds may obtain dominance. Therefore, the advantage of the organic conditions may be for such taller varieties as the ones from OE group. However, in the literature sources there is a note that the old varieties and landraces are not always acceptable for production in organic conditions. As demonstrated by previous research results by A. Kokare and L. Legzdiņa (2006), landraces have low-yield potential and poor lodging resistance, which can cause additional problems in more favourable growing conditions with the relatively higher yield levels. ML and MA varieties have medium tall straw, which results in better resistance to lodging. They have a higher tillering rate in comparison with OE varieties. ML varieties developed faster in the spring and reached the same plant height as OE at tillering stage. As a result the soil covering and competitiveness with weeds during the tillering stage was within the level of OE varieties. Bertholdsson et al.

(2005) indicate that the morphological and biological traits, such as flag leaf attitude, rapid growth in early development stage, tillering ability, the plant height, etc., negatively correlate with the yield. Results of our research show that the length of flag leaf, width of flag leaf, and the plant height before harvest within conventional environments C1 and C2 had a tendency to negative correlation with the yield (Table 4). In the organic conditions O1 there was a significant negative correlation between leaf parameters and yield. This could be partly explained by the OE intensity group variety`s relatively tall plants and low yield potential in general. Under favourable growing conditions they produced lower yields in comparison with other varieties (Kokare et al., 2009). However, in O2 growing conditions, a significant positive correlation between yield and plant height was observed (Table 4) indicating that selection by those traits can improve not only competitive ability with weeds but yield as well.

Other studies confirm that the correlation with the yield in conventional and organic conditions may vary (Ostergard and Jensen, 2004). In a location where it is not possible to meet all the agrotechnical requirements and which is regarded as atypical, it is important to select genotypes with higher biomass (taller plants with long and broad leaves). This is in addition to improving the competitiveness against weeds, which would also provide an acceptable level of harvest.

These values of the length of flag leaf, width of flag leaf, and the plant height before harvest in organic O1 and O2 conditions poorly correlated with each other (Table 5). A closer correlation was observed between organic (O1) and conventional (C1) conditions. This suggests that the selection of genotypes for organic farms with favourable growing conditions by these traits may be implemented in conventional conditions with medium input level as well.

Conclusions

1. Under organic conditions such traits as erect plant habit in tillering stage, development speed and plant height at the beginning of stem elongation stage, which provides good soil shading and hence the competitiveness against weeds at the beginning of the vegetation period, are important.
2. Higher productive tillering ability was observed for genotypes with planophile growth habit in the tillering stage, but they had slower development speed which resulted in lower soil shading.
3. Selection of genotypes by such traits as growth habit in the tillering stage, development speed in tillering stage, plant height at the beginning of stem elongation, length of flag leaf and the width of flag leaf, and the plant height before harvest may as well take place in conventional conditions.
4. In order to breed varieties for environments with lower yield level, selection for traits providing soil shading ability is more essential.
5. From varieties included in this study, the most suitable genotypes for organic conditions were ML and MA.

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EFFECT OF DIFFERENT PLANT PRODUCTION METHODS ON YIELD AND QUALITY OF WINTER WHEAT 'PORTAL' IN 2009

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Abstract. The yield and quality (volume weight, 1000 kernel weight, protein content, falling number, gluten content, gluten index, gluten content in dry matter) of winter wheat was studied in variety 'Portal'. The wheat was part of the five-year crop rotation experiment where red clover (*Trifolium pratense* L.), winter wheat (*Triticum aestivum* L.), peas (*Pisum sativum* L.), potato (*Solanum tuberosum* L.) and barley (*Hordeum vulgare* L.) were following each other. There were two production variants which followed the crop rotation. In one variant mineral fertilizers and pesticides were used, and the other variant was conversion to organic without any synthetic agrochemicals. In mineral fertilizing variant, on the background of P25 and K95 kg ha^{-1} the N amount varied from 0 to 150 kg ha^{-1} and herbicide Mustang (preparation norm 0.5 L ha^{-1}, active substance florasulam, 6.25 g L^{-1}; 2.4-D, 300 g L^{-1}), insecticide Fastac 50 (preparation norm 0.2 L ha^{-1}, active substance 50 g L^{-1} alpha-cypermethrin) fungicide Falcon EC 460 (preparation norm 0.4 L ha^{-1}, active substances 167 g tebuconazole, 250 g spiroxamine, 43 g triadimenol) and growth regulator Moddus (preparation norm 0.4 L ha^{-1}, active substance 250 g L^{-1} trinexapac-ethyl) were used. In conversion to organic the winter wheat grains which followed the red clovers after effect had higher volume weight, 1000 kernel weight and gluten index compared to the variants where mineral fertilizers were used. The yield, protein content, falling number and gluten content in dry matter increased with increase of the amount of mineral N. The wet gluten content was significantly higher compared to the other variants where the N amount was 50 kg ha^{-1}.
Key words: gluten, volume weight, 1000 kernel weight, falling number, protein, *Triticum aestivum* L.

Introduction

Wheat (*Triticum*) is one of the most cultivated cereal in the world. Winter wheat (*Triticum aestivum* L.) is prevalent in areas where conditions are favorable for wintering like in Estonia. The past several years winter wheat is becoming more competitive with spring wheat. The main reason is higher winter wheat yields. There also is the advantage of lower seed and herbicide costs (Swenson, 2006).

In organic farming, the yields remain lower but the crop quality is usually better. The interest in organic farming is stimulated not only by the concern about stable and well-balanced further development of the economy but also by increasing consumer awareness of food safety and quality. Currently, the demand for organically produced cereals, including wheat, still exceeds supply (Cesevičienė et al., 2009).

Leguminous crops are reserving up to 200-300 kg ha^{-1} of nitrogen per year (Tonitto et al., 2006). Red clover (*Trifolium pratense* L.) is most cultivated legume in Estonia which is used as a green manure. Growing clover in crop rotation decreases the occurrence of disease-causing pathogens in subsequent crops (Cook et al., 1987) and enriches the soil with atmospheric nitrogen and with soil organics.

Nitrogen deficiency is the main cause why the yields are low and the plant growth is inhibited. On cereals it also leads to a low protein content in kernels. Excessive fertilization with nitrogen extends the plant growth period and reduces resistance to diseases and pests (Luik et al., 2008). Knowing the nitrogen demand is important not only from economic viewpoint, but also from environmental one, because over-supplying of nitrogen causes leaching of nitrates (Addiscott et al., 1991).

The volume weight is most widely used criteria of cereal quality. It shows the grain weight per unit volume, usually per litre (g L^{-1}) or per hectolitre (g hL^{-1}) (Tamm et al., 2008). It is influenced by many factors, including fungal infection, insect damage, kernel shape and density, foreign materials, broken and shrivelled kernels, agronomic practice, and the climatic and weather conditions (Gaines et al., 1997). The protein fraction and gluten are known to play the most essential role for baking the bread. Mature wheat grains contain 8-20% (80-200 g kg^{-1}) of proteins (Kuktaitė, 2004). The wheat grain protein content is influenced by the climate, cultivar, nitrogen application rate, nitrogen application timing, seeding rate, and soil fertility (Geleta et al., 2002). The optimum gluten index is 60-90 (Talgre et al., 2009). The Hagberg Falling Number measurement is widely used for assessing the baking quality of wheat flour. Falling number value of 350 seconds or longer indicates low enzyme activity and very high quality wheat. As the amount of enzyme activity increases, the falling number decreases. Values below 200 indicate high levels of enzyme activity (Sorenson, 2006) and poor quality of flour. 1000 kernel weight, measure of the size of a grain, depends on the variety of genetic characteristics, on growth conditions, and on fertilization (Koppel and Ess, 2007).

Table 1

Average monthly temperatures (ºC) and precipitation (mm) in Estonia during the vegetation period

Month	Temperatures, ºC		Precipitation, mm	
	Average of 2009*	Average of 1966-1998**	Sum of 2009*	Average Sum of 1966-1998**
April	5.3	4.2	14.2	33.0
May	11.5	11.6	13.4	55.0
June	13.8	15.1	137.4	66.0
July	16.9	16.7	54.6	72.0
August	15.4	15.6	89.2	79.0
April-August	12.6	12.6	308.8	305.0

* according to the Eerika weather station
** (Jaagus, 1999)

The aim of this research was to investigate how the red clovers after-effect and different N fertilizer amounts are influencing the winter wheat yield and quality.

Materials and Methods

Field trials with the winter wheat variety 'Portal' (bred in Germany) were carried out on the experimental fields of the Department of Field Crops and Grassland Husbandry, Estonian University of Life Sciences. There were five treatments – second-year conversion to organic (Conv. Organic CR), $N_0P_0K_0$, $N_{50}P_{25}K_{95}$, $N_{100}P_{25}K_{95}$ and $N_{150}P_{25}K_{95}$. The wheat was part of the five-year crop rotation experiment where red clover, winter wheat, peas (*Pisum sativum* L.), potato (*Solanum tuberosum* L.) and barley (*Hordeum vulgare* L.) were following each other. Wheat was sown according to the norm 175 kg ha^{-1}, 450 germinate able seeds per $1m^2$. Fields were fertilized with different fertilizers: Kemira Grow How Power N:P:K – 5:14:28, and AN 34.4 N: P:K – 34:0:0. The variants that had received $N_0P_0K_0$ and mineral fertilizers, were sprayed with herbicide Mustang, insecticide Fastac 50, fungicide Falcon EC 460, and growth regulator Moddus. The experiments were laid out in four replications. The size of each test plot was 60 m^2. The soil of the experimental field was *Stagnic Luvisol* by WRB (2002) classification (Deckers et al., 2002), the texture of which is sandy loam with a humus layer of 20-30 cm.

The soil analyses were carried out at the laboratories of the Department of Soil Science and Agrochemistry, Estonian University of Life Sciences. The trial soil was slightly acidic – pH KCl 6.0; carbon – 13.8 g kg^{-1}; mobile phosphorus - 0.103 g kg^{-1} (AL); mobile potassium – 0.179 g kg^{-1} (AL); calcium – 0.980 g kg^{-1}; magnesium – 0.164 g kg^{-1} and nitrogen – 1.29 g kg^{-1} of soil.

Compared to the average temperatures of many years, the average temperatures in 2009 were a bit colder. April, May and July had less precipitation compared to the average of 1966-1998, but there was abundant rainfall in June and August (Table 1).

The yield was determined by weighing after harvesting and drying the grains. Yield data and grain quality parameters were adjusted to 14% moisture content. Yield, 1000 kernel weight, protein content, volume weight, falling number, wet gluten content, gluten index and gluten content in dry matter were calculated as average of 4 replications. 1000 kernels were counted manually and then weighted. In order to get the protein content percentage total nitrogen content was determined from oven-dried samples by the dry combustion method in a varioMAX CNS elemental analyzer (ELEMENTAR, Germany), and then for getting the protein content percentage the number from dry combustion method was multiplied with the coefficient 6.25. Volume weight was measured with the 1-litre measuring cylinder. Wet gluten content, gluten index and gluten content in dry matter were determined by ICC standard methods 137/1 and 155. Falling number was determined by ICC standardmethod 107/1. Gluten index is found by centrifuging wet gluten. Wet gluten is placed to the sieves which are put into a centrifuge. Liquid gluten is then separated from the solid gluten by centrifuging. Solid gluten is used to calculate the gluten index percent from the wet gluten.

Experimental data were processed by Statistica 7.0 software (Anova, Fisher LSD test) (Statsoft, 2005).

Results and Discussion

Wheat is a valuable crop in organic farming, and much effort has been put into optimizing the yield and quality of organically grown wheat. In many areas, farmers prefer to grow wheat, and in order to achieve the highest possible yield and quality, they grow it after the best pre-crops in the crop rotations (Sarunaite et al., 2009).

The lower yields of conversion to organically grown wheat is the result of inadequate nutrient content in the soil.

In 2009. The averaged yields of winter wheat

'Portal' were 4-6 t ha^{-1} (Fig. 1). Agronomically the most effective fertilizer amount was 50 kg of N ha^{-1}. Greater N amounts decreased the winter wheat yields. However, the fertilization gave higher average yields compared to the variant of conversion to organically grown wheat and $N_0P_0K_0$. The leaves from fertilized variants were capable for longer photosynthesis period and therefore were able to grow longer period which led to an increased yield.

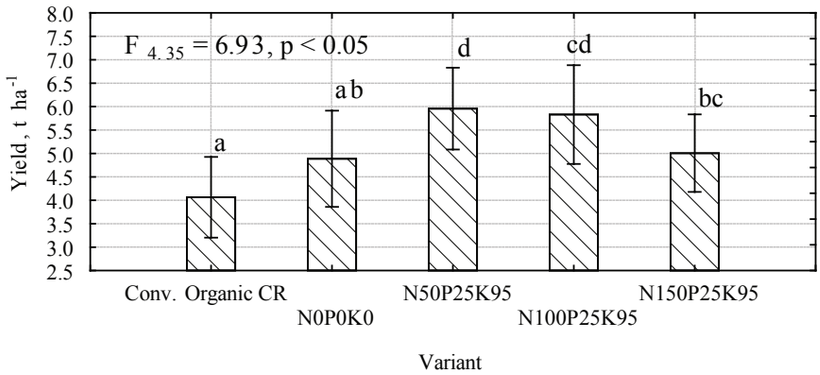

Figure 1. Average yield of winter wheat 'Portal' in 2009, t ha^{-1}. Means followed by a different letter in the same row are significantly different ($P < 0.05$).

The average 1000 kernel weight as well as volume weight were highest in the variant of conversion to organic growing (Table 2). Fertilization decreased the 1000 kernel weight and volume weight so that the variant $N_{150}P_{25}K_{95}$ had the smallest 1000 kernel weight and volume weight.

The previous experiments have shown that fertilization increases the Hagberg falling number (Lloveras et al., 2001; Kindred et al., 2005). Winter wheat 'Portal' average falling numbers in 2009 were between 412.8 s in the variant of conversion to organic growing and 469.1 s in $N_{150}P_{25}K_{95}$. As in previous experiments, the falling number increased with the increase of the amount of nitrogen.

The wet gluten is a mixture of flour and water. In 2009, the highest wet gluten content was in variant $N_{50}P_{25}K_{95}$ (264 g kg^{-1}) (Table 3). It was significantly higher compared to the other variants.

Gluten index over 90 decreases the flexibility and elasticity of wet gluten, which is unfavourable for a ready-to-use product (Talgre et al., 2009). In our research, the gluten index varied between 82.1 ($N_{150}P_{25}K_{95}$) and 88.7 (conversion to organic). The higher mineral fertilizer amounts decreased the gluten index in winter wheat 'Portal' flour in 2009.

After drying the wet gluten in dry chamber, the remaining dry matter contents were higher in the variants which got mineral fertilizers (Table 3). The highest gluten content in dry matter was in the variant $N_{100}P_{25}K_{95}$ but the lowest in the variant of conversion to organic growing which followed the crop rotation and was not treated with any chemicals and mineral fertilizers.

High grain protein content is essential for bread wheat cultivars in order to get high-quality bread. Average grain protein content of winter wheat is 12-14% (120-140 g kg^{-1}) (Koppel and Ess, 2007). Higher N amounts are increasing the grain protein content (Nakano et al., 2008). The average protein content was the highest in variants $N_{100}P_{25}K_{95}$, and $N_{150}P_{25}K_{95}$ and remained the lowest in variants of conversion

Table 2

Winter wheat 'Portal' average 1000 kernel weight, g, volume weight, g L^{-1} and falling number, sec, in 2009. Means followed by a different letter in the same row are significantly different ($P < 0.05$)

Variant	1000 kernel weight, g	Volume weight, g L^{-1}	Falling number, sec
Conv. Organic CR	45.9a	750.1a	412.8a
$N_0P_0K_0$	43.9ab	743.1a	434.1ab
$N_{50}P_{25}K_{95}$	41.5bc	741.3ab	436.8ab
$N_{100}P_{25}K_{95}$	39.2c	729.1b	439.8b
$N_{150}P_{25}K_{95}$	35.8d	712.1c	469.1c

Table 3

Winter wheat 'Portal' average wet gluten content, g kg^{-1}, gluten index, and gluten content in dry matter, g kg^{-1} in 2009. Means followed by a different letter in the same row are significantly different ($P < 0.05$)

Variant	Wet gluten content, g kg^{-1}	Gluten index	Gluten content in dry matter, g kg^{-1}
Conv. Organic CR	246ab	88.7a	305a
$N_0P_0K_0$	248ab	86.7ab	309a
$N_{50}P_{25}K_{95}$	264a	84.5ab	345b
$N_{100}P_{25}K_{95}$	247ab	85.3ab	362c
$N_{150}P_{25}K_{95}$	242b	82.1b	359c

to organic growing, $N_0P_0K_0$ and $N_{50}P_{25}K_{95}$ (Fig. 2). Increase by the N amount increased the protein content in grains.

Conclusions

Crop rotation helps to improve and provide higher yields and soil fertility, therefore it is an important measure in organic farming. In conversion to organic growing, the winter wheat which followed the red clover grains had higher volume weight, 1000 kernel weight and gluten index compared to the variants where mineral fertilizers were used. The yield, protein content, falling number and gluten content in dry matter increased with increase of the amount of mineral N. The wet gluten content was significantly higher compared to the other variants where the N amount was 50 kg ha^{-1}. At the present experiment the optimal amounts of nitrogen for conventional farming were 50-100 kg ha^{-1}.

It can be concluded that quality of winter wheat depends on both crop rotation and mineral fertilization. But if the main purpose is higher yields, the winter wheat should receive adequate amounts by fertilizers.

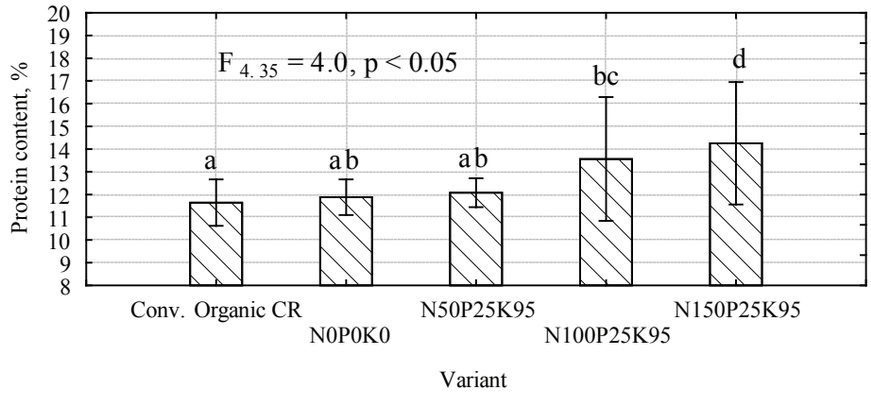

Figure 2. Average protein content in winter wheat 'Portal' in 2009, %. Means followed by a different letter in the same row are significantly different ($P < 0.05$).

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EFFECT OF DIFFERENCES IN SOIL MOISTURE ON WINTER WHEAT YIELD

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Abstract. It is important to define which of the plant growth factors determines the yield level. During recent years in Latvia, in most cases it was moisture. Grain yield level mostly depends on meteorological conditions during the tillering stage. Plants do not utilize all water from precipitation. Rain water efficacy depends on soil granulometric composition and content of humus. Humus content could be considered as regulated factor. Important is also distribution of precipitation during the vegetation period as well as run-off of rain water which depends on micro-relief, soil tillage type, and direction in accordance with the slope gradient. It is very important for cereal growing in what conditions tillering is done, because during that time productive stems as well as ear sprouts are formed. The aim of this research was to determine the effect of soil moisture on the growth and development of winter wheat *Triticum aestivum* L. Field trials were carried out during 2005-2007 in Kurpnieki field at the Research and Study farm „Vecauce" of the Latvia University of Agriculture. A total of 47 points (distributed as a grid of 50×50 m) were selected for sampling in the winter wheat field. In both experimental years, the increasing soil moisture in spring had a significant positive effect on the flag leaf area, which, in its turn, increased also the level of grain yield. Partial correlation analysis showed that exclusion of organic matter content and altitude above the sea level as factors, changes soil moisture at different layers of the soil insignificantly.
Key words: cereal development, precision field management, soil moisture content, winter wheat.

Introduction

Soil moisture is one of the most important factors contributing to the compaction of soil. When moisture in soil increases, strength of uncultivated soil rapidly deteriorates. The same load packs more wet soil than dry. Wet soil load affects deeper than dry soil. When the soil pores are filled with water (saturated soil), soil compacts only where the water is out of the pores (Scaffer et al., 2006).

The modern technique allows using the images from aero-photo for general soil observation. They can be useful to determine differences in the field and to find problems. These images inform about the soil mainly in indirect way through the symptoms of cultivated plants (Florinsky and Kuryakova, 2000).

Variability of soil fertility is explained by soil physical conditions: type, compaction, organic matter, and moisture content. On-the-go soil sensors are designed to test changes in the soil (Adamchuk and Christenson, 2005).

It is important to define which of the plant growth factors determines the yield level. During recent years in Latvia, in most cases it was moisture. Grain yield level most by depends on meteorological conditions during the tillering stage. Plants do not utilize all water from precipitation. Rain water efficacy depends on soil mechanical composition and content of humus. Important is also distribution of precipitation during the vegetation period as well as run-off of rain water which depends on micro-relief, soil tillage type, and direction in accordance with the slope gradient. Usage of soil moisture is highly dependent on the dynamics of development of crop root system. Essential is the initial development stage because very often in Latvia moisture deficit occurs in late April and early May, when there is not enough precipitation and soil surface dries quickly. Groundwaters through capillary rise up slowly and reache field surface only when it is not deeper than 0.40-0.80 m in the ground (Lapins, 1997). The aim of this research was to determine the effect of soil moisture on the growth and development of winter wheat.

Materials and Methods

Field trials were carried out during 2005-2007 in Kurpnieki field (latitude: N 56º 28´, longitude: E 22º 53´) at the Research and Study farm „Vecauce" of the Latvia University of Agriculture.

Soil characteristics: predominant soil type - sod podzolic loam soil, humus content - 14-91 g kg^{-1} (by Tyurin's method), soil reaction - pH_{KCl} 6.0-7.4, phosphorus content - 102-394 mg kg^{-1}, and potassium content -102-333 mg kg^{-1} (by Egner-Riehm method). Relief - wavy terrain, area with explicit macro-relief. The field had a drainage system.

The same agrotechnology for growing of winter wheat variety 'Tarso' was used on the entire field: forecrop winter oilseed rape *Brassica napus* ssp. *Oleifera*; soil tillage before drilling soil deep loosening at the depth of 0.35-0.50 m, and following soil ploughing at the depth of 0.18 m. Drilling of winter wheat was done with combined drilling-soil tillage equipment with a vertical power harrow, using 400 germinate able seeds per m^2. Fertilizers: in autumn - $N_6P_{26}K_{30}$ at the rate of 300 kg ha^{-1}; in spring - ammonium nitrate (N_{34}) two times at the rate of

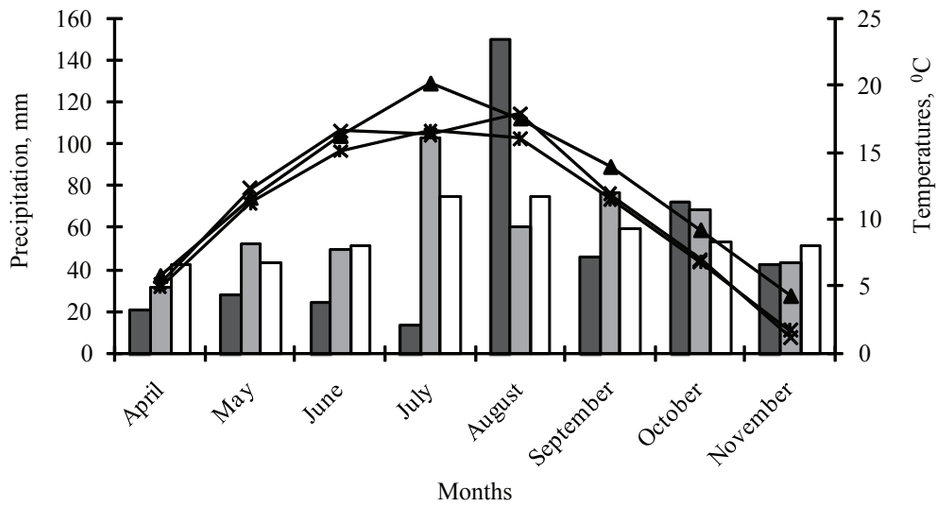

Figure 1. The average day and night air temperatures and precipitations in years 2006 and 2007, °C (according to Vecauce Metpole and long term to Dobele HMS):

200 kg ha^{-1}. Weed control and fungicide application was done according to the needs.

Winter wheat variety 'Tarso' was grown in 2006 and 2007. The agrotechnology used in wheat cultivation was equal in the whole field and in both trial years. A total of 47 points (distributed as a grid of 50×50 m) for sampling were selected in the winter wheat field. All points were attached to their geographic coordinates. The coordinates of observation points were defined by GPS receiver Garmin IQ 3600 using AGROCOM software AgroMAP Professional that allows to find the coordinates by accuracy of ±3 m, as well as to determine the field boundaries. Information from Garmin IQ 3600 was transferred into a computer and processed by the program AgroMAP Professional. Data characterizing growth and development of winter wheat (number of leafs, total weight of plant, mass of roots, the length of roots, coefficient of tillering, and area of flag leaf) were determined from 10 plants in each sampling point two times in autumn at winter wheat growth stage BBCH 11-12, and in spring at growth stage BBCH 25-29. Soil moisture content, %, was measured with Eijkelkamp Agrisearch Equipment instrument in 0.00-0.45 m soil layers, 3 replications in each sampling point. Flag leaf area was determined by using a specialized computer program WinFOLIA in growth stage BBCH 37-39. Samples for organic matter content were taken from the depth of 0.20 m using a probe. Sampling was done after harvesting on August 14, 2006, in 3 replicates in each sampling point. The samples were analyzed in the certified laboratory VSIA „Agroķīmisko pētījumu centrs" („Agrochemical Research Centre"), using local standard method LV ST ZM 80-97. The yield was harvested by combine CLASS LEXION 420. Mapping was created using specialized software AgroMAP. Data analysis was performed using a mathematical descriptive statistics, correlation and partial correlation analysis.

Meteorological conditions varied in the research years, and the main indices - average daily temperature and precipitation - are shown in Figure 1. The observed average air temperatures were above the long-term average in both trial years, especially in the second part of the year 2006.

The average temperature of July 2006 was by 3.5 ºC higher than the long-term observed. Alongside with insufficient amount of precipitation it caused rapid ripening and early harvesting of winter wheat compared with long-time observed harvesting time. The sum of precipitation was low in both trial years, but during the period April-August it was lower in the year 2006 if compared to 2007, despite the high amount of precipitation in August 2006 (Figure 1).

Results and Discussion

Importance of soil moisture, as one of partly regulated plant growth factors, will be described in connection with the growth and development of winter wheat for the yields in 2006 and 2007. The results showed that differences in soil moisture were significant only in the autumn of 2006 which was characterized by low amount of precipitation (Table 1). In trials in Lithuania it was found that the greatest soil moisture variation for all soil tillage systems was in the 0.00-0.05 m topsoil layer, which is more affected by the meteorological conditions.

From the beginning to end of the crop growing season, in a loam soil, in conventional tillage system, moisture content in the ploughlayer declined on average by 29.9%, in reduced tillage system - by

Table 1

Soil moisture effect on winter wheat development in the autumn of 2006 and 2007

Indices	Moisture, %, 0.00-0.05 m		Moisture, %, 0.20-0.25 m	
	r_{yx}	p-value	r_{yx}	p-value
2006				
Total weight of plant, g	-0.278	0.095	-0.233	0.164
Length of roots, cm	-0.401*	0.013	-0.409*	0.011
Number of leaves	-0.381*	0.019	-0.273	0.101
2007				
Length of roots, cm	-0.108	0.469	-0.010	0.942
Total weight of a plant, g	-0.100	0.499	-0.178	0.229

*p< 0.05.

38.8%, and in direct drilling system - by 37.4%, whereas in a sandy loam soil - by 32.4%, 29.8% and 17.2%, respectively, i. e., was nearly twice as low as in a loam soil. When direct drilling was applied, the soil absorbed moisture more slowly, and under droughty conditions the soil was able to retain moisture in the ploughlayer longer. Soil moisture depends on the chosen soil tillage system, and application of direct drilling can be one of the ways of economical use of moisture (Kadžiene, 2009).

Assessment of soil moisture in spring 2006 showed that moisture content at the 0.00-0.05 m soil layer has significant positive effect on the coefficient of tillering, area of flag leaf, and winter wheat yield ($p<0.05$), but at the 0.20-0.25 m deep soil layer on the area of flag leaf, and winter wheat yield (Table 2).

The results show that soil moisture at both tested soil layers has significant ($p<0.01$) positive effect on the area of flag leaf in both trial years. Effect on winter wheat yield was significant ($p<0.05$) for soil moisture at the depth of 0.20-0.25 m in both trial years, but significance of moisture content at the top soil layer on winter wheat yield was found only in the year 2006 (Table 2). A significant effect of soil moisture at the top soil layer on winter wheat grain yield was found also in trials with soil deep loosening (Dinaburga, 2007).

Correlation analysis in the year 2006 showed that at the yield level below 7.00 t ha^{-1}, significant positive effect on winter wheat yield soil moisture content in autumn at the depth of 0.00-0.05 m had ($p<0.05$). In places where the yield was above 7.00 t ha^{-1}, soil moisture showed significant effect in autumn at 0.20-0.25 m depth and in spring at 0.40-0.45 m depth ($p<0.05$), but significant effect with higher probability ($p<0.01$) showed soil moisture in spring at 0.00-0.05 and 0.20-0.25 m depth (Figure 2). Whereas in the year 2007, significant positive effect to winter wheat yield

Table 2

Soil moisture effect on winter wheat yield and its formation in 2006 and 2007

Indices	Moisture, %, at 0.00-0.05 m		Moisture, %, at 0.20-0.25 m	
	r_{yx}	p-value	r_{yx}	p-value
2006				
Coefficient of tillering	0.312*	0.032	0.195	0.186
Total weight of a plant	0.038	0.796	0.038	0.799
Mass of roots	-0.096	0.520	-0.140	0.346
Area of flag leaf	0.464**	0.001	0.535**	0.000
Yield	0.470**	0.004	0.370*	0.028
2007				
Coefficient of tillering	0.056	0.707	0.039	0.791
Total weight of a plant	0.161	0.278	0.179	0.227
Mass of roots	0.233	0.114	0.268	0.068
Area of flag leaf	0.418**	0.003	0.420**	0.003
Yield	0.228	0.122	0.299*	0.040

*p< 0.05; **p<0.01.

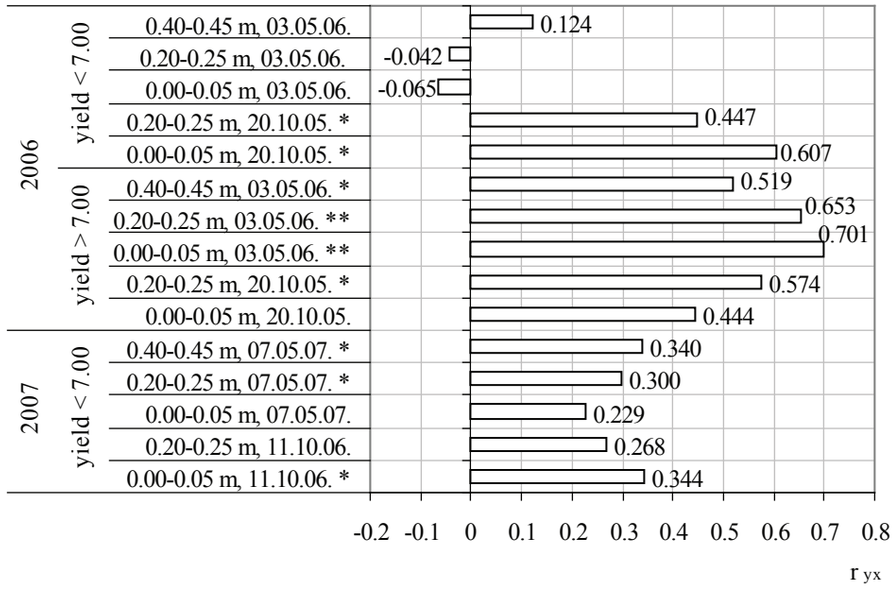

Figure 2. Correlation between soil moisture at various depths of soil and winter wheat grain yield at different levels; *p< 0.05; ** p<0.01.

showed soil moisture content in autumn at 0.00-0.05 m depth (similar with year 2006) and moisture content in spring at 0.20-0.25 and 0.40-0.45 m depth ($p<0.05$). The influence of soil moisture in subsoil layer (at the depth of 0.40-0.45 m) or winter wheat yield has been found also in trials with soil deep loosening, and the obtained coherence was described by equation: $y = -0.654x + 24.708$ (Dinaburga, 2007).

Correlation analysis between soil moisture and area of flag leaf shows significant effect of moisture only at subsoil layer in places with winter wheat yield level above 7.00 t ha^{-1} in the year 2006. But in the year 2007, the effect of moisture in soil was significant with high probability ($p<0.01$) in all tested layers of soil both in autumn and spring (Figure 3).

The area of winter wheat flag leaf had a significant positive effect on the grain yield at both considered yield levels; in 2006 the only difference was in the probability level: at the yield level below 7.00 t ha^{-1}, the probability level was higher, but correlation was insignificant in the year 2007 (Figure 4). A significant positive effect ($r_{yx} = 0.532$) of the area of flag leaf

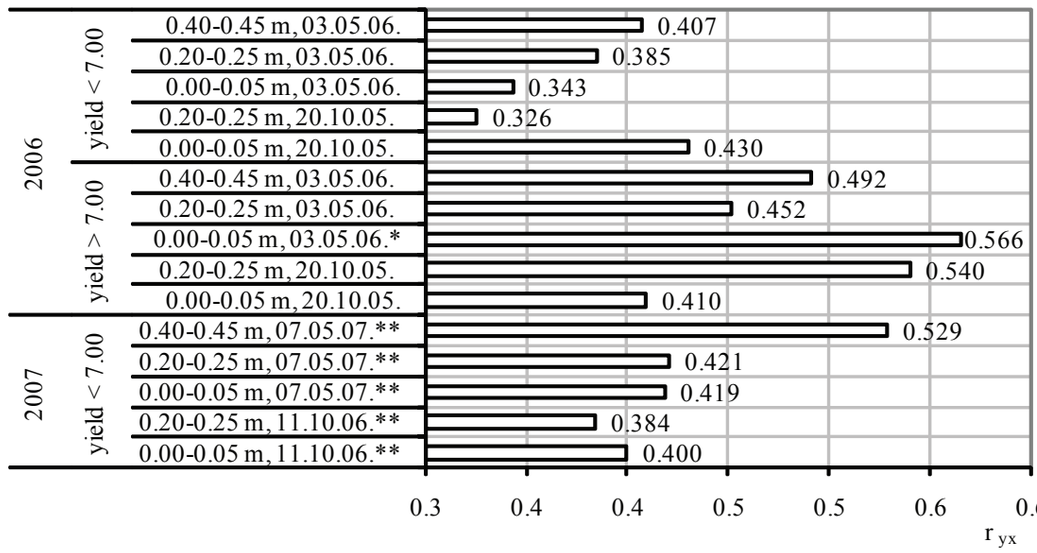

Figure 3. Correlation between soil moisture at various depths of soil and area of wheat flag leaf at different levels of winter wheat yield; *p< 0.05; ** p<0.01.

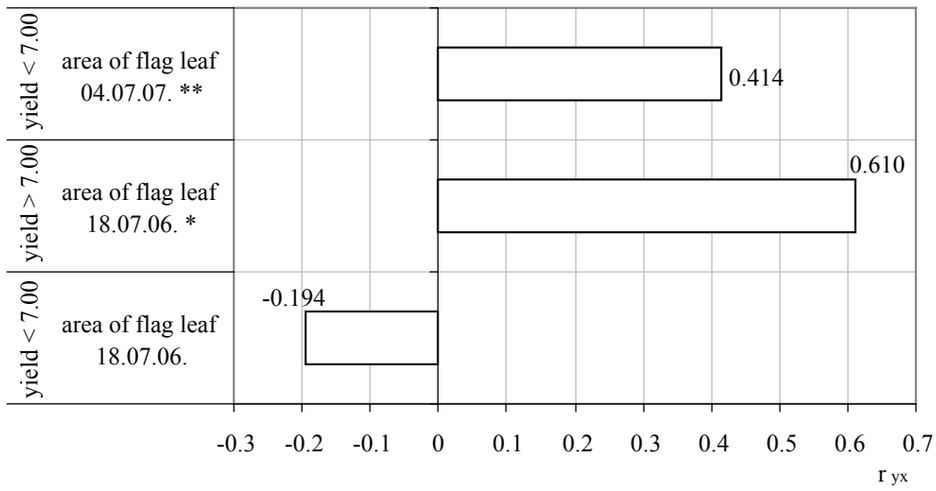

Figure 4. Correlation between the area of flag leaf and winter wheat grain yield at different yield levels; *p< 0.05; ** p<0.01.

on wheat yield has been found also in other trials (Dinaburga, 2007).

In the trials with soil deep loosening it has been found that only 18.1% of winter wheat yield changes can be explained by moisture differences at the depth of 0.20-0.25 m, and 30.0% of changes by moisture content at the depth of 0.40-0.45 m (Dinaburga, 2007).

Partial correlation analysis showed that exclusion of the effect of organic matter content and relative height above the sea level causes no significant changes in conclusions about soil moisture influence on the formation of winter wheat yield. The only important difference concerns soil moisture at the top soil in autumn - the relationship becomes significant if partial correlation analysis is used (Figure 5).

Also other researchers have found that the greatest influence on variability of the yield components is exerted by moisture and by penetration resistance of soil. Decreased soil moisture mainly brings about a reduction in the number of ears on a unit of area (Weber et al., 2004).

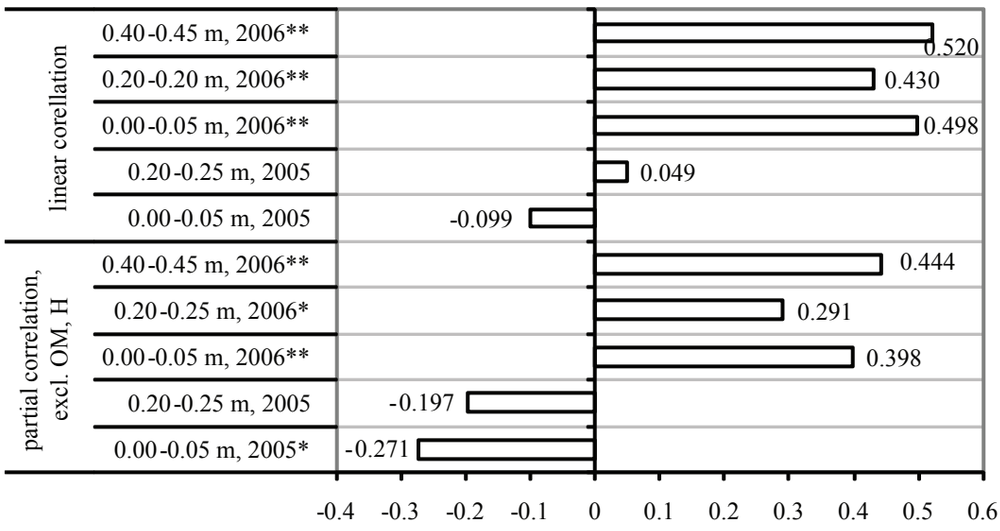

Figure 5. Coefficients of linear correlation, r_{yx} and partial correlation, between winter wheat grain yield, t ha^{-1}, (y) and soil moisture content, %, (x); *p< 0.05, ** p<0.01; OM - organic matter content (mg kg^{-1}); H - relative height above the sea level, m.

Conclusions

1. Effect of soil moisture in autumn on parameters characterizing growth and development of winter wheat was significant only in the autumn of 2006 when low amount of precipitation was observed.
2. Soil moisture in spring had significant ($p<0.01$) positive effect on the area of flag leaf at both tested soil layers 0.00-0.05 m and 0.20-0.25 m.
3. Soil moisture effect to winter wheat yield was significant ($p<0.05$) for soil moisture at the depth of 0.20-0.25 m in both trial years, but significance of moisture content at the top soil layer on winter wheat yield was found only in year 2006.
4. Analysis of correlation between soil moisture and area of flag leaf showed significant effect of moisture at subsoil layer in places with winter wheat yield level above 7.00 t ha^{-1}.
5. Partial correlation analysis showed that exclusion of the effect of organic matter content and relative height above the sea level makes no significant changes in conclusions about soil moisture influence on the formation of winter wheat yield.

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BIOETHANOL OUTCOME FROM WINTER RYE, TRITICALE AND WHEAT DEPENDING ON N-FERTILIZER RATE

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Abstract. Grain after enzymatic treatment, which is a starch-containing raw material, is used for ethanol production. Bioethanol production in Latvia began in 2006. Extraction of biofuels is a clean process, because the byproduct is used in various sectors of the economy. The bioethanol in Latvia was derived primarily from winter wheat, winter rye, and winter triticale. The objective of the research is to determine the different nitrogen fertiliser rates required for winter cereal crop yields and bioethanol outcome. The trials were carried out from 2005 to 2008 in Agricultural Science Centre of Latgale (Latvia). The method (ethanol outcome) is based on fermentation of saccharified cereal samples by yeast *Saccharomyces cerevisiae* followed by the calculation of ethanol outcome and speed of fermentation. The highest starch content was in winter wheat and winter triticale grain, but the lowest - in winter rye grains. A close negative correlation ($p<0.05$) was found for winter triticale and winter wheat between the ethanol outcome and thousand grain weight. Production of bioethanol from rye starch content is used with full utilisation of grain. The winter wheat has the largest ethanol outcome from one hectare.
Key words: bioethanol, N fertilizer rate, winter wheat, winter rye, winter triticale.

Introduction

Bioethanol is ethanol which is produced through fermentation of the biomass for use as a fuel. The grain after enzymatic treatment is starch-containing raw material which is used for bioethanol production (Enerģētisko..., 2007; Kalniņš, 2009). As bioethanol can be obtained from grain, it counts as a renewable fuel. One litre of ethanol replaced about 0.65-0.75 litre of gasoline (Kalniņš, 2009) or the energy value of the coefficient for bioethanol - 32% less than fossil petrol (Kalniņš, 2006).

Cars in Latvia were powered by biogasoline before the Second World War. The fuel from the gasoline and ethanol was called the latol. According to A. Kalniņš (Kalniņš, 2009) 70% of gasoline and 30% of ethanol were in the biogasoline in winter-time, and 50% of gasoline and 50% of ethanol were in summer-time.

The use of grain is increasing the production of bioethanol in the world (Булаткин, 2009; Kalniņš, 2009). Bioethanol can also be obtained from low quality grain (Технология..., 1981; Enerģētisko..., 2007). According to the International Grain Council (GC) estimates, in the financial year 2007/08, around 96 million t of grain were used for ethanol production or by 32% more than in the previous year (Kalniņš, 2009). The more ethanol is produced, the lower its cost (Биоэтанол, 2006). This is due to the improvement of the growing and processing technologies. The area for grain cultivation varies Latvia, and winter wheat is grown. In 2010 32 thousand tonne bioethanol productions need 26 thousand ha cereal aria (Benfelde, 2005).

The fermenting grains are used for bioethanol production in Latvia. The main by-product of distilling dregs is liquid of distillation residue, which is used for cattle forage, or as substrata for biogas production (Технология..., 1981; Enerģētisko..., 2007; Kalniņš, 2009). In Sweden, from 2.65 kg of wheat the following products can be produced: one litre of ethanol E100, 0.85 kg of protein for cattle feed, and 0.7 kg of carbon dioxide in carbonated beverages and food cooling (Kalniņš, 2009).

Ethanol production in Latvia began in 2006 (Latvijas..., 2010). The data show that 48% in 2006 of ethanol production in Latvia was exported, but in 2007 and 2008 the export reached already 99%. It means that the production of ethanol is not consumed domestically.

Bioethanol can be obtained from various plants: mainly from corn in the USA, from sugar cane in Brazil, and from grain of wheat, triticale, and rye in Latvia (Grosvalds and Alksnis, 2009; Kalniņš, 2009). Sunlight is also an important and cheap source of energy that plants use for photosynthesis. It is important to increase the yield of plants. Nitrogen is one of the most important elements for crop yield and quality (Jermušs and Vigovskis, 2002).

The objective of the research was to determine the influence of different nitrogen fertiliser rates on the production of winter cereal yields and bioethanol outcome.

Materials and Methods

Winter rye (*Secale cereale* L.), winter wheat (*Triticum aestivum* L.), and winter triticale (*Triticosecale* Wittm) were cultivated for the investigation in 2004/2005, 2006/2007 and 2007/2008. Winter cereals did not survived in winter of 2005/2006. The used winter rye varieties were 'Kaupo' (Latvia), 'Amilo' (Poland), and 'Walett' (Poland), winter wheat varieties – 'Stava' (Sweden), 'Harnesk' (Sweden), 'Bjorke' (Sweden), and 'SW Maxi' (Germany), winter triticale – 'Lamberto' (Poland), and 'Falmoro' (Sweden). The influence of these varieties was not analysed in the research. The field trials were conducted on sod-podzolic sandy loam soil: organic matter content – 27 g kg^{-1} (Tyurin's method), pH 6.7, P – 67.64 mg kg^{-1} of soil, and K – 76.37 mg kg^{-1} of soil (DL method). The

soil parameters were fit for winter cereal cultivation. The pre-crop was bare fallow.

The winter cereals in the trial were sown on 18 September 2004, 14 September 2006, and 14 September 2007. The seeding rate was 450 germinate able seeds per m^2. The field experiment was carried out using a randomised block design. The area of a trial plot was 20 m^2 (2 m × 10 m), 4 replicates. N fertiliser variants were N_{30}, N_{60}, N_{90}, N_{60+30}, and N_{90+30} for winter rye: N_{60}, N_{90}, N_{120}, N_{90+30}, and N_{90+60} for winter triticale: and N_{60}, N_{60+30}, N_{90+30}, N_{90+60}, and $N_{90+60+30}$ for winter wheat. The herbicides and fungicides were used at the plant growth stage (GS) 26-32. The growth regulator was used at GS 32-49. N fertiliser (ammonium nitrate) was applied at GS 20-29 - in spring after renewal of the vegetation growth. 2nd nitrogen fertilizer rate was applied at GS 30-32, 3rd rate – at GS 51-53.

Protein content, starch content, and volume weight of winter grains were determined with *Infratec1241*. 1000 grain weight was determined by standard method LV ST ZM 43-95. Quality and harvest traits were calculated at 100% purity and 14% moisture level.

Ethanol fermentation was carried out with different parameters of evaluation for the winter species. The method is based on fermentation of a saccharified wheat sample by yeast *Saccharomyces cerevisiae* followed by the calculation of ethanol output and speed of fermentation (Технология..., 1981; Enerģētisko..., 2007; Vigants et al., 2008). The ethanol outcome for the 2008 harvest was determined in the Laboratory of Institute of Microbiology and Biotechnology of the University of Latvia.

Work procedure:

1) Inoculum's preparation. The used *Saccharomyces cerevisae* strain was maintained on an agar medium. The inoculum for fermentation was prepared by transferring the yeast strains from the agar on the liquid malt medium and by cultivating for 24 hours at +30 °C (Vigants et al., 2008). Liquid malt medium for inoculums propagation: sucrose, 100.0 g L^{-1}, $(NH_4)_2SO_4$ 1.6 g L^{-1}, KH_2PO_4 2.5 g L^{-1}, $MgSO_4$ x $7H_2O$ 1.0 g L^{-1}, yeast extract 5.0 g L^{-1}, malt extract 50.0 g L^{-1}. The volume was made up with distilled water to 1 L, the initial pH of the medium was 5.35-4.55.

2) Saccharification. The sample of 20 g of ground cereals and 80 g of water was taken and mixed. Liquefaction of the sample was done by 54 µL of α-amylase preparation *Talzyme AL90* (JPBiotechnology) for 40 min at 90 °C. Then pH was adjusted to 5 and the mixture was cooled to 60 °C. Saccharification was done by 400 µL of glucoamylase *Talzyme GL60* (JPBiotechnology) for 40 min at 60 °C. Liquefaction and saccharification were performed by constant stirring of the sample.

3) Fermentation. After saccharification the sample was cooled to 30 °C. They 2 mL of *Saccharomyces cerevisae* inoculum were added, and batch fermentation in the flasks was carried out at 30 °C for 1-2 days. The flask weight changes were measured during the fermentation. When the weight became constant (which indicates the end of fermentation), a sample was taken and ethanol concentration was determined by gas chromatography.

4) Ethanol from the theoretical result was calculated from the starch data (Технология..., 1981):

$$c_{gly\cos e}(\%, w/w) = \frac{c_{starch}(\%, w/w) \cdot 180.16}{162.16} \quad , (1)$$

$C_6H_{12}O_6 \rightarrow 2CO_2 + 2C_2H_5OH + 118$ kJ
(Технология..., 1981; Enerģētisko..., 2007)

$$c_{eth.theor}(\%, w/w) = \frac{c_{gly\cos e}(\%, w/w) \cdot M_{gly\cos e}}{K \cdot 2 \cdot M_{ethanol}} \quad , (2)$$

where K – the dilution factor, which in our case it is 5 (fermentation medium: 20% of corn, 80% of water); *M* – molar mass for ethanol and glucose.

$$c_{et(\%\,from\ theoretical)} =$$

$$= \frac{(c_{eth.theor}(\%, w/w) - c_{ethanol}(\%, w/w)) \cdot 100\%}{c_{eth.theor}(\%, w/w)} \quad , (3)$$

Ethanol outcome from grain calculated formula No. 4.

$$(g_{\ ethanol} \text{ on } g_{\ grain}) = c_{eth.}(\%, w/w) / C_{\ grain} (\ g/100\ g\ ). \quad (4)$$

5) Determination of ethanol. Ethanol content was determined by gas chromatography *CHROM 4*, the thermostat temperature 80 ºC, the evaporator temperature of 200 ºC, with a flame-ionization detector. Content: A 1.2 m length x 3.0 mm (inner diameter) filled column, filling: Inerton AW-HMDS + 5% PEG, carrier gas: helium (Vigants et al., 2008).

Mathematical data processing analysis was used from ANOVA.

The weather conditions during the trial years were different.

The dry and cool weather delayed winter vegetation to the 3rd ten-day period of April in 2005. In, June the weather conditions were favourable for the growth and development of winter cereals. Also in July the weather contributed to a normal maturation of winter cereals. Excessive moisture content of grain during the ripening period negatively affected the quality of the grain.

In 2007, the winter cereals endured in the winter relatively well. In May, the sufficient rainfall positively affected plant growth and development. In June the rainfall was 69% of the long-term average, but in July it adversely affected the plant development and grains began to germinate in the ears.

The rainfall and the air temperature of the stage of cereal growing in tufts (GS 20-29) were more than the long-term average in the vegetation period of 2007/2008.

In the third ten-day period of May 2008, the air temperature was close to the possibility long- term average, but there was no rain. The rainfall was half of the long-term average. In winter cereals, the

stalks of the lower leaves became sallow. In July the average daily temperature corresponded to the long-term average. The amount of precipitation in the 1st and 3rd ten-day period of July was only 40% of the long-term average, but in the second ten-day period it was 173% of the long-term average. In the first ten-day period of August, the daily temperature was 15.8 °C, close to the long-term average, but the rainfall was 86% of the long-term average.

Results and Discussion

The influence of N fertilizer rates for food grains on the bioethanol outcome is analyzed in this paper.

Nitrogen fertiliser rates N_{60} kg ha^{-1} and N_{90+30} kg ha^{-1} which were compared, were the same for all winter cereal species. The highest average yield per all N treatments was obtained from winter wheat (Table 1). The highest yield was obtained when applying nitrogen rates N_{90+30} kg ha^{-1} for winter rye, N_{90+60} kg ha^{-1} for winter triticale, and $N_{90+60+30}$ kg ha^{-1} for winter wheat. The total nutrient demand for wheat is greater than for other cereals (Jermušs and Vigovskis, 2002).

Since ethanol is obtained from starch, the starch content should be as large as possible. The highest starch content in our trials was determined in winter triticale grain (Table 1), but the lowest – in winter rye. Our results correspond to the results of other investigations (Шаршунов et al., 2009). Also other studies confirme that in Latvia the production of bioethanol is the most advantageous from wheat (Bonāts and Sīviņš, 1978; Enerģētisko..., 2007; Grosvalds and Alksnis, 2009). The time of nitrogen fertiliser application affects also the starch content (Bonāts and Sīviņš, 1978). Significant fluctuations in the starch content over the years (Table 1) could be explained by the meteorological conditions during the growing season. The relatively high rainfall and relatively low temperature during the growing season increase the starch content in grains (Bonāts and Sīviņš, 1978). In our research the cultivation year and N fertilizer rates had significant influence on the winter cereal yield and starch content. The same conclusions have been made also by other researchers (Gaile and Kopmanis, 2002).

Table 1

Quality indicators for winter cereals

Nitrogen fertiliser rates*, kg ha^{-1}	Winter rye			Winter triticale			Winter wheat		
	2005*	2007*	2008*	2005*	2007*	2008*	2005*	2007*	2008*
Harvest yield, t ha^{-1}									
N30	5.47	6.83	7.33	-	-	-	-	-	-
N60	6.09	7.02	7.52	9.07	6.87	8.41	8.06	7.54	10.03
N90	6.66	7.68	8.10	9.23	7.20	8.73	-	-	-
N120	-	-	-	9.58	750	8,86	-	-	-
N60+N30	7.01	8.05	8.55	-	-	-	8.77	8.01	10.59
N90+N30	7.42	8.84	9.05	9.70	7.44	9.02	9.27	8.52	10.75
N90+N60	-	-	-	9.91	8.02	9.40	9.85	8.98	11.17
N90+N60+N30	-	-	-	-	-	-	10.25	9.34	11.38
Average	6.5	7.7	8.1	9.5	7.4	8.9	9.2	8.5	10.8
$LSD_{0.05A}$	0.04			0.03			0.03		
$LSD_{0.05C}$	0.05			0.04			0.03		
$LSD_{0.05ABC}$	0.12			0.09			0.12		
Starch, g kg^{-1}									
N30	613	619	643	-	-	-	-	-	-
N60	616	629	639	673	673	712	672	575	697
N90	609	621	634	676	675	707	-	-	-
N120	-	-	-	665	670	706	-	-	-
N60+N30	612	618	633	-	-	-	674	574	691
N90+N30	611	617	625	676	666	703	667	575	693
N90+N60	-	-	-	670	668	705	662	573	690
N90+N60+N30	-	-	-	-	-	-	656	569	686
Average	612	619	635	672	670	706	666	573	691
$LSD_{0.05A}$	19			11			7		
$LSD_{0.05C}$	24			14			9		
$LSD_{0.05ABC}$	58			34			31		

* Factor A – year; Factor B – variety; Factor C – fertilizer rate

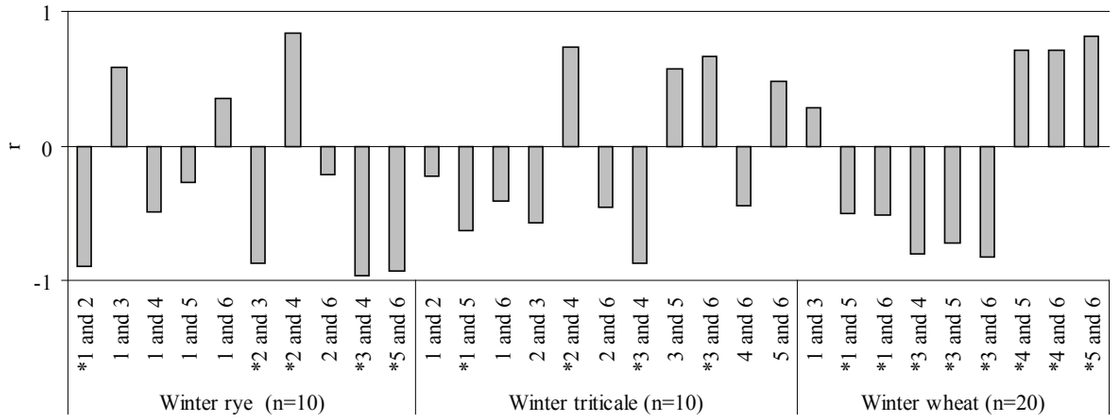

Figure 1. The interaction between average yield and average quality indices of winter cereal in 2008: * ($p<0.05$), 1 – g by ethanol g^{-1} of grain, 2 – yield, 3 – starch, 4 – protein content, 5 – 1000 grain weight, 6 – volume weight.

For bioethanol production, raw material composition characteristics are very important. After starch the amount of protein is the next important component for bioethanol production. Proteins are alcohol yeast nutrients, which increase the quality and outcome of alcohol (Шаршунов et al., 2009). Correlation analysis (r) showed that there was a strong ($p<0.05$) negative correlation between starch and protein content in grain (Fig. 1) for winter cereals (Linina and Ruza, 2005; Ruzgas and Plycevaitene, 2005). A close ($p<0.05$) negative correlation was found for winter triticale and winter wheat between the ethanol and 1000 grain weight, because ethanol is produced from grains with a higher 1000 grain weight. A weak ($p>0.05$) positive correlation was found between ethanol and starch content in grain.

The starch content in winter grain is different, therefore the theoretical outcome of ethanol is also different. This explains the high level of the theoretical ethanol rye yield (Fig. 2), which, in its turn, that is explained by the differences between rye and wheat grains.

Our test results showed (Fig. 3) that for production of one tonne of bioethanol on average 3.2 tonne of rye, 3.1 tonne of triticale, and 3.2 tonne of wheat are required. It can be said that production of one tonne of bioethanol requires approximately 3.7 tonne of grain or 1 m^3 of bioethanol needed about 2.8 tonne of grain (Kalniņš, 2009). Areas of winter wheat and winter triticale will be smaller if we take one tonne of bioethanol (Биоэтанол, 2006).

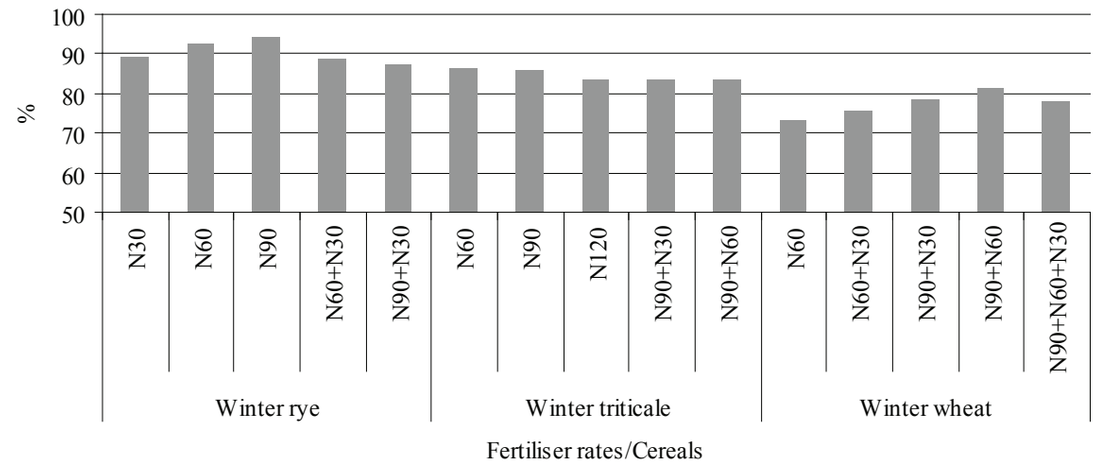

Figure 2. Average ethanol outcome from the grain harvested in 2008, % of theoretical (calculated from real starch content data).

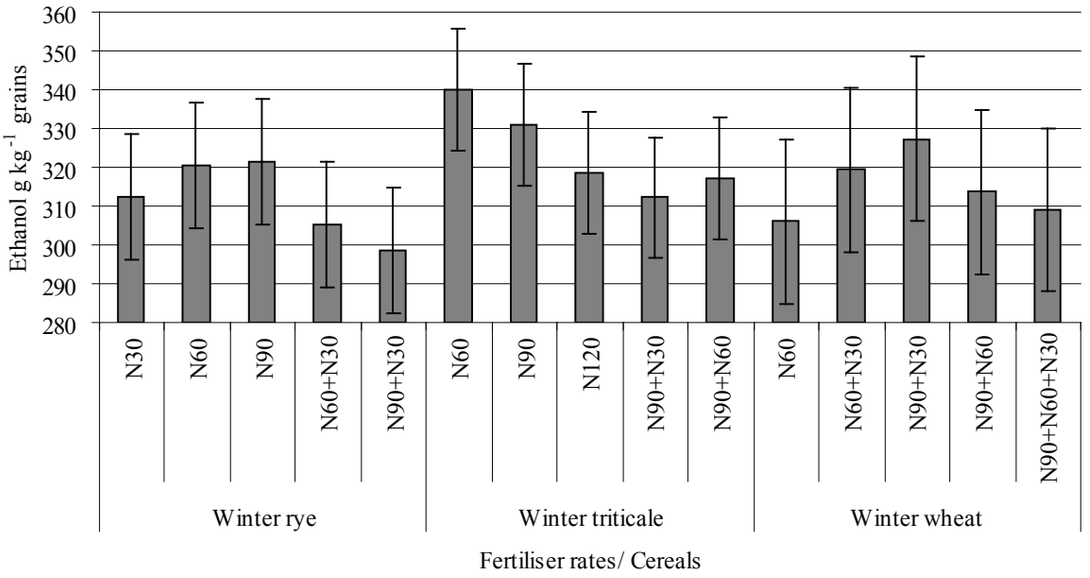

Figure 3. Average ethanol outcome, g kg^{-1} of grain in the laboratory from the 2008 harvest.

The proportion for the factors influencing on the ethanol produced in the laboratory g g^{-1} grain from the 2008 harvest follows ($p<0.05$) was the following: nitrogen fertiliser rate for winter rye 36% and winter triticale 16%, interaction between variety and nitrogen fertiliser rate for winter triticale 21%. For winter wheat all these factors were not important ($p>0.05$).

Although rye starch content in bioethanol has the highest value (Fig. 2), the results of ethanol per hectare of wheat were higher (Fig. 4). In other investigations, one litre (0.7 kg) of ethanol production required 2.57 kg of wheat, 2.73 kg of rye, and 2.67 kg of triticale (Kalniņš, 2006).

One of the most important indicators is the ethanol outcome from one hectare. Comparing nitrogen fertiliser rates N_{60} kg ha^{-1} and N_{90+30} kg ha^{-1}, it can be seen, that winter wheat was the most responsive to nitrogen application (Fig. 4).

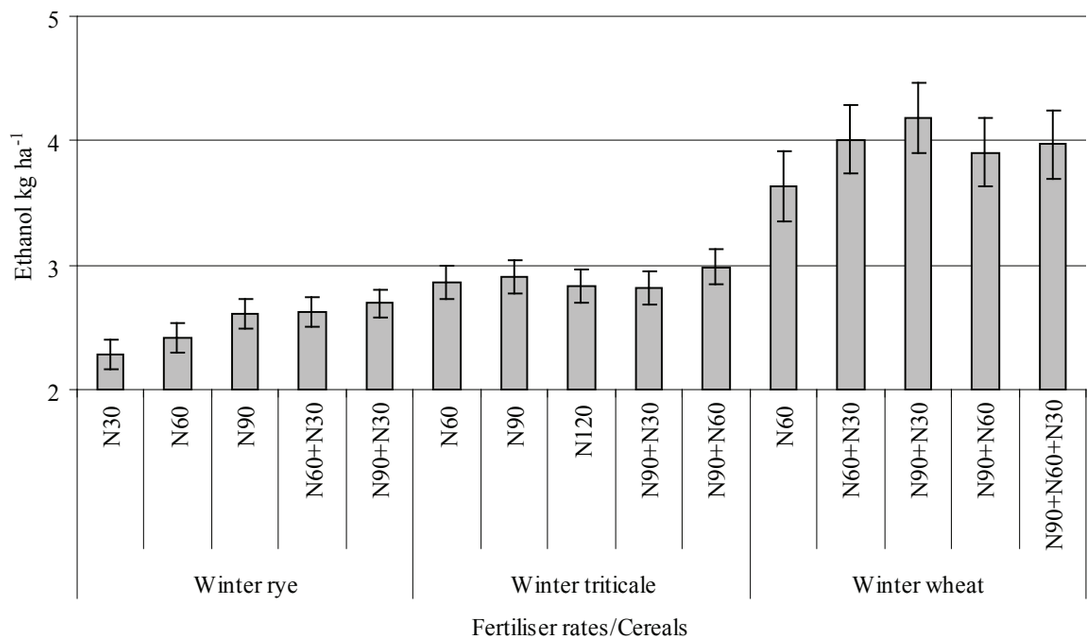

Figure 4. Ethanol outcome from one hectare in 2008, kg ha^{-1}.

In assessing the various factors influencing the ethanol outcome from one hectare, it was found that nitrogen fertiliser rate had a significant ($p<0.05$) influence on the ethanol outcome from winter rye (75.0%) and winter wheat (22.6%), but significant ($p<0.05$) variety and N fertiliser rate interaction effect was found only for winter triticale (19.8%).

Now in Latvia growed till cereal varieties with great protein and gluten content. They are used for food production. Besides the food grains have a higher purchase price. Therefore it is important to find the optimal N fertilizer rates for obtaining large yields as well as grain with a high starch content.

Conclusions

1. Nitrogen fertiliser rates N_{60} kg ha^{-1} and N_{90+30} kg ha^{-1} which were compared were the same for all winter cereal species. The highest yield was obtained from winter wheat.
2. The highest starch content was found in winter triticale grain, and the lowest – in winter rye grain.
3. Correlation analysis showed that there was a strong ($p<0.05$) negative correlation between starch content and protein content in winter cereal grain. A close ($p<0.05$) negative correlation was for winter triticale and winter wheat between the ethanol and 1000 grain weight.
4. The production of one tonne of bioethanol required 3.2 tonne of rye, 3.1 tonne of triticale, and 3.2 tonne of wheat. In order to obtain one tonne of bioethanol, winter wheat and winter triticale needed the smallest areas.
5. The proportion of factors ($p<0.05$) influencing the ethanol produced in the laboratory g g^{-1} of grain, from the 2008 harvest was the following: nitrogen fertiliser rate for winter rye - 36%, and for winter triticale - 16%, interaction between variety and nitrogen fertiliser rate for winter triticale – 21%. For winter wheat all these factors were not important ($p>0.05$).
6. In assessing the various factors influencing the ethanol outcome from one hectare, it was found that nitrogen fertiliser rate had a significant ($p<0.05$) influence on the ethanol outcome from winter rye (75.0%) and winter wheat (22.6%).

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IMPACT OF SOME AGROECOLOGICAL FACTORS ON WINTER OILSEED RAPE (*BRASSICA NAPUS* L.) PLANT DENSITY

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Abstract. Winter rape (*Brassica napus* L.) significance among field crops is unchangeable for last decade in Latvia. Rape plant development in autumn, plant wintering and plant yielding is partly influenced by plant density. The aim of our research, started in autumn 2007 in the Research and Study farm "Vecauce", was to investigate the influence of agroecological factors (sowing date, sowing rate and fungicide (as growth regulator in autumn) application) on two type (line and F_1) winter rape varieties' plant density in autumn, in spring and at harvesting. Plant density in autumn, in spring and during harvesting was determined also by meteorological parameters as air temperature and precipitation. Oilseed rape field germination was observed from 84% sown on 1st of August in 2007 till 67% sown on 1st of August in 2008. A significant impact ($p<0.05$) of the sowing rate was noted for plant winterhardiness (in points) for variety 'Californium' in both years: 2008 and 2009. Fungicide application effect on plant survival during winter was observed for treatments with greater plants density. A very little average plant number decrease per m^2 during summer growth period was noted for all treatments in both years - within 1 to 4% in some cases. The tendency was observed that final plant density at harvest if greater sowing rates had been used, was never high on average for 'Californium' from 75 plants per 1 m^2 (120 germinable seeds per m^2) to 60 plants per 1 m^2 (100 germinable seeds per m^2) in 2009. Correlations between plant density at harvest time and seed yield were found.
Key words: winter rape, sowing date and rate, growth regulator, plant density changes.

Introduction

Significance of rape (*Brassica napus* L.) among field crops in Latvia is stable, and its proportion has increased during the last 15 years. The area under rape during very last years has shown some stabilization: 99 600 ha in 2007, 83 000 ha in 2008, and 93 900 ha in 2009. On-going rapeseed export and capacity increase of Latvia biofuel factories' production is a guarantee for stable rape growing development in Latvia. Latvia, especially central region, has suitable soils and meteorological conditions for winter rape cultivation. The area sown with winter rape is increasing (from 43% in 2005 to 66% in 2007 from the total rape sowing area) in central Latvia region - Zemgale (Central Statistical Bureau of Latvia).

Plant survival during winter and adverse spring is one of the key factors for successful growing of winter oil-seed rape. Wintering of rape depends on two aspects: (1) meteorological conditions for rape growth during autumn before wintering and (2) the plant development stage in the autumn, which could be affected by the growing manner including used cultivar, and agro-meteorological factors.

Rape seed germination is affected by soil moisture and temperature (Kondra et al., 1983; Rapacz, 1998a; Rapacz et al., 1998; Diepenbrock, 2000). Soil moisture is critical as it affects how quickly water penetrates the seed. Dry and cold seedbed will result in reduced and delayed germination, reduced rate of seedling emergence and may inhibit germination altogether until a rain occurs. Important rape plant development functions such as evapotranspiration, photosynthesis, water and nutrient absorption and other biological and chemical activities are regulated by temperature (Rapacz, 1998b). Rape is a relatively cool season crop, in that its best growth occurs above 12 °C and below 30 °C (optimal from 18 °C to 22 °C). Also water is essential for plant growth. The amounts and duration of rainfall cannot be controlled and may be a limiting factor to crop growth unless irrigation is applied, and too much or too little water at any particular growth stage reduces yield potential (Good et al., 1993). Other factors such as light (day length), nutrition and variety also play a role to plant development in autumn, but they generally are of secondary importance (Thomas, 2003).

The above mentioned factors can affect rape plant development and plant density in autumn. Plant density has the greatest effect on seed yield and the yield components of individual plants. Optimum crop density for successful wintering is reported 30 to 70 plants m^{-2} (Leach et al., 1999; Velicka, 2003). Our previous research showed that application of a growth regulator affected the number of leaves per plant and the root-neck diameter, and decreased the height of growth-point of winter rape, thus favouring winterhardiness of the crop (Balodis et al., 2008; Balodis et al., 2009).

In agro-ecological conditions of Latvia, data on winter rape plant density changes from autumn through winter and the following summer growth period is little documented.

The aim of currently described section of our research was to investigate the influence of agroecological factors (sowing date, sowing rate and fungicide (as growth regulator in autumn) application as well as moisture supply and temperature) on two type winter rape varieties' plant density changes from sowing till harvesting.

Materials and Methods

The investigations were carried out on winter oilseed rape (*Brassica napus* ssp. oleifera) plants. Three- factor field trials using two type winter rape

cultivars (line 'Californium' and hybrid 'Excalibur') were carried out in the Research and Study farm 'Vecauce' of Latvia University of Agriculture starting from 2007/2008 and continuing till 2009/2010; the paper is focused on plant density results for seasons 2007/2008 and 2008/2009.

Factor A - sowing date:

- 1st – called 1st August (exactly 2nd August – 2007; 1st August – 2008),
- 2nd – called 10th August (exactly 10th August – 2007; 11th August – 2008),
- 3rd – called 20th August (exactly 20th August – 2007; 21st August – 2008),
- 4th – called 1st September (exactly 31st August – 2007; 30th August – 2008),
- 5th – called 10th September (exactly 10th September – 2007; 9th September – 2008).

Factor B – fungicide application (B1 – control, without fungicide; B2 - fungicide applied as growth regulator). Fungicide application scheme: dose (0.5 L ha^{-1}) of fungicide juventus 90 s.c. (metconasol 90 g L^{-1}) was applied at the 4-6 leaves stage (for rape sown on 1st August - on 30th August 2007, on 8th September 2008; sown on 10th August – on 12th September 2007, on 13th September in 2008; sown on 20th August – 27th September 2007, on 8th October in 2008); no fungicide was applied to rape sown on fourth and fifth sowing dates, because rape plants did not achieve the necessary stage for fungicide application for growth regulation at the first ten-day period of October.

Factor C – four different sowing rates for each cultivar: 120, 100, 80, and 60 germinable seeds per 1 m^2 – 'Californium'; 80, 60, 40, and 20 germinable seeds per 1 m^2 – 'Excalibur'. A treated seed was used.

Soil at the trials' site was strongly altered by cultivation loam with pH KCl = 7.2 to 7.4; content of available for plants K was 169 to 194 mg kg^{-1} and P - 100 to 115 mg kg^{-1}; humus content - 32 to 38 g kg^{-1}. Pre-crop was cereal mixture for silage in both years. Herbicide raundap gold s.c. (gliphosate 450 g L^{-1}), 3.0 L ha^{-1}, was used two weeks before traditional soil tillage with mould-board ploughing. Rototilling was used before sowing. The crop was fertilized with a complex mineral fertilizer at the rate of N 12 to 28 kg ha^{-1}, P 18 to 30 kg ha^{-1}, and K 79 to 103 kg ha^{-1} before sowing depending on a year. Sowing was done according to the previously described design. Weeds were controlled using herbicide butisan star s.c. (metasachlor 333 g L^{-1} + kvinmerac 83 g L^{-1}), 2.5 L ha^{-1}, when the rape was fully germinated in plots of first three sowing dates in 2007 and 2008. For plots of 4th and 5th sowing date, the herbicide was not used in autumn 2007 (lontrel 300 s.c. (clopiralid 300 g L^{-1})), 0.5 L ha^{-1}, was used in spring 2008), but in autumn 2008 butisan star s.c. was used directly after sowing at previously mentioned rate.

Rape plant density was determined by counting plants in autumn after full emergence had occurred, in spring after renewal of vegetative growth, and exactly after harvesting in one constant 0.5 m^2 area of each plot. Winterhardiness of plants was evaluated using two approaches:

1) condition of plants in plots was visually evaluated in autumn and then – again visually - in spring. Visual assessment of every plot according to methodology used in the official test of 'Value for cultivation and use', was given:
   - 9 points (100% of plants survived),
   - 7 points (up to 25% of plants dead),
   - 5 points (up to 50% of plants dead),
   - 3 points (up to 75% of plants dead),
   - 1 point (100% of plants dead);
2) winterhardiness was calculated as percentage of survived plants from those counted in autumn.

ANOVA procedures were used for processing the experimental data. Also correlation analysis was used.

Mean air temperatures in August and September 2007 (17.9 °C and 11.9 °C respectively) were higher than in 2008 (16.4 °C and 10.6 °C). Air temperature only in the first ten-day period of September 2008 was higher than that in 2007 (14.1 °C and 11.2 °C respectively, Figure 1.)

Precipitation in autumn 2007 was apportioned, and soil moisture was appropriate for successful seed germination. The period before 1st August was rich in precipitation thus ensuring soil moisture for seed germination also for the next sowing time. Second ten-day period of September was warm and rainy, which had an effect on good seedling germination even for late sown (10th of September) plants (Figure 1).

Different was autumn 2008, when the first significant rain for seed germination was recorded only on 14th August that negatively affected seed germination of rape sown on 1st August. Also the ten-day period before 1st sowing time was extremely dry (Figure 1). A long-lasting rain was recorded from 21st August to 29th August (totally 45.6 mm), which made some difficulties for successful rape drilling on 3rd sowing date. Weather in September 2008 was cool and dry (only 1 mm of rainfall in the second decade, and totally 14 mm of rainfall per month). Summarizing meteorological conditions of both autumns of the research years, it can be said that meteorological conditions were considerably different. Unusually long-lasting autumns and warm winters (2007/2008 and 2008/2009) were observed for both research years.

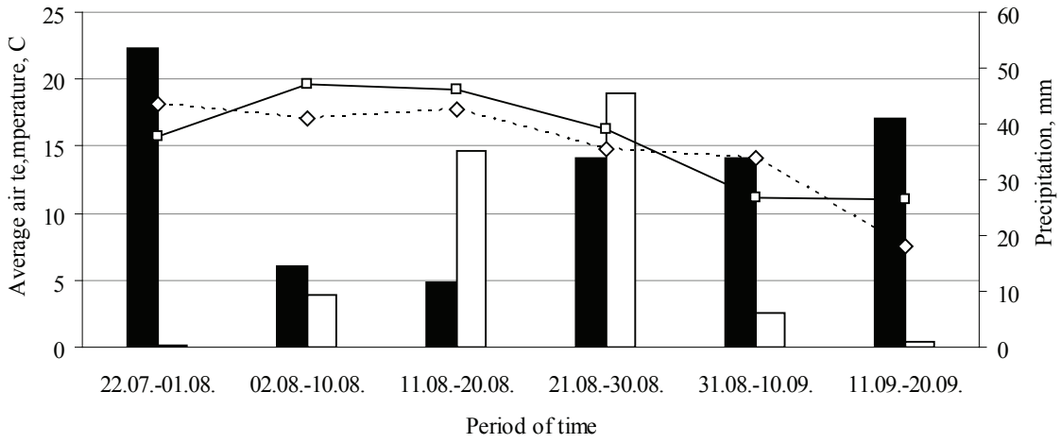

Figure 1. The average air temperature and precipitation in RSF 'Vecauce' in autumn 2007 and 2008 (■ - precipitation in 2007; □ - precipitation in 2008; —— - average air temperature in 2007; – – – - average air temperature in 2008).

Frosts and particular temperature fluctuations that can damage plant growth in spring were not observed in both trial years. Plant damage (central bud and leaves) during winter 2007/2008 was damaged by wild doe for rape sown in first two sowing dates; the level of plant injury did not cause additional plant death in spring 2008. Pools were observed in plots on 1st and 2nd sowing date in spring 2009, which influenced plant survival during winter. Summer for both trial years was without untypical meteorological conditions that could cause greater plant death during summer vegetation.

Results and Discussion

Oilseed rape germination and development during autumn

The emergence of seedlings depends on moisture, temperature, and the seedbed quality. Our observations showed that appropriate soil moisture and productive precipitations were very significant for successful seed field-germination. Oilseed rape sown in the year 2007 had more even field germination (from 98% sown on 20th of August to 84% sown on 1st of August) (Figure 2); exception was rape sown on 1st of September 2007 – 63%. Rapeseed field germination was highly influenced by soil moisture, especially in 2008 when it was affected by drought in the beginning of August when soil humidity was insufficient. Rainfall period in the third decade of August affected drilling quality on 3rd (in 2008) and 4th (in 2007) sowing dates. For those sowing dates rape germination was influenced mainly by drilling quality (which in turn was affected by excessive soil moisture) in both trial years. Better average field germination was observed in the second (10th August) and third (20th August) sown plots in both trial years. Overly high field germination was observed on the 3rd sowing date (10th August) – 105% - and on the 4th (1st September) sowing date in 2008 – 114%. That could be the result of excellent seedbed quality, soil moisture, and temperature conditions, but in addition the reason could be inaccurate germination test result in combination with some technical inaccuracy of the seeder. Our results agree with Diepenbrock (2000) that percentage germination of rapeseed in a standard test is correlating poorly with field performance. Kondra et al. (1983) found that seed germination of rape seed at different temperatures varies a little, which also agrees with our observations of moisture importance for seed germination.

Research results from Canada (Thomas, 2003) have shown that temperature is one of the most important environmental factors regulating growth and development of rape. Our results confirm that rape plant development is considerably different in each trial year. Our previous research results (in detail described in Balodis et al., 2009) showed that sowing date was the main factor which had a strong and significant impact on biometrical parameters of rape plants in autumn. Mainly is early sowing connected with a higher temperature during plant autumn development (Figure 1). Early sowings are often characterised by profuse autumn growth which sets the base for excessive canopy growth in spring. Sowing later can reduce canopy size in spring, but unsuitable late sowing results in small plants which are most vulnerable during wintering. An earlier sowing date significantly increased height of growth point, root neck diameter, plant and root mass, and main root length ($p<0.05$) for both cultivars.

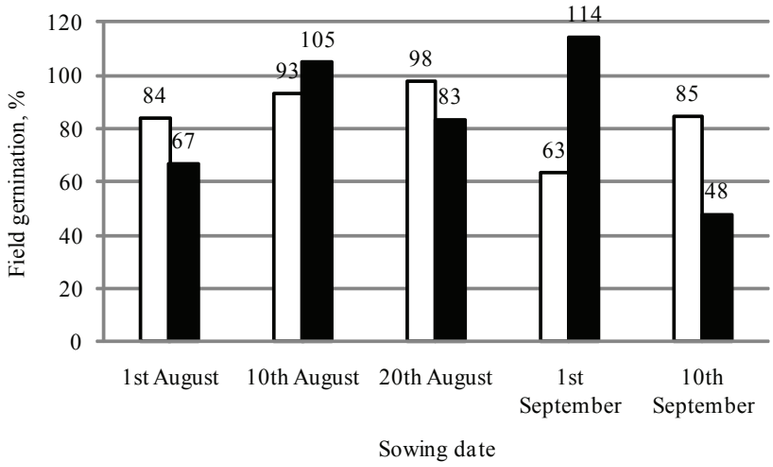

Figure 2. Average (per all sowing rates and both cultivars) winter rape seed field germination depending on the sowing date in 2007/2008 and 2008/2009, % from sown germinable seeds (□ - 2007, ■ - 2008).

Analyses of winter rape growing in Lithuania have shown a highly significant relationship between the leaf area and the air temperature expressed as growing degree-days (GDD) (Sidlauskas et al., 1999). GDD parameters that characterize winter rape autumn growth have to be studied deeper in future also in Latvia's conditions.

Lithuanian researchers found that air temperature was of particular significance in the determination of the leaf area. At 552 °C GDD, only the area of 5 primary leaves reached a plateau, which means that the maximum leaf size was obtained. Marked differences to leaf area were observed between the sowing dates. Due to higher air temperature, earlier sown plants developed a bigger leaf area (Sidlauskas et al., 1999).

The day length closer to the end of the vegetative growth decreases in Latvia (from 16 h 14 min on 1st August to 13 h 12 min on 10th September), which influences plant ability to accumulate necessary chemical and physical components for successful wintering and vigorous spring growth. Day length differences also ensures sharply different plant development during autumn (Thomas, 2003).

Oilseed rape plant density in spring and its winterhardiness

Winter oilseed rape winterhardiness or survival of plants till spring is the most important characteristic for the cultivar used in such conditions as in Latvia where winters with sharp temperature fluctuations, black frost and other adverse factors may occur. Possibility to improve rape wintering using any growth regulation in autumn is little documented in Latvia. Some references in the literature show (Gaveliene et al., 2005) that use of growth regulators (auxin analogues) can improve wintering of winter oilseed rape. Also our previous study results showed that fungicide treatment affected winterhardiness which was evaluated in points (1st approach of winterhardiness evaluation) in some winters (Balodis et al., 2008). Winterhardiness evaluated in points was observed within 8 to 9 points in spring of 2008 and 2009 which were excellent wintering results. Fungicide treatment did not increased ($p>0.05$) winterhardiness (in points) in both years for both cultivars. A significant impact ($p<0.05$) of the sowing rate was noted only for winterhardiness (in points) of 'Californium' in both trial years.

Two-year experimental results showed contrary results of winterhardiness depending on the evaluation method. After winter 2007/2008, a essential correlation between the results of the visual method (evaluation in points) and the survived plants calculated as percentage from those established in plots in previous autumn was not observed ($r= 0.112<r_{0.05}=0.444$ for 'Californium', n=20; $r=/-0.083/<r_{0.05}=0.444$ for 'Excalibur', n=20; $r=0.087<r_{0.05}=0.310$ for both cultivars together, n=40). A correlation was found between the same parameters in the year 2008/2009 ($r= 0.597>r_{0.05}=0.444$ for 'Californium', n=20; $r= 0.473>r_{0.05}=0.310$ for both cultivars, n=40), but was not observed for 'Excalibur' $r=0.431<r_{0.05}=0.444$, n=20. It should be pointed out that the manner of observing winterhardiness has to be improved and discussed in winter rape growing sphere in Latvia. Winters were untypically warm and winterhardiness (in points) was similar in both years; however, another growth factors influenced yield results between the years.

From the two-year results (2007/2008-2008/2009) it was evident that plant death during winter was higher when higher was plant density in autumn or when initially higher sowing rate was used. Total average number of perished plants per winter was noted from 31 plants (30% from the plant number in autumn) for cultivar 'Californium' when sown at the rate of 120 germinable seeds per 1 m^2 in the year 2008 till totally survived plants for cultivar 'Excalibur' when sown at the rate of 20 germinable seeds per 1 m^2 in the year 2008 year, but 26 plants (28% from plant number in autumn) for cultivar 'Californium' when

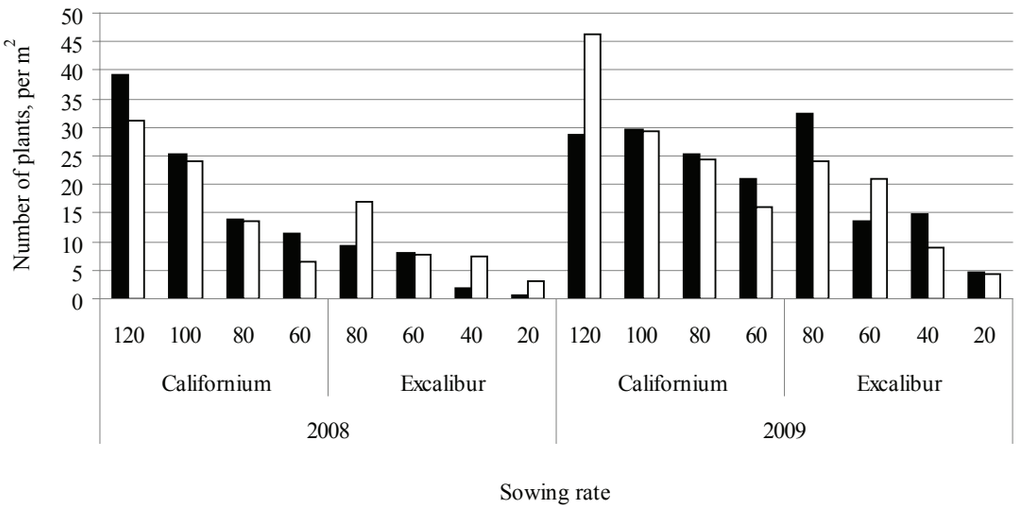

Figure 3. The impact of fungicide application on the average number of dead plants from three sowing dates (1st August; 10th August; 20th August) during winters 2007/2008 and 2008/2009 (■ - control without fungicide; □ - application of fungicide juventus 90).

sown at the rate of 120 germinable seeds per 1 m^2 in the year 2009 till 3 plants (18% from the plant number in autumn) for cultivar 'Excalibur' when sown at the rate of 20 germinable seeds per 1 m^2 in the year 2009. Fungicide application as growth regulator in autumn improved rape plant vitality during winter and in the subsequent spring. An important fungicide application effect on plant survival was observed for plots with greater density (see 'Californium' in 2007/2008 and 2008/2009, Figure 3); exception was 'Californium' with the sowing rate of 120 germinable seeds per m^2 in 2008/2009, which was influenced by pools on the plots. Air temperatures were appropriate for autumn growth and hardening for winter period. Also M. Rapacz and F. Janowiak (1998) reported that lower day temperatures in autumn considerably improved the frost resistance of plants. Winter conditions in 2008/2009 were similar to those of 2007/2008, but the wet and cold spring of 2009 was worse for plant survival if compared to the winter of 2007/2008 and spring of 2008. Due to this on average more plants perished during the winter of 2008/2009.

A very little decrease in average plant number per 1 m^2 during summer growth period was observed for all sowing dates in both years - mainly it was within 0% to 4% with only some exceptions: for 'Californium' 24% plant decrease in 2008 when 100 germinable seeds per m^2 were sown in on the 5th sowing date (2007), and for 'Excalibur' 19% plant decrease during summer 2009 when 60 germinable seeds per m^2 were sown on the 1st sowing date (2008). An effect of sowing date effect on plant decrease during summer was not observed. On the one hand, it is completely clear that in our two-year experiments mainly winter conditions decreased plant density per 1 m^2, but, on the other hand, the winter oilseed rape sowing rate is an important factor that can stabilize on optimal plant density for high seed yields.

Oil-seed rape plant density at harvest time, and yield

Sufficient total plant density at harvest time is the most important base for high winter rape seed yields. Hence, in spite of other meteorological and agronomical factors, winter rape yield can be affected also by the used sowing rate. The tendency was observed that the final rape plant density when higher sowing rates were used was not particularly high - on average for 'Californium' from 75 plants per 1 m^2 (120 germinable seeds per m^2 were sown) to 60 plants per 1 m^2 (100 germinable seeds per m^2 were sown) in 2009 (Table 1). A tendency was noted that average plant density more decreased at earliest and latest sowing dates (Table 1). Also 1st of September is considered to be a little too late sowing date for Latvia, however, the average plant density at harvest was highest for this sowing time in 2009, which was affected by the unexpected extra germination (explained in subsection *Oilseed rape germination and development during autumn*) (Table 1). Final plant densities obtained in our experiment were optimal for a good yield that is reported to be 30 to 70 plants m^{-2} (Leach et al., 1999; Velicka, 2003). An exception is 'Excalibur', when sown only 20 germinable seeds per 1 m^2. There are also other factors like sowing rate that can influence the rape seed yield. Our field experiment results showed that the sowing rate affected the plant density at harvest time significantly ($p<0.05$) for 'Californium' and 'Excalibur' in 2008 and 2009. Results from Germany show that there is not significant seed yield increase for much higher sowing rates than the optimal, but importance of optimal and appropriate sowing rates for different sowing dates is also emphasized (Шпаар, 2007). After all, correlations had been found between the plant density at harvest time and the sowing rate ('Californium' - $r=0.616>r_{0.05}=0.444$, 'Excalibur' –

Table 1

The influence of sowing date and sowing rate on winter rape plant density during harvesting of fungicide - untreated cultivars 'Californium' and 'Excalibur' in the years 2008 and 2009, plants per 1 m^2

Sowing date	'Californium', sowing rate, germinable seeds per 1 m^2								
	120		100		80		60		Average
	2008	2009	2008	2009	2008	2009	2008	2009	
01-Aug	77	26	66	27	43	14	42	11	38
10-Aug	71	74	61	57	46	42	39	46	54
20-Aug	79	85	75	65	72	40	45	33	62
01-Sep	41	139	44	114	42	77	26	54	67
10-Sep	45	51	46	40	31	25	31	20	36
Average	62	75	58	60	47	39	36	33	x
Sowing date	'Excalibur', sowing rate, germinable seeds per m^2								
	80		60		40		20		Average
	2008	2009	2008	2009	2008	2009	2008	2009	
01-Aug	57	27	54	27	35	12	17	8	30
10-Aug	51	63	44	39	38	22	24	17	37
20-Aug	60	73	54	45	35	29	18	9	40
01-Sep	41	98	44	52	42	39	26	20	45
10-Sep	45	51	46	40	31	25	31	20	36
Average	51	62	48	41	36	25	23	15	x

$r=0.870>r_{0.05}=0.444$ in 2008; 'Californium' - $r=0.515>r_{0.05}=0.444$, 'Excalibur' - $r=0.792>r_{0.05}=0.444$ in 2009, n=20).

Still, there are no fixed optimal sowing rates in Latvia's conditions, but our previous research about winter rape yield structural elements in 2008 showed correlation between the plant density at harvest time and the seed yield ('Californium' - $r=0.689>r_{0.05}=0.444$; 'Excalibur' - $r=0.600>r_{0.05}=0.444$, n=20, Balodis et al; 2009.), which agrees with W. Diepenbrock (2000) and R. Velicka (2003). It was found that the effect of fungicide as growth application (in autumn) on the total plant density at harvest time was not significant in both trial years ($p<0.05$).

Highest rape seed yields in 2008 were observed for 'Californium', at the sowing rate of 100 germinable seeds per m^2 (7.19 t ha^{-1}), and for 'Excalibur', at the sowing rate of 80 germinable seeds per m^2 (7.42 t ha^{-1}), sown on 10th and 20th August respectively. Similar in one aspect was the year 2009 when higher seed yields were provided by the same sowing rates for each cultivar ('Californium' - 4.94 t ha^{-1}, and 'Excalibur' – 5.49 t ha^{-1}), but both were sown on 1st September. The sowing rate as an initial reason of plant density at harvest significantly ($p<0.05$) affected the seed yield in 2008 for both cultivars (for 'Excalibur' by 15%, and for 'Californium' by 9%), and for 'Excalibur' by 38% in 2009. An interesting tendency was found that for almost all sowing dates the highest seed yields were noted when the highest sowing rates were used, exceptions were 'Californium' with the sowing rate of 100 germinable seeds per 1 m^2 sown on 1st and 20th August 2007, and 'Excalibur' with the sowing rate of 60 germinable seeds per 1 m^2 sown on 1st September 2007. Affirmation that plant density is an important element that influences the seed yield, was confirmed by the correlations found between plant density at harvest time and seed yield ('Californium' - $r=0.662>r_{0.05}=0.444$, not found for 'Excalibur' in 2008; 'Californium' - $r=0.829>r_{0.05}=0.444$, and for 'Excalibur' - $r=0.637>r_{0.05}=0.444$ in 2009, n=20). If this research is continued at least for the next two years it will be possible to find out the most suitable sowing rate for each sowing date in particular conditions.

Conclusions

1. Our research results confirmed that oilseed rape field germination was mainly affected by soil moisture and productive precipitation. On average, field germination was observed from 84% to 98% with some exceptions when adverse conditions - mainly lack of moisture or too much moisture - occurred.
2. Main periods in which most decrease of initially by sowing rate designed plant number was observed, were field-germination and wintering. More plants perished during winter when higher was plant density in autumn or in its turn – when initially higher sowing rate was used. A tendency that fungicide application (as growth regulator in

autumn) can affect plant survival during winter was noted for treatments with greater plant density. The effect of fungicide application on total plant density at harvest time was not significant in both trial years.

3. Excellent winterhardiness results were noted (from 8 to 9 points) in both trial years when it was evaluated visually in points, but only some correlations between this result (winterhardiness in points) and exact percentage of the survived plants during winter were found. Besides, high rapeseed yields were obtained in spite of contradictory winterhardiness results. Winterhardiness assessment manner has to be improved and discussed in Latvia.
4. Final plant density at harvest is an important factor that influences the seed yield. If this research is continued at least for the next two years it will be possible to find out the most suitable sowing rate for each sowing date in particular conditions.

Acknowledgements

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EFFECT OF SILAGING ON CHEMICAL COMPOSITION OF MAIZE SUBSTRATE FOR BIOGAS PRODUCTION

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Abstract. Maize (*Zea mays* L.*)* is one of the agricultural plants that is suitable substrate for biogas production. Fresh as well as ensiled maize can be used as biogas substrate. Practically, mainly maize silage is used because fresh maize is available only for short term during the vegetative period. The paper is aimed to determine the impact of ensiling on substrate composition of maize hybrids. A field trial was carried out in the Research and Study farm "Vecauce" of the Latvia University of Agriculture (LLU) in 2008 and 2009. Ten (in 2008) to eleven (2009) maize hybrids with different maturity rating according to FAO numbers (FAO 180 – 340) were harvested at three different times beginning on 5 September at fourteen-day intervals. Chopped maize samples of 1-3 kg were ensiled in plastic silos. Samples of fresh and ensiled (for at least 90 days) maize were analyzed detecting 14 parameters in the Scientific Laboratory of Agronomy Research of LLU using standard methods.
Our results showed that ensilaging influence on organic dry matter and hemi-cellulose content was not substantial. High ($p<0.05$) correlation between organic dry matter content of fresh and ensiled samples was noted.
The crude protein, crude fiber, ash and cellulose concentration was greater in the ensiled samples if compared with fresh maize samples in 2008 and 2009. After ensilaging process neutral detergent fiber (NDF) concentration was higher compared with fresh samples in 2009 but lower in 2008.
Key words: maize hybrid, maize silage, biogas, chemical composition.

Introduction

Renewable energy sources draw attention all over the world because they are sustainable, improve the environmental quality and provide new job opportunities in rural areas. In Europe, there is a significant increase in biomass cultivation for bioenergy purpose, especially for biogas production via anaerobic digestion. The growing number of biogas plants causes an increasing demand for crops as a feedstock for agricultural biogas plants in both mono- and co-digestion processes. Most of the crop species and fertilization levels investigated supply more energy, in the form of electricity, compared to the energy used for the production. In 2020, renewable resources shall cover 20% of the primary energy demand within the European Union (Heiermann et al., 2009a).

The use of energy crops as substrate has increased. The predominant and probably most used crop for biogas production is maize. Maize is considered to have the highest yield potential of field crops grown in Central Europe (Cus et al., 2009). Maize is renowned as an outstanding crop for silage. It produces high yields of high energy forage and is easily ensiled.

The biogas yield depends essentially on the chemical composition of the used substrates. Researchers have mentioned that a substrate for an anaerobic digester can consist of a very wide range of bio-degradable materials but generally does not include materials high in lignin as these are very slow to break down under anaerobic conditions.

The value of a crop as a substrate for anaerobic digestion depends on its biomass yield capacity compared to the effort for cultivation and on its ability to produce biogas with high methane content (50–65%). From this point of view, the most suitable plant species are those rich in easily degradable carbohydrates, such as sugar and protein matter and poor in hemicelluloses and lignin which have a low biodegradability (Heiermann et al., 2009b).

Since the growing season is relatively short, and the seasonal availability of plant material promotes the need to store the substrate, this might change its characteristics (water-soluble carbohydrates are fermented to acids and alcohols) and losses might be introduced (Angelidaki et al., 2010). Important factor that affects the energy output from crops is ensiling. The ensiling process is based on lactic acid bacteria converting carbohydrates into preserving organic acids under anaerobic conditions. The keys to good silage making are to harvest at the right plant growth stage, to achieve the correct chop length of the forage, to exclude the oxygen from the silage, and to seal it well (Neureiter et al., 2005). M. Heiermann et al. (2004) mentioned that in general, the ensiled whole crop cereals investigated lead to higher biogas yields and records show a faster digestion process compared to the fresh substrates tested. Many authors have previously reported various crops stored as silage without additives to have equal or higher methane potentials than fresh crops (Lehtomäki, 2006). Higher methane potentials of silages compared to fresh material can be interpreted as the result of partially degrading of structural polysaccharides of plant cells during ensiling. Therefore, ensiling can be regarded as a pre-treatment to biogas production.

J.J. Combs et al. (1993) reported that differences occurred in fresh as well as ensiled samples. Fresh samples had greater dry matter, NDF, hemicellulose, cellulose, and ash content than did the ensiled samples. Lignin and crude protein content were lower for fresh than for ensiled samples. Also A. Lehtomäki (2006)

found that dry matter (DM) and organic dry matter (ODM) concentrations of stored crops were in general lower than those of fresh materials.

Several reports have indicated an increase in concentrations of fiber and crude protein (CP) and a decrease in digestibility when maize forage is ensiled (Darby et al., 2002; Cherney et al., 2007).

Methane production from organic substrates mainly depends on their content of substances that can be degraded to CH_4 and CO_2. Composition and biodegradability are key factors for methane yield from energy crops. Crude protein, crude fat, crude fiber, cellulose, hemicellulose, starch and sugar markedly influence methane formation (Amon et al., 2007). Hybrid selection is one of the most important management practices that can affect the substrate quality of maize silage. Hybrids with a high protein, fat, cellulose, hemicellulose, and starch content and with a high potential for biomass production are especially suitable for anaerobic digestion. Crude fiber does not give much methane (Amon et al., 2004). Maize hybrids have been developed with improved composition quality which results in higher energy intake and thus higher biogas production.

In Latvia, the interest about energy from alternative energy resources and especially for biogas increases (Adamovics et al., 2008). The Ministry of Environment of Latvia has developed a program for biogas production and utilization in the period of 2007-2011. The greatest potential of biogas production is related to agricultural sector: from 13 million m^3 of the biogas produced in 2011 ~ 64% should be produced using substrates from agriculture. The first biogas production project in agriculture is already realized in the Research and Study Farm (RSF) "Vecauce" of the Latvia University of Agriculture in 2008 and animal manure together with plant (mainly maize) biomass are used as substrate. Only some research papers up to date reported on particularities of biomass production on purpose for biogas generation via anaerobic digestion in Latvia. Maize composition depending on hybrid and harvesting time also is little documented, but quality changes in result of ensiling when modern hybrids are used are not documented at all in Latvia.

The paper is aimed to determine the impact of ensiling on substrate composition of maize hybrids.

Materials and Methods

A three factor field trial was carried out during 2008 - 2009 in the Research and Study farm "Vecauce" (latitude: N 56° 28´, longtitude: E 22° 53´) of the Latvia University of Agriculture. Trials were arranged in four replication randomized blocks with plot size of 16.8 m^2. Row width was 0.7 m. Planted population density was 82000 plants per ha. Original seed of ten maize hybrids (Factor A) in 2008 and of eleven maize hybrids in 2009 with different maturity rating defined by FAO number were used: Tango* (standard, FAO 210), Target (FAO 180), Estelle (FAO 200), Salgado* (FAO 200), Silas* (FAO 210), Turini (FAO 220), Ceklad* (FAO 235), Celio* (FAO 250), Cemet* (FAO 260), Celido* (FAO 270), Ronaldinio (FAO 240), Fernandez (FAO 260), KX A8151 (FAO 250), and Cefran (FAO 340). The seven asterisked hybrids were used in both trial years. Soil at the site was strongly altered by cultivation: sand loam with pH KCl – 6.7, available for plants content of P – 112 mg kg^{-1}; K – 99 mg kg^{-1}, and humus content – 19 g kg^{-1}. Traditional soil tillage was used: mould-board ploughing in previous fall, cultivation and rototilling before sowing in spring. The following fertilizers were given: 34 kg ha^{-1} P, 75 kg ha^{-1} K, 148 kg ha^{-1} N (18+70+60). Maize was sown on May 6. Planting was carried out by a hand handled planter at a 3-4 cm depth. Weeds were controlled by spraying herbicides: arrat d.g. 200 g ha^{-1} (triosulfuron 250 g kg^{-1}; dicamba 500 g kg^{-1}) and titus 25 d.g. 30-50 g ha^{-1} (rimsulfuron 250 g kg^{-1}) together with surfactant were applied when maize reached 3-6 leaves stage (1st decade of June in both years). In addition, lontrel s.c. 0.4 L ha^{-1} (clopiralid 300 g L^{-1}) together with estet e.c. 0.5 L ha^{-1} (2.4 - D 600 g L^{-1}) were applied in 2009 for control of *Tussilago farfara* and *Artemisia vulgaris*. Mechanical weeding was used for the remainder of weeds in later maize development stages (in July of both years). Harvesting was done at three different times beginning on 5 September in 2008, and on 4 September in 2009 at fourteen-day intervals. Yield was accounted from 0.7 m^2 at first and second harvest times, and from 8.4 m^2 at last harvest time. Samples were taken for every hybrid from every replication, but all one-hybrid samples were integrated and subsamples formed: for analyses of fresh maize and for ensiling. Maize was chopped after harvest, prior to the ensiling process. Particle size was 0.5-1.0 cm. Chopped maize samples of the size of 1-3 kg were ensiled in plastic silos. Silage was analyzed when at least 90-days long ensiling process was accomplished.

Composition of fresh and ensiled maize were analyzed for all hybrids using standard methods: DM (samples were dried up to constant weight at 105 °C) and ODM (calculated from DM and ash content) content of naturally wet material; all other components were calculated in g kg^{-1} on DM basis: total N (by Kjeldahl method), crude protein (by multiplying total N with the coefficient 6.25; ISO 5983), crude fiber (ISO 5498:1981), cellulose (calculated from NDF and ADF), hemi-cellulose (calculated from NDF and ADF), lignin (calculated from ADF), crude fat (ISO 6492:1999), neutral detergent fiber (NDF) (LVS EN ISO 16472:2006), acid detergent fiber (ADF) (Forage analyses, USA, method 4.1:1993), crude ash (XA) (ISO 5984), total carbon (C) (CS – 500 method). For silage, also pH KCl was detected (GOST 26180: 1984). Results were statistically analyzed using analysis of variance, correlation, T test and descriptive statistics. Sum of precipitation during the same period was 230 mm in 2008, and 327 mm in 2009.

June and September of 2008 were cool, and the season in general was dry if compared with long-term average data; sum of active temperature was 1943 °C.

Table 1

The average day and night temperature and precipitation during maize growing season in 2008 and 2009 in comparison with long term average

Month	Long-term average temperature	Temperature, ºC		Long-term average precipitation	Precipitation, mm	
		2008	2009		2008	2009
May	11.2	11.3	11.0	43	24.2	18.0
June	15.1	14.6	13.7	51	44.2	95.0
July	16.6	17.1	17.1	75	56.8	136.0
August	16.0	16.4	15.8	75	90.2	38.8
September	11.5	10.6	12.9	59	14.8	39.8

Start of the season (May, June) in 2009 was too cold (Table 1) and unsuitable for the development of maize, but average day and night temperature in September was warmer if compared with long-term average data, active temperature sum per season was 2037 °C.

Results and Discussion

Losses of obtained maize fresh material were minimal (0.8% in 2008, and 0.6% in 2009) during the ensiling process.

The ODM content is an important factor influencing the microbial population and activity during ensilaging. Maize was harvested at three different times in course of the vegetation period. DM and ODM content substantially ($p<0.05$) depended on harvest time and increased from September 4-5 till October 3-5 (specific data depends on the trial year). Maize hybrid influence on average ODM content was substantial ($p<0.05$) in analyzed fresh and ensiled samples in both years.

Average ODM content per all treatments was not substantially ($p>0.05$) higher in 2008 (224.6 g kg^{-1}) as compared with 2009 (214.5 g kg^{-1}).

There was a significant correlation between ODM content of fresh and ensiled maize samples ($r = 0.89 > r_{0.05}=0.25$, $p < 0.05$) (Figure 1). Maize hybrid preservation influence on ODM content was not substantial ($p=0.22$). The range of ODM content of maize hybrids per both years and three harvest times (from early September till early October) was wide - from 158.7 g kg^{-1} ('Celido' (fresh) on second harvest time in 2009) to 301.8 g kg^{-1} ('Silas' (ensiled) on last harvest time in 2009).

Scientists reported that the typical range of pH for maize silage is 3.7 to 4.2 (Cherney et al., 2007). Our results showed that values of pH ranged from 3.47 to 4.47 analyzed in both years. There was a high correlation ($r=0.78$; $r_{0.05}=0.30$) between ODM content and pH values of silage samples. The average pH value of ensiled maize was highest on the last harvest date. The pH values of maize decreased from early to late maturity.

There was a significant ($p<0.05$) impact of conditions in the vegetation period on crude ash content of fresh maize samples. A higher ash content per all treatments, 44.6 g kg^{-1}, was noted in 2009 as compared with 39.2 g kg^{-1} in 2008. The crude ash content decreased during plant development. The highest ash content was noted at the first harvest date: 42.9 g kg^{-1} in 2008, and 51.1 g kg^{-1} in 2009. The ash content was significantly lower ($p<0.05$) in fresh samples than in ensiled samples in both years. After ensilaging process, the average per all treatments crude ash content increased from 40.9 to 42.7 g kg^{-1}. A significant ($p<0.05$) impact of the used hybrid

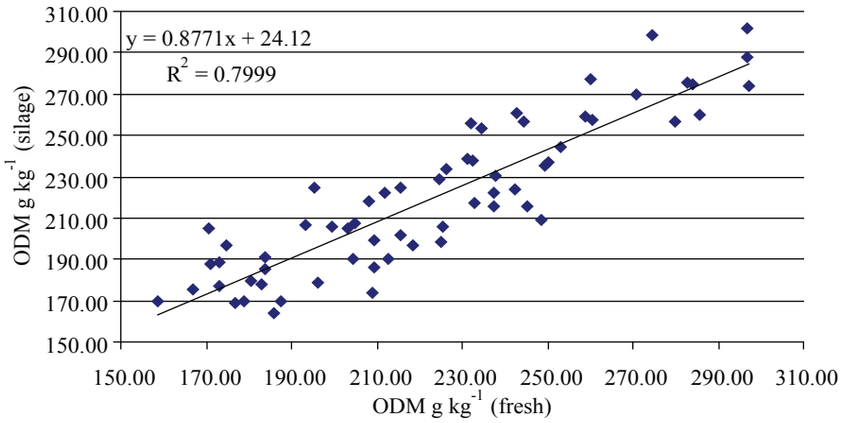

Figure 1. The relationship between ODM content, g kg^{-1}, of fresh maize (x) and ensiled maize (y) samples in both years.

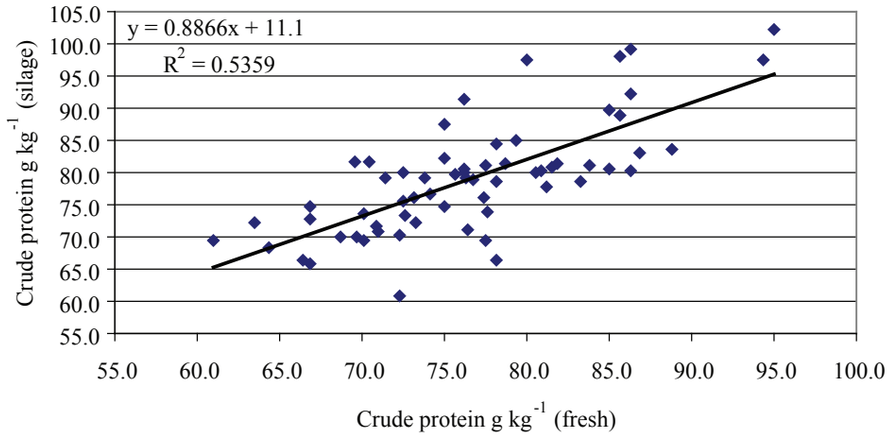

Figure 2. The relationship between crude protein content, g kg^{-1}, of the fresh maize (x) and ensiled maize (y) samples in both years.

on ash content was noted. Correlation between ash concentrations of fresh and ensiled samples was significant ($r = 0.86 > r_{0,05}=0.25$; $p < 0.05$). Scientists have reported that fresh whole-plant samples had greater ($p < 0.05$) insoluble ash than did ensiled samples (Combs et al., 1993).

Crude protein (CP) of ensiled samples highly correlated ($r = 0.77$) with that of fresh samples, but CP was significantly ($p<0.05$) greater in the ensiled samples (Figure 2). Average crude protein concentration per all variants and both years in fresh samples was 76.5 g kg^{-1}, but in ensiled samples - 78.9 g kg^{-1}.

The crude protein concentration decreased during plant development. The highest crude protein content, 91.85 g kg^{-1}, (ensiled sample) was noted on 4 September in 2009, but the lowest, 69.07 g kg^{-1}, (fresh sample) - on 3 October in 2008. There were significant ($p<0.05$) differences in crude protein content in the fresh and ensiled maize samples between the harvest times in both years. D. J. R. Cherney (2007) found increase in protein and fiber and decrease in digestibility for ensiled versus fresh material. I. Filya (2004) mentioned that hemi-cellulose content did not change consistently throughout the growth period of maize but the crude protein content decreased. M. Darby and J. G. Lauer (2002) reported stover quality decrease as harvest dates progressed through the growing season: crude protein ranged from 100 to 67 g kg^{-1} between the first and last harvest date.

Mean difference of average NDF content between fresh and ensiled samples was 7.37 g kg^{-1} in 2008. The NDF content of three maize hybrids increased during the ensiling process (Figure 3). The largest NDF content reduction, 23.2 g kg^{-1}, was noted for hybrid 'Target'. There was significant NDF content of specific hybrids deviation if compared with mean deviation. The vegetation period impact on NDF content was significant ($p<0.05$). The highest NDF content was in 2009. The NDF concentration decreased during plant development. Comparing the results, there were differences in NDF content of ensiled samples between the both years. The NDF content increased during ensilaging in 2009, but decreased in 2008. M. Darby and J. Lauer (2002) observed lower NDF in ensiled samples. I. Filya (2004) reported that the original concentrations of NDF and ADF in the whole crop maize were reduced in the ensiled maize, at least partly because maize is rich in hemi-cellulose.

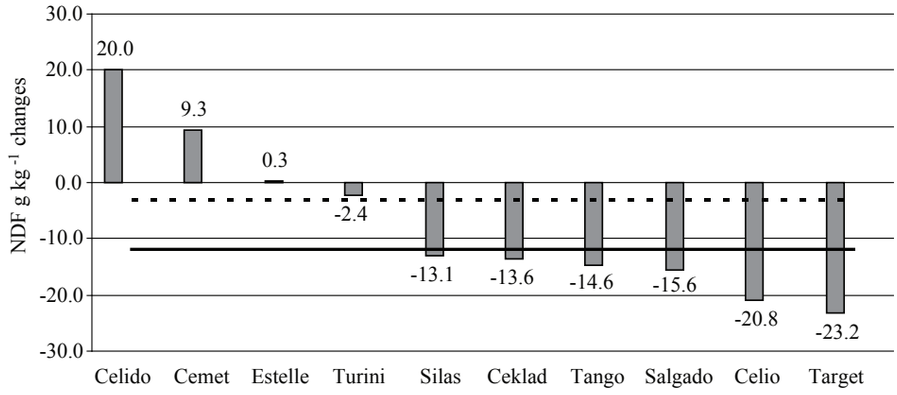

Figure 3. Changes in NDF content after ensiling process in 2008.
NDF g kg^{-1} —— Average + Sx - - - Average - Sx

Table 2

The Average Content of Chemical Components of Ten Maize Hybrids, g kg^{-1}

Year	Harvest date	Treatment	Crude fiber	Crude fat	Cellulose	Lignin	ADF
2008	5th September	Fresh	232.3	15.9	240.4	10.3	250.7
		Ensiled	255.4	25.4	272.5	8.4	280.1
	19th September	Fresh	208.8	19.7	234.6	8.9	243.5
		Ensiled	229.2	23.9	245.1	6.5	252.2
	3rd October	Fresh	202.7	22.3	225.3	8.9	233.2
		Ensiled	216.5	23.2	227.0	6.7	231.6
2009	4th September	Fresh	238.7	9.6	284.2	9.8	294.0
		Ensiled	275.3	16.4	309.4	8.4	318.3
	21st September	Fresh	219.7	14.4	263.0	8.2	271.3
		Ensiled	240.9	20.8	267.7	6.7	274.4
	5th October	Fresh	224.5	18.4	276.1	8.7	285.6
		Ensiled	231.4	22.8	283.2	7.0	273.3

J. Halgerson et al. (2006), on the other hand noted increased NDF in ensiled samples at one site, but not another in a Minnesota study. Lower NDF of ensiled samples than fresh samples might be expected, as there would be same very digestible NDF that could be utilized during the early stages of fermentation (Cherney et al., 2007). The correlation between fresh and ensiled hybrids for NDF was still high ($r=0.76$; $r_{0.05}=0.25$) in our experiment.

A marketed relevance was not noticed between harvest dates and hemi-cellulose content. The highest hemi-cellulose content was achieved in 2009 as compared with 2008. There were no significant ($p=0.28$) differences in hemi-cellulose content between fresh and ensiled samples. After the ensilaging process, the content of hemi-cellulose ranged from 159.6 to 315.5 g kg^{-1}. A significant influence of the used hybrid was noted for the content of hemi-cellulose ($p<0.05$), but influence of the harvest date was not noted in 2008. But harvest date significantly ($p<0.05$) influenced the content of hemi-cellulose in 2009. I. Filya (2004) found that the hemi-cellulose content did not change consistently throughout the growth period of maize. According to findings of T. Amon et al. (2004), cellulose content declined in the course of the vegetation period, but that of hemi–cellulose and starch increased.

Table 2 gives average chemical content of maize hybrids at different harvesting times in both years. Crude fat concentration of fresh samples increased during plant development in both years. The crude fiber concentration of fresh and ensiled samples decreased during plant development in 2008. Crude fiber did not give much methane (Amon et al., 2004).

The cellulose lignin and ADF concentration of fresh and ensiled (except lignin content of ensiled samples) samples decreased during plant development in 2008.

No marked relevance was noticed between harvest dates and crude fiber, lignin and ADF content of fresh maize samples in 2009.

The crude fiber, crude fat and cellulose concentration were greater in the ensiled samples if compared with fresh samples in both years. The lignin content decreased during ensilaging process in both years. Contradictory results were obtained on ensilaging effect on the content of ADF.

Conclusions

1. This publication focused on the evaluation of differences in chemical composition between fresh and ensiled maize hybrids. Our results showed strong maize hybrid influence on the average ODM content in both trial years. ODM content decreased during ensilaging, but the influence was not substantial at the 95% probability level. A high correlation between ODM content of fresh and ensiled samples was noted.
2. The crude protein concentration decreased during plant development, and was greater in the ensiled samples, which could markedly influence methane formation. The ash content was lower in fresh samples than in ensiled samples in both years.
3. The results also demonstrated that NDF content increased during ensilaging process in 2009, but decreased in 2008. The study showed that differences in hemi-cellulose content between fresh and ensiled samples were not significant. The crude fiber, crude fat and cellulose concentrations increased, but the lignin content decreased after ensilaging process in both years.

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PRECOCITY OF SOUR CHERRY CULTIVARS INFLUENCED BY USING WOODCHIP MULCH AND DRIP IRRIGATION

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Abstract. Precocity of fruit plants enables early obtaining of yield and income. But precocity can be influenced by drought. In some cases precocity is related to insufficient growth and insufficient yield in the succiding years. The precocity of sour cherries was investigated in the trial established at the Latvia State Institute of Fruit Growing. Soil moisture treatments - woodchip mulch and drip irrigation as well as cultivars 'Bulatnikovskaya', 'Desertnaya Morozovoi', 'Latvijas Zemais', 'Orlica', 'Shokoladnica', 'Tamaris', and 'Zentenes' were used as trial factors. The flowering and yielding was observed in 2008 and 2009. Flowering, fruit set, fruit mass, and yield were characterized in order to evaluate precocity of cherries in different soil moisture treatment variants. The use of drip irrigation did not influence the yield of sour cherries, but the use of woodchip mulch decreased the yield in the third growing year. The cultivar 'Tamaris' was the most precocious, and the cultivar 'Bulatnikovskaya' was the most productive.
Key words: flowering, fruit set, fruit mass, yield.

Introduction

Precocity is defined as the ability to develop or mature unusually early. The term "precocity" is used in horticulture in order to characterize the yielding ability of young fruit trees. In general, precocity is a desirable trait of fruit plants, which enables early obtaining of yield and income.

The precocity was controlled endogenously - the significant differences of early yielding among cultivars were observed in numerous investigations (Blazek, 1985; Christensen, 1986). Precocity is an important trait of cultivars, especially if they are grown in combination with vigorous seedling rootstocks.

Precocity is influenced by several factors. The use of rootstocks which advance early yielding is on a large scale practiced method in apple growing. Intensive orchard systems with precicious and no vigorous scion–rootstock combinations are introduced also in sweet cherry growing. But such scion-rootstock combinations often tend to overcrop already in the first growing years. It leads to insufficient vegetative growth, decreased fruit size, and insufficient total yield. Therefore the precocity has to be decreased: the yielding has to be delayed and vegetative growth has to be increased in the first growing years (Lang, 2001).

Vegetative growth and yielding can be influenced by enwironmental factors too, inter alia drought. D.R. Layne and J.A. Flore have observed growing and development of sour cherry organs depending on the supply of nutrients. Plant organs have been observed as sinks for nutrients. Sink strength is determined as a capacity of sink tissues to utilize photoassimilates (Wilson, 1972, cited by Flore and Layne, 1999). Sink strength of plant organs varies during their development. Vegetative organs have more sink strength than the generative during the time of intensive growth (Flore and Layne, 1999). So shoots have priority of nutrient supply in spring and beginning of summer. If drought occurs at the time of active vegetative growth, stressed roots produce growth inhibitors and growth is decreased (Davies et al., 1990), therefore proportion of photoassimilates supplied to generative organs increases. Influence of drought on generative organs is not equal: promotion of sour cherry flower bud differentiation (Diaz et al., 1981) and increased fruit set of grapevines (Wang, 2001) has been observed as well as no affecting of grapevine yield (Ndung'u et al., 1996) or little affecting of sour cherry fruits (Flore and Layne, 1999). Severe drought stress significantly affects differentiation of flower buds of sweet cherries (Engin, 2008). Sink strength of fruits is the greatest during the final swell of fruits, but this effect is inconsistent. Drought during this period affects both shoot and fruit growth (Sams and Flore, 1983).

Yield can be increased using drought stress during periods of rapid shoot growth, and managing irrigation during rapid fruit growth (Jerie et al., 1989). Such manipulations in the first growing years can influence also precocity.

In the world, greatest part of sour cherry yield is harvested mechanically and processed (Brown et al., 1996). Therefore productivity of the trees is especially important. Mainly it is provided by choosing high yielding cultivars as 'Schattenmorelle' in the West Europe. As mentioned before, high productivity not always is related with precocity. The growing technology is known, where sour cherries have been treated by growth stimulators in the first growing years. It has resulted in delayed but higher yielding (Looney, 1996). On the other hand, precocity is advisable for sour cherries too. Early yielding sour cherry cultivar has been one of the aims in Sweden's breeding program (Trajkovski, 1996). Beside other traits, the precocity of sour cherry scion-rootstock combinations has been investigated in USA (Perry et al., 1996) and Hungary (Hrotkó, 2008).

Sour cherries in Latvia have not been harvested mechanically yet. Precocity is an important trait in

Latvia's conditions now: due to economical situation growers especially need fast income from their gardens. However, yielding of young trees has to be balanced with vegetative growth to provide sufficient yield in the following years. Furthermore, it must be appointed that only mechanically harvested sour cherries can be competitive for processing industry due to lower yielding costs. Therefore introduction of mechanical cherry harvesting is advisable also in Latvia. Information about sour cherry yielding dynamics is needed for planning of yield managment both for mechanically or by hand harvested cherries. The aim of the research was to evaluate the influence of woodchip mulch and drip irrigation on the precocity of sour cherry cultivars. Results of this investigation characterize the yielding of local and introduced sour cherry cultivars in the first growing years and their response to use of woodchip mulch and drip irrigation.

Materials and Methods

The trial was established at the Latvia State Institute of Fruit Growing in Dobele in 2007. Effects of two factors were determined in this trial. The treatments were arranged in a split plot design: the method of soil moisture treatment – on the main plots, the cultivars – on the split plots. The factor – method of soil moisture treatment had three variants:

- using of woodchip mulch in the tree strips, thickness of woodchip layer was 10 cm.
- drip irrigation which moistens 1 m wide tree strips.
- control – neither mulch nor irrigation in the tree strips.

There were three replications both per mulch and control variants, and four replications per the irrigation variant. Seven sour cherry plants (one of each cultivar) were planted in every replication.

The factor – cultivar of sour cherries had the following variants:

- local cultivars 'Latvijas Zemais' (medium vigour) and 'Zentenes' (strong vigour);
- introduced cultivars, which were originated in Russia - 'Tamaris' (week vigour), 'Bulatnikovskaya', 'Desertnaja Morozovoi', 'Orlica', and 'Shokoladnica' (all medium vigorous).

The trial was established on clay Podzoluvisol soil which was slightly acid – pH 6.4. The content of P was 53 mg kg^{-1}, content of K was 124 mg kg^{-1}.

Sour cherries were planted in spring of 2007 at distances of 4 × 4 m. The fertilizers were given yearly as 12 g m^{-2} of N, 5 g m^{-2} of P, and 10 g m^{-2} of K in the tree strips. Nitrogen fertilizer was given in the spring (in April), phosphorus and potassium ferilizers were given in the autumn.

Weeds were controlled both by removing them and spraying with herbicide Basta® (soluble concentrate, active ingredient glufosinate-ammonium - 200 g L^{-1}) in 1 m wide strips along the trees. Perennial grasses were sown in the space between strips.

Soil moisture was measured with device Theta Probe type ML2x once during 7–11 days. In the variant with drip irrigation, soil moisture was provided at about 70 per cent of the field water capacity. In Dobele, the total amount of precipitation during the period of active vegetation was 373 mm in 2007, 191 mm in 2008, and 275 mm in 2009. The weather conditions were dry in May and July of 2008, also in May of 2009, hydrotermal coefficient was 0.3–0.4 in these months (these indices are based on data of Latvian Agency of Enviroment, Geology and Meteorology). In 2007, sour cherries were irrigated 9 times and the total amount of water was 647 L per a 4 m long tree strip. In 2008 and 2009, irrigation was done 12 times and the total amount of water was 996 L and 876 L per a 4 m long tree strip, respectively.

In the second growing year (2008), flowering and yielding were evaluated by using points from 0 (no flowers or fruits) to 5 (abundant flowering or more than 20 fruits).

In the third growing year (2009), flowering was characterized as flower density (FD) – a number of flowers per cm of shoot length. Fruit set, crop density, and fruit mass (g) were determined to analyze the yield formation. Fruit set is the ratio of fruit and flower number expressed in percents. Crop density was characterized as a number of fruits per cm of shoot length. The yield was characterized as total amount of fruits (kg) per tree. The data were statistically processed using analysis of variance and Duncan's test or Dunett T3 test for *post hoc* analysis (in cases with equal variances or not equal variances, respectively). Mann–Whitney U test was used in cases when data distribution differed from normal.

Results and Discussion

Flowering and yielding in 2008

Sour cherry trees flowered at first in the second growing year. There were no significant differences in flowering among woodchip mulch, drip irrigation and control variants. On average, the flowering ranged from 2.7 points in control variant to 3.0 points in woodchip mulch and drip irrigation variants (Figure 1). Nevertheless, the yield in woodchip mulch variant (1.6 points on average) was a little less than in other variants (2.3–2.5 points on average). These differences were not significant too.

The flowering of sour cherry cultivars differed significantly ($p<0.05$). The flowering of cultivars 'Tamaris' and 'Desertnaya Morozovoi' was significantly more abundant than flowering of the other cultivars (Table 1). No significant differences in flowering among soil moisture adjustment variants for each cultivar separately were observed in 2008.

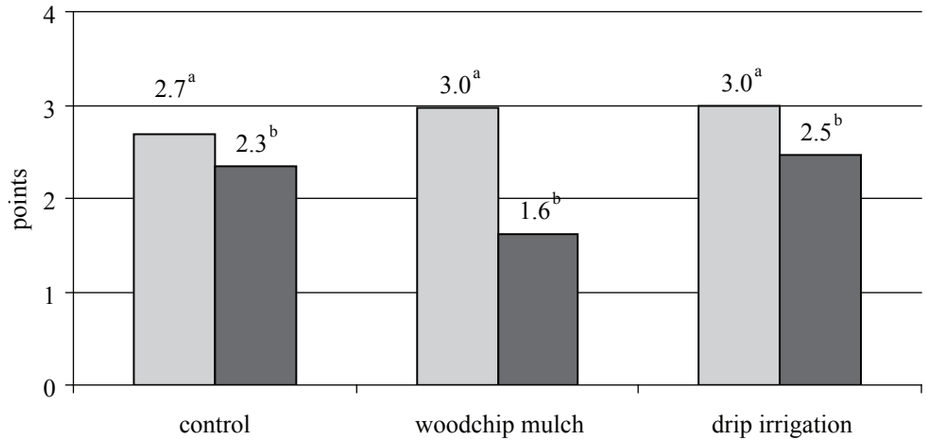

Figure 1. Flowering and yielding of sour cherries in 2008: ☐ flowering, ■ yield.
Means within the figure marked with the same letter did not differ significantly at $p < 0.05$.

The yield of sour cherries obtained in the second growing year was not economicaly essential, but it indicated precocity of cultivars and their response to use of woodchip mulch and drip irrigation. The abundant flowering of cultivar 'Tamaris' led to the significantly highest yield (4.4 points). The yielding of cultivar 'Desertnaya Morozovoi' was intermediate (2.2 points) without regard to abundant flowering. Obviously, fruit set of these cultivars differed significantly.

Similar situation was described in Germany. Cultivars 'Schattenmorelle' and 'Morina' had a comparetivaly high fruit set rating during three investigation years (6.5–6.6 points on average), but rate of flowering of cultivar 'Morina' was lower than that of other cultivars (7.4 points on average) (Schuster and Wolfram, 2005). Whereas cultivars 'Karneol' and 'Köröser' had a higher rate of flowering (8.1–8.2 points). Their fruit set gradually increased (from 4.2 and 2.5 points to 5.7 and 3.7 points respectively) but did not reach the level of fruit set of cultivars 'Schattenmorelle' and 'Morina'.

In our research the cultivar 'Zentenes' yielded less than the all other cultivars. Yielding of cultivar 'Zentenes' differed significantly from other cultivars except cultivar 'Orlica'. The cultivar 'Shokoladnica' had a significantly lower yield in mulch variant (0.6 points on average) compared to drip irrigation variant (4.5 points on average). The yield of cultivar 'Shokoladnica' in control variant (1.2 points on average) did not differ significantly from other variants.

Flowering in 2009

In general, no significant differences in flowering among woodchip mulch, drip irrigation, and control variants were observed in 2009. On average, flower density (FD) was 1.3 in mulch, 1.5 in irrigation, 1.6 in control. Cultivar 'Zentenes' had the most pronounced tendency ($p=0.1$) to decrease the flowering in woodchip mulch variant (the average FD 0.7) comparing to control and drip irrigation variants (the average FD 1.1). Though, in general, the influence of soil moisture adjustment variants to flowering did not depend on sour cherry cultivars, the effect of interaction was insignificant ($p=0.9$).

Although differences were numerically small and insignificant at $p<0.05$ level, results partially agreed with hypothesis of J.A. Flore: drought and decreased growth during maturity of sour cherry fruits advanced differentiation of flower buds because in this time differentiation began (Flore, 1994, cited by Flore and

Table 1

Flowering and Yielding of Sour Cherry Cultivars in 2008

Cultivar	Flowering (0–5 points)	Yielding (0-5 points)
'Zentenes'	2.0 [a]	0.5 [a]
'Orlica'	1.5 [a]	1.4 [ab]
'Latvijas Zemais'	3.0 [a]	2.0 [b]
'Shokoladnica'	2.5 [a]	2.1 [b]
'Desertnaya Morozovoi'	4.5 [b]	2.2 [b]
'Bulatnikovskaya'	2.6 [a]	2.5 [b]
'Tamaris'	4.5 [b]	4.4 [c]

Means in the column marked with the same letter did not differ significantly at $p<0.05$.

Layne, 1999). In Latvia, sour cherry fruits mattured in July. The weather was dry in July of 2008 – total amount of precipitiation was 21.2 mm and hydrotermal coefficient was 0.3 in this month. Soil in control variant was dryer than in woodchip mulch and drip irrigation variants. Possible, it was one of the reasons of higher flower density in control variant in 2009. Regarding to growth and flowering competition, vegetative growth of cultivar 'Zentenes' in control variant was smaller than in the mulch variant in 2008 (Feldmane, 2009), but there were no significant relations with flower density in 2009 ($r = -0.2$, $p=0.3$).

Differences in the flowering among cultivars were significant. Cultivars 'Tamaris' and 'Bulatnikovskaya' had a significantly higher flower density than culivars 'Latvijas Zemais', 'Zentenes', 'Orlica' ($p<0.05$) (Figure 2).

Fruit set in 2009

No significant differences in fruit set among woodchip mulch, drip irrigation, and control variant were observed. The average fruit set ranged from 17% in drip irrigation variant to 19% in control variant.

Like previously, fruit set significantly differed depending on the cultivar. Sour cherry cultivar 'Zentenes' had the lowest fruit set – 8% on average. It differed significantly from the fruit set of cultivars 'Shokoladnica' (18%), 'Orlica', 'Bulatnikovskaya', and 'Tamaris' (the average fruit set of these three cultivars was 21%). Fruit set of cultivars 'Desertnaya Morozovoi' and 'Latvijas Zemais' was 15% and 18% respectively, which did not differ significantly from the other cultivars.

A similar fruit set of cultivars originated in Russia was determined in the research in Germany. Fruit set of seven Russian sour cherry cultivars ranged from 17.6% to 25.1% (Schuster and Wolfram, 2004). Cultivar 'Ostheimer' (synonym of 'Latvijas Zemais') had a low fruit set in Germany conditions - 7.4%. Productive cultivar 'Schattenmorelle' had higher fruit set (30%) than cultivars in our research. However total yield depends on flower quantity as well as on fruit set and fruit mass.

Quantity of fruits was a result of flowering and fruit set. Accordingly, there were no significant differences in crop density among woodchip mulch, drip irrigation, and control variant – on average 0.3 fruits per 1 cm shoot length. The effect of soil moisture adjustment variants to crop density did not depend on sour cherry cultivars – the effect of interaction was insignificant ($p=0.9$). Cultivars 'Tamaris' and 'Bulatnikovskaya' had the highest crop density – 0.6 and 0.4, respectively ($p<0.05$). Cultivars 'Zentenes' and 'Latvijas Zemais' had a lower crop density (0.1) than cultivars 'Desertnaya Morozovoi', 'Shokoladnica', and 'Orlica' (0.2 – 0.3) but without significant differences.

Fruit mass in 2009

There were no significant differences ($p < 0.05$) in fruit mass between sour cherries in control, drip irrigation, and woodchip mulch variants for all cultivars together as well as separately. Most of the cultivars had a tendency to develop the biggest fruits in the variant with drip irrigation. However, cultivars 'Latvijas Zemais' and 'Tamaris' had the biggest fruits in the control variant, but 'Shokoladnica' – in the variant with woodchip mulch.

The results of fruit mass were in agreement with the observations of C. E. Sams and J. A. Flore – drought during final swelling of fruits decreased their mass (Sams and Flore, 1983). There was rich amount of precipitations in time of fruit swelling – in July of 2009. Soil moisture was optimal or above optimum level in woodchip mulch variant and drip irrigation variant, as well as in the control variant. Therefore fruits in the control variant were not significantly smaller regardless of drought weather conditions in May.

Fruit mass of various cultivars differed significantly. Cultivar 'Latvijas Zemais' had the significantly lowest fruit mass (2.7 g). Cultivar 'Tamaris' had the significantly highest fruit mass (5.6 g) (Figure 2). Fruit mass of other cultivars ranged from 4 g (cultivar 'Shokoladnica') to 5 g (cultivar 'Zentenes').

Similar distribution of sour cherry fruit mass was shown in the report about cultivars grown in Denmark (Christensen, 1986). Most of the observed cultivars had a fruit mass of 4 - 5 g (for example, cultivar 'Nefris' had fruit mass of 4.8 g). The lowest fruit mass in that study was 2.8 g (cultivar 'Marasca Siva Uspravna'), the highest – 5.7 g (cultivar 'Crisane 2'). These data were obtained also from young trees, but there were no references about irrigation or mulching.

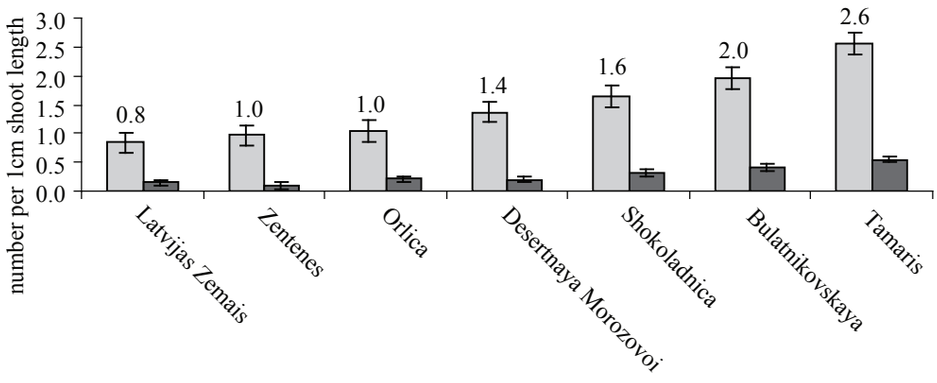

Figure 2. Flower density and crop density of sour cherry cultivars in 2009: □ flowers, ■ fruits.

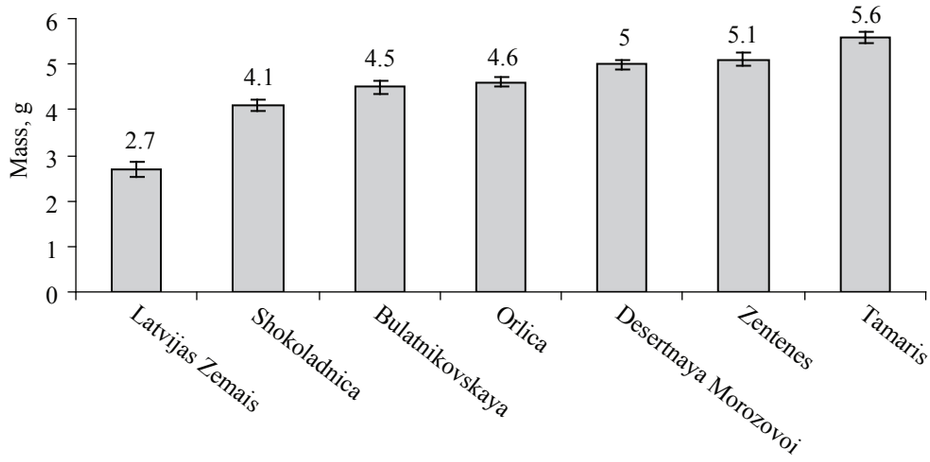

Figure 3. Fruit mass of sour cherry cultivars in 2009.

Fruit mass of new best sour cherry selections at Dresden-Pillnitz was essentially higher - 6.6 – 7.8 g (Schuster and Wolfram, 2005).Though, smaller sour cherry fruits were appropriate for the U. S. processing market - fruit mass should be higher than 3.5 g (Iezonni and Sebolt, 2005).

Yield in 2009

The yield of sour cherries was significantly influenced both by soil moisture adjustment variant and by the cultivar ($p<0.05$). Cherry yield in the variant with woodchip mulch was significantly smaller than in control – on average 0.7 and 1.4 kg per tree respectively. Sour cherry yield in the variant with drip irrigation (on average 1.2 kg per tree) did not differ significantly from the control variant. The effect of mulch to yield was similar among the cultivars, and the effect of interaction was insignificant ($p=0.9$). It seems that insignificant differences in flower density and fruit set as well as fruit drop in June resulted in a significantly decreased yield in the mulch variant. The results obtained by E. Rubauskis confirm with the results of this study: apples in the first yielding year had lower yield in the sawdust mulch variant than those in the control (Rubauskis, 2005)

In general, cultivar 'Bulatnikovskaya' had a higher yield than the other cultivars. Yielding of cultivar 'Bulatnikovskaya' differed significantly from other cultivars with exception to cultivar 'Shokoladnica' (Figure 3). Yield differences of cultivars 'Bulatnikovskaya' and 'Shokoladnica' were not significant at level $p<0.05$. The average yield of cultivar 'Bulatnikovskaya' was 2 kg per tree, but the average yield of other cultivars ranged from 0.6 kg (cultivar 'Zentenes') to 1.2 kg per tree (cultivar 'Shokoladnica'). The yield of cultivar 'Tamaris' was intermediate (0.8 kg per tree) despite the high fruit number per shoot and high fruit mass. Precocity of this cultivar did not result in high total yield due to low vigour of the tree.

Similarly, in J. Christensen's investigation, the first essential yield of sour cherries was obtained in the third growing year (Christensen, 1986). The average yield ranged from 0.4 kg per tree (cultivar 'Schwäbischen Weinweichsel') to 4.7 kg (cultivar 'Nefris'). The yield

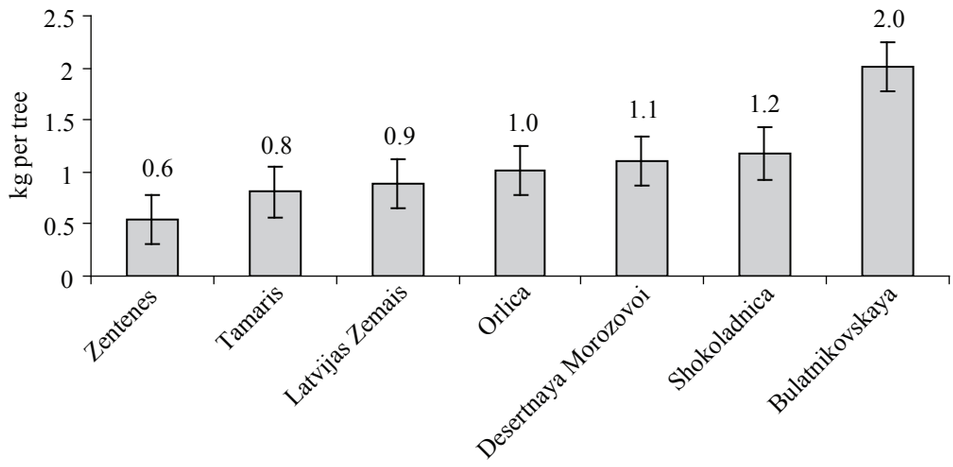

Figure 4. Yield of sour cherry cultivars in 2009.

Table 2

Yield of Sour Cherry Cultivars in Woodchip Mulch, Drip Irrigation, and Control Variants

Cultivar	Yield, kg per tree		
	woodchip mulch	drip irrigation	control
'Zentenes'	0.3[a]	0.5[a]	0.8[ab]
'Orlica'	0.3[a]	1.3[ab]	1.4[b]
'Tamaris'	0.5[ab]	1.0[ab]	1.0[ab]
'Shokoladnica'	0.7[ab]	1.3[ab]	1.6[ab]
'Latvijas Zemais'	0.8[ab]	0.7[ab]	1.3[ab]
'Desertnaya Morozovoi'	0.9[ab]	1.1[ab]	1.2[ab]
'Bulatnikovskaya'	1.9[b]	2.3[b]	2.3[ab]

Means within the table marked with the same letter did not differ significantly at $p < 0.05$

of cultivar 'Bulatnikovskaya' in our research was similar to the yield of some 'Stevnsbär' clones and cultivar 'Kelleris 14' (clone 4562) (2.0 – 2.6 kg per tree).

In our research, the yielding was observed also separately for soil moisture adjustment variants and cultivars in order to specify differences among them. The effect of the cultivar was most pronounced in wetter soil conditions – in woodchip mulch and drip irrigation variants. So the cultivar 'Bulatnikovskaya' had a significantly higher yield than cultivars 'Zentenes' and 'Orlica' in the woodchip mulch variant and drip irrigation variant ($p<0.05$) (Table 2). Differences between the yield of sour cherry cultivars were not significant in the control variant.

Cultivar 'Orlica' responded to soil moisture treatment variants more than other cultivars. This cultivar had a significantly higher yield in the control variant compared to in the variant with woodchip mulch. Differences among soil moisture adjustment variants were not significant for other cultivars if they were observed separately.

Conclusions

1. Sour cherry yielding was not significantly influenced by use of drip irrigation in the second and third growing year.
2. Sour cherry yielding was not significantly influenced by use of woodchip mulch in the second growing year, but yielding was significantly decreased in the third growing year.
3. Precocity and yielding varied significantly depending on the cultivar:
   a) sour cherry cultivar 'Tamaris' had the most pronounced precocity in the second and third growing year related with significantly more abundant flowering, higher fruit set, higher fruit mass, and bigger amount of fruits per shoot length than the other cultivars;
   b) sour cherry cultivar 'Bulatnikovskaya' was one of most precocious cultivars – it had abundant flowering, high fruit set, and significantly higher yield than all other cultivars in the third growing year.

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NITROGEN REMOVAL WITH APPLE–TREE FRUITS

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Abstract. The investigation was done at the Latvia State Institute of Fruit-Growing in Dobele in 2009, on the basis of an established field experiment planted in 1997 with apple (*Malus domestica* Borh.) cultivar `Melba` (rootstock B9) trees spaced at 1.5 × 4 m distances. Three different treatments of soil moisture management were compared: control, sawdust mulch and fertigation. Soil of the experimental plot was Haplic Luvisol (Hypereutric), sandy loam, interspaced with Cutanic Luvisol, sandy loam. Organic matter – 25 g kg^{-1}, soil reaction pH – 6.5. Plant available P was 130.9, K – 157.7, and Mg – 102.2 mg kg^{-1}. The aim of the investigation was to determine nitrogen removal with fruit yield taking into consideration the used soil moisture regulation method – sawdust mulch or fertigation. The applied soil moisture regulation methods (mulch and fertigation) had significant influence on the content of dry matter in apple fruits ($p<0.05$). The highest content of dry matter was found in the control treatment. A significantly higher nitrogen concentration (47 g kg^{-1}) in apple dry matter was in the control treatment, whereas in mulch and fertigation treatments nitrogen concentrations were lower (36 and 42 g kg^{-1}). The highest nitrogen concentration in dry matter was found in fruits with the biggest mass ($r=0.61$). A negative significant ($p<0.05$) correlation was found between nitrogen concentration and trunk diameter ($r=-0.85$), and between nitrogen concentration and yield ($r=-0.84$). Removal of N was 24.4 kg ha^{-1} in the control, 22.3 kg ha^{-1} in the mulch, and 25.0 kg ha^{-1} in the fertigation treatment.
Key words: fertigation, mulch, nutrient remove

Introduction

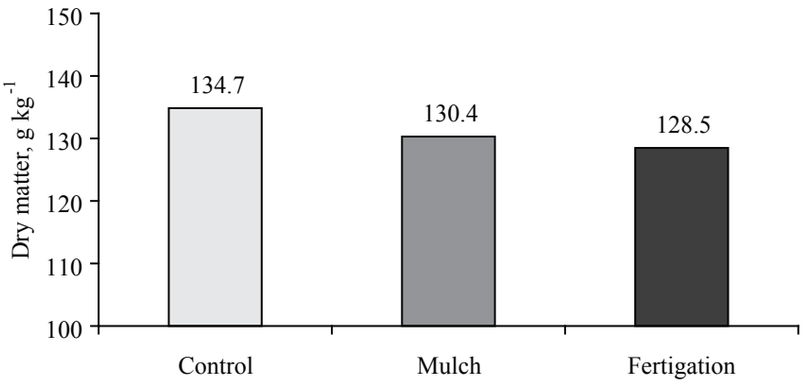
Harvesting of yield irreversibly removes plant nutrients from the orchard. Nitrogen, a biologically active and important nutrient, also is leaving the orchard, therefore and should be replaced by fertilizers for the next growing seasons. Nitrogen is consumed in relatively large quantities and is necessary for many life functions of the trees, such as growth of shoots, setting of buds and fruits, and fruit development. Nitrogen deficiency for apple–trees results in several negative consequences: decreases growth of shoots leaves become light green or yellowish, which in turn negatively influences the intensity of photosynthesis (Fallahi et al., 2001; Cmelik et al., 2006).

The main task of fertilization is to provide that part of plant nutrients which the plants need in order to obtain a good quality yield, but which cannot be supplied by soil. Yet, if the amount of nutrients turned in eith fertilizers exceeds the loss of nutrients, this is harmful both to plants and the environment. Independent of the form of N in fertilizers, when in soil it soon transforms into nitrate $N\text{-}NO_3^-$ form and is washed out, polluting ground water and water bodies (Dong et al., 2005). Development of integrated fruit growing in Latvia makes some restrictions for use of mineral fertilizers. These restrictions are fixed in regulations of the Latvia Council of Ministers No. 531 and No. 406, which are worked out on the basis of EU guidelines Nr. 91/676 EEK (Nitrate directive) as well as on the fruit and berry integrated production guidelines which provide the measures for recording of used fertilizers and mechanisms of control. The rapidly increasing price for mineral fertilizers also stimulates the producers, without any loss of yield and income, to choose more rational growing technologies, choice of a sustainable fertilizing system. Regulations require the farmers to compose annual fertilizing plans based on nutrient removal, therefore relevant data sets should be developed taking into consideration the modern technologies of orchard crop growing. Some part of fertilizer use recommendations have been worked out in 1960s -1970s – they are more applicable for apple-trees on vigorous rootstocks. Since 1990-s apple-trees have been grown on dwarfing or dwarf rootstocks, which have a morphologically different root system, as well as different growing technologies have been used in plantations. Despite the fact that average amount of precipitation is quite satisfactory and sometimes even too wet, there are periodical water deficit in soil. Therefore some fruit growers are interested to implement any methods of soil moisture regulation, such as mulching of tree strips as well as establishment of different irrigation systems which may affect not only nutrient turnover but also their removal. The applied method of soil moisture control and plant nutrient management works parallel, and therefore key figures of fertilizer planning like nutrient requirement and removal is important and should be validated periodically. Therefore the aim of this investigation was to determine nitrogen removal with (*Malus domestica* Borh.) fruit yield taking into consideration the used soil moisture regulation method – sawdust mulch or drip irrigation with fertilizers (fertigation).

Materials and Methods

The investigation was carried out at the Latvia State Institute of Fruit – Growing, Dobele, in 2009. It was done on the basis of a field trial planted with one year maiden trees in 1997 (Rubauskis et al., 2004). The planting distance of trees was 1.5 × 4 m. The agrochemical investigations were done for trees of cultivar 'Melba' on the dwarf rootstock B 9. The canopy of trees was trained as slender spindle. The obtained average yield per year was 20 t ha^{-1}.

The meteorological data were collected by a`Lufft`



* significantly different ($p<0.05$)
Figure 1. The content of dry matter in apple fruits (g kg^{-1}).

meteorological-station placed at the institute. The climate situation was as following in 2009: the period of vegetation, when air temperature is 5 ºC or higher was 204 days (average of long-term observations 135–145 days); the average air temperature was 8.1 ºC (long-term average 5.5 ºC) and annual precipitation was 531 mm (long-term average 560 mm), however, it should be pointed out that precipitations in vegetation the period was only 312 mm. Soil of the experimental plot was Haplic Luvisol (Hypereutric) interspaced with Cutanic Luvisol, sandy loam. Organic matter content in soil was 25 g kg^{-1} (according to Tyurin method, wet combustion), soil reaction pH – 6.5 (in 1*M* KCl suspension, potentiometrically). Plant-available P was 130.9 mg kg^{-1}, K – 157.7 mg kg^{-1}, and Mg – 102.2 mg kg^{-1} (according to Egner–Riehm or DL method).

The following treatments of soil moisture regulation in tree strips (1 m wide) were compared: (1) control – no regulation methods; (2) sawdust mulch; and (3) fertigation, e.g., drip irrigation in combination with fertilizers. In the mulch treatment, soil surface was covered with a 10–20 cm layer of sawdust, which was renewed every three years for three times. In the irrigation treatment, 'Den' type pipelines with built–in drippers, spaced 0.38 cm apart, were used. The irrigation provided effective moistening of a 1m wide zone in sandy loam soil or about 25% from the orchard area. In 2009, for trees with irrigation, additional 353 liters of water were provided, in 12 applications. In the alleyways (3 m wide) grasses *Lolium perenne* L., and *Poa pratensis* L. in proportion 1:3, were sown. Some weeds such as white clover *(Trifolium repens*) and dandelion *(Taraxacum officinale)* were also spread out into the grass lawn. In 2009, the trees were provided with 9 g of N and 10 g of K using ammonium and potassium nitrates. In the control and mulch treatments they were provided once during the flowering of apple-trees, but in the fertigation treatment – bi–weekly (3 times) expanded into 6 weeks after flowering of apple-trees. The grass in alleyways was not fertilized.

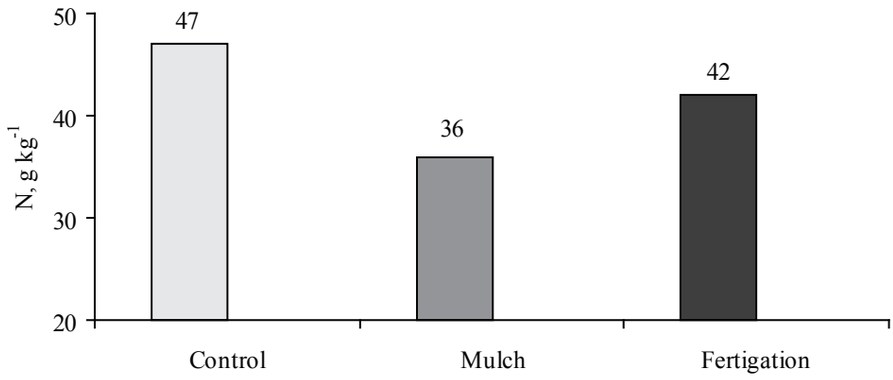
The fruits were harvested on August 24. Fruit samples were taken as 1 kg of randomly chosen fruits from each treatment. In the fruit samples of dry matter amount (ISO 6496) and total nitrogen (Kjeldahl method, wet digestion) were determined. The removal of nutrients was calculated as kilograms per hectare area (kg ha^{-1}) (Kārkliņš, 1988). The results of the investigation were analyzed using analysis of variance *ANOVA*, as well as descriptive statistics *(Descriptic statisti)* and correlation.

Results and Discussion

The applied methods of soil moisture regulation – mulch or fertigation – had significant ($p<0.05$) influence on the content of dry matter in apple fruits (Figure 1).

The lowest content of dry matter was in the fertigation treatment – 128.5 g kg^{-1}, but the highest – 134.7 g kg^{-1} in the control treatment. These differences may be explained by the different moisture supply in the treatments. Other researchers (Nagy et al., 2006) point out that apple–trees which have higher available soil moisture supply contain less dry matter in biomass. This might indicate that apple-trees grown with mulch or fertigation had more suitable moisture situation than those growth in the control treatment, which, in its turn may indicate that apples during their growth and development had higher water intake in these treatments. The content of dry matter did not differ significantly ($p>0.05$) between the mulch and fertigation treatments. The investigation showed that the control treatment hard relatively higher data variation ($S_{\bar{x}} = 6.2$). In mulch and fertigation treatments the data variation was 2 times lower. Variation of data possibly indicates variation of moisture situation during apple growth. Mulch and fertigation provide an optimal moisture regime so the moisture in plants is supplied regularly, while in the control treatment moisture supply is unstable, therefore apple trees as well as fruits often may lack it (Evans and Proebsting, 1985; Rubauskis, 2005).

Results of the investigation demonstrate that the nitrogen concentration in plants was significantly influenced by the used soil moisture regulation treatments – sawdust mulch and fertigation ($p<0.05$) (Figure 2).



* significantly different ($p<0.05$)
Figure 2. The content of nitrogen in apple fruit dry matter (g kg^{-1}).

The highest concentration of nitrogen in fruits was in the control treatment, in fertigation treatment it was lower by 11%, but in the mulch treatment – by 24%; the difference was significant ($p<0.05$). These data do not contradict with the results obtained by other researchers (Dris et al., 1998) who have found that in early-ripening apple cultivars (like 'Melba') nitrogen concentration is 40 to 70 g kg^{-1}. The significantly lower nitrogen content in the mulch treatment may be explained by the fact that nitrogen which during decomposition of sawdust is used by microorganisms for their life functions has not yet been fully released and the immobilization process continues. It has been established that if the organic matter at the beginning of decomposition has ratio of C:N up to 20, then mineralization exceeds immobilization, but if this ratio is over 30, then immobilization dominates over mineralization (Wickramasinghe et al., 1985). In sawdust, depending on its origin (deciduous trees or conifers, and of tree species), the C:N ratio may reach even 400 (Shengzuo et al., 2008), so it is possible that the decaying process of sawdust has not been finished. There are various, even contradictory observations about the length of decay of sawdust. Some researchers (Haynes and Goh, 1980) have found that sawdust decomposes during 2–3 years, but others (Shengzuo et al., 2008) have established that decomposition may last until 7–8 years depending on conditions. Nitrogen in plants is dominating as an organic compound, and it is found also in a mineral form either as NO_3^- or NH_4^+ ions (Dong et al., 2005). In this study only total N was determined in apple fruits. In moist soil, nitrogen is more easily available to plants (Thakur and Shekhar, 1982), and it is possible that, if fertigation is applied, nitrogen uptake could be higher. This is indicated by our data and also by data of other authors (Parchomchuk et al., 1994; Malaguti et al., 2006).

Several correlations were found between nitrogen concentration in dry matter and yield level (Table 1). Acomparatively close but non-significant correlation ($p>0.05$) was found between average fruit mass and nitrogen concentration in fruits. The higher was the fruit mass, the higher was the nitrogen concentration ($r = 0.61$). This complies with the results obtained by other researchers (El-Boray et al., 2006) who investigated the uptake of nitrogen by apple-trees and fruits at certain N concentrations in soil. A significant and close correlation ($p<0.05$) was found between the content of dry matter and per cent of fallen apples ($r = 0.85$). This means that the part of fallen apples was larger from trees which had higher content of dry matter in fruits. It was already mentioned that significantly highest content of dry matter was found in apples of the control treatment (Figure 1).

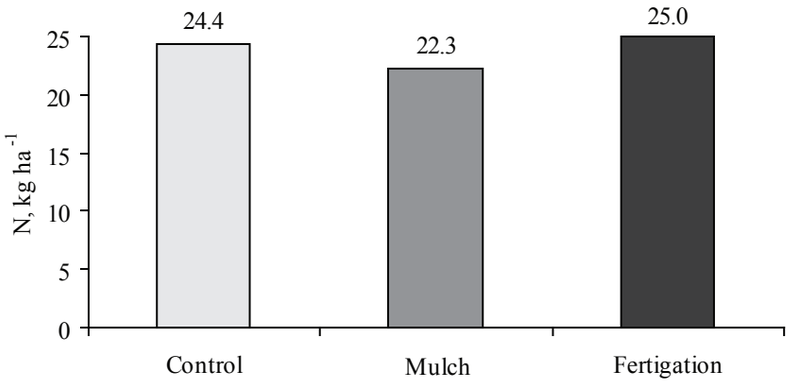
This shows that soil moisture deficit consed premature fruit drop. To give professional explanation of the results, additional research is needed. A medium close, non-significant correlation ($r = 0.58$) was found between the share of non-standard fruits and the content of dry matter.

Table 1

Results of correlation analysis

Parameter	Average fruit mass, g	Fallen fruits, %	Non-standard fruits, %	Trunk diameter, cm	Yield, t ha^{-1}	N, % in dry matter
N, % in dry matter	0.61	0.43	0.40	-0.86**	-0.74*	1
Dry matter, %	0.55	0.85*	0.58	-0.62*	-0.56	0.30

* correlation significant at $p<0.05$
** correlation significant at $p<0.01$



* significantly different ($p<0.05$)
Figure 3. Removal of nitrogen by apples, kg ha^{-1}.

Next, the bigger was the tree trunk diameter, the lower was the nitrogen concentration in fruits ($r = -0.86$), wich may mean that the more nitrogen is used by the tree for biomass growth, the less is contained in fruits. Results of the investigation show that with the increase of fruit yield the nitrogen concentration in the fruits decreases ($r=-0.74$). This may signify that a tree has some certain limit in possible uptake of nutrients. To confirm this, additional research is necessary and correlation must be found also for a year of low yield.

Although concentration of nitrogen in fruits was significantly higher in the control treatment (Figure 2), removal of this element (kg per ha) with the fruit yield was similar ($p>0.05$) in all treatments (Figure 3). In 2009 it was 22.3 kg from the mulch treatment, 10.9% more from the control treatment, and 11.2% more from the fertigation treatment compared with mulch treatment.

In the previous study (Surikova and Kārkliņš, 2009), showed that removal of nitrogen with vegetative parts during summer pruning was 16.64 kg of nitrogen from 1 ha of apple orchard in control treatment, 30.48 kg in mulch treatment, and 17.66 kg in fertigation treatment. So together with the yield, without applying soil moisture treatments, nitrogen removal was 31.04 kg ha^{-1}, which does not significantly differ from the data obtained in Latvia during 1960s – 1970s (Dimza and Gross, 1994), when nitrogen removal for apple cultivar 'Antonovka' on seedling rootstocks was 30.3 kg ha^{-1} (including branches removed by tree pruning). Similar nitrogen removal was found also in the fertigation treatment in our experiment – 32.66 kg ha^{-1}, but, by mulching tree strips, nitrogen removal increased up to 70% and reached 52.79%.

Conclusions

1. The applied soil moisture regulation methods (mulch and fertigation) had influence on the dry matter content in apple fruits. A significantly higher ($p<0.05$) nitrogen concentration (47 g kg^{-1}) was in the fruit dry matter of the control treatment, while in the mulch and fertigation treatments nitrogen concentrations were similar (36 and 42 g kg^{-1}).
2. The highest nitrogen concentration was in fruits with the biggest mass ($r=0.61$). A significantly negative correlation ($p<0.05$) was found between nitrogen concentration and tree trunk diameter ($r=-0.85$) and between nitrogen concentration and yield ($r=-0.84$).
3. Removal of N with apple fruits was 24.4 kg ha^{-1} in the control treatment, 22.3 kg ha^{-1} in the mulch, and 25 kg ha^{-1} in the fertigation treatment ($p>0.05$).

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PRELIMINARY OBSERVATIONS OF PHENOLOGY DEVELOPMENT, YIELD AND YIELD QUALITY OF SOME HIGHBUSH BLUEBERRY CULTIVARS IN LATVIA

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Abstract. Cultivars of northern highbush blueberries (*Vaccinium corymbosum* L.) are the most suitable for areas with long, cold winter, because they require the greatest number of winter chilling hours. The blueberries are excellent sources of phytochemicals that are believed to have a significant biological activity. The experiment was done at the Institute of Agrobiotechnology, Latvia University of Agriculture, Jelgava, in the year 2009. The aim of the experiment was to evaluate phenological development of 9 highbush blueberry cultivars, their yield, fruit weight and biochemical composition: total anthocyanins, total phenols, ascorbic acid, titratable acids, and soluble solids. The obtained data showed correlation between duration of flowering and fruit ripening; between harvest and end of vegetation period of the highbush blueberry. The phenological development was dependent not only on cultivars, but also on the effective air temperature (above + 5 ºC). The research results allow to assess the phenology development of blueberry cultivars and predict the fruit ripening time in conditions of Latvia. The most productive cultivars were 'Patriot' and 'Northland' The biggest single berry weight presented the cultivars 'Chippewa' and 'Bluejay'. The cultivar 'Jersey' had the highest content of titrable acid, anthocyanins, and ascorbic acid. The highest phenol content was detected in highbush blueberry cultivars 'Spartan' and 'Bluecrop'.
Key words: *Vaccinium corymbosum* L., vegetation period, flowering and fruit ripening time, biochemical composition.

Introduction

Highbush blueberries (*Vaccinium corymbosum* L.) are native to North America (latitudes 40 to 45º N), Latvia is located in latitudes 55 to 58 º N, but with similar climate conditions. Highbush blueberries are an upright, 2 m high, crown-forming bush. Fruits, ranging from 3 – 20 mm in diameter, are blue-black berries with many seeds. More than 50 cultivars of highbush blueberry have been developed in North America, primarily based on selections for commercially valuable fruit characteristics and seasonality. The blueberry cultivars require from 120 to 160 growing degree days to ripen fruit.

Northern highbush blueberries require a great number of winter chilling hours and therefore are most suitable for areas with long, cold winters. The northern blueberries have a long period of apparent dormancy during winter; they need an average of 750 hours of temperature below 7 ºC before growth can begin. In general, the more chilling hours, the better, and temperatures that range from 2 to 9 ºC are most effective in promoting good and strong growth of a plant and which will give abundant fruit in the future. (Gough, 1994; Trehane, 2004). If the chilling received during the winter is not sufficient to break dormancy then growth cannot be resumed (Wang and Buta, 1997).

Blueberries is an excellent source of phytochemicals substance that is believed to have significant biological activity in the human body (Schmidt et al., 2005).

In the literature (Gough, 1994; Trehane, 2004) it has been described that the temperature at which blueberry buds are injured depends primarily on their development stage, but does not mention the length of stage of development to blueberry cultivars.

The aim of this experiment was to compare the phenological development, including flowering and fruit ripening length and times, and yield quality of some highbush blueberry cultivars in conditions of Latvia.

Materials and Methods

The experiment was done at the Institute of Agrobiotechnology of the Latvia University of Agriculture (LLU), Jelgava. Plants were observed during the year 2009. Seven-year-old plants highbush blueberry cultivars 'Spartan, 'Patriot', 'Chippewa', 'Northland', 'Blueray', 'Bluecrop', 'Jersey', 'Bluejay', and 'Barkeley' were evaluated in a study site. The cultivars were grouped by vegetation period: early season 'Spartan' and 'Patriot'; early midseason 'Northland' and 'Bluejay'; midseason 'Chippewa', 'Berkeley', 'Bluecrop', and 'Blueray'; late midseason 'Jersey' (Hancock and Hanson, 2001).

The trial was carried out an experimental plot on sandy soil, the plants were planted in the furrow that filled with peat (the peat pH were 4.5). Planting distance was 3 × 1 m. 17 complex fertilizer was given: 20 g m^{-2} ($N:P_2O_5:K_2O$ 8:7:21, with microelements Mg 1.6%, S 15.9%, B 0.03%, Cu 0.01%, Fe 0.2%, Mn 0.2%, Mo 0.002%, Zn 0.01%). Fertilizer was given 2 times per season: the first time at the beginning of vegetation, the second time at the end of flowering. In spring, peat was used as mulch (a 5-cm layer). No regular irrigation system was used in the trial.

The phenological development of highbush blueberry, which was defined using blueberry growth stages by Mark Longstroth from Michigan State University (Longstroth, 2008): start of vegetation (leaf development), flower-bud break, beginning of fruit growth (first fruits visible at raceme base), and harvest time (blueberries are picked several times as the fruit ripens). The highbush blueberry fruits were collected gradually, so the total yield was picked at

three to five times from the end of July 2009 to the end of August 2009.

The sum of the effective air temperature was determined using data of the Latvian Environment, geology and meteorology agency. The sum of the effective air temperature is the average daily air temperature in Celsium degrees above + 5 °C, which is the average temperature of the start of vegetative growth.

In general, the year 2009 in Latvia can be described as warm and wet enough. The average annual air temperature was + 6.5 °C (0.7 °C above the average of long term), annual rainfall - 753 mm (114% of the long term average). Winter 2008/2009 was characterized by the stability of warmth and moisture. The year 2009 was dryer than the long term average. In Jelgava, the lowest temperature was observed June 18 (- 0.2 ºC). The 1st decade of August was the driest time of the summer of 2009, when total rainfall was only 5% of the average, and precipitation made 127% of the long term average. Later the air temperature fluctuated around the long term average, and frost was not observed until early October.

The following yield and yield quality parameters were determined: yield (kg per bush), single fruit weight (g) and biochemical composition: total anthocyanins (mg 100^{-1}), total phenol (mg 100^{-1}), ascorbic acid (mg 100^{-1}), titratable acid (%), and soluble solids (%). The samples of berries were analyzed after freezing and frozen storage. The chemical composition analyses were carried out at the Latvia State Institute of Fruit-Growing. Total anthocyanins were determined by spectrophotometric method at a wavelength of 535 nm. Total phenol content was determined by spectrophotometric method at a wavelength of 765 nm. The content of ascorbic acid was determined with iodine method. Total titrable acids were determined by titration (LVS EN 12147:2001A). The content of soluble solids was determined by refractometer (LVS EN 12143:2001A).

All measurements were made in three replications. The data were statistically processed using analysis of variance (ANOVA), and standard error. Correlation coefficients were also calculated.

Results and Discussion

They year 2009 in Latvia generally was warm and wet enough. The vegetation period of most of the highbush blueberry cultivars started when the average air temperature was above +5 °C and the sum of effective air temperature was 26 °C (Table 1). The difference in the vegetation period between the earliest and latest cultivars was 17 days. The first cultivar was ‘Spartan’ (for this cultivar, the vegetation period started on 2nd April, when average air temperature in last five days was 3 °C). Beginning of flowering started at the end of the second decade of April, when the sum of effective air temperature was 145 °C. The sum of positive temperature of the flowering period was 559 °C.

Table 1

The average air temperature and the sum of effective temperature between phenological phase changes of the highbush blueberry, 2009

Phenological phase	Average air temperature, °C	Sum of effective air temperature, °C
Bud break (beginning of vegetation period)	7.3	26
Beginning of vegetation period – beginning of flowering	7.8	145
Beginning of flowering – beginning of fruit ripening	11.5	559
Beginning of fruit ripening - full ripeness	15.0	965
Full ripeness - end of vegetation period	11.2	101
Length of vegetation period	12.3	1606

The results obtained in the field trials indicate that the length of the vegetation period of highbush blueberry varied from 167 to 181 days (Table 2). In assessing the development of phenological phases there were significant differences observed between highbush blueberry cultivars ($F_{crit.}$=2.24>F_{fakt}=0.19).

The beginning of flowering is a very important parameter because of the possible damage to flowers by late spring frost. Beginning of flowering was evaluated on April 19 for cultivars ‘Spartan’, ‘Chippewa’, ‘Patriot’, ‘Northland’, ‘Blueray’ and ‘Bluejay’, and on May 3 for cultivars ‘Jersey’ and ‘Berkeley’. Difference between beginning of the vegetation period and start of the flowering was from 17 to 31 days. The best incomes are usually obtained for fruit ripening very early or very late in season. It is difficult to secure high fruit quality in cultivars ripening at either extremes because vigor is often low or modest in the earliest cultivars, and both early and late cultivars ripen under more variable environmental conditions than midseason cultivars. In the first stage of floral development, frost tolerance is critical even in areas that do not have severe winter temperatures (Galletta and Ballington, 1996).

Beginning of fruit ripening started on the 41st day (Table 2) after beginning of flowering for cultivars ‘Spartan’, ‘Patriot’, ‘Chippewa’, and ‘Bluecrop’, and on the 51st day for cultivars ‘Blueray’ and ’Bluejay’. The time of fruit ripening fluctuated from 56 to 66 days (the longest fruit ripening period was observed for cultivar ‘Bluecrop’ – 66 days).

Table 2

Days between the phenological phases of highbush blueberry cultivars

Cultivars	Beginning of vegetation period – beginning of flowering	Beginning of flowering - beginning of fruit ripening	Beginning of fruit ripening - full ripeness	Full ripeness - end of vegetation period	Full vegetation period
Spartan	17	41	64	59	181
Patriot	17	41	64	64	175
Northland	17	46	59	59	170
Bluejay	17	51	61	57	176
Chippewa	17	41	64	64	175
Blueray	17	51	61	55	173
Bluecrop	22	41	66	55	167
Barkeley	31	42	60	53	169
Jersey	31	42	56	55	167
$LSD_{0.05}$	9.2	6.7	5.3	6.4	7.3
S %	29	10	5	7	3

The coefficient of linear correlation (Table 3) demonstrates that there is a significant, negative correlation (r = -0.64) between duration of the flowering and fruit ripening of the highbush blueberry. In other researcher it has been observed that flowering time significantly correlates with harvest time in highbush blueberries (Galletta and Ballington, 1996). Gough (1994) was noted that there is neither correlation between the time a flower opens and the ripening of its fruit, nor any pattern of berry ripening within a cluster.

The yield of observed cultivars ranged from 5.99 to 0.106 kg per bush (Table 4). The most productive cultivars were 'Patriot' (5.99 kg per bush) and 'Northland' (5.26 kg), but an insignificant yield was to 'Barkeley' (only 106 g per bush), which was the reason for the small amount of racemes in the bush. The cultivars 'Chippewa' (1.8 g) and 'Bluejay' (1.6 g) gave the largest average single berry weight whereas 'Berkeley', 'Jersey' and 'Northland' presented 1.1 g of single berry weight. In natural *Vaccinium* populations, fruit size and flavour vary independently. Traditionally, large fruit has been more appealing to the producer and consumer, and is much easer to harvest and handle than small fruit, where hand labor is involved. Large fruit bring a premium on the market, and fruit size is

Table 3

Correlation between the lengths of different phenological phases

Indices	Beginning of vegetation period – beginning of flowering				
Beginning of flowering - beginning of fruit ripening	-0.34	Beginning of flowering - beginning of fruit ripening			
Beginning of fruit ripening - full ripeness	-0.53	-0.32	Beginning of fruit ripening - full ripeness		
Full ripeness - end of vegetation period	**-0.64**	-0.29	0.43	Full ripeness - end of vegetation period	
Full vegetation period	-0.68	0.11	0.42	0.55	Full vegetation period

*Underlined values significant at p=0.05

Table 4

Average yield and berry weight of highbush blueberry cultivars

Cultivars		Yield, kg per bush	Single fruit, g
Early season	Spartan	4.31	1.30
	Patriot	**5.99**	**1.55**
Early midseason	Chippewa	4.12	**1.79**
	Northland	**5.26**	1.08
Midseason	Blueray	0.76	1.30
	Bluecrop	3.47	1.31
	Jersey	0.93	1.12
	Bluejay	2.04	1.55
Late midseason	Barkeley	0.11	1.06
	p-value	0.04	

the principal grading criterion in marketing highbush fruit (Galletta and Ballington, 1996; Giongo et al., 2006). Compared with information in the literature, the single berry weight of highbush blueberry in Latvian conditions is lower. For example, the single berry weight of cultivar 'Spartan' are 2 g, 'Jersey' 1.7 g and 'Blueray' 2.2 g (Giongo et al., 2006). Berry size, however, is affected somewhat by the location of the fruit on the bush (Gough, 1994).

The content of titrable acid in berries of highbush blueberry cultivars differed between 0.58% in 'Northland' and 1.09% in 'Bluejay' (Table 5). These results are lower than in other blueberry cultivars, for example, the titrable acid content in cultivar 'Chandler' was 1.35% in 2008 (Kampuse et al., 2009). It is important for processing to find blueberry cultivars with the highest acidity therefore the cultivars 'Chandler' could be more suitable for the production of juice and other preserves (Kampuse et al., 2009). The titrable acids content in most of the cultivars did not exceed 1% and is low compared to other berry species.

The soluble solids content in the cultivars differed between 8.53% ('Barkeley') and 12.54% ('Jersey', 'Spartan', and 'Bluecrop') (Table 5). The soluble solids content in some blueberry cultivars grown in Latvia is lower than in other growing regions due to more rainy and colder climate. For example the soluble solids content in berries of cultivar 'Berkeley' was on average only 8.53%, which is notably lower compared to the data mentioned in the literature (Saftner et al., 2008). Prior et al. (1998) have indicated that soluble solids content in lowbush blueberries is 14.3%; they found much wider scope of soluble solids content in particular cultivars of highbush blueberry: 10 – 19%.

The total phenol of highbush blueberry cultivars varied from 76.80 to 118.73 mg 100 g^{-1}. The cultivars with the highest phenol content were 'Northland' and

Table 5

Average yield, berry weight, and biochemical content of highbush blueberry cultivars

Cultivars		Total phenol, mg 100 g^{-1}	Total antocyanin, mg 100 g^{-1}	Ascorbic acid, mg 100g^{-1}	Titrable acid, %	Soluble solids, %
Early season	Spartan	108.95 ±0.20	**381.14** ± 6.22	7.81 ± 0.73	0.82 ± 0.00	**12.53** ± 0.01
	Patriot	97.65 ± 4.84	234.78 ± 11.99	**11.83** ± 0.37	0.77 ± 0.00	10.74 ± 0.12
Early midseason	Chippewa	76.80 ± 4.36	230.75 ± 7.93	8.25 ± 0.16	0.59 ± 0.00	11.05 ± 0.05
	Northland	**118.73** ± 7.35	272.96 ± 10.37	7.36 ± 0.46	0.58 ± 0.00	9.56 ± 0.08
Midseason	Blueray	111.24 ± 3.93	337.92 ± 5.73	9.17 ± 0.28	0.91 ± 0.03	10.85 ± 0.05
	Bluecrop	91.72 ± 1.27	**362.17** ± 9.76	8.15 ± 0.31	0.87 ± 0.06	12.48 ± 0.13
	Jersey	**113.76** ± 5.70	261.73 ± 5.74	**10.78** ± 0.74	**0.97** ± 0.09	**12.54** ± 0.05
	Bluejay	87.40 ± 1.95	220.09 ± 9.6	7.01± 0.80	**1.09** ± 0.03	11.91 ± 0.08
Late midseason	Barkeley	99.03 ± 2.14	244.58 ± 3.21	8.13 ± 1.44	0.62 ± 0.03	8.53 ± 0.05

'Jersey' (on average 118.73 and 113.76 mg 100 g^{-1}, respectively).

The total antocyanin content of highbush blueberries differed from 226 to 381 mg 100 g^{-1}. The highest antocyanin content was detected in highbush blueberry cultivars 'Spartan' and 'Bluecrop' (on average 381.14 and 362.17 mg 100 g^{-1}, respectively).

The ascorbic acid content in highbush blueberries differed from 7.01 to 11.83 mg 100 g^{-1}. The highest content of ascorbic acid was in cultivars 'Patriot' and 'Jersey' (on average 11.83 and 10.78 mg 100 g^{-1}, respectively). It should be noted that the ascorbic acid content in highbush blueberry cultivars is low compare to other berry cultivars (currants, strawberries, etc.) (Kampuse et al., 2009)

Conclusions

1. In 2009, the vegetation period of blueberry cultivars began on the 2nd April and was from 167 to 181 days. Flowering time was from 31 to 46 days from the beginning of yhe vegetation period. The time of fruit ripening fluctuated from 56 to 66 days.
2. The most productive cultivars were 'Patriot' (5.99 kg per bush) and 'Northland' (5.26 kg). The cultivars 'Chippewa' (1.8 g) and 'Bluejay' (1.6 g) presented the biggest single berry weight.
3. The cultivar 'Jersey' had the highest titrable acid content (1.09%), the highest anthocyanins content (113.76 mg 100 g^{-1}), and the highest content of ascorbic acid (10.78 mg 100g^{-1}).
4. The highest phenol content was detected in highbush blueberry cultivars 'Spartan' and 'Bluecrop' (on average 381.14 and 362.17 mg 100 g^{-1}, respectively).

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PHENOTYPIC ANALYSIS OF HYBRIDS AND THEIR PARENTS IN LILIUM SPP. BREEDING

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Abstract. The lily (*Lilium* L.), a vegetative propagated perennial crop, is one of the economically most important flower bulb plants. It is cultivated worldwide as a cut flower, garden plant and pot plant. Lilies, 'the aristocrats of the garden', are very popular for their elegant flowers and stately habit, and offer the gardener an enormous range of colors, forms, and fragrances. The aim of this research was to evaluate the parent plants with the hybrids according to their morphological characteristics: plant height, flower diameter and inflorescence: number of flowers. In a trial, fourteen cross combinations were analyzed in 2006 – 2007. The evaluation was conducted in a lily breeding and growing farm 'Puķulejas', located in Saulkrasti, region of Riga, Latvia. The results showed that, if there were no significant differences in plant height between the parent plants, the hybrids were shorter than their parents. Male parents with short stems produced hybrids with shorter stems. Parental selections of medium and large number of flowers produced hybrids with comparatively large numbers of flowers on a stem, but if parents of different number of flowers were selected, in hybrids the smaller count dominated or prevailed. Parental selections of flowers with small and medium flower diameter showed heterosis effect on flower diameter. In crosses where the parental cultivars had different flower diameter, large or medium flowers prevailed.
Keywords: crosses, morphological characteristics, traits.

Introduction

Used worldwide as an ornamental flower, the lily (*Lilium* L.) is a perennial bulb plant. The lily belongs to the *Liliaceae* plant family, which comprises about 100 lily species (Beattie and White, 1993; Straathof et al., 1993). The genus is scattered all over the Northern Hemisphere (North America, Europe and Asia). Their distribution is one reason for the great adaptability of lilies in the garden (McRae, 1998). Another characteristic that has made lilies among the foremost garden flowers is the great variation of size and flower form, color and fragrance, habitat preference, and flowering time (Van Holsteijn, 1994).

Every year breeders have to select and to analyse several thousands of plants. This labour-consuming process is burdened by a modificative variability. In production of lily hybrids, plant height is considered a very important trait (Van Holsteijn, 1994; Grassotti et al., 1990). For genomes of pot-growing plant cultivars, small-sized genes are used; for cut-flower cultivars, genetic material for taller plants is selected; for garden cultivars, plants of medium height with rather durable stems are considered most suitable. Because genetics in lily breeding is very important, understanding the principles of genetics can save much time, trouble and effort. These principles can help breeders to predict what can be expected from a particular cross and also if there is a good chance to get a desired combination of a certain trait in a plant. In some crosses there is an incomplete dominance, in which a hybrid is 'in between' or intermediate forms of its parents. It is usually easy to see when incomplete dominance is occurring, but sometimes it is difficult to tell how many genes are involved (McRae-Freeman, 1980). In developing new hybrids, outdoor factors are an important consideration, so cultivars suitable for outdoor growing could be produced (De Hertogh, 1996; Thomas, 1986).

The research goal of these cross combinations was to evaluate the parent plants together with the hybrids according to their morphological characteristics: plant height, number of flowers and flower diameter, so that it may be possible to estimate the outcome of the trial and to select the best hybrids.

Materials and Methods

Plant Material and Growing Conditions

Bulbs of 21 Asiatic lily cultivars and four Trumpet lily cultivars were used as parent plant material, as well as hybrids of 14 cross combinations. The evaluation was conducted in a lily breeding and growing farm 'Puķulejas', located in Saulkrasti, region of Riga, Latvia. According to the International Union for the Protection of New Varieties of Plants (UPOV) requirements, 10 bulbs of commercial size (4 cm diameter) from each cultivar and also hybrid populations were used in four replications. Bulbs were planted in 1 m wide beds, 15 cm apart rows, 10 cm depth, and 20 cm between rows. Two free rows separated parent plants from hybrid populations. Parent cultivars included in cross combinations are presented in Table 1.

Table 1

Parent cultivars included in cross combinations

Cross no.	Parentage	
	♀	♂
Asiatic lily hybrids		
K-1	Nakts Tango	H-7-92
K-2	Nakts Tango	Cha-Cha
K-3	Cha-Cha	My Joe Ann
K-4	Cha-Cha	Silly Girl
K-5	Baltais Lacis	Shirley
K-6	Tirreno	Honey Pink
K-7	Herrold	Solstice
K-8	Compass	Gran Paradiso
K-9	Nepal	Alisa
K-10	Patricija	Magic Eye
K-11	Orfejs	Aristo
K-12	Olga	Arabeska
Trumpet lily hybrids		
K-13	Zemgale	White Henryi
K-14	Ekzotika	*L. henryi* var. *citrinum*

Lilies were cultivated in the field conditions without the use of fungicides for two seasons, 2006 - 2007. The soil was podzolic sandy, well prepared with pH KCl = 6.7, content of nitrogen (N) = 366 mg kg^{-1}, phosphorus (P_2O_5) = 239 mg kg^{-1}, potassium (K_2O) = 250 mg kg^{-1} (determined by Egner-Rhiem), magnesium Mg = 617 mg kg^{-1} (determined by the photometry method), and the humus content 60 g kg^{-1} (determined by Tyurin's method). Additional fertilizing was done according to the soil analysis. At the beginning of vegetation season in April, lilies were fertilised with ammonium nitrate at the rate of 4 kg 100 m^{-2}, in May – with calcium nitrate at the rate of 3 kg 100 m^{-2}, before flowering – in early of July with Hydro complex (NPK 12-11-18+ microelements) at the rate of 4 kg 100 m^{-2}, and after flowering – in early August with complex fertilisers with reduced amount of nitrogen (NPK 6-21-32), at the rate of 4 kg 100 m^{-2}.

Evaluation: plant height, number of flowers and flower diameter

Parent plants and hybrids were estimated individually by the plant height (cm), number of flowers (count), and flower diameter (cm). In the estimation of hybrids in relation to parents, the following conditions were observed:

- if the numerical value of a hybrid is in the range between the values of both parent plants, and this difference is significant, this position of a hybrid is called intermediate (IM) (the numerical value of a trait is intermediate);
- if the produced hybrids show significant improvements over parent plants, such a hybrid position is called heterosis (H);
- if the numerical value of a hybrid is in the range between numerical values of both parents and there are no significant differences detected, such a position is called prevalence (P) (the numerical value of a trait is closer to one of the parents);
- if the numerical value of a hybrid is significantly lower than the numerical values of both parents, it is called negative transgression (NT);
- if the numerical value of a hybrid is equal to that of one of the parents, or does not significantly differ from the parents, such a hybrid position is called dominance (D).

The obtained data were analyzed by heterogenous complex dispersion method; the hybrids produced were compared to parent plants using Fisher's criterion (F); phenotypic variability of the studied traits was characterized by the coefficient of variation (V%) according to the method by J.Guzhov (1978): < 10% – low, 10 – 20% – medium, > 20% – high.

Meteorological conditions

April of **2006** was mostly cold, the average temperature was 5.5 °C which was 0.8 °C below the average. The spring frosts occurred in the third decade of April – with night temperature two degrees below zero, which adversely affected the development of young sprouts. The average monthly temperature in May was 11.4 °C – slightly below the average. The average monthly temperature in June was 15 °C – slightly below the average temperature. The first decade of June was without precipitation. July was hot and sunny with little precipitation (23.5%), which required additional watering. The average monthly temperature was 20 °C, i.e. for 0.6 °C higher than the average temperature of many years. The weather of the year 2006 was favourable for growing lilies.

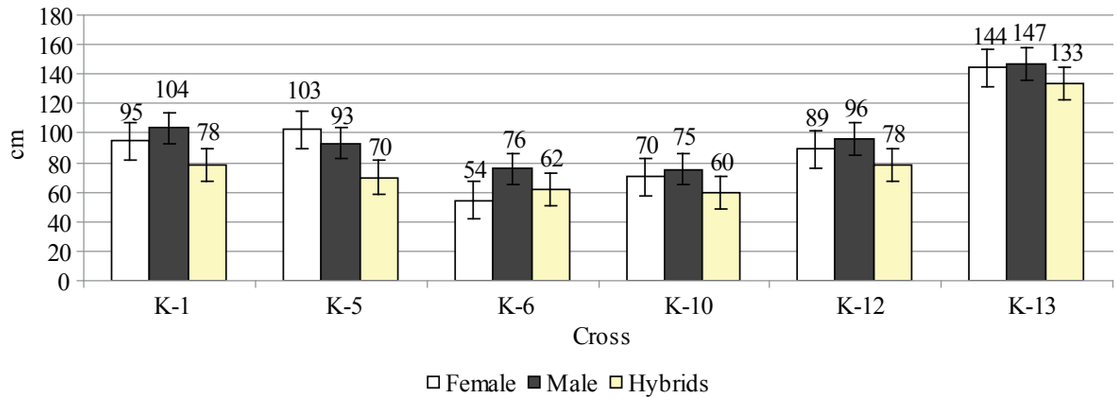

Figure 1. Crosses between parent plants similar in height.

April of **2007** was cold and cloudy with precipitation in the first half of the month, and warm and sunny – in the second, which favoured fast development of lilies. The third decade in May was very warm – 5.1 °C above the average. Precipitation was 42.9 mm that complied with mean observations. The weather conditions were favourable for fast development of lilies. The June was warm and with much precipitation. Heavy rains observed in the first and third decade in July, therefore leaves and flowers of lilies were damaged by the parasitic fungus *Botrytis spp*.

Results and Discussion

Plant height

By plant height, lilies were divided into three groups: 1) short (S) – lower than 80 cm; 2) medium (M) – ranging from 80 to 110 cm; 3) tall (T) – with stems exceeding 110 cm.

Figure 1 shows that crossing parent plants similar in height with plants of not significant differences in height (K-1, K-10, K-12, K-13, $F_{fact.} < F_{0.05}$), resulted in hybrids produced in cross combinations ((K-10 (S × S), K-1 and K-12 (M × M)), that were significantly shorter when compared to both parent plants selected in the cross, i.e., there was noted a negative transgression (NT).

In all cases there were significant differences in height between parent plants ($F_{fact.} > F_{0.05}$). Cross between short-growing female parent 'Cha-Cha' (58 cm) and medium tall male parents ('My Joe Ann' and 'Silly Girl') resulted in hybrids (K-3, K-4) – intermediates between both parents. When medium tall-stem female parents ('Nakts Tango', 'Herrold', 'Nepal', 'Orfejs') and short-stem male parents ('Cha-Cha', 'Solstice', 'Alisa', 'Aristo') were selected, in one of the combinations (K-2) the produced hybrid was an intermediate, in two combinations short–stem hybrids were prevalent (K-7, K-9), and in one combination – dominant (K-11). It suggests that a short–growing cultivar selected as a male parent contributes to the production of short–growing progeny. Short-growing hybrids were dominant (K-8, K-14) when crossing plants of medium and tall cultivars (Figure 2).

The trait 'plant height' was characterized by great phenotypic variability. For parent plants, the coefficient of variation for this trait was recorded in the range from 3.4 to 12.4%, and for hybrids – from 10.9 to 20.4%. These results confirm that the range of variability for plant height is quite high (V% = 20.4) as it has been reported by Grassoti et al. (1990).

The number of flowers

The productivity of a cultivar is determined by the number of flowers. The more flowers on a stem, the more attractive is the plant with a longer flowering time. Parent plants included in cross combinations were characterized by a small (S) number of flowers

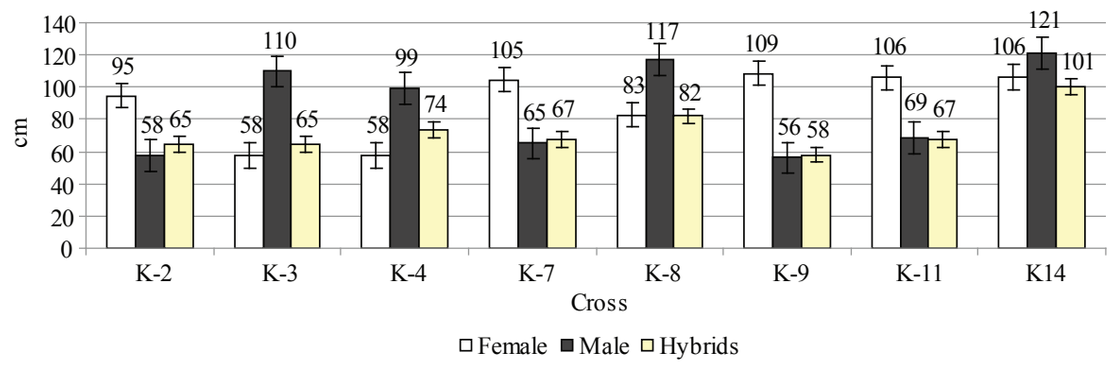

Figure 2. Crosses between parent plants different in height (difference is significant $F_{fact.} > F_{0.05}$).

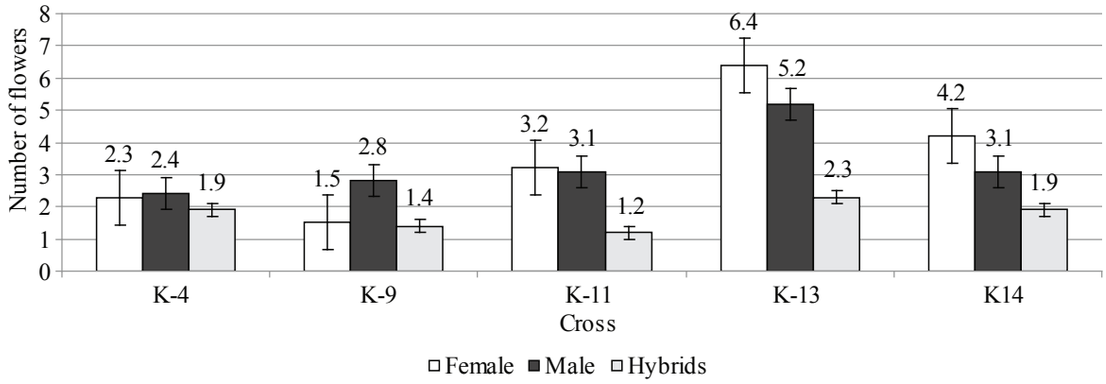

Figure 3. Crosses between parent plants similar by number of flowers:

(< 3), medium (M) number of flowers (3 – 5), and numerous (N) flowers (> 5) on a stem. Crosses between parent plants that have a small number of flowers (S × S) (as in combinations K-4 ('Cha-Cha' × 'Silly Girl') and K-9 ('Nepal' × 'Alisa')) resulted in hybrids with even smaller number of flowers when compared to a parent plant with the smallest number of flowers on a stem. This difference is not significant ($F_{fact.} < F_{0.05}$), and, in this case, the trait of 'small number of flowers' could be regarded as dominant. Parent plants that have medium number of flowers (K-11, K-14) and plants that have numerous flowers (K-13) on a stem produced hybrids with a smaller number of flowers than their parent plants, i.e., there was noted NT (Figure 3).

Figure 4 indicates – when choosing cultivars with a small number of flowers as female parents and male parents with a medium number of flowers (S × M) on a stem, the trait of 'a small number of flowers' was dominant (K-7) or prevalent (K-6) in the produced hybrids. In a case where female parent had a medium number of flowers and the male parent had a small number of flowers (M × S), NT was noted in one of the combinations (K-10). In the other combination (K-12), the dominant trait was a 'medium number of flowers per stem'. A small number of flowers on a stem was prevalent in hybrids obtained in crosses between cultivars with a small number of flowers and cultivars with many flowers (S × N), as in combination K-3 ('Cha-Cha' × 'My Joe Ann'), or vice versa (N × S), as in combination K-2 ('Nakts Tango' × 'Cha-Cha').

Hybrids with a comparatively greater average number of flowers (3.0 – 4.2) on a stem were produced in crosses that included parent plants with a medium number of flowers and parents with many flowers on a stem (M × N, N × M) – even if NT was noted for the combination K-5 and medium number of flowers was dominant in combination K-1. The hybrids from the cross K-8 ('Compass' × 'Gran Paradiso') were intermediates between parents and were characterized by medium number of flowers (4.2).

The selected trait 'number of flowers' was characterized by a great phenotypic variability. For parent plants, the coefficient of variation for this trait was recorded in the range from 15.1 to 49.2%, and for hybrids – from 34.2 to 59.3%. The variability of these results is higher than the range of variability for the number of flowers (V % = 28.6) as it has been reported by Grassoti et al. (1990), which means that the large variability in the 'number of flowers' indicates that through these selected parents a selection programm could supply interesting results (Grassoti et al., 1990).

Within combinations, the fluctuations between the minimum and the highest possible number of flowers were in the range from 2 to 10. High phenotypic variability of the number of flowers on a stem enables the selection of medium- and multiflorous forms (at least 3 and more flowers per stem) in each cross

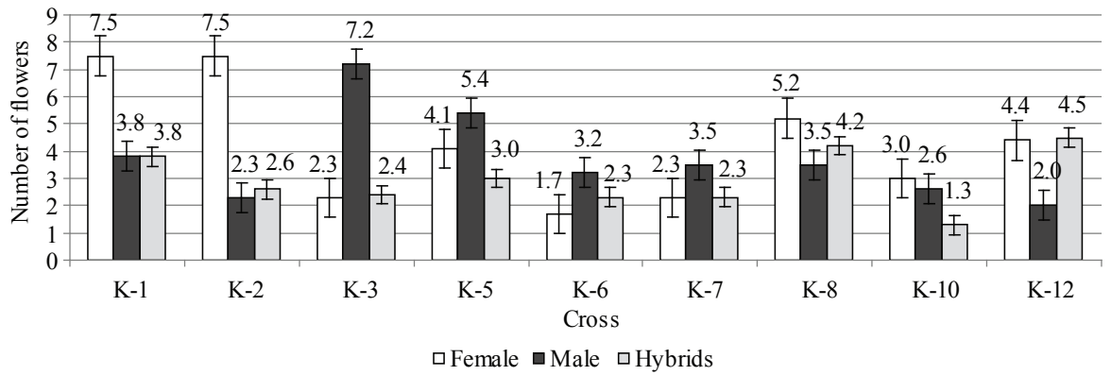

Figure 4. Crosses between parent plants different by number of flowers.

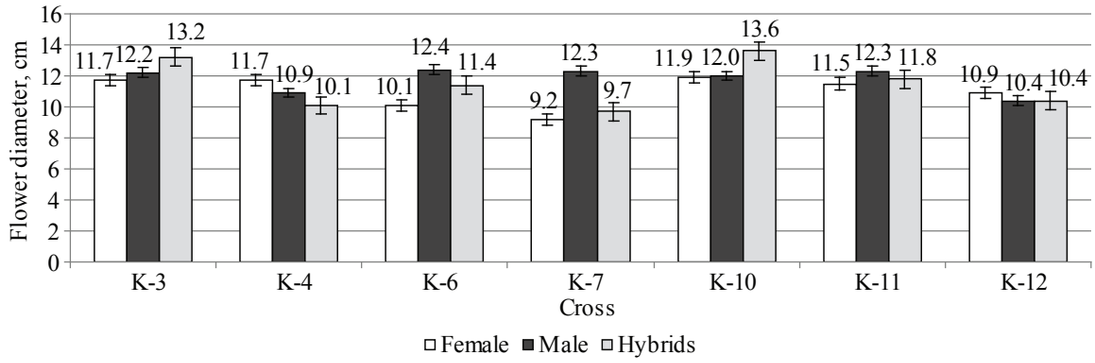

Figure 5. Crosses between parent plants similar by flower diameter.

combination. According to the plant model of garden lilies, there should be at least three flowers per stem, obtained from a bulb of three to four centimetres in diameter (Thomas, 1986).

Flower diameter

The general attractiveness of lilies is determined by flower diameter. The cultivars of parent plants involved in the crosses were characterized as 1) small-blossomed (S) with flowers less than 10 cm in diameter (< 10 cm); 2) medium (M) diameter (10 - 14 cm); 3) large-bloom (L) lilies (diameter >14 cm).

Crosses between small–blossomed parents with not significant differences in flower diameter produced hybrids with heterosis effect showing up in combination K-7, i.e., flower diameter in hybrids was larger: 133.7% when compared to female parent 'Herrold' (9.2 cm), and 126.8% when compared to male parent 'Solstice' (9.7 cm).

Crosses with flowers of medium diameter (M × M) resulted in hybrids with heterosis effect showing up in combination K-6. In hybrids, the flower diameter was 12.4 cm, (121.8%) when compared to female parent 'Tirreno' (10.1 cm), and 108.8% when compared to male parent 'Honey Pink' (11.4 cm). In combination K-4 ('Cha-Cha' × 'Silly Girl') there was noted an intermediate. The dominance of comparatively small flower diameter was noted in combinations K-10 and K-12, and prevalence was observed in combination K-3 (Figure 5).

Crossing the parents with small and medium size flowers, no matter what the forms of the female and the male parent were ((S × M, M × S), as in combinations K-2 and K-9), resulted in hybrids with the largest flower diameter prevalent; but in one case of these crosses (K-1), in the hybrids small-blossomed flowers prevailed.

When crossing cultivars with medium or large-diameter flowers (M × L, L × M), the produced hybrids were intermediates between both parents ((K-5, K-14) and large-bloom plants (K-13)), and medium-sized flowers (K-8) were dominant (Figure 6).

'Flower diameter' as a trait is characterized by the lowest level of phenotypic variability. The average values of the coefficient of variation were 4.9% (2.9 - 12.3%) for parent plants, and 10.9% (6.8 - 15.9%) for hybrid seedlings. In hybrid populations, the fluctuations between minimum and maximum flower diameter were in the range of 3 to 6 cm. It facilitates plant selection with different flower diameter in each cross combination. Not significant difference between two–year results was confirmed at 95% significance level thus indicating that 'flower diameter' is a stable inherited trait, and selection by this trait can be made in the first year of development. These results confirm that the 'flower diameter' could serve as a varietal trait (De Hertogh, 1996).

Conclusions

1. Not significant diversity in plant height between parents in cross combinations has resulted in hybrids significantly lower in plant height than that of both parents, i.e., there has been noted negative transgression. Parents with a significant difference in plant height in crosses produce hybrids shorter

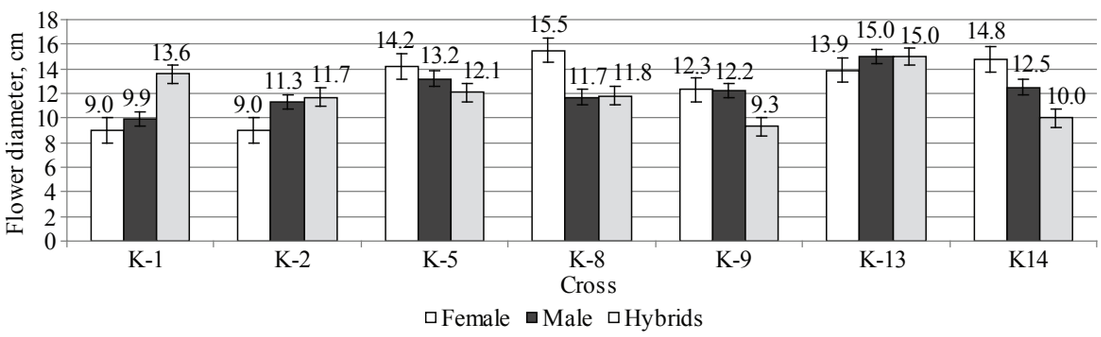

Figure 6. Crosses between parent plants different by flower diameter.

in stem if the male parent is a short-stem cultivar. If a short-stem female parent is selected, the produced hybrids are intermediates between both parent plants.

2. Lily cultivars with conditionally equal number of flowers in cross combinations have produced hybrids which do not vary significantly from their parents – even if negative transgression has been observed in some cases. The smaller number of flowers is dominant or prevalent when parents with a diverse number of flowers on a stem are selected. Small-blossom parents reciprocally crossed have produced hybrids with a noted heterosis effect on flower diameter. Dominance of both the largest and the smallest flower diameter has been found in hybrids produced by cross parents with medium-sized flowers. In hybrids, the prevalence of the largest flower diameter or an intermediate has been noted in combinations of cultivars with different flower diameter.
3. For further testing according to UPOV requirements, the selected and propagated hybrid material is given in charge of Plant Variety Testing of the State Plant Protection Service of the Ministry of Agriculture, Republic of Latvia.

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ANALYSIS OF KAPPA-CASEIN (*CSN3*) ALLELES IN LATVIAN BROWN AND LATVIAN BLUE BREED POPULATIONS

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Abstract. Genotypes of *CSN3* gene were detected in 71 individuals of Latvian Blue breed and in 30 individuals of Latvian Brown breed using a PCR-RFLP method. Animals were chosen at random from each heard. This study investigated the alleles A and B of *CSN3* gene, while determining the allele and genotype frequencies and Hardy-Weinberg equilibrium proportions in both populations. The results showed that in our analyzed samples from 71 Latvian Blue breed only 7 animals (10%) had the genotype BB, and in analyzed samples from 30 Latvian Brown breed only 3 had the genotype BB (10%). Frequencies of *CSN3* genotypes AA, AB, and BB correspond to Hardy-Weinberg equilibrium proportions and populations in genetic equilibrium. A wide variation in the B allele frequency among Latvian Blue and Latvian Brown breeds was found suggesting that molecular selection for animals carrying the allele B of *CSN3* could impact breeding programs for dairy production in Latvia.
Key words: kappa-casein alleles, cows, genetic structure of population.

Introduction

Caseins are milk proteins secreted by mammary gland cells. They constitute about 78-82% of bovine milk proteins and are subdivided into four main groups: αS1-casein, αS2-casein, β-casein, and κ-casein (Eigel et al., 1984; Rohallah et al., 2007). These proteins and their genetic variants have been extensively studied and reported as an important factor associated with lactation performance, milk composition, and cheese yield efficiency (Aleandri et al., 1990). Bovine casein is encoded by a 200 kb DNA fragment located at chromosome (Chr) 6 q31-33 (Ferretti et al., 1990) arranged in the order of αS1, αS2, β, and κ. *CSN3* fragment spans the 13 kb DNA sequence divided into five exons and intervening sequences, and constitutes about 25% of the casein fraction (Alexander et al., 1988; Lien and Rogne, 1993; Martin et al., 2002). The αS1-, β-, and αS2-casein genes are the most closely linked and form an evolutionarily related family, whereas the *CSN3* gene is at least 70 kb away from them (Ferretti et al., 1990).

CSN3 has been extensively studied for its role in stabilizing the casein micelles and its influence on the manufacturing properties of milk. For several breeds, the genetic variability in the *CSN3* locus has been reported each with a different allelic frequency based on genetic diversity among breeds. Various allelic variants have been described for *CSN3* gene in different cattle breeds, which include A, B, C, E, F, G, H, I, and AI (see review by Soria et al., 2003). Among these, variants A and B are most commonly found and variant B is predominantly concerned with processing properties of milk and has better lactodynographic properties (Lin et al., 1992). In variant B, due to a single base mutation in the *CSN3* locus, isoleucine substitutes threonine and aspartic acid is substituted by alanine (Pinder et al., 1991).

Selection for the allele B of *CSN3* gene is integrated into cattle breeding programs in many countries, and it also should be done in Latvia too. The research of the milk protein genes is needed to obtain the information of the GAS program development in Latvia and to promote the cows' milk protein breeding in Latvia. This research helps to create conditions for single gene or QTL assisted breeding methodologies for dairy cattle breeding of milk protein, their use of other economically important traits in the breeding perfection.

Materials and Methods

Animals were chosen at random from each heard. The blood was taken from the jugular vein and was collected in K3-EDTA coated sterile vaccutainers and stored at -200 °C until used for DNA extraction. DNA was extracted using the Fermentas Genomic DNA Purification Kit # KO512. The *CSN3* alleles were identified using the PCR-RFLP (Polymerase Chain Reaction and Restriction Fragment Length Polymorphism) method in accordance with methodology provided by J.F. Medrano and E. Aguilar-Cordova (1990) and G.E. Sulimova (2007). We used primers: *SGE* 5'-TAT CAT TTA TGG CCA TTG GAC CA-3' and *SGO* 5'- CTT CTT TGA TGT CTC CTT AGA GTT-3'from methodology of G.E. Sulimova (2007). The amplification was carried out in Applied Biosystems 2720 Thermal Cycler with the following amplification conditions: 95 °C for 2 min (initial denaturation), then followed 35 cycles with denaturation at 95 °C for 30 sec, annealing at 55 °C for 40 sec, and extension at 72 °C for 30 sec with a final extension step of 72 °C for 7 min. Samples of PCR products (25 µl) were digested with *HindIII* and *HinfI* endonucleases according to the manufacturer's recommendations (Fermentas). Restrictive fragments that were obtained this manner were separated in a 2% agarose gel with ethidium bromide (10 µl EtBr 100 ml^{-1} of 2% agarose gel). Electrophoresis on 2% agarose gel was used for visualisation of the restricted DNA bands (60V, 150 min) in 0.5X TBE buffer. Research was done in the Laboratory of the Molecular Genetic Researches of the Faculty of Agriculture of LLU.

The data on cows milk performance were acquired from the LDC (Latvia data centre) system database.

Table 1

Bovine *CSN3* genotype frequencies in Latvian Blue and Latvian Brown breed populations

Genotype	Latvian Blue breed		Latvian Brown breed	
	Number of genotype	Frequency of genotype	Number of genotypes	Frequency of genotype
AA	33	0.465	14	0.467
AB	31	0.437	13	0.433
BB	7	0.099	3	0.100

Allele frequencies were calculated by using the appropriate diallele locus expressions, where allele A relative frequency was designated as p, and the relative frequency of B allele as q. We obtained the p and q expressions:

$$p = \frac{2D + H}{2N} \quad (1) \quad \text{and} \quad q = \frac{2R + H}{2N} \quad (2),$$

where:

D, H, R - the number of individuals with genotypes AA, AB, and BB;
N - total number of animals in the analysis;
2N - total number of alleles in the analysis.

Calculations were made with Microsoft Office Excel 2007 standard package assistance, but computer program package TFPGA was used as a population genetic basis of the accuracy of testing (Miller, 1997).

Results and Discussion

In the analysis we found that *CSN3* (κ-casein gene) genotype distributions are very similar in both of the tested populations (Table 1).

In both analyzed populations we found practically similar allele frequencies: 0.683 for allele A, and 0.317 for allele B. It was seen that the frequency of k-casein allele A in both populations was two times higher than the frequency of k-casein allele B. Examining the population genetic equilibrium in these alleles, it was found that in both populations frequencies of genotypes correspond to Hardy-Weinberg equilibrium proportions:

$$(0.683 + 0.317)^2 = 0.466 + 0.433 + 0.100.$$

Comparing κ-casein A and B allele frequency of Latvian Brown breed cows (Table 2) in the samples of our analysis (n = 30) and recently published data (L. Paura et al., 2009), we found that in the population could have been a change and increased desirable allele B frequency from 0.167- 0.184 to 0.316, i.e. about 0.132 or 0.149.

The fact that the alleles A and B frequencies in both analyzed populations (Latvian Blue and Latvian Brown breed) turned out to be virtually identical, and could indicate that the factors which affect the frequency of both populations are identical, and the favourable allele is allele A. This factor should be put on the clearance of subsequent studies, particularly because of the selection process would be desirable to reduce the frequency of allele A and increase frequency of desirable allele B and frequency of the genotype BB. In our analyzed samples from 71 Latvian Blue breed only 7 animals (10%) had the genotype BB, and in analyzed samples from 30 Latvian Brown breed only 3 had the genotype BB (10%). Our sample data is analyzed within the framework we tried to determine the genotypes AA, AB and BB, as influence factors on cow productivity in the first standard lactation. However, our sample volume proved insufficient to speak of statistically significant results. We can only point to possible trends.

Table 2

***CSN3* alleles A and B and the corresponding genotype frequency analysis of Latvian Brown breed**

Genotypes and genes	Our results Cows, n=30			(Paura et al., 2009) Bulls, n=19			(Paura et al., 2009) Cows, n=30		
	De facto	HW	±	De facto	HW	±	De facto	HW	±
AA	0.467	0.468	0.001	0.632	0.666	0.034	0.733	0.694	0.039
AB	0.433	0.432	0.001	0.368	0.300	0.068	0.200	0.278	0.078
BB	0.100	0.100	0	-	0.034	0.034	-	0.028	0.028
A	0.684	-	-	0.816	-	-	0.833	-	-
B	0.316	-	-	0.184	-	-	0.167	-	-

HW – Hardy - Weinberg equilibrium

Table 3

Analysis of milk characteristics estimated for the 1st lactation depending on genetic variant of *CSN3* in the investigated population

Genotype	Number of genotype	Milk yield, kg	Fat content, %	Protein content, %
Latvian Blue breed				
AA	28	4225.2±211.73	4.24±0.108	3.22±0.046
AB	21	3712.6±217.56	4.33±0.111	3.29±0.047
BB	5	4592.8±430.42	3.98±0.220	3.29±0.093
Latvian Brown breed				
AA	12	4129.2±305.46	4.23±0.103	3.09±0.076
AB	14	4489.5±371.79	4.55±0.126	3.27±0.093
BB	3	3801.2±981.52	4.00±0.332	3.56±0.244

The study of animals used for yield data in the 1st standard lactation (Table 3) had the following limits: milk yield ranging from 3713 kg to 4592 kg, fat content 3.98-4.50%, and milk protein content 3.09-3.56%.

By the analysis of *CSN3* different genotype effects on cow productivity characteristics, we obtained that sample volumes were still insufficient. However, the genotype BB probably tended to prevent the milk fat content expression. As a test of possible effects of genotypes on milk protein content we can note that the Latvian Brown breed where the difference between AA and BB genotype cows average protein content of the 0.47 and $t_{emp.} = 1.839 < t_{teor.\ (0.05,13)} = 2.16$, which is already close to $\alpha = 0.05$ requirements.

We found references in the literature about the positive effect of κ-casein BB genotype on dairy cow milk dietary and technological properties. If such a relationship could be found in our breed populations, probably it would be purposeful to increase the frequency of B allele in our cattle populations. If we view that so far these allele frequencies are not selective controlled and the results identified a gene drift, then the question remains, however, which resulted in A allele frequency prevalence, which we found in the data.

Conclusions

1. DNA samples (n = 101) of Latvian Blue and Latvian Brown breeds, the examinatition found that *CSN3* A and B allele frequencies are 0.683 and 0.317, and both populations are practically same.
2. Frequency of k-casein allele A more than twice times higher than the frequency of *CSN3* allele B and the reason which caused the prevalence of k-casein allele A is not known yet.
3. Frequencies of *CSN3* genotypes AA, AB, and BB (Latvian Blue breed, n=71, 0.465, 0.437, 0.099, and Latvian Brown breed, n = 30, 0.467, 0.433, 0.100) correspond to Hardy-Weinberg equilibrium proportions: $(0.683+0.317)^2=0.466+0.433+0.100$ and populations in genetic equilibrium.

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EXPERIMENTAL INVESTIGATION OF SOLAR ENERGY COLLECTOR ON PRODUCTION CAPACITY OF HOT WATER

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Abstract. Pollution of atmosphere and decrease of fossil fuels stimulates people to search for an alternative energy sources for production of energy, both electrical and heat. In Latvia it is possible to use almost all alternative energy sources for production of energy, including solar energy that becomes more relevant year by year. Solar radiation in direct way makes no hazardous, but, as the intensity of solar radiation in geographical location of Latvia is comparatively low, the utilization of it demands relatively expensive equipment. Production of energy from other sources of energy also demands certain investments as well as the purchase of fuel, maintenance of equipment and presence of different manipulators. In practice several types of solar collector constructions with efficiency from 30 up to 75% exist and expenses vary in the wide range. The average number of sunshine hours in Latvia is about 1800 hours yearly. Nevertheless solar collectors for water heating in Latvia are used. Mostly flat-plate collectors are used whose efficiency often is not sufficient and water has to be additionally warmed-up. The aim of our investigation is to study operation of evacuated tube collector in conditions characteristic to Latvia. It is stated that it is feasible to use evacuated tube collector for water heating in Latvia, especially from March till October. During winter period solar collector can be used as additional energy source for water heating.
Key words: solar radiation, solar energy collector, temperature rate, distribution, characteristic.

Introduction

Solar energy can be used in three basic technological processes Tiwari G.N. (2006): chemical, electrical and thermal (Fig.1). Chemical process, through photosynthesis, maintains life on earth by producing food and converting CO_2 to O_2. Electrical process, using photovoltaic converters, provides power for spacecraft and is used in many terrestrial applications. Thermal process can be used to provide much of thermal energy required for solar water heating and building heating. Another form of converted solar radiation is mechanical energy as wind and water steams Weiss W., Themessl A. (1996).

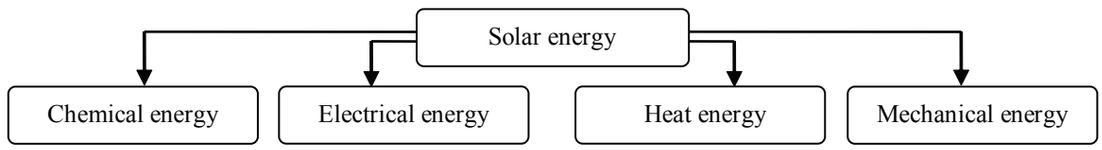

Figure 1. Conversion of Solar Radiation to other Energy Forms Jesko Z. (2008).

The most important and most expensive single component of an active solar energy system is the collector field, which may be performed in several versions, from constructions of solar collectors, till collector configuration. *Solar collector* is a mechanical device which captures the radiant solar energy and converts it to useful thermal energy Jesko Z. (2008).

The objective of the research is to establish the temperature character of warmed water in the solar collector and distribution of day numbers in groups by temperature range for each moth separately and for the year in general.

To investigate possibilities of utilization of solar energy for water heating throughout the year, an evacuated tube solar collector with area of 3 m^2 is mounted on the roof of the building of the Research Institute of Agricultural Machinery of Latvia University of Agriculture in Ulbroka, Riga region. Solar collector is not equipped with registration equipment of produced or/and consumed water quantity. Because of that for completely evaluation of warm water supply the logger HOBO H08 is used, which has registered temperature of water in storage tank of the solar collector during the period of a year. For that the temperature sensor of the logger HOBO H08 is placed into a temperature sensor socket of warmed water storage tank. Value of temperature was registered with an interval of 15 minutes for each month separately. From acquired graphs (see below) it is possible to consider the length of corresponding solar energy collector working time and water temperature in storage tank, as well as for how many degrees it has increased. Processing obtained data in software MS Office Excel, i.e., achieving empirical distribution in groups after temperature range (amplitude), it is possible to get collateral duration for several temperature diapasons. Similarly, when quantity and temperature increase of water in storage tank is obatined, it is possible to calculate quantity of heat energy Q_c produced by solar collector during given period Харченко Н.В. (1991).

$$\eta_c = \frac{Q_c}{Q_g A_c} , \quad (1)$$

where η_c – efficiency of solar collector;
Q_c – quantity of heat energy produced by solar collector, W·h;
Q_g – quantity of heat energy arrived at 1m^2 of solar collector area, W·h·m^{-2};
A_c – are of solar collector, m^{-2}.

$$Q_c = mC_p(T_2 - T_1) , \quad (2)$$

where m – mass of water in storage tank, kg;
C_p – specific heat capacity of operating liquid, W·h·kg^{-1}·K^{-1}
T_1 – initial temperature of water in storage tank, K;
T_2 – finish temperature of water in storage tank, K.

Efficiency of solar collector can be obtained from effective optical efficiency of solar collector and effective coefficient of heat loss in accordance with

$$\eta_c = \frac{\eta_o - k_c(T_1 - T_a)}{Q_s} , \quad (3)$$

where η_o – effective optical efficiency of solar collector;
k_c – effective coefficient of heat loss, W·m^{-2}·K^{-1}
T_a – ambient temperature, K;
Q_s – power of global solar radiation reaching surface of solar collector, W·m^{-2}.

During the year power of global radiation and charge power of electrical battery produced by solar cell was registered. In way of integration of diagrams of global radiation or produced power of solar cell with diagrams of warmed water in storage tank it is possible to discuss producing capacity of solar collector depending on power of solar radiation.

Materials and Methods

It is easier to understand structure and operational principles of solar energy collector device when the functional scheme of system has been examined in advance (Fig.2). Obviously the most important assembly of solar energy collector system is a storage tank 15 of heated water by which two contours incorporated are: *a contour of solar energy collector*, whose parts mutually are connected to copper pipes, through which the liquid heat carrier circulates during operation, and energy obtained from the solar energy collector conveys to the storage tank and *the contour of warmed water*, whose parts also are connected to pipes and through which heated water is drained to a consumer.

The contour of solar energy collector, in the direction from solar energy collector and moving towards the direction of a heat carrier flow consists of: a solar energy collector 1, by which in the output of heat carrier temperature sensor 7 and air valve 18 are inserted; a thermometer 4 built-in solar divicone 3; heat exchangers 25 and 26; a block of valves for fill in heat carrier 18; one-way valve (non-return valve) equipped with indicator of flow intensity 7; a heat carrier circulation pump 6 and a thermometer 5. As mentioned before, all these parts are connected by copper pipes. These pipes by the use of block of valves for filling in heat carrier 18 and the air valve 9 under pressure heat carrier are filled and circulate through this contour during operation of the circulation pump 28. Devices of operation and safety control are connected to the contour: a safety-valve 10, a manometer 11, an expansion tank 12 and an overflow container 13. Elements of solar energy system that during operation might be replaced, for example, a circulation pump, are equipped with disjunctive valves and performance of elements exchange could be performed without deflation of heat carrier.

The contour of heated water consists of a one-way valve 24, an expansion tank 27, a manometer 28, a storage tank 15 and a mixing valve 23 (not installed), pipes and valves 22 by which the storage tank with cold water supply is connected and distribution canal of heated water 20. When some valve of water distribution is open, cold water through valves 22 and 24 flow in a storage tank and through a cold-warm water mixing valve press out warmed water. For operation in automatical regime the system is equipped with a programmable device 17.

Operation principles of solar energy system in automatic regime are as follows: when the sun is heating a heat carrier and the temperature of sensor 8 exceeds the temperature of sensor 19 for several degrees, e.g., 6 °C (adjusted value by manufacturer), the control equipment 17 turns on a circulation pump, that draws a warmed heat carrier through heat exchangers 25 and 26 where the heat carrier provides heat to water located in the storage tank. The temperature of heat carrier decreases when the pump is operating. Circulation pump turns off when temperature difference between sensors 8 and 9 becomes less than 4 °C (adjusted value by manufacturer). The process of system operation restarts if the heat carrier becomes warm again. Switching on and switching of the temperature of circulation pump is controllable. It has to be denoted that due to the fact that the sensor 8 is located a little bit further from a solar energy collector, the temperature in the collector contour measured by thermometer 4 at first increases when the circulation pump is switched on and, when the highest temperature is achieved, it begins to decrease until the circulation pump turns off. Such a location of the sensor 8 brings a periodical operation regime of the system.

Adjusted values of relevant operating parameters:

maximum water temperature in storage tank, °C 60;
minimum turn of circulation pump, % from nominal ones 30;
difference of circulation pump switch-on temperature, T_k-T_{tv}, °C 6;
difference of circulation pump switch-off temperature, T_k-T_{tv}, °C 4,

where T_k – temperature in collector sensor 8, K;
T_{tv} – temperature in heated water sensor in storage tank 19, K.

Examined solar energy collector system is located in the premises (block of engineers) of the Research Institute of Agricultural Machinery of Latvia University of Agriculture and 7 sinks and a shower are connected to it. Approximately 40 personas have to exploit it. Major part of piping is equipped with heat insulation. Solar energy collector is situated on the roof, but the equipment of solar energy system is located in the premises on 3rd floor.

As mentioned earlier, the solar energy solar system is not equipped with registration devices of produced and consumed energy. That is why, for discussion about efficiency of system, it was equipped with a logger HOBO H08, whose temperature sensor 16 was inserted into 2nd (unused) socket of a storage tank. HOBO H08 is a measuring device available with a computer, performing data registration without any assistance, i.e., without a link to computer. Computer is needed to adjust parameters for data registration and transfer of data saved into the logger to the computer. Further data can be saved, processed and presented into necessary form.

In order to achieve advances goal, it is provided to register two temperatures – water temperature T_w in a storage tank and ambient temperature T_a of room, where the storage tank is located. Recording two parameters, i.e., two temperatures with an interval of 15 minutes, memory range of HOBO H08 is enough for 33 days. It means that registered data by HOBO H08 have to be transformed to computer once a month and, thus, monthly graphs for temperatures T_w and T_a can be achieved. It is clear that depending on water consumption, season and meteorological conditions, water temperature T_w into the storage tank will vary and it's numerical values will be hardly predictable. In order to consider the character of produced warm

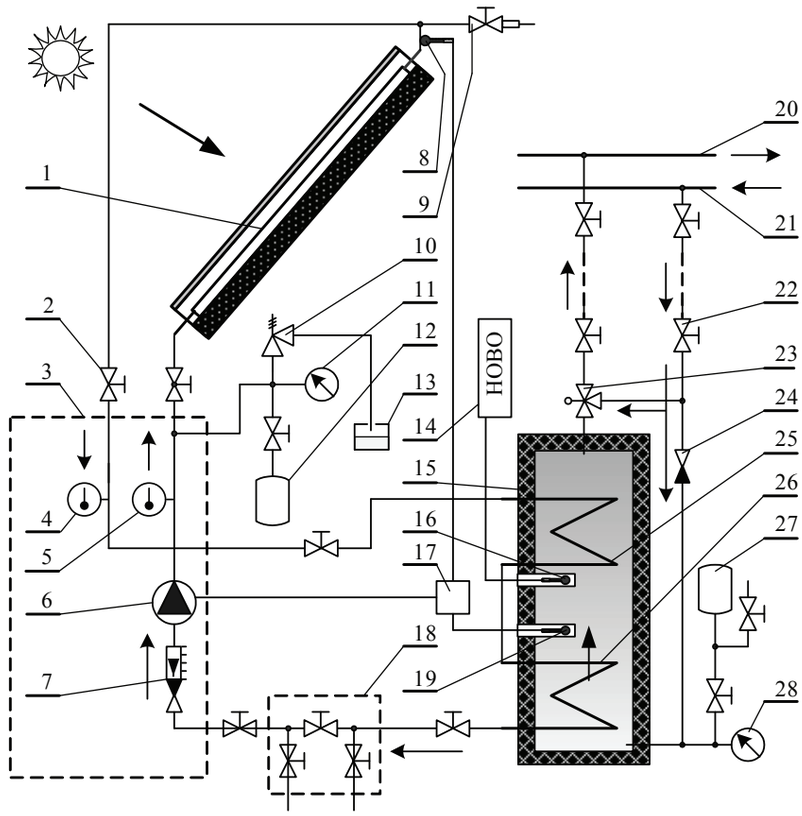

Figure 2. Functional scheme of solar energy collector Vitosol 200 SD: 1-solar energy collector Vitosol 200 SD; 2, 22–valves; 3-solar divicone; 4, 5-termometers; 6-circulation pump; 7-one-way valve; 8, 16, 19-temperature sensors; 9-air valve; 10-safery valve; 11, 28-manometers; 12, 27-expension tanks; 13-overflow container; 14-data register device; 15-storage tank; 17-programmable device; 18- block of valves; 20-distribution canal; 21-cold water intake; 23-mixing valve; 24-one-way valve; 25, 26-heat exchangers.

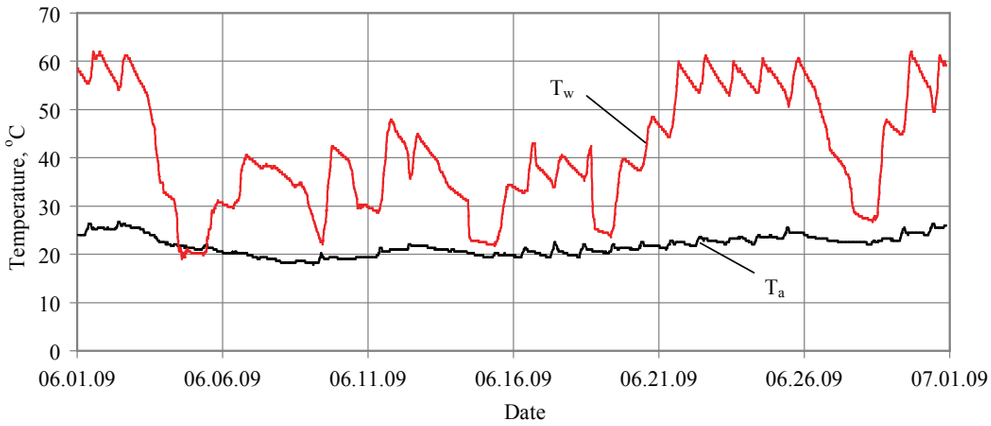

Figure 3. Ambient temperature T_a and water temperature in storage tank T_w during June.

water by the solar energy collector, it is assumed that temperature range from 15 to 65 °C should be divided into groups of 5 °C and for each group of temperature range duration for each month and year in general should be determined. From mathematical viewpoint it means that there has to been found empirical distribution of temperatures in groups after range.

Results and Discussion

Processing data registered by HOBO H08, diagrams of water temperatures of water in the storage tank T_w and ambient space T_a for December 2008 and 11 months of 2009 (see Figures 3 and 4) are obtained. Distribution of day numbers in groups according to temperature diapasons (Table 1) is calculated.

If water heated by solar energy reaches temperature of 40 °C (according to norms 55 °C ± 5 °C) then we can assume that water heating was started in the 3rd part of February, when during two days water temperature in the storage tank was achieved over 40 °C and water temperature in the storage tank increased to 27 °C. Beneficial conditions for water heating with solar collector are in March, when water in the storage tank gets heated over 50 °C.

While analyzing obtained data, it is possible to deduce that qualitative heating of water starts in April and continues till the end of September. During these six months the solar collector can operate as the main water heater. During this period water temperature in the storage tank often achieves adjusted 60 °C when water heating has been stopped instead of sufficient solar radiation for further water heating. That is why, when the solar energy system with a mixing valve 23 (Fig.2) is equipped, it would be possible to store more heat energy in the storage tank and reduce temperature drop during disadvantaged periods. Furthermore, in September water temperature in the storage tank achieved limit of 60 °C.

In October power of solar radiation steadily decreases and that is why water in the storage tank newer reaches more than 40 °C. From November till the end of February downtime of the solar energy system sets in when power and duration of solar radiation is not sufficient to heat water in the storage tank for something considerable.

Table 1 summarizes distribution of day numbers after diapasons with step of 5 °C of limits from 15 to 65 °C. From the table it is seen that during the year warm water in the storage tank had the following temperatures: 35-35 °C – 176.6 days (48.4% from 365 days); 40-45 °C – 125.4 days (34.4%); 50-55 °C – 72.4 days (19.8%).

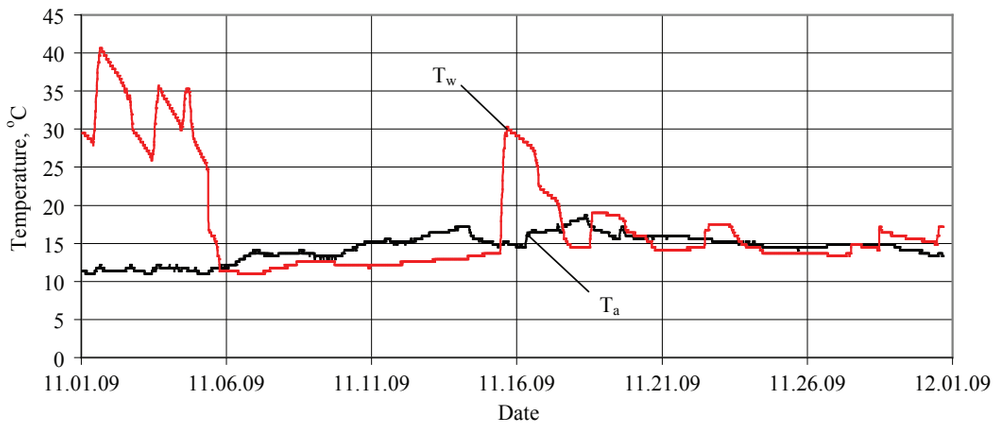

Figure 4. Ambient temperature T_a and water temperature in storage tank T_w during November.

Table 1

Distribution of day numbers after temperature rate in the storage tank of the solar energy collector system during years (2008) and 2009

Month	Number of days with temperature of heated water in storage tank greater than: °C									
	15	20	25	30	35	40	45	50	55	60
January	7.4	3.1								
February	16	9.6	4	1.9	0.8	0.2				
March	23.4	18.6	15	10.4	6.2	5	2.4	0.9		
April		27.9	26.7	25.5	24.7	23.1	18.6	14	6.2	0.8
May		30.5	30.4	30	28.1	22	17.4	14	7.7	0.8
June		29.8	27	24.1	19.8	14.2	11	8.8	6.5	1.
July		31	30.8	30.2	27.9	24.9	21	17.3	10.7	1.3
August		31	30.8	28.7	25.9	23.6	18.2	12.5	5.8	0.1
September	29.8	27.6	23.9	19.9	16.6	12.2	8.3	4.8	1.7	
October	17.8	9.9	4.9	3.3	2.1					
November	12.4	6.3	5.5	2.6	1.2	0.1				
(December)	2.2	1.9	0.8							
Total	109	227.2	199.8	176.6	153.3	125.3	96.9	72.3	38.6	4
% from 365	29.9	62.2	54.7	48.4	42	34.3	26.5	19.8	10.6	1.1

Conclusions

1. During the period from April till September the solar energy collector can operate as the main energy source for water heating. If hot water with temperature higher than 40 °C is needed, an additional energy source, for example, electrical water heater has to be used.
2. From November till the end of February downtime of the solar energy system sets in when power and duration of solar radiation is not sufficient to heat water in storage tank for something considerable.
3. In March and October the solar energy collector can work as an additional water heater.

Acknowledgement

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USE OF HEAT EXCHANGERS IN VENTILATION SYSTEMS OF PIGSTIES IN DEPENDENCE ON OUTSIDE AIR TEMPERATURE

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Abstract. To raise the level of energy efficiency of the ventilation systems of pigsties it is useful to introduce heat exchangers. A heat exchanger is a device built for efficient heat transfer from one medium to another. In the case when a heat exchanger is used in the ventilation systems of premises of pigsties, clean and cold air (in the autumn-winter period) gets amount of heat energy from unclean but warm inside air. Respiration of pigs and the processes occurring on the surface of manure cause the generation of carbon dioxide and ammonia, which are considered harmful gases not only for people and animals but also for the equipment, high relative humidity that occurs also during respiration of pigs influences working conditions as well.
The article deals with experimental results obtained from the experiments about plate counter-flow heat exchanger models with plastic cellular boards (HE PVC) and plastic plates (WVT-120K) as heat transfer surfaces. Operational parameters describing the energy efficiency of heat exchangers were calculated – power of recovered heat energy (kW), heat transfer coefficient ($Wm^{-2}\,°C^{-1}$), and coefficient of efficiency by recovered heat.
Due to better operational parameters of experimental recuperative outflow heat exchanger (HE PVC) simulation and analysis of its exploitation during 4 seasons (December/January) depending on outside air temperature in the article are made as well. The possibility of development of ESA heat exchanger construction with built-in tubular electrical heater to preheat outside air (at outside air temperatures below -15 °C) has been carried out in the research.
Key words: heat exchanger, pigsty, ventilation system, tubular electrical heater.

Introduction

Researches about possibilities of recuperation of heat energy from the polluted outflow air are performed in Germany and nowadays several companies offer appropriate recuperative outflow air heat exchangers. According to constructional details polluted outflow air heat exchangers are classified as: tubular heat exchangers-double pipe, shell and tube, coiled tube; plate heat exchangers-gasketed, spiral, plate coil, lamella; extended surface heat exchangers-tube-fin, plate-fin; regenerators-fixed matrix, rotary (Kuppan, 2000). The basic flow arrangements of the airs in a heat exchanger are: Parallel-flow, Counter-flow, and Cross-flow. The choice of a particular flow arrangement depends on the required exchanger effectiveness, air flow paths, packaging envelope, allowable thermal stresses, temperature levels, and other design criteria. When selecting a heat exchanger for a given task, the following points must be considered: materials of construction; operating pressure and temperature, temperature program, and temperature driving force; flow rates; flow arrangements; performance parameters - thermal effectiveness and pressure drops; fouling tendencies; types and phases of maters; maintenance, inspection, cleaning, extension, and repair possibilities; overall economy; fabrication techniques; intended applications.

In Latvia in livestock breeding heat exchangers are not widely used, mainly because of shortage of experience of the use of heat exchangers in our climatic conditions. Experimental heat exchanger (HE-PVC) is made of polyvinylchloride (PVC) cellular boards, which are set in a definite distance by means of wooden lathes. Through the space between boards unclean warm outflow air is blown, but fresh warming up air (Ilsters et al., 2007) is blown through the cellular board hollows.

The aim of the research is, firstly, to simulate and analyze exploitation parameters of recuperative outflow heat exchanger (HE-PVC) during 4 seasons (December/January), which are calculated depending on outside air temperature using operational parameters which were determined experimentally taking into consideration conditions of production before; secondly, to analyze the effect of use of outflow air heat exchanger on heat balance in pigsty that depends on outside air temperature and development of construction of heat exchanger.

Materials and Methods

Results obtained in previous years were used for calculation and analysis. Experiments in conditions of production were carried out in a pigsty with 1.5 brick thick walls where 500 fattened pigs are kept. Specific heat loss through the structure was 1.5 $W\,m^{-2}\,°K^{-1}$ per unit of floor area. Prior to that there was no source of energy for fresh air preheating. The test was carried out using experimental recuperative counter flow heat exchanger HE-PVC and a heat exchanger WVT-120K produced in Germany.

The main technical parameters of the HE-PVC and industrially produced WVT-120K in Table 1 are given.

Table 1

Main technical parameters of the experimental heat exchanger and industrially produced WVT-120K

Indexes	**HE-PVC**	**WVT – 120K**
Type	Counter flow, plate	
Location	horizontal	vertical
Distance between plates, m	0.010	0.025
Heat transfer area, m^2	100	52 (calculated)
Productivity of heat flows, $m^3\ h^{-1}$	2200	under 4800
Power of heat energy at ΔT=30 °C, kW	under 17	under 27

Doing analysis of the heat balance of pigsty, it is important to determine the outside air temperature when the heat deficiency appears, and it is necessary to determine the value of heat deficiency when the outside temperature goes below par as well. It is possible to define the value of the outside air temperature T_d using the following formula:

$$T_d = -T_c - 2T_c \times Q_d \times (Q_{com} - Q_{vap})^{-1} \quad (1)$$

where T_c inside air temperature, °C;

Q_d heat deficiency at negative outside temperature value, when $\Delta T = 2T_c = -2T_{out}$, W;

Q_{com} total power of heat losses, W;

Q_{vap} energy needed for liquid evaporation from the floor, W.

Results and Discussion

During the experiment in years 2006 and 2007 in February there were traditional Latvian winter weather conditions, when temperature dropped below -20 °C and at the same time there were thaw periods as well. Detailed analysis of experimental heat exchangers was made at negative temperatures when fresh air heating is required more.

Efficiency of the WVT-120K heat exchanger varied depending on outside air temperature: at outside temperature above -10 °C productivity of ventilators was on average 3500 $m^3\ h^{-1}$, but below -10 °C – from 2200 till 2500 $m^3\ h^{-1}$. Therefore, at lower temperatures it was not possible to achieve designed power of the WVT-120K heat exchanger, even though a heat transfer coefficient was comparatively high. As the distance of heat transfer plates of WVT-120K heat exchanger is 2.5 times greater (comparing with HE-PVC), outflow air gives less amount of heat energy to inflow air. At the same time greater distance protects heat transfer plates from icing more effectively when icing of the experimental heat exchanger HE-PVC starts at outside air temperature about -15 °C. The obtained results show that structure of WVT-120K heat exchanger with appurtenant technical parameters is more suitable for the use in pigsties with superior heat insulation of boundary constructions. Vertical performance of WVT-120K heat exchanger (that contributes to refinement of heat transfer) plates and equipping heat exchanger with axial ventilators (which are operated with small power monophase engines) is appreciated. The amount of recovered heat energy in relation to total eliminated amount of heat essentially influences the heat balance in a pigsty, and that, at what outside air temperature the delivering of additional heat has to be started (Ilsters et al., 2008).

The inside air temperature, changes of initial and end temperature of both air flows, the power of recovered heat energy depending on outside air temperature at Fig.1 are shown. At the lowering of the outside air temperature the small decrease in inside air temperature took place. It means that free heat eliminated by pigs and recovered by the use of heat exchangers only partially covered increased heat losses through the boundary constructions of the building and delivered out amount of heat by the way of ventilation. A calculation has showed that if the inside air temperature in the pigsty is kept constant, the power of recovered heat energy increases. An interrupted line shows its amount in Fig.2. In our experimental investigation the heat transferring plates started to be covered with ice when the outside air temperature was below -15 °C, and after that there was a fast decrease in the power of recovered heat energy; the heat exchanger has to be switched off. As low temperatures occur quite often in our climatic conditions, it is important to ensure a normal operation of heat exchanger by the use of, for example, air pre-heaters (Ilsters et al., 2007).

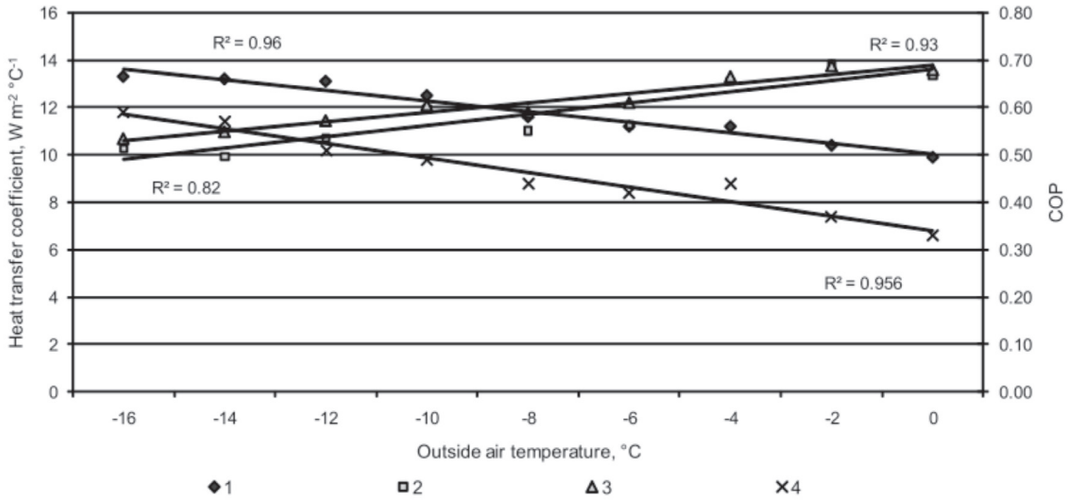

Figure 1. Operational parameters describing the experimental heat exchanger according to experimental results: 1 – power of recovered heat energy, kW; 2 – heat transfer coefficient, Wm^{-2} $°C^{-1}$; 3 – coefficient of efficiency by temperature; 4 - coefficient of efficiency by recovered heat.

Average values of operational parameters (that depends on outside air temperature) of experimental heat exchangers HE-PVC and WVT-120K during the experiment were obtained. Power of recuperated heat from outside airflow of experimental heat exchanger increased from 9.4 kW till 12.6 kW during the temperature drop from 0 °C till -15 °C, in the case of WVT-120K heat exchanger power of recuperated heat from outside airflow increased from 9.7 kW till 13.6 kW. In relation to heat transfer coefficient the situation is the same – coefficient of experimental heat exchanger increased from 13.3 W m^{-2} $°C^{-1}$ till 10.1 W m^{-2} $°C^{-1}$ during the temperature drop from 0 °C till -15 °C, in the case of WVT-120K heat exchanger heat transfer coefficient decreased from 24.0 W m^{-2} $°C^{-1}$ till 20.2 W m^{-2} $°C^{-1}$. Value of the coefficient of performance (COP) by recovered heat of experimental heat exchanger fluctuated between 0.54 and 0.88, the

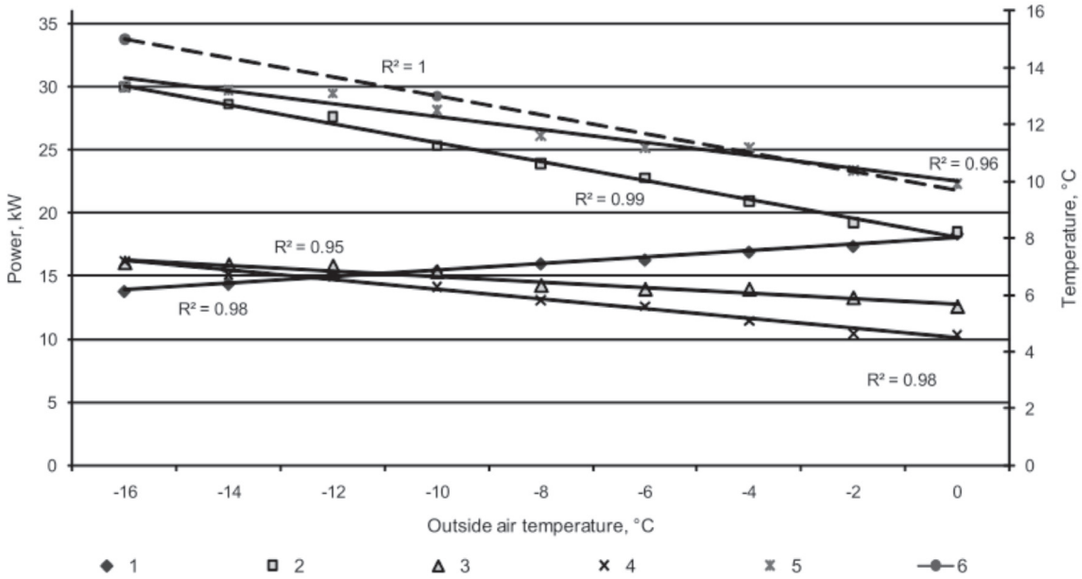

Figure 2. Pigsty inside temperature, the change of initial and end temperature of both air flows, and the power of recovered heat energy depending on outside air temperature at the period of investigation:
1 – temperature in the pigsty; 2 – difference in inside and outside air temperature;
3 – increase in inflow air temperature; 4 – decrease in outflow air temperature;
5 – power of recovered heat energy; 6 – power of calculated recovered heat energy.

value of the COP by recovered heat of WVT-120K heat exchanger varied between 0.23 and 0.40 respectively due to outside air temperature decrease. Better heat recuperation of HE-PVC heat exchanger is achieved mainly due to a smaller distance between heat transfer plates (Ilsters et al., 2007).

After analysis of operational parameters, we can make a conclusion, that the use of ESA heat exchanger is more suitable for weather conditions in Latvia than WVT-120K heat exchanger. It was necessary to make the analysis of efficiency of the use of the experimental heat exchanger during 4 seasons (December/January). The calculations were made using operational parameters of the heat exchanger which were determined experimentally before and outside air temperature during definite seasons.

In Table 2 and Table 3 the value of necessary heat energy for the coverage of heat deficiency in the pigsty of 500 fattened pigs in December 2006/January 2007, December 2007/January 2008, December 2008/January 2009, December 2009/January 2010 are presented. The data is given at a heat loss level through the building constructions 1.5 W m^{-2} K^{-1} (referred to the floor area). With a reference to formula 1 we can calculate temperature when heat deficiency in the pigsty occurs and it is -5 °C if we do not use the heat exchanger to preheat inflow air, -12 °C if we use the heat exchanger for preheating. For the calculation of economy of costs for heating, the price of electricity was taken as 0.1 € kWh^{-1}.

As we can see from the Table 2 and Table 3, the biggest percentage economy (taking into account that it is necessary to use the heat exchanger only when temperature drops till -5 °C) of heat energy required for heat balance in the pigsty appears during January 2010; since the weather is very cold, we need to use all possible amount of feasible recovered heat energy by heat exchanger per month. Unfortunately it is usefully to use the heat exchanger only from -5 °C till -15 °C (-5 °C is calculated temperature when deficiency of the heat energy in the pigsty appears, and -15 °C is the temperature when boards of the heat exchanger still remain unfrozen). For this reason decrease of amount of heat energy required for provision of comfortable temperature in the pigsty per January 2010 was only 6642 kWh (average outside air temperature -11.3 ±4.6 °C), compared to December 2009 it was for 25% less. While efficiency of the use of heat exchanger by expediently used amount of possibly recovered heat energy in December 2010 is only 49% (average outside air temperature -2.0±6 °C). The efficiency of the use of heat exchangers depends on the value of outside air temperature and its fluctuations. If we compare January 2007, December 2007, December 2008 we can see the tendency that the bigger fluctuations at the same average outside air temperature per month are, the bigger efficiency of the use of heat exchanger is.

If we compare 4 winter seasons, the smallest economy of heat energy consumption is during the season 2006/2007, because of non-typically warm winter months, when the requirement of preheating of inflow air was not required, and it was 7155 kWh. Nearly the same decrease of energy consumption occurred during the 2007/2008 and 2008/2009, and it was about 1100 kWh.

The biggest decrease of heat energy – 15559 kWh was during 2009/2010, when the coldest weather lasted for quite a long period. There is still a possibility to improve construction of ESA with a built-in tubular electrical heater to preheat the inflow air (when it is less than -15 °C) up to -15 °C. We can make the calculations of possible economy of heat energy in case when a tubular electrical heater is built in. In case of January 2010 the amount of heat energy needed to preheat inflow air with the tubular electrical heater is 2010 kWh, but amount of possible energy recovery is 17000 kWh. If we talk about money, there is a possible economy of about 1494 €.

Table 2

The economy of heat energy for the heat coverage of heat deficiency in the pigsty of 500 fattened pigs in December 2006/2007 and January 2007/2008

Parameters	Dec.06	Jan.07	Dec.07	Jan.08
feasible amount of recovered heat energy by heat exchanger per month, kWh	11724	15182	16129	17544
average temperature, °C	4.34±2.7	0.72±5.0	0.82±2.6	-1.19±4.3
economy of heat energy using the heat exchanger, kWh	298	6857	4776	6267
economy of heat energy using the heat exchanger, %	3	45	30	36
decrease of heat expenses in a month, €	30	686	478	627

Table 3

The economy of heat energy for the heat coverage of heat deficiency in the pigsty of 500 fattened pigs in December 2007/2008 and January 2008/2009

Parameters	Dec.08	Jan.09	Dec.09	Jan.10
feasible amount of recovered heat energy by heat exchanger per month, kWh	16486	18276	18209	6642
average temperature, °C	0.81±2.2	-1.60±4.0	-2.00±6.0	-11.30±4.6
economy of heat energy using the heat exchanger, kWh	2658	7696	8917	6642
economy of heat energy using the heat exchanger, %	16	42	49	100
decrease of heat expenses in a month, €.	266	770	892	664

Conclusions

1. In weather conditions of Latvia it is important to achieve heat transfer as complete as possible, widening the interval of coverage of heat deficit in the direction of lower outside air temperature to -30 °C.
2. The efficiency of the use of heat exchangers depends on the value of outside air temperature and its fluctuations. Results show that in January 2007, - 0.72±5.0 °C is the biggest possible economy of 6857 kWh, compared to the month with nearly the same outside air temperature - December 2007 - 0.82±2.6 °C, December 2008 -0.81±2.2 °C.
3. It is advisable to improve the construction of HE-PVC with a built-in tubular electrical pre-heater and make the experiments in the conditions of production. In case of January 2010, the calculated amount of heat energy needed to preheat inflow air with a tubular electrical heater is 2010 kWh, but amount of possible energy recovery is 17000 kWh. If we talk about money, there is a possible economy of about 1494 €.

Acknowledgement

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EMISSION REDUCTION POTENTIAL OF USING BIOFUELS

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Abstract. One of the primary reasons for expanding the production and use of biofuels is the potential environmental benefit that can be obtained from replacing fossil fuels with fuels derived from renewable biomass resources. This investigation examines the impact of biofuels on the environment directly from the practical view point analysing how the internal combustion engine emission concentration changes using the most common first-generation biofuels in Latvia – biodiesel, rapeseed oil and bioethanol. Laboratory experiments were performed on a chassis dynamometer *Mustang MD1750*, but the content of exhaust gases components was determined by the *AVL SESAM FTIR* measurement system. Investigation shows that the trends of different exhaust gas component changes, which would be the same for all investigated fuels, don't exist, i.e., each vehicle and biofuel type or blend is particular and has to be analysed separately. In comparison with fossil diesel, running the car *VW Golf* on rapeseed oil the average reduction of NO_x was 10%, but SO_2 – 59%. The CO, CO_2, unburned hydrocarbon and mechanical particle emissions were higher. Running the car *Opel Vectra* on biodiesel the amount of NO_x in comparison with fossil diesel increased in average by about 12%, the amount of mechanical particles and unburned hydrocarbons decreased quite significantly, but just a small increase of CO and SO_2 was observed. Testing the car *VW Passat* on gasoline-bioethanol blends increase of the bioethanol content in the fuel blend increased also the NO_x content in exhaust gases, but the content of CO, CO_2 and NH_3 decreased.
Key words: biofuels, biodiesel, rapeseed oil, bioethanol, exhaust emissions.

Introduction

Liquid biofuels made from biomass are attracting increasing interest worldwide. The major drivers of biofuel development are changes in world oil prices, care about energy security, and concerns about climate change from greenhouse gas (GHG) emissions, caused primarily by the burning of fossil fuels. For developing countries the production of biofuels is also the way to stimulate rural development, create work places, and save foreign currency. Transportation, including emissions from the production of transport fuels is responsible for about 27% of total energy-related GHG emissions in the USA (including 42% of carbon dioxide emissions) and 28% of total emissions in the European Union (EU) (Biofuels for Transport..., 2007).

For petroleum products, such as gasoline and diesel, a life-cycle analysis of the climate impact includes all GHG emissions associated with the production, transportation, refining, storage, distribution and retail of oil; the fuelling of a vehicle; and the evaporative and exhaust emissions using the fuel in a vehicle. For biofuels, the stages to be considered include the planting and harvesting of crops; processing the raw materials into biofuel; transporting the raw materials and the final fuel; storing, distributing and retailing biofuel; and, finally, the impacts of fuelling a vehicle and the evaporative and exhaust emissions resulting from combustion.

The climate impact of biofuels depends mainly on their fossil energy balance, i.e., how much energy the biofuels contain versus how much fossil fuel energy was required to produce them. In opposition to fossil fuels, which contain carbon stored beneath the Earth's surface, biofuels have the potential to be 'carbon neutral'. This is because biofuels are produced from biomass, and exactly the same amount of carbon dioxide (CO_2) that is absorbed from the atmosphere by the plants through photosynthesis is emitted during combustion. As an example the research carried out in Denmark can be mentioned. The research results show that rapeseed oil fuel has strongly positive energy balance and rapeseed oil is genuinely CO_2 neutral because the rape straw alone gives a CO_2 saving which by far exceeds the total CO_2 emission from cultivation and processing (Bugge, 2000).

With the exception of a few investigations that report associated increases in GHG emissions, most studies find a significant reduction in global warming emissions from biofuels relatively to conventional transport fuels. Ethanol has the greatest air-quality benefits where vehicle fleets are old, as is often the case in developing countries. It helps to reduce the exhaust emissions of carbon monoxide and hydrocarbons, especially in cold climates. Biodiesel reduces emissions of carbon monoxide, hydrocarbons, and particulate matter, but can slightly increase emissions of nitrogen oxides (Kojima and Johnson, 2006).

However, figures vary widely due to differing assumptions about factors such as assumptions about the feedstock used, crop management (including use of fertiliser and tilling of soil), crop yields, the relative efficiencies of gasoline and ethanol (diesel and biodiesel, including blends, have about the same vehicle efficiency), and the methodologies used to calculate total life-cycle emissions. Most studies consider emissions of CO_2, nitrous oxide (N_2O) and methane, but many omit other components of exhaust gases.

This investigation examines the impact of biofuels on the environment directly from the practical viewpoint analysing how the internal combustion engine emission concentration changes using the most common first-generation biofuels in Latvia – biodiesel

Table 1

The main technical data of experiment objects

Vehicle	VW Golf 	VW Passat 	Opel Vectra 
Year of manufacture	1992	1997	1998
Engine characteristics	Diesel engine, capacity – 1896cc, turbo diesel, output power – 55 kW at 4200 rpm	Otto engine, capacity – 1781cc, 20 valves, output power – 92 kW at 5800 rpm	Diesel engine, capacity – 1994cc, turbo diesel, output power – 60 kW at 4300 rpm
Fuel system	Modified for use of rapeseed oil	Standard fuel system, lambda – closed loop control	Standard diesel fuel system, Bosch VP44 injection pump
Used fuels	Fossil diesel, rapeseed oil	Fuel mixtures from A95(E0) to E85	Fossil diesel, biodiesel
Test modes	Idling; constant speeds – 50, 90, 110 km^{-1}; cycles – IM240 and 'Jelgava'		

(rapeseed methyl ester), rapeseed oil and bioethanol. The actuality of use of these biofuels confirms also the results of European Commission supported project 'Clean Views on Clean Fuels', that conventional biofuels – straight vegetable oil (SVO), biodiesel and bioethanol remain the lowest cost options until 2020 with a gradually increasing market share for future biofuels based on lignocelluloses (Wakker et al., 2005).

Materials and Methods

As three different biofuels has been studied, three different cars were chosen for experiments. The first of them is *VW Golf* equipped with a 1.9-liter diesel engine, the second one – *VW Passat* with a 1.8-liter spark ignition engine, but the third – *Opel Vectra* with the 2.0-liter diesel engine. The main technical data of experiment objects as well as used fuels and test modes are given in the Table 1.

The choice of experiment objects is based on the fact that these models represent some of the most popular cars in Latvia in both – diesel and spark ignition engine classes. Besides that selected automotive engines are wide spread not only in specific cars, but also in other car brands and models.

The *VW Passat*, which is being used for the investigation of bioethanol fuel mixture use, is equipped with a standard Otto engine without any rebuilding or adaptation for biofuel use. Electronic engine management system based on a closed lambda loop control provides certain mixture enrichment, increasing the ethanol concentration in ethanol-gasoline fuel blend.

The *VW Golf* is equipped with a standard diesel engine, but is adapted to run on rapeseed oil. The car was modified using *ELSBETT* one-tank conversion kit at the Scientific Laboratory of Biofuels (Latvia University of Agriculture) during recent investigations (Dukulis et al., 2009a).

Laboratory experiments were performed on a chassis dynamometer *Mustang MD1750*, but the content of the exhaust gases components was determined by the *AVL SESAM FTIR* multicomponent exhaust gas measurement system. The main technical data of used equipment are given in the Table 2.

Experiments were carried out based on the experimental methodology developed by the authors (Dukulis et al., 2009b), which estimates the duration on each experiment, repetition times, methods of data processing and evaluation etc. The test modes used in

Table 2

The main technical data of used equipment

Measurement equipment	Chassis dynamometer	Emission measurement system
Model	*Mustang MD1750*	*AVL SESAM FTIR*
Measuring characteristics	Maximum measurement capability – 1287 kW; peak absorption – 294 kW; maximum measurement speed – 362 km h^{-1}; maximum axle weight – 4535 kg	Measurable components: up to 25 gases (CO_2, CO, H_2O, SO_2, NO, NO_2, N_2O, NH_3, CH_4, C_2H_4 etc.); response time – 1 second; measurement method – optical

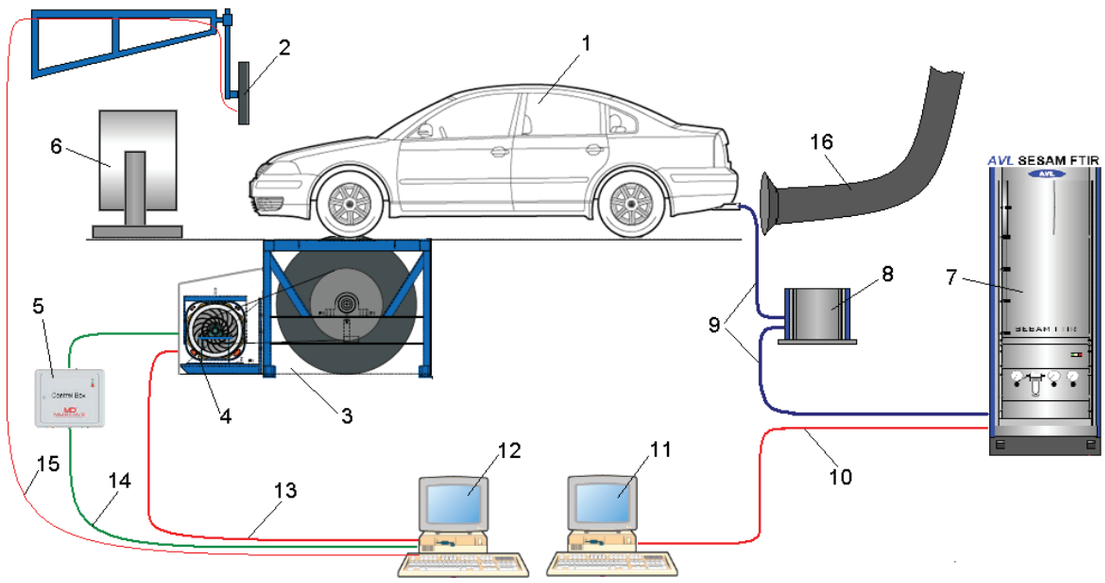

Figure 1. Block diagram for the emission test routines: 1 – tested car; 2 – simulation screen; 3 – chassis dynamometer *Mustang MD-1750*; 4 – power absorber unit; 5 – dynamometer control box; 6 – air blower; 7 – multicomponent exhaust gas measurement system *AVL SESAM FTIR*; 8 – heated filter; 9 – heated gas line; 10 – AVL date communication cable; 11 – PC with special AVL software; 12 – Mustang chassis dyno control and data recording PC; 13 – dyno date communication cable; 14 – dyno control circuit; 15 – screen communication cable; 16 – exhaust extraction pipe.

these tests were: idling; driving at constant speeds of 50, 90 and 110 km^{-1}; cycles – IM240 and 'Jelgava'. The IM240 cycle is a chassis dynamometer schedule used for emission testing of in-use light duty vehicles in inspection and maintenance programs implemented in a number of countries. It is a short, 240 second test representing a 3.1 km route with an average speed of 47.3 km h^{-1} and a maximum speed of 91.2 km h^{-1}. The 'Jelgava' cycle is a driving schedule that represents the real driving conditions in the city Jelgava (360 seconds, 2.3 km, average speed – 23.3 km h^{-1}, maximum speed – 50.0 km h^{-1}) and is developed at the Scientific Laboratory of Biofuels (Dukulis and Pirs, 2009c).

For comparison of exhaust emission content fossil diesel fuel, A95 gasoline, biodiesel, rapeseed oil and bioethanol blend with gasoline were used in these experiments. The block diagram for the emission test routines is shown in Figure 1.

Results and Discussion

Measurements at constant regimes were performed for 60 seconds with the reading step of 1 second (for driving cycles accordingly to the cycle duration). Three repetitions were made in each testing mode. The content of all 25 exhaust gas components was fixed but more detailed analysis was done only for the most essential emission components for each fuel type, i.e., for diesel, biodiesel and rapeseed oil – total nitrogen oxides (NO_x), carbon monoxide (CO), carbon dioxide (CO_2), unburned hydrocarbons (HCDiesel), sulphur dioxide (SO_2) and mechanical particles (MP), but for gasoline and bioethanol blend with gasoline – NO_x, CO, CO_2, ammonia (NH_3) and unburned hydrocarbons (HCGasoline).

Figure 2 shows exhaust emission comparison performing *VW Golf* simulation tests using rapeseed oil and fossil diesel fuel, but Figure 3 – *Opel Vectra* simulation test results using biodiesel and fossil diesel fuel.

In comparison with fossil diesel, running on rapeseed oil the average reduction of NO_x was 10% and decrease of NO_x was observed in all driving modes. The amount of SO_2 in exhaust gases was also lower by an average of 59%. The CO, CO_2, unburned hydrocarbon and mechanical particle emissions were higher with pure rapeseed oil fuel comparing with fossil diesel fuel. If CO_2 increase (compared to fossil diesel fuel by an average of 5%) is irrelevant, because the plants (in this case, rape) take it back from the atmosphere and consume in growth process providing a neutral carbon balance, then the mechanical particle amount, compared to fossil diesel fuel, increased approximately 2.7 times, CO – 1.2 times, but the unburned hydrocarbons – 1.8 times. The injection timing in these tests was set as it is required by the vehicle specification for fossil diesel, i.e., 10.5°. As more detailed investigations of rapeseed oil and one-tank system usage (Dukulis et al., 2010) show, the content of different exhaust gas components can be improved by changing the ignition timing. For example, the optimal injection timing, running the car *VW Golf 1.9TD* on rapeseed oil fuel is 18.5°, because there is the power and torque peak, the

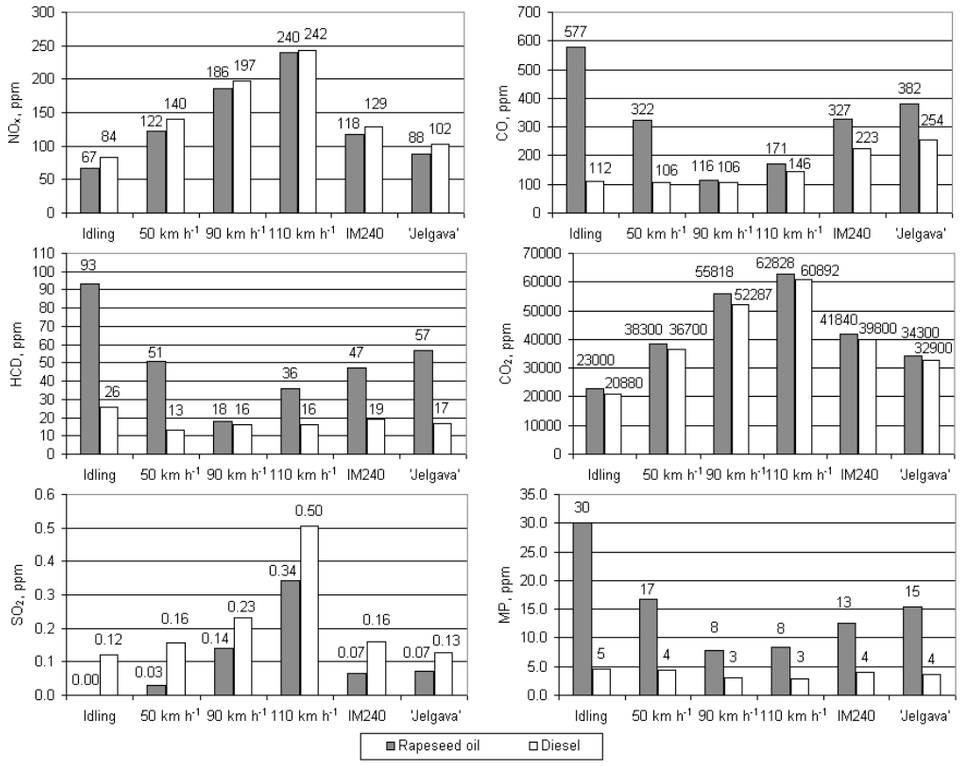

Figure 2. Exhaust emission comparison performing *VW Golf* simulation tests.

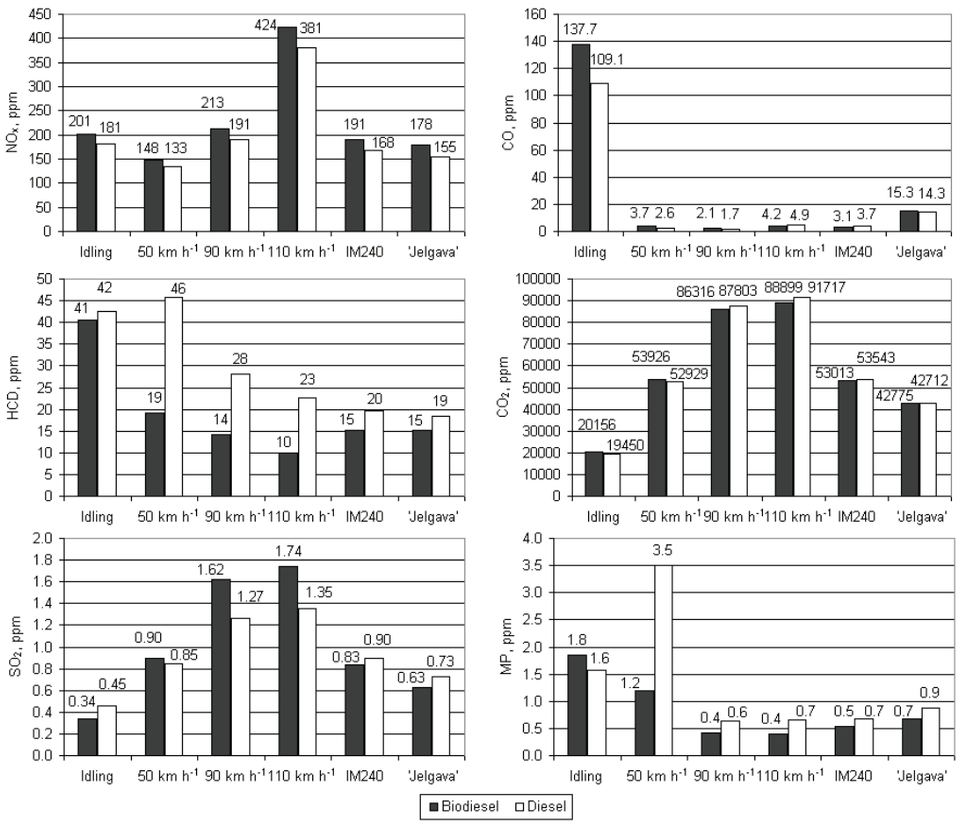

Figure 3. Exhaust emission comparison performing *Opel Vectra* simulation tests.

minimum CO content in exhaust gases (reduced about 35% comparing with the 10.5° injection timing), significantly reduced unburned hydrocarbon (66%) and mechanical particle (68%) content.

Running on biodiesel in all driving modes the amount of NO_x in comparison with fossil diesel was increased (in average by about 12%), but as the analysis of other investigations show, the increase of NO_x is unavoidable disadvantage of biodiesel fuel use. Using biodiesel quite significantly (compared to fossil diesel fuel by an average of 30%), the amount of mechanical particles and unburned hydrocarbons was decreased. The content of CO_2 in exhaust gases stayed approximately the same, but a small increase of CO and SO_2 was observed. It should be noted that the change tendencies in different test modes slightly varied, for example, in idling the amount of mechanical particles using biodiesel in comparison with fossil diesel was higher, but in all driving modes – lower.

Figure 4 shows exhaust emission comparison performing *VW Passat* simulation tests using fossil gasoline A95 and gasoline-bioethanol blends. Mixing the corresponding proportions the following experimental fuel blends were used: A95 or E0 (pure gasoline), E10, E20, E30, E40, E50 and E85.

Analyses of the obtained measurement results show that increasing the bioethanol content in the fuel blend in all test modes except idling increase NO_x content in exhaust gases. The most significant increase of NO_x is in driving cycle modes, when a load and speed are variable. Conversely the content of CO, CO_2 and NH_3 decreases increasing the ethanol content of the fuel mixture. When using E85 fuel, the CO content in exhaust gases at variable load and speed modes reduces significantly, and its value is close to 0. Also the reduction of CO_2, which is the key component of greenhouse effect, is very important. So, for example, performing IM240 and 'Jelgava' cycle imitations the reduction of CO_2 compared to fossil fuel is about 3% using E50, and about 9% using E85 fuel.

Total unburned hydrocarbon emissions for a particular car at all test modes and using different fuels are relatively small, and their changes at different ethanol concentrations are not clearly identifiable.

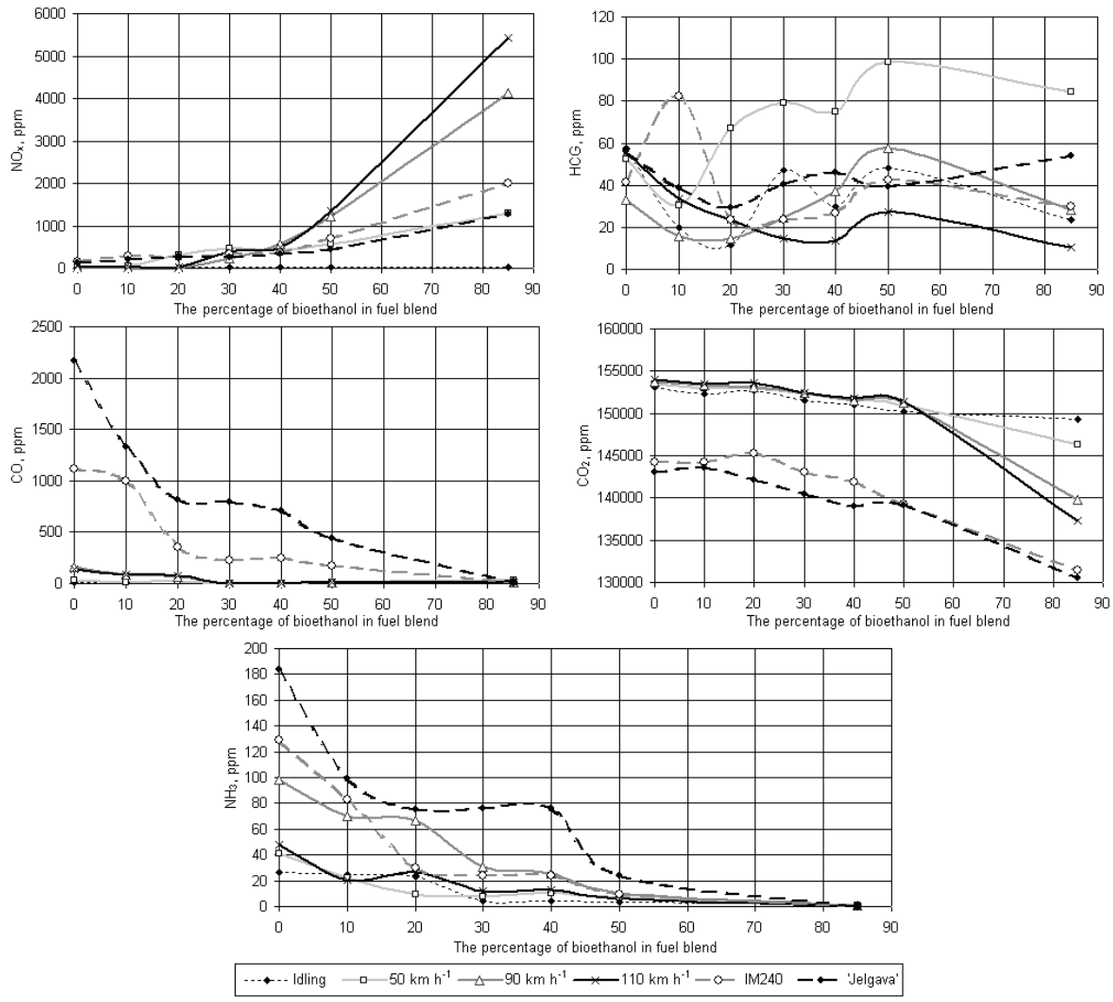

Figure 4. Exhaust emission comparison performing *VW Passat* simulation tests.

Conclusions

1. Investigation of the use of the most common first-generation biofuels in Latvia – biodiesel, rapeseed oil and bioethanol – show that the trends of different exhaust gas component changes, which would be the same for all investigated fuels, don't exist, i.e., each vehicle and biofuel type or blend is particular and has to be analysed separately.
2. In comparison with fossil diesel, running the car *VW Golf 1.9TD* on rapeseed oil the average reduction of NO_x was 10%. The amount of SO_2 in exhaust gases was also lower by an average of 59%. The CO, CO_2, unburned hydrocarbon and mechanical particle emissions were higher with pure rapeseed oil fuel compared to fossil diesel fuel.
3. Running the car *Opel Vectra* on biodiesel, the amount of NO_x in comparison with fossil diesel increased on average by about 12%. Using biodiesel, the amount of mechanical particles and unburned hydrocarbons decreased quite significantly. The content of CO_2 in exhaust gases stayed approximately the same, but a small increase of CO and SO_2 was observed.
4. Running the car *VW Passat* on gasoline-bioethanol blends and increasing the bioethanol content in the fuel blend in all test modes except idling, also increased the NO_x content in exhaust gases. The content of CO, CO_2 and NH_3 was decreased.
5. Since not all the contents of harmful components using biofuels decrease, to evaluate the effectiveness of their use, more detailed studies of engine construction, regulation and conversion kit impact on exhaust gases have to be carried out. To perform the complete fossil fuel and biofuel life-cycle analysis in further studies, it would be useful to evaluate detailed exhaust emissions during biofuel production corresponding to peculiarities of Latvia.

Acknowledgements

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THE INFLUENCE OF BIOMASS TYPE ON QUANTITATIVE AND QUALITATIVE INDICATORS OF BIOGAS

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Abstract. The utilization of traditional fossil fuels (oil, gas) as primary energy resources causes a destabilization of the eco-environmental situation in the world. Latvia has to significantly decrease energy imports from its neighbouring countries. This can be achieved by using high-quality local primary renewable energy sources. One of the solutions is to utilize anaerobic fermentation for biogas production. This process can be ensured by utilizing manure, food waste as well as energy biomass - wood, grass and maize. Biogas is utilized as a primary energy source in a cogeneration plant which is a combined cycle plant for electricity and heat power production. Microcogeneration plant for farming household needs would ensure an independent power supply, in case the overall electrical network is in a state of emergency. In order to ensure optimal biogas yield, which, in turn, would ensure a stable operation of the microcogeneration plant, it is important to know the parameters and quality of the biomass that have been filled in bioreactor. This research deals with the influence of the linkage biomass type on the qualitative and quantitative indicators of biogas. As a result, it has been found that biomass type affected the methane percentage in biogas greatly. The methane content of biogas independent with biomass type was diminished from 65% (galega) to 44.5% (fresh sawdust), but biogas yield decreased from 0.627 m^3 kg_{VSd}^{-1} (galega) to 0.185 m^3 kg_{VSd}^{-1} (fresh sawdust).
Key words: biogas, biogas yield, methane concentration, galega.

Introduction

The utilization of traditional fossil fuels (oil, gas) as primary energy resources causes destabilization of the eco-environmental situation in the world. One of the ways to decrease the global warming effect is to utilize renewable energy sources such as biomass, Sun and wind energy. Using environmentally friendly local energy sources, for example, manure, biomass energy and wood, would reduce the harmful emissions into the atmosphere. These primary energy sources could be used for biogas (a mixture of methane and carbon dioxide) production. Methane produces more heat (891 kJ mol^{-1}) per weight mass unit (16.0 g mol^{-1}) than other complex hydrocarbons. In this context, methane is usually known as natural gas, and has an energy content of 39 MJ m^{-3} (Dubrovskis et al., 2009). Biogas could be burned in cogeneration plants, combined cycle for electricity and heat power production. Optimization of biogas production technology is necessary for optimal use of this plant in farming household.

Latvia has a large potential of biomass for biogas production (table 1).

For providing a successful operation of a biogas plant, economical and technical aspects are important. The maximum effect of biogas, which is achieved by fermentation of biomass, can require a long setting time of biomass and an appropriate volume in the bioreactor. In practice, the selection of the bioreactor volume and type is based on the compromise between a maximum yield of biogas and a justifiable investment. In this case, goal of work is to find the optimal biomass type which to refill in bioreactor.

Table 1

Potential resources for biogas production (Dubrovskis et al., 2008)

No.	Resource	Dry biomass, t $year^{-1}$	Biogas, mill.m^3 $year^{-1}$	Energy, MWh $year^{-1}$
1.	Cow manure	107300	32.2	193300
2.	Pig manure	44400	20	118830
3.	Poultry manure	43160	21.6	129480
4.	Food waste	57500	23	138000
5.	Waste water treatment	23000	7	42000
6.	Landfills	400000	23	138000
7.	Crop residue, grass	80000 (800000*)	20 (200*)	120000 (1200000*)
8.	Silage	20000	8	64000
9.	Slaughterhouse wastes	3500	2.1	12600

*in the case, where energetic grass is specially grown for energy purposes

Figure 1. Equipment for biogas yield investigation in the biogas laboratory.

Materials and Methods

The determination of biomass parameters as well as quantitative and qualitative indicators of biogas took place in the biogas laboratory (LUA Faculty of Engineering, Institute of Agricultural Energetics). There are three stands in the Laboratory of biogas, each having four bioreactors filled at once.

Each bioreactor was of 0.005 m^3 volume and equipped with heating devices for automated regulation of temperature 38±1 °C in the bioreactors. The bioreactors were equipped with sensors for automated registering of pH and gas volume data in the computer.

Biomass need to dilute with water before filling in bioreactor. Fermentation process would happen biomass add inoculum (fermented cow manure). Proportion show in table 2. A linkage of biomass with different composition ratios has been investigated during the experiment. The inoculum is an integral ingredient in the biomass. The inoculum has necessary microbacteria which are intended to facilitate the process of anaerobic fermentation. The analysis of biomass was carried out before it was loaded into the bioreactor. The total quantity of dry matter percentage was determined by the drying of biomass, but the degradation of organic matter was determined by using the method of De Boever. Content of organic matter and pH value was measured both before and after the 60-day fermentation period using standardized methods accepted in Latvia.

The biomass properties have been analyzed for organic matter, total solids, organic solids and moisture content before filling in and after extracting out of the bioreactors. The accuracy of the measurement is as follows: ±0.02 for pH value; ±0.0025 l for the volume of gas; ±0.1 °C for the temperature of biomass.

Results and Discussion

The experimental data are shown in table 2. Investigation results show that biomass type choice is an important at quantitative and qualitative indicators of biogas promotion.

Table 2

Biomass and biogas parameters

	Total substrate weight, kg	Total dry matter, %	Organic dry matter, %	Biogas yield, m^3 kg_{VSd}^{-1}	CH_4, %	Methane yield, m^3 kg_{VSd}^{-1}
Pig manure 50% + peat 25% + inoculum 25%	1.580		7.03	0.124	63.41	0.078
Galega 75% + cow manure 25%	3.294	5.8	3.40	0.627	61.00	0.382
Pig manure 50% + sawdust 25% + inoculum 25%	1.617		8.08	0.157	65.26	0.103
Galega 50%+ inoculum 50%	3.593	9.4	7.20	0.535	57.80	0.309
Galega 25% + inoculum 75%	3.624	12.6	10.50	0.436	56.10	0.245
Cow manure	4.120	14.7	12.80	0.411	53.20	0.219
Chicken litter manure 34% + slaughterhouse wastes 32% + inoculum 34%	2.900	33.7	33.50	0.354	52.00	0.184
Chicken litter manure 50% + inoculum 50%	2.230	45.1	33.50	0.202	49.00	0.099
Old sawdust 76% + inoculum 24%	3.364	33.8	32.70	0.200	45.50	0.091
Fresh sawdust 77% + inoculum 23%	3.534	45.1	33.35	0.185	44.50	0.082

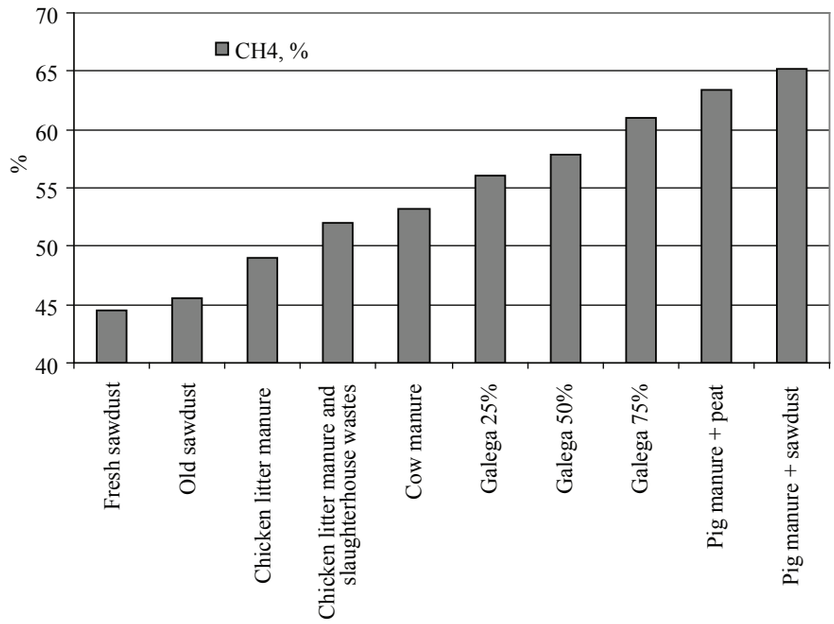

Figure 2. Methane concentration in biogas depending on biomass type.

The highest methane content in biogas was achieved fermenting pig manure with peat and sawdust 65-63%, but fermenting fresh sawdust methane content decreased to 45%.

Biogas quantity from one kilogram of volatile matter (kg_{VSd}) produced during the experiment, shows that this parameter also depends on biomass type. The obtained data show that only the biogas yield by fermentation of pig manure is low and does not correspond to the general pattern. Based on the study of the leading researcher of the LUA, Faculty of Engineering, Institute of Agricultural Energetics V. Dubrovskis (Дубровский and Виестур, 1988), this can be explained by too low loading of bioreactor or there could be a high ammonia (NH_3) concentration in the pig manure. The highest biogas yield from 1 kg_{VSd} was achieved by the fermentation of galega – 0.627 $m^3\ kg_{VSd}^{-1}$, but fermenting fresh sawdust biogas yield has decreased to 0.185 $m^3\ kg_{VSd}^{-1}$.

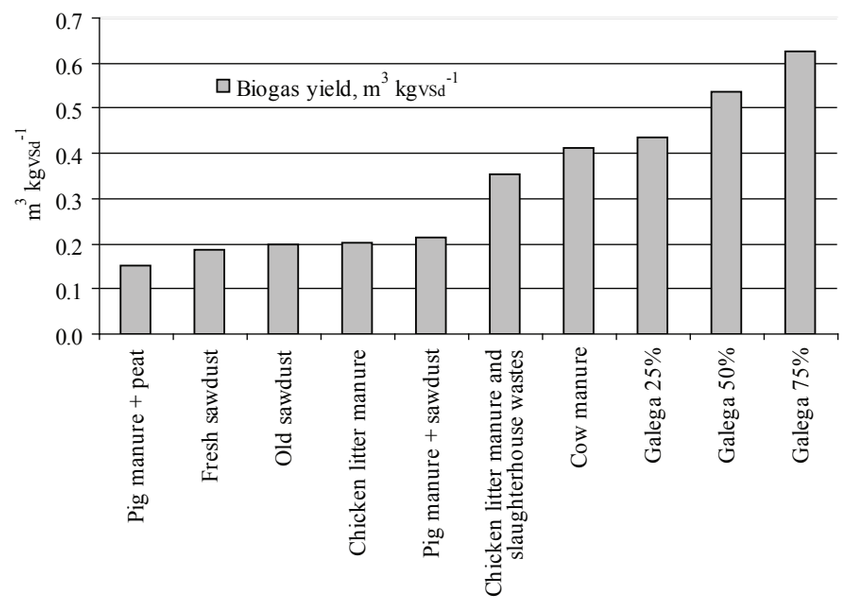

Figure 3. Biogas yield depending on biomass type.

Conclusions

1. Biogas quantitative and qualitative indicators have been the best in galega fermentation. The methane content of biogas was 61% and biogas yield was 0.627 m^3 kg_{VSd}^{-1}. In this case, the biogas purification from unnecessary gases (CO_2, NH_4) can consume less power. Consequently, it is advisable to build the biogas plant closer to the gas supply system.
2. Investigation proves that the biogas yield depends on biomass type. Potential of biogas in Latvia is such that highest biogas yield has been achieved fermenting galega (0.627 m^3 kg_{VSd}^{-1}), but the lowest yield has been at fermenting fresh sawdust (0.185 m^3 kg_{VSd}^{-1}).
3. One of the indicators of the quality of biogas - methane content in biogas also shows that it depends on biomass type. Methane content of biogas is the highest - 65% fermenting pig manure, but fermenting drier biomass (fresh sawdust) methane content of biogas - the lowest to 45%.

Acknowledgements

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INVESTIGATION OF PRESSURE OSCILLATION IN HYDRAULIC HITCH-SYSTEM

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Abstract. This paper presents results of pressure oscillation investigation in hydraulic hitch-system of a tractor Claas Ares ATX 557 during the motion during artificial roughness road test. During experiments oscillation at different driving speed, tyre pressure and hitch-system oscillation damping (turned on/off) were investigated. Tractor hydraulic hitch-system was equipped with a pressure sensor Wika Transmitter ECO-1 and data processing software PicoLog. Results of experiments present maximum pressure peak of 210 bar in the tractor hydraulic system when hydraulic hitch-system oscillation damping system at driving speed 8 km·h^{-1} is not used and system pressure peak reduces to value of 180 bar if the hydraulic hitch-system oscillation damping is used.

Key words: pressure peak, pressure sensor, oscillation, tractor hydraulic hitch-system.

Introduction

The tractor hydraulic system is characterized by a pressure pulse effect, which reduces the hydraulic system components service life, especially the lifetime of hydraulic hoses. The pressure pulse in hydraulic hoses arises when the tractor moves along a rough terrain with mounted soil cultivation tools on hydraulic hitch-system. Hose durability (number of cycles T until the break-up) can be determined by formula (OST 23.I.82-82):

$$T = 43\alpha \cdot \left(\frac{p_f}{p_s} \right)^{-7.7},$$

where T - number of cycles until the break-up;
α - hose characteristic coefficient; α ≈ 1;
p_f - maximum actual pressure in pulse, MPa;
p_s - failure pressure, MPa.

Therefore, a tractor hydraulic hitch-system pressure pulse amplitude reduction is important to extend metal construction life time, because high-pressure pulse values in hydraulic hitch-system causes high power and tension values in metal constructions. Pressure oscillations damping in hydraulic systems can reduce overall system oscillation and improve the implement steering ability. The main task of experiment is to determine changes of the tractor Claas Area 557 ATX hitch-system hydraulic pressure pulse operating with a hitch-system oscillation damper or without it at various driving speeds and tyre pressure.

Materials and Methods

Experiments have been performed in the Faculty of Engineering of Latvia University of Agriculture. In the experiment the tractor Claas Area 557 ATX with engine power of 102 hp was used. Before measurements the tractor - was fitted with LEMKEN short disk harrow and LEMKEN rubber rings roller (specifications are given in Table 1).

Table 1

Main Specifications of the Mounted Implements

LEMKEN short disk harrow 8/300	
discs number/ Ø (mm)	24/465
working width, cm	300
weight without roller, kg	805
LEMKEN rubber rings roller GRW 600	
diameter, mm	600
weight (3m large), kg	623

In order to study the dynamic oscillation of the tractor hydraulic hitch-system, in experiments-artificial road roughness was used (see Fig.1). Road roughness is established according to rural conditions. Total road length is 24 meters which is divided into 12 equal parts by 2 meters each, respectively. Road roughness is constructed from wood planks with dimensions 150 x 50 x 1000 mm. Planks are connected with steel angle shaped bars 40 x 40 x 3 mm by screw-bolts M10 x 1.5. Roughness columns are placed in two parallel rows with 1.8 m distance on asphalt road surface.

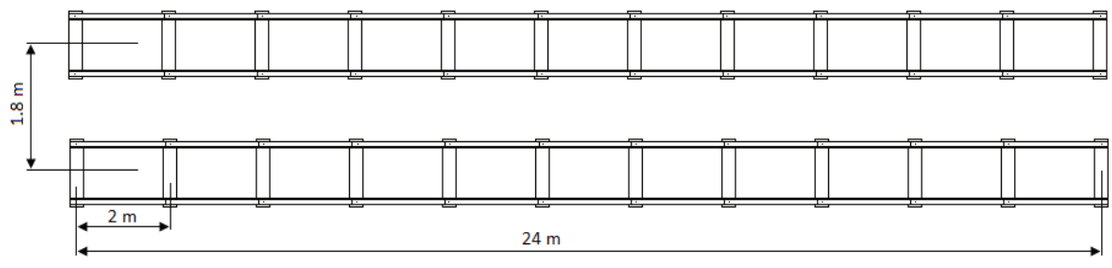


Figure 1. Artificial Roughness Test Road.

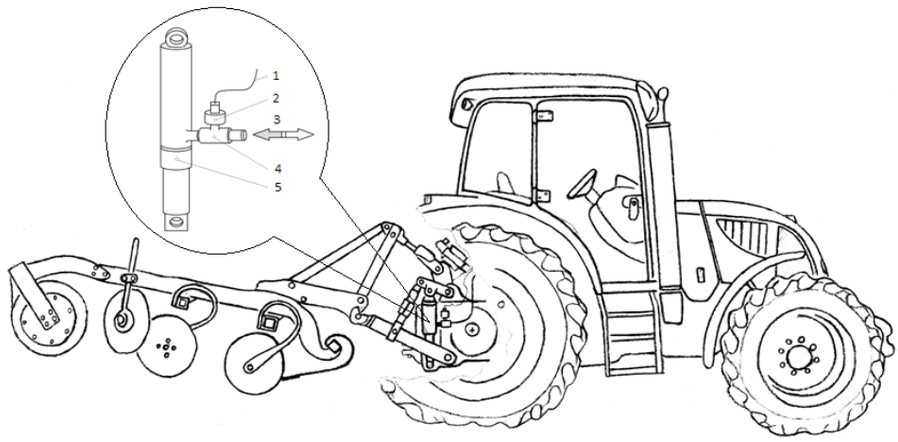


Figure 2. Pressure Sensor Wika Transmitter ECO-1 Mounted on Tractor Hydraulic Hitch-System:
1– data cable; 2 – pressure sensor Wika Transmitter ECO-1; 3 – hydraulic flow direction;
4 – T-piece (reducer); 5 – hydraulic cylinder.

The current tractor hydraulic hitch-system is equipped with pressure oscillation sensor Wika Transmitter ECO-1 (Fig.2). The sensor is chosen such that its own oscillation frequency is 10 times higher than the frequency of measured pressure changes (Kaķītis et al., 2008). The sensor parameters are given in Table 2.

Table 2

Main Specification of the Mounted Pressure Sensor Wika Transmitter ECO-1

Parameter	Values
pressure range	0 ... 400 bar
operating temperature	- 60 ... + 100 °C
output current	4 ... 20 mA
inlet intensity	DC 10 ... 30 V

For data acquisition and processing software offered by the company PicoTechnology is used, provided for devices manufactured by different companies Kaķītis A. (2008). Using a universal data collection and processing device PicoLog, the hydraulic pressure oscillation in the tractor hydraulic hitch-system is measured. PicoLog gauge connection scheme in Fig.3 is shown.

Because of influence of road roughness, the tractor and its hydraulic hitch-system are oscillated with the determined frequency according to driving speed. System oscillations are transferred to the hydro cylinder 8 (Fig.3) of hydraulic system. Pressure pulses are immediate and for a short time. Pressure pulses are measured with a pressure sensor 6 attached to the tractor hydraulic hitch-system hydro cylinder 8. In order to conect it to the pipes, it is needed to create a T-piece (reducer), in which an appropriate pressure sensor could be screwed in. A pressure converter receives and converts liquid (oil) pressure in proportional electrical signal. Data obtained by the pressure sensor are transmitted to the central processing unit 3. The control unit is fed from the common network with voltage of 12 V. In the next step modified data are transmitted to the laptop 1. For data recording and processing subprogram PLW Recorder is used. The acquired data values are saved in *.txt format for further processing. Data are processed by Microsoft Excel data analysis software.

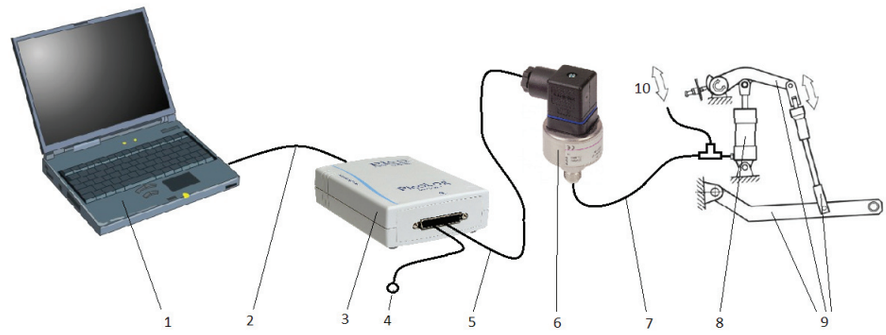


Figure 3. Installation Scheme of Measuring Device PicoLog:
1 – computer with PicoTehnology software; 2 – data cable; 3 – Pico transducer; 4 – DC (12V);
5 – data cabel (4...20 mA); 6 – pressure sensor; 7 – connection tube to hydraulic cylinder; 8 – hydraulic cylinder; 9 – hydraulic hitch-system; 10 – hydraulic oil flow.

Air pressures in tyres are selected from the tractor manual which provides different tyre pressures for different operating conditions. For example, for cultivation tyre pressure is provided 0.8 - 0.9 bar, but for transportation it is 1.1 - 1.2 bar Claas (2007).

Each measurement is repeated three times at certain gears and engine revs. From the acquired data only the maximum value is taken into account, which characterizing the greatest pressure of the tractor hydraulic hitch-system.

Results and Discussion

While changing the tractor hydraulic hitch-system oscillation damping position (switch on or off) and driving speed from 3 – 14 km·h^{-1}, various hydraulic hitch-system oscillation characteristics are acquired, which may create the pressure pulse in hydraulic system.

Hydraulic pressure curve is divided into three stages (Fig.4 a, b):

1. beginning of oscillation (beginning of movement);
2. oscillation in stationary regime (during which the maximum hydraulic hitch-system pressure occurs);
3. ending of oscillation (phase in which oscillations are terminated).

Fig.4 (a and b) demonstrate how hydraulic pressure in the hydraulic hitch-system changes in at all three stages at driving speed of 7.8 km·h^{-1}. Initial operation pressures in the hydraulic hitch-system is 100 - 120 bar, which is achieved from mounted implement total load on hydro cylinder.

The maximum pressure oscillation amplitude is observed at driving speed of 7.8 km·h^{-1} and pressure in tyres of 1.2 bar.

Position b in Fig.4 describes changes of pressure curves at the tyre pressure of 1.2 bar: if the the hydraulic hitch-system oscillation damping system is not used, then maximum average pressure of the hydraulic hitch-system reaches 210 bar, but, when the hydraulic hitch-system oscillation damping system is used, the pressure pulse reduces to 180 bar.

Reducing the tractor tyre pressure from 1.2 to 0.8 bar, the maximum mean value of pressure in the hydraulic hitch-system reaches 180 bar, but, when the additional pressure oscillation damper system is used, then the system pressure does not exceed 150 bar (Fig.4 a).

Looking at two graphs in Fig. 4, it can be concluded that reducing tire pressure from 1.2 to 0.8 bar and in addition using the tractor hydraulic hitch-system oscillation damping option, the hydraulic pressures about 60 bar can be reduced. Due to the influence of hydraulic pressure, pulse controllability of aggregate deteriorates. By reducing the hydraulic pressure pulse it is possible to prolong the service life of the common system and to improve steering ability of aggregate.

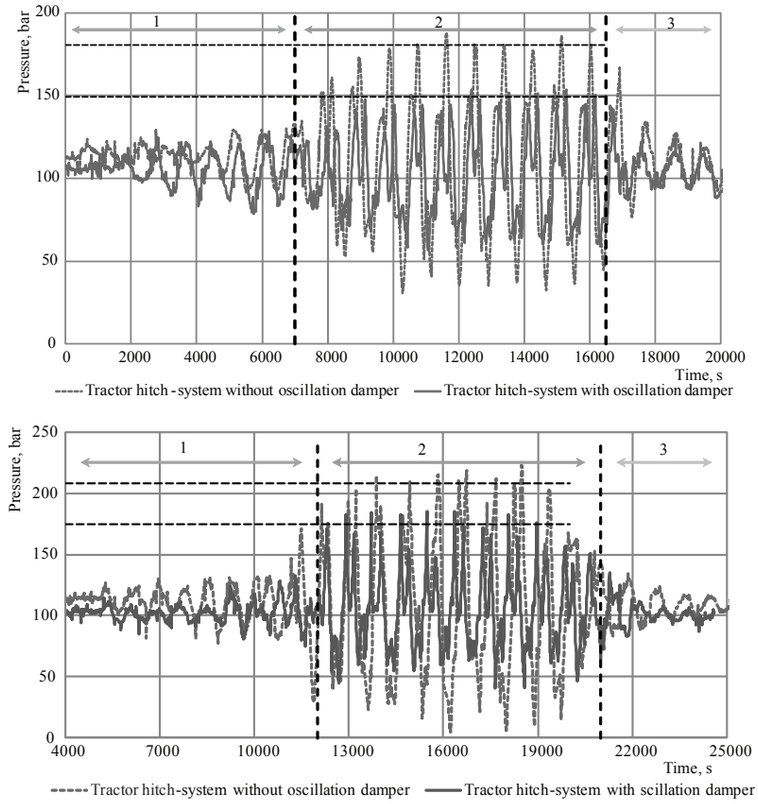


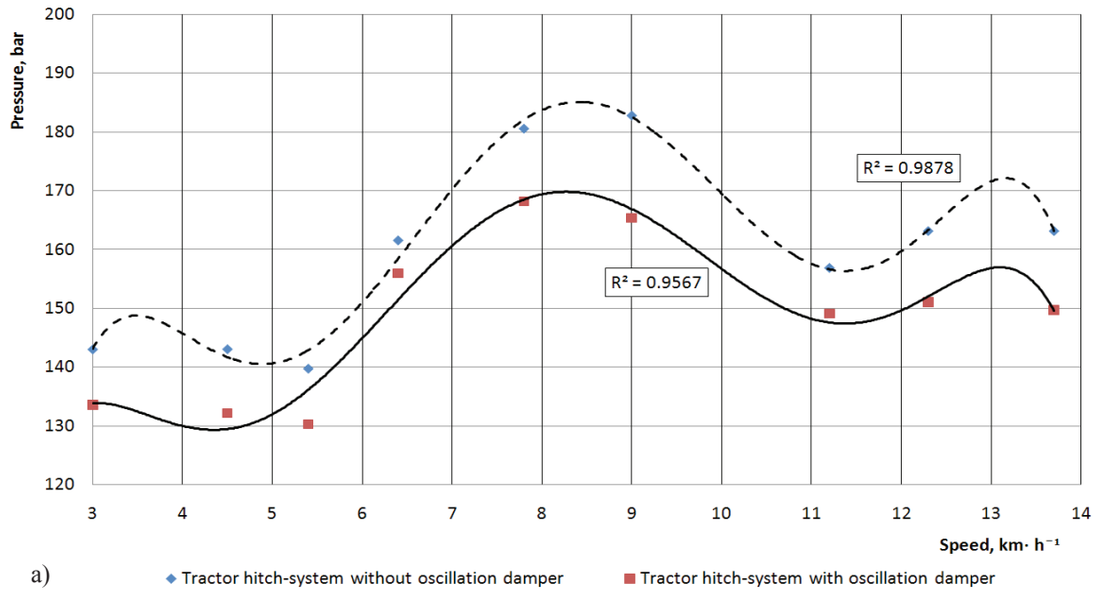
Figure 4. Pressure Peak in Tractor Hydraulic Hitch-System at Different Tyre Pressures:
a – measurement at tyre pressure 0.8 bar; b – measurement at tyre pressure 1.2 bar; 1– oscillation begins; 2 – oscillation in stationary regime; 3 – finishing oscillation.

In Fig.5 (a, b) common average values of the system pressure at different motion speeds and tyre pressures are described. Testing the- tractor on artificial roughness test road with air pressure in tyres of 1.2 bar, confirms that to transportation regime of aggregate at, and driving speed of 5 km·h^{-1} (see Fig.5 b), and steering ability of the tractor were good, and pressure in the hydraulic hitch-system was in limits 140 – 170 bar.

By gradual increase of driving speed from 5 to 8 km·h^{-1} - pressure of hydraulic hitch-system increased as well and achieved 210 bar. Steering ability of the tractor worsened, and it was observed that front wheels were torn by the road surface. At driving speed of 8 km·h^{-1} the tractor was impossible to steer.

Bu gradual increase of driving speed from 8 to 11 km·h^{-1} - pressure in hydraulic hitch-system decreased to 140 – 150 bar and steering ability of the tractor improved, but by exceeding driving speed over 13 km·h^{-1}, steering ability has become worsened.

Exploiting damping option of the tractor hydraulic hitch-system, common pressure oscillation in the tractor hydraulic hitch-system has decreased for approximately 20 bar, but steering ability of the tractor has not been substantially improved.



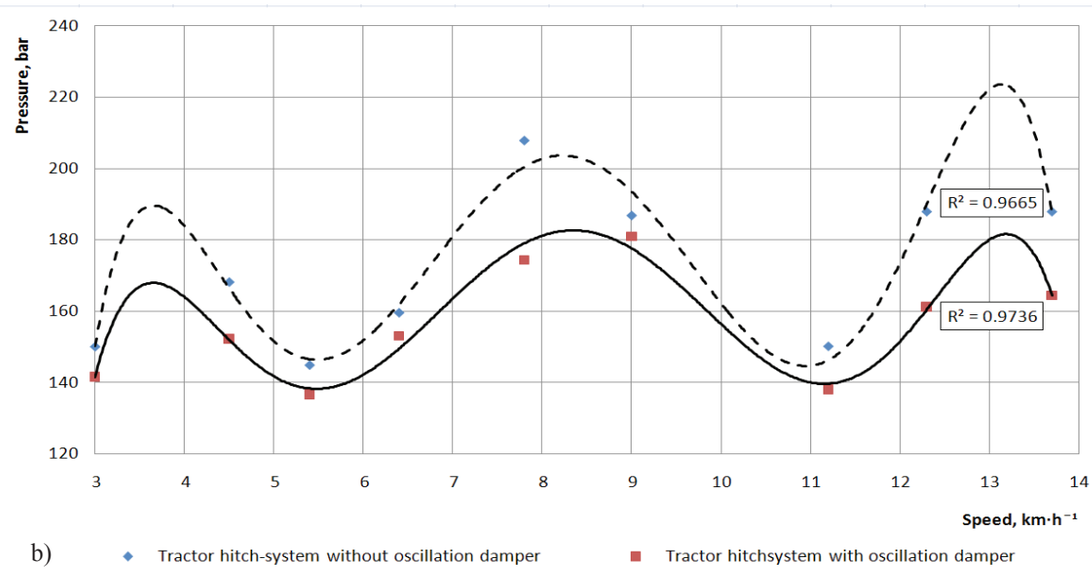


Figure 5. Tractor Hitch-System Oscillation at Different Speed:
a – measurement at tyre pressure 0.8 bar; b – measurement at tyre pressure 1.2 bar.

Reducing the tyre pressure from 1.2 to 0.8 bar that, corresponds to the required soil processing pressure, and additionally employing damping option of the tractor hydraulic hitch-system, the steering ability of the tractor substantially increases and during movement at maximum pressure of hydraulic hitch-system it corresponds to 170 bar (Fig.5 a), front wheel tearing from the road surface has not been observed. When driving speed exceeds over 13 $km \cdot h^{-1}$, the steering ability worsens.

Conclusions

1. Decreasing the hydraulic hitch-system pressure impulse range is substantial for amplification of service life of metal constructions because a high leap of pressure in the hydraulic system corresponds to high forces and tension values in metal constructions.
2. Maximum range of pressure oscillation is observed at driving speed of 7.8 $km \cdot h^{-1}$ and tyre pressure of 1.2 bar.
3. Oscillation of pressure can be reduced by exploitation of oscillation damper that can be established on the tractor instrument panel. If the oscillation damper is not used, then at driving speed of 7.8 $km \cdot h^{-1}$ the pressure oscillation in hydraulic system reaches 210 bar but, when the oscillation damper is used, the pressure in hydraulic system decreases to 180 bar (at tyre pressure 1.2 bar).
4. Decreasing tyre pressure from 1.2 to 0.8 bar, the maximum average values of pressure in hydraulic hitch-system reach 180 bar but, if an additional oscillation damper is used, pressure in the hydraulic system does not exceed 150 bar.
5. By decreasing tyre pressure of aggregate and exploiting the option of oscillation damper of the tractor hydraulic hitch-system, it is possible to decrease the pressure of hydraulic hitch-system for 50 bar and improve steering ability of tractor.
6. For hydraulic hitch-system longer service it would be necessary to reduce the hydraulic pressure oscillation to a greater range.

Acknowledgement

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DIVERSITY OF *LACTOBACILLUS SPP.* IN KRIEVIJAS CHEESE

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Abstract. Dairy ecosystems have been developing in thousands of years giving us wonderful collection of different types of cheese varieties. On the other hand, modern technology provokes the loss of variability (Adamberg et al., 2007). The only way to keep biological processes under control is to study physiology of microorganisms, and relationships between them systematically, especially non-starter lactic acid bacteria (NSLAB). The number of non-starter lactic acid bacteria increases during cheese ripening and may constitute a dominant population in the mature cheese. The NSLAB diversity, their metabolism and interactions with starter bacteria have an effect on the ripening process of the cheese.
The aim of the present study was to investigate the diversity of *Lactobacillus spp.* during ripening of Krievijas cheese.
The diversity of different profiles varied among the cheese samples matured at different temperatures, and the results indicated a correlation between NSLAB species and aroma development (Miķelsone et al., 2009). A higher metabolism of *Lactobacillus curvatus* at 12 °C leads to faster depletion of nutrients and approaching the last two phases of microorganism growth curve. However, in each of the cheeses, the microflora had a tendency to be dominated by one *Lactobacillus* profile at the end of maturation.
Key words: NSLAB, *Lactobacillus spp.,* ripening temperature, Krievijas cheese.

Introduction

Ripened cheese varieties of Latvia are traditionally made with a mesophilic starter, and non-starter facultative heterofermentative *Lactobacillus spp*. contribute to the development of quality and sensory properties of cheeses during ripening. In spite of not being added deliberately to the milk, heterofermentative lactobacilli grow to high numbers in many hard and semi-hard cheeses. The non-starter microflora has been studied during the last decade in the Norwegian cheeses Jarslberg and Norvegia (Lindenberg et al., 1996), the Swedish Herrgärd (Antonsson et al., 2001), Greve (Lindenberg et al., 1996) and Präst (Østlie et al., 2005), the Danish Danbo cheese (Antonsson et al., 2003; Christiansen et al., 2005), and the Estonian Eesti Kuldne, Eesti, Saare and Arensburg cheeses (Kask et al., 2003).

Non-starter lactic acid bacteria may have different roles in cheese ripening including contribution to flavour development and controlling activities of detrimental bacteria (Ärdo, 2007). Their effects are strain-specific, strongly affected by the technological context and probably by the interactions with other microorganisms in cheese. Some of them also have a potential as probiotics. Diversity of the NSLAB strains in cheese may therefore be of importance for a satisfactory aroma development during ripening (Steele et al., 2006).

The aim of the present study was to investigate the diversity of *Lactobacillus spp.* during ripening of Krievijas cheese.

Materials and Methods

Krievijas cheese (SC "Smiltenes piens") (salt content - 1.5-2.5%, protein content – 17%, fat content – 28.2%, and fat content in dry matter – 50%) was chosen for further analysis.

The cheese was made, cut and wrapped at the factory using traditional Krievijas cheese technology, and afterwards was divided into two groups for further examination at the Laboratory of Microbiology of the Latvia University of Agriculture. The first group was matured at 6 °C, and the second - at 12 °C at the same relative humidity of 85%. Samples of both groups were checked on the first day and then on the15th, 30th, 45th and 60th day of ripening. In all cases LAB colony forming units (cfu) and identification of *Lactobacillus* species were analyzed.

The following scheme was used for the analysis: 10 g of analysed cheese samples were diluted in 90 ml of saline solution, and samples were homogenized in a bag Mixer 400 MI. Serial dilutions of each cheese sample (1:1 000, 1: 10 000) in saline were made, then plated onto MRS agar and incubated for 48 hours at 37 °C. Calculation of cfu was performed by ACOLYTE colony counter. The grown cultures were examined microscopically and seeded onto MRS agar at the same conditions as previousely for multiplication.

Lactobacillus spp. colonies were identified on the basis of carbohydrate patterns with API 50 CHL system (BioMerieux, Mareyl'Etoile, France) as recommended by the manufacturer. The APILAB plus version 4.0 program (BioMerieux) was used to analyse the fermentation profiles obtained with the API 50 CHL kit. Gram staining was performed as described by Gregersen, and catalase reaction was determined by transferring fresh colonies from a Petri dish to a glass slide and by adding 5% of H_2O_2.

Results and Discussion

Starter bacteria until two weeks of age dominated in all cheese samples (Table 1).

Table 1

Species of lactic acid bacteria in Krievijas cheese samples during ripening

Ripening time, days	Species and ripening temperature	
	6 °C	12 °C
Unripened	*Lactococcus lactis ssp.lactis 2, Lactococcus lactis ssp.lactis 1, Lactobacillus curvatus*	
15	*Lactococcus lactis ssp.lactis 2, Lactococcus lactis ssp.lactis 1, Lactobacillus curvatus*	*Lactobacillus plantarum 1*
30	*Lactobacillus curvatus*	*Lactobacillus plantarum 1*
45	*Lactobacillus helveticus*	*Lactobacillus plantarum 1*
60	*Lactobacillus curvatus*	*Lactobacillus plantarum 1*

Three *Lactobacillus* species were found in the Krievijas cheese. The NSLAB species were identified as *Lactobacillus curvatus, Lactobacillus plantarum,* and *Lactobacillus helveticus*. *Lactobacillus helveticus* was detected only after 45 days of ripening. Only two of studied species were found in the mature cheese. Recent studies have shown that the non-starter microflora of Cheddar cheese is dominated by mesophilic lactobacilli, especially *Lactobacillus casei*, *Lactobacillus paracasei*, *Lactobacillus plantarum,* and *Lactobacillus curvatus* (Fox et al., 2004). As Krievijas cheese has some similarities with Cheddar cheese production, such as intensification of acidification process during the treatment of cheese grains, the diversity of NSLAB should be analogous.

Although Krievijas cheese samples were matured at different temperatures, the NSLAB varied in diversity, number, and dominance. The microbiological composition in cheese changed during ripening. Although the different species have different growth characteristics (specific growth rate, acidification ability, and final cell count), they are well adapted to changing environmental parameters of cheese ripening (carbohydrate limitation, low temperatures, and water activity).

The changes in cheese pH during ripening are shown in Figure 1.

The study showed that during the ripening of Krievijas cheese there was a relatively low increase in pH - 5.42 - 5.49 at the end of cheese maturation. pH of classical Krievijas cheese is 5.25-5.35. Differences in pH between the analysed and classical Krievijas cheeses depends on the activity of starter bacteria, the concentration and distribution of NaCl in cheese, maturation temperature and availability of nutrients for *Lactobacillus* species in cheese (Ozola et al., 2002). pH is very closely connected with the amount of colony forming units in cheese.

The total amount of lactic acid bacteria reached its maximum number of 10^7-10^8 cfu g^{-1} of cheese. Smith (2003) found that NSLAB from the beginning of ripening to population of 10^7 cfu g^{-1} grow over the first 10-20 weeks of ripening, and then their population remains relatively constant. Beresford (2001) has

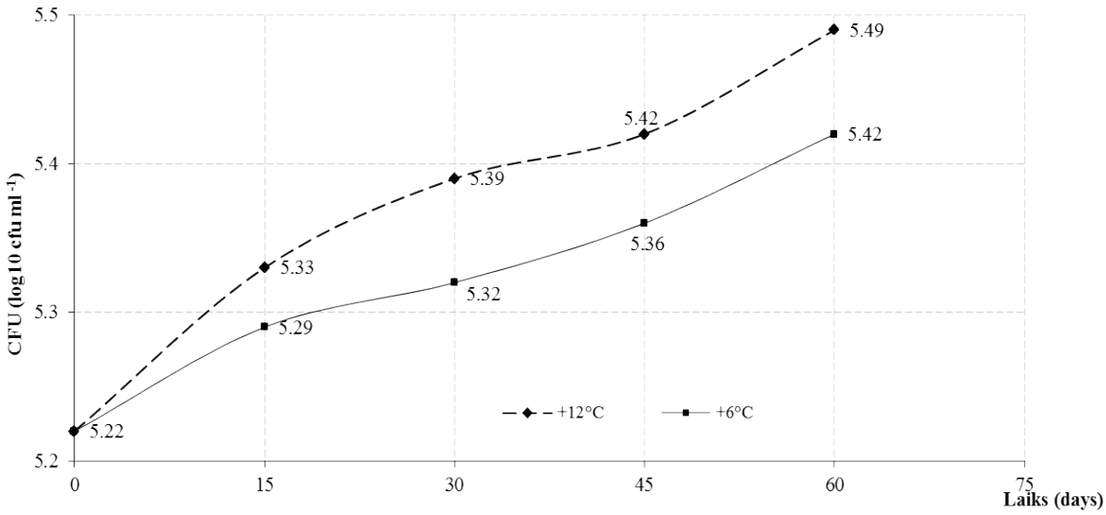

Figure 1. The changes in pH during Krievijas cheese ripening.

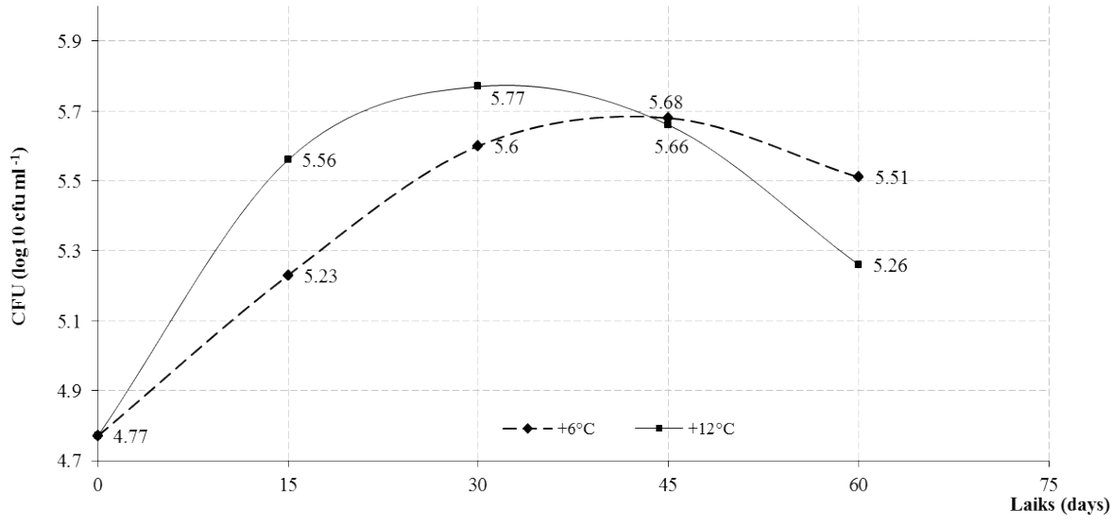

Figure 2. The dynamics of NSLAB in Krievijas cheese during ripening.

indicated that NSLAB reached their maximum value after 6-8 weeks; other authors mention that up to 3 months are needed for NSLAB to reach 10^7-10^8 cfu g^{-1} (Fox et al., 2004). However, all the authors have a common opinion that growth of NSLAB is tightly dependent on the ripening temperature (Crow, 2001; Shakel-Ur-Rehman et al., 2000; Fenelon et al., 1999). The amount of non-starter lactic acid bacteria was relatively low through all ripening period (Figure 2) for Krievijas cheese.

The samples ripened at 12 °C, more rapidly approached the stationary phase of lactic acid bacteria than those ripened at 6 °C. Both samples reached the peak after 30 and 45 days respectively. More sharp elevation of the curve for the sample ripened at 12 °C could be explained by intensification of exponential phase due to the higher ripening temperature; besides, depletion of nutrients comes faster than for the samples ripened at 6 °C. After 30 days of growth the curve reached the stationary phase, and the concentration of lactic acid bacteria fell down intensively. The total amount of LAB in the cheese ranged from $1.60 \cdot 10^5$ to $3.55 \cdot 10^5$ cfu g^{-1}. It indicates that cheese samples also had a weak aroma development, which is in accordance with earlier studies of the numbers of NSLAB versus aroma development during ripening (Antonsson et al., 2001).

Cheese samples also had a lower number of different profiles, which further suggested a relationship between low strain diversity and aroma development of Krievijas cheese during ripening (Miķelsone et al., 2009). However, in each of the cheeses, the microflora had a tendency to be dominated by one *Lactobacillus* profile.

Conclusions

1. The diversity of *Lactobacillus curvatus*, *Lactobacillus plantarum,* and *Lactobacillus helveticus* varied among the cheese samples matured at different temperatures.
2. A higher metabolism of *Lactobacillus curvatus* at 12 °C leads to faster depletion of nutrients and approaching of the last two phases of the growth curve of lactic acid bacteria.
3. However, in each of the cheeses, the microflora had a tendency to be dominated by one *Lactobacillus* profile at the end of ripening.

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COMPARISON OF SPECTROPHOTOMETRIC METHODS FOR ASSESSMENT OF OXIDATION OF MILK FAT

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Abstract. Three spectrophotometric methods were compared for the assessment of milk fat oxidation: the AOCS (American Oil Chemists' Society) 2-thiobarbituric acid (TBA) value direct method, the TBA method of Aristova, and the modified method for the estimation of total carbonyl compounds in oils of Endo et al. Two cow milk fat samples – a fresh fat, obtained from fresh milk, and a rancid fat, obtained from a rancid commercial butter by means of high-speed centrifugation (15.871 × *g*, 30 minutes at 40 °C) – were analyzed several times in a 22-day period after sample preparation.
A positive linear correlation between absorbance and storage time was established in all used methods.
The method of Aristova is appropriate for exact determination of oxidation products. This method had rather good R^2 value (0.85) of the fresh fat sample results; however, the coefficient of variation (45.78%) showed rather large deviations between parallel analyses.
The methods of AOCS and Endo et al. can be used for the assessment of the dynamics of fat oxidation by reporting absorbance values from the assay directly. By these methods rather high R^2 values (0.86, 0.93) of the fresh fat sample results were obtained; the coefficients of variation also were satisfactory (10.13%, 11.12%). The last two methods are cheaper and require smaller sample amounts (< 0.2 g), while the method of AOCS is the most time-consuming method.
Key words: fat deterioration, malonaldehyde, TBARS, carbonyl compounds, rancidity, TBA method.

Introduction

The oxidative stability of milk and dairy products is of concern to the dairy industry. The oxidation processes can result in strong off-flavours and in deterioration of nutritional quality of dairy products, making them unacceptable to consumers (Hawemose et al., 2004). The reaction of lipids with oxygen results in flavour deterioration and creates serious problems in storage stability (Day, 1960). Inhibiting the progress of lipid oxidation is a key factor in maintaining the product quality and extending the shelf-life. Autoxidation is one of the chief processes by which lipids degrade. In addition to autoxidation, lipid oxidation can proceed along a photooxidation route or a lipoxygenase route. These differ from autoxidation at the initiation stage only. Food lipids possess an inherent stability to oxidation, which is influenced by the presence of antioxidants and pro-oxidants. After a period of relative stability (induction period), lipid oxidation becomes autocatalytic and rancidity develops. The length of the induction period is crucial for the shelf-life estimation of oils, fats and of fatty foods (O'Connor and O'Brien, 2006; Pegg, 2005).

Lipid oxidation is essentially a free-radical chain reaction involving initiation, propagation and termination stages. Unsaturated fatty acids are very susceptible to oxidation. The cause of the autoxidation is the particular reactivity with oxygen of the methylene group, situated between two double bonds. The speed of autoxidation increases with the amount of double bonds in fatty acid molecule. Unsaturated fatty acids are oxidized to form odorless, tasteless hydroperoxides. These are unstable and degrade by entering into numerous and complex breakdown and interaction mechanisms responsible for the production of myriad compounds of various molecular weights, flavour thresholds and biological significance. Since milk fat contains different minor unsaturated fatty acids, very many carbonyl products may be produced during autoxidation. Hence, the overall flavour produced during the autoxidation of milk fat is a combination of many flavours imparted by individual carbonyls present in minute concentrations (O'Connor and O'Brien, 2006; Gunstone, 1996).

One of the important polyunsaturated fatty acids in milk fat is linolenic acid. As reported by Hawemose et al. (2004), its content is depending on the type of the cow forage and in their investigation was approximately 0.2-0.7% from total fatty acids. A scheme revealing the photooxidation development of the triene group of linolenic acid is given as an example of the oxidation processes (Fig. 1).

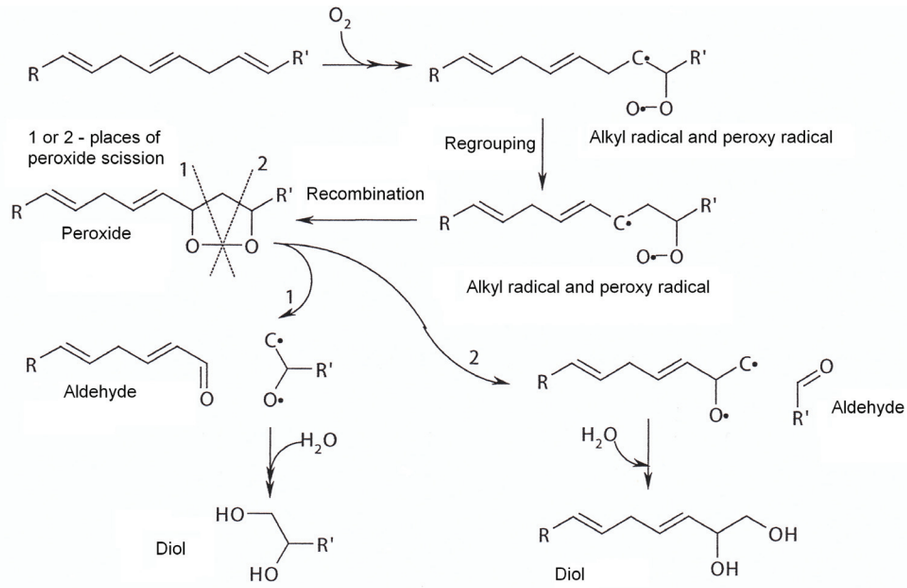

Figure 1. The scheme of the photooxidation processes of the triene group of the linolenic acid.

Among the various families of flavour compounds of dairy products, carbonyl compounds have been studied extensively. Some of them are naturally in milk fat, and some others are produced during heat treatment and autoxidation of milk fat. The flavour of dairy products depends on the nature and amounts of these compounds. Aldehydes are the main secondary oxidation products that are largely responsible for the development of undesirable taste and flavour which characterizes rancid fat. At elevated temperatures these products are further degraded to short-chain acids (tertiary oxidation products) (Bhat et al., 1981; Gunstone, 1996).

The changes in the concentration of carbonyl compounds and consequently the intensity of oxidation processes can be monitored by simple spectrophotometric techniques or by more sophisticated instrumental analysis, for example, methods using high-performance liquid chromatography (HPLC) or methods involving static and dynamic gas chromatography, so called 'electronic nose' apparatus and electron spin resonance (ESR) analysis (MacGibbon and Taylor, 2006; Pegg, 2005). The selection of methods is essential for the right monitoring of lipid oxidation processes. Advantages of spectrophotometric methods are that they are faster, cheaper and simpler regarding the necessary laboratory apparatus, personnel and reagents.

As a marker for lipid oxidation serves a relatively stable dialdehyde ($OHCCH_2CHO$) – malonaldehyde (MDA) (Vattem and Shetty, 2007). The measurement of MDA has been performed by the thiobarbituric acid (TBA) test. Methods involving TBA as an analytical reagent are widely used to follow the progress of oxidation in dairy products. These methods are based on the condensation of two molecules of TBA with one molecule of the lipid oxidation end-product, MDA, resulting in the formation of a red or pink-colored complex that can be determined spectrophotometrically with maximum absorbance at 532 nm (MacGibbon and Taylor, 2006). The reaction scheme is given in Figure 2.

MDA formation and accumulation in foodstuffs depend on the degree of unsaturation of polyunsaturated fatty acids, MDA precursors from non-lipid origin, types of oxidation catalysts, conditions, etc. Of these factors, the degree of fatty acid unsaturation markedly influences the extent and amount of MDA. With increasing level of unsaturation, more MDA will be generated during autoxidation. Consequently, TBA values cannot be used as an indicator of lipid oxidation when comparisons of oxidation are being made between foods with significantly different fatty acid compositions. Heat and/or strong acid are thought to be essential for the liberation of MDA from precursors or bound forms, for condensation with TBA, and for

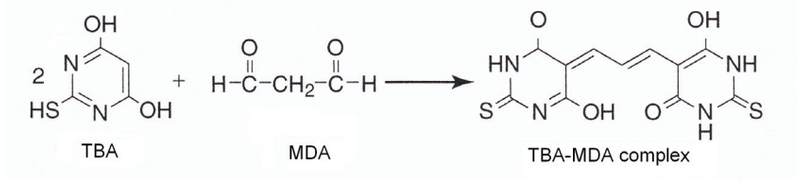

Figure 2. The reaction of the malonaldehyde with the thiobarbituric acid (Pegg, 2004).

maximal color development (Pegg, 2005; Seljeskog et al., 2006).

However, compounds other than MDA, such as alkanals, alkenals and dienals, conjugated aldehydes and hydroperoxydes, may react with TBA. Due to uncertainty concerning the exact identity of compounds that can react with TBA, the ambiguous term 'thiobarbituric acid reactive substances' (TBARS) is now commonly used in lieu of TBA number or value (Vattem and Shetty, 2007; Pegg, 2005). The kinds of TBARS produced depend on the substrate and oxidation conditions. Because these compounds may undergo extensive modification at advanced stages of oxidation, the TBA test is useful as a measure of lipid oxidation only during the initial stages of oxidation. The test is most useful for detecting incipient oxidation of lipids rich in methylene interrupted, three or more, double bonds. The color formation is empirical, as the color yield from each fatty acid varies (Pomeranz and Meloan, 1994). Probably this is the reason why different wavelengths are specified measuring TBARS: 532-535 nm (Vattem and Shetty, 2007), 536 nm (Инихов и Брио, 1971), and, as written by Miller and Miller (1998), the pink chromogen absorbs at 450, 530, and 538 nm.

Another important aspect is autoxidation of the sample during the analysis. As during the assay, especially the heating step, the autoxidation processes can be in progress consequently giving artifactually high analytical results, several scientists are advising the addition of antioxidants to the reaction mixture (Pegg, 2005). Still in biological samples, MDA can also react with proteins and give abnormally low values in an oxidizing system (Vattem and Shetty, 2007).

Because of many uncertainties, the appropriateness of the TBA assay for milk has been questioned and it needs empirical observations for each method and type of samples (MacGibbon and Taylor, 2006).

Many workers have attempted to make quantitative evaluations of rancidity through the use of carbonyl tests. They are based on the reaction of carbonyl moiety with 2, 4-dinitrophenylhydrazine (DNPH), followed by the reaction of the resulting hydrazones with alkali which are turned into quinoidals colored wine-red (see Fig. 3).

Variations in methodology for the choice of solvent, wavelength, or workup in which to analyze the quinoidal ion exist in literature. As with the TBA test, the carbonyl content in oils is usually monitored at discrete time intervals during storage and the contents of saturated, unsaturated and total carbonyls are compared against one another or with different chemical indices. The carbonyl compound method has been criticized because hydroperoxides decompose under the conditions of the test and thus possibly interfere with the quantitative determination of the carbonyls. Several modifications of this procedure have been offered to overcome this problem, but no ideal solution has been found (Pegg, 2005).

In this investigation three spectrophotometric methods were compared to choose the most appropriate for the assessment of oxidation of milk fats:

- the AOCS (American Oil Chemists' Society) Official Method Cd 19-90 (reapproved 2009) – 2-Thiobarbituric Acid Value Direct Method for the determination of TBA reactive substances (TBARS) in oils or lipid extracts (Pegg, 2005);
- the method of Aristova that uses TBA for the determination of oxidation degree of milk lipids (Инихов и Брио, 1971); and
- the method of Endo et al. (2003).

The method of AOCS is based on the protocol reported by Pokorny and Dieffenbacher (1989) and permits the direct determination of TBARS in oils and fats without preliminary isolation of secondary oxidation products. It is applicable to animal and vegetable fats and oils. The method of Endo et al. is used for the determination of total carbonyl compounds in frying oils and was derived from the Japan Oil Chemist's Society Standard Method and other similar conventional methods as of Henick et al. Japan scientists Endo et al. modified this method using less toxic 1-butanol as a solvent in place of benzene and reduced the volumes of reagents (Endo et al., 2003).

The aim of this study was to compare the above mentioned spectrophotometric methods to clarify their suitability for the assessment of oxidation processes of milk fat.

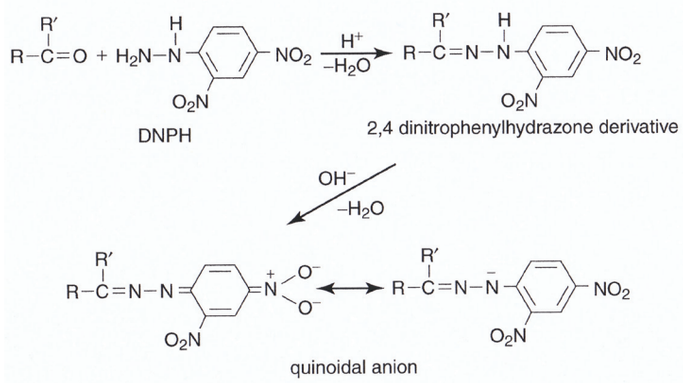

Figure 3. The reaction of a carbonyl with the DNPH to yield quinoidals colored wine-red (Pegg, 2005).

Materials and Methods

The experiments were carried out in the Research Laboratory of Biochemistry and Microbiology of the Research Institute of Biotechnology and Veterinary Medicine 'Sigra' of the Latvia University of Agriculture in winter 2010.

Two samples of milk fat were used in this investigation.

Fat sample preparation. The first sample was made from fresh cow milk obtained from a conventional dairy farm. At first milk was centrifuged in a separator, and cream was obtained. The second sample was commercial butter with expired shelf-life (approximately 50 days old, stored in a refrigerator) and had rancid flavor. The pure fat part for experiments was isolated by the centrifugation from cream and butter in a centrifuge (15.871 × *g*, 30 min. and 40 °C). Fat samples were divided into small portions (1 or 3 g) in Eppendorf or glass tubes (semi-filled, not special atmosphere was applied) for each day and type of analysis and were stored refrigerated at temperature of 4-6 °C. Samples were analyzed 6-7 times within a 22-day period after sample preparation (by the methods of AOCS and Aristova – in days 5, 7, 9, 12, 15, 17, and 22; by the method of Endo et al. – in days 5, 8, 11, 14, 16, 17, and 22). Before testing, fat was heated till 40 °C and mixed thoroughly.

Analytical reagents. 2-tiobarbituric acid (TBA) (purity >=98%), 1,1,3,3 – tetramethoxypropane (TMP) (purity 99%), 2,4-dinitrophenylhydrazine (DNPH), and butylated hydroxytoluene (BHT) were purchased from Sigma-Aldrich; 1-butanol was of analytical grade containing water < 0.5% from Lab-Scan Analytical Sciences, Poland; isooctane was of pestician grade from Lab-Scan Analytical Sciences, Ireland. All other reagents were of analytical grade: glacial acetic acid was from Stanchem, Poland; potassium hydroxide was from Lachema, Czech Republic. Absolute ethanol was from Lako, Latvia.

The method of AOCS. A fat sample of 10-40 mg was put into a 20 ml test tube, recording accurate weight, and dissolved in 4.95 ml of 1-butanol (with 50 mg of BHT in 100 ml). Blank trial was made taking 5 ml of 1-butanol. Five ml of TBA reagent (prepared by dissolving 0.2 g of TBA in 1-butanol in a 100-ml volumetric flask by means of careful heating till 75 °C) were added. Tubes were sealed, mixed by vortex mixer and heated in 95 °C water bath for 2 h, then cooled till room temperature. Absorbance readings at 532 nm were taken, using plastic cuvettes (path lengths – 1.00 ± 0.01 cm). Calibration curves were made with series of 0.02 m*M* 1,1,3,3-tetramethoxypropane (TMP) solution aliquots (0.2 to 2.0 ml), adding 1-butanol to each tube till 5 ml total. Addition of TBA and further steps were performed as described previously.

The method of Aristova. A fat sample of 3 g was put into a 20-ml test tube and dissolved in 10 ml of isooctane. Blank trial was made taking 3 ml of distilled water instead of fat. 10 ml of TBA reagent (prepared by dissolving 0.67 g of TBA in distilled water in a 100 ml volumetric flask by means of careful heating till 60 °C and US bath to succeed the dissolution of TBA; after cooling till room temperature, TBA solution was mixed with glacial acetic acid in ratio 1:1) were added. Tubes were sealed, mixed for 4 min. The lower layer was separated and heated in boiling water bath for 30 min. Then tubes were cooled till room temperature, and the solution was filtered through a paper filter. Absorbance was read at 536 nm using glass cuvettes (path lengths – 1.00 ± 0.01 cm). Calibration curve was made with series of 0.02 m*M* TMP solution aliquots (0.02 to 0.2 ml). To each tube with TMP solution, distilled water was added till 3 ml total. Addition of TBA and further steps were performed as described previously.

The method of Endo et al. A fat sample of 5-20 mg (10µl) was put into a 10-ml test tube recording accurate weight and was dissolved in 990 µl of 1-butanol. One ml of 2,4-DNPH solution (prepared by dissolving 50 mg of 2,4-DNPH in 100 ml of 1-butanol containing 3.5 ml of concentrated HCl) was added, the tube was sealed and mixed by vortex mixer, then heated in 40 °C water bath for 20 min., and cooled till room temperature. Then 8 ml of KOH solution in 1-butanol (0.8 g l^{-1}) were added, the tubes were shaken and centrifuged at 845 x *g*, 5 min. at 20 °C. The absorbance of the upper layer was measured at 420 nm, using glass cuvettes (path lengths – 1.00 ± 0.01 cm).

In the methods of AOCS and Endo et al., modifications were made to reduce the amounts of reagents. In the method of Aristova, filtration was applied to get transparent solution, suitable for absorbance readings, because there were droplets of isooctane and fat remained into the reaction solution after the first separation.

All analyses were made in triplicate employing the same apparatus – spectrophotometer Spectro 2000 RSP from Labomed, Inc., USA, and taking at least four readings of absorbance for each sample.

The results were calculated, and analyzed, and graphs were made using MS Office program Excel.

Results and Discussion

The first principal difference of the three spectrophotometric methods chosen for the comparison is the spectrum of the secondary oxidation products detected and revealing the processes of fat oxidation. The methods of AOCS and Aristova are based on the reactions of TBA reactive substances, while the method of Endo et al. is based on the reactions of a larger spectrum of substances – total carbonyl compounds. The different chemicals used as reagents and solvents yielding different colour nuances are the basic reason why different wave lengths are used for the measurements.

The second principal difference is in the expression and interpretation of the results obtained by measurements. In the practice there are two ways for the assessment of lipid oxidation. The first approach is the exact determination of analyte concentration in the product, by making stationary measurements reflecting its quality in one exact

point of time. The second practice is to observe the dynamics of the products' quality in longer period of time, by reporting absorbance values from the assay directly, as do some researchers to avoid having to construct a standard curve and to perform recovery experiments, if it is not necessary to know the exact concentrations of oxidation products (Pegg, 2005; Pokorny and Dieffenbacher, 1989). In the first case, the results, calculated as concentration in grams, moles and like, can be used to compare the quantities of the oxidation products, estimated by different methods or to compare accuracies of different methods. In the second case it is possible to observe the progress and intensity of oxidation processes in the limits of one separate method, furthermore comparisons can be made between the results if equal conditions (analytical reagents, types of the samples, etc.) are used.

In this investigation, the determination of secondary oxidation products could be made in the method of Aristova. The exact concentrations of TBARS expressed as μg per 1 g of fat were obtained by means of the calibration curve. It was found that in the rancid fat sample it was 13.8–27.0 μg, but in the fresh lipid sample - 0.1–7.5 μg per 1 g of fat.

In the method of AOCS, the calibration curve was not obtained although trials were repeated several times. Commonly 1,1,3,3-tetramethoxipropane (TMP) or its ethyl analog is used as a standard. In the presence of an acid solution, these chemicals are hydrolyzed to MDA and therefore can be used to obtain TBARS calibration curve; one mole of MDA is released for each mole of TMP (Pegg, 2005). In the method of AOCS, acid is not added, but in the method of Aristova the medium of reaction is more acidic due to the glacial acetic acid. Another probable factor can be the presence of water. It is known that MDA has hydrophilic nature. In the method of Aristova there is the presence of the water, while in the method AOCS – is not. So, probably MDA can be liberated from TMP reagent and reacted with TBA easier in environment, which is in the method Aristova.

The results obtained from all the three methods are shown as absorbance readings in Figs 4 - 6.

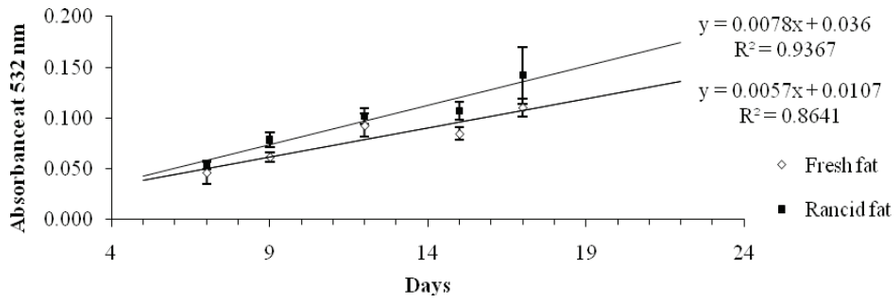

Figure 4. Results of the method of AOCS.

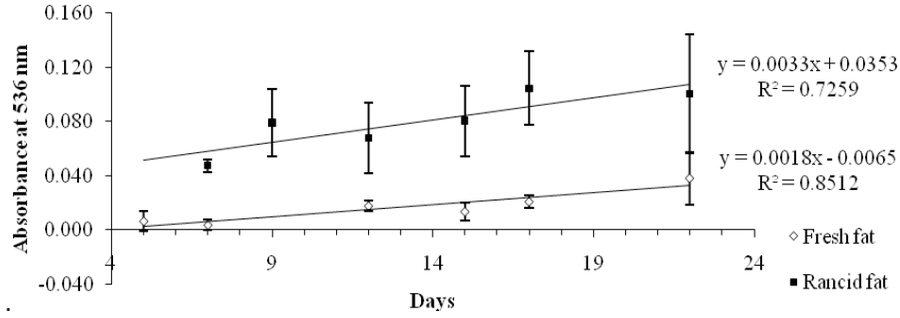

Figure 5. Results of the method of Aristova.

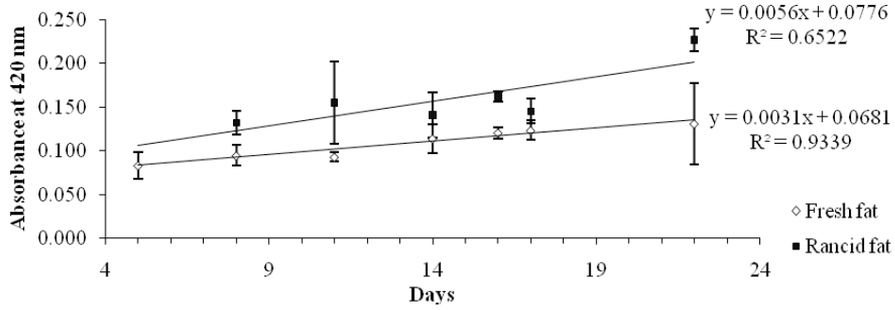

Figure 6. Results of the method of Endo et al.

Table 1

Comparison of the principal parameters of the methods

Parameters for method comparison	Symbol, units	Method					
		AOCS		Aristova		Endo et al.	
		fresh fat	rancid fat	fresh fat	rancid fat	fresh fat	rancid fat
Coefficient of determination	R^2	**0.86**	0.94	**0.85**	0.73	**0.93**	0.65
Coefficient of variation [a]	%	**10.13**	9.76	**45.78**	26.60	**11.12**	9.72
Time consumption [b]	h: min.	3:00		1:45		1:30	
Expenses of chemicals [b]	LVL	0.56		1.08		0.91	
Sample amount [b]	g	0.03 - 0.12		9.00		0.02 - 0.06	

[a] Calculated within parallel analyses.
[b] Calculated for 3 parallel analyses of 1 sample (not included time or expenses for making calibration curve).

It is important to remember that all the three test procedures differ in sample amounts, used solvents and reagents, heating temperatures, wavelengths of spectrophotometrical measurements, and lead to different levels of secondary oxidation products recovered from an identical sample (Pegg, 2005). In this case, when the exact concentrations of the secondary oxidation products are not determined in all the methods, its accuracy cannot be compared directly by absorbance readings, carbonyl or TBARS values.

However, graphical images can serve to compare how similarly all methods are representing the development of oxidation processes in lipid samples.

All methods used in this experiment show that the absorbance of the rancid fat sample is significantly higher ($p = 0.05$) than that of the fresh fat. Concentrations of secondary oxidation products are increasing linearly in both fat samples; however, the slopes of the trendlines show that the speed of the secondary oxidation products development is higher in the rancid fat sample. The summary of the results is given in Table 1.

Further only the results of fresh fat were analyzed for method comparison, as in practice the monitoring of the oxidation processes is more important for the fresh fat.

As seen from Table 1, the R^2 values of the results obtained by all three methods are rather high (AOCS – 0.86, Aristova – 0.85, and Endo et al. – 0.93). Deviations within parallel analyses of each sample were compared by the coefficient of variation, expressed as percentage, also called 'the relative standard deviation', which is often preferred since it generally has the advantage of being constant at every level of concentration of the quantity to be measured (Grappin, 1996). The methods of AOCS and Endo et al. show rather good coefficients of variation (10.13%, 11.12%), while the method of Aristova has the greatest variation (45.78%). Possibly it can be explained with the fact that absorbance readings for fresh fat in the method of Aristova are very low, near zero. As known, low absorbance values give particularly high errors. Smaller errors could be obtained, if the absorbance of the measured light is within the interval of 0.2-1.4. It should be considered that this method originally was prescribed for the measurements on photo-electro colorimeter using cuvette with longer path length, yet in this experiment measurements were made on the spectrophotometer. The readings on both apparatus can be different. If readings are taken on spectrophotometer, greater absorbance values can be obtained by preparing the solution with higher analyte concentration.

Time consumption, expenses of chemicals and necessary sample amounts also were calculated to compare the methods and, as seen in Table 1, the fastest is the method of Endo et al., while the method of AOCS is the most time consuming. Regarding analytical reagent expenses, the last method is the cheapest while other two methods are in the same level and, in general, are more cheaper, compared to sophisticated analytical techniques. The method of Aristova needs the biggest amount of the sample (9 g), though that is not always easy obtainable. Other two methods need considerably smaller amounts of the sample (< 0.2 g), but care must be taken to the very accurate weighting technique.

Conclusions

1. The positive linear correlation between absorbance and storage time was established in all used methods.
2. The methods of AOCS and Endo et al. are suitable for the assessment of the dynamics of fat oxidation by reporting absorbance values from the assay directly.
3. The Aristova method can be used for exact determination of oxidation product concentrations.
4. The methods of AOCS and Endo et al. are cheaper and require smaller sample amounts (< 0.2 g) compared with Aristova method (9 g), while the method of AOCS is the most time-consuming method.

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THE DYNAMICS OF VITAMINS C AND E IN BARLEY PRODUCTS DURING MALTING

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Abstract. Barley is a key ingredient in beer production. The aim of the current research was to study the dynamics of vitamin C and vitamin E in flaky and hull-less barley grains during steeping, germinating, and kilning.
The research was accomplished on hull-less barley (two lines – '3528' and '3537') and flaky barley selected in Latvia in 2009 with a germination capacity above 95%. The grains were steeped, germinated and kilned using traditional malt production technology. During research the vitamin content was analysed using standard methods: vitamin C by EN 14130:2003, and vitamin E by AOAC 971.30.
The content of vitamin C increased during steeping in flaky barley grains till 0.23 mg 100 g^{-1}, but in hull-less barley grains: line '3537' till 0.47 mg 100 g^{-1}, and line '3528' till 0.30 mg 100 g^{-1}. During germination vitamin C content in flaky barley increased by 68%, in hull-less barley: line '3528' – by 82%, and line '3537' – by 57%. The content of vitamin C in the analysed malt samples was 0.35-0.38 mg 100 g^{-1}. The content of vitamin E in all barley samples was similar after grain steeping. The content of vitamin E was 3.9 times higher in flaky barley, but in hull-less barley lines: '3528' – 4.1, and '3537' – 4.5 times higher compared with its initial content after germination. After grain kilning, the content of vitamin E decreased in all barley grain samples.
The results show that using some cultivars of hull-less barley for malt production, it is possible to obtain a higher content of vitamins C and E in the end-product.
Key words: barley, hull-less barley, malt, germination, vitamins C and E.

Introduction

Barley (*Hordeum vulgaris L.*) is a common staple in human and animal diets. Part of the grass family, barley grows in over 100 countries and is one of the most popular cereal crops, surpassed only by wheat, corn, and rice. Although barley is fairly adaptable and can be grown in many regions, it is a tender grain, and care must be taken in all stages of its growth and harvest. According to a recent study, eating whole-grain barley can regulate blood sugar (i.e. can reduce blood glucose response to a meal) for up to 10 hours after consumption compared with white or even whole-grain wheat, which has a similar glycemic index. The effect was attributed to colonic fermentation of indigestible carbohydrates (Nilsson et al., 2006).

A large part of the remainder is used for malting, for which barley is the best suited grain. It is a key ingredient in beer and whisky production. Barley has been retained as the primary cereal of choice, not the least, because it retains its husk during threshing and this traditionally forms the filter bed through which the wort is collected in the brewery (Bamforth, 2006). Within the barley malting process, the enzymes activity increases and as a result starch degradation occurs and fermentable sugars are synthesized. Additionally there is a synthesis of the enzymes which degrade the cell walls and much of the protein in the starchy endosperm, thereby softening the grain and making it more millable (Bamforth, 2006).

Barley and malt are rich in substances that are growth factors for yeasts and vitamins for humans. Barley used in malt production should contain high amounts of extract substances (at least 80.0%) and low amounts of proteins (not more than 11.5%).

In the food industry, hull-less barley (*Hordeum vulgaris L.*) is acknowledged as more valuable and more economical, compared with covered barley. The hull-less barley has an elevated ß-glucans content. Soluble dietary fiber, mainly ß-glucans, provides the formation of viscosity; as a result, digestion, cholesterol and fat absorption are decreased (Bhatty, 1999; Belicka and Bleidere, 2005). Selected hull-less barley varieties are able to pass flaky barley criteria: moreover, the amount of extract substances in hull-less barley is higher by 4-5% compared to malting barley.

Vitamin C (ascorbic acid) is a well-known antioxidant. The main function of vitamin C as a radical producer is to provide the regenerating system for tocopherol (Dewick, 2006). Ascorbic acid can act as a synergist with tocopherol by regenerating or restoring their antioxidant properties. (Niki, 1987). Ascorbic acid and its esterified derivatives may also function as oxygen scavengers. Vitamin C declines in the heating process (Pokorny, 2001).

Vitamin E is also a major biological antioxidant, it quenches free radicals and acts as a terminator of lipid peroxidation, particularly in membranes that contain highly unsaturated fatty acids. Barley is one of the best sources of raw food material, containing a high concentration of tocols. A higher content of natural antioxidant tocopherol and tocotrienols, as well as vitamin E, was established in barley grain, compared with wheat and rye grain (Shewry, 1993).

Germinated grains have already been used in food since ancient times because germination is one of the processing methods for increasing the grain nutritive value. The gross chemical changes observed during

malting are the net result of the degradation of reserve substances, the interconversion of materials in the living tissues, the flux of materials to the embryo, and their incorporation into the growing tissues, the synthesis of new materials and the losses occasioned by leaching during steeping, oxidation to carbon dioxide and water, and the separation of the rootlets (Briggs et al., 1981). The content of vitamin E increases and vitamin C synthesizes during the grain germination. It leads to the hypothesis that the amount of C and E vitamins in hull-less barley varieties is significantly higher compared to traditional barley.

Germination of grains and external modification are stopped by kilning. Analysing the sources of literature, it can be concluded that more attention has been paid to the efforts of not destroying the enzymes during kilning of the prepared malt for beer production, and less attention has been paid to saving the content of vitamins.

After analysing the scientific data from the literature, the aim of the current research was to study changes in vitamins C and E in flaky and hull-less barley grains during barley steeping, germinating, and kilning.

Materials and Methods

The research was carried out on hull-less barley (two lines '3528' and '3537') and flaky barley (one line 'Rolands') grains, which were selected in Latvia in 2009, with germination capacity above 95%. The following technology was used for malt production from the tested grains: washing and steeping of grains (H_2O t=17±2 °C) till moisture content in grains reached 38 - 40%. Then the grains were put in germination trays where a wet laboratory paper was extended, and then the grains were covered with the same wet paper. The grains were kept for 6 days at 18-20 °C for germination. The changes in the concentration of vitamins C and E were monitored over the germination period. An 8 hour kilning of the germinated grains was carried out in a laboratory kiln. Grains in a small layer were put on sieves in a chamber with controlled hot air circulation at the temperature from +50 °C to +105 °C till a constant moisture content was achieved in the grains (5±1%).

The content of vitamin C was determined by the standard method EN 14130:2003 "Foodstuffs – Determination of vitamin C by HLPC".

The analyses of vitamin E content were carried out by using the AOAC Official Method 971.30 "α-Tocopherol and α-Tocopheryl Acetate in Foods and Feeds" standard colorimetric method (1971-1972). The term "vitamin E" is the generic descriptor for all tocol and tocotrienol derivatives that exibit qualitatively the biological activity of α-tocopherol (Ball, 2006). The figures are created by using MS Excel programme.

Results and Discussion

Cereals are considered only moderate sources of vitamin E, providing 0.6–2.3 mg kg^{-1} of α-tocopherol. Since cereals contain great amount of tocotrienols with very low vitamin E activity, they are more valuable as sources of tocotrienols and tocopherols than vitamin E (She et al., 2000).

The dynamics of vitamin E was analysed during the current research in several barley cultivators starting form non-processed barley to ready malt. It was found that the content of vitamin E in non-processed flaky and hull-less barley was similar. The content of vitamin in two types of hull-less barley grains was identical: 4.03 mg 100 g^{-1}. The content of vitamin E in flaky barley was lower by 0.27 mg 100 g^{-1} compared with hull-less barley grains, which mainly could be explained by the individuality of the barley cultivar. The volume of the endosperm of hull-less barley grain is larger than that of flaky barley. It is known that the endosperm contains many nutritional compounds of grains, such as albumens, vitamins (including vitamin E), carbohydrates, mineral substances, and others.

During grain steeping process, the increase in vitamin E content in flaky and hull-less barley grain types '3528' and '3537' was similar: the content of vitamin increased 1.7 (6.5 mg 100 g^{-1}), 1.8 (7.37 mg 100 g^{-1}), and 2.0 (8.17 mg 100^{-1}) times respectively compared with the initial vitamin content in non-processed grains. Such changes could be explained by the beginning of a biochemical reaction in the grains.

The results of our experiments prove that more intensive synthesis of vitamin E was detected in grains after 2 days of germination. The content of vitamin E increased in flaky barley grains 1.75 times, and in two hull-less barley grain lines - 1.60 times (Fig. 1) compared with the vitamin E content in steeped grains, which indicates intensive growing processes as a result of chemical and biochemical reactions. Increases in vitamin E were detected also after 4 days of germination. After finishing the germination process on the 6^{th}, day the content of vitamin E in flaky barley was 3.9 times (14.8 mg 100 g^{-1}), in hull-less barley grain line '3528' – 4.1 times (16.56 mg 100 g^{-1}), and in hull-less barley grain line '3537' – 4.5 times (18.27 mg 100 g^{-1}) higher compared with the initial vitamin E content in non-processed grains. Such increases could be explained with the modulation of the plant growth. Therefore it is possible to foresee that more suitable for malt production will be hull-less barley grain line '3537', because the content of vitamin E in germinated hull-less barley grains was the highest (Fig. 1).

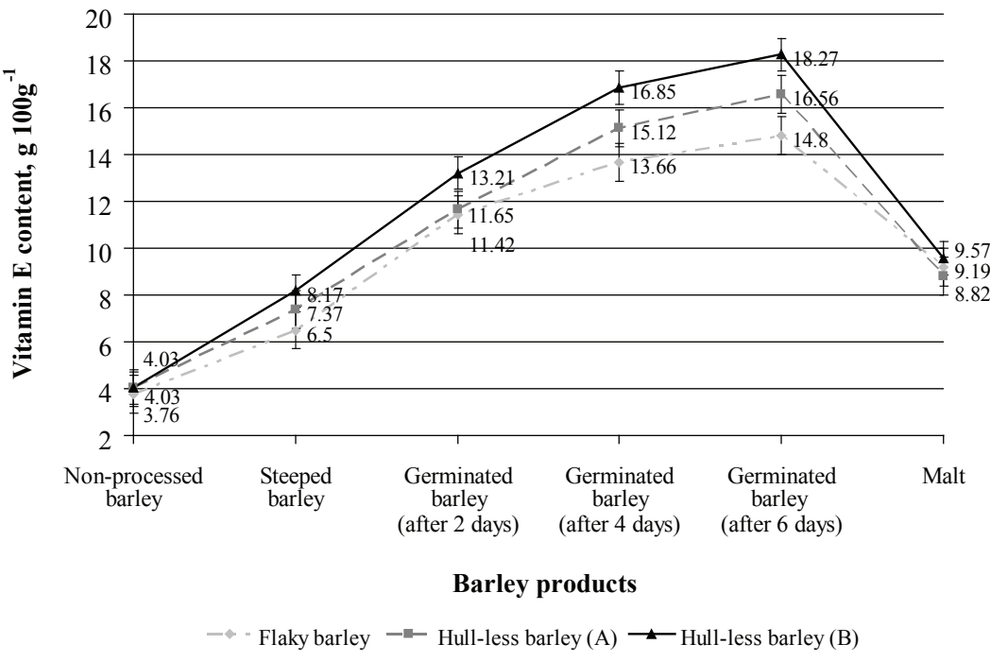

Figure 1. The dynamics of vitamin E content (in dry matter) in barley products.

After grain kilning at temperatures of +50 °C to +105 °C in a time period of 8 h, the content of vitamin E decreased in all barley grain samples: in flaky barley grains 1.6 times (to 9.19 mg 100 g^{-1}), and in hull-less barley grains 1.9 times. The content of vitamin E in hull-less barley grain was (in line '3528' - to 8.82 mg 100 g^{-1}, in line '3537' – to 9.57 mg 100 g^{-1}). The scientific literature data indicate the possible vitamin E resistance to elevated processing temperatures, but the results of our research prove that decreases in vitamin E in grains during processing can be influenced by an elevated processing temperature and the presence of oxygen. Therefore more suitable for malt production is hull-less barley grain line '3537', where the content of vitamin E after kilning was 0.75 mg 100 g^{-1} higher than in hull-less barley grain line '3528', and 0.38 mg 100 g^{-1} higher than in flaky barley grains.

The scientific literature data prove that the content of vitamin C is not detected in grains. Vitamin C synthesizes during grain germination. In our research, the dynamics of vitamin C was analysed in several barley products. In non-processed flaky barley and hull-less barley grain line '3528' it was not detected. In the dry matter of non-processed hull-less barley grain line '3537', vitamin C was detected at the amount of 0.19 mg 100 g^{-1} (Fig. 2), which could be explained by possible germination process beginning in the grains during storage.

The results suggest that, vitamin C synthesis begins during grain steeping for 24±1 h in water ambiance. It can be explained by the increase of moisture content in grains, which is a positive factor for enzymes activation, therefore the synthesis process of vitamin C starts. In addition, the vitamin C synthesise occur during the grain activation time (Rakcejeva and Skudra, 2006). The content of vitamin C was synthesized during the steeping process in flaky barley grains till 0.23 mg 100 g^{-1}, in hull-less barley grain line '3537' till 0.47 mg 100 g^{-1}, and in hull-less barley grain line '3528' till 0.30 mg 100 g^{-1} in dry matter, compared with the vitamin C content in non-processed grains. Therefore more intensive synthesis of vitamin C was found in hull-less barley grains from line '3537'.

Further germination caused a progressive increase in vitamin C in all barley samples after 2 and 4 days. This finding is in agreement with a well-known fact that biosynthesis of vitamin C in grains takes place during germination and seems to be directly involved in the modulation of plant growth, including the early stage of embryo germination (Plaza et al., 2003). After 4 days of germination, maximal vitamin C content increases were found in the tested grain samples (Fig. 2). During the research it was found that vitamin C content in flaky barley increased by 68%, in hull-less barley line '3528' – by 82%, and in hull-less barley line '3537' – by 57%, respectively, after germination for 96 h at 20±2 °C in the dark.

The content of ascorbic acid decreased in all samples of barley grains (Fig. 2) after germination for 6 days, possibly because the grains started to use vitamin C for providing the life processes and for the same growth and development. Therefore, after germination for 6 days, the content of the ascorbic acid in all grain samples was very similar - 0.5 mg 100 g^{-1} in dry matter.

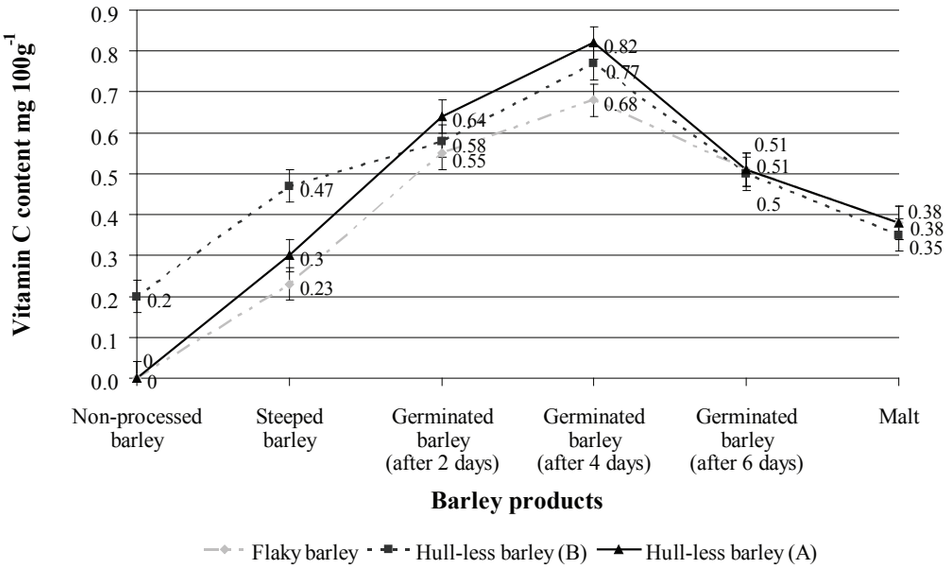

Figure 2. The dynamics of vitamin C content (in dry matter) in barley products.

Ascorbic acid decreases during grain drying at elevated temperatures in the air and oxygen ambiance are not avoided. Decreases in vitamin C in all grain samples were similar – 1.3 times lower compared with its content in grains germinated for 6 days. The content of vitamin C in dried grain samples was 0.35–0.38 mg 100 g^{-1}. The obtained data prove that vitamin C is not resistant to light, temperature and oxygen (Ball, 2006).

Conclusions

1. The content of vitamin C increased in flaky barley grains during steeping till 0.23 mg 100 g^{-1}, in hull-less barley grain line '3537' till 0.47 mg 100 g^{-1}, and in hull-less barley grain line '3528' till 0.30 mg 100 g^{-1}.
2. Grain germination caused a progressive increase in vitamin C content in all barley samples: in flaky barley by 68%, in hull-less barley line '3528' – by 82%, and in hull-less barley line '3537' – by 57%.
3. The content of vitamin C in dried grain samples decreased during kilning and was 0.35 – 0.38 mg 100 g^{-1}.
4. After grain steeping, the content of vitamin E in flaky barley and hull-less barley grain line '3528' and '3537' was similar.
5. The amount of vitamin E in flaky barley was 3.9 times, in hull-less barley grain line '3528' – 4.1 times and in hull-less barley grain line '3537' – 4.5 times higher compared with its initial vitamin E content in grains after germination.
6. After grain kilning, the content of vitamin E decreased in all barley grain samples: in flaky barley grains 1.6 times (to 9.19 mg 100 g^{-1}), and in hull-less barley grains 1.9 times.
7. The obtained results show that use of hull-less barley line '3537' in malt production allows to obtain by 0.38 mg 100 g^{-1} higher content of vitamin E in the end products compared to use of flaky barley grains.

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ANTIRADICAL ACTIVITY OF RYE BREAD DURING BAKING

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Abstract. Nowadays one of most urgent problems is the production of healthy food which ensures normal maintenance of the human body. Rye bread is one of the bases of wholesome food. Every year scientists in Europe and other countries carry out investigations on the nutritional impact of processing rye. Interesting questions arise regarding changes in the biologically active compounds in rye bread as a result of processed. Connate antiradical activity (ARA) of rye bread affects free radicals by scavenging or donating electrons, which suggests that the ARA of rye bread averts attacks of free radicals on human cells. Therefore it is important to use antioxidants containing products with antiradical activity in human diets.
The aim of this study was to analyse the impact of baking processing on rye bread antiradical activity. The object of the study was Latvian traditional rye bread baked in a commercial bakery, made with scald, and baked in a clay-floor firewood oven. The antiradical activity of rye bread crumb and crust was measured by using free radical 2.2-diphenyl-1-picrylhydrazyl (DPPH).
It was concluded in the research that rye flour type 1740 has antiradical activity by scavenging 67 $\mu mol \cdot 100g^{-1}$ DM of the stable free radical DPPH, which is 13% more than the dough antiradical activity of the same flour. The antiradical activity of the crust is 49.6% more than the antiradical activity of the crumb. Baking time has no significant impact on changes in the antiradical activity of rye bread crumb.
Key words: rye bread, antioxidants, antiradical activity.

Introduction

Free radicals have high chemical activity. They are natural metabolic products which are constantly being synthesized in the metabolism of the human body, and where they are inactivated by enzymatic reactions in the human body. Adverse environmental factors such as mental stress, drugs, or radiation may lead to an uncontrolled synthesis of free radicals, and a disorder in the metabolic self-regulation of living organisms.

Antioxidants are naturally occurring chemical compounds which inhibit, scavenge or repair free radicals in human organisms, plants and animals. Rye bread in Latvia is rich with dietary fibre, vitamins, polyphenols, and mineral substances, and is one of the antioxidant sources in human diet. Antioxidants are biologically active substances (vitamins, trace elements, and enzymes) with different chemical structures which prevent or preclude the detrimental oxidation process of tissues, inhibiting the evolution of a variety of sickness. Investigations presented evidence that antioxidants decrease the level of blood cholesterol, diminish atherosclerosis, heart disease and genetic risk of all cancers, ease the progress of pulmonary diseases (bronchitis, asthma), and slow down senescence (Kujala, 1999; Temple, 2000). In reaction to stress, human organisms themselves generate antioxidants for counteracting the free radicals which can damage cells. The free radicals can emerge as a result of inimical environmental factors (atmospheric pollution, radiation, ultra-violet light, pesticides), as well as being the result of some medicines. They damage cells, and cause dangerous diseases, including cancer. To avert this, the free radicals must be linked in stable compounds by using antioxidants. As the amount of antioxidants synthesized by the human organism is not enough for counteracting all free radicals, diets must be improved with antioxidants.

Natural antioxidants are located in almost all foodstuffs. One of these is rye used for flour production which is further used for dough making and bread baking. Rye contains such antioxidants as polyphenols, lignans, phytoestrogens, tocols, flavons, as well as trace elements like selenium, zinc, copper, and iron. Polyphenols include five groups of compounds: benzoic and cinnamic acid derivatives, flavonoids, tannins, and stilbenes (Rice-Evans et al., 1996). Their action relies on scavenging of free radicals initializing the oxidation process or chelating the metal ions catalyzing the oxidation process, lowering or stopping the oxidation enzymes' activity and by activation of antioxidant enzymes (Bravo, 1998). Bread baked from rye flour is one source of antioxidants in the human diet. The influence of processing on the quantity and activity of antioxidants in rye bread has been only occasionally investigated. Heat processing is known to affect nutrients in foods, but its effect on phytochemical content and antioxidant activity is not well investigated. Results from other food systems show that thermal treatment significantly reduces concentration of natural antioxidants (Fares et al., 2010). Recent literature has underlined that grain fractions–bran and flour–have different antioxidant capacities (Liyana-Pathirana and Shahidi, 2007). Cereal processing including the baking process results in a loss of antioxidants, but it has also been described as a synthesis of new antioxidants, especially the Maillard reaction with a new type of antioxidant pronyl-L-lysin formed during the baking process (Lindenmeier and Hofman, 2004). Thereby overall antioxidant properties of food products are maintained or even enhanced. German researchers have come to the conclusion that baking parameters may significantly enhance the level of antioxidants in bread, and that crust antioxidant capacity is higher than crumb antioxidant capacity, but different baking times

have no clear impact (Lindhauer, 2008; Lindhauer et al., 2009). This was the reason for undertaking the baking process of Latvian rye bread in our study. In this research, the antiradical activity (ARA) is evaluated by measuring the scavenging activity of examined rye flour, dough and rye bread at different baking times against stable free radical- DPPH (2.2-diphenyl-1-picrylhydrazyl hydrate) and expressed as an amount of DPPH scavenged by sample.

Materials and Methods

The research was realized in the Research Laboratory of Molecular Genetics of the Institute of Soil and Plant Sciences of the Latvia University of Agriculture in 2010. The rye bread samples used in the research were derived from the production process at 'Lāči', a commercial bakery: the flour– a 1740 type; the dough– prepared by scalding and fermented with natural starter; the bread– a 1kg loaf baked on a clay-floor with various baking times– 45, 60 and 75 minutes at the temperature of 220 °C. Dough samples were frozen to stop fermentation. Slices, 1 cm in width, were cut from the centre of the loaf. A top-crust sample, 0.7 cm in width, was separated from the crumb of a loaf slice. A down-crust was separated from the crumb of the bottom of a loaf slice. Dry matter of samples was obtained by using an infrared radiation drying equipment (ЭСПИ-3, Russia) at the temperature of 30-45 °C. The antiradical activity was determined by using a slightly modified DPPH method (Miliauskas et al., 2004), as described below, which measures the radical-scavenging activity or antiradical activity of the sample against stable free radical-DPPH. The samples of flour, dough and bread were extracted with ethanol. Extraction was applied by shaking flasks with 3 g of a sample material and 6 ml of 80% ethanol in a shaking machine for 2 h, after wards the samples were centrifuged at 8000 rpm for 3 minutes.

Antiradical activity of samples` extracts against stable DPPH (Sigma-Aldrich Chemie, molecular weight 394, 32) was determined by spectrophotometer (Perkin Elmer Lambda 25). The solution of DPPH in methanol was $15 \cdot 10^{-6}$ M. Three ml of this DPPH solution were mixed with 300 µl extract of solution in microcuvettes. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The samples were kept in the dark for 5 minutes at room temperature then the decrease in absorption was measured at 517 nm on a UV/VIS spectrophotometer. The blank sample contained the same amount of ethanol–300 µl, and 3 ml of DPPH solution- were prepared. The antiradical activity was calculated as difference of concentration of DPPH in the blank sample and the remaining concentration of the sample after 5 minutes of its reaction with DPPH (Roginsky and Lissi, 2005). Statistical analyses of the results were performed by SPSS program (Arhipova and Bāliņa, 2003).

Results and Discussion

In has been determined that rye bread flour type 1740 – the basic raw material for rye bread baking - has antiradical activity. Flour can inactivate 66.8 µmol of the radical DPPH per 100 g of dry matter (DM). It is in agreement with the findings by Lindenmeier M. and Hofmann T. (2004), showing that the primary element of baking-flour already contains natural antioxidative potential such as phenols, vitamins, and other biologically active compounds. The next phase of producing bread-dough can inactivate 57.8 µmol of DPPH per 100 g of dry matter, meaning that antiradical activity is 13.5% lower than in flour (Figure 1).

Figure 1. Antiradical activity of flour and dough.

This is due to the fact that in the dough fermentation process, not only starch, but also biologically active compounds with antioxidative capacity are used for lactic acid and yeast bacteria metabolism. During the process of rye bread baking, the antiradical activity of crumb is from 45.5 to 48.4 µmol·100 g^{-1} of dry matter (Figure 2).

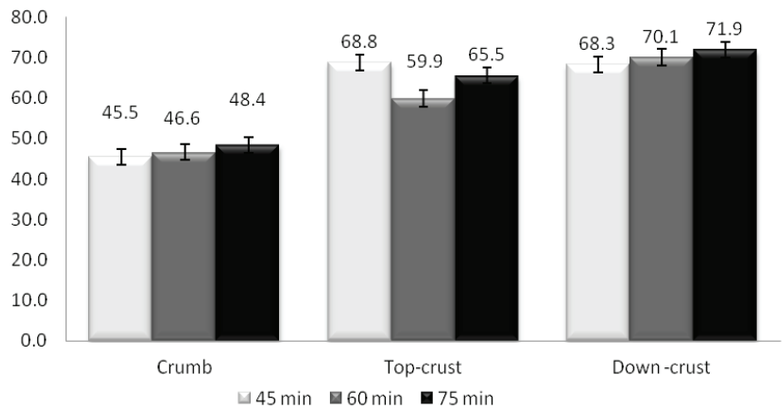

Figure 2. Crumb, top-crust and down-crust ARA activity at a different baking time.

A baking time of 45, 60, or 75 minutes has no significant impact (p=0.986) on crumb antiradical activity. However, in a comparing between a sample baked for 45 minutes with a bread sample baked 75 for minutes, it was observed that the bread sample with the longest baking time (75 min) had 6.3% higher ARA, except top-crust at baking time 45 minutes. The temperature of the bread crumb at the end of baking did not exceed 100 °C, and the crust temperature usually was higher but was not measured in this experiment (Figure 2).

This trend is also observed in Lindenmeier et al. (2002) findings that high baking temperature increases the antioxidative capacity of bread products. The antiradical activity of rye bread top-crust baked for 45 minutes reaches 68.3 µmol·100 g^{-1} of DM, but slightly decreases when baked for 75 minutes– 65.5 µmol·100 g^{-1} of DM. The decrease is 4%, and is not statistically significant (p=0.689), which is explained by the fact that rye bread with a prolonged baking time (75 min) has fewer hydrophilic antioxidants than normally (60 min) baked bread (Lindhauer et al., 2008). The ARA of top-crust in comparison with the ARA of crumb is increased by 38.2%.

This research shows that the ARA of down-crust increases in a statistically significant quantity as a result of prolonged baking time (p=0.00). The ARA of down-crust at all researched baking times is, on average, 70.1 µmol·100 g^{-1} of DM, which compared with the ARA of a crumb, is an increase of 21.4%. This is the ARA added value of the baking process on rye bread. In rye bread, baked for 75 minutes, the antiradical activity of the down-crust is higher than that of the crumb by 48.5%. Comparing slices of the loaf, it is apparent that the ARA value of the down-crust is higher than that of the top-crust. This should be due to a higher temperature impact during baking. The surface of the top-crust is in contact with the oven air, and here the impact of convection between the air and the surface is less than on the down-crust which is in contact with the hot oven clay-floor. Linear dependence between bread crumb, top-crust, down crust, and antiradical activity (r=0.6) was calculated.

In Latvia it is necessary to improve the methods for antiradical activity determination. The ARA is influenced not only by processing (baking time) but also by materials, dough preparation methods, and additives. Currently there is no scientific literature or data available about antiradical activity of Latvian bread. Further investigations are necessary.

Conclusions

Rye flour, type 1740, has antiradical activity by scavenging 67 µmol·100 g^{-1} DM of the stable free radical DPPH, which is 13% more than the dough antiradical activity of the same flour. The antiradical activity of the crust is 49.6% more than the antiradical activity of the crumb, and baking time has no significant impact on this ratio. Baking time (45, 60, and 75 min) has no significant impact on changes in the antiradical activity of the rye bread crumb. The greater antiradical activity apparent in the down-crust of the rye bread loaf is accounted for by more intensive Maillard reactions during thermal processes.

Acknowledgements

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THE CONTENT OF CARBOHYDRATES AND ORGANIC ACIDS IN ORGANICALLY GROWN VEGETABLES AND DRIED VEGETABLE PRODUCTS

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Abstract. The content of carbohydrates (fructose, glucose, and sucrose) and organic acids (citric acid, succinic acid, malic acid, sorbic acid, and ascorbic acid (vitamin C)) in organically grown vegetables (carrots, parsnips, celeriacs, and red beets) and dried vegetable products were analyzed by the HPLC reverse phase and ion exchange methods.
The main carbohydrate detected in fresh vegetables invariably was sucrose (1.29 – 11.17 g 100 g^{-1}). Fructose and glucose were found in similar ranges (0.09 – 2.47 g 100 g^{-1}, 0.14 – 3.02 g 100 g^{-1} respectively). A similar distribution of carbohydrates was determined in dried products. The highest fructose and glucose contents were found in fresh carrots - 2.47 g 100 g^{-1} and 3.02 g 100 g^{-1} respectively; but in dried carrot products – 14.53 g 100 g^{-1} and 15.43 g 100 g^{-1}. High amounts of sucrose were found in red beets: 11.17 g 100 g^{-1} in fresh samples, and 48.50 g 100 g^{-1} in dried products.
Malic and citric acids were detected as the main acids of fresh vegetables. Highest citric acid amount was in red beets (266.19 mg 100 g^{-1}), whereas celeriacs and parsnips had a high malic acid content.
The results from this study demonstrated variability and concentration range of carbohydrates and organic acids, wherewith the organoleptic profile of the products. Dried products are considered as a good source of energy.
Key words: acidity, sugar content, moisture, vegetables, high performance liquid chromatography.

Introduction

The determination of organic acids and sugars in foods is very important – their presence and relative ratio, in fact, can affect the chemical and sensorial characteristics of the matrix (e.g., pH, total acidity, microbial stability, sweetness), can provide precious information on food wholesomeness, as well as possibilities of optimizing some selected technological processes (Chinnici et al., 2004).

During the last few years, not only in Latvia, but also all over the world the society's interest about organic farming and the demand for organically grown products have increased. There is a number of scientific data demonstrating that organic vegetables and fruits contain more compounds with antioxidant properties than the produce from conventional farming (Bourn and Prescott, 2002; Hecke et al., 2006; Kazimierczak et al., 2008). Although a number of studies has been carried out, the information on composition of vegetables, coming from organic farming, is not sufficient.

It is known that the process of dehydration is related with losses of different vitamins and biologically active compounds; most vitamins such as A, C, B_1 are heat sensitive, some (e.g., A and C) are also sensitive to oxidative degradation (Sablani, 2006).

Various methods have been published for the determination of sugars, organic acids, and vitamin C; however, high performance liquid chromatography (HPLC) methods are mainly used (Romero Rodriguez et al., 1992; Vazquez Oderiz et al., 1994; Shui and Leong, 2002; Kafkas et al., 2006).

Therefore, the objective of this study was to analyze the content of carbohydrates and organic acids in organically grown vegetables and dried vegetable products by HPLC methods, and to compare the differences in vitamin C content in fresh and dried vegetables.

Materials and Methods

For the study, carrots (*Daucus carota L.*) 'Nantes 4', parsnips (*Pastinaca eusativa L.*), celeriacs (*Apium graveolens L. var. rapaceum*) 'Edward', and red beets (*Beta vulgaris L. var. cruenta Alef.*) 'Bordo' were chosen and were gathered in the certified organic farm 'Lušēni', rural municipal Nītaure, Cēsu district in autumn 2009. The samples were grinded in chips (1 – 2 cm) and stored at < -18 ºC temperature until analyses. The storage period was not longer than 1 month.

The dried products of the vegetables were prepared in the organic farm 'Lušēni': grinded in chips (1 – 5 cm), dehydrated in a convection air dryer at 40 ºC till fixed moisture, stored at ambient temperature, and packed in low – density polyethylene (LDPE) bags. Temperature selection of the dehydration regime (40 ºC) was set by application of technical resources used in the organic farm 'Lušēni'.

Equipment

The principle for determination of the moisture content: drying of a test portion at the temperature 103 ± 2 ºC to constant mass, and weighing to determine the loss mass. Equipment used: thermostat Nabertherm Program Controller S27 (Nabertherm, Germany).

A high performance liquid chromatography (HPLC) reverse phase separation method with refractive index detector (RID) for determination of carbohydrates, and an ion exchange method with ultraviolet visible (UV/VIS) spectrophotometric detector for determination of organic acids and vitamin C were used.

The equipment used included Shimadzu HPLC Prominence chromatograph that consisted of the pump LC-20AD, degasser DGU-20A5, auto sampler SIL-20A, column oven CTO-20A, UV/VIS detector SPD-20A, RID RID-10A, communications bus module CBM-20A, interfaced to a personal computer running

Shimadzu LabSolutions, LCsolution Version 1.21 SP1 software.

Separation of carbohydrates was carried out on Supelcosil™ 250 4.6 mm LC-NH2 column (particle size – 5 µm), of organic acids and vitamin C – on Ostion 250 8 mm LG-KS H^+ column (particle size – 10 µm).

Chemicals

Demi-water (18 MΩ cm^{-1}) was prepared by using deionization apparatus UHQ-II-MK3 Elga (USF Elga, UK), D(-)-fructose (≥ 99.0%, Merck), D(+)-glucose (Merck), sucrose (Merck), citric acid (p.a., Lach-Ner), succinic acid (≥ 99.5%, Sigma-Aldrich), DL-malic acid (≥ 99.0%, Sigma-Aldrich), L(+)-ascorbic acid (vitamin C) (p.a., Reag.ACS, Reag.ISO, Reag. Ph.Eur., Sigma-Aldrich), sorbic acid (≥ 99%, Sigma-Aldrich), zinc acetate dihydrate (≥ 99%, puriss, Sigma-Aldrich), potassium hexacyanoferrate (II) trihydrate (p.a., ACS, ISO, Scharlau), acetic acid (99.5%, p.a., Standard), acetonitrile (HPLC grade, min. 99.8%, Sigma-Aldrich), sulphuric acid (95%, p.a., Chempur), phosphoric acid (85%, Polskie odezynniki chemiczne S.A.).

Determination of carbohydrates and chromatographic conditions

The in-house method ML-M-6 'Determination of sucrose, fructose, glucose, maltose and lactose by HPLC' of the State Revenue Service National Customs Board Customs Laboratory for the analysis of carbohydrates was used.

Working conditions: the mobile phase – 80% aqueous acetonitrile. The flow rate was 1 ml min^{-1}, column temperature – 30 ºC, RID cell temperature – 40 ºC, and injection volume 10 µl. Calibration curve was acquired after two repeated HPLC runs of 7 standard solutions containing each compound.

Test portions of 10 g of homogenized fresh vegetables and 1 g of homogenized dried vegetables of up to 0.1 mg were weighted in 50 ml volumetric flasks, and circa 20 ml of demi-water was added. Then the portion were heated in water bath at 60 ºC for 20 minutes, cooled to ambient temperature, clarified with 1 ml of Carrez I and Carrez II, homogenized and diluted to 50 ml. Solutions were filtered through fluted filter (DP 503 125, Albet) and membrane filter (0.20 µm, Sartorius) before the injection into the HPLC system.

Quantifications were performed in triplicate and were based on peak area measurements.

Determination of organic acids and chromatographic conditions

The determination of organic acids and vitamin C was based on methods reported by Romero Rodriguez et al. (1992) and Vazques Oderiz et al. (1994) by adding some modifications.

Working conditions: the mobile phase – demi-water acidified to pH 2.2 with sulphuric acid. The flow rate was 0.4 ml min^{-1}, column temperature – 30 ºC, UV/VIS detection was at 215 nm for organic acids and at 245 nm for vitamin C, and injection volume – 10 µl. Calibration curve was acquired after two repeated HPLC runs of 5 standard solutions containing each compound.

To 10 g of homogenized fresh vegetables and 1 g of homogenized dried vegetables (up to 0.1 mg), 30 ml of 4.5% phosphoric acid were added, and then stirred with magnetic stirrer (IKA Labortechnik, Germany) for 15 minutes. The solutions were filtered (DP 503 125, Albet) in 50 ml volumetric flasks and filled up with phosphoric acid solution. An aliquot of the acid extract was then filtered through membrane filter (0.20 µm, Sartorius) prior to injection into the chromatographic system.

Quantifications were performed in triplicate and were based on peak area measurements.

The mean values and standard deviations were calculated by Microsoft Excel. If the precision had two components (standard deviations), the total standard deviation was calculated as a square root of sum of the standard deviations squares.

Results and Discussion

The highest moisture content was found in carrots, the lowest – in parsnips, but in red beets and celeriacs the moisture was in similar ranges. Our results were close to the information found by Souci et al. (2008), where the moisture content in fresh carrots was within the range of 87.5 – 92.1 g 100 g^{-1}, but in dried carrots – 4.0 – 14.6 g 100 g^{-1}. In Souci et al. (2008) investigations, the moisture content for other vegetables was determined only for fresh products: parsnips (76.0 – 78.7 g 100 g^{-1}) and, red beets (82.9 – 91.7 g 100 g^{-1}). In our research, the moisture content in fresh celeriacs was lower (87.3 – 90.5 g 100 g^{-1}). The moisture content results are presented in Table 1.

Table 1

Moisture content of fresh and dried vegetables

No.	Type of vegetables		Moisture content, g 100 g^{-1}
1.	Carrots	fresh	88.80±0.48*
		dried	14.18±0.48
2.	Celeriacs	fresh	84.43±0.49
		dried	10.03±0.11
3.	Parsnips	fresh	78.66±0.48
		dried	9.02±0.39
4.	Red beets	fresh	82.95±0.03
		dried	10.53±0.16

* – values were expressed as mean ± standard deviation

Sucrose was the main carbohydrate in organic grown vegetables – carrots, celeriacs, parsnips, and red beets (1.29 – 11.17 g 100 g^{-1}). Fructose and glucose were found in similar ranges (0.09 – 2.47 g 100 g^{-1} and 0.14 – 3.02 g 100 g^{-1} respectively). The highest fructose and glucose contents were detected in fresh carrots – 2.47 g 100 g^{-1} and 3.02 g 100 g^{-1} respectively, but

Table 2

The content of carbohydrates (g 100 g^{-1}) in fresh and dried vegetables

No.	Type of vegetables		Fructose	Glucose	Sucrose
1.	Carrots	fresh	2.47±0.12*	3.02±0.13	1.29±0.09
		dried	14.53±0.70	15.43±1.16	18.37±0.41
2.	Celeriacs	fresh	0.18±0.05	1.54±0.04	5.37±0.29
		dried	1.20±0.12	12.51±0.11	18.84±0.80
3.	Parsnips	fresh	0.79±0.04	0.89±0.04	8.53±0.20
		dried	6.71±0.39	6.98±0.51	28.58±0.98
4.	Red beets	fresh	0.09±0.01	0.14±0.02	11.17±0.29
		dried	0.37±0.02	0.99±0.12	48.50±0.30

* – values were expressed as mean ± standard deviation

in dried samples – 14.53 g 100 g^{-1} and 15.43 g 100 g^{-1} respectively. In red beets, the content of fructose and glucose was low, but sucrose content was the highest – 11.17 g 100 g^{-1} in fresh samples, and 48.50 g 100 g^{-1} in dried samples.

Comparing with the data by Souci et al. (2008) on carbohydrates content in fresh vegetables (fructose – 0.84 – 1.96 g 100 g^{-1}; glucose – 0.84 – 1.71 g 100 g^{-1}; sucrose – 1.55 – 4.17 g 100 g^{-1}), our research showed that the fructose and glucose content in carrots was higher, but sucrose content – lower. Fructose and glucose content in parsnips and red beets reported in literature were close to our values, whereas sucrose content was lower than our data (in parsnips – 2.49 – 2.81 g 100 g^{-1}, in red beets – 6.12 – 8.88 g 100 g^{-1}). The differences between the measured results and literature data could be explained by various factors – specific character of cultivars, weather and agronomic conditions. The carbohydrate content is presented in Table 2.

Comparing the results of carbohydrate content in fresh and dried vegetables calculated on the dry matter content, a slight common trend was found – during the dehydration, the content of fructose and glucose increased, but sucrose – decreased, except in carrots. This probably reflects to the enzymatic or chemical hydrolysis of sucrose to glucose and fructose, which occurs as a result of disruption of the cellular structure during drying. The carbohydrate content calculated on the dry matter content is given in Table 3.

The main acids in fresh vegetables invariably were malic and citric acids. Ascorbic acid (vitamin C) was found in the lowest concentrations. Citric acid in red beets was detected in the highest concentrations (266.19 mg 100 g^{-1}), whereas malic acid was the most abundant in celeriacs and parsnips.

In our research, acid content in fresh vegetables appeared mostly to be higher than that reported by Souci et al. (2008), for example, citric acid concentration in red beets – 266.19 mg 100 g^{-1} (according to Souci et al. (2008) – 195 mg 100 g^{-1}). Succinic acid – 23.51 mg 100 g^{-1} (according to Souci et al. (2008) – 10 mg 100 g^{-1}), whereas citric acid content in parsnips (59.86 mg 100 g^{-1}) was lower than in the Souci's et al. (2008) research (130 mg 100 g^{-1}). The results on the content of organic acids and vitamin C in fresh and dried vegetables are not included in this paper.

Comparing the results of the organic acid and vitamin C content in fresh and dried vegetables calculated on the dry matter content, a decrease in

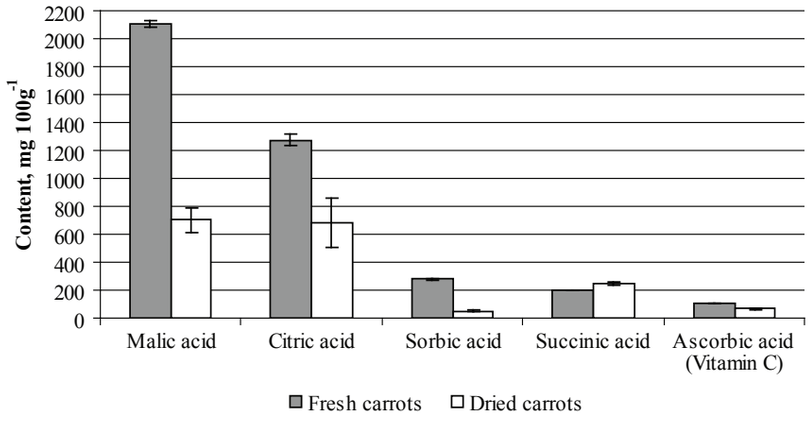
Table 3

The content of carbohydrates (g 100 g^{-1}) in fresh and dried vegetables calculated on the dry matter content

No.	Type of vegetables		Fructose	Glucose	Sucrose
1.	Carrots	fresh	22.05±0.49*	26.96±0.50	11.52±0.49
		dried	16.93±0.85	17.98±1.26	21.41±0.63
2.	Celeriacs	fresh	1.16±0.49	9.89±0.49	34.49±0.57
		dried	1.33±0.16	13.90±0.16	20.94±0.81
3.	Parsnips	fresh	3.70±0.48	4.17±0.48	39.97±0.52
		dried	7.38±0.55	7.67±0.64	31.41±1.05
4.	Red beets	fresh	0.53±0.03	0.82±0.04	65.51±0.29
		dried	0.41±0.16	1.11±0.20	54.21±0.34

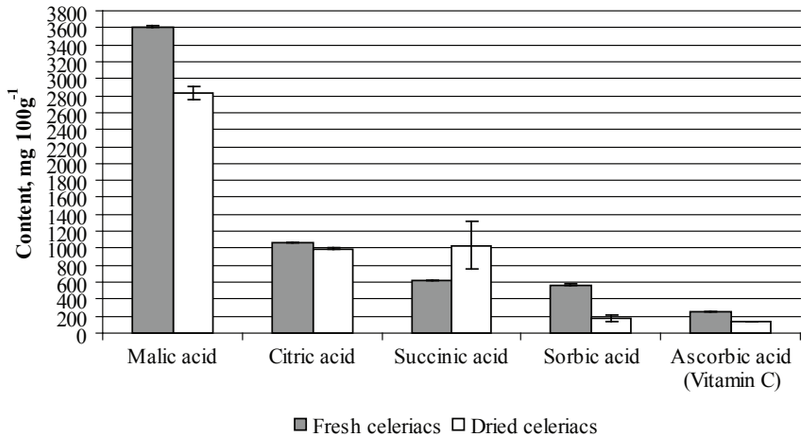
* – values were expressed as mean ± standard deviation

the content of organic acids and vitamin C during the dehydration with some exclusion was observed. But increase in succinic acid was detected in all types of vegetables. The content of organic acids and vitamin C in fresh and dried vegetables calculated on the dry matter content is presented in Figures 1 – 4.



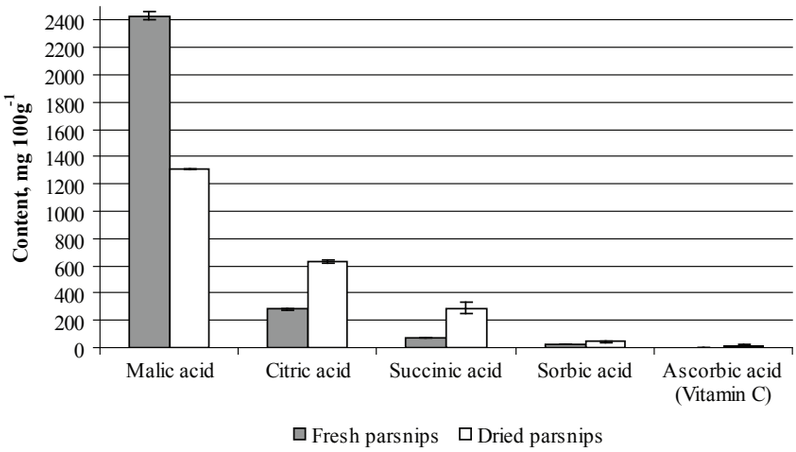
Values were expressed as mean ± standard deviation

Figure 1. The content of organic acids and vitamin C calculated on the dry matter content in carrots.



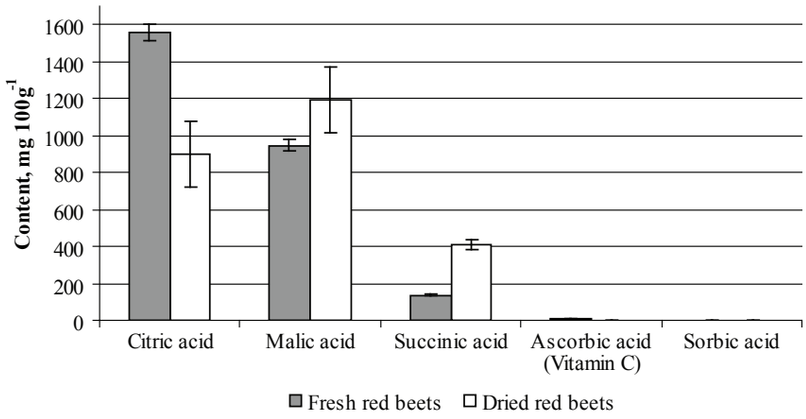
Values were expressed as mean ± standard deviation

Figure 2. The content of organic acids and vitamin C calculated on the dry matter content in celeriacs.



Values were expressed as mean ± standard deviation

Figure 3. The content of organic acids and vitamin C calculated on the dry matter content in parsnips.



Values were expressed as mean ± standard deviation

Figure 4. The content of organic acids and vitamin C calculated on the dry matter content in red beets.

Conclusions

1. This study yielded the information on contents of carbohydrates, organic acids, int. al., vitamin C in organically grown vegetables (carrots, parsnips, celeriacs and red beets) and dried vegetable products.
2. Among the detected carbohydrates (fructose, glucose, sucrose), sucrose was found to be the most abundant in fresh vegetables (1.29 – 11.17 g 100 g^{-1}).
3. In dried products, fructose and glucose content was higher, but sucrose content – lower.
4. Dried vegetables are rich in carbohydrates, therefore they are considered to be a good source of energy.
5. As for the acids, malic and citric acids dominated in fresh vegetables; a similar distribution of acids was observed also in dried products.
6. A decrease in organic acids and vitamin C, except succinic acid, during the dehydration was found. Vitamin C content in parsnips after dehydration is found to be higher.

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DYNAMICS OF SUGARS COMPOSITION IN BERRIES

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Abstract. The major sugars in six cultivars grown in Latvia are analysed. The content of glucose, fructose and sucrose in strawberry, blackcurrant and redcurrant berries was determined by applying the method of high performance liquid chromatography (HPLC). By using the obtained calibration curves, concentrations of diverse sugars were found in the analyzed samples. Fructose or fruit-sugar, the same as glucose, is mainly the source of energy in the human body though it is decomposed faster and easier than glucose. In all the researched berry cultivars, the amount of fructose was the largest, particularly in blackcurrants. Six strawberry, black- and redcurrant cultivars were evaluated as raw materials for industrial production. The investigation involved chemical and sensory analyses of fresh berries. The results indicate that pH and sugars of fresh berries influence their quality. Furthermore, high level of sugar adversely affects taste stability. Taste degradation in berries is parallel to flavour deterioration. It is concluded that the main emphasis should be given to the properties of fresh berries.
Key words: berries, glucose, fructose, sucrose, potential of hydrogen.

Introduction

In order to optimize humans' health and decrease emergence of diseases caused by food, new food-related strategies are necessary, which are connected with a link between nutrition and metabolism. American and British researchers consider that if fruits and vegetables prevail in the diet, possibility to fall ill with cardiovascular diseases is reduced for 40%. In Latvia's climatic conditions research will enable to use local fruit and berry production more extensively and comprehensively. Their use in nutrition will eliminate seasonal nature and imbalance. In order to improve continuous supply of Latvian inhabitants with locally produced good quality products of fruits and berries, it is necessary to carry out their complex study, including also study of chemical content (Petrova et al., 2005).

In botany, a berry is a simple fruit having seeds and pulp produced from a single ovary; the ovary can be inferior or superior. The garden strawberry is a common plant of the genus *Fragaria* which is cultivated worldwide for its fruit, the (common) strawberry (Moore and Ballington, 1990). The fruit is highly appreciated not only for its characteristic aroma but also for its bright red colour, and it is consumed in large quantities - either fresh, or in prepared foods such as preserves, fruit juice, pies, ice creams, milk shake, etc. Artificial strawberry aroma is also widely used in all sorts of industrialized food products (Skrede, 1982).

Blackcurrant and redcurrant berries are of raw material importance for human alimentary (Laugale, 2007). Aroma, color and ascorbic acid content are the most important quality parameters of these berries (Laugale and Bite, 2008). Together these fruits account for more than 90% of soft fruit production for the industry (Brennan, 1990). During the process of ripening enzymes in berries react with their substrates: starch is decomposed to glucose which partly isomerizes as fructose. From the viewpoint of physiological effect, the most important biologically active substances are carbohydrates, organic acids, vitamins, mineral substances, fibre, etc. Carbohydrates are a component of every live cell. They are the main component of plant dry matter (50-80%). In food carbohydrates are the source of energy.

Carbohydrates are the major source of fuel for the body and individual cells. They are composed of carbon, hydrogen and oxygen.

Physical state of food products often is compared with physical-chemical characteristics of pure water, although water quality of different solutions and products is quite variable and depends on the impact of dissolved substances to transition of water phases.

Those carbohydrates come from the plant-based foods that you eat. You can either use carbohydrates right away for your energy needs or your body can convert them into fat to use later. There are three types of carbohydrates: sugars, starches, fibre (Moore and Ballington, 1990).

The body begins the process of breaking carbohydrates down into their individual monosaccharides almost before we start to eat them.

High sugar fruits are still low in calories and very high in nutrition. They don't need to be avoided, unless you are in the initial stages of a low carbohydrate diet.

The major sugars are identified as glucose, fructose and sucrose. All three increased during ripening; sucrose was found to be in the greatest concentration throughout, with fructose the predominant reducing sugar. Acidity loss was shown by decreasing titratable acidity and increasing pH values (Viljakainen et al., 2002).

Under the influence of fructose, activity of enzymes necessary for metabolism of carbohydrates is increased. In the human body, fructose is able to change into glycogen without the involvement of hormone insulin contrary to glucose, for the use of which insulin is necessary. These properties of fructose are important in the case of some diets when fructose is more recommendable than glucose.

By carrying out comparative researches with different kinds of carbohydrates in nutrition it is ascertained that fructose more than other carbohydrates increases fat content in blood and liver.

Enlarged amount of sucrose in nutrition increases fat and cholesterol content in blood more than respective amount of glucose; from all types of carbohydrates, the compound carbohydrate – starch - has the least impact on fat content in blood. It is considered that unfavourable impact of sucrose on fat metabolism mainly depends on fructose, which sucrose molecule contains, as well as it is connected with relatively fast absorption of sucrose from small intestines.

The aim of the research is related to usage of fresh berries in further heat treatment.

Materials and Methods

Experiments were carried out at the laboratories of the Faculty of Food Technology by using berry cultivars available for consumption. The following cultivars were used in the research: strawberries 'Polka' and 'Honeoye', currants 'Red Lake' and 'Primus', blackcurrants 'Silmu' and 'Titania'. The content of glucose, fructose and sucrose in strawberry, blackcurrant and redcurrant berries was determined by applying the method of high performance liquid chromatography (HPLC). The method is based on the fact that the chromatographic separation of glucose, fructose and sucrose is based on their delayed time differences (Kūka, 2008), which enables to carry out quality and quantity analysis of the sample. Liquid chromatography is a method of separating and analyzing mixtures of substances in which the mobile phase is a liquid. By the method of liquid chromatography it is possible to separate fixed and thermally non-persistent substances. In the liquid chromatography, separation of substances takes place in a constant temperature which most often is close to room temperature. In the liquid chromatography, the mixture to be separated is forced through columns and the mobile phase is let in. By injecting the sample in the HPLC, with the mobile phase it flows to the column. In order to guarantee high efficiency, correct injection of the sample, its quantity and volume have essential importance. After the extraction of carbohydrates they are determined by using refraction detector which monitors composition of elution flown out of the column. The signal coming from the detector is electronic; therefore it can be processed by using electronic data processing systems. For determination of carbohydrates in berries, light refraction coefficient detector is used. It is all-purpose and can be used in cases when the substance does not absorb UV radiation, for example, in determination of carbohydrates. The pikes are identified according to the time of a particular carbohydrate flowing out from the chromatography column. Composition of carbohydrates is calculated by using standard method according to the pike square (Kūka, 2008).

Fundamentals of the standard method are construction of calibration curve by using sugar standard-solutions of diverse concentrations. By using the obtained calibration curves, concentrations of diverse sugars are found in the analyzed sample. Using potentialities promoted by Shimadzu LC-20 Prominence with light refraction coefficient detector of the RID-10A brand liquid chromatography software, analyses of the obtained results of the calibration curve and the analyzed sample is carried out automatically.

The research was made according to the standard method.

The reagents used: glucose, fructose, maltose, sucrose, acetonitrile and deionised water.

Preparation of the basic solution – weighed out 2±0.0002 g of fructose, glucose, maltose and sucrose in 10 ml volumetric flasks, filled with deionised water up to the mark and mixed thoroughly (concentration of the obtained mass was 0.2 g mL^{-1}).

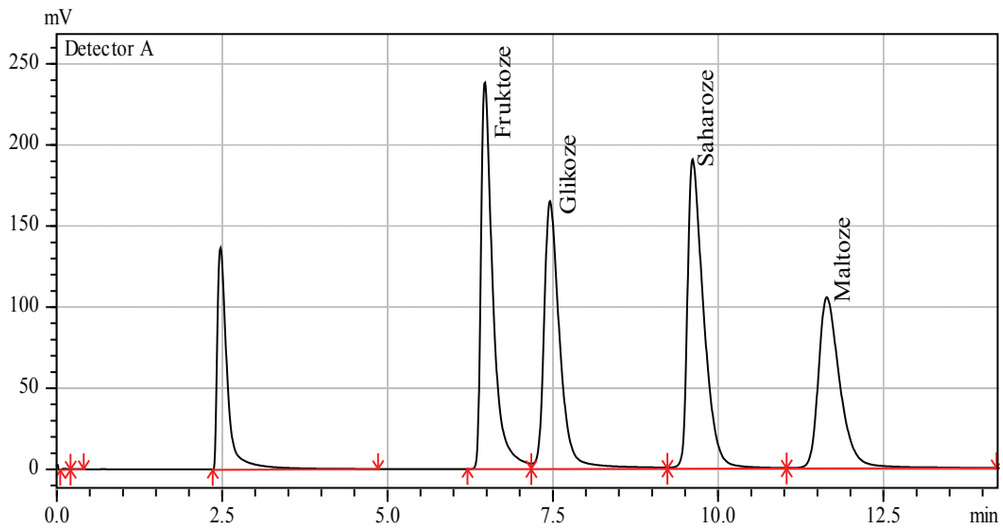

Figure 1. Standard-curve of carbohydrates for carrying out chromatography analysis.

Parameters of the method:

- column: amino column (- NH_2);
- isocratic regime;
- temperature of the column and detector: 25 °C;
- mobile phase: acetonitrile/water (80:20 V/V);
- capacity of the injection sample: 10 µl;
- total time of the analysis (glucose, fructose, sucrose, maltose): up to 30 minutes;
- rate of the flow: 1.3 mL min^{-1}.

Liquid chromatography of the carbohydrate calibration:

the chromatography data processing system fixes the composition of glucose, fructose, sucrose, maltose in berry by comparing the berry chromatography with the chromatography of sugar standard-solution mentioned above.

Composition of carbohydrates in the analyzed samples was calculated in the form of percentage expression. The following formula was used in calculations:

$$W\,\% = \frac{CxV}{m} \times 100, \qquad (1)$$

where

C – concentration read, g L^{-1};

V – capacity of the extraction solution (total), L;

m – weighed mass, g

Results and Discussion

Consumers try to choose as fresh and good looking products as possible and products that are familiar and known to be safe. The consumer has the right to obtain safe food. The strawberry, a fruit that features a fragrantly sweet flavour, is the most popular type of berry fruit in the world (Lefever et al., 2004). While there are more than 600 cultivars of strawberries that differ in flavour, size and texture, one can usually identify a strawberry by its red flesh that has yellow seeds piercing its surface, and the small, regal, green leafy cap and stem that adorn its crown (Petrova et al., 2005). In addition to strawberries that are cultivated, there are also varieties that grow wild. These are much smaller in size, but feature a more intense flavour (Deuel, 1996). The fruit of the blackcurrant (scientific name *Ribes nigrum* has an extraordinary high vitamin C content (302% of the Daily Value per 100 g), good level of potassium, phosphorus, iron and vitamin B5, as well as broad range of other essential nutrients (Kampuse et al., 2002). Other phytochemicals in the fruit (polyphenols/anthocyanins) have been demonstrated in laboratory experiments with potential to inhibit inflammation mechanisms suspected to be at the origin of heart disease, cancer, microbial infections or neurological disorders like Alzheimer's disease. Blackcurrant seed oil is also rich in many nutrients, especially in vitamin E and several unsaturated fatty acids including alpha-linolenic acid and gamma-linolenic acid. European raw blackcurrants content: carbohydrates – 15.4 g, sugar – 7.9 (Laugale, 2007). The researched cultivars were characterised by productivity and self-fruitfulness. Clusters were short; berries were very large and large, average levelled by size, did not fall; tearing of berries was dry; taste was good – sour-sweet. Redcurrants are for culinary use: juice, jellies and purees. Cultivars are selected for the clarity of juice, size of berry and productivity. The sector of soft fruits and cherries for processing faced a difficult 2004 year with low producer prices for several products (in particular blackcurrants, strawberries and sour cherries) in several Member States. Low prices affected also several products in 2005 (in particular strawberries and blackcurrants).

The largest dry matter part of blackcurrant and redcurrant chemical composition is made by carbohydrates, which are there in the form of sugars, and they make approximately equal proportions in these berries (Kampuse et al., 2002). Sugars are the most important energy and flavour substances of many berries and fruits. Energy value of berries and fruits depends on the quantity of sugars, but flavour – on the quantity and also on the composition of sugars (Laugale and Bite, 2008).

When analyzing berry samples we found out that the most significant carbohydrates are glucose and fructose.

Fructose is a simple sugar (monosaccharide) found in many foods. It is also one of the three most important blood sugars, the other two being glucose.

Glucose is a simple carbohydrate, or *sugar*. It is very important because cells in an organism use it to provide energy. Its chemical formula is $C_6H_{12}O_6$. This means it has 6 carbon atoms, 12 hydrogen atoms, and 6 oxygen atoms bonded together.

Glucose and fructose composition of berries depends on the ripeness stage of berries. If the berries are in full- ripeness, then glucose and fructose composition is higher. In unripe berries carbohydrates are mainly found in the form of starch, which by the increasing ripeness transform into glucose, fructose and sucrose.

The most significant carbohydrates of berries included in this experiment are shown in Fig. 2.

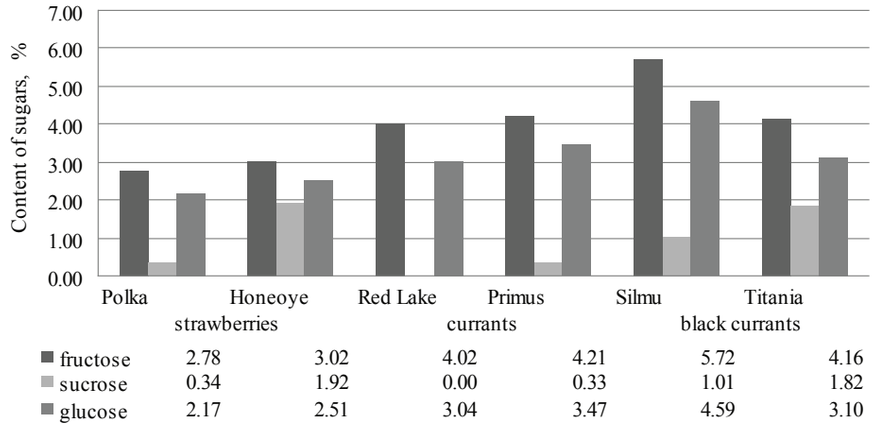

Figure 2. Composition of sugars in berries.

The results illustrated in figure 2 confirm that amount of carbohydrates in berries fluctuates from 0.00 to 5.72%. The results also show that the variety of berries influences the amount of sugars found in berries. It is particularly typical between the strawberry cultivars 'Polka' and 'Honeoye' and blackcurrants cultivars 'Silmu' and 'Titania'. The dynamics of sugars in berries shown in figure 2 indicates that the most important carbohydrates of berries are glucose and fructose. The amount of fructose in researched strawberry, blackcurrant and red currant cultivars was the highest. Fructose is the sweetest of the carbohydrates. It means that blackcurrants were the sweetest of all the researched berries. In its turn amount of sucrose was the lowest in all the researched cultivars of berries. By comparing kinds of berries we found out that in red currants the amount of sucrose was the lowest, and in currant cultivar 'Red Lake' there was no sucrose at all. It proves that the results of the research confirm to the data mentioned in the scientific literature (Brennan, 1990).

In strawberries, blackcurrants and redcurrants monosaccharides glucose and fructose were found more than sucrose and these monosaccharides in all these berries were approximately in equal proportions. Disaccharide sucrose was found in very little amounts. The least amount of monosaccharides was found in redcurrants, compared to blackcurrants. In some cultivars they are not found at all (Kampuse et al., 2002). According to different data of scientific literature, quantity of sugars in blackcurrants is different. According to the data of Western scientific literature, the content of sugars in fresh blackcurrants is 6.3%, but according to the data of Russian scientific literature – 7.3-12.0% (Laugale, 2007).

The medium of food products influences possibilities of different chemical reaction processes. Results of the research of pH of berries confirm the presence of acids. Potential of hydrogen (pH) is a scale of acidity from 0 to 14, which shows how acidic or alkaline a substance is. The lower the pH, the more acidic is the solution. The higher the pH, the more alkaline the solution. The prevailing acidity of fresh berries ranges from pH 3 to 5. Acidity decreases during ripening when organic acids are used in the synthesis of sugars (Viljakainen et al., 2002). The researched pH of strawberry, blackcurrant and redcurrant medium is shown in the figure 3.

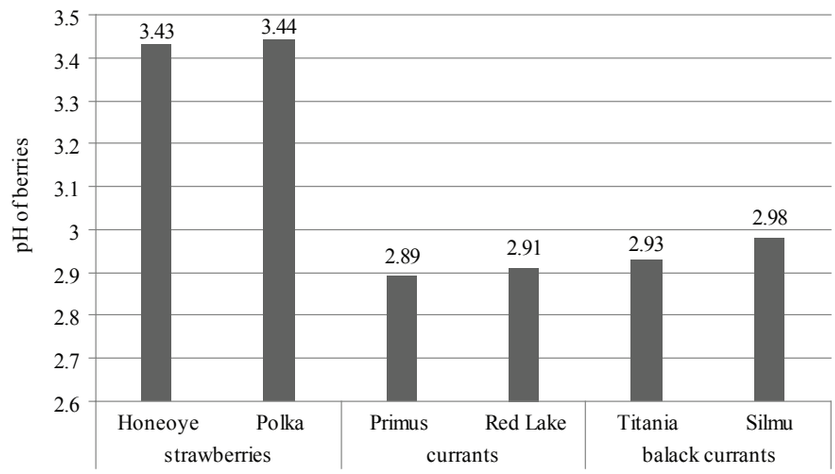

Figure 3. pH concentration in berries.

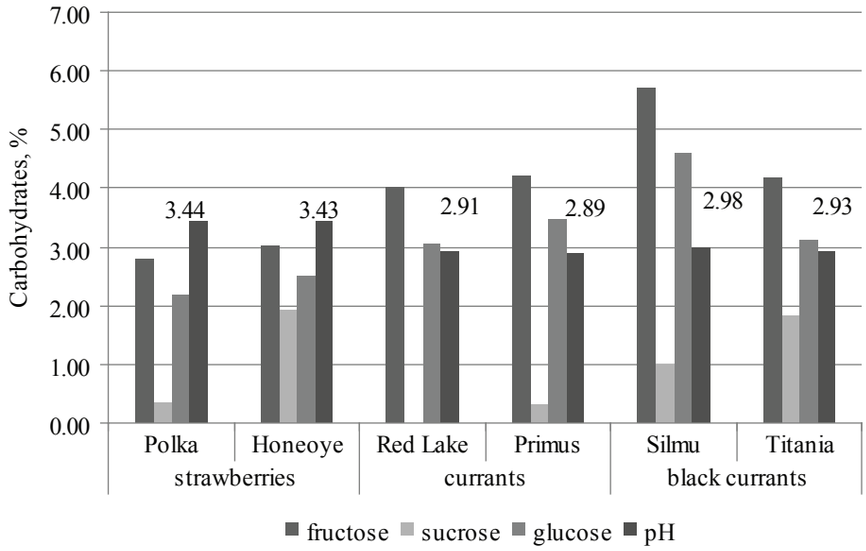

Figure 4. Comparison of pH and sugars in berries.

The results indicate that pH of analyzed berry samples ranges from 2.89 to 3.44. The increased acidity facilitates decomposition of carbohydrates present in berries. Taste of berries is influenced by pH, it states whether the berries are sour. pH is a value depending on temperature a lot, therefore with temperature changes also pH amount cahanges. If we compare types and cultivars of berries it can be concluded that there are no essential differences.

Increased acidity facilitates decomposition of carbohydrates in berries (glucose, sucrose and fructose). Taste of berries depends on pH. The characteristic sour taste of berries is formed by organic acids. The sour taste depends on the quality and quantity composition of organic acids - they in certain proportions, together with sugars and flavourings, determine sensory properties and quality of berries. Citric acid is found most in redcurrants and blackcurrants, which is confirmed also by diverse sources of scientific literature (Viljakainen et al., 2002). Besides, in blackcurrants are found also malic acid and a bit of oxalic acid. In redcurrants, malic acid is found in very negligible quantities and oxalic acid practically is not found at all. Historical information and facts about consumption of sweet products prove that people have always enjoyed sweet taste. Enjoying sweet taste is hereditary to people.

When analyzing pH concentration among types and cultivars of berries shown in figure 3, it can be concluded that pH found in strawberries is the highest. Taste of berries depends on pH. It indicates that there is more soury medium in researched cultivars ‘Polka’ and ‘Honey’ than in blackcurrants and redcurrants. Comparison of pH and sugars in berries is shown in figure 4.

The major sugars are identified as glucose, fructose and sucrose. All three increase during ripening; sucrose is found to be in the greatest concentration throughout, with fructose the predominant reducing sugar. Acidity loss is shown by decreasing titratable acidity and increasing pH values.

Berries are also analyzed for pH. Data are presented both on a concentration basis (mg/g of fresh weight) and on a per berry basis (mg/ berry).

The sugars and organic acids present in the pulp at various stages of ripeness are analysed by HPLC. Ripening is associated with a loss in firmness, peel chlorophyll and pulp acidity, with increasing soluble solids and total sugars.

Carbohydrates in foods that are digested and absorbed in the small intestine provide energy to body tissues mainly in the form of glucose and, to some extent, fructose. Fruits and berries are the principal sources of major dietary antioxidants, and there is now compelling evidence to support the assertion that beneficial effects result from consuming larger amounts of these foods.

Conclusions

1. The largest dry matter part of strawberry, currant and blackcurrant chemical composition is made by carbohydrates, which are found there in the form of sugars – fructose, glucose, and a bit of sucrose.
2. In all the researched berry cultivars the mount of fructose was the largest, particularly in blackcurrants, but the amount of sucrose was the least; in the redcurrant cultivar ‘Red Lake’ it was not found at all.
3. Taste of berries depends on quality and quantity composition of organic acids, they in certain proportions together with sugars and flavourings influence sensory properties and quality of berries.
4. When anallyzing pH concentration in different types and cultivars of berries, pH level found in strawberries was the highest.

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CHANGES IN PHYSICALLY-CHEMICAL AND MICROBIOLOGICAL PARAMETERS OF LATVIAN WILD CRANBERRIES DURING CONVECTIVE DRYING

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Abstract
The research focuses on the study of physically-chemical and microbiological parameter changes in Latvian wild cranberries during convective drying. The research was accomplished on fresh Latvian wild cranberries. The following quality parameters were controlled during the experiments: moisture content (oven-dry method), content of vitamin C (LVS EN 14130:2003), content of polyphenol (HPLC), preparation of samples for microbiological testing (LVS EN ISO 7218:2007), enumeration of yeasts and moulds (ISO 21527-2:2008(E)), counting of lactic acid bacteria (LVS ISO 13721:1995), and total plate count (LVS EN ISO 4833:2003A). The research detected the following optimal convective drying parameters of wild cranberries: temperature – +50±1 °C and drying time – 20.3 hours. The moisture content of dried berries equalled to 9.0±0.1%. The content of vitamin C decreased 1.9 times in wild cranberries dried at the temperature of +50±1 °C compared with the content of vitamin C in non-dried cranberries and was 13.05 mg 100 g^{-1} in dry matter. The results of current experiments show, that the content of such polyphenols as gallic, caffeic, and epicatechin acids decreased 9.70, 9.90, and 11.68 times, respectively, during the treatment at temperatures up to +50±1 °C compared with the initial content of these compounds in non-dried berries. The drying temperature substantially influences the microflora development in cranberries. It is possible to decrease significantly the content of LAB, mould, and fungi in berries provided the drying temperature of the wild cranberries does not exceed +50±1 °C, thus prolonging the ready product shelf-life.
Key words: cranberries, convective drying, vitamin C, polyphenols, mould, yeasts.

Introduction

Cranberries are a group of evergreen dwarf shrubs or trailing vines in the genus *Vaccinium* subgenus *Oxycoccos*, or, in some treatments, in the distinct genus *Oxycoccos*. Traditionally they are found in acidic bogs throughout the cooler parts of the world. A cousin of the blueberry, this very tart, bright red berry can still be found growing wild as a shrub, but when cultivated, is grown on low trailing vines in great sandy bogs. Even Latvian swamps are protected from drainage, so that species survival is guaranteed in the future. It is one of the most marketable berries in autumn, which competes with both autumn strawberries, and the artificially cultivated cranberries, or red-bilberries (Поплева, 2000).

Fresh cranberries, which contain high amount of beneficial nutrients, are at their peak from October through December, just in time to add their festive hue, tart tangy flavour and numerous health protective effects. Cranberry juice and dried or frozen cranberries might be consumed after the end of cranberries short fresh season (Cranberries, 2010). Cranberries contain triterpinoids, a range of different organic acids, sugars, basic alkaloids, and anthocyanin dyes. Moisture content in cranberries is approximately 88%; sugar content of cranberries ranges from 5.25 to 7.13 g 100 g^{-1}, where glucose comprises approximately 80% and fructose – 20%. The different organic acids found in cranberries include catechin, glucuornic acid, ascorbic acid, benzoic acid, malic acid, quinic acid, leptosine glycosides, and, of course, citric acid (Birulis, 2008; Bruvere, 2006; Ripa, 1992).

Cranberries also contain vitamin C (as evidenced by the presence of citric acid) and phytochemicals. The importance of vitamin C in the diet is well understood and scientifically proved. Vitamin C is also an important antioxidant. It is important to recall that the antioxidants β-carotene and vitamin E protect water soluble substances from oxidising agents; vitamin C protects water soluble substances the same way. Vitamin C is also involved in the metabolism of several amino acids (DeBruyne, 2008; Birulis, 2008; Bruvere, 2006). Vitamin C is stable, since the pH value of a product is about 4, yet this vitamin is unstable due to product processing / storage conditions: air oxygen, light, and temperature above +80 °C (Belitz, 2004; Henry and Chapman, 2002).

Many small fruit phenolic compounds are good sources of natural antioxidants and have inhibitory effects on mutagenesis and carcinogenesis. During the past decades, extensive analytical research has been carried out on the separation and determination of phenolic constituents in various fresh fruit products and environmental samples. The unique antibacterial activities of cranberry implicate that cranberry may possess a very different flavonoid and phenolic composition from other kinds of fruits. An efficient separation and quantitation method is essential for understanding the components of flavonoid and phenolic antioxidants in cranberry and their health benefits. Phenolic compounds are not temperature resistant; therefore treatment at elevated temperatures will negatively influence polyphenol compound activity (Chen et al., 2001).

Latvian climate is very suitable for growing cranberries, though several climatic conditions as, for example, temperature variations, rain, winds, elevated air humidity, and others also contribute to the

development processes of several diseases. Diseases may cause damage to shoots, leaves, flowers, roots, and berries, thus most of the harvest is subject to qualitative and quantitative factors. Rotting of berries sometimes develops during their growth, but mainly after get hearing the harvest collection (Ripa, 1996).

The microbial biota of land-grown berries may be expected to reflect the soils in which they are grown, although exceptions occur. The actinomycetes (gram-positive branching forms) are the most abundant bacteria in stable soils, yet they are rarely reported on berries. On the contrary, the lactic acid bacteria are rarely found in the soil, but they are significant part of the bacterial biota of plants and plant products. The overall exposure of plant products to the environment provides many opportunities for contamination by microorganisms. The protective cover of berries and the possession of some pH values below which many organisms cannot grow are important factors in the microbiology of the products (Jay et al., 2005).

The main microflora of berries will contain small amount of moulds, yeasts, and lactic acid bacteria (LAB). However, yeasts may be viewed as being unicellular fungi in contrast to the moulds, which are multi cellular; moulds are filamentous fungi that grow in the form of a tangled mass that spreads rapidly and may cover several inches in area; and the LAB is composed of 13 genera of gram-positive bacteria (Jay et al., 2005).

Through the past few years, there has been an increasing demand for high - quality dried berries. These dried fruits are widely used in the bakery industry for a variety of processed products including muffin mixes, breakfast cereals, yogurts, sauces, and snack bars (Beaudry, 2001).

Decrease of water activity and increase of treatment temperature during drying are no acceptable conditions for microorganisms growing and development; activity was retained only by spores.

Fruits and vegetables can be dried using hot air as the drying medium, which is often found as the simplest and most economical method. In hot air drying, four main factors can affect the rate and total time of drying: the physical properties of the food (particle size and geometry), the physical arrangement of the food with air (cross flow, through flow, tray load), the physical properties of the air (temperature, humidity, velocity), and the design characteristics of the drying equipment (Jayaraman et al., 1995).

The aim of the present research was developed after the analysis of the literature: to study changes in physically-chemical and microbiological parameters of Latvian wild cranberries during convective drying. The following tasks were advanced to achieve the set aim:

- to determine experimentally parameters for wild Latvian cranberry convective drying: temperature and drying time;
- to determine the changes in content of vitamin C and polyphenol compounds in cranberries during drying at different temperatures;
- to evaluate changes in microbiological parameters in cranberries during convective drying at different temperatures.

Materials and Methods

The research was accomplished on fresh Latvian wild cranberries harvested in Vidzeme region greenwood bogs in 2009.

During the experiments, berries were cut prior to drying and dried in a chamber with controlled hot air circulation at various temperatures: +30 – +90 °C with the interval of +10 °C. Berries were placed on a perforated sieve (diameter - 0.185 m), with the diameter of a hole – 0.002 m, the sieve area – 0.030 m^2, and load - 6.700 kg m^{-2}. Cranberries were dried till a constant moisture content of 9.0±0.1% for storage time extension.

During the experiments, the following quality parameters of cranberries were controlled using standard methods:

- moisture content was determined using an oven-dry method, where 3.00±0.01 g of berries were cut prior to drying in an oven-drier "Precisa XM105E" till a constant weight (with sensibility ±0.01g) at the temperature of +105±1 °C (Temminghoff and Houba, 2004);
- vitamin C content was determined according to the standard method LVS EN 14130:2003 "Foodstuffs – Determination of Vitamin C by HPLC";
- phenolic compounds were determined using a high-performance liquid chromatography (HPLC) with UV detection (at 280 nm) (Berregi et al., 2003);
- preparation of samples for microbiological testing was conducted in accordance with the standard method LVS EN ISO 7218:2007;
- enumeration of yeasts and moulds in cranberries was analysed according to the standard method ISO 21527-2:2008(E);
- lactic acid bacteria counting was accomplished according to the standard method LVS ISO 13721:1995; and
- total plate count was analysed according to the standard method LVS EN ISO 4833:2003A;

Microsoft Excel software was used for the research purpose to calculate mean values and standard deviations of the mathematical data used in the research. The research includes five reiterations.

Results and Discussion

The moisture content is the main parameter of food products which influences the storage time. It is known that the shelf life of products with high moisture content is shorter than that of products with lower moisture content. It was determined that the initial moisture content of non-dried wild cranberries is 82.03±0.10%. Therefore it is possible to forecast shorter shelf life for such berries. Thereby cranberries were dried in several temperature conditions ranging from +30±1 °C to +90±1 °C till reaching the moisture

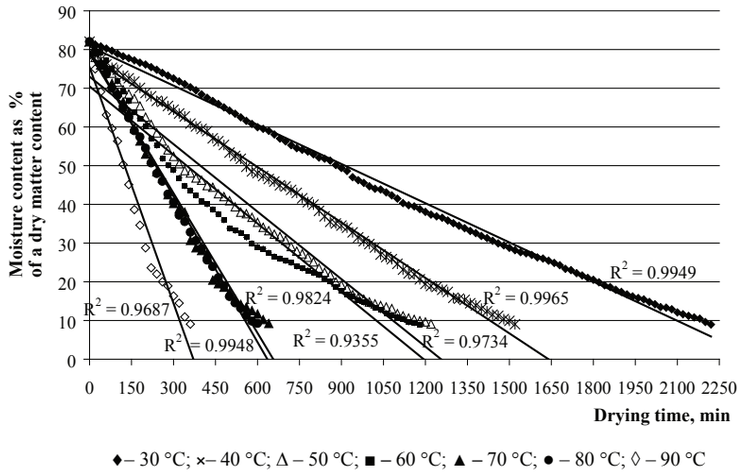

Figure 1. Decrease in the moisture content on a dry matter basis of cranberries in the drying process.

content of 9.00±0.10%. The results of the experiments show that the extended drying time is 37 h at the drying temperature of +30±1 °C and, the short drying time is 6 h and 20 min at the temperature of +90 °C. The chemical composition of cranberries, especially their acidity, mainly influences the drying time. A linear interconnection (Figure 1) was found between the moisture content applying to a dry matter, temperature and the drying time. It means that there exists a correlation between the moisture changes, temperature, and drying time. During the experiments it was found that after drying berries for 4h at +30±1 °C a decrease of moisture content was only by one times lower yonder if drying were occurred at +90±1 °C – the moisture content decrease by four times. It could be explained with the rapid moisture evaporation during processing at the elevated temperatures. After a 9-h-drying, the moisture content of cranberries processed at the temperature of +30±1 °C decreased 1.2 times, while in cranberries dried at the temperature of +90±1 °C the moisture content was 9.00±0.10%. The drying time of cranberries at the temperatures of +80±1 °C and +70±1 °C was found to be very similar (Figure 1), i.e., 10 h, and 10 h and 40 min respectively.

Vitamin C is the least stable of all vitamins and is easily destroyed during processing and storage. The most harmful factors to vitamin C content are the availability of oxygen, prolonged heating in the presence of oxygen, and exposure to light (Cruz et al., 2007). The initial content of vitamin C in wild non-dried cranberries was found to be to 13.05±0.20 mg 100 g^{-1} of fresh weight. Vitamin C is one of most significant compounds in berries; therefore the main task of drying is to maximally keep its quantity. During the experiments it was ascertained that the amount of vitamin C had decreased approximately 12.0 times during processing at the temperature of +90±1 °C (Figure 2). Such results are not acceptable for the development of a product with an elevated nutritive value; therefore other conditions should be search for. The content of vitamin C decreased 1.2 times during processing at the temperature of +30±1 °C, 1.5 times – at +40±1 °C, 1.9 times – at +50±1 °C, 3.0 times – at +60±1 °C, 5.1 times – at +70±1 °C, and 11.0 times – at the temperature of +80±1 °C. Vitamin C was destroyed mainly because berries were processed at elevated temperatures during convective drying and in presence of oxygen. A linear interconnection (Figure 2) was found between the decrease in vitamin C content and the drying time, which means that there exists a correlation between the changes in vitamin C content and the drying time. As the optimal drying parameters of cranberries the temperature not higher than +50±1 °C (the drying time 20.3h) will be acceptable, because if processing temperature increase more than +50±1 °C, the decrease of Vitamin C content is relevant.

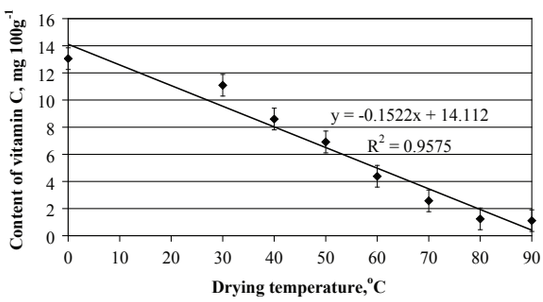

Figure 2. Decrease in the vitamin C content (in dry matter) in cranberries in the convective drying process.

Berries include notable sources of polyphenols. Industrial food processing affects the polyphenol content too (Macheix and Fleuriet, 1998). Our research demonstrated that the content of gallic and caffeic acids in non-dried wild cranberries was higher than the content of catechin and epicatechin acids (Figure 3).

The amount of catechin acid in wild non-dried cranberries was 10.0 times lower the amount of caffeic acid, which could be explained by the individuality of

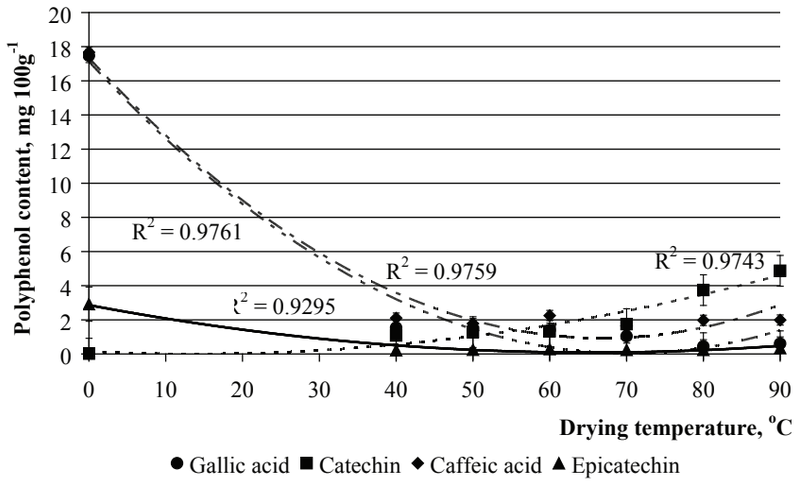

Figure 3. Changes in the polyphenol compounds (in dry matter) in berries during the convective drying.

the cranberries chemical composition. Mathematical data processing showed that there was no linear interconnection between the processing temperature and the changes in phenolic compounds. Therefore polynomial interconnection provides a possibility of foreseeing changes in the pholyphenol content in cranberries during the convective drying. During the experiments it was proved that the processing temperature substantially influences the content of measured polyphenols in cranberries. The content of polyphenols decreased during processing at the temperatures of up to +50±1 °C: gallic, caffeic and epicatechin acids decreased 9.70, 9.90, and 11.68 times respectively compared with their initial content in non-dried berries. Hydroxylgroup oxidation in polyphenol compounds mainly explain the increase in the content of polyphenols during the convective drying of cranberries at the temperature above +70±1 °C (Figure 3). During processing at relatively low temperatures the energetic barrier of activisation energy was stable. Oxidation occurred with benzol ring in compounds, as a result di- and tri- phenols developed from monophenols. Of course, the main factor influencing such changes is pH value of cranberries.

The population of bacteria found on small fruits vary widely. The initial count of predominant microflora is about 10^5 cfu g^{-1}, although low numbers of moulds and yeasts are also present (Jonger, 2005). Appropriate care should start in the bog environment. Contamination may result, for example, from soil, rains, animal contact, etc.

In our research, total count of microorganisms was investigated in convective dried cranberries at several temperatures. The presence of microorganisms was not found if cranberries were dried at the temperature of +70 – 90±1 °C (Figure 4). Therefore vegetative cells of the microorganisms may be damaged during the convective drying.

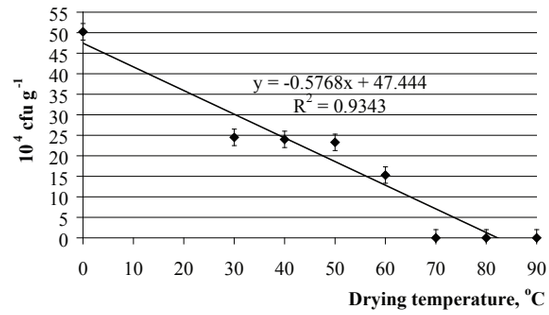

Figure 4. Decreases in the total count of microorganisms in cranberries during the convective drying.

Total count of microorganisms in non-dried wild cranberries was two times higher compared with the same parameters if cranberries were dried at the temperature of +30±1 °C, and three times higher if cranberries were dried at the temperature of +60±1 °C. Decrease in water activity and increase in processing temperature during drying are not acceptable conditions for the growth and development of microorganisms; activity was retained only by spores.

One of the most common species of the genus *Aspergillus* a *Aspergillus niger* fungi was found during the microscopy of non-dried wild cranberries tested microflora. The spores are widespread, and are often associated with organic materials and soil. Since soil is the natural habitat for *Aspergillus niger*, long-term survival in the environment is expected. Human exposures via dermal contact and ingestion routes and environmental exposures may occur at the discharge site because of the establishment of *Aspergillus niger* within the soil (Aspergillus…, 1997).

The lactic acid bacteria (LAB) gram-positive, acid-tolerant, generally non-sporulating, non-respiring rod or cocci associated by their common metabolic and

physiological characteristics were controlled in wild cranberries during drying. It was ascertained that the increase of drying temperature inactivated LAB in the tested berries. The content of LAB decreased two times in cranberries processed at the temperature of +30±1 °C compared with the LAB content in non-dried berries, since the elevated processing temperature negatively influences LAB activity. Whereas the content of LAB in berries processed at the temperature of +50±1 °C decreased 13 times. During convective drying, the LAB were not detected at the temperature above +60±1 °C.

Mucor mucedo were detected in non-processed cranberries and cranberries dried at the temperatures of +30 – 50±1 °C. The contamination is explained by the presence of these microorganisms in the air during cranberries growing and short-term storage. The research results show that it is possible to eliminate the content of these microorganisms during processing using elevated temperatures above +50±1 °C.

It is known that both yeasts and moulds cause various degrees of deterioration and decomposition of foods. Moisture and warmth are the most preferable conditions for the development of mould and yeasts. Though, it is possible to decrease the presence of such microorganisms in processed food due to the decrease in the moisture content in food and the increase in the treatment temperature. During our research it was determined that wild cranberries convectively dried at the temperature of +80 – +90±1 °C were not contaminated with the mentioned microflora, as yeasts and moulds are not heat resistant. The content of yeasts and moulds decreased 7 times in cranberries dried at the temperature of +70±1 °C compared with non-dried berries.

Yeasts and *Penicillium italicum* were detected in the microflora of wild cranberries. *Penicillium italicum* is one of the most common causes of fungal spoilage in berries from moss and substratum. The development of such microorganisms could be prevented applying the control of the storage conditions of cranberries.

As the results of experiments show the changes of Vitamin C content are not relevant if drying temperature of cranberry does not exceed +30±1 °C. The content of vitamin C decreased 1.2 times compared with its initial content. Under such conditions the drying time is very long – 39.0 h, which is not economically advantageous. At the drying temperature of +50±1 °C, the changes in vitamin C are not very significant since its content decreases 1.9 times and the drying time is shorter – 20.3 h. Therefore the optimal drying temperature for cranberries is +50±1 °C, excluding consideration of the dynamics of microorganisms. The total count of microorganisms in cranberries dried at the temperatures of +30±1 °C and +50±1 °C was approximately two times lower compared with the initial count of microorganisms in non-dried berries. It is possible to decrease significantly the content of LAB, mould, and fungi in berries if the drying temperature of the wild cranberries does not exceed +50±1 °C, thus prolonging the ready product shelf-life.

Conclusions

1. Optimal convective drying parameters of wild cranberries in a drier with controlled hot air circulation are the following: temperature – +50±1 °C, and drying time – 20.3 hours. The moisture content of dried berries makes 9.0±0.1%.
2. The study ascertained that vitamin C is labile to light, extremes in temperature, and oxygen. The vitamin C content decreased 1.9 times in wild cranberries dried at the temperature of +50±1 °C, compared with its content in non-dried cranberries – 13.05 mg $100g^{-1}$ in dry matter.
3. The study shows changes in the polyphenol content in wild cranberries due to the influence of hot air drying at different temperatures. Even the content of polyphenols decreases during the processing at temperatures of up to +50±1 °C: gallic, caffeic, and epicatechin acids decrease 9.70, 9.90, and 11.68 times respectively compared with the initial content of these compounds in non-dried berries.
4. The drying temperatures substantially influence microflora development in cranberries. It is possible to decrease significantly the content of LAB, mould and fungi in berries if the drying temperature is of wild cranberries +50±1 °C, thus prolonging the ready product shelf-life.

Acknowledgements

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SENSORY AND QUALITATIVE INDICES (HARDNESS AND COLOUR) EVALUATION OF CAKES WITH JERUSALEM ARTICHOKE (*HELIANTHUS TUBEROSUS L.*) POWDER

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Abstract. Jerusalem artichoke powder (JAP) from Jerusalem artichoke (*Helianthus tuberous* L.) roots is a nutritive valuable product that can be used for a healthy product development. The influence of JAP on the quality and degree of liking of cakes was investigated. Sensory evaluation of cakes was realized in two stages: the first - to define the quality attributes of the cakes with JAP from the point of view of the experts, the second – to find out the consumers' views on the cakes, whose quality has been improved in accordance with the recommendations of the experts. As a control sample, the classic home cake was evaluated. Control sample was compared with the cakes, where the amount of wheat flour prescribed by recipe was partly substituted with JAP at different concentrations. Experts evaluated cakes and defined the highest acceptable concentrations of JAP in cakes. For the consumers it was offered to assess the cakes with JAP at concentration 30% and with different taste and aroma enhancers. Cakes with 30% of JAP additive were the most favoured by the sensory properties: aroma, texture, softness elasticity, porosity and softness, surface crust, appearance, colour, shape, and size. Results of analysis of variance showed that there no significant differences in the degree of liking between the cakes with JAP and the cakes with taste and aroma enhancer additives ($p > 0.05$). There had been performed hardness analysis and colour measurement for cakes with JAP. The observed results declare high influence of aroma and taste enhancer on cakes structure and colour.
Key words: cake, Jerusalem artichoke powder, sensory analysis.

Introduction

Jerusalem artichoke powder (JAP) made from Jerusalem artichoke roots is a valuable product, rich in inulin, as well as vitamins and minerals. Using 3–11 grams of inulin per day may improve intestinal activity, as inulin stimulates '*Bifidobacterium*' development, improves fat exchange, reduces blood cholesterol and improves calcium absorption, thereby decreasing risk of osteoporosis (Bekers et al., 2004).

To influence human health positively, JAP can be added to favourite and may be not so healthy foods, including pastry products, which consumption in Latvia is increasing every year and in 2008 was 6.6 kg per household member as shown by the Central Statistical Bureau data (Consumption of food products average per household member per year, 2010).

As one of the most popular pastries are cakes that can be enriched with JAP, thus producing a healthy product. JAP increases the nutritional value of cakes substantially, reducing 'empty' calorie ingesting and giving also benefit for health.

Enrichment of daily products, including cakes has become popular. Earlier studies showed that it is possible to replace typical component of cake by a valuable product, for example, wheat flour can partly be substituted by almond flour (Jia et al., 2008), rice flour (Sae – Eaw et al., 2007) or barley flour, etc.; butter can be substituted by gum or olive oil, etc.; full cream milk - by skimmed milk; sugar - by sucrose syrup or encapsulated aspartame, or extract of stevia (Abdel-Salam et al., 2009), etc. The cake enrichment has a specified purpose, for example, fat reducing, sweets decrease (Jia et al., 2008; Wetzel et al., 1997), calorie quantity decrease (Abdel-Salam et al., 2009) etc.

Sometimes a new ingredient improves the quality of the cake – for example, unique aroma or taste, appetizing colour, excellent texture and softness, enlarged volume. But the most common reason for enrichment of cakes is nutritional improvement with vitamins, minerals, dietary fiber, valuable bacteria, and other health-enhancing substances. For example, cakes with dietary fiber as lupin, oat fiber, soy polysaccharides, etc. provide users with high amount of dietary fiber, vitamins A, B_1, B_2, B_6, B_{12}, E, D_3, nicotinamide, and folic acid, etc. (Wittig de Penna et al., 2003). Sometimes, for enrichment of cakes specific components are used to make cakes useful for people with health problems – overweight, allergy, diabetics, etc. The diabetics should use low - calorie functional yogurt cakes, because the obtained results showed a decrease in urea and aspartateaminotransaminase values (Abdel-Salam et al., 2009). For example, cakes with rice flour are suitable for allergic people, as rice flour has non - allergenic properties, is free of sodium, cholesterol, and gluten (Sae – Eaw et al., 2007).

The above-mentioned beneficial effects on health may also be observed with Jerusalem artichoke. The full potential of Jerusalem artichoke has not been used, so it has perspectives in future.

Consumers should eat the cakes with JAP in the highest acceptable concentration so to gain the highest benefit for health. Addition of JAP in cakes is not, of course, unlimited; it is limited by the technological factors (Bicāne, 2007) and the sensory requirements to cakes (Linden, 1995).

The aim of current research was to investigate the influence of JAP on the quality and degree of liking of cakes.

Materials and Methods

Experiments have been carried out in the Laboratory of Food Sensory Evaluation, Laboratory of

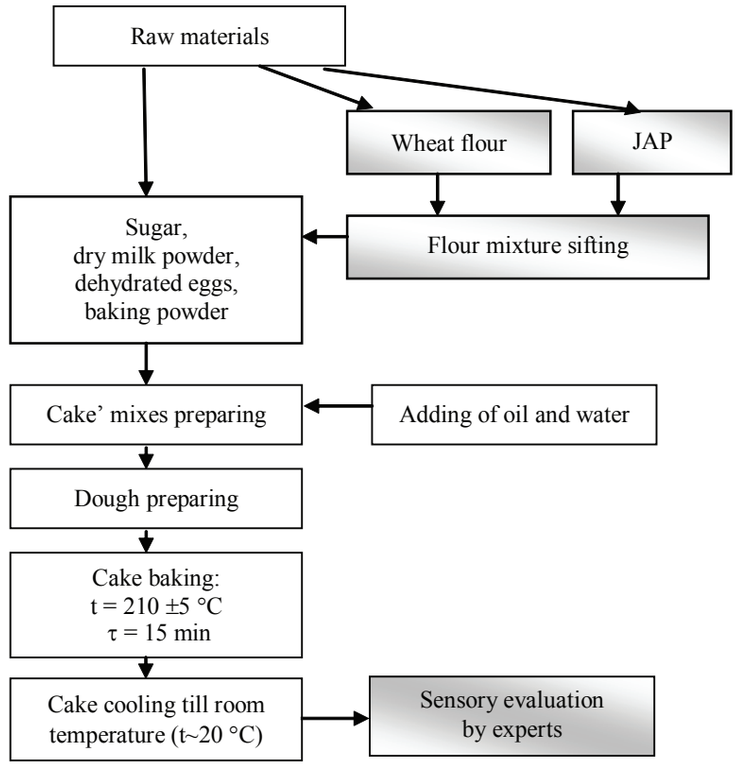

Figure 1. Technological scheme of preparing cakes for sensory evaluation by experts.

Food Analysis, and Research Laboratory of Packaging Material Properties at the Department of Food Technology of the Latvia University of Agriculture.

Sensory evaluation of cakes was realised in two stages: the first - the quality attribute of the cakes with JAP was defined from the point of view of the experts; the second, to find out consumers opinions about the cakes with JAP and aroma enhancers. Texture analysis and colour measurement was performed for improved cakes. Figure 1 shows technological scheme of preparing cakes for sensory evaluation by experts, but Figure 2 - technological scheme of preparing cakes for sensory evaluation by consumers.

Cake preparation.

For cake production the following materials were used: JAP, wheat flour - type 405C, sugar, dry milk powder, dehydrated eggs, drinking water, vegetable oil, and baking powder.

In the first stage of the experiment the following materials were used: the classic home cake mixture served as a control sample, but in other samples the classic cake ingredient – wheat flour –was partly replaced by JAP at concentrations of 10, 20, 30, 40, and 50% (Figure 1).

In the second stage of the experiment, the following ingredients were used: JAP, wheat flour - type 405C, sugar, dry milk powder, dehydrated eggs, drinking water, vegetable oil, baking powder, and a variety of flavours – vanilla powder, banana pure concentrate, mocha concentrate, cocoa powder, lemon and rum essences (Figure 2).

The experimental cakes were baked in an electric oven at the temperature of 210±5 °C for 15 minutes.

After cooling till room temperature (t~20 °C), the samples were used for analysis.

Colour measurement.

The colour of cakes with JAP supplemented with varied aroma enhancers was determined by measuring with the Tristimulus colorimeter *Color Tec PCM/PSM* (Accuracy Microsensors, Inc). The CIE Lab colour value included L*a*b* system, where L* determined lightness - darkness, a* – redness - greenness, b* – yellowness – blueness. Colours were measured in crumb of cake in the middle of slices. It was carried out at least ten readings in three replicates for each cake type.

Evaluation of hardness.

Hardness of crumb for cake with JAP and varied aroma enhancers was determined using a texture analyzer TA.XT.plus (Stable Micro Systems Ltd). The method is based on compression test (Pre-Test speed and Test speed were 1 mm sec^{-1}, Post-Test speed was 10 mm sec^{-1}), using 25 mm DIA CYLINDER ALUMINIUM (type P/25) measuring the hardness of the cakes. The TA.XT.plus texture analyzer was equipped with a load cell of 50 kg. The results were expressed as maximum force in Newtons (N). In these tests Trigger force was 0.04903 N. The outcome was determined from approximately ten readings of three replications for each cake type.

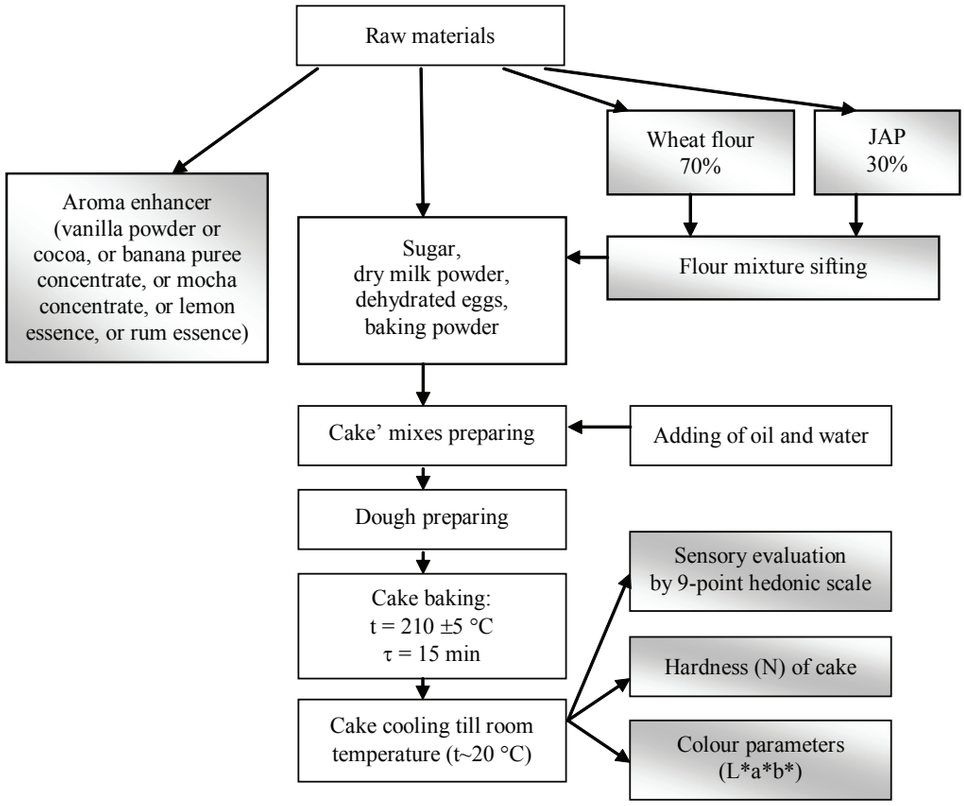

Figure 2. Technological scheme of preparing cakes for sensory evaluation by consumers.

Sensory evaluation - Quantitative Descriptive Analysis.

The sensory profiles of cakes with JAP were evaluated by experts (n = 5). Descriptors, description and references were constructed by a panel. The descriptors included appearance (colour, shape, and size), porosity and softness, surface of crust, texture, softness elasticity, crumbliness, aroma, and taste. The structured 5 - point scale (0 – insufficient quality, 5 – very good quality) was used in scoring the quality of each attribute (Strautniece, 2004). All experts were offered six coded samples: control - a classic home cake, and the rest of cakes with different concentrations (10%, 20%, 30%, 40%, and 50%) of JAP.

Sensory evaluation - Acceptability test.

The acceptability on taste and overall liking of the cakes with JAP and varied aroma enhancers was evaluated by 50 male (n = 15) and female (n = 35) panellists of the age between 21 and 67.

Each panellist was served with 6 samples (cakes with JAP at the concentration of 30% in variety of cake flavours) in a randomized serving sequence. The improved cakes were offered to consumers for sensory evaluation using a 9-point hedonic scale to determine consumer degree of liking of each kind of samples, i.e., 9 – extremely like, 5 – neither like nor dislike, and 1 – extremely dislike (Meilgaard et al., 1991; Strautniece, 2004).

Statistical analysis.

The results were processed by mathematical and statistical methods. Data were processed using one - way analysis of variance (ANOVA) and two-factor without replication analysis of variance (General Linear Model) using the statistical analysis software SPSS 14.0 for Windows; significance was defined at $p<0.05$.

Results and Discussion

In sensory evaluation of cakes with JAP experts defined the flavour profile and the highest acceptable concentration of JAP. The obtained results showed that the cakes with JAP have a good score. The results of experts' evaluation are shown in the star diagram (Figure 3). Star diagram describes cakes using a range of attributes - taste, aroma, texture, softness elasticity and porosity, surface crust, and appearance – colour, shape, size.

As the experts' evaluation showed, JAP amount had almost no influence on the aroma, porosity, softness, and texture and crumb elasticity of cakes - the high quality (5-point evaluation) for all samples. The aroma enhancers often made the aroma too intense, but cakes with JAP preserved the pleasant and characteristic aroma. The quality of appearance of cakes decreased proportionally to increase of the JAP concentration. The taste became worse, colour is got darker, shape

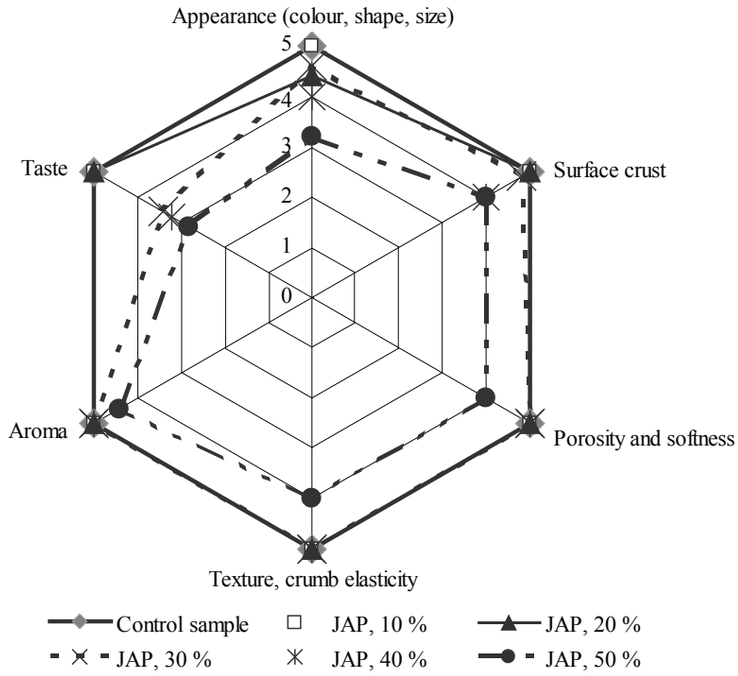

Figure 3. The results of expert evaluation of cakes with JAP using a structured 5 - point scale.

flowed out, and size decreased with increasing of concentrations of JAP in cakes (Figure 3).

The experts determined that the highest acceptable concentration of JAP in cakes is 30%. When increasing content of JAP the cakes needed to be improved with other product that is important for an excellent and enjoyable taste in cakes. If the amount of JAP additive exceeded 30%, the flow ability of dough increased too much, this changed the cake's shape and reduced the volume and size. This could be explained with increase in the amount of fibres in cakes. In that case, experts recommended improving the taste of cakes using several additives.

As Jerusalem artichoke is not known to all people, according to the experts' advice cakes with JAP were supplemented by different additives - flavouring, essences, concentrates, etc.

Results of analysis of variance showed that $F_{calculated}$ = 0.81 does not exceed $F_{critical}$ = 1.40, therefore there do not exist significant differences between cake samples in the degree of liking. That means that the consumers liked all cakes with JAP similarly (Table 1).

The acceptance test for all samples has been done by a hedonic scale and results ranged from 6 till 7, it means, 'little like' – 'average like'. Overall sensory quality of cakes was 6.82, the highest result was 7.00 – for cakes with banana flavour, but cakes with cocoa flavour had the lowest rating – score 6.32 (Figure 4). The assessment of the cakes suggests that people could accept the new product.

Table 1

Results of analysis of variance of cake samples evaluated using hedonic scale

Source of variation	Degree of freedom, df	Sum of squares, SS	Mean squares, MS	Variance ratio, F
Cakes with JAP	6	14966.75	2494.46	74.45
Panellists	49	1321.76	26.97	0.81
Error	294	9850.96	33.51	
Total	349	26139.47		

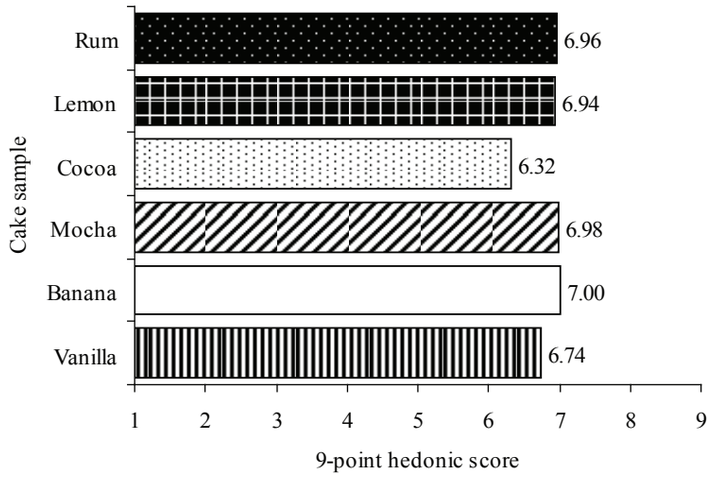

Figure 4. The results of consumer evaluation of cakes with JAP and varied aroma enhancers using a structured 9-point hedonic scale.

General Linear Model tests showed that gender and age has no significant influence on the sensory evaluation of cakes ($p<0.05$).

Figure 5 demonstrates the average opinion of males and females on cakes with JAP and various ingredients (vanilla and cocoa powder, banana puree concentrate, and mocha concentrate, lemon and rum essence). In general, men gave higher sensory evaluation rating for cakes with JAP than women.

Mocha and rum taste women liked the best, but most women have highly evaluated cakes with bananas and lemon taste. Men stayed loyal to their favourite cakes flavours; they gave the highest scores to the cake with rum and mocha taste, which could be connected to liking of their favourite drink – rum and coffee. Women have stricter requirements for food, so their rating for cakes is lower. Women most approved mocha flavour, and then followed cakes with vanilla flavour. Both women and men choose fewer samples with cocoa taste. Among of all cakes enriched with JAP, vanilla taste was the most favourite for both genders.

The results of textural analyses (hardness, N) are presented in Figure 6. The cake without JAP and aroma enhancer was used as a control sample. The varied aroma enhancers substantially influenced the hardness of cakes with JAP. The results of the experiment showed that the aroma enhancers affect the structure

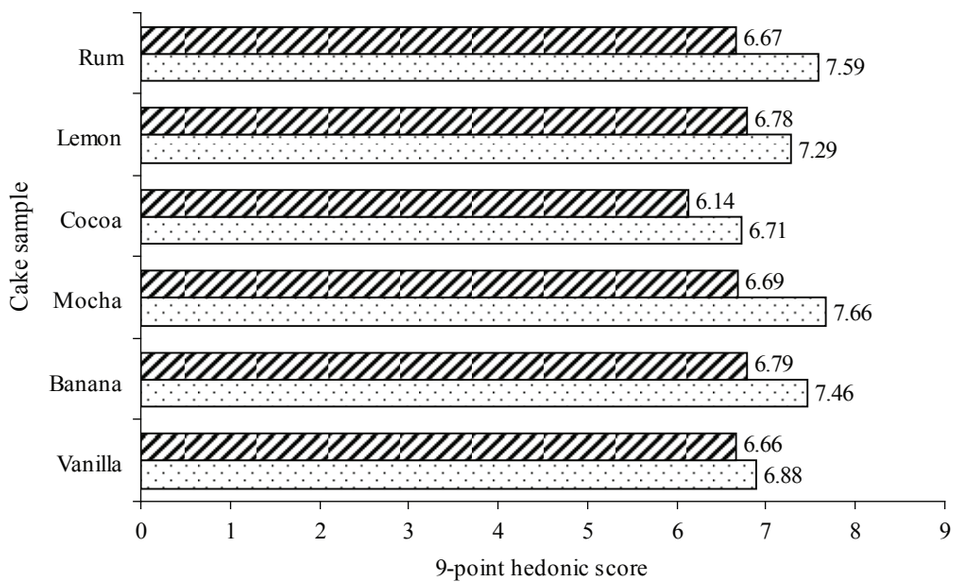

Figure 5. The results of male and female evaluation of cakes with JAP and vanilla or cocoa powder, banana puree concentrate, mocha concentrate, lemon or rum essence by using a structured 9-point hedonic scale (◪ male ⊡ female).

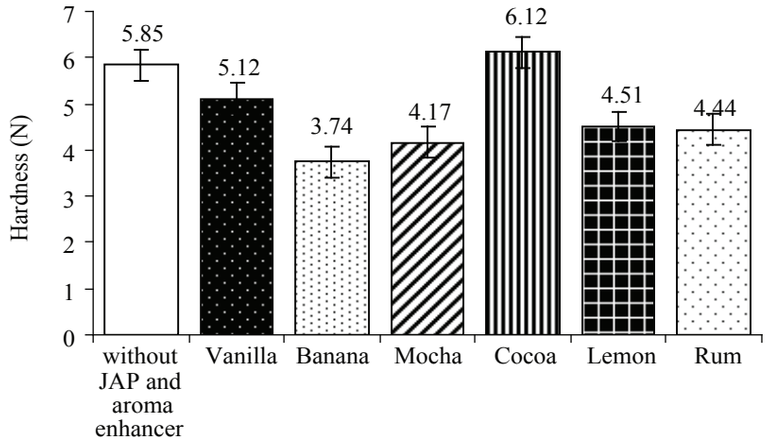

Figure 6. Hardness of the crumb of cakes with JAP and varied aroma enhancers.

of the cake, for example, fruit concentrate addition - banana puree concentrate- makes the cakes more airy and softer than the samples with other enhancers (Figure 6). The cocoa and vanilla in powder form makes the cake's structure harder. The control sample and the sample with cocoa had the highest hardness; nevertheless, the structure of the cakes with cocoa was excellent, porosity was regular, and volume of cakes increased. The results of hardness were similar in samples with essences (lemon and rum).

Changes in colour intensity (L*a*b*) in the samples is demonstrated in Figure 7. Addition of aroma enhancer resulted in the highest effect on colour components. Colour component L* shows that samples with banana puree concentrate is lighter than other samples; then followed cakes with rum essence, lemon essence and vanilla sugar. The darkest colour is for cakes with cocoa, which is three times darker than sample with banana.

Colour component a* show at samples red or green colour. The samples with mocha and cocoa had positive value which indicates the presence of red colour. All other samples had a little bit green colour.

The yellow colour observed in all samples except one – sample with cocoa. The sample with cocoa had three times lower yellowness as other samples that is shown by the b* value in Figure 7.

The results of variance shows that colour components L*, a*, b* has been significantly affected by taste and aroma enhancers (p<0.01), because $F_{calculated}$ =33.39>$F_{critical}$ =6.36.

Conclusions

1. The results of this study indicate that JAP and aroma enhancer do not influence significantly (p>0.05) sensory quality, but it has high influence on cake texture and colour components.
2. Content of JAP amount till 50% has no significant influence only on such sensory properties as cakes aroma, porosity, hardness and crumb elasticity.
3. With increasing concentrations of JAP in cakes, unwanted changes were observed in cakes quality

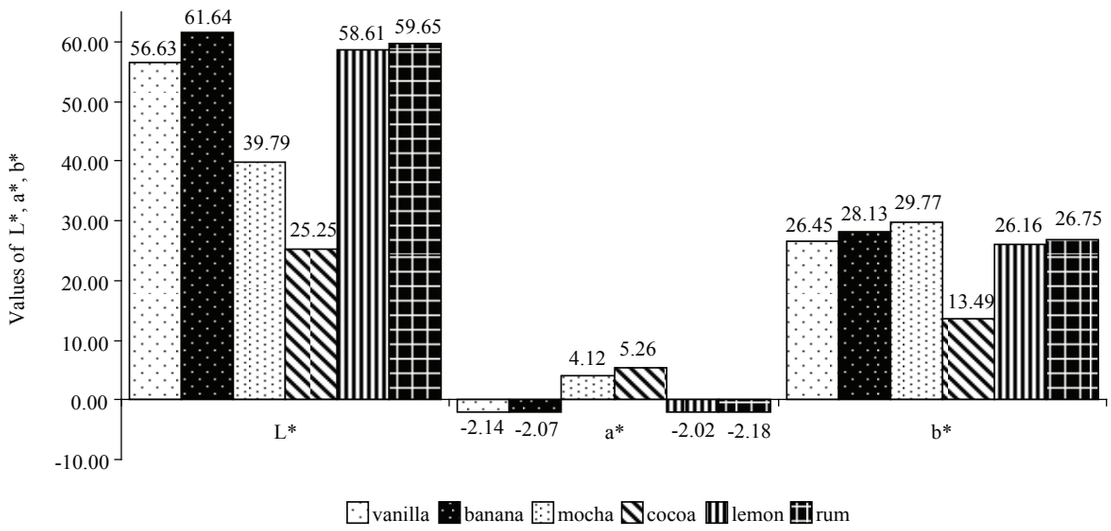

Figure 7. Results of colour analyses for cakes with JAP and taste and aroma enhancers.

- taste became worse, cake colour got darker, shape flowed out and cake volume was low.

4. The cakes retain good quality if amount of JAP does not exceed 30% of total amount of flour.
5. In the hedonic evaluation all samples received score from 6 to 7, its means, 'little like' – 'average like'.

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THE EVALUATION OF SENSORY PHYSICAL AND CHEMICAL PROPERTIES OF PEARS GROWN IN LATVIA

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Abstract. The pears (*Pyrus communis*) are one of most popular fruits that consumers willingly use as fresh produce. On the market place, consumer mainly pays attention to per's externals, size, colour, as well as to fruit taste, substantially influenced by degree of readiness, which in its turn has affect on the chemical composition of pears. The physical, chemical and sensory indices are significant when fruits have been used for fresh-cut fruit salad preparation.
All tests have been carried out at Latvia State Institute of Fruit Growing, Dobele in year 2009-2010. The objective of this study was to evaluate the physical, chemical and organoleptical properties of winter cultivars of pears grown in Latvia. The object of the research was 13 cultivars of pears: 'Delta', 'Latgale', 'Tayushchaya', 'Talgarskaya', 'BP 8965', 'Elektra', 'Janvarskaya', 'Beta', 'Bere Kiyevskaya', 'Eckehard', 'Belorusskaya Pozdnaya', 'Erika', and 'Conference' and as a control commercial cultivar. The pears were analysed and their parameters were measured: average mass and diameter of fruits, flesh firmness, colour of flesh, titratable acid (TA) and soluble solids content (SSC) were determined. Sensory analyses were carried out by 9-point hedonic line scale method.
Soluble solids content (°Brix) of pear fruits grown in Latvia is within 9.6 - 14.5°Brix. The titratable acidity (TA) is insignificant (0.05-0.26%). The average mean mass of one fruit depending on a cultivar, is 174 g, the flesh firmnes at the maturity stage is on average 10.7 to 26.5N. The fruits of cultivars 'Tayushchaya'; 'Elktra', and 'Bere kiyevskaya' by their sensory indices were evaluated as the best.
Key words: pear, physical and chemical sensory an evaluation, colour.

Introduction

Pears contain a large amount of fibres, pectin and sugars, and have energetic and dietary value. 95% of total fruit mass is flesh with integrated sugar and acid balance. The pear fruits contain mineral substances, microelements, vitamins C, P, little B_1, B_6 ,and B_9 wich is important in synthesis of amino acids (methionin, serin) (Janick and Paul, 2006). Pears contain biologically active compounds having positive influence on the function of human organism, improves the metabolism, lowers the level of cholesterol in the blood, builds on immunity (Силина, 1996; Криворот, 2001).

Insignificant amount of pears have been offered on the European market for fresh consumption. Cultivar 'Abate Fetel' occupies 36% range of all pear production in Europe (Predieri and Gatti, 2009). The cultivar'Conference' is mainly cultivated in Spain, Belgium and Netherlands taking up 30% of the total fresh fruit market. The cultivar 'Rosha' is prevalent mainly in Portugal (Mazzotti et al., 1999; Fragata et al., 2007). The cultivar 'William' make 10-12% total fresh fruit amount (Bianco, 2007), and it is possible to purchase fruits of on the market in Latvia.

The content of individual sugars, organic acids, main and trace elements are very important as they determine the internal quality of fruits. Consumers like pears with good organoleptic properties, high nutritional value, and healthful substances. The fruits have to be at the proper biological degree of ripeness, with characteristic quality indices for each variety (colour, aroma, taste, and flash firmness) (Криворот, 2001). The content of individual sugars, organic acids, main and trace elements is very important as they determine the internal quality of fruits. The fruits containing high quantities of sugars and organic acids together with the optimal mineral contents are of better quality and therefore more suitable for a longer storage (Hudina and Štampar, 2000). To obtain adequate quality the pears could be maturated before distribution on the market (Gorny et al., 2000). The quality indices of pears, like taste, firmness, texture of pulp, soluble dry matter and titrated acid content, on a number of factors – cultivar, climatic conditions, harvesting time, and growth zone. The experiments carried out in Poland indicate difference in the quality of variety's 'Erika' fruits depending on harvesting season (Wawrzyńczak et al., 2006). The qualitative indices of pear cultivar 'Conference' have an impact of geographical origin. The optimum firmness and texture of fruits grown in Italy is 5.56 N, in Netherlands it is somewhat higher – 6.8 N, whereas in Spain have pears sold remarkably higher compression force – 14.31 N, the soluble solids content of the fruits above - mentioned cultivars is equivalent – on average 15 °Brix (Baia et al., 2009; Baldwina, 2009; Paz et al., 2009; Bianco et al., 2007; Maas, 2007).

The visual appearance of fresh fruits, especially after cutting in segments, is one of the first quality determinants made by the consumer. The colorimeter is used to quantify the colour and to differentiate quality among cultivars of fruits.

The enzymatic browning is a major problem in the food industry to ensure the quality of fresh-cut fruit

produce (John et al., 2002). It occurs at the cut surface in the presence of oxygen when the enzyme polyphenol oxidaze (PPO) converts phenolic compounds into dark coloured pigments. Traditionally, enzymatic browning has been quantified using browning indicators or using a biochemical index, for example, the polyphenol oxidase activity or physical indicator characterised by surface colour (Quevedo et al., 2009; Lamikanra, 2002).

The assessment of quality indices of pear cultivars grown in Latvia has not been accomplished until now. The objective of this study was to evaluate the physical, chemical and organoleptical properties of winter cultivars of pears grown in Latvia.

Materials and Methods

All tests have been carried out at the fruit and berry processing department of the Latvia State Institute of Fruit Growing (LVAI), Dobele. As object of the research, fruits of 13 pear cultivars grown in LVAI: 'Delta', 'Latgale', 'Tayushchaya', 'Talgarskaya', 'BP 8965', 'Elektra', 'Janvarskaya', 'Beta', 'Bere Kijevskaya', 'Eckehard', 'Belorusskaya Pozdnaya', 'Erika', and 'Conference' as a control commercial cultivar were selected. The pears were stored at the temperature of +1.0 ºC, RH 90%. All chemical and physical properties of each pear cultivar were ivestigated on randomly selected 10 fruit samples. Before testing, the pears were stored at the temperature of +16 to +18 ºC. The maturation period for each cultivar was dependent on fruit harvesting time and individual features of cultivars:

- 'Elektra' ,'Janvarskaya', and 'Konference' – 2 days;
- 'Belorussskaya Pozdnaya' and 'Delta', 'Beta', 'Talgarskaya Krasavica', and BP 89652 – 5 days;
- 'Bere kiyevskaja', 'Tayushchaya', and 'Eckehard' – 6 days; and
- 'Erika' and 'Latgale' – 7 days.

Physical, chemical and sensory analyses were performed.

Physical properties

- Flesh firmness (N) was determined by digital penetrometer TR 53205 according to LVS EN 1131:2001 nozzle diameter - 8 mm, penetration depth -10 mm, speed of measurement - 600 mm min^{-1}. Pealed fruit flash firmness was measured on the diametrically opposing sides of the fruit.
- The average mass of one fruit (g) determined by 3 recurrent weighings of 10 pears in each using calibrated electronic scale 'Vibra', precision ±0.5 g.
- The mean diameter of one fruit was determined by calibration method in 3 repetitions, 10 fruits measurement; the diapason of measuring 30-90 mm.
- The colour of pear flesh samples was measured using a portal Tristimulus Colorimeter, wich had been calibreated with a standard a white plate Y – 92.15, x – 0.3623 and y – 0.3434). The colorimeter generates a composit, three color parameters in the CIELAB color systems L*, a*, b* uniform color space. The color coordinates are: L* – the lightness coordinate (0 – black, 100 – white), a* – the red/green coordinate (-a - greenness, +a - redness) are two chromatic components which range from -120 to +120 (Mac Dougal, 2002). b* – the yellow/blue coordinate, with +b* indicating yellow, and -b* indicating blue. The L*, a* and b*. The hue angle h°, chroma C*, and Browning index (BI) characterise the colour changes in fruits (Oms-Oliu et al., 2008; Perez-Gago et al., 2006; Clarka et al., 2002). The hue angle h° is determined by formula:

$$h^{\circ} = \arctan\frac{b^*}{a^*} \ . \quad (1)$$

The depth of colour chroma C* measures colour saturation or intensity and could be calculated by formula:

$$C^* = \sqrt{a^{*2} + b^{*2}} \ . \quad (2)$$

Browning index (BI) can be calculated by formula:

$$BI = \frac{100(x - 0.31)}{0.172} \ , \quad (3)$$

where x is calculated as

$$x = \frac{a^* + 1.75^*}{5.645L^* + a^* - 3.012b^*} \ . \quad (4)$$

The colour was determined on the surface of peeled pear segments. The measurements were repeated on 20 randomly selected locations on each sample.

Chemical analyses.

Soluble solids content (°Brix) was determined according to LVS EN 12147:2001 using refracometer ATAGO;

titratable acidity (TA) was determined according to LVS EN 12147:2001.

Sensory analyses.

- Sensory evaluation of pears was carried out by 20 trained panellists. A 9-point hedonic scale was used to determine degree of acceptance among the cultivars of pears (ISO 4121:1987). Panellists evaluated taste, sweetness, acidity, graininess, firmness, consistency and colour of fruit flesh.

Statistical analysis.

Data processing was carried out by the following univariate and multivariate methods of statistical analysis: analysis of variance, correlation analysis, principal component analysis and using SPSS 15 software package. Sheffe criterion was used to clarify significant differences ($p<0.05$) among the studied samples in the analysis of variance. Closeness of the relationship between the parameters was determined by analysis of Pearson correlation coefficient.

Results and Discussion

The research showed that the average values of the geometric mean diameter and mean mass of one fruit on the cultivar (Table 1),and there is a medium close relationship (r =0.583) between the diameter and average mass ± of the fruit. The cultivars 'Tayushchaya', 'Latgale', 'BP8965', 'Elektra', and 'Belorusskaya Pozdnaya' are mutually disparate ($p<0.05$). The average mean mass of the cultivar 'Konference' fruits grown in Latvia is 168 g, however in other European countries it is different: in Spain depending on harvesting yield it is 103.7 g (2001) and 165.3g (2002), in Italy – on average 175 g, in Netherlands may reach 217-234 g (Bianco et al., 2007; Maas, 2007). The mean diameter of pears cultivated in Latvia varies from 59 mm (cultivar 'Talgarskya Krasavica') to 73.5 mm (cultivar BP 8965). The research in Germany (Stehr, 2003) informs about cultivar 'Konference', having diameter of fruits 60.1-64.3 mm, wich is smaller than that 'Conference pears grown in Latvia in 2009 (70.5 mm). Flesh firmness of the investigated pear cultivars differ as well. The fruits of cultivar 'Tayushchaya', 'Bere kiyevskya', and 'Erika' have milder and softer flesh firmness (10.7, 10.7, and 12.1 N respectively), while cultivars 'Latgale' are grainy and hard (26.5 N). The fruits of cultivars BP 8965 'Talgarskya krasavica', 'Elektra' and 'Eckehard' have fine-grained structure as well as and flesh firmness among them don't disparate ($p<0.05$). The flesh firmness of cultivar 'Konference' fruits grown in Germany and Italy (Cambiaghi et al., 2003) is analogons (9 N and 9.1 N respectivly), however, the firmness of pears grown in Latvia in 2009 is higher (20.9 N). Scientists (Wawrzyńczak et al., 2006) report that flesh firmness of pears of 'Erika' cultivar grown in Poland differ depending on harvesting season (36.29 N in 2004 and. 35.30 N in 2005). Obviously, the flesh firmness of 'Erika' pears grown those in Poland is 3 times higher than that 'Erika' pears grown in Latvia in 2009 (12.1 N).

The soluble solids content (SSC) of pears is disparate ($p<0.05$) and depends on the cultivar, for example, within 9.6 °Brix for cultivar 'Belorusskaya Pozdnaya', and 14.5 °Brix for 'Beta' (Table 2). The fruits of cultivar 'Erika' grown in Latvia are on average sweet, and their soluble solids content (SSC) is 12.6 °Brix. The SSC of cultivar 'Erika' fruits are similar in Poland and in Latvia. The researchers in Poland (Wawrzyńczak et al., 2006) report about SSC 13.7 °Brix in the year 2004, and slightly less – 12.3 °Brix in 2005. The SSC of cultivar 'Konference' fruits grown in Latvia is 12.2 °Brix wich does not differ the pears from it grown in Italy (12 °Brix) (Cambiaghi et al., 2003). Titratable acid (TA) content of cultivars 'Conference' and 'Erika' is 0.10 and 0.05% respectively,wich is less than that established in Poland for the same cultivars (Wawrzyńczak et al., 2006). Fruits of cultivar 'Erika' grown in Poland have at an average 4 times higher TA content (0.24%). One of the taste characteristics of fresh fruits is the ratio SSC/TA. The results obtained for pears grown in Latvia indicate this ratio greater than that of the pears grown in Poland: for cultivar 'Erika', the ratio SSC/TA was 58.3 in Poland (Wawrzyńczaket et al., 2006), but Latvia is 257.5.

Table 1

The characteristics of pear physical indices (mean ± SD)

Cultivars	Diameter, mm	Weight, g	Flesh firmness, N
'Conference'	70.50 ± 2.84^{cd}	167.91 ± 5.03^{de}	20.91 ± 0.94^{def}
'Belorusskaya Pozdnaya'	59.50 ± 3.16^{ab}	122.94 ± 4.94^{a}	21.94 ± 1.58^{defg}
'Bere Kiyevskya'	71.00 ± 2.36^{cd}	202.12 ± 3.60^{gh}	10.74 ± 2.98^{a}
'Beta'	64.00 ± 3.69^{abc}	151.71 ± 3.61^{bc}	17.27 ± 2.32^{bcd}
BP 8965	73.50 ± 6.69^{d}	233.93 ± 3.23^{i}	18.11 ± 1.50^{bcde}
'Eckehard'	69.00 ± 3.94^{cd}	175.55 ± 6.82^{ef}	22.75 ± 2.60^{efg}
'Elektra'	67.50 ± 5.68^{bcd}	207.81 ± 3.39^{h}	14.35 ± 2.60^{abc}
'Erika'	68.50 ± 2.42^{cd}	181.63 ± 4.29^{f}	26.47 ± 1.79^{g}
'Delta'	64.50 ± 4.86^{abc}	160.76 ± 5.91^{cd}	12.08 ± 3.77^{a}
'Janvarskaya'	70.50 ± 3.16^{cd}	197.04 ± 4.92^{g}	13.4 ± 1.48^{ab}
'Latgale'	71.50 ± 2.42^{cd}	167.15 ± 2.93^{de}	25.51 ± 1.73^{fg}
'Tayushchaya'	65.00 ± 3.69^{abc}	146.84 ± 4.13^{b}	10.69 ± 2.27^{a}
'Talgarskya Krasavica'	59.00 ± 3.69^{a}	156.15 ± 4.99^{bc}	19.02 ± 2.18^{cde}

Differences of ^{abcdefg} means ± SD followed by a different letter within a column are significant ($P<0.05$).

Table 2

The characteristics of pear chemical indices (Mean ± SD)

Cultivars	SSC, °Brix	TA, %	SSC/TA ratio
'Conference'	12.19± 0.10^{f}	0.10 ± 0.00^{b}	204.16 ± 24.89^{e}
'Belorusskaya Pozdnaya'	9.58 ± 0.09^{a}	0.10 ± 0.00^{b}	128.97± 20.55^{b}
'Bere Kiyevskya'	14.52± 0.10^{g}	0.10 ± 0.00^{b}	159.81 ± 1.21^{d}
'Beta'	13.93 ± 0.31^{g}	0.20 ± 0.00^{d}	87.24 ± 0.75^{b}
BP 8965	12.75 ± 0.16^{h}	0.15 ± 0.00^{c}	84.02 ± 14.92^{b}
'Eckehard'	10.59 ± 0.09^{b}	0.10 ± 0.00^{b}	77.65 ± 3.74^{b}
'Elektra'	9.84 ± 0.09^{a}	0.10 ± 0.00^{b}	79.04 ± 2.03^{b}
'Erika'	9.72 ± 0.24^{a}	0.26 ± 0.05^{e}	37.50± 2.85^{a}
'Delta'	12.61 ± 0.29^{h}	0.05 ± 0.00^{a}	257.45 ± 3.13^{f}
'Janvarskaya'	11.35 ± 0.08^{c}	0.20 ± 0.00^{d}	53.89 ± 8.32^{a}
'Latgale'	12.61 ± 0.09^{h}	0.10 ± 0.05^{b}	144.67 ± 1.83^{cd}
'Tayushchaya'	11.98± 0.15^{df}	0.20 ± 0.00^{d}	75.95 ± 0.56^{b}
'Talgarskya Krasavica'	11.71 ± 0.16^{d}	0.10 ± 0.01^{b}	92.18 ± 4.87^{b}

Differences of ^{abcdefg} means ± SD followed by a different letter within a column are significant (P<0.05).

Sensory aspect is the most important to consumers looking for the best pear cultivars for fresh consumption. A 9-point hedonic scale was used to determine which cultivar of pears was preferred by the panellists. The results indicate that pear cultivars 'Elektra', 'Bere Kiyevskaya', and 'Tayushchaya' were accepted by higher scores 7.0, 6.9, and 6.8 respectively. The cultivars 'Latgale' and 'Janvarskaya' with scores from 3.1 to 4.8 were rated as unacceptable, the results didiffered from all other analysed pear cultivars (p<0.05).

Figures 1. show the linescale the six main evaluated qualities: colour, taste, acidity, sweetness, firmness and graininess. The highest valuation received fruits with highest soluble solids content: 'Beta', 'Bere Kiyevskaya', and 'Tayshchaya' pears from 11.7 to 14.5 °Brix. Whereas the fruits of cultivars 'Latgale' and 'Janvarskaya' achieved the least sensory features. An interconnection between the sweetness and taste was calculated, and an average close correlation (r = 0.605) was found.

Pear cultivar fruits grown in Latvia showed an insignificant is titratable acid content. The experts assessed this parameter as unimportant and suggested in not to use it in future. Cultivars 'Erika' un 'Conference' in accordance with acidity were evaluated as more enjoyable.

Cultivars 'Erika' and 'Conference' by acidity were evaluated as more enjoyable. The pear fruits have high SSC, good taste properties, but they don't have typical aroma. In most of the pear cultivar fruits, flesh firmness influences the taste. Best of all the

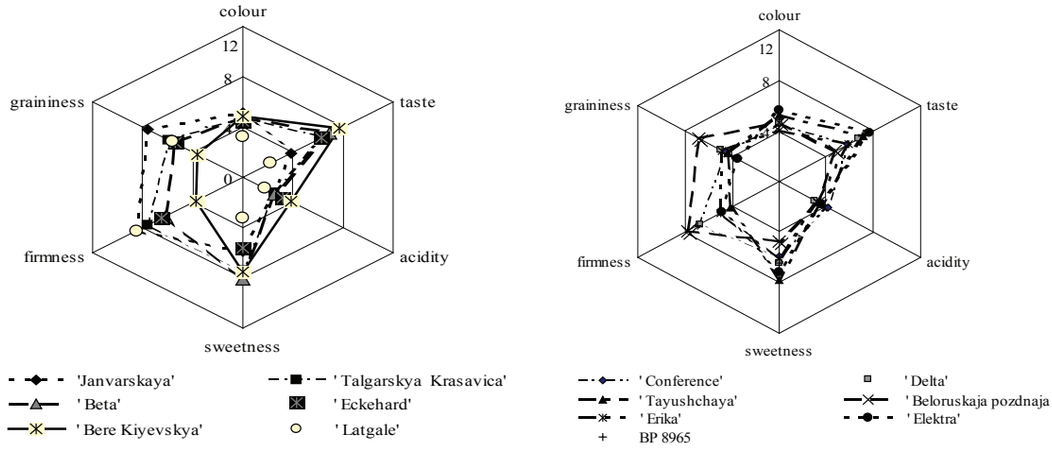

Figure 1. Sensory profiles of pear cultivars evaluated by panellists.

panellists liked small-grained pear fruits with mild firmness. The results of line scale evaluation indicate that pear cultivars 'Elektra', 'Bere Kiyevskaya', 'Tayushchaya', and BP 8965 were accepted by higher score. The mathematical calculations allow to draw conclusion low correlation exists (r = 0.313) between flesh firmness and graininess.

The colour is one of parameters describing organoleptical features of fruits. Fruits of cultivar 'Elektra', achieved the highest valuation by colour, whereas 'Latgale' had the lowest sensory evaluation.

The results of colour evaluation are summarised in Table 3. Values of colour parameters a* and b* were used to compute values for hue angle h° and chroma C*, two parameters that are effective for describing visual colour appearance. The main properties of fruit flesh colour changes – hue angle h°, depth of colour C*, and browning index -were assessed and calculated. It is found a disparity among fruit flesh colour features of all pear cultivars ($p<0.05$) was found.

L* mean values ranged from 41.80±1.48 (cultivar 'Beta') to 50.90±2.09 (cultivar 'Elektra'). The highest L* value means the highest pear flesh quality, because the enzymatic browning in this case is less (Quevedo et al., 2000). Cultivar 'Elektra' with fine-grained firmness and white flesh achieved the highest L* mean value. a* mean values ranged from 6.30±1.21 (greener) ('Delta') to 9.30±2.06 (more red colour) ('Conference'). Whereas mean b* values ranged from -1.4±2.48 ('Delta' and 'Elektra') to -6.2±3.2 ('Conference') (Table 3). Fruits of cultivars 'Conference' and 'Delta' showed significant differences compared to other investigated cultivars.

The chroma value measures the colour saturation or intensity. A higher C* value is indicative of a brighter red colour. Cultivar 'Conference' had the highest C* value (11.10±3.22), but cultivar 'Delta' showed the lowest C* value (7.70±1.89) among the investigated samples. Evaluating by C* value, fruits of cultivar 'Delta' shoved significant differences among all investigated cultivars ($p<0.05$). It has been established that of fresh cut pears the h^o value is related with browning index (BI) (Oms-Oliu et al., 2008; Perez-Gago et al., 2006). The degree of readiness substantially influences the BI. The pear fruits harvested too early after maturation will show higher h^o and BI as well (Quevedo et al., 2009; Oms-Oliu et al., 2008).

Appearance, flavour, texture, and nutritional value are four attributes considered by consumers when making food choices. Appearance which is significantly impacted by colour is one of the first attributes used by consumers in evaluating food quality. Enzymatic browning is a major problem in the production of fresh-cut fruit wedges and shelf life determination (Toivonen and Brummell, 2008). Colour may be influenced by naturally occurring pigments such as chlorophylls, carotenoids and anthocyanins in food, or by pigments resulting from both enzymatic and non-enzymatic reactions. Enzymatic browning is one of the most important colour reactions that affect fruits. It is catalysed by the enzyme polyphenol oxidise (1, 2 benzenediol; oxygen oxidoreductase) which is also referred to as phenoloxidise, phenolase, monophenol oxidise, diphenol oxidise, and tyrosinase. Enzymatic browning is one of the most studied reactions in fruits, vegetables, and seafoods. Researchers in the fields of food science, horticulture, plant and postharvest physiology, microbiology, and even insect and crustacean physiology have studied this reaction because of the diversity of its impact in these systems (Quevedo et al., 2009; Lamikanra, 2002).

Table 3

The main properties of colour changes in pear flesh

Cultivars	Colour values					
	L*	a*	b*	h°	C*	BI
'Conference'	46.30±2.76^{cde}	9.30±2.06^{b}	-6.2±3.2^{a}	31.20±5.67^{ab}	11.10±3.22^{a}	3.10±1.49^{a}
'Belorusskaya Pozdnaya'	47.80±3.15^{abcd}	7.60 ±1.85^{b}	-2.6±3.16^{a}	19.10±5.23^{ab}	8.30±2.7^{a}	7.30±4.53^{ab}
'Bere Kiyevskya'	46.00±2.22	8.60±1.55^{ab}	-4.30± 1.63^{ab}	24.10±6.64^{a}	9.60±1.90^{a}	5.30±2.48^{ab}
'Beta'	41.80±1.48^{ef}	7.60 ±1.98^{ab}	-4.0±2.51^{ab}	23.50±5.41^{a}	8.60±2.73^{a}	4.10±1.46^{ab}
BP 8965	47.30± 0.93^{de}	8.20±1.31^{ab}	-4.2±1.32^{a}	22.70±4.29^{ab}	9.20±1.39^{a}	7.40±2.37^{a}
'Eckehard'	45.20±2.04^{a}	7.90±2.60^{ab}	-2.6±4.48^{a}	18.60±4.71^{ab}	8.70±2.83^{a}	12.00± 4.18^{a}
'Elektra'	50.90±2.09^{abcd}	7.70±2.57^{ab}	-1.4±2.48^{a}	11.70±3.64^{ab}	8.00±2.87^{a}	8.30±3.77^{a}
'Delta'	43.70±2.05^{ab}	6.30±1.21^{ab}	-1.4±2.02^{a}	32.60±6.84^{ab}	7.70±1.89^{a}	3.40±1.78^{a}
'Janvarskaya'	43.50±4.06^{de}	7.80±2.36^{ab}	-3.6±4.21^{a}	22.10±5.14^{ab}	8.70±3.68^{a}	6.40±3.22^{a}
'Latgale'	43.30± 2.46^{ef}	8.00 ± 2.53^{ab}	-4.6±2.96^{a}	24.30 ± 6.17^{ab}	9.30 ± 3.51^{a}	4.60 ± 1.01^{a}
'Erika'	43.60± 1.80^{abc}	8.20±1.77^{a}	-4.5±3.19^{b}	25.00 ±6.74^{b}	9.30 ±3.06^{a}	2.80±1.67^{b}
'Tayushchaya'	46.10± 1.37^{ef}	8.80±1.25^{ab}	-5.0±1.5^{ab}	26.30±6.49^{ab}	10.10±1.72^{a}	3.70±1.03^{ab}
'Talgarskya Krasavica'	43.10±2.11^{ef}	9.00±1.56^{ab}	-4.90±2.62^{ab}	26.40±5.36^{ab}	10.10±2.55^{a}	4.00±1.92^{ab}

Differences of ^{abcdefg} means ± SD followed by a different letter within a column are significant ($P<0.05$).

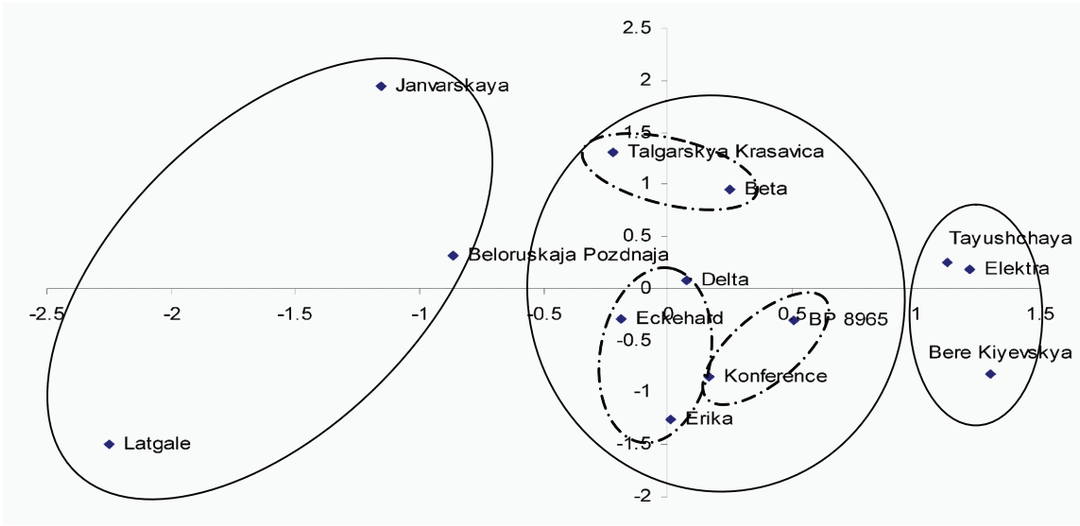

Figure 2. Liking groups of pear sensory evaluation.

A Dimension Reduction Factorial analysis was performed to assess the effectiveness of any pear cultivar for fresh consumption. The average valuables of evaluated pears can be seen in Figure 2. All panellists tasted pear cultivars that were divided into three groups. The highest judgment of sensory properties achieved cultivars: 'Tayushchaya'; 'Elektra', and 'Bere Kiyevskaya'. The fruits of cultivars 'Delta', 'Talgarskaya Krasavica', 'BP 8965', 'Beta', 'Eckehard', 'Erika' and 'Conference' achieved lower appreciation, while fruits of cultivars 'Latgale', 'Janvarskaya', and 'Belorusskaya Pozdnaya' were assessed as the worst.

Conclusions

1. Soluble solids content (°Brix) in Latvia grown pear fruits is within 9.6 - 14.5°Brix. The titratable acidity is insignificant (0.05-0.26%). The average mean mass of one fruit dependending on the cultivar is 174 g, the flesh firmness at the maturity stage is on average 10.7 to 26.5N. The fruits of cultivars 'Tayushchaya'; 'Elektra' and 'Bere Kiyevskaya' were evaluated as the tastiest by the hedonic scale.
2. The experimentally obtained results of analyses proved that for fresh fruit consumption as well as for fresh-cut fruit salad production are recommended the cultivars with mild and soft taste 'Tajushaya', 'Bere Kiyevskaya' are recommended; their flesh firmness is 10.7 N, soluble solids content - 11.71 and 13.9 °Brix, and ratio of soluble solids content and acids - 92.18 and 159.81 respectevely. The pear cultivars 'Elektra', 'Eckehard', and BP 8965 with small-grained flesh firmness of 14.4-22.8 N, soluble solids content of 9.8 – 12.8 °Brix, and ratio of soluble solids content and acids of 77.7-84.0, can be used as well.

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THE CHANGES OF THE SUGARS AND SORBITOL IN ROWANBERRIES AND CHOKEBERRIES AFTER FREEZING AND THAWING

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Abstract. Both the rowanberries (*Sorbus*) and the chokeberries (*Aronia*) belong to the family *Rosaceae*. The berries of these cultivars are suitable for production of health-food products. The main components in the dry matter of fruits are carbohydrates, primarily sugars. The aim of this experiment was to determine the content of simple sugars and sorbitol in different fresh rowanberry cultivars and chokeberries and to compare the composition of these compounds after freezing and thawing of berries.
The experiments were done in the Customs laboratory of the National Customs Board, State Revenue Service (Latvia). The rowanberries were picked in the Pure Horticultural Research centre, and black chokeberries were obtained from farm 'Ceplīši' (rural municipality of Pure). The contents of dry matter and soluble solids were analyzed in fresh berries, and the contents of sugars and sorbitol were analyzed both in fresh berries and in berries after freezing and thawing of 13 different cultivars of rowanberries and one black chokeberry. For determination of the soluble solids content, the refractometric method was used, and for determination of the glucose, fructose, sucrose and sorbitol content, high performance liquid chromatography was used.
The results showed that rowanberries and chokeberries contained 3.24-7.24 g 100 g^{-1} of sorbitol which was the dominant compound in all investigated berries. The highest content of sorbitol was found in the wild rowanberries and rowanberries *Sorbus aucuparia* var. *sibirica*, but fruits of rowanberry×pear 'Alaya Krupnaya' contained the least content of sorbitol. After freezing and thawing of berries, the content of sugars and sorbitol decreased by 5-23% with some exclusion.
Key words: rowanberry, chokeberry, dry matter, soluble solids, sugars, sorbitol.

Introduction

The rowanberries (*Sorbus*) and the chokeberries (*Aronia*) belong to the subfamily *Maloideae* of the family *Rosaceae*. These cultivars are closely related and their berries have been promoted as a health-food or can be a source for health-promoting products. Rowanberries have been traditionally used for jellies and jams, but their wider use as food ingredients has been less popular because of their bitter taste; though they can be dehydrated and ground into powder to be mixed in porridges or bread dough (Hukkanen et al., 2006; Poyrazoğlu, 2004). Fruits of *Aronia* are used to produce fruit syrup, fruit juice, soft spreads, fruit jellies and tea. Chokeberries are also used to make food colourants (Ara, 2002; Kulling and Rawel, 2008).

Rowanberries are small orange-red "fruits" of a rowantree (also known as mountain ash) and grow in the northern part of Europe. The ripe wild rowanberries (*Sorbus aucuparia)* are picked in the autumn and they are eatable, but very tart in flavour although contain lots of sugar. Freezing causes the bitter-tart berries to turn sweeter and more suitable for eating (Poyrazoğlu, 2004). Sweeter and less astringent than wild rowanberries are different cultivars of sweet rowanberries and hybrids with other species. Comparison of the data obtained to wild rowanberries sugar content indicated that cultivars of the sweet rowanberries are really the sweetest – sugar content in their fruits were considerably (1.2-2.1 times) higher (Navys, 2001). A breeding program of the sweet rowanberries was started by the famous botanist Ivan Michurin in Russia more than a hundred years ago. The hybrid cultivars were developed by cross-breeding rowan with *Malus, Pyrus, Aronia,* and *Mespilus* (Hukkanen et al., 2006).

Chokeberries are small, dark violet fruits, but because of their astringency are not favoured as 'table fruits' (Skupień et al., 2008). Chokeberries (*Aronia spp.*) are native to eastern North America and were introduced to European botanical gardens and interchanged between European countries. Large-scale cultivation started in the late 1940s in Siberia and soon spread all over Russia from the Baltic Sea in the west to eastern Russia. Black chokeberry was known in German as 'appleberry', and in Russian as 'ryabina chernoplodnaya' (Jeppsson, 2008). The genus name *Aronia* has been replacing the common name, chokeberry. The other common names for *Aronia* besides chokeberries include black apple berry and rowanberries, whereby in the latter case the hybrids of rowanberry with *Aronia* are likely to be addressed (Kulling and Rawel, 2008).

The berries consist mostly of water, and the main components in the dry matter are carbohydrates, primarily sugars. Rowanberries and chokeberries contain glucose and fructose, which are abundant in the human diet, and sugar alcohol such as sorbitol, which is naturally occurring. The sugar composition of different fruits is helpful in determining the authenticity of fruit juices and concentrates. Individual fruits have characteristic patterns related to their sorbitol content, glucose:fructose ratio, and sucrose content, which are influenced by the variety, season, or geographic origin only to a small degree (Wrolstad and Shallenberger, 1981).

According to food composition and nutrition tables by Souci et al. (2008), sweet rowanberry *S. aucuparia L.* var. *edulis* contains on average 9300 mg of reducing sugars, 150 mg of sucrose, and 8500 mg of sorbitol per 100 g of edible portion. Other authors have reported that rowanberries contain 6.2 g per 100 g

of free sugars (int.al., fructose – 2.5 g 100 g^{-1}, glucose – 3.7 g 100 g^{-1}, and sucrose – traces) (Varo et al., 1984). Mäkinen and Söderling (1980) have established by gas chromatography that rowanberries *S. aucuparia* contain sugar alcohols – sorbitol and mannitol (up to 5.3 and 0.38 mg per 1.0 g of fresh weight, respectively). They were observed that the level of sorbitol tended to increase slightly during ripening process of the rowanberry. It has been found that the berries of *Aronia* contain 6.91 g 100 g^{-1} of total sugar (Skupień et al., 2008), or the sum of glucose and fructose in chokeberries varies between 13-17.6 g 100 g^{-1} of fresh weight, sucrose has not been detectable (Kulling and Rawel, 2008). The content of sorbitol enzymatically has been determined to be 80 g L^{-1} in fresh pressed juice of *Aronia* berries (Ara, 2002). When the fruits of *Aronia melanocarpa* are ripened to full maturity, they can contain up to 20% of sugar, but simultaneous oxidation of organic molecules in the fruit produces unattractive brown compound (Scott and Skirvin, 2007).

Freezing is one of the best methods for long-term storage of fruits and for preservation of the original colour, flavour, and nutritive value. Fresh fruits, when harvested, continue to undergo chemical, biochemical, and physical changes. The freezing process reduces the rate of these degradation reactions and inhibits the microbiological activity. The quality of frozen fruits is closely related to freezing and thawing processes and is very dependent on other factors such as the type of fruit, varietal characteristics, stage of maturity, and the rate of freezing. The rate of freezing and the formation of small ice crystals in freezing are critical to minimise tissue damage and drip loss in thawing. Thawing generally occurs more slowly than freezing (Li and Sun, 2002; Ancos et al., 2006).

There is no published data about the effect of freezing, freezing storage and thawing process on simple sugars and sorbitol content in rowanberries and chokeberries, and there is, however, little published information on the content of individual sugars and sorbitol in these berries.

The purpose of this investigation was to quantify the content of simple sugars (fructose, glucose, and sucrose) and sorbitol in different rowanberry cultivars and chokeberries, and to compare the composition of these compounds after freezing and thawing of berries.

Materials and Methods

Samples of berries

We investigated berries from 13 rowanberries and their hybrid cultivars and from one black chokeberry cultivar grown in Latvia, for their dry matter, content of soluble solids, and content of simple sugars (glucose, fructose, sucrose) and sorbitol. The rowanberries and their hybrid fruits were picked in the Pure Horticultural Research centre (HRC) collection of genetic resources in September 2009, and the black chokeberry fruits were obtained from farm "Ceplīši" (rural municipality of Pure). The wild rowanberry *Sorbus aucuparia*, sweet rowanberry cultivars *S. aucuparia* var. *fructolutea*, *S. aucuparia* var. *sibirica* (syn. *uralensis*), 'Businka', 'Michurinskaya Krasnaya', 'Moravica', 'Moravskaya Krupnoplodnaya CGL', 'Rosina', *Sorbus arranensis,* rowanberry×pear 'Alaya Krupnaya' (hybrid of *S. aucuparia*×*Pyrus* sp.), rowanberry×pear 'Titan' (hybrid of *S. aucuparia*×*Pyrus* sp.×*Malus* sp./*Pyrus* sp.), rowanberry×hawthorn 'Granatnaya' (hybrid of *S. aucuparia*×*Crataegus sanguine*), rowanberry×chokeberry 'Burka' (hybrid of *S. aucuparia*×*Sorbaronia alpina* (syn. *Sorbus alpina*)), and black chokeberry *Aronia melanocarpa* (origin from Michurin's collection) were included in to the investigations.

The samples of rowanberries and chokeberries after harvesting were packed in plastic bags, frozen, and kept at -20±2 °C until analyses for 6 months. Berries were thawed for 24 hours at +4 °C in the refrigerator before use. Thawing experiments were performed with 100-g portions of berry samples.

Determination of dry matter

Dry matter of fresh berries was determined by a gravimetric method. An aliquot of samples (5-10 g) was dried at 97 °C overnight (16 h) according to Mattila et al. (2006). For analysis, oven TR60 (Naberthern) and analytical scales BP-210s (Sartorius) were used. Measurements were carried out in three replications.

Determination of the content of soluble solids

Fresh berry samples (100 g) were homogenised in a manual blender (Braun). Homogenate of berries was used for making a juice, which was further used for determination of the soluble solids content. The content of soluble solids (expressed as ºBrix) for rowanberries and chokeberries was determined at the temperature +20 °C with a digital refractometer RX-5000CX (Atago) according to ISO 2173:2003. Measurements were carried out in five replications.

Extraction and HPLC analysis of simple sugars and sorbitol

Fresh or thawed berries (100 g) were homogenised in a manual blender (Braun). The test portion of samples (5 g) was weighed into a volumetric flask (50 ml), and water (~ 20 ml) was added. The mixture was heated at 60 °C for 20 min in a water bath. The sample was cooled to ambient temperature and homogenised by shaking. For clarification, Carrez I (1 ml) and Carrez II (1 ml) was added, and then the sample was shaken. Afterwards the sample was diluted to the mark with water, homogenised, and filtered through a paper filter DP 503 125 (Albet). The sample extracts were collected and filtered through a membrane filter with pore size of 0.2 µm (Sartorius) and the filtrate was put in to vials.

Extracts of berries were analysed for the content of individual sugars (fructose, glucose, sucrose) and sorbitol using HPLC Prominence (Shimadzu, Japan) equipped with Sypelcosil™ LC-NH_2 column (250 x 4.6 mm, particle size – 5 µm) and autosampler SIL-20A. Sugars and sorbitol were detected with a refractive index detector RID-10A (Shimadzu), and the data were acquired and processed using Shimadzu LabSolutions software (LCsolution Version 1.21 SP1). Column temperature was 30 °C, and acetonitrile:water, 80:20 (v/v) was used as eluent. The flow rate was 1.0 ml min^{-1}. Injection volume of samples was 10 µl. Calibration

Table 1

Concentration ranges and calibration equations of reference compounds used for calibration of the HPLC analysis

	Fructose	Glucose	Sucrose	Sorbitol
Concentration range, g L^{-1}	0.10-20.11	0.10-20.04	0.10-20.10	0.10-20.17
Calibration equation	$y=1.295\cdot10^{-5}\cdot x+0$	$y=1.228\cdot10^{-5}\cdot x+0$	$y=1.196\cdot10^{-5}\cdot x+0$	$y=1.073\cdot10^{-5}\cdot x+0$
R^2	0.99980	0.99997	0.99992	0.99994

curve was acquired after two repeated HPLC runs of 7 standard solutions of reference compounds (Table 1). Quantifications of individual sugars and sorbitol content of berries were performed in duplicate and were based on peak area measurements.

Data analysis

All values of parameters were expressed as mean and standard error. The results were statistically evaluated by two-factor analysis of variance (ANOVA). Statistical differences with *p*-values under 0.05 were considered as significant.

Results and Discussion

The content of dry matter and soluble solids

The content of dry matter and soluble solids in fresh rowanberries and chokeberries is shown in Table 2. The average dry matter content in the wild rowanberries was 24.99%, and in the chokeberries – 22.74%. These values are greater than reported by Mattila et al. (2006) (21.0% and 18.5%, respectively). As we had not more information about the content of dry matter of rowanberry cultivars, it was difficult to make any conclusions for these differences.

Table 2

The content of dry matter and soluble solids of fresh rowanberries and chokeberries

Cultivar	Dry matter, % (n=3)	Soluble solids, ºBrix (n=5)	Soluble solids (ºBrix) as reported previously
S. aucuparia var. *sibirica*	27.57±1.05*	25.58±0.10	-
Sorbus arranensis	25.16±0.29	19.66±0.17	-
Rowanberry×chokeberry 'Burka'	20.81±0.29	19.51±0.06	12.4 (Jeppsson, 2000) 26.72 (Петров, 1957)
Wild rowanberry	24.99±0.19	19.11±0.02	18 (Kampuss et al., 2009) 29.33 (Петров, 1957)
Black chokeberry	22.74±0.16	18.73±0.02	15.7-18.3 (Jeppsson, 2000) 19.5-33.53 (Петров, 1957)
Rowanberry×pear 'Titan'	19.06±0.40	18.45±0.02	11.9 (Hukkanen et al., 2006) 16.2 (Jeppsson, 2000) 25.43 (Петров, 1957)
'Rosina'	21.94±1.39	16.43±0.01	15 (Kampuss et al., 2009) 19.1 (Hukkanen et al., 2006)
S. aucuparia var. *fructolutea*	19.35±0.06	16.43±0.01	-
Rowanberry×hawthorn 'Granatnaya'	20.75±0.16	16.12±0.03	15 (Kampuss et al., 2009) 12.6 (Hukkanen et al., 2006) 25.11 (Петров, 1957)
'Michurinskaya Krasnaya'	18.74±0.38	15.47±0.02	-
'Moravskaya Krupnoplodnaya CGL'	18.86±0.52	15.32±0.04	-
'Businka'	19.11±0.46	14.86±0.02	-
Rowanberry×pear 'Alaya Krupnaya'	16.30±0.30	13.72±0.03	13 (Kampuss et al., 2009) 13 (Hukkanen et al., 2006)
'Moravica'	18.85±0.73	12.05±0.01	-

* Values are expressed as mean ± standard error.

The content of soluble solids in fresh rowanberries differed between 12.06±0.02 ºBrix ('Moravica') and 25.56 ±0.23 ºBrix (*S. aucuparia* var. *sibirica*), and in fresh black chokeberries – 18.74±0.05 ºBrix. Wild rowanberries contained 19.11±0.02 ºBrix of soluble solids.

According to previous investigations (see Table 2), it can be concluded that the content of soluble solids of rowanberries and chokeberries can fluctuate greatly in different regions, different years and cultivars.

The content of simple sugars and sorbitol

The content of simple sugars (glucose, fructose, and sucrose) and sorbitol is a very important parameter that determines the quality of berries. The amounts of sorbitol, glucose, fructose, and the sum of these compounds in fresh rowanberries and chokeberries are given in Figures 1, 2, 3 and 4, respectively. The content of sucrose in the investigated berries was not detectable. The differences among mean concentrations of fructose, glucose, and sorbitol in rowanberry cultivars were statistically significant ($p<0.05$).

As shown in Figure 1, sorbitol was the dominant compound compared with simple sugars in all investigated berries. The sorbitol concentration of fresh rowanberries varied between 3.24±0.02 g 100 g^{-1} (rowanberry×pear 'Alaya Krupnaya') and 7.24±0.20 g 100 g^{-1} (wild rowanberry), but in berries after frozen storage and thawing, the highest value was obtained to *S. aucuparia* var. *sibirica* (2.56±0.02 g 100 g^{-1}) but the lowest value – to rowanberry×pear 'Alaya Krupnaya' (2.56±0.02 g 100 g^{-1}). Black chokeberries contained 5.51±0.14 g 100 g^{-1} of sorbitol in both fresh berries and berries after frozen storage and thawing.

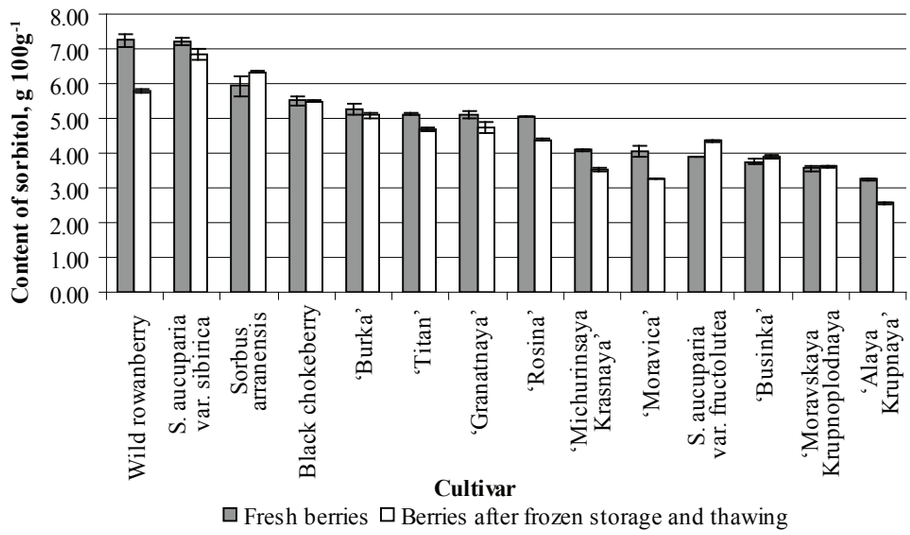

Figure 1. The sorbitol content in fresh berries and berries after frozen storage and thawing. Values are expressed as mean ± standard error.

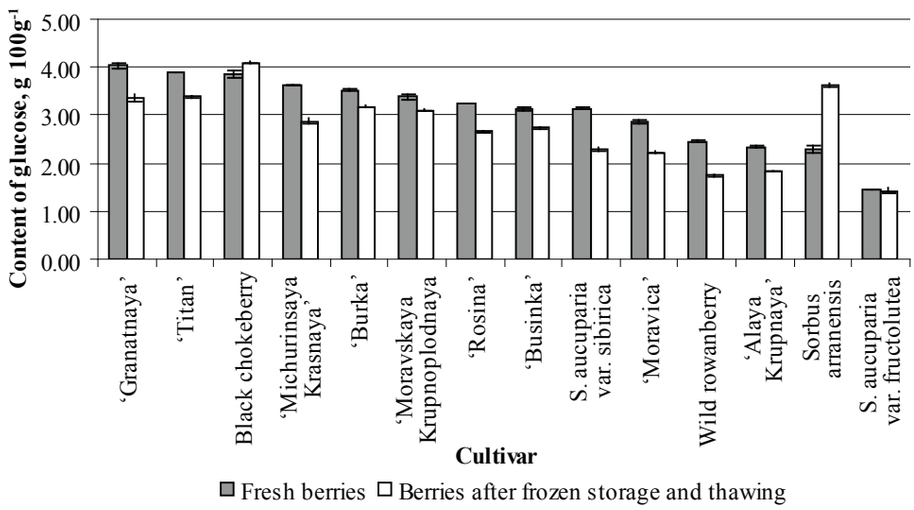

Figure 2. The glucose content in fresh berries and berries after frozen storage and thawing. Values are expressed as mean ± standard error.

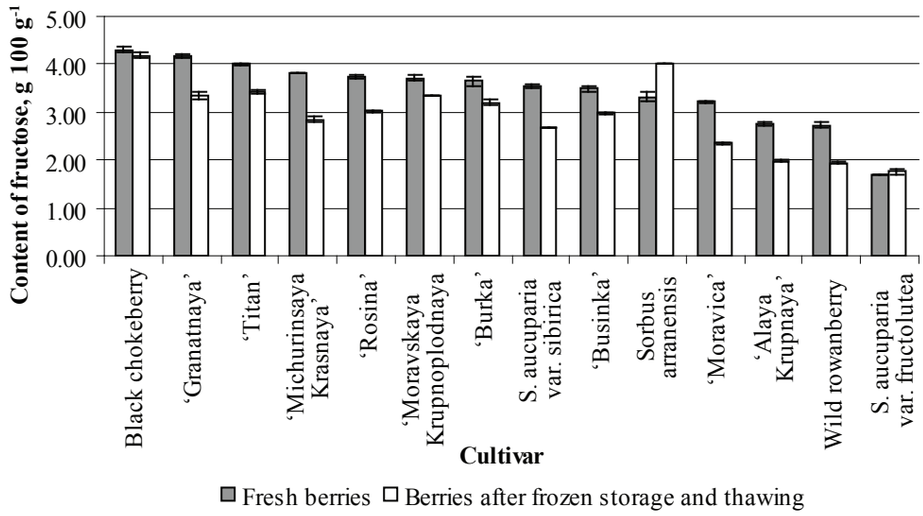

Figure 3. The fructose content in fresh berries and berries after frozen storage and thawing. Values are expressed as mean ± standard error.

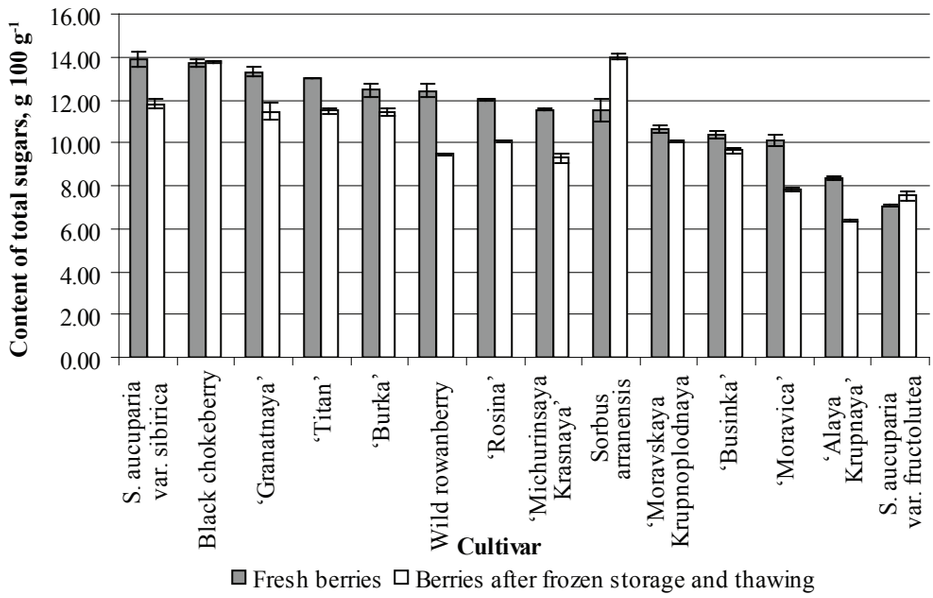

Figure 4. The total sugars content in fresh berries and berries after frozen storage and thawing. Values are expressed as mean ± standard error.

The content of glucose of sweet rowanberries varied from 1.45±0.01 g 100 g^{-1} (*S. aucuparia* var. *fructolutea*) to 4.03±0.06 g 100 g^{-1} (rowanberry×hawthorn 'Granatnaya') (Figure 2), and the content of fructose – from 1.70±0.01 g 100 g^{-1} to 4.17±0.04 g 100 g^{-1} in the same cultivars (Figure 3). Wild rowanberries contained 2.46±0.02 g 100 g^{-1} of glucose and 2.73±0.05 g 100 g^{-1} of fructose, but black chokeberries – 3.86±0.08 g 100 g^{-1} of glucose and 4.31±0.05 g 100 g^{-1} of fructose (Figures 2 and 3). A decrease in the content of simple sugars (glucose and fructose) after frozen storage and thawing with some exclusion was observed.

According to the sum of glucose, fructose and sorbitol, the highest value was obtained to *S. aucuparia* var. *sibirica* (13.89±0.12 g 100 g^{-1}), and to black chokeberry – 13.69±0.32 g 100 g^{-1} (Figure 4). The sum of concentrations of glucose, fructose, and sorbitol among rowanberry cultivars was statistically significant ($p=0.001$).

Different results of the sugar content of rowanberry cultivars (within range of 5.8-7.7%) planted in the Botanical garden of Vilnius University were obtained from Navys (2001), but the author did not report which method was used for analysis. According to the data of Russian researchers, the content of total sugars in cultivars of rowanberries fluctuated from 4.29-9.45% (Петров, 1957) to 9.4-10.2% (Каталог, 2001), and fruits of black chokeberries contained 9.44-13.25% of sugars

(Петров, 1957). Based on these values we noticed that the data of sugar composition were variable. The results reflected that there must be genetic variation among the rowanberry cultivars according to their individual sugars. In addition, the different climatic conditions during the vegetation, geographic zone, and biological features of a variety may also be a reason to affect the sugar composition.

A similar composition of simple sugars and sorbitol was obtained after freezing and thawing of the investigated rowanberries and chokeberries. The results are given in Figures 1, 2, 3 and 4. Generally, the content of sugars decreased during the freezing and thawing period of berries. We observed that the content of individual compounds and the sum of glucose, fructose and sorbitol of berries after frozen storage and thawing was increased in fruits of the rowanberries *Sorbus arranensis*. The differences in concentrations of mean fructose, glucose, sorbitol and the sum of these compounds among the fresh berries and berries after freezing and thawing were found statistically significant ($p < 0.05$).

After the evaluation of the results detected by HPLC method for simple sugars and sorbitol determination, it was established that peaks of glucose and sorbitol were close. Brandao et al. (1980) also have reported that the analysis of fruits of the *Rosaceae* family, where sorbitol occurs in the presence of its parent sugar glucose and other monosaccharides, had proved difficult. The methods of sample preparation were discussed related to the determination of underivatised sugars. For sample preparation and sugar extraction water and samples heated at 60 ºC were used. Whereas Richmond et al. (1981) have reported that water is obviously an excellent sugar extractant, but is non-selective in that polysaccharides and proteins are soluble to different extents. A commonly-used sugar extraction solvent was 80% aqueous ethanol, which provides a good compromise between efficiency and selectivity.

Conclusions

1. The content of soluble solids in fresh rowanberries differed between 12.05 and 25.58 ºBrix. The highest content of dry matter (27.57%) and soluble solids (25.58 ºBrix) was in the rowanberries *S. aucuparia* var. *sibirica*.
2. The results of the research show large variability in sugars and sorbitol composition between different rowanberry cultivars.
3. Sorbitol was found to be the most abundant in all cultivars of rowanberries and black chokeberries, but sucrose was not detectable. The content of sorbitol in fresh rowanberries differed between 3.24 and 7.24 g 100 g^{-1} of fresh weight. The highest content of sorbitol was in the wild rowanberries and rowanberries *S. aucuparia* var. *sibirica* (7.24 and 7.20 g 100 g^{-1}, respectively).
4. The highest content of glucose was in the hybrid of rowanberry × hawthorn 'Granatnaya' and in the hybrid of rowanberry × pear 'Titan' (3.37-4.03 g 100 g^{-1}) in both fresh berries and berries after frozen storage and thawing. The highest content of fructose was 3.36-4.17 g 100 g^{-1} for the same cultivars.
5. Black chokeberries contained 5.51 g 100 g^{-1} of sorbitol, 3.86-4.10 g 100 g^{-1} of glucose, and 4.19-4.31 g 100 g^{-1} of fructose in both fresh berries and berries after frozen storage and thawing.
6. A decrease in the content of simple sugars (glucose and fructose) and sorbitol in rowanberries and chokeberries after frozen storage and thawing with some exclusion was observed.

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PHYSICAL-CHEMICAL EVALUATION OF *SOUS VIDE* COOKED PARENT STOCK HEN BREAST MEAT DURING REFRIGERATED STORAGE

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Abstract. The aim of this study was to evaluate physical and chemical changes in *sous vide* cooked marinated parent stock hen breast meat during refrigerated storage.
Sous vide method is suitable for preparation of ready-to-eat meat products satisfying consumer demand for convenience and safety. Meat tenderness is recognized as important quality attribute of meats.
The study involved preparation of the raw material, vacuum packaging of the products in polyamide/polyethylene (PA/PE) film pouches, marinating for 20 hours, *sous vide* cooking and chilling in ice water, followed by storage at 3.0±0.5 °C. Each sample containing a chicken portion or chicken mix with shredded carrots, sea buckthorn sauce and spices was analysed during storage. Water activity, pH, and moisture were assessed during storage. Hardness (shear force) measurement was used as an instrumental method for meat tenderness evaluation. The differences between two studied products were noted regarding all studied parameters – water activity, pH, moisture content, and hardness. The differences mainly were determined by the properties of carrot and sea buckthorn additive, which generally have higher moisture content and lower pH. The researched parameters were well preserved during refrigerated storage for 28 days.
Key words: *sous vide* cooking, hen breast, hardness, storage.

Introduction

Convenience, improved eating qualities such as tenderness, juiciness, and flavour along with safety and prolonged shelf life have important impact on the consumers' overall judgement of the food product. Extended shelf-life cook-chill foods offer a solution to conflicting requirements - they allow minimal heating to preserve freshness and extended shelf-life suitable for a production line approach (Rodgers, 2003). Vacuum cooking developed in France in the mid - 1970s called – *sous vide* processing which includes vacuum packaging food before applying low (below 100 °C) temperature thermal processing and storing under chill conditions (0 – 3 °C) is considered to offer enhanced quality and extended shelf life (Creed and Reeve, 1998; Armstrong and McIlveen, 2000).

It is widely recognized that meat tenderness is the most significant factor in consumers' satisfaction (Barbanti and Pasquini, 2005; Kong et al., 2008). The improvement of tenderness in meats is mainly caused by changes in structure of connective tissues solubilised by heat, while at the same time heat-denaturation of myofiblrillar proteins generally causes meats toughening (Palka and Daun, 1999). Marinating is a traditional culinary technique that increases water binding capacity of meats thus reducing cooking losses, tenderizing meat and improving juiciness (Brabanti and Pasquini, 2005; Froning and Sackett, 1985; Quiao, 2002).

The aim of this study was to evaluate physical and chemical changes in *sous vide* cooked marinated parent stock hen breast meat during refrigerated storage.

Materials and Methods

Parent stock hen fillets of the cross Ross 308 were used for the study. The average age of slaughtered parent stock hens was 60 weeks; an average carcass weight of a bird was 3.1±0.5 kg. The slaughter and primary treatment was performed at a meat processing plant (line Stork PMT). Parent stock hens were stunned, then killed with a knife, bled for 3.37 minutes and scalded in a steam bath at 60.2±0.2 °C for 3.47 minutes, defeathered, eviscered, and chilled for 100 minutes at 1±0.5 °C. Three carcasses were randomly selected for separating a fillet *[musculus pectoralis]* 1.5 hours after *post-mortem*. The obtained products were packed in polyethylene bags and refrigerated at the temperature of 1±0.5 °C for 24 hours prior to *sous vide* cooking.

Two types of *sous vide* cooked poultry meat products were studied: hen breast fillets marinated in species and wine, and hen breast fillets with shredded carrots in sea buckthorn sauce and spice-wine marinade. Each sample containing chicken portion in size of approximately130 grams and mix of various vegetables, fruits and/or spices was vacuum packed and sealed in a chamber type machine MULTIVAC A 300/16 in polyamide/polyethylene (PA/PE) film pouches (size of 200x300 mm, film thickness – 60 μm) with barrier properties. Prepared samples were marinated for 20 hours, followed by *sous vide* cooking in a water bath "Clifton Food Range". After heat treatment the samples were chilled in ice water and stored at 3.0±0.5 °C.

The following parameters were analysed at the Faculty of Food Technology of the Latvia University of Agriculture on the 0^{th}, 7^{th}, 10^{th}, 14^{th}, 21^{st} and 28^{th} day of storage in triplicates for two randomly selected samples:

- moisture content - by method LVS ISO 1442:1997;
- water activity (AquaLab Lite, Decagon Inc, ASV);
- pH – using 3510pH Meter – JENWAY (Barloworld Scientific Ltd., Essex, UK);
- hardness – by TA.XT.Plus Texture Analyser (Stable Microsystems Ltd., Surrey, England) and software Texture Exponent 32.

For calibration the verification of water activity meter AquaLab Lite a standard a_w 0.984±0.003 KCl 0.5 molal solution was used. The sample for water activity measurement was homogenized prior to measurement. Approximately 2±0.05 g of a homogenized product were placed in a measuring chamber. Analyses were performed in triplicates.

Approximately 50 g of the product were homogenized, and 5 g of the obtained sample was weight in a glass container, adding 50 ml of distilled water for pH measurement. Presented results are the average of triplicate measurement.

The hardness of cooked chicken was determined by the Stable Miro Systems TA.XTplus Texture Analyser with load cell of 50 kg. Samples prior to test were divided in a size to fit on a platform, then positioned under the knife blade to assure cut along the meat fibres. The test speed of knife blade was set at 2 mm s^{-1}. The measurement was triggered automatically at 0.05 N. The maximum force required for sample compression was calculated as an average of 10 measurements.

Results and Discussion

The changes in water activity in the hen fillet *[musculus pectoralis]* were observed during refrigerated storage and are presented in Fig. 1.

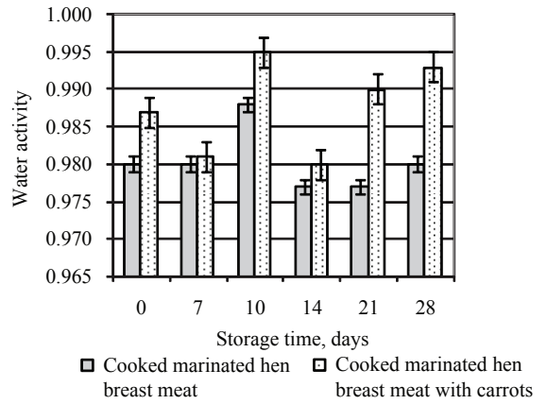

Figure 1. Water activity in cooked marinated hen breast meat without and with carrots.

A comparatively higher water activity was observed in cooked marinated hen breast meat with carrots. At the beginning of the experiment, before the storage of *sous vide* cooked products, water activity in the sample with carrots was by 0.7% higher compared to the sample without carrots, but after 28 days of storage it was by 1.3% higher. It can be explained by higher water activity of cooked carrots and sea buckthorn sauce being added in the sample. This may cause more rapid spoilage of the product although reduced pH 5.2 – 5.5 of the sample due to the added ingredients may have protective impact. When *sous vide* cooking is used, the shelf life is significantly longer compared to the traditional cook-chill technology. Generally, cook-chill products are 'unprotected' having high a_w and pH, low salt content, and no preservatives (Rodgers, 2003).

The tendency of slight increase in water activity was noted by the end of the researched storage period. The product containing carrots did not show consistent measurements during the storage time – at the beginning there was a slight decrease which was followed by an increase.

The pH value in the hen fillet *[musculus pectoralis]* was observed during refrigerated storage and is presented in Fig. 2.

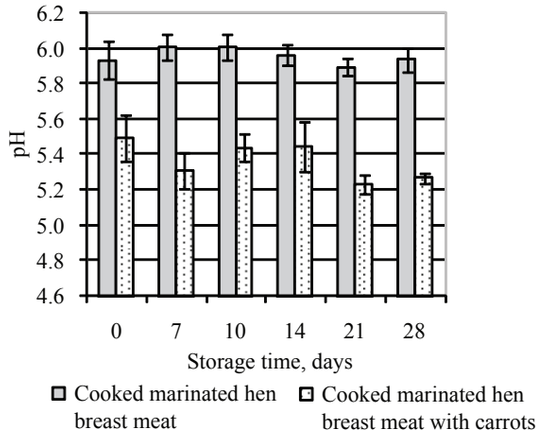

Figure 2. pH comparison between cooked marinated hen breast meat without and with carrots.

In pH in raw marinated hen breast pH was 5.79, and in marinated hen breast with carrots - 5.5. After cooking, the pH value of meat remained approximately at the same level. Product samples without carrots had higher pH. During 28 days of storage, no significant changes were found in pH of the researched poultry products.

The changes in moisture content of the hen fillet *[musculus pectoralis]* were observed during refrigerated storage and are presented in Fig. 3.

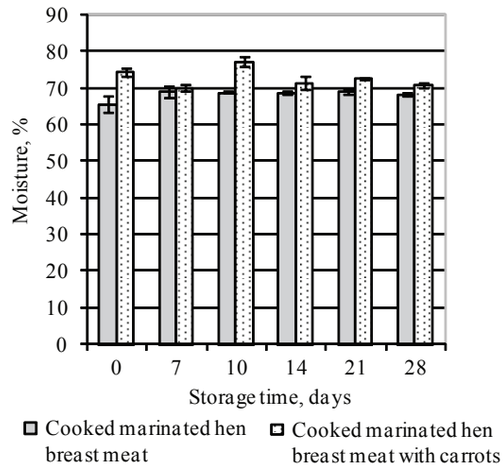

Figure 3. Moisture content in *sous vide* cooked marinated hen breast meat without and with carrots.

Moisture content in *sous vide* cooked marinated hen breast fillet with carrots was higher than in the product without carrots. At the beginning of the storage, moisture content in the sample containing carrots was by 13.5% higher than in the sample without carrots. During the researched storage period, moisture in hen breast meat prepared without carrots was quite consistent – no significant differences were observed. Whereas moisture content in the product containing carrots was periodically increasing and decreasing, although the changes were not significant. It can be explained by the heterogeneity of the sample.

During heating, the different meat proteins denature and cause structural changes in the meat, such as destruction of cell membranes, shrinkage of meat fibers, aggregation and gel formation of myofibrilar and sarcoplasmic proteins, and shrinkage and solubilisation of the connective tissue (Tornberg, 2005). The changes in the hardness during the cooking can be determined by the mechanical rigidity of perimysium in the space endomysium–perimysium, whereas the shortening of endomysium supposes water loss of the muscle (Palka, 1999; Liu et al., 1996). *Sous vide* cooking parameters selected for the current study were selected basing on the previous experiments which provided good product sensory properties - acceptable meat hardness, juiciness, flavour, and colour (Sturmovica et al., 2009).

In the current research, the changes in hardness in the hen fillet *[musculus pectoralis]* were studied during refrigerated storage and are presented in Fig. 4.

Hardness of *sous vide* cooked hen breast meat did not change significantly during the storage period, although tendency of a very slight decrease was observed. The hardness of meat sample containing carrots demonstrated fluctuations up and down, although it was within the range of the standard deviation. A slightly higher hardness was noted in the meat samples cooked together with carrots, although the difference was not significant.

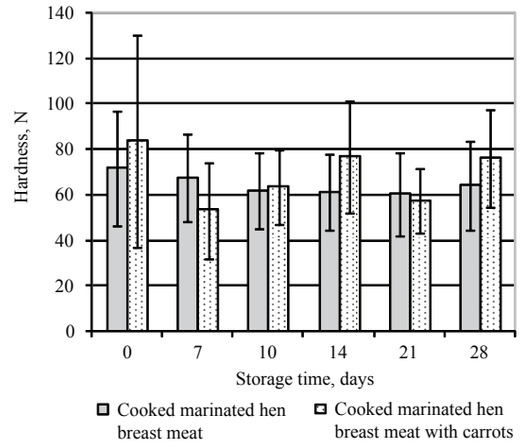

Figure 4. Hardness of the marinated hen breast meat cooked without and with carrots.

Physical-chemical parameters in *sous vide* cooked hen breast meat without carrot additive were better retained during the researched storage time.

Conclusions

In both studied products, differences were noted in all studied parameters – water activity, pH, moisture content, weight loss, and hardness. The differences mainly were determined by the properties of the added carrots and sea buckthorn which generally have a higher moisture content and lower pH. The researched parameters were well retained during the refrigerated storage at the 3.0±0.5 °C temperature.

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PROMISING EUROPEAN UNION SUPPORTED INITIATIVES WITHIN PRIVATE FOREST SECTOR IN LATVIA

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Abstract. The aim of this paper is to evaluate all kinds of experiences with attractive EU funding for Latvia's private forest sector in the past and to generate new proposals for effective acquisition in near future. Latvian institution may learn from these experiences in order to improve their (acquisition) role in project proposals for the private forest sector. Consequently, the relatively underdeveloped Latvian private forest sector will get its highly needed support for better performance, and progress towards other, leading EU forest sectors. The possibilities for attractive financial EU support are reviewed through both historic experiences with the EU's 'Rural Development Program for 2007 – 2013' and the EU grants, designated for forestry projects. An extensive round of interviews is held with state forest owners and experts involved in forest management programmes and other dedicated EU forest funding initiatives. Following the expert interviews, two key indicators are developed to evaluate the support from the EU. Contacts with the EU countries (the Netherlands, Belgium, Finland and Poland) where similar forestry activities have been done as in Latvia were made. Research done in this work is unique as according to the author's information these countries do not have similar research related to the EU funds efficiency and Rural Development Program for 2007 – 2013. The successfulness of Latvian applications for the EU funding is one major indicator for further fund raising. The EU supported investments generally have higher economic results in comparison with their non EU funded counterparts.
Key words: EU funds, private forest owner.

Introduction

The forest resource must be managed in accordance with sustainable principle as well as in state forest and in private sector (Latvian Forest policy, 1998). One of the chances to manage sustainable forest properties is to attract finance from the EU funds in Rural Development Program for 2007-2013. The chance to attract finance for management started in the year 2000 with payments for areas of agricultural lands, afforestation activities and compensations for aridity (Rural Support service, 2008). From the years 2004 – 2006 forest owners had a possibility from the EU funds to set up private forest owner`s organisations for successful management of their properties (Ministry of Agriculture, 2006). Nowadays forest owners have a chance to get finance for forestry activities within the framework of Rural Development Program for 2007-2013. The estimation of used and potentially supported forestry activities from EU funds in the period 2007 - 2013 can give a view in perfection of Regulations issued by the Cabinet of Ministers (Rural Support service, 2008). The support programme of forest economical value improvement as well as afforestation of agricultural lands and activities for infrastructure has been assessed. Many institutions such as State Forest Service Consultation Service centre, Rural Support Service, Forest Department of Ministry of Agriculture in receipt EU funds financing as well as in status of physical and legal persons from the state and private sphere are involved. There are approximately 150 thousand private forest owners in Latvia who are physical people (Latvian Forest Owners association, 2009). Forest policy is influenced by many interest groups which take part in the EU funds finance allocation and estimation including institutions controlling all processes. Information and controlling function is carried out by State Forest Service and Rural Support Service, consulting is performed by Consulting Service Centre and private forest owner`s organisations. At present there are six regulations issued by the Cabinet of Ministers regarding forest activities which should be improved for better approach in issuing forestry projects. The aim of research is to estimate the EU funds for management of forest properties and work out proposals for successful finance acquisition.

Materials and Methods

A theoretical working approach is incorporated for reviewing political documents and laws of Rural development program 2007 - 2013 as well as other applicable EU regulations for the Latvian forest sector. The extra materials of conferences, scientific literature and Latvian newspapers which publish information of forest activities and its results were considered. The principle of SWOT was used to evaluate the mechanism of the EU funds implementation and for working out the proposals of further actions for private forest owners how to issue projects avoiding bureaucracy.

The empirical evidence of results is based on questionnaires of private forest owners and expert interviews with the following state institutions:

1. Rural Support Service;
2. Consultation Service Centre;
3. Ministry of Agriculture Forest Department;
4. Forest management enterprises;
5. Private forest owners, subdivided into
   - Forest owners who submitted projects for the EU funds Rural Development Program 2007-2013;
   - Forest owners who did not submit projects for the EU funds Rural Development Program 2007-2013.

Table 1

Number of respondents in regions of Latvia

Region	Number of forest owners who submitted projects for the EU funds	Number of forest owners who did not submit projects for the EU funds	Number of experts in state institutions	Number of enterprises	Total
Vidzeme	72	6	19	2	99
Zemgale	8	1	7	1	17
Latgale	25	5	11	2	43
Kurzeme	70	4	11	1	86
Total	**175**	**16**	**48**	**6**	**245**

Specific questionnaires were drafted to gain the goal of research. For each interest group there were specific issues according to work specification (see for groups of interest).

1. Rural Support Service experts' questionnaire contains points on main private forest owner's faults in issuing and submitting projects.
2. Questionnaire for Consultation Service Centre was created to find out the major demand of forest activities.
3. Forest Department of Ministry of Agriculture officials were interviewed to enquire problems which were in the process for preparing laws and regulations for the EU funds forestry activities.
4. Questions for forest owners were formed to clear up the reasons for not submitting projects in the EU funds.
5. A special questionnaire was developed for private forest owners who handed in projects to clarify the efficiency of funds financial attraction.

Interviews were done in March, 2009 with experts from Rural Support Service, Consultation Service Centre and Forest Department of Ministry of Agriculture when the first round of EU funds was closed and estimation of projects already started. Rural Support Service centre experts` opinion is important because they accept and evaluate submitted EU forestry projects. Consultation Service Centre is an institution which helps to prepare documents for forestry projects and elaborate field works in property. Private forest owners` opinions were surveyed by anonymous query and by telephone. Forest owners were surveyed in one district of each region of Latvia. The district in each region has been selected according to one main criteria – where the number of most registered forest owners in workshop time was the greatest. Few criteria (age, sex, distribution of forest properties area, occupation) were used for providing forest owners` social portrait. Findings were compared to average private forest owners` ratios and the results of previous researches. The acquired information was used in SWOT analysis principle and in further researches on private forest owners` activities in Latvia.

The number of respondents in each interest group was different because everyone had different work specification. The target audience depended on regions of Latvia distribution, and forest owners 'had to answer questions (Table 1).

The first projects were estimated in January, 2009 by Rural Support Service. Earlier information was not accessible because the EU funds were not opened yet. Finance deficiency was the barrier to get opinion of more forest owners. The active opinions of private forest owner`s were made in seminars and compared with data obtained before in earlier researches. Responsiveness of respondents was low because many people did not want to waste time for questionnaires. There were many inquiries (6%) which were not used in further research because questionnaires were incomplete. Forest owners did not want to spend more than 10 minutes per query; therefore, there were a minimal number of questions in questionnaires.

Results and Discussion

Forest owners` age and gender

Respondents who attended workshops were 85% men and 15% women. Dominating age was 40 - 50, private forest owners of age between 20- 30 and 70- 80 years were less. Forest owners (10%) who submitted project applications for the EU funds forestry activities were of age between 40 -50 years. All submitters were men. The age of potential target audience is between 40 - 60 years. Most of active forest owners were of age 40 - 50 years according to recently research (Vilkriste, 2007). It confirms the author`s findings. Project applications were submitted by forest owners (2- 3%) of age over 65 years. It means that workshops were attended by younger forest owners than average indices in Latvia. Results of author research purport that projects were written by younger forest owners, too. Lots of projects in forest sector were written by men (80%) according to research (Vilkriste, 2007). Consultation Service Centre experts mentioned that a great number of projects were written by men (85%). Even State Land Service (2002) data shows that 56% of forest owners are men in forest owner's structure. Statistic analysis showed that probability is not substantial ($F_{fact.}=2.28<F_{crit.}=4.19$). Project application rejection/ supporting does not depend on respondents` age. There is a need to think about information

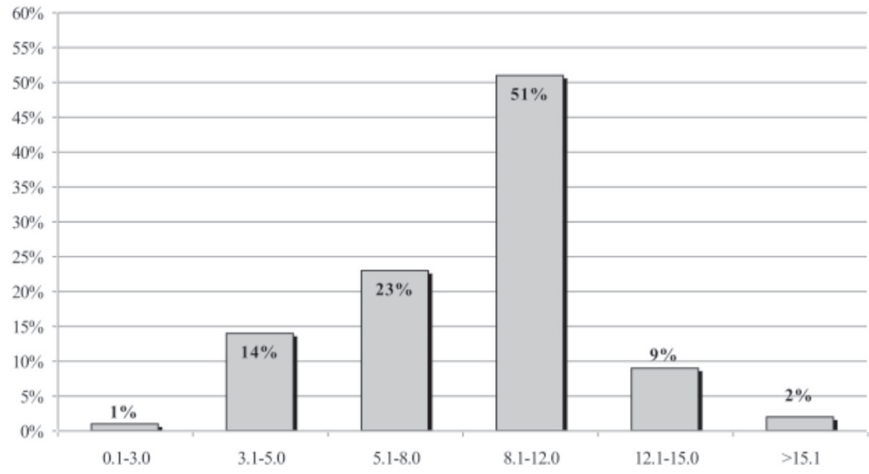

Figure 1. Distribution of respondents` forest area.

channels that can enchain forest owners - women if in future informative campaigns related to the EU funds are organised.

The distribution of forest area in respondents` properties

Those forest owners who are interested in submitting projects for the EU funds property possess areas of 40 - 50 ha. Average area of property is 7.1 ha according to researches (Vilkriste, 2008) but in data basis of State Land Service (2002) - 8.3 ha. According to the author's questionnaire, the average forest property area is 6 - 8 ha. Respondents (89%) had a territory less than 12 ha. Statistic analysis showed that probability is not substantial ($F_{fact.}$=2.05 <$F_{crit.}$=4.19). Project application rejection/ supporting does not depend from respondents` properties area. The difference is not substantial between average forest property in Latvia and territories announced for the EU funds. The area of average forest properties decreased in the year 2004 and 2008 (State Land service, (2004/2008).

The average property area of respondents is 10 ha. Properties areas more than 8 ha are predominant (Figure 1). Approximately 20% of respondents had more than 10 ha of forest in the property.

Forest owners` education

The professions of respondents are the following - doctors (2%), drainage engineers (4%), insurance agents (3%), forest owners (35%) and foresters (48%). Other respondents were pensioners (8%). Respondents` education (35%) is connected with forest management but 65% have other profession. In Latvia 25% people are with higher education according to data of Central Statistics Department (2008). Respondents (75%) have higher education in forest sector (according to the results of author). It means that forest owners with higher education draft and submit projects for the EU funds. Forest owners (25%) address private forest owner`s organisations or Consultation Service Centre to prepare documentation for project application. The number of forest owners without appropriate knowledge in forest management has increased for 12% in seven years time (Vilkriste, 2008). Statistic analysis showed that probability is substantial ($F_{fact.}$=3.48<$F_{crit.}$=1.47). Project application rejection/ supporting depends on respondents` education. The private forest sector owners in Latvia gained back their properties 15 years ago. Other European countries already had forest management traditions and higher level of knowledge in forestry than in Latvia.

Forest owners` motivation to receive EU funds financial support

950 forest owners took part in submitting forestry projects for the EU finance support in the autumn 2008 (State Land service, 2008). The main reason for submitting forestry projects was easily received money from Europe. Other part of forest owners wanted to receive support for the situation improvement in property. Some forest owners would take part in writing forestry projects next time because they had not prepared all documents for the first round of the EU funds. Forest owners are planning activities in the forest by themselves. More than one third of respondents took part in the second round of receiving financial support than in previous one. More forest owners will take part in the third round of the EU funds financial support. Activities of writing projects for the EU funds are low if compared to total amount forest owners in Latvia who are approximately 150 thousand (Latvian Forest Owners association, 2009). Forest owners often want to submit projects for new stands clearing (44%), for buying equipment for forest works (26%) and afforestation of agricultural lands (30%). Lots of forest owners do not have properties in areas where preventive measures need to be taken. Respondents (30%) submitted projects in the first EU funds support round, but 68% private forest owners will issue projects for the next round. Only 2% forest owners will not take part in the EU financing programme for forestry activities (Figure 2).

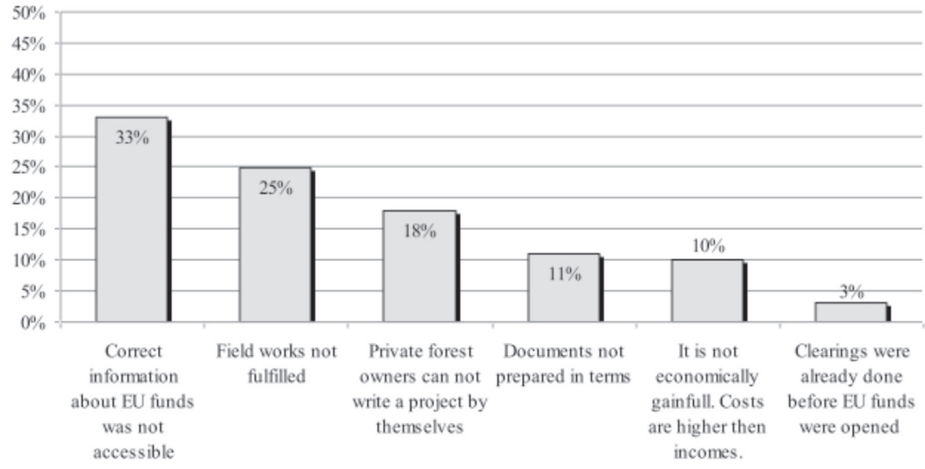

Figure 2. Reasons for not submitting projects in the EU fund.

Almost all private forest owners (99%) stressed that attention has to be paid on most popular forestry activities for the next EU financial years. Financial support had already been allocated from the EU for drought and afforestation from the year 2000 till 2006. Respondents pointed out that aims were not achieved completely (45%) and for the EU funds support in 'Rural Development Program 2007 - 2013' they will not be reached either.

Information sources about the EU funds activities

Information on the EU funds has an important role. It must be not only accessible for forest owners but also easily understandable for each target group. Information about financial projects is available by specialists working in forests and on the Internet. There were enough details to draft projects, but the other part pointed lack of necessary data. Information on submitted documents (34%) and field works (35%) was not enough accessible. The access to the information was proper. Forest owners (39%) got information about the EU funds in workshops and trainings, regional Rural Support Services, newspapers and brochures (Vilkriste, 2007). Greater part of respondents considers that amount of information was enough. Some of them stressed that information was devised for large forest property owners. Information structure has been changed by the author`s obtained data. Most of forest owners would like to get information on TV, media and the Internet (12%) and from employees (78%) working in forest sector. The role of media and the Internet decreased, but importance of consultations increased. The author thinks that employees working in forest sector and providing consultations to forest owners should be educated more substantially, so information on the EU activities will be easily accessible. Focus should be placed on practical issues of receiving EU support if seminars for forest owners are organised. Meetings should be held after working hours to attract active forest owner`s group.

Evaluation of forest owners` knowledge on the EU fund financial support

Forest owners` questionnaire (Vilkriste, 2007) showed lack of knowledge on supported afforestation activity of agriculture lands for the EU funds. 69% of respondents mentioned willingness to afforest non - used agricultural lands, but only 5% did it (State Land Service, 2002). It means that forest owners have potential areas for afforestation, but anyway they do not use accessible EU financial support completely. Forest owners need consultations on receiving EU financial support according to researches (Vilkriste, 2007). Respondents draw attention to problems with unskilled employees and lack of equipment. The author thinks that explanation for low forest owners` activity is their passive actions.

Most popular activity of the EU funds is agricultural lands afforestation where calculated sums for submitted projects are more than granted. Finance is left over in other activities. Leader line occupies afforestation agriculture lands activity because conditions are easy for issuing projects and benefits are more. The author found out that in other activities it is easy to draft projects. For example, 8 documents need to be collected in the activity "Forest economical value" in a short time, but transformation permission which has been prepared in activity agricultural lands afforestation should be received. The forest owner must have forestry education and knowledge on harmonisation and confirmation of documents to issue specific project application.

Every forest owner calculates the expenses and benefits before drafting and submitting project. Financial support receipt from the EU funds for small properties with the forest area less than 4 ha is not profitable. Drafting of project is more expensive than economical benefit from the EU funds. For example, the EU financial programme gives support - 105 EUR ha^{-1}. Submission of the project by forest owners is not financially gainful, if the project application has been issued by Consultation Service

Centre specialists whose cost rate is approximately 85 EUR ha^{-1} with VAT. Thus, after the project confirmation the forest owners do not have enough money to pay for new stands clearing. The author thinks that Consultation Service uncertain service fee is one of the reasons of delay in the use of their specialists. Consultation Service Centre should have be rated for hectare not for hour. Besides, forest owners have to spend time to compile project documentation and visit different institutions in several cities as well as pay extra money for travels and notices. The forest owner should have the start capital if he receives 50% financial support from the EU funds. These are reasons why forest owners do not draft project applications for the EU funds.

EU funds efficiency

Consultation Service Centre employees must issue forestry projects in 98% cases, but only 3 - 4 forest owners drafted applications themselves. All submitted projects of respondents were accepted and supported. The activities in which forest owners will take part in the next rounds of the EU 'Rural Development Program 2007 - 2013' are the following - new stands clearing (35%), buying equipment for field works (34%) and afforestation of agricultural lands (31%). 35% of forests owners assert that support will be received in amount of 50%, but 65% respondents think that support will be collected 100%. There were two main goals to get the EU financial support - to improve situation in property (56%) and just take part in acquisition of funds (44%).

Forest owners estimated the EU funds efficiency from different aspects. Bureaucratic demands and long term of projects review that is almost 5 months by Rural Support Service inner procedures was evaluated as the lowest aspect. The most important reason in bureaucratic process was mentioned that correct and complete information has not been within access. Documents were very hard to collate in time. Immediate help in eliminating mistakes in forest management plan and checking for errors in forest was evaluated as a positive aspect. Rural Support Service specialists instantly discovered mistakes in the project application and helped to prevent them. Many forest owners did not submit applications in time because one month is a short term to do it. It was mentioned as a negative aspect. Part of respondents has already done clearings in their forests without the EU financial support. In general, the EU financial support to forest owners is captivating, but bureaucratic burden is too high. Forest owners assert that bureaucratic demands are high if completed work in the forest has been controlled twice. Few state institutions (State Forest service and Rural Support Service) control submitted area for the EU funds at the same time. Control is performed a few times before and after project application submission. Double control is unnecessary, it only increases administrative costs. It is enough, if foresters carry out control function. Forest owners admit that the situation is understandable and economically non-profitable as the state institution employees working hours and travel costs doubled. Foresters found out first mistakes in the forest when the owners added old area stretches to the project. Stretches should be new related to a suitable area size.

Policy was created especially for a private forest sector. Created politics sectors' activities should be controlled to use offered economical benefits in forestry (Krott, 2005). One of the basic principles defined in Latvian forest policy is forest owners training and consultation. The author thinks that the financial support must be given to a particular competent state institution for trainings of private forest owners. Public relations are hard work (Krott, 2005), and information instruments will be effective only if they hard in sufficient quantity. It is necessary to keep up practical knowledge of forest owners, their competence exchange and increasing society cognition (Broussard, 2008).

Even pessimistic forest owners assert that the EU finance is important and requisite according to research in the year 2008 (Vilkriste, 2008). Forest owners who already had positive cooperation with the EU funds mentioned that the EU funds are a great chance to invest capital in property. Many forest owners have used EU financial support and are planning to do it in future as well.

The most effective acquisition of money funds will be in case if finance is given to small forest owners who do afforestation of agricultural lands. The result will be better if financial support is not given to wood processors and businessmen (Bertomeu, 2008). Acquisition of the EU funds will be more effective, if one of political directions could be providing necessary information for forest owners. Every appropriately trained forest owner will pass his knowledge further to other person (Smorfitt, 2008).

Consultation Service Centre specialists` opinion

In Consultation Service Centre half of employees' duties pertain project drafting and consulting private forest owners on the EU financial support. Private forest owners have different attitude to the EU financial support; therefore, it was important to understand positive and negative aspects in projects issuing. Forest owners' attitude was positive in the following several aspects:

- If forest owners are interested to get maximal benefit from the EU funds (23%);
- If forest owners draft project themselves (30%);
- If forest owners give right data for project writing which are not checked (28%);
- If forest owners give the recommendation to other forest owner on Consultation Service Centre employees well done work (19%).

Consultation Service Centre employees' stress that there should be issued (86%) unnecessary information in projects. There is a problem to get information on attached documents (2%) as well as private forest owners do not want to put efforts to improve project application. The most popular forest activities are the following - new stands clearing (35%) and buying

equipment for forestry works (32%). Project drafting and evaluating time have been noted as a typical mistake. Private forest owners did not have time to come and speak with Consultation Service Centre employees. Sometimes forest owners' information was wrong. Consultation Service Centre employees think that chances to get the EU financial support is 50% because forestry project writers are different and those respondents whose project documents have already been issued today will prepare them right. Some submitters had problems to fill in project applications themselves. Consultation Service Centre employees (52%) think that more attention should be paid to afforestation of agricultural lands and preventive measures, if they draft projects for the EU funds. The most popular projects' declining reasons were the following: wrongly filled in application forms (53%), lack of project documents (28%), insufficient EU finances (11%) and inobservance of projects preparation and submission conditions (8%).

For the consultant it is important to understand, whether the forest owner just wants to receive financial support from the EU and will not perform any activities in the forest or it is just the opposite. Consultation Service Centre project drafters' stress that the forest owner wants to get maximal finance support to his property in any case. Most forest owners want to use all accessible forestry activities according to the EU funds, Activity of project issuing depends on the number of forest owners in districts and the size of property. There is conformity between the number of forest properties and an average size of property. No projects for this reason in Jelgava and Dobele districts have been drafted, but the level of activities in Saldus, Kuldīga and Cēsis districts has been high.

Bureaucratic demands are high in receiving the EU support. Even foresters assert that the number of documents is too large. For example, project description should not be issued because all actions have been already mentioned in the forest management plan. Consultation Service Centre specialists should often consult forest owners and use service fees of piece-work that is common nowadays. The author suggests that the first hour of consultation could be free of charge and you get some brochures on the EU funds. Support is profitable for large property forest owners, if they have decided to submit project for the support from the EU funds. In this case it is profitable to have consultants service which is of high quality in Consultation Service Centre. Every project submitter must evaluate financial situation himself, and decide whether he needs consultant service. As small forest property owners admit, they do not use the service of Consultation Service Centre specialists in preparing documents and drafting projects due to their high costs. Research of market and social institute was performed in the year 2008. Forest owners (49%) used third person's service in the following activities: in harvesting use 29%, new stands clearing - 28% and consultation for forest management activities - 2% (Market and social research centre, 2008). Forest owners trust state institutions as advisers, but finally, the owner is one who is the decision maker (Vilkriste, 2008).

The opinion of Forest Department of Ministry of Agriculture, Rural Support service and State Forest Service

Foresters were asked to give most common private forest owners failures in field works. Relevant faults were the following: wrongly marked forest area (20%) and incorrectly collated forest management plan (23%). Forest owners did not understand regulations of the EU funds correctly. Marked forest area should not be related to State Forest service data basis. Respondents could mark the area in the forest which they wanted to submit for the EU financial support.

Forest department of Ministry of Agriculture respondents as a negative aspect mentioned that activities of the EU funds have been opened later than it was planed.

Most popular problems were wrongly calculated support sums (72%) in submitting projects. Forest owners did not want to do field work in forest just receive the money from the EU funds (15%). The information was wrong (7%) in projects and areas for clearings pointed out incorrectly (6%). Rural Support Service specialists turned down project applications and asked to recalculate sums of submitted areas for the EU funds financial support. The author thinks that Rural Support Service specialists must do calculation for submitted areas. Documents have been accepted firstly in regional Rural Support Service and secondly, the central administration reviews all projects in details. Lack of documents in project appeared in checking the document pack for the second time. The author thinks that Rural Support Service employees should demand notes for projects to other state institutions by themselves.

Conclusions

1. Supported directions in forestry are shown in Rural Development Program 2007 - 2013. As prior activities, forest economical value improvement and afforestation of agricultural lands are set out.
2. For project processing teaching aids and guidelines were made, but applications still had mistakes related to wrongly calculated forestry areas and lack of complete pack of important documents. After dedicated support, Rural Support Service needs additional time to correct information. Calculation of the project should be done by Rural Support Service, based on feedback from private forest sector.
3. In most cases forest owners do not understand EU funds conditions and criteria of appropriate area choice.
4. Lack of experience in submitting proposals was a major reason for rejection of proposals. It makes project costs higher and project issuing unprofitable.

5. Cooperation and information exchange should be promoted between different Latvian institutions (less documents).
6. Highly active focussing groups are those of forest owners having higher education, aged 40 -50 years, close to their properties (areas>10 ha). Specific forest ownergroups should be formed. They could improve knowledge and expertise in workshops and seminars after working time.

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APPLICATION OF DISCOUNT RATE IN FORESTRY

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Abstract. Appropriate discount rate for forestry is still an issue for the forest economy. Therefore, it is significant to understand the importance of discounting, and how costs of forestry production process are valued. Forest regeneration and new stands tending costs are considered as investments; they are related to each particular stand. For that reason, the goal of this study was to compare how the value of this investment is growing at the bank deposit or 1 ha forest stand. The study examined the growth rate of two different tree species value with young forest stand establishment and tending investments depositing in the bank or the growth rate of compound interests. As discounting formula derived from a compounding formula, it would allow to find an appropriate discount rate for each timber species. High rates of compound interest cause a very rapid and unrealistic future value increase, while low rates cause slow value increase for a very long period represented by a common forest rotation; for that reason high discount rates used in forestry are not rational. Taking into account an income from intermediate cuttings, the value of timber species in comparison with the deposit significantly improves. Suitable discount rate determination in forest assessment is mainly determined by the species of wood productivity and cutting age. As it is hard to find two identical forest properties, each situation should be considered individually, taking into account and understanding the relationship between a growth rate of timber and compound interests, thus choosing an appropriate discount rate.
Key words: compound interest, discount rate, growth rate of standing timber value.

Introduction

Forestry often has a relatively long production cycle, and operations of a forest business require certain investments. Investor, when investing money, wants a certain return from capital because the value of future capital is completely different compared to money which is received today. Investor deposits certain amount of money in a bank at fixed interest rate, and at a definite number of years would obtain increase of initially invested sum, but how large interest of profit could be gained by investing in forestry, remains a question for discussion.

To calculate the future value of the amount invested, the compounding formula is used, while in the present capital value calculations from expected future income discounting is used; the interest rate used in the calculation is called the discount rate. Discounting formula can be derived from the compounding formula. Discounting reduces the future value, while the compounding creates the opposite effect (Pearse, 1990).

Different opinions about determination of the discount rate are found in the literature. In New Zealand Forest Valuation Standards (Anonymous, 1996) it has been argued that a rate of zero should be applied when assessing forestry investments, while rates in excess of 20% are also found. It is difficult to find a consequent use of the discount rate because one investor may conservatively project no stumpage-price increase and use a low-risk discount rate while other may project optimistically high prices using a large discount rate to reflect the resulting risk. Another approach is to add a 2 or 3 percentage-point risk premium to the average real risk-free long-term government bond interest rate (Klemperer, 1996). The regime of lower discount rate is more suitable to forestry than to other investments - the high discount rates are typical for short-term projects, but the discount rates in the long-term projects gradually decrease (Price, 2002; 2005). While in researches and reports available in Latvian language, Iesalnieks (2001) is the only one who notes that discount rate should be from 2-5% and emphasizes that discount rate larger than 10% is unrealistic in long term view in forestry, but this issue has not been analysed deeper. The author failed to find any straight justification for the most appropriate discount rate determination in written records available. In European Commission (1999) the results of European forest valuation are published by European Framework for Integrated Environmental and Economic Accounting for Forests. In this document it is stated that the discount rate may be equal to the natural growth rate of standing timber without any explanation how to determine this natural growth rate or without any argumentation why such a size of discount rate is chosen. Brukas et al. (2001) analyzed the potential impact on Lithuania forests and economy of adopting different discount rates and the classical Faustmann approach for pine, spruce, oak, birch and aspen. The main conclusion of this research is that adoption of discount rates values above 5% would make forestry unprofitable. It was noted that profitability could affect price changes and choice of species (faster and slower growing) and site type. The above mentioned results from other authors proof of the difficulty associated with the choice of discount rate in forestry.

Therefore, the aim of this study is to compare the

growth rate of different tree species value with the growth rate of compound interests. As the discounting, that reduces the future value, resulting from the compounding formula, and then using the compound interests, which increase or 'grow up' cash value, just as timber is growing, we can find a suitable discount rate for each tree type that meets the maximum growth rate of timber achieved by standing timber.

Materials and Methods

In the research, the data obtained from the forest management planning information system 'Forest Expert' developed by the Latvia University of Agriculture, Forest Faculty, were used. 1 ha of pine and birch stand total stock and its possible structure of assortment and value every 10 years until cutting age is reached or when it is cut at clear cutting was modelled in this program. In this information system prices of assortment (roundwood - large, medium, thin; pulpwood and firewood) and costs were used such as they were in 2007 according to the Latvia University of Agriculture price monitoring data. The intermediate cuts and their intensities were estimated according Dubrovskis (2007) specially developed equations in the program 'Forest Expert' maximizing forest stand expectation value. In the Excel the particular tree species theoretical value was modelled, calculated by the above mentioned information system and certain amount of money necessary for particular species regeneration and tending in 1 ha, depositing at a bank at interest rates from 1-10%.

Term of deposit was chosen when it is possible to harvest the timber species according to cutting regulations in Latvia that is for pine - 101 years, and birch - 71 years. In the calculations and graphs for convenience the period for all wood species has been reduced by one year. In Latvia five site indexes are used for characteristics of forest stand productivity 1st (highest) to 5th (lowest). In this research, in each chart the growth rate of tree species was examined just by two site indexes – one with a higher site index (1st) and the other with the worst site index (3rd). To calculate the total amount interest added which is obtained after depositing an amount necessary for the 1 ha stand creation and tending for a certain period, the formula described by London mathematical practitioner Richard Witt in 1613 was used (Lewin, 1970) (1):

$$V_n = V_0 (1 + r)^n \quad (1)$$

where, V_n – sum, which is the return with interest;
V_0 – credit sum, which has been invested;
n – number of years;
r – interest rate in decimal.

In calculations the deposit at the bank with payment available at maturity and with the capitalisation of interest rate is assumed, that is, the interest is calculated not only from initial deposit, but also compound interest on the earned interest, with 1-3 money withdrawals, which correspond to the intermediate cuts of particular species, to ensure comparability in terms of revenues. Investments in 1 ha spruce or birch stand were estimated according to the author's surveys for services of Latvian enterprises – soil preparation, plants and planting costs, new stands tending.

Results were summarized in Excel table as well as systematised and analysed. The scientific method of induction and deduction was used. The scientific induction method was used in order to make general statements or coherences from separate facts, while the deductive method was used to draw conclusions, systematise and theoretically justify the author's personal research.

Results and Discussion

According to the theory, low interest rates cause a very slow increase and may be applied for some reason to the investments that run for a very long period. High rates cause a very rapid increase, which may quickly pass the bounds of possible attainment in practice. As the timber grows slowly in the long term, then theoretically the growth rate of timber curve should correspond more to low interest rates. Therefore, we would examine further the size of the deposit at different interest rates and the grow rate of various timber species value in the graphs are.

Figure 1 represents the pine with 1st site index and pine with the 3rd site index value until cutting age as well as 600 LVL deposit growth at different interest rates, which is identical to the amount required for the creation of 1ha pine stand (soil preparation – 70 LVL, planting – 80 LVL, new stands tending – 90 LVL, plants – 360 LVL). In this case, the pine with the 3rd site index is possible to be cut at 90 years according to diameter. As it can be seen from the graph, the pine with the 1st site index reaching the age of cutting in terms of value is slightly above 3%, while the pine with the 3rd site index practically crosses with the 3% curve. This figure does not take into account the revenue from intermediate cuts in relation to deposit, so in the next figures changing relations between the pine with the 1st and 3rd site index and deposit would be examined.

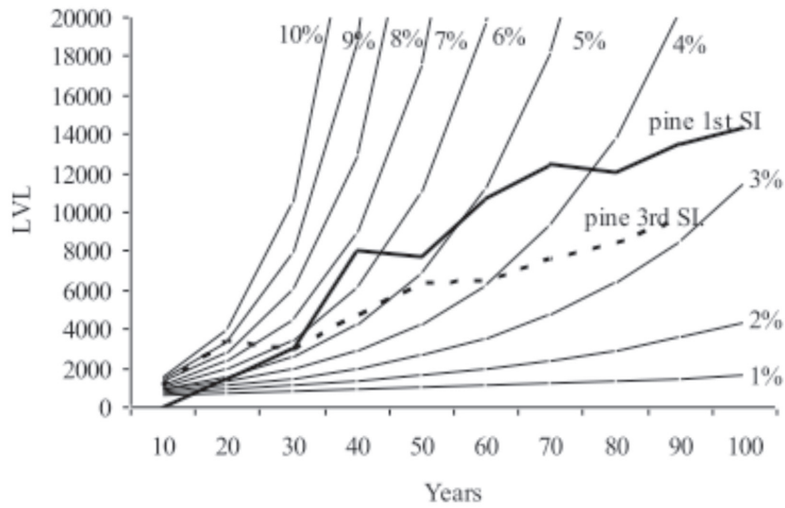

Figure 1. The growth rate of pine with the 1st and 3rd site index (mentioned as SI) value in comparison to the growth rate of 600 LVL deposit.

It is possible to realize up to three intermediate cuts in the age of 20, 40 and 70 years for the pine with 1st site index, there is possibility to obtain income before the clear cut. Thus, from 600 LVL deposit in the bank, which is identical to the sum for 1 ha pine young forest stand development, there should be withdrawal of the same size of the sum that is income from intermediate cuts in 20, 40 and 70 years. In Figure 2 the correlation between the pine with the 1st site index value and 600 LVL deposit at different interest rates, taking into account the income from intermediate cuts is represented. As a result, in 20 years the sum in the deposit would decrease, in 40 years from the deposit at interest rates from 1-4% it is not even possible to withdraw such a large amount, as could be gained from the second intermediate cut for the pine with the 1st site index, because the deposit 600 LVL at the 1% interest rate would be 732.11 LVL in 20 years. If it is a withdrawal of 212 LVL as much as it is obtained from the first intermediate cut, then the sum of 520.11 LVL would remain in the bank account. In 40 years income from the second intermediate cut is 2753 LVL, but bank deposit at the 1% interest rate has risen only till 634.64 LVL. If such an amount of money were withdrawn, nothing would remain in the bank account, but the value of the standing timber would continue to grow. Similarly, it is also with a deposit at interest rates of 2, 3, and 4%. As a result, the pine with the 1st site index value is well above the deposit at interest rate 5%. Pine with the 1st site index at age of 70 years crosses with the deposit's curve at 6% and reaches the equivalent value.

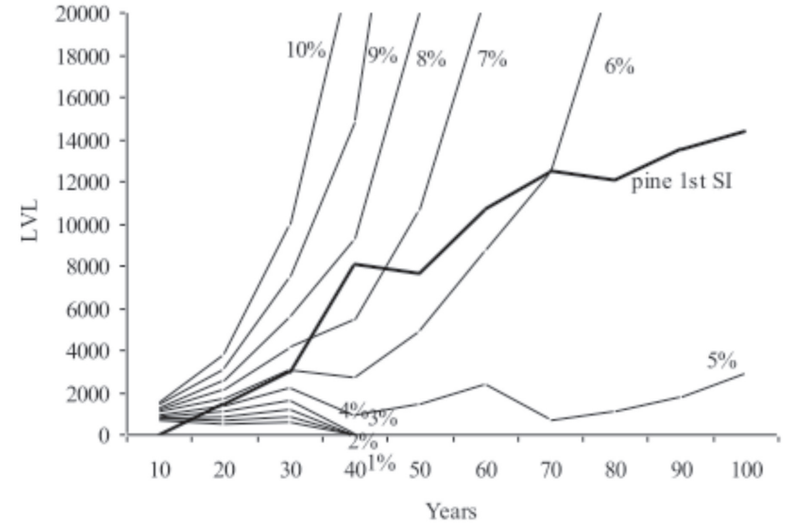

Figure 2. The growth rate of pine with the 1st site index value in comparison to the growth rate of 600 LVL deposit (including intermediate cuts).

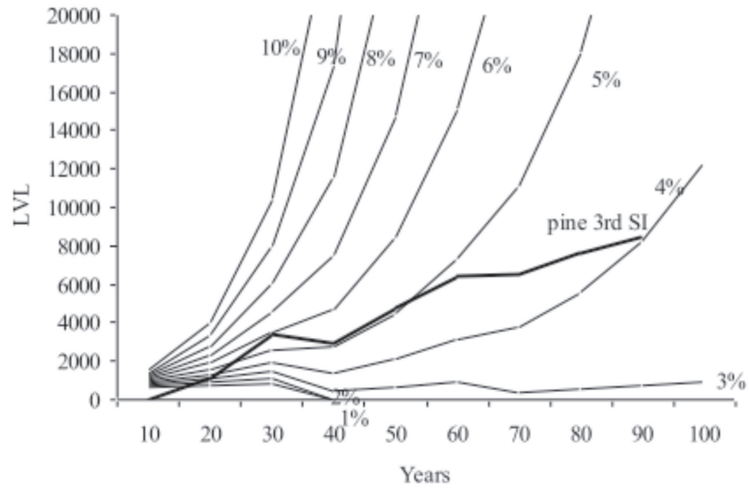

Figure 3. The growth rate of the pine with the 3rd site index value in comparison to the growth rate of 600 LVL deposit (including intermediate cuts).

While for the pine with the 3rd site index (see Fig. 3), which has two intermediate cuts in the age of 20 and 70 years, crosses with deposit curve at 4% when it has reached its clear cut. There for increasing its value by 1% compared to the situation where the intermediate cuts are not taken into account.

Figure 4 shows the birch with the 1st site index and the birch with the 3rd site index value till cutting age as well as 480 LVL deposit growth at interest rates from 1-10% which is identical to the amount required for the creation of 1ha birch stand (soil preparation – 70 LVL, planting – 80 LVL, new stands tending – 90 LVL, plants - 240 LVL). As it can be seen from the graph, the birch with the 1st site index reaching the age of cutting crosses deposit curve at 4%, while the birch with the 3rd site index is slightly above the 2% curve. This figure does not take into account the revenue from intermediate cuts in relation to deposit. Further it would be examined the change of the value of birch with the 1st and 3rd site index if the above mentioned income is taken into account.

It is possible to perform up to two intermediate cuts in the age of 40 and 60 years for the birch with 1st site index. Thus, from 480 LVL deposit in the bank, which is identical to the sum for 1 ha birch young forest stand development, the same size of the sum that constitutes income from intermediate cuts in 40 and 60 years should be withdrawn. As a result (see Fig.5), in 40 years the sum in the deposit would decrease, but at interest rates from 1 and 2% it is not even possible to withdraw such a large amount as it was obtained in intermediate cuts. In 60 years from the deposit at 3% interest rate it is also impossible to remove such a large amount, as derived from the second intermediate cut for the birch with the 1st site index. As a result, the birch with the 1st site index value is above the deposit rate at 5%.

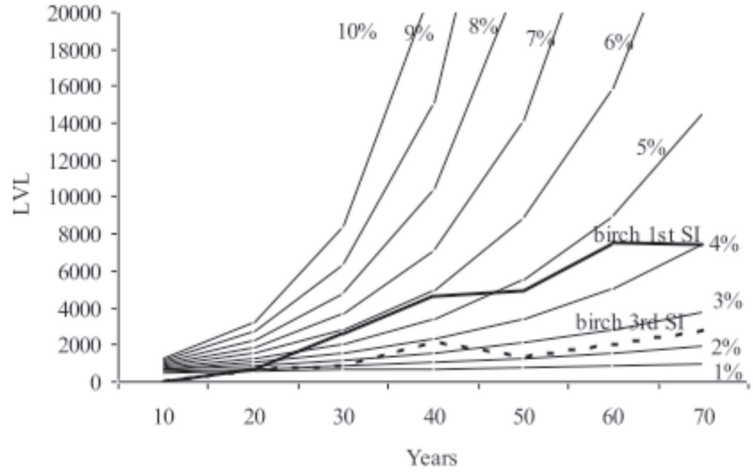

Figure 4. The growth rate of a birch with the 1st and 3rd site index value in comparison to the growth rate of 480 LVL deposit.

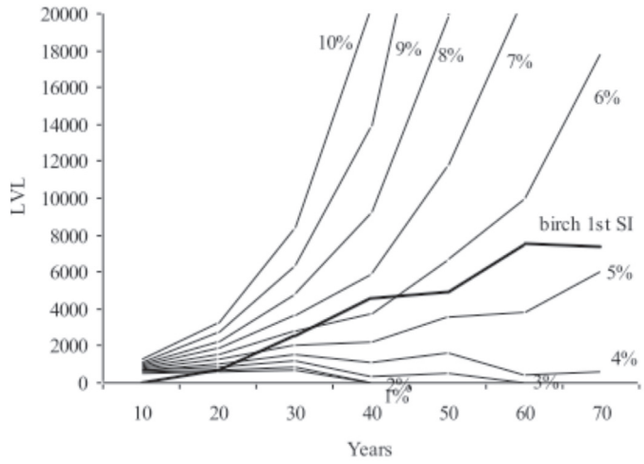

Figure 5. The growth rate of a birch with the 1st site index value in comparison to the growth rate of 480 LVL deposit (including intermediate cuts).

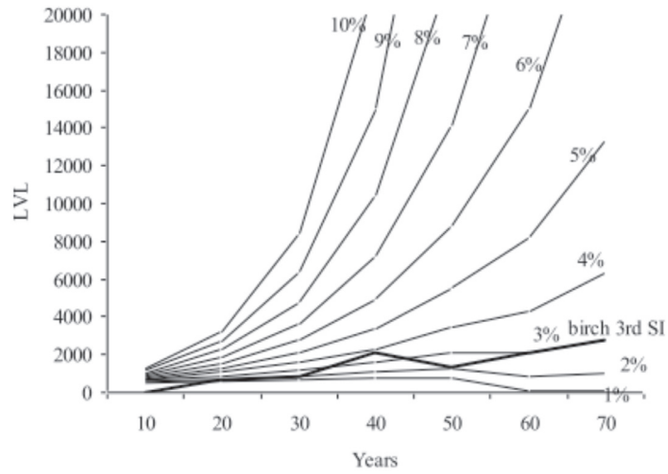

Figure 6. The growth rate of a birch with the 3rd site index value in comparison to the growth rate of 480 LVL deposit (including intermediate cuts).

The birch with the 3rd site index in Figure 6 having one intermediate cut in the age of 40 years, crosses with deposit curve at 3% (reaching the same value).

According graphs analysis it can be concluded that the growth rate of timber and the growth rate of compound interests are different, if the timber is growing quite steadily in its all life cycle while at the beginning compound interests are growing very little and then there is a sharp jump. The lower interest rates increase very slowly and slightly; while high interest rates increase very fast (see Tab.1.).

Table 1

The growth rate of a birch compared to the growth rate of 480 LVL deposit at 5 and 10% (including intermediate cuts), LVL

Years	480 LVL at 5%	480 LVL at 10%	birch with the 1st site index value*
20	1273.58	3229.20	696.37
30	2074.53	8375.71	2567.43
40	2165.19	20510.44	4601.38
50	3526.87	53198.81	4887.43
60	3771.91	136011.00	7523.62
70	6144.00	352777.51	7394.26

* Source: 'Forest Expert' data

For example, if we discount the birch with the 1st site index the value of which is reaching its cutting age of 70 years is 7394.26 LVL with discount rate 5%, we would obtain the value of standing timber 1040.46 LVL at 20 years. This almost reaches the growth rate of 480 LVL which at this period would be 1273.58 LVL. While discounting with the 10%, we would get the value of timber 62.99 LVL in 20 years that is less than growth rate of 480 LVL which is 3229.2 LVL. We are sharply decreasing value of standing timber when discounting with large interest rates, for example, investments in 1 ha birch young stand development and tending value in 70 years discount to 20 years with discount rate 10%, it decreases 117 times, while the natural growth of birch value has increased only 10.6 times during the same period.

If we discount the values of above mentioned timber species with lower interest rates, they would exceed the growth rate of deposit up to a certain size of interest rate, but for larger interest rates, the value would be much lower because it does not meet the growth rate of timber. Therefore, choosing the size of discount rate, the future revenues from the timber, as well as from alternative investments in order to choose the most appropriate discount rate in each specific situation should be assessed. As it is hard to find two identical forest properties, then each situation should be considered individually, taking into account and understanding the relationship between growth rate of timber value and a growth rate of compound interests, thus, choosing an appropriate discount rate.

This research confirms other authors' assumptions and calculations that discount rate larger than 5% would make forestry unprofitable business. Also, with this research it has been proved that size of discount rate could affect choice of species and species productivity which depend on site type. It also should be noted that the research was performed taking into account timber prices when they were quite high in the market; in addition, the changes in the market standing timber assortment prices and total value of stand would change and that can reduce the interest rate. In the calculations the average interest rate for the entire period was used because the growth rate is different in various stand ages. Further research in this field is necessary to exactly justify the discount rate size for each tree species.

Conclusions

1. It can be concluded that if the income from intermediate cuts is taken into account, it significantly improves the value of particular timber species in comparison to the deposit.
2. Examined species correspond to the growth rate of deposit at interest rates which are from 2-5%.
3. Low interest rates correspond more to the growth rate of timber although the compound interests and timber growth curve dynamics is different; therefore, the appropriate discount rate should be chosen.
4. In the discount rate selection dominant tree species, their yield performance and cutting age, as well as the risk characteristics which is not examined in this study should also be taken into consideration.

Acknowledgments

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CHARACTERIZATION OF NATURALLY AFFORESTED FARMLANDS IN LATVIA

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Abstract. Reduction of agricultural production has led to abandonment of farmlands in Latvia. According to the Central Statistical Bureau, 1.4 million ha of lands, including 0.6 million ha of farmlands were set aside in 2008. Most of them transform into forests; however, information about the afforestation has been limited until recent years. The first field measurement based evaluation of forest stands on abandoned farmlands was implemented within the scope of the National statistical forest inventory (NSFI). The NSFI covers forests, farmlands, settlements, wetlands and other lands, providing valuable information about the land use and forest resources. According to the NSFI, total area of naturally afforested farmlands is 257,850 ± 3,606 ha with growing stock of 2,870.364 ± 239,088 m³. Naturally afforested lands occupy 4% of the total country area. The highest share of naturally afforested lands is in Ludza (11% of the total area) and Krāslava districts (9% of the total area). Birch (Betula pendula Roth) and grey alder (Alnus incana (L.) Moench) are the most common dominant tree species in naturally afforested areas (77,092 ± 1,861 ha and 40,285 ± 1,395 ha, respectively). In relation to the Kyoto protocol it is important to separate lands afforested before and after 1990. The total area of lands afforested after 1990 in Latvia is 170,890 ± 2,862 ha with total growing stock of 1,367.427 ± 125,482 m³ and annual increment of timber volume – 122,530 ± 10,513 m³.
Key words: naturally afforested farmlands, stand characteristics, management options.

Introduction

Forest inventory is the systematic collection of data and forest information to follow up forest growth and get an estimate of the value and possible uses of the timber. Important things to measure and note during forest inventory are: species, diameter at breast height (DBH), height (H), site type, age and defects. Calculated data are the number of trees per ha, the basal area, the volume of timber wood in an area, and the value of the timber. Other reasons for inventories are determination of potential fire hazards, forest health conditions as well as evaluation of biodiversity (Brack, 1997). The forest inventory originated in Europe in the 18th century out of a fear that wood (biofuel) would run out. In the early 19th century, foresters estimated the volume and stocking of small forests by running their eye over it. Larger forests were divided into smaller sections that were individually estimated by the same method. Development of forest inventory techniques brought new relationships between DBH, H and volume. By the end of 19th century, the surveys were conducted through sample-based methods involving statistics. In the 20th century, the statistical method of sampling became well established and commonly used, and better methods, such as unequal probability sampling, came in practice. As soon as computer technologies became available, sampling and assessment became more efficient and the results – more convenient to use (Brack, 1997; Zviedre, 2007).

Today, the most common type of inventory uses a random sampling technique, which groups similar forests into one category based on age, stand structure, species, and location. The next step is measurement of equally distanced circular plots. There are several different types of plots, but the most common types are fixed and variable radius plots. In a fixed radius, the forester finds the centre of a plot and measures every tree within a certain distance away from that point. Variable radius plots are used for inventory of volume. Using this method, an angle is created and projected from the centre of the plot, and all trees that are larger than the projected angle are measured. Today advanced GIS and remote sensing technologies come into forest inventory practise, providing more data about the stand and timber quality and reducing costs of the inventory (Kepner and Edmonds, 2002).

Traditionally, forest information has been collected through user-driven national forest inventories (NFIs). The Nordic countries began sample-based inventories in 1920s, whereas some countries began them early in the 1980's or 1990's. Today, the sampling inventories are carried out in most of the European Union (EU) member countries; however, the tradition in Eastern Europe has been gathering data by aggregating stand inventories originally designed for management planning purposes. Many of the Eastern European countries have recently changed their system towards statistically sampling based NFIs or maintain both systems (Brack, 1997).

The National Statistical Forest Inventory (NSFI) was introduced in Latvia in 2004 in accordance with Regulation of Cabinet of Ministers of Republic of Latvia No. 169 (15.04.2003.) 'Regulations on State Forest Register' (Anonymous, 2003). 'The methodology for the performance of the forest statistical inventory and calculation of secondary parameters of a forest stand' is approved by The Minister of Agriculture (Instruction No. 10 from 17.03.2004.) (Anonymous, 2004). NSFI is performed by The Latvian State Forestry Research Institute 'Silava' (Anonymous, 2007).

The aim of the NSFI is to get quick and precise information about forest resources to satisfy needs of national and international statistics, to control dynamics of forest area, to get precise information about structure and dynamics of wood resources, to

evaluate effectiveness of usage of resources and forest ecosystem and accumulate historical information about development of forest stands. Simultaneously continuous control of the whole land area of the country is performed to ensuring observation of the dynamics of land property and evaluation of naturally or artificially afforested land (Anonymous, 2006). The first round (5 years) of the NSFI was completed in 2008 providing a complete picture of forest resources in Latvia including exhaustive information about the actual afforestation rate. Results of the inventory which relate to afforestation in Latvia are published in The Latvia's National Inventory Report Under UNFCCC and the Kyoto Protocol (NIR, 2010).

The Kyoto Protocol is an international agreement linked to the United Nations Framework Convention on Climate Change (UNFCCC). The major feature of the Kyoto Protocol is that it sets targets for 37 industrialised countries and the European community for reducing greenhouse gas (GHG) emissions to an average of 5% against 1990 levels over the 5 year period between 2008 and 2012. Latvia has to reduce GHG emissions by 8%. Under the Treaty, countries must meet their targets primarily through national measures. However, the Kyoto Protocol offers an additional means of meeting their targets by 3 market-based mechanisms from which Emissions trading or 'the carbon market' is the most visible nowadays. The Article 3.3. of the Kyoto protocol notes that the net changes in GHG emissions resulting from direct human-induced land-use change and forestry activities, limited to afforestation, reforestation and deforestation since 1990 shall be used to meet the commitments under this Article of each Party included in The Annex I of the Kyoto protocol (Anonymous, 1998). In other words, countries limited by current accounting approach in Land Use, Land Use Change and Forestry (LULUCF) sector ('gross – net' method with 3 % CAP on forest management related GHG) marked in the decision 11/CP.7 (Anonymous, 2002) can account all removals of CO_2 due to afforestation. Latvia has very small CAP (6.23 mill. tons of CO_2 in 5 years period); therefore, afforestation is not a key issue in GHG accounting in the first commitment period; however, the role of these lands can increase in following commitment periods due to maturing and harvesting of forest lands being forests before 1990 (Anonymous, 2010).

The scope of the study is evaluation of naturally afforested farmlands, including area, distribution, growing stock, dominant tree species and forest site types as well as to estimate the climate change mitigation potential of the afforested farmlands.

Materials and Methods

The NSFI database summarises the results of calculations of the secondary forest inventory data (such as weighted average diameter at breast height and height of trees, basal area, number of trees per ha, growing stock and increment) in every NSFI plot and sectors of plots.

The first step of calculation is a selection of plots and the sectors belonging to the 62nd and 64th NSFI's internal categories of land use, respectively, afforested farmlands and forests on farmlands (1 470 records in total). The second and the most important step of calculation is estimation of area represented by the selected plots. Total number of the NSFI plots in Latvia is 26 863 (500 m^2 each) including 16 166 permanent plots, 5 348 temporary plots and 5 349 so called "stump plots". The last group were excluded from calculations of area of the afforested lands as well as from all further calculations as they do not provide any quantitative information about characteristics of forest. Similarly, plots and sectors with a mark "artificial afforestation" were excluded from the calculation of area of naturally afforested lands, except calculation of lands relevant to the activities mentioned in Article 3.3. of the Kyoto protocol. Area by full single plot is calculated according to the equation (1):

$$A = \frac{C}{B} = \frac{6{,}456{,}181.4580}{(16{,}166 + 5{,}348)} = 300.092, \textit{where}$$

A - area represented by a single plot (ha);

B - total number of permanent and temporary sample plots;

C - total country area (ha). (1)

Total area of afforested lands is calculated using equation (2):

$$D = A * \frac{E}{F} = 300.092 * \frac{E}{500}, \textit{where}$$

D - area of the afforested lands (ha);

E - area of the temporary and permanent plots belonging to land use categories 62 and 64 (m^2);

F - area of a single sample plot (m^2). (2)

Area represented by each sample plot and sector is calculated using equation (3):

$$G = \frac{A}{F} * H = \frac{300.092}{500} * H, \textit{where}$$

G - area represented by the particular sample plot or sector (ha);

H - area of the particular sample plot or sector (m^2). (3)

Table 1

Biomass expansion factors used for calculations of biomass and carbon stock

Basic wood density, tons m^{-3}	0.5 ($t_{d.m.}$ m^{-3})
Biomass expansion factor for conversion of merchantable biomass to above-ground biomass	1.30 (dimensionless)
Biomass expansion factor for conversion of above-ground biomass to total biomass	0.32 (dimensionless)
Fraction of carbon in dry matter, tons of C per ton of biomass	0.5

Growing stock and annual increment of timber is calculated by multiplying of the growing stock and the increment by the area represented by a particular sample plot or the sector. Note that stock of undergrowth and advance growth is not taken into account in calculations of growing stock to avoid overestimation.

Other parameters estimated within the scope of this study and important for bioenergy production, and evaluation of carbon stock changes are total above-ground biomass (expressed in dry tons) and total carbon stock in above- and under-ground biomass. Biomass expansion factors for recalculations are borrowed from the National GHG inventory (Anonymous, 2010 and Penman et al., 2003), consequently both values, biomass and carbon stock are calculated from volume of timber (Table 1).

The land value is calculated according to weighted average price of forest and agricultural lands sold in 2008. The weighting factor is an area of land sold in each former administrative district. A source of information is public statistical reports provided by the State Land Service. Vegetation is not considered in calculation of land value.

Standard error of mean expressed in per cents is used to characterize uncertainty level of calculations. All calculations were done by the OpenOffice.org Calc spreadsheet software using built in DataPilot module for the data analysis and calculations of uncertainties.

Results and Discussion

According to the results of the first round of the NSFI, the total area of naturally afforested lands in Latvia is 257,850 ± 3,606 ha with total growing stock of timber 2,870.364 ± 239,088 m³ and total stock of aboveground biomass – 2,175,794 ± 168,096 tons. Total carbon stock sequestrated in above- and under-ground biomass is 1,436.024 ± 110,943 tons. These results are relevant to data obtained during the preliminary evaluation of the NSFI's data (Liepins et al., 2008). The standard error of mean is considerably small – 1.4% for total area and 8.3% for total timber stock; therefore, these data can be utilised in evaluation of status of naturally afforested areas; however, more detailed evaluation (by species per administrative unit) may lead to considerable uncertainties in calculations.

Total area of artificially afforested lands at the same time is 14,820 ± 634 ha with total growing stock 87,802 ± 48,288 m³.

Calculated value of naturally afforested lands is about 343,512.877 LVL (1,322 LVL ha^{-1}). It is according to weighted average price of forest and agricultural lands sold in 2008; however, this assumption can lead to significant overestimations, because it is based on price of lands being sold and does not take into account different limitations which may affect land price, for instance, irregular woody vegetation without market value or accessibility issues caused by lack or collapse of roads.

Natural afforestation took place most intensively in South Eastern regions of the country and in Jelgava district. The "champion" of natural afforestation is former Ludza district where natural afforestation is found in 11% of the total area. The smallest share of naturally afforested lands is in Jēkabpils and Ventspils districts (Figure 1).

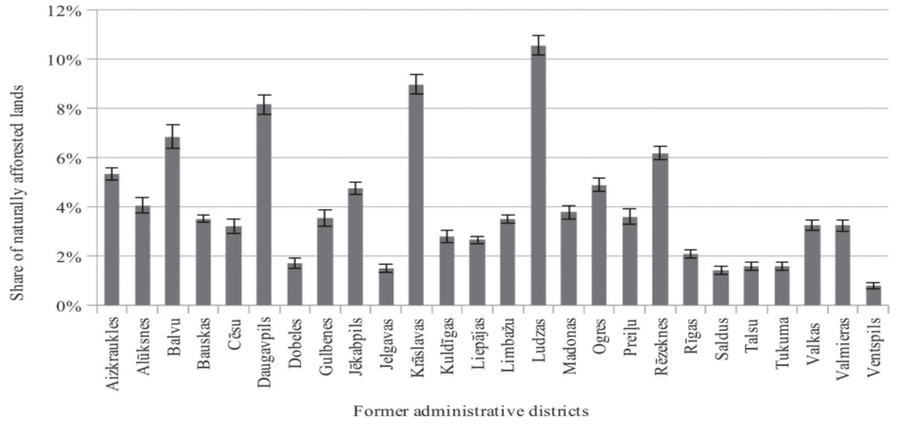

Figure 1. Share of naturally afforested areas from total area of former administrative districts.

Table 2

Distribution of area and growing stock between different groups of land owners

Owner	Area, ha	Growing stock of timber, m³	Above ground biomass, tons	Sequestrated carbon, tons
LVM	3,111 ± 300	26,682 ± 9,886	29,165 ± 11,698	19,249 ± 7,721
Other state organisations	550 ± 50	12,469 ± 12,341	8,122 ± 8,038	5,361 ± 5,305
Municipalities	290 ± 109	35,413 ± 2,861	23,129 ± 1,749	15,265 ± 1,154
Companies	781 ± 299	8,405 ± 9,187	5,493 ± 6,018	3,625 ± 3,972
Private persons	253,118 ± 3,777	2,787.395 ± 252,369	2,109.885 ± 177,440	1,392.524 ± 117,110

The majority of naturally afforested lands according to the Land register belong to private persons (253,118 ± 3,777 ha with total growing stock 2,787.395 ± 252,369 m³) which is not surprising if taking into account that afforested lands according to the National GHG inventory are mainly abandoned farmlands (NIR, 2010). Considerably smaller area of naturally afforested lands is managed by the Joint stock company 'Latvia state forests' (LVM), other types of ownership are represented by few sample plots or their sectors (Table 2).

The stand inventory data are measured in stands, where average height of dominant tree species is at least 2 m, therefore, considerable area of naturally afforested areas with smaller trees (61,338 ± 981 ha) cannot be further evaluated. From this point and further on only naturally afforested areas with the stand measurement data (196,512 ± 3,055 ha) are evaluated (Table 5).

The most common dominant tree species in naturally afforested areas is birch with total area 77,092 ± 1,861 ha (Table 3) and total growing stock 902,274 ± 107,287 m³ (Table 6). The most of naturally afforested lands originated from seeds (187,133 ± 3,005 ha).

A distribution of growing stock and biomass follows the distribution of area of dominant species for birch and grey alder, but for other species it is contravention – the highest growing stock found in black alder dominant stands but the smallest – in Scots pine dominant stands (Table 4). Reasons for such contravention are not evaluated within the scope of this study, however, it might be assumed, that black alder occupies the most fertile soils and Scots pine

Table 3

Distribution of area according to dominant species and origin of stands

Dominant tree species	Natural coppices	Seeds	Total
Birch (*Betula pendula* Roth)	2,901 ± 297[1]	74,191 ± 1,838	77,092 ± 1,861
Grey alder (*Alnus incana* (L.) Moench)	3,099 ± 400	37,186 ± 1,339	40,285 ± 1,395
Scots pine (*Pinus sylvestris* L.)	-	20,564 ± 803	20,564 ± 803
Aspen (*Populus tremula* L.)	304 ± 58	12,121 ± 900	12,425 ± 901
Norway spruce (*Picea abies* (L.) H.Karst.)	-	9,703 ± 787	9,703 ± 787
Black alder (*Alnus glutinosa* L.)	-	9,173 ± 605	9,386 ± 605
Other species (oak, ash, willows)	2,862 ± 151	24,196 ± 944	27,058 ± 966
Total	9,379 ± 541	187,133 ± 3,005	196,512 ± 3,055

[1] Initial afforestation took place by other species by natural copies, birch in all cases originated from seeds.

Table 4

Distribution of growing stock according to dominant species

Dominant tree species	Growing stock, m³	Aboveground biomass, tons	Sequestrated carbon, tons
Birch	902,274 ± 107,287	682,586 ± 74,023	450,507 ± 21,874
Grey alder	831,231 ± 104,046	594,605 ± 70,745	392,439 ± 14,777
Black alder	65,054 ± 19,734	65,921 ± 21,151	43,508 ± 2,356
Norway spruce	45,702 ± 22,077	30,774 ± 14,357	20,311 ± 2,387
Aspen	304,543 ± 104,767	234,941 ± 75,969	155,061 ± 7,373
Scots pine	133,748 ± 44,697	94,281 ± 29,184	62,225 ± 4,612
Other species	587,812 ± 79,453	472,687 ± 56,063	311,973 ± 8,905
Total	2,870.364 ± 208,109	2,175.794 ± 146,316	1,436.024 ± 64,989

Table 5

Distribution of area and growing stock according to age classes

Age class	Area, ha	Growing stock, m³	Above ground biomass, tons	Sequestrated carbon, tons
1...10	101,117 ± 2,251	249,972 ± 38,498	200,837 ± 20,166	132,553 ± 20,166
11...20	66,113 ± 1,741	1,258.813 ± 108,496	957,702 ± 58,892	632,083 ± 58,892
21...30	17,796 ± 925	706,765 ± 94,603	552,705 ± 52,654	364,785 ± 52,654
31...40	7,934 ± 514	424,784 ± 84,569	303,391 ± 42,155	200,238 ± 42,155
41...50	1,343 ± 229	133,483 ± 32,092	95,204 ± 15,882	62,835 ± 15,882
51...60	355 ± 45	35,504 ± 12,480	24,942 ± 5,071	16,462 ± 5,071

Table 6

Distribution of annual increment of growing stock in m³ according to age class[2]

Age class	Birch	Grey alder	Black alder	Norway spruce	Aspen	Scots pine	Other species
1...10	13,684 ± 2,987	9,865 ± 2,352	284 ± 117	71 ± 15	2,760 ± 905	419 ± 93	3,300 ± 1,881
11...20	35,343 ± 4,240	38,654 ± 6,026	1,503 ± 633	1,713 ± 545	4,413 ± 2,135	5,493 ± 1403	17,662 ± 3,215
21...30	8,955 ± 2,454	16,246 ± 3,677	1,560 ± 464	224 ± 117	16,637 ± 5,419	3,371 ± 3,326	14,477 ± 2,978
31...40	12,206 ± 4,015	2,310 ± 807	-	-	-	-	10,186 ± 2,390
41...50	6,271 ± 1,291	-	725 ± 573	-	-	-	-
51...60	-	-	-	-	-	-	-
Total	76,954 ± 8,495	67,076 ± 8,378	4,556 ± 1,240	3,219 ± 1,218	27,403 ± 8,475	11,690 ± 3,979	47,416 ± 5,636

2 Cells represented by a single sample plot cannot be statistically evaluated marked with '-'.

comes to the poorest soils.

Most forests on naturally afforested lands are 1...10 years old (101,117 ± 2,251 ha); however, the highest growing stock and above ground biomass is located in 11...20 years old forests (1,258.813 ± 108,496 m³). The oldest forests represented by several NSFI plots or sectors of plots belong to 51...60 years age group (Table 5). Forests on farmlands and forests on forest lands in mature forests are separated by presence of stumps and old laying dead trees from previous generation, which are characteristic to the forest lands but not for afforested farmlands.

Important value for characterisation of forest lands is annual increment of timber volume and, consequently biomass and carbon stock. Average annual increment of timber volume according to the data of the first round of the NSFI measurements (2004...2008) of naturally afforested lands was 238,313 ± 16,439 m³. The highest total increment is in 11...20 years old grey alder and birch dominant stands, respectively, 38,654 ± 6,026 m³ and 35,343 ± 4,240 m³ (Table 6).

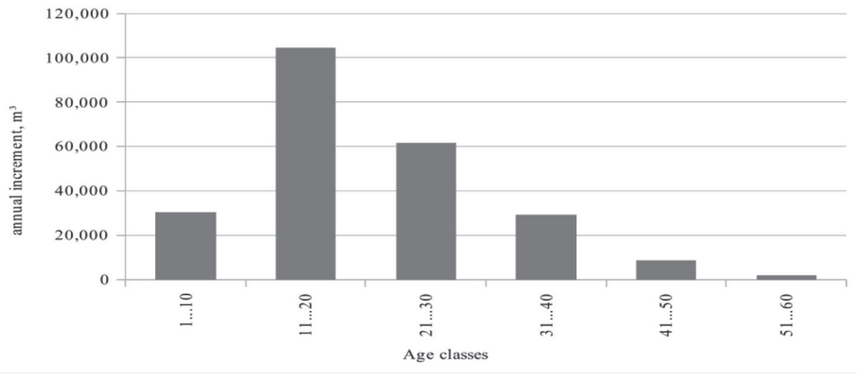

Figure 2. Distribution of total annual increment of growing stock according age classes.

In total figures distribution of annual increment according to the dominant tree species follows to distribution of growing stock. Similarly, the highest increment is found in 11...20 years old stands (Figure 2).

Total area of naturally and artificially afforested lands relating to activities mentioned in the Article 3.3. of the Kyoto protocol in Latvia is 170,890 ± 2,862 ha with total sequestrated carbon stock in above- and under-ground biomass 685,847 ± 58,652 tons and annual increment of timber volume 122,530 ± 10,513 m³ (Table 7). Mean annual increment of timber volume in forest lands remaining forests at the same time was 25.29 mill. ± 0.26 mill. m³. It means that afforested lands currently contribute about 0.5% of increment in forest lands remaining forests.

Mean annual increment of timber volume on these lands is 1.04 ± 0.09 m³ ha^{-1} (Table 9). Mean annual increment on forest lands remaining forests in age classes 1...20 years according to the NSFI is 1.79 ± 0.20 m³ ha^{-1}; therefore, it is important to support proper management of afforested lands to secure increase of increment at least to the level of other forest lands.

Table 7

Characteristics of areas afforested after 1989

Characteristics	Value
Area, ha	170,890 ± 2,862
Total growing stock, m³	1,367.427 ± 125,482
Annual increment of timber, m³	122,530 ± 10,513
Average increment of timber, m³ ha^{-1}	1.04 ± 0.09
Total biomass stock, tons	1,371.693 ± 117,305
Total sequestrated carbon, tons	685,847 ± 58,652

Conclusions

1. The NSFI provides comprehensive information about naturally afforested areas. Level of uncertainties is reasonable – 1.4% for total area and 8.3% for total timber stock. However, splitting of these values to calculate, for instance, area of birch dominant stands in particular former districts may lead to considerable overestimation or afforested area and growing stock.
2. The total area of naturally afforested lands in Latvia is 257,850 ± 3,606 ha or 4% of the country area with total growing stock – 2,870.364 ± 239,088 m³ and total amount of sequestrated carbon – 1,436.024 ± 110,943 tons. The most intensive afforestation took place in South Eastern part of the country reaching in some cases more than 20% of the initial area of farmlands.
3. The most common dominant tree species on naturally afforested lands are birch and grey alder (77,092 ± 1,861 ha and 40,285 ± 1,395 ha, respectively). Distribution of growing stock of birch and grey alder follows to the area distribution, but for other species comparably the highest growing stock is characteristic for black alder (65,054 ± 19,734 m³).
4. Total area of lands afforested (naturally and artificially) after 1990 in Latvia is 170,890 ± 2,862 ha with total carbon stock – 685,847 ± 58,652 tons and annual increment of timber volume – 122,530 ± 10,513 m³.
5. Contribution to reduction of the net GHG emissions of afforested lands corresponds only to 0.5% of total net emissions from the forest lands remaining forests, however in future this contribution will considerably increase because these forests will mature.
6. To secure higher timber volume increments, it is important to introduce proper silvicultural measures so that annual increment on afforested areas reaches at least the level of the increment on forest lands remaining forests.

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FACTORS AFFECTING HARVESTER PRODUCTIVITY IN FOREST THINNING IN LATVIA

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Abstract. One of the key factors affecting the formation of high-quality forest is thinning. Latvia's yearly budget of commercial thinning is not met; therefore, there are plenty of forests which are not thinned as planned and this leads to declining quality of forest stands. The timber processing industry needs significant excluded volume of timber, but forest owners are unable to maximize profits in future. Extensive logging of commercial thinning is hindered by economic factors and forestry regulations. Logging business is not satisfied with the productivity indicates and restricting forestry and environmental laws.
The world's leading forest engineering firm offers a variety of forest harvester design and structural solutions. The first real use of commercial harvesters in commercial thinning in Latvia's conditions revealed several problems. The most important one is how to increase harvester productivity, while ensuring the quality of residual stands. To increase effectiveness of the logging machines and the proportion of commercial thinning, it is necessary to explore main factors affecting harvester productivity. This work aims to clarify the main pressures hampering harvester productivity and evaluate the quality of remaining trees in commercial thinning in Latvia. The main results are that during night productivity of harvester decreases by 12%, while marking trees to be cut down with fluorescent color increases productivity of harvester at night by 15%. Productivity of harvester in thinning depends on average volume of harvested trees. These results of the work were obtained through various experiments on twelve different commercial thinning areas in Latvia.
Key words: harvester productivity, thinning operation.

Introduction

One of the key factors affecting the formation of high-quality forest is thinning. Thinning potential of today is not fully realized because it is complicated and hard work. Latvian yearly budget accumulating of commercial thinning timber volume is not met, quality of forest stands is declining since the forest industry does not receive significant volume of timber, and forest owners are not able to maximize profits. Latvian thinning produced is about 20% of the total felling volume. Average commercial thinning area is 2.6 ha and an average production of timber from 1 ha of commercial thinning is about 40 m³.

Today, only about 50% of the total timber stock in thinning timber volume in the country is produced by harvester-type machines. The remaining amount is harvested using conventional approach of logging with chainsaws and forwarding with specialized forwarders (Osis, 2008). Until the year 2005, it was not possible to use harvesters in commercial thinning in Latvia due to restrictions in regulations of Cabinet of Ministers No. 217. According to these regulations, area of strip roads should not exceed 12% of the total area of a stand. Consequently, the distances between the technological corridors should not be less than 30 m. Harvester boom cannot cover all of the area between the two corridors during thinning. On March 15, 2005, as a result of scientific research, significant changes were introduced into Regulations of Cabinet of Ministers No. 217 allowing use of up to 20% of stand for technology corridors. Consequently, the strip roads can be installed every 20 m, which significantly increases efficiency of the harvesters in commercial thinning, securing that all trees marked for removal can be reached by the crane securing implementation of both, silvicultural targets of forest owner and commercial targets of logging company.

Despite the above mentioned, broad utilization of harvesters in commercial thinning is also hindered by economic factors and forestry restrictions. Logging companies are not satisfied with the productivity performance of logging machines.

Productivity of harvesters in commercial thinning is expressed as volume of timber (cubic meters) harvested per unit of time (per hour).

Similar research in the direction of the year 2007 was carried out by Japanese scientists. This group of researchers studied the performance of small harvester head in a thinning operation.

To increase the productivity of logging machines in commercial thinning, it is necessary to explore main factors affecting productivity of harvester. This work aims to clarify factors influencing the productivity of harvesters and evaluate the quality of remaining trees in commercial thinning in Latvia.

Materials and Methods

Mechanical logging is based on the use of specialized machinery, which, depending on the type of felling (clear-felling or thinning) uses logging machinery with appropriate specifications. So, for example, in clear-felling large harvesters with higher performance and productivity characteristics are mainly used, but commercial thinning characteristics indicating usability of a harvester are dimensions, size and weight.

Commercial thinning is aimed to improve the quality of stands, providing remaining trees with

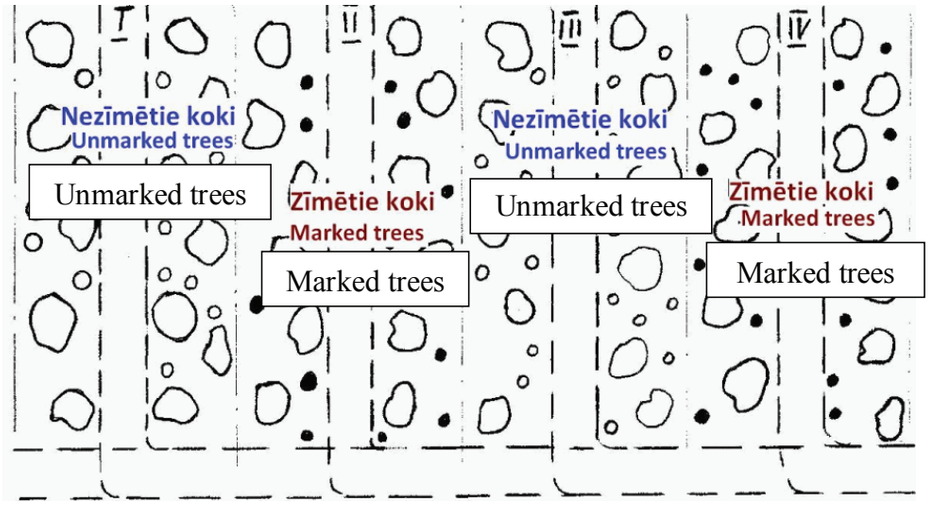

Figure 1. Marked trees felling scheme.

adequate growing space in order to maximize revenues from forest production. Prerequisite for achieving a perfect forest stand is thinning quality (Iwaoka and Aruga, 2007).

Studies of professional literature and similar studies in Scandinavia as well as the daily work of monitoring the operation of mechanical tree logging in commercial thinning has formed the author's perception that the main factors affecting labor productivity are:

- Stands thickness (number of trees per hectare);
- Average volume of harvested trees;
- Dominant tree species;
- Stand structure (number of species);
- Stem form of harvested trees;
- Stand type (soil resistance);
- Harvesting time (during daylight hours or night work);
- Operator's skill and knowledge in forestry.

Taking into account all these factors together, the "harvester productivity accumulating thinning" becomes a complex multi-variable. To estimate which of these factors is more important, the tests were conducted. In the summer of 2008 and 2009, harvester productivity studies were performed in 12 different stands. Felling was selected on geographically different points in the Joint Stock Company "Latvia State Forests" managed area and in MPS Jelgava district.

In all of the selected felling areas the time studies of crane cycle were implemented using full operation in time for a wood processing approach. Time studies were implemented during daytime and at night. It was not carried out during twilight hours, which are perceived as 30 min before and after the sunset and 30 min before and after the sunrise. Each time study was 1 to 3 hours long with the same operators on each site. All steps of time studies for each tree including species of tree were recorded in the table. If processing time exceeded onaverage by 50%, the reason for that was documented. In that way it was possible to detect the number of hampering factors and the length of delay they cause.

Time studies were done by a professional sports "CASIO" stopwatch. Time consumption for processing of every tree was fixed separately for each working element:

$$tc = ta + tb + tc + td + te, \quad (1)$$

where:

ta - harvester mechanism for channeling cutting tree (a cutting tree is chosen);
tb – catching tree cutting ;
tc - processing and cutting in necessary length;
td – ranging and ordering of assortment piles;
te - placing of tops and branches into strip roads.

Separate study was carried out in specially prepared stands where prior to harvesting, trees were marked by luminescent color and an operator had to cut down only marked trees in prepared areas and decide, which trees to cut according to good practice guides and regulations in unmarked area. Marked trees felling scheme is shown in Fig.1.

This work was carried out during night time using the same operators in each felling area, fixing time of tree processing and tree species.

After felling, basal area of remaining stand was measured in different places. For this purpose electronic remote measurement device "DLEISO BOSCH" was used. Using this device, sample plots (100 m² plot with R = 5.64 m) were established. Distribution of plots in stand is shown in Fig. 2.

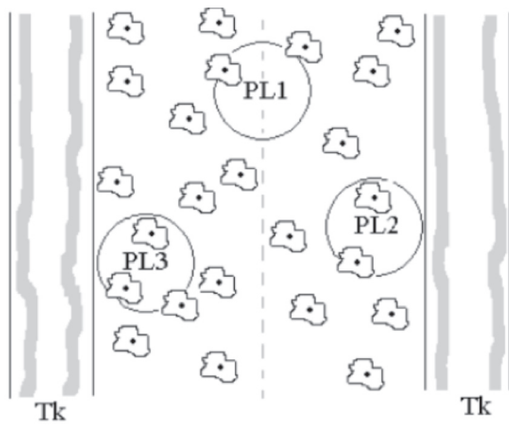

Figure 2. Sample plot placement scheme.

On an axis that is perpendicular to the technological corridors, three sample plots were established:

- The central sample plot in the middle between technological corridors - PL1;
- Two (right and left) sample plots to the left and right from the central sample plot - PL2 and PL3.

Tk - technological corridor.

The plots on the left and right side overlap partially with the central plot. Every tree in distance up to 5.64 m from the center of sample plots is accounted and measured. Diameter and species of the remaining trees are recorded in table. Plots are distributed evenly along the stand, so that the area of plots covers 15 – 20% of the felling area.

Along with the basal area of plot damages and other standing tree quality remarks were estimated and recorded including:

a) Mechanically damaged trees;
b) Trees with poor stem form (high sinuosity, visible stem defects);
c) Damages of crown of trees (dry top, crowns with the essential defects, which impair the viability of trees);
d) Dry trees.

The main damages of remaining trees are usually bark damages, incidental cuts, broken or damaged roots. Joint Stock Company "Latvia State Forests" in the internal regulations has stated that damage is any permanent damage of bark starting from 15 cm² area or roots broken up to 70 cm away from the tree and with diameter at least 2 cm or more as well as incidental cuttings in stems if they cover at least 10% of diameter of atree or 1 / 5 of circumference tree at the cutting place. –The number of damaged trees after thinning should not exceed 6% of the number of remaining trees (Latvijas valsts meži, 2007). Quality of remaining trees and share of damaged trees is relatively simple to calculate mathematically.

Remaining trees with poor stem form or trunk defects were usually found by visual inspection. Then, share of poor quality trees was calculated per hectare or expressed as a percentage. Bad trunk shape is usually attributed to trees whose trunks are crooked or tortuous. Further, they could be divided like in case of evaluation of round-wood assortments - unilateral and multilateral deformations, or forestry – "C" or "S" type curve in the trunk. During evaluation of trees with a poor stem form, the evaluator has to be able to estimate how many such trees grow in stand after thinning and how many of them grew there before. It is quite often that 2/3 of trees in stand has poor stem form. In such cases it is impossible to remove all trees with a bad shape of stem because then the stand will become too sparse, and it would not fulfill quality requirements for commercial thinning. Another case is when there are only few trees with a bad shape in the stand, and the operator didn't find them during thinning process because the operator is unable to inspect trees from all sides. During evaluation of quality of remaining trees in the stand, the share of trees with various inadequacies was calculated.

Inadequacies of crowns of remaining trees are trees with a dry top or without it. Such trees are always considered as non-perspective, and they should not be between remaining trees. An exception could be made only in case if these trees meet requirements for the environmental trees. In practice operators of harvesters don't find such trees working in night shift. Results of evaluation were expressed as number of trees per hectare and the percentage of remaining trees.

Dry and semi-dry trees are dead, and they can become a nest of pests or diseases; therefore, they should be harvested during commercial thinning. Exceptional cases could be small dimension trees with 6 - 10 cm in diameter, which don't affect growth of remaining trees. Also, an exception could be an isolated old branched dead tree, which could serve as an ecological tree in future. In recent years Latvia's forests suffer from bark beetle (*Ips typographus L.*) damages; therefore, there might be a plenty of dead trees with this type of damage. In such cases the operator should carefully choose trees to harvest to avoid over-cutting of stand. Otherwise, instead of commercial thinning sanitary clear-cut might be necessary. Dry or dying trees growing in measurement plots were counted and expressed as the number of trees per hectare and the percentage from remaining trees.

Results and Discussion

All measurement and results of field calculations were entered into electronic tables of the computer program Microsoft Excel. These data were grouped, sorted and statistically processed (Arhipova and Bāliņa, 2003). All experimental objects were located in dry forests; therefore, the effect of soil carrying capacity was not investigated during the study.

Results of time studies were sorted according to lighting conditions – data obtained during daytime and results from night shifts. For each group calculation of secondary values was done, including the average weighted values, standard deviation, minimum and maximum values. Evaluation of results obtained in 12 stands demonstrated that during night time productivity decreases by 12%. The largest increases

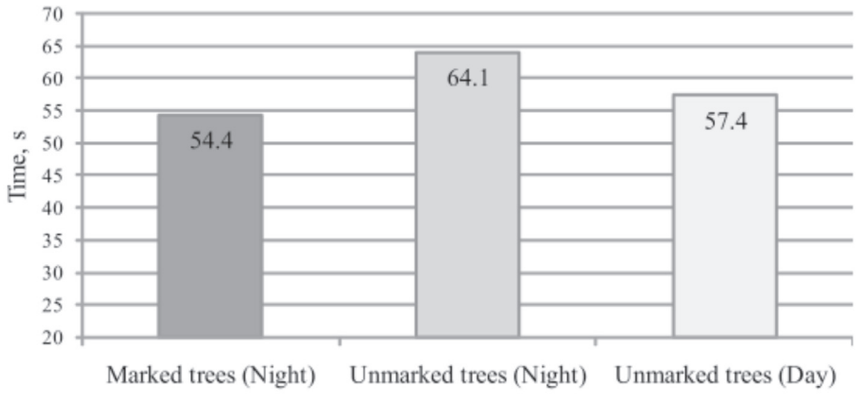

Figure 3. Time spent for processing of a single tree working in daylight and during night separately for marked and unmarked trees.

of time consumption were observed in trials where the harvester operator had to choose tree to cut. This is due to effect caused by artificial searchlights of the harvester which make it more complicated to the operator to evaluate characteristics of trees. The second largest increase in time consumption is associated with cutting to length when the operator has to focus on quality of assortment. Results of productivity estimates in daytime and after dark are shown in Figure 3.

The next step of evaluation of results of time studies was the comparison of results obtained in trials with marked and non-marked trees. Note that this study was implemented only during the night shift using the same operators for all studies. The result of these time studies demonstrates that tree marking increases productivity by 15 %. Results of this experiment are presented in Figure 3. It is remarkable that productivity working with marked trees during night time could be even higher than during daytime working with unmarked trees. This can be explained by the fact that the operator needs more time to choose a tree to cut. However, the study did not include estimation of time consumption for marking trees. During the measurement of sample plots, it was found out that 5% of marked trees were left to grow during thinning because they were not accessible.

Problematic trees having trunks with atypical wreaths, branches and dimensions affect productivity of harvester quite seriously. According to the results of time studies, processing of such trees takes 1.5 to 3 times longer than doing the same of standard trees. Sometimes problems arise with logging of such trees, but more commonly it is with pruning and cutting to lengths. This is mainly due to the technical inadequacy of harvesters for harvesting such type of trees.

Another important factor affecting productivity of the harvesting is dominant tree species and structure of stand - number of species to harvest (Osis, 2004). It is well known that productivity in pure coniferous stands will be always much higher than in deciduous stands with a similar dimension of trees. This is due to the limited operator's knowledge in forestry as well as due to technical and technological characteristics of harvesters affecting productivity of processing of different tree species. Taking in account that only three of twelve studied stands contained admixture of deciduous trees, it would not be correct to compare productivity depending on species.

Productivity of the harvester depending on the volume of timber was investigated by the team of authors from the Latvian State Forestry Research Institute "Silava". The results are shown in Figure 4.

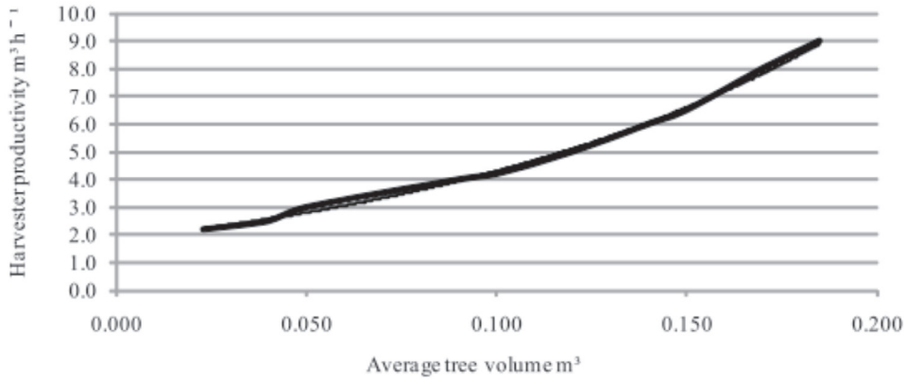

Figure 4. Productivity of harvester according to the average wood volume.

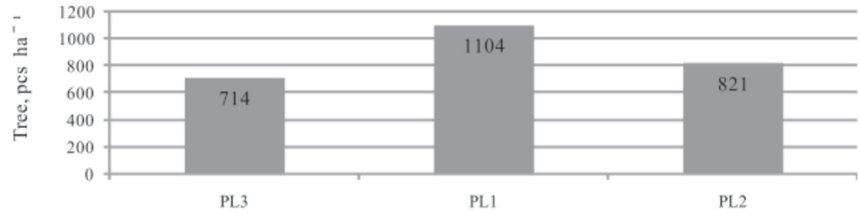

Figure 5. Uniformity of remaining trees after thinning.
PL1, PL2 and PL3 - plot location, see Figure 2.

Obtained results demonstrate that productivity of harvesting increases with increasing of average volume of trees in the stand, which correlates with earlier obtained results (Lazdāns, 2006). Working in stands with larger trees, the harvester's productivity will be higher.

Results of the experiment demonstrating density of remaining trees after thinning at various distances from the technology corridor are shown in Figure 5. Layout of the experimental plot has already been shown in Figure 2.

The results obtained in the study show that the stand has smaller basal area closer to the technological corridors, but stands become denser away from technological corridors. From good silviculture point of view, the stands should be thinned equally in all areas without respect to difference from strip roads; however, specifics of mechanical operations and sometimes hastiness of operators result in improper distribution of trees across the stand.

Quality of remaining trees in sample plots is summarized in Table 1.

Table 1 shows the average health status of trees in 12 evaluated stands after thinning. It should be considered before final conclusions are made that an environmentally friendly forestry guideline should be preserved for future ecological trees already during the last commercial thinning. This also applies to a small share of dead wood that should remain in the stand after thinning if it doesn't affect health of future trees.

Table 1

Quality characteristics of remaining stands

Characteristics	%
Dry trees	1.5
Defects in crowns	2.7
Defects of trunks	3.0
Mechanical damages	4.2
Healthy trees	88.6
Total	100

Conclusions

1. Atnight hour's productivity of harvester decreases by 12%, while marking of trees to cut with fluorescent color increase productivity of harvester in night hours by 15%.
2. Productivity of the harvester in thinning depends on the average volume of harvested trees. As soon as the volume of trees increases, the productivity of the harvester increases as well.
3. Mechanical harvesting of non-standard trees takes 1.5 to 3 times more than harvesting of ordinary trees.
4. Closer to the technological corridors stands are thinned more intensively than in more distant areas; the most common reasons for such a difference are insufficient accessibility of trees in areas located away from the strip roads and hastiness of the operator.

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THE WOOD RESOURCE AVAILABILITY INFLUENCING FACTORS IN PRIVATE FORESTS IN LATVIA

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Abstract. The forests and woods are the main resources in Latvia. It is vital to understand the amount of wood resources that is being extracted from private forests now and in future in order to be able to predict the forest sector development and growth.
The study objective was to identify the key factors in the wood resource availability and assess their role in private forests. In October 2009, the study was conducted to appraise the private forest owners the operational results and find out the future plans in connection to wood resource extraction. In the study a random sampling method was applied, where in the respondent selection the State Forest Service database information was used. In the study the respondent interviewing method CATI with computerized telephone interviews was used. In the selection of affecting factors the expert's method was applied.
The study showed that only 25.40% of all private forest owners claimed that there was wood resources extraction in forests, but 29.50% of all private forest owners claimed that they were planning to perform the wood resource extraction in 2010. If the timber selling prices increased by more than 10 LVL per m^3 in 2010, then 38% of all respondents claimed that they would increase the wood resource extraction in forests. The study data show that 30% of all private forest owners admit that there are growing stands in the forests, but they can not be harvested, because road infrastructure is poorly developed or there are no roads in the forests.
Key words: wood resource, private forest, Latvia.

Introduction

Concerning the territory, Latvia is a small country, but there is a large number of private forest owners - about 150 000, thus creating a fragmented wood resources supply from private forests (Meža nozares…, 2009). In recent years, Latvian wood resource availability question has become very topical, because the market condition has changed and the competition has increased in the domestic and global markets.

In Europe and other parts of the world, the scientific research on trends of the private forest sector, on predictions and on opportunities as well as onresources already started in the previous century (Dennis…, 1989; Järvelainen…, 1983; Karppinen…, 1995; Kuuluvainen…, 1991; Roos…, 1996). In Latvia, the forest owners surveys were carried out within the framework of international cooperation projects in 1996 (Development…, 1996). But this is the first study, which has analysed and studied the private forest owners sector in Latvia in depth. Currently the main data sources on the private forest sector in the country are the National Forest Service, Ministry of Agriculture who prepare statements and reports, as well as special publications in various journals and newspapers.

The study objective was to identify the key factors in wood resource availability and assess their role in private forests. The wood resource is a raw material that is derived from tree trunk, less often from the twigs and roots. Rational wood resource exploitation affects this resource availability. According to the statistical inventory data, there are approximately 3.26 million hectares of land in Latvia which are covered by forests of agricultural territories that meet the criteria related to being into forestland (Forest sector…, 2008). The forest statistical inventory data (April 1, 2009) show that 47% of the total forest areas are the state forests, but 53% - private forests. During the last 70 years, the forest area in Latvia has almost doubled, while the volume of wood has increased to 630 million m^3 after forest inventory statistical data (Meža nozare…, 2009, Figure 1).

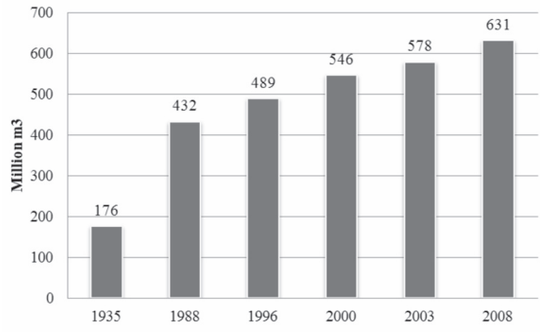

Figure 1. Total wood amount according to the data of the Ministry of Agriculture, million m^3, 2009.

The economic activity in the state forest is carried out according to sustainable forest management principles. The wood resource extraction in the state forests is performed systematically and regularly; as a result, the wood resource availability planning and forecasting process is easier (Forest sector…, 2008). The amount of wood that is cut down in Latvia`s forests each year remains quite stable – between 10 and 11 million m^3 of wood resource each year. In 2009, 10.73 million m^3 of wood were cut – 7.73 million in the state-owned forests, and 3.00 million in the forests owned by private owners, local government, or other types of owners (Koku ciršana…, 2010; Figure 2).

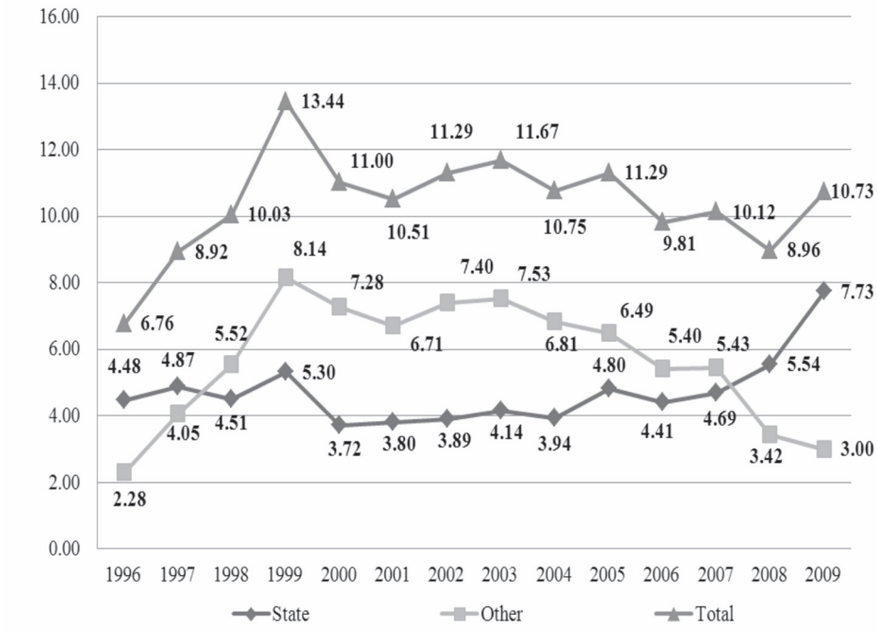

Figure 2. Timber cutting in state and private forests according to the data of the State Forest Service, million m^3, 2009.

In recent years, the wood resource mostly comes from the state forests, that is two times more than in the private forests, although nearly half of the forest areas is owned by private forest owners. The wood resource extraction in the private forests takes place according to free market conditions, where the final products are sold by the market prices when the price of supply matches the demand price. During the past five years the wood resource extraction in the private forests has decreased, especially during the period of 2007 – 2009.

Therefore, a detailed study of factors which operate in free market conditions and influence the wood resource availability directly in the private forests, is necessary to be carried out in order to understand better the processes and trends in this sector. The wood resource extraction must be balanced between the state forests and private forests, since it is determined in the Latvian forest policy guidelines that long- term intensive exploitation in one of these sectors is no allowed.

Materials and Methods

The study target group is the Latvia`s forest owners. The planned sample size was 1000 respondents, where the whole forest owners group includes 600 respondents (the 1st target group), but the forest owner group planning to carry timber felling in 2009, includes 400 respondents (the 2nd target group). The achieved sample size was 907 respondents, where from the first target group the responses were received from 583 respondents, but from the second target group - from 324 respondents. In the study a random sampling method was used, where in the respondent selection the State Forest Service database information was applied. In the study, the respondent interviewing method CATI (Computer Assisted Telephone Interviews) with computerized telephone interviews was used (Kvantitatīvās pētījuma…, 2010). In the selection of affecting factors the expert's method was used.

In the forest sector and related sectors the expert method is often used since the current trends and processes can be understood better and assessed only by experts of forest sector. In the study five experts from the forest industry were interviewed, and each expert determined the value of each factor affecting the timber resource availability. The affecting factors were assessed according to 5-point system (two decimal places), from one to five points where one – is the lowest assessment, but five - is the highest assessment. Further, according to expert assessments the average value was calculated. In the study 12 factors affecting the wood resource availability had been identified. They are the economic activities, the timber selling prices, the geographical position, the legislation and policy, the knowledge and experience, the infrastructure, the climatic conditions, the imported wood resource, lack of funding, the wood resource extraction place, the forest quality and the product markets. As a result, the following four main factors were selected: the economic activity, the wood selling prices, the infrastructure and the funding attraction.

Results and Discussion

For data processing the statistics treatment methods are used: descriptive statistics and data inter-comparison. Since this is the first study on the private forest owners, the study results can be used as the reference for other studies, the mutual data comparison

and evaluation. The study results are always with a certain degree of statistical error probability. It has been taken into account whilst analyzing and interpreting the study results. If these differences are included in the statistical error limits or less, then they can be regarded as minor.

The statistical error is calculated by the following formula (Meža nozares…, 2009):

$$SE = q \times \sqrt{\pi} \times (100 - \pi) / n$$

where:

SE – statistical error;

q – the coefficient that at 95% probability is equal to 1.96;

π – in the study achieved responses of the percentage breakdown;

n – the number of respondents.

To determine the statistical measurement error, unweighted number of respondents in the group and the results as a percentage are given. Using these values, the section can find the measurement error limits of +/- percentage with 95% probability. For example, if the study result from the surveyed Latvian forest owners (respondents n = 583) the target group of 20.0% is obtained and which is the affirmative attitude towards the judgment. Then the probability of 95% can be said that the statistical measurement error is + / - 3.2% range. It follows that the group, which identifies itself with this judgment, is from 16.8% to 23.2%.

Using the study results, the factors affecting the wood resource availability in private forests and the decision to carry out / not to carry out wood extraction in these forests can be judged. From the study results it can be concluded that the key affecting factors whether to carry or not to carry out the wood extraction in private forests are the following: low economic activity from the private forest owner side, the low timber selling prices, the road infrastructure deficiency and the funding deficiency.

The economic activity

For this study, the economic activity is understood as the wood felling (the timber sales) from the private forests (Ķirsons, 2009). A stable wood resource extraction in the private forests can provide the timber industry with necessary resources, so it is important to understand further private forest owner activities in 2009 and 2010.

According to the obtained results of the study, only 25.40% of all private forest owners claimed that in nine months (in 2009) the wood resource extraction in forests was performed, 61.40% claimed that the timber felling was done before 2009, but 12.20% of all private forest owners claimed that they had never done the wood resource extraction in forests (Figure 3).

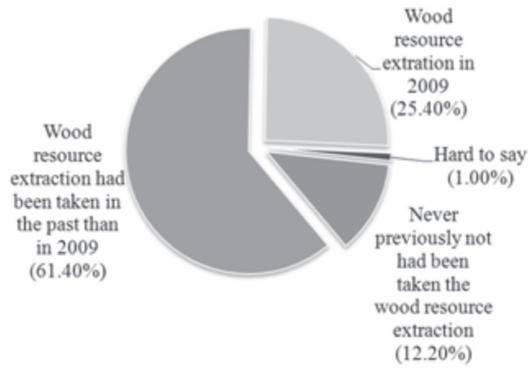

Figure 3. Wood resource extraction, %.

The private forest owners, who according to the data of the State Forest Service had planned to carry out the wood resource extraction constituted 54.00% of all respondents and confirmed that they had carried out the wood extraction in forests, but 45.70% respondents claimed that the wood resource extraction was not carried out in 2009 (Figure 4).

Most frequently mentioned causes for wood resource extraction in the private forests in 2009 are as follows:

1. 38% of all respondents claimed that the firewood or timber resource was needed for their own needs;
2. 29% of all respondents confirmed that the wood resource extraction was done in accordance with the forest inventory plan;
3. 21% of private forest owners claimed that the income of wood resource extraction was intended to cover the household expenses.

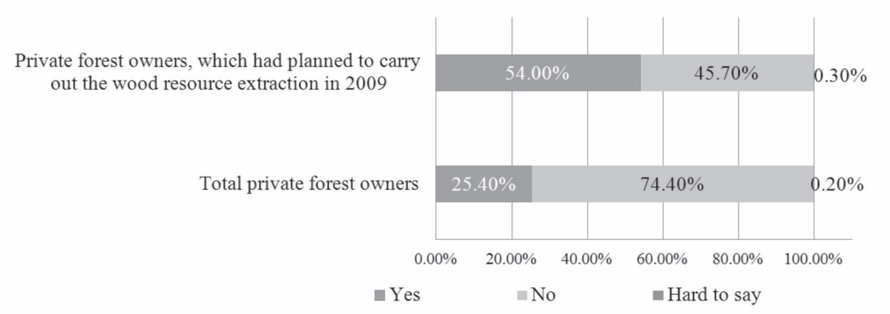

Figure 4. Wood resource extraction in 2009, %.

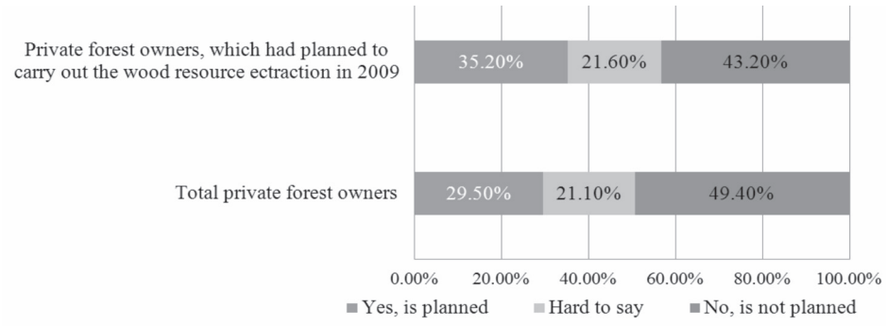

Figure 5. Wood resource extraction in 2010, %.

A little less frequently, respondents indicated that the money obtained from the wood resource extraction they invested in the non-forestry business (15% of all private forest owners), credit or debt settlements (12%), but 12% of all respondents mentioned other reasons.

An important factor of wood resource availability is planned activities in connection with the wood resource extraction in the private forests. The study shows, that 29.50% of all private forest owners claimed that they are planning to perform the wood resource extraction in 2010, 49.40% of all respondents had just thought about wood resource extraction in forests. But 21.10% of all respondents had not made their decision about wood resource extraction in forests yet. 35% of all respondents, who planned to perform the wood resource extraction in 2009, plan to do it also in 2010, but 43% of all respondents are not planning the wood resource extraction, and 22% of private forest owners do not know what they will do (Figure 5). The research results show that a large proportion of the private forest owner does not intend to perform the timber felling in forests, which significantly may influence the wood supply for timber industry.

To understand the overall forest sector development, it is important to understand the size of wood amount that could be expected from the private forests. The study shows that 35% of all private forest owners claimed that the wood resource extraction volumes will amount to 100 m^3, 10% of all respondents said that it would range from 101 to 250 m^3, but 19% of all private forest owners claimed that the volumes would amount from 251 to 500 m^3 and just 12% of all respondents claimed that would be more than 500 m^3 from forest (Figure 6).

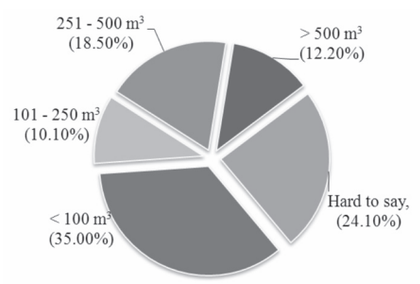

Figure 6. Potential wood resources volumes in 2010, %.

Most frequently mentioned causes why the wood resource extraction will not be carried out in 2010 are as follows:

1. 34% of all private forest owners claimed that the low prices in the timber markets are predicted;
2. 24% of all respondents claimed that there are no growing stands in their forests;
3. 15% of all forest owners said that the income from forest in coming years would not be necessary;
4. 13% of all respondents claimed that they were not sure about the sufficiency of timber resources volume in forests;
5. 5% of all private forest owners claimed that they could not do anything, because the neighboring forest has been cleared.

Timber selling prices

In the availability of wood resource the timber selling prices that are determined by the economic activity in the private forests is an important factor. Therefore, it is important to clarify the private forest owner motivation if the timber selling prices increase. If the timber selling prices increased by more than 10 LVL per m^3 in 2010, then 38% of all respondents claimed that they would increase the wood resource extraction in forests. Slightly less frequently, 27% of all private forest owners claimed that, if the price increased in the range of 6 to 10 LVL per m^3 then they would be willing to increase the wood resource extraction. But 8% of all respondents claimed that, if the price rose by 3 to 5 LVL per m^3 then they would be willing to increase the timber felling in forests (Figure 7).

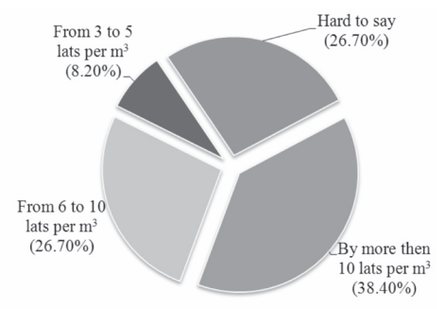

Figure 7. Timber price and its influence on the wood resource extraction from private forests, LVL.

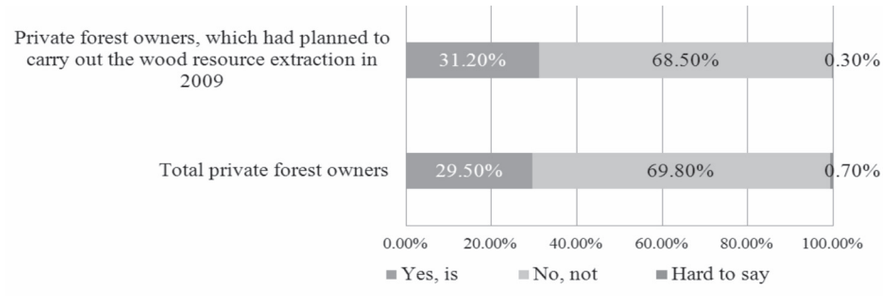

Figure 8. Road infrastructure in private forests, %.

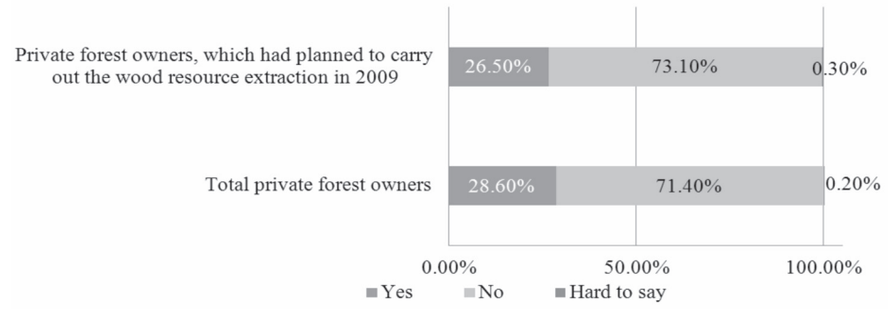

Figure 9. EU funds attraction in private forests, %.

Road infrastructure in private forests

For obtaining of additional wood resource from the private forests, it is important to identify the current situation of the road infrastructure and its impact on the wood resource extraction (Ķirsons, 2009c). The study data show that 30% of all private forest owners said that there are growing stands in their forests, but they cannot be felled, because the road infrastructure is poorly developed or there are no roads in the forests (Figure 8).

European Union funds attraction

Forest management requires substantial financial resources so that the private forest owners can recover just after several years, therefore, an important factor in wood resource extraction is the EU (European Union) financial involvement in forest management (Ķirsons, 2009a). The study data show that 28.60% of all private forest owners claimed that they used the EU funding for forest management (Figure 9).

The study findings show that 75.40% of the EU funding go to young stands cleaning, but 23.40% of the EU funding are used for afforestation of agricultural land. But 11.9% of the EU funding go to the worthless thinning in forests and just 2.4% - go to the drainage system renovation.

Conclusions

1. An important factor in the wood resource availability is the low economic activity in the private forests during nine months in 2009. Only 25.40% of all private forest owners claimed that there was wood resources extraction in forests, but 61.40% - claimed that the timber felling had been done before 2009. But 29.50% of all private forest owners claimed that they planned to perform the wood resource extraction in 2010 and 49.40% of all respondents had just thought about wood resource extraction in forests. This situation can be promoting the timber resources deficit in the local market, in Latvia.
2. If the timber selling prices increased by more than 10 LVL per m^3 in 2010, then 38% of all respondents claimed that they would increase the wood resource extraction in forests. Slightly less frequently, 27% of all private forest owners claimed that, if the price increased in the range of 6 to 10 LVL per m^3 then they would be willing to increase the wood resource extraction. Such a situation has arisen because the private forest owners are willing to get the same price that it was in 2007.
3. The study data show that 30% of all private forest owners said that there are growing stands in their forests, but they cannot be felled, because of poor road infrastructure or even lack of roads. This percentage is very high, so this problem needs solution as fast as possible.
4. An important factor in wood resource extraction is the EU (European Union) financial involvement in forest management and 28.60% of all private forest owners claimed that they have used the EU funding for forest management. 75.40% of the EU funding go to young stands cleaning, but 23.40% of the EU funding are used for the afforestation of agricultural land.

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ESTIMATION OF FOREST PARAMETERS USING THE NON-PARAMETRIC TECHNIQUES AND SATELLITE IMAGES AT COMPARTMENT LEVEL

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Abstract. This paper discusses the use of medium resolution Landsat TM satellite images to support conventional approaches of Lithuanian forest inventory practices. Estimation accuracies achieved using just field measured sample plots, Landsat TM satellite images and two non-parametric k-nearest neighbor and most similar neighbor estimators were studied at a level of compartments. 19 mature forest areas, prepared for final felling with GPS measured borders and all trees callipered, were used for validation. Notably higher estimation accuracies were achieved using field sample plots distributed through the whole forest area studied than just ones located on mature forest stands. The root mean square deviations in estimating compartment-wise volume of growing stock per 1 ha was around 27-28% if the best variant of estimation approach was used. Possible influence of the accuracy in locating the borders of validation areas on the estimations is discussed in the paper, too.
Key words: forest inventory, satellite images, k-nearest neighbor, most similar neighbor.

Introduction

Current forest inventory practices in Lithuania can be divided into three broad approaches – stand-wise inventory, National forest inventory (NFI) by sampling methods and pre-harvesting inventory of forest compartment (Kuliešis, 2008). Even the NFI plays important role in providing forest statistics for various objectives, the main source of forest related information in the country that is used for tactical and operational forestry planning remains the stand-wise inventory. There are numerous issues in the approaches used for stand-wise inventories. Stand-wise forest inventory usually lacks methods for error assessment of the large area estimates and suffers from possible bias of visual estimates (Tomppo, 2005), even though they are supported by some measurements. Forest management planning, especially when it regards the amounts of forest to be harvested during the main felling, requires reliable information on the resources. Sampling based inventories of mature forests were started in several forest enterprises in Lithuania in 2008-2009 (Kasperavičius, 2009). There were several methodological approaches for estimating volume of growing stock per 1 ha used within the frames of such inventories (Jonikavičius et al., 2009). The prevailing solution was to locate statistically required systematic sample of fixed area circular field plots in order to provide total volume of growing stock estimates for the whole forest enterprise. However, the sample size was focused on the expected error level for forests of the whole forest enterprise, therefore resulting in unacceptable accuracies for areas several times smaller than the forest enterprise. Landsat TM satellite images and the non-parametric k-Nearest Neighbor estimation (kNN) solution were alternatively used for one forest enterprise, keeping the same field measured sample as a reference, to provide volume of growing stock statistics on the level of forest district and prevailing tree species (Jonikavičius and Mozgeris, 2009). This experiment was based on the assumption that the spatial variation in forests is often such that field measurements in a certain area can also be used in neighbouring areas by employing a relevant extrapolating, or the so called 'information borrowing' technique (Tomppo, 2005). Main outcomes of the experiment were that stable and relatively low (around 2%) standard estimation errors of volume of growing stock per 1 ha of mature stands were obtained for different area units (i.e. forest enterprise, forest districts, etc.). However, the main objective of a stand-wise forest inventory – improvement estimations on forest compartment level – remained undisclosed in the mentioned study. Thus, the aim of current paper is to introduce the results of an attempt to go down with the volume of growing stock estimations using remotely sensed images and limited sample of field measurements for a compartment, which is considered as a basic stand-wise forest inventory and management planning unit.

This topic remains rather weakly studied in the literature. The most current and methodologically closest to our approach was the attempt by the USA scientist to use forest inventory sample plot data, Landsat TM satellite imagery, and the k-Nearest Neighbors technique to produce stand-level estimates (McRoberts, 2008). He found that estimates of stand-level stem density and basal area means were within confidence intervals for validation estimates for around 90% of stands. Other research projects were using potentially more accurate and therefore more expensive remotely sensed data or dealing with rather simple forest conditions. Previously, the approach was mainly to use aerial image and historical forest inventory records to get forest compartment-level estimates (Moeur and Stage, 1995; Temesgen et al., 2003). More recently, aerial images are used together or independently with data, derived using laser scanning (Næsset, 1997, 2004; Means et al., 1999; Holmström et al., 2001; Muinonen et al., 2001; Holmgren, 2004; Eskelson et al., 2008). Our choice to use Landsat TM satellite images as the remotely sensed data set to support volume of growing stock estimations for

mature forests within the frames of stand-wise forest inventories was motivated by the objective to have cheap and fully automated solution in addition to the use of color-infrared aerial images, sample plot based and visual inventories, applied conventionally in Lithuania (Mozgeris et al., 2008).

Materials and Methods

Dubrava forest which is in the center of Lithuania near Kaunas (E 24°4' and N 54°50') was chosen as the study object (Fig. 1). This forest has been serving as a research polygon for the Lithuanian Forest Research institute, Forest inventory and Management Planning institute, Forest faculty of Lithuanian University of Agriculture and Kaunas Forestry College already for many years. A well developed network of field observations is available for the area, and it is maintained continuously. Moreover, most of Lithuanian forest conditions are present within this forest.

457 circular field sample plots were established and measured in Dubrava forest in 2008-2009. Polar coordinates of each tree, tree species, diameter, crown type, height for sample trees (not less than 1-3 sample trees for each forest element), etc. Volumes of growing stock for all trees (including the volumes of stems, branches and volume distributions by merchantability classes) were later estimated utilizing the methods operational in Lithuanian forest inventories (Bikuviene et al., 2009) and used to get plot-wise estimates. Plot center coordinates were measured using electronic tacheometer (Leica Smart Station GPS 1200 Series) with accuracy of at least 0.1 m. As the auxiliary data set we acquired Landsat LT scene from 2007 09 17, scene ID 187/022. Satellite image was processed to the geocoded products using ground control points measured on topographic maps and in the field, stored in Lithuanian coordinate system LKS94.

Conventional stand-wise inventory data (borders of compartments and associated attributes) were considered as insufficient quality data set to be used for validation. Therefore, data on 19 areas, assigned for final felling (hereinafter FFA) for the period between satellite image acquisition date and the summer of 2009, were received from Dubrava state experimental forest enterprise (Fig. 1.b). Borders of these areas were measured using GPS (accuracy of locating the vertices of at least 2 m) and every tree inside the area was callipered following the requirements of pre-harvesting inventory of forest compartment (REFF). Total volume of such final felling area and the volume of growing stock per hectare were calculated with the expected root mean square error amounting 10%. Average area of FFA was 1.39 ha, average volume of growing stock per 1 ha – 394.5 m^3, total volume of growing stock – 9373.2 m^3. The forests of on FFA were rather mixed - average tree species distribution of main storey was as follows: pine – 39%, spruce – 36%, birch – 12%, black alder – 6%, oak – 4% and other tree species – 3%. There were 4 FFA's with significant 2^{nd} storey. Naturally, all stands were mature.

Several approaches were used to estimate the volume of growing stock per 1 hectare as well as the total volume of growing stock (as a product of mean volume of growing stock per 1 ha and the area of FFA) based on sample plot and Landsat TM data as well as the continuous field view concept in representing forests geographically (Mozgeris 2008a; 2008b). The continuous field view assumes that the real world is a series of continuous maps or layers, each of them representing the variability of a certain attribute over the Earth's surface. For each given attribute, a unique value should be recorded at every location or point inside the forest. Forest information is organized using a grid of systematically distributed virtual samples or points corresponding to pixels in a raster data model. Such points were distributed using 30x30 m, 16x16 m and 8x8 m schemes in this study. Two non-parametric estimation techniques were used to get volumes of growing stock for each of such point

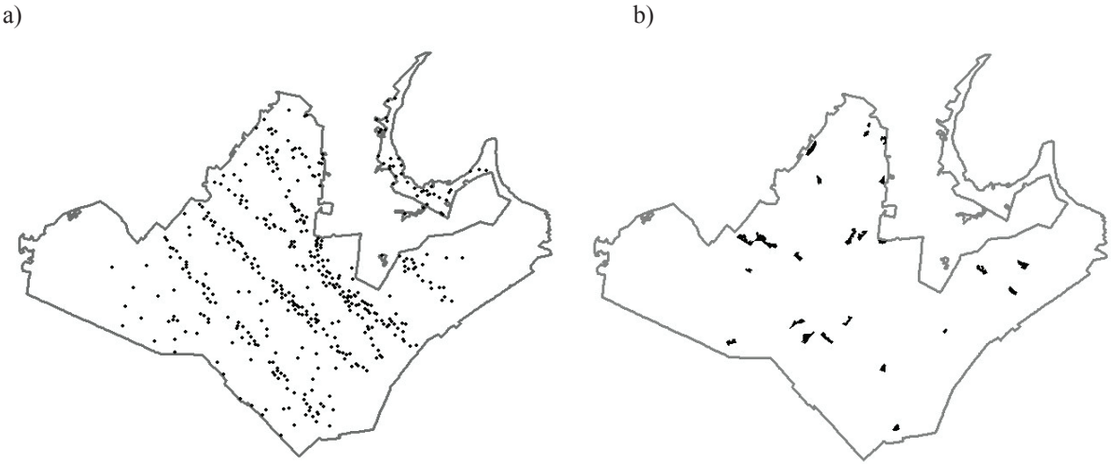

Figure 1. Object of this research: a) location of sample plots, b) location of final felling areas.

using satellite images and the data field estimated on the sample plots: (i) the *k*-nearest neighbor method (Tomppo, 1993) or multi-dimensional version of inverse distance weighted technique familiar to the majority of GIS users, hereinafter referred to as kNN_{10} (k=10), and (ii) the most similar neighbor method (Crookston et al., 2002), which performs a canonical correlation analysis between auxiliary and field measured information data to guide the selection of measurements to impute (MSN_1 and MSN_{10}, with 1 and 10 most similar neighbors used respectively). Detailed descriptions on these techniques may be found in numerous references (e.g. Moeur and Stage, 1995; Tomppo, 2005). Variables available for all the observations were referred to as X-variables (the ones, extracted from satellite images) and the field measured variables (volume of growing stock) were referred to as Y-variables. All field plots measured in Dubrava forest were used to get the Y-variables (this choice is referred as ALL later) and just field plots measured in mature and over-mature forests only (M).

Two methods were used to extract satellite image band-wise digital number values (X-variables) – nearest neighbor (NN) and bilinear interpolation (BIL). Having volumes of growing stock estimated for every "virtual sample point" and assuming that they were unbiased (Mozgeris et al., 2007; Mozgeris, 2008), volume of growing stock estimation for FFA was rather straight-forward and based on averaging the values of "virtual sample points" belonging to a certain FFA. To study the influence of a border "virtual sample points" and potentially reducing the impact of positioning errors both in FFA borders and satellite image, the core area of FFAs was gradually reduced using buffering towards the centers and excluding border points. The following buffer zones were applied: 6, 12, 18 and 24 m.

Volumes of growing stock of FFAs, estimated based on individual tree callipering, were used to validate the modeled ones. There were several statistics used for validation: differences in total and average volumes of growing stock for all FFAs, expressed as percentage of total value coming from FFA's field survey, respectively D_T and D_{1ha}, share of FFAs with the modeled (M_{MOD}) and validation (M_{VAL})volume of growing stock differences larger by 10% and 20% than the validation characteristic (referred to as SH_{10} an SH_{20}, respectively), and the root mean square deviation (RMSD), expressed as percentage of total values coming from FFA's field survey, too:

$$RMSD = \sqrt{\frac{1}{19}\sum_{n=1}^{19}(M_{MOD,n} - M_{VAL,n})^2}. \qquad (1)$$

The Most Similar Neighbor (Moeur and Stage, 1995; Crookston et al., 2002) program was used for non-parametric estimation. Standard GIS, photogrammetric and statistical processing packages (ArcGIS, PCI Geomatica, MS Excel, etc.) were used to prepare, manipulate and analyze the input and output data.

Results and Discussion

Summarized results for all 19 final felling areas are provided in Table 1. It should be noted, that most of the statistics are expressed as percentages of total values coming from FFA's field survey in order to have comparisons with the results from similar studies possible. No matter the estimation tactics, the use of all field sample plots to get the Y-variables resulted in better accuracies than the option to take only sample plots from mature and over-mature stands. This could be considered as the message for the inventories of mature forest carried out in Lithuania, especially using remotely sensed data to get statistically reliable estimates for relatively smaller area unit. The nature of compartments is such that they may contain relatively small amounts e.g. of trees from younger, not being accounted for using field sample plots from mature stands only. Mean volume of growing stock per 1 ha was increased by approximately 10% and the total volume of growing stock by 20% and more if using just field measured sample plots for estimation, i.e. there was no negative bias detected. For sure, the number of field sample plots is important within the frames of two-phase sampling and using non-parametric estimators – increasing the number estimation accuracies tend to increase (Mozgeris, 2000; Mozgeris et al., 2007). However, the minimum amount of such plots resulting in acceptable accuracies is reported to be around 200 (Mozgeris, 1996; Tokola and Heikkila, 1997). Here, the number of field sample plots established in mature and over-mature stands was 190. Thus, the subsequent analyses concentrated on the results achieved using all field sample plots to get the Y-variables (Y_{ALL}), even some of them could be located on younger stands.

The mean volume of growing stock per 1 ha seems to be estimated rather accurately. If 10 nearest or most similar neighbors were used (methods kNN_{10} and MSN_{10}), there was some underestimation (-0.7 – -2.6%) of the parameter observed. Choosing one most similar neighbor resulted in (except some cases) even less differences in mean volumes of growing stock per 1 ha. Total volumes on all 19 FFAs were overestimated by some 5-8%. Lowest root mean square differences were achieved using k-nearest neighbor estimator. However, the differences if most similar neighbor estimator with 10 most similar neighbors was used were rather small. The use of 1 most similar neighbor instead of ten resulted in 1-2% larger RMSDs.

Densification of the network of "virtual sample points" resulted in slightly decreased RMSDs only when mowing down from 30x30 m schemes. There were practically no differences in estimation quality between two methods used to extract satellite image band-wise digital number values (to get the X-variables). However, the nearest neighbor resulted in somewhat larger shares of individual FFAs with the volume of growing stock deviations under 20%.

Table 1

Results of analyses for 19 final felling areas*

Extraction of X-variables	Field plots used	Schemes of virtual sample point distribution														
		30x30					16x16					8x8				
		D_T	D_{1ha}	RMSD	SH_{10}	SH_{20}	D_T	D_{1ha}	RMSD	SH_{10}	SH_{20}	D_T	D_{1ha}	RMSD	SH_{10}	SH_{20}
MSN_1																
X_{NN}	Y_{ALL}	6.2	-1.5	30.8	26	68	6.4	-1.4	29.5	37	68	6.3	-1.4	29.5	37	67
	Y_M	28.1	9.7	29.7	26	37	18.8	10.1	28.7	16	42	18.6	9.9	29.5	16	37
X_{BIL}	Y_{ALL}	12.5	4.3	34.5	26	32	8.4	0.5	30.0	32	53	8.3	0.3	31.0	32	47
	Y_M	25.3	10.9	32.8	5	21	19.1	10.4	31.6	11	32	18.8	10.1	31.1	11	37
MSN_{10}																
X_{NN}	Y_{ALL}	5.5	-2.3	28.6	32	58	5.1	-2.6	28.1	32	53	5.1	-2.6	28.0	37	53
	Y_M	26.8	9.6	31.9	16	37	18.2	9.5	31.9	21	47	18.1	9.5	31.3	21	42
X_{BIL}	Y_{ALL}	7.1	-0.7	28.7	32	53	5.5	-2.2	28.1	32	47	5.7	-2.1	28.6	42	47
	Y_M	25.8	9.4	31.7	16	42	17.6	9.0	31.7	11	42	17.9	9.2	31.2	16	47
kNN_{10}																
X_{NN}	Y_{ALL}	5.9	-1.8	27.9	42	53	6.0	-1.8	28.0	42	53	6.1	-1.7	27.6	42	58
	Y_M	27.4	12.1	31.4	5	37	20.0	11.3	31.0	0	37	20.3	11.5	31.0	11	37
X_{BIL}	Y_{ALL}	6.4	-1.4	28.0	37	53	-2.1	-1.4	27.8	42	53	5.6	-2.2	28.4	42	53
	Y_M	25.0	11.8	32.7	0	32	20.9	12.1	32.4	0	37	20.9	12.1	32.2	0	37

* see the text for interpretation of abbreviations

The root mean square deviations achieved in this study were around 27-28% if the best variants of estimation approach were used. Only less than half of individual FFAs were with the modeled volume of growing stock deviations from the validation one under 10% and only slightly more than half – under 20%. Achieved accuracies are lower than the ones described in literature with using more advanced sources of remotely sensed information or received for rather simple, e.g. Scandinavian forest conditions (Holmström et al., 2001; Holmgren, 2004; Wallerman and Holmgren, 2007). However, they are compatible with the results achieved using just satellite images for rather diverse forest conditions in the USA (McRoberts, 2008), even basal area is used there as the target variable. So, only summary statistics, such as mean volume of growing stock per 1 ha for all final felling areas seems to be applicable operationally at this stage or research. However, there are many signals that rather large number of significant individual deviations may be resulted not by the methods or source data used in this study, but by the quality of validation material. Borders of compartments within the frames of stand-wise inventories are usually not even discussed in terms of positional accuracy; however, they may result in significant volume of growing stock errors (Mozgeris, 1995). GPS-surveyed borders of final felling areas are traditionally considered to be precise, too. Nevertheless, even they surround accurately measured trees for a certain area, outlines of such area may be shifted and therefore may disagree with other geographical data sets, such as e.g. satellite images. Volume of growing stock estimation for FFA was based on averaging the values of "virtual sample points" belonging to a certain FFA. Volume of growing stock estimates at a "virtual sample point level" is usually unbiased and estimated with a root mean square error 40-43% if similar approaches are used (Mozgeris, 2000; Mozgeris et al., 2007), but the point-wise deviations usually have random spatial behavior within the same forest stand. Spatial autocorrelation of point-wise deviations (point-wise volume of growing stock per 1 ha estimate minus the volume of growing stock per 1 ha for the whole final felling area) was studied using gradually reduced core area of the FFA. Core area of FFAs was reduced buffering away from the borders of FFAs towards the center, and only the "virtual sample points" belonging to "new" FFAs were accounted for the spatial autocorrelation. Moran's I and associated Z score value to evaluate the statistical significance of tendencies toward clustering were calculated for the FFAs. There was practically always less than 1% likelihood that the clustered pattern of volume of growing stock estimation residuals could be the result of random chance for with smaller buffer distances and the Z scores were steadily decreasing enlarging the buffers (Fig. 2). With the largest buffer distances, which practically eliminated the border pixels of the satellite image, the patterns became neither clustered nor dispersed.

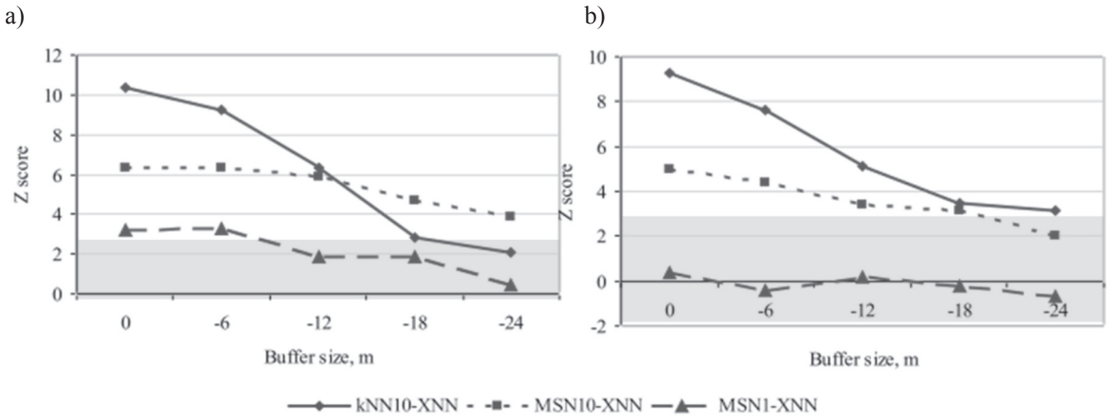

Figure. 2. Statistical significance of the statement that the clustered pattern of volume of growing stock per 1 ha estimation residuals for 30x30 m "virtual samples points" could not be the result of random chance, depending on the buffer distances used to reduce the core area of FFA. The light gray shade indicates the critical values for 0.01 significance level; a) nearest neighbor method was used to extract satellite image band-wise digital number values, b) bilinear interpolation was used to extract satellite image band-wise digital number values.

Table 2

Results of analyses for reduced number final felling areas*

Validation statistics	Estimation method					
	MSN_1-X_{NN}	MSN_{10}-X_{NN}	kNN_{10}-X_{NN}	MSN_1-X_{BIL}	MSN_{10}-X_{BIL}	kNN_{10}-X_{BIL}
3 FFAs with largest residuals removed						
RMSD, m^3	63.8	85.6	86.4	90.6	87.8	88.7
RMSD, %	17.0	22.8	23.0	24.1	23.4	23.6
SH_{10}	44	44	50	38	50	50
SH_{20}	75	63	69	57	56	63
6 FFAs with largest residuals removed						
RMSD, m^3	48.9	66.9	70.7	74.0	73.8	73.6
RMSD, %	13.2	18.1	19.1	20.0	20.0	19.9
SH_{10}	54	54	62	46	62	62
SH_{20}	92	77	85	69	69	77

* see the text for interpretation of abbreviations

When some FFAs with largest differences in modeled and validation volumes of growing stock were removed from the analyses, validation statistics improved notably (Table 2). With just three removed problematic areas, there were practically two thirds of remaining FFAs with modeled and validation volume of growing stock deviations under 20%. This is still unacceptable for the pre-harvesting inventory of the forest compartment, however very promising for the stand-wise forest inventories, especially bearing in mind that practically no extra costs are involved (if the stand-wise inventory continues as the combination of conventional approaches and sampling-based surveys of mature stands).

Conclusions

The following conclusions may be drawn from this study. They most likely will be refined continuing this research and focusing on the issues described above. Thus:

1. Higher estimation accuracies are achieved using the field sample plots distributed through the whole forest area studied than just ones located on mature forest stands.
2. The mean volume of growing stock per 1 ha for the whole study object is underestimated by -0.7 – -2.6%, with no obvious influence of a proper estimation approach. Total volumes of growing stock are overestimated by some 5-8%.

3. There are no significant differences between results using different non-parametric estimation techniques observed
4. The root mean square deviations in estimating compartment-wise volume of growing stock per 1 ha are around 27-28% if the best variant of estimation approach is used.
5. Spatial autocorrelation of the volume of growing stock estimation residuals on a 30x30 m grid tends to decrease when removing grid points closest to the border of a compartment.

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TESTING THE SIMULTANEOUS USE OF LASER SCANNING AND AERIAL IMAGE DATA FOR ESTIMATION OF TREE CROWN DENSITY

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Abstract. This paper introduces the first test results to use laser scanning and high resolution digital color infrared aerial image data to estimate average tree crown density at a sample plot level. General methodological framework based on two-phase sampling schemes, non-parametric estimators and satellite images as the auxiliary data sets was adopted for the use with airborne data sources. More than 400 circular sample plots were established and measured in a special research forest area near Kaunas, the central part of Lithuania. The tree crown density was visually estimated for every coniferous tree belonging to the 500 m^2 plot together with other conventional forest parameters. Two variants of digital color infrared aerial images (ground sampling density 15 and 40 cm), LiDAR point clouds, based on 1 point/m^2 scanning density and two phase sampling approach with non-parametric k-nearest neighbor and most similar neighbor estimators were used to test the accuracies of tree crown density estimation at a sample plot level. Reliable estimates were found to be possible on pure coniferous stands only. Average tree crown density was estimated with the root mean square error around 17.5-18% at a sample plot level, bearing in mind average crown density around 64% for the whole study area. The estimates were unbiased. Integration of laser scanning based variables with the ones available from digital aerial images resulted in lowest estimation root mean square errors. Laser scanning based variables used as the auxiliary data set independently resulted in better estimation errors than the variables available from digital color infrared images.
Key words: sample plot, crown density, LiDAR, aerial image, non parametric methods.

Introduction

Research and as well as operational application of remote sensing techniques in Lithuanian forest inventory was mainly focused on aerial photography solutions in last century (Mozgeris et al., 2008). Aerial photographs were studied as an information source to support the delineation of forest compartments, using general methodological requirement: objective division of forest into compartments is possible only when stand parameters, which can be directly estimated on aerial photographs – such as tree species composition, height, density – are used (Mozgeris and Daniulis, 1997; Daniulis and Deltuvas, 1998; Daniulis and Deltuvas, 2000; Brukas et al., 1999). The evaluation of forest health conditions was left practically behind the scope of research interests during the last decade, in not taking into account some research on the opportunities of color infrared aerial images for estimation the forest health conditions using enhanced method of analytical interpretation (Daniulis et al., 1993). However, there are many successful examples on the use of airborne remote sensing for evaluation of forest or tree health conditions (Hildebrandt, 1993; Zawila-Niedzwiecki, 1996; Daniulis, 1998). Some previous research carried out in Lithuania identified the importance of airborne and satellite remotely sensed images in cartographic modeling of tree crown defoliation (Mozgeris and Augustaitis, 1999; Augustaitis and Mozgeris, 2003). More recently, the relationships between the radiometric and textural characteristics available from digital color infrared aerial images and the tree crown defoliation at a single tree level were reported by Augustaitis et al. (2009). Nevertheless, the general status of research focused on the use of remote sensing for forest health assessment remains rather low and requires special focus, especially having in mind some methodological framework for the use of remote sensing for forest inventories in general being developed in the country.

Main feature of such framework is: the continuous surfaces of key forest characteristics are derived from remotely sensed images and forest field sample plot estimates using parametric and non-parametric estimators within the frames of two-phase sampling schemes (Mozgeris, 2008; Mozgeris, 2009a). This approach originates from Scandinavia and is used by many countries, especially in their National forest inventories by sampling methods (e.g. Tomppo, 1993; Nilsson, 1997; Tomppo et al., 1999; Gjertsen et al., 2000; Franco-Lopez et al., 2001). Different outputs can be generated using different array of auxiliary information, based on similar processing mechanisms (Mozgeris, 2009b). However, the non-parametric methods are recommended as an alternative to the traditional approaches based on regression models as they retain the full range of variation of the data as well as the covariance structure of the population (Moeur et al., 1995). Whole array of forest characteristics is estimated at once, eliminating the need to develop separate models for specific forest attribute and specific set of remotely sensed information (Tomppo, 2005). Estimation of conventional forest stand-wise characteristics, such as growing stock, basal area, height, diameter, species composition, etc. with the use of various auxiliary data sources, such as SPOT 4 HRVIR, Landsat-5 TM, aerial images, was quite well investigated in Lithuania during the last decade. Main conclusion was that the quality of auxiliary data is of main importance for the estimation accuracy, while the implementation tactics, estimators (for sure, if the settings are appropriate) play just a secondary role (Mozgeris and Jonikavicius, 2007). Integration

of several sources of auxiliary information did always result in improved estimation accuracies (Mozgeris, 2008).

Laser scanning or LiDAR (Light Detection and Ranging) is getting more and more important as an option to get remotely sensed information on the forests. Various forest parameters – volume, basal area, height, diameter, number of trees, etc. – are evaluated using LiDAR based techniques (Næsset, 2002). 3D tree models are developed and analyzed (e.g. Harding et al., 2001; Hodgson and Bresnahan, 2004). The first research results on the use of LiDAR to evaluate the tree crown structures were published by Lefsky et al. (2002) and Lim et al. (2003). Laser scanning was reported as a useful tool to get the Leaf Are Index (Solberg et al., 2005) and, using repeated data acquisitions, defoliation (Solberg et al., 2006).

Current study, described in this paper, is a part of a larger scale project aimed at development of operational methods for using laser scanning in Lithuanian forest inventories. Here we aim to test the usability of approaches, originally developed for traditional forest inventory related attributes and satellite images, to estimate other forest characteristics. As such characteristics we took the tree crown density or defoliation, which is a direct indicator of tree health condition (e.g. Augustaitis et al., 2007). This attribute is easy to estimate during field sample plot surveys along with other forest characteristics practically with no extra cost. Laser scanning data and digital color infrared aerial images are used as the sources of auxiliary information here. Potentially, these types of auxiliary information are superior to that ones used in previous studies in Lithuania; however, they are expected to play an important role in forest inventories in the nearest future.

Materials and Methods

The test area (Dubrava forest) is situated in the central part of Lithuania (24°4'E and 54°50'N) with practically all country's forest conditions present here. This area was scanned using ALTM3100 instrument with shooting density 1 point per m^2 (resulting in more than 2 responses per square meter) in the summer of 2008 (Fig. 1). Standard basic processing techniques were applied to get LiDAR point clouds by Finnish company Blom Kartta Oy. Digital aerial photography was carried out the same season to get color infrared aerial images with up to 90% overlap between the images using UltraCam digital camera and 15-40 cm image ground sampling density. All images were orthorectified. More than 400 circular field plots (area 500 m^2) were established and measured within half a year after acquiring the remotely sensed data. Standard dendrometric characteristics were measured for trees on the plot: polar coordinates of each tree, tree species, diameter, crown type, height for sample trees (not less than 1-3 sample trees for each forest element), etc. Volumes for all trees (including the volumes of stems, branches and volume distributions by merchantability classes) were later estimated utilizing the methods operational in Lithuanian forest inventories (Bikuviene et al., 2009) and used to get plot-wise estimates, including the shares of different tree species. Coordinates (X, Y and Z) of the center of each sample plot were measured using electronic tacheometer (Leica Smart Station GPS 1200 Series) with accuracy not worse than 0.1 m.

Figure 1. Canopy height model and locations of field sample plots within the test area: ● ——— Sample plot.

Crown density for all coniferous trees was visually estimated in the field following the methodology of forest health monitoring (Tallent-Halsell, 1994). Only coniferous trees were taken into account, thus influencing the approaches used later for current study.

The non-parametric estimation technique was used to test the usability of laser scanning and digital aerial image data to estimate the tree crown density, which could be considered as an inverse version of tree crown defoliation, i.e. the indicator, commonly used to evaluate forest health conditions. All sample plots were described using two types of variables: X-variables, originating from laser point clouds and digital aerial images and Y-variables, based on field estimates. There were 169 various X-variables based on laser point cloud and 90 based on digital image data calculated for each plot (pf95, pf90, pl95, pl40, pl95/pf99, pl90/pf99, pf80/pf95, pl99, pi60, pi95, normalized differences and ratios of some percentiles, indices of vertical density, ratios of digital numbers of aerial images – red/green, green/near infrared channels. pfXX means corresponding percentile of the first pulse response, plXX – corresponding percentile of the last response, piXX – the percentile of response intensity). The approach was to use all possible variables without focusing on some of them, as the computation techniques enabled managing them all together and there was no objective to study them separately.

Average crown density of coniferous trees belonging to the sample plot was used as the Y-variable. As only coniferous trees were visually estimated in respect of crown density in the field, sample plots were grouped according to the share of coniferous trees using the following threshold values: 95, 90, 85, 80, 75 and 70%, i.e. the share coniferous trees making up corresponding value or higher. All subsequent analyses are based on these groups. There were no less than 100 sample plots in such a group.

Two non-parametric estimation techniques were used to estimate the plot-wise average crown density using the whole array of remotely sensed auxiliary variables: (i) the *k*-nearest neighbor method (Tomppo, 1993) or multi-dimensional version of inverse distance weighted technique familiar to the majority of GIS users, hereinafter referred to as kNN, and (ii) the most similar neighbor method (Crookston et al., 2002), which performs a canonical correlation analysis between auxiliary and field measured information data to guide the selection of measurements to impute (MSN). Detailed descriptions on these techniques may be found in numerous references (e.g. Moeur and Stage, 1995; Tomppo, 2005). The choice of these methods was motivated by several reasons: they are easy to implement and to use operationally, they are well documented in numerous references, and they have resulted in best outputs in our previous studies (Mozgeris, 2009). However, MSN is potentially even more attractive than kNN, as it does not require the optimization of settings for the estimator. As all plots contained both X and Y variables, a type of cross-validation technique was used to validate the estimation quality (based on selecting the second most similar/nearest neighbor among the reference observations). The following statistics referring to the imputation residuals were used for validation (Crookston et al., 2002): the root-mean-squared error (RMSE) of the residual values, the mean residual, the t-Ratio and the probability of a greater t-Ratio. The t-Ratio is calculated as:

$$t = \frac{s_d}{s_{\bar{d}}},$$

where: s_d - the standard deviation of differences $s_d = \sqrt{\frac{\sum_i (d_i - \bar{d})^2}{n-1}}$, (1)

$s_{\bar{d}}$ - the standard deviation of the mean difference $s_{\bar{d}} = \frac{s_d}{\sqrt{n}}$. (2)

t-Ratio was computed for a paired comparison under the null hypothesis that the mean of the residuals is zero. A large t-Ratio and a low probability of getting a larger one indicated that the imputed values are significantly different than the observed. The root mean square error was expressed as the percentage of mean observed crown density value on a subset of sample plots.

The Most Similar Neighbor (Moeur and Stage, 1995; Crookston et al., 2002) program was used for both non-parametric estimation and validation of the plot-wise crown density figures according to numerous laser scanning and aerial image based auxiliary variables. Standard GIS, photogrammetric and laser data and statistical processing packages (ArcGIS, TerraScan, MS Excel, etc.) were used to prepare, manipulate and analyze the input and output data.

Results and Discussion

Although two non-parametric estimation methods were assumed to be used for this study, the most similar neighbor estimation was sometimes problematic due to relatively small amount of sample plots used for calculations. The main issue in selecting the kNN method as the non-parametric estimation technique is a little bit more complicated operational use of this method. Both estimation techniques were later compared using reduced number of X-variables.

There were practically no significant differences detected between mean observed and imputed crown density values using both types of auxiliary variables (LiDAR and aerial image based) – Table 1. However, the imputed values where somewhat lower than the observed ones when using aerial image based variables, especially when considering relatively more pure coniferous stands.

Table 1

Validation statistics referring to the estimation bias

Share of coniferous trees, %	Number of plots	Whole stand					The main storey only				
		Crown density		Residuals			Crown density		Residuals		
		Mean	Standard deviation	Mean	t-Ratio	P>|t|	Mean	Standard deviation	Mean	t-Ratio	P>|t|
LiDAR variables only											
95	100	63.782	15.355	0.659	0.36	0.72	63.526	15.183	0.725	0.402	0.689
90	138	62.952	15.93	-0.55	-0.345	0.73	62.89	15.716	-0.606	-0.374	0.709
85	170	63.387	16.541	-0.67	-0.397	0.692	63.599	16.505	-0.548	-0.324	0.747
80	197	63.222	16.462	-0.666	-0.422	0.674	63.527	16.491	-0.577	-0.365	0.716
75	218	63.963	16.226	-0.142	-0.095	0.924	64.215	16.251	-0.199	-0.134	0.893
70	231	63.885	16.123	0.67	0.464	0.643	64.208	16.114	0.576	0.405	0.686
Aerial image variables only											
95	100	63.782	15.355	3.933	1.96	0.053	63.526	15.183	4.039	2.016	0.047
90	138	62.952	15.93	2.226	1.242	0.216	62.89	15.716	2.279	1.266	0.208
85	170	63.387	16.541	1.889	1.125	0.262	63.599	16.505	2.061	1.199	0.232
80	197	63.222	16.462	1.981	1.298	0.196	63.527	16.491	2.117	1.349	0.179
75	218	63.963	16.226	1.224	0.887	0.376	64.215	16.251	1.328	0.936	0.35
70	231	63.885	16.123	0.496	0.371	0.711	64.208	16.114	0.505	0.367	0.714
LiDAR and aerial image variables together											
95	100	63.782	15.355	0.81	0.453	0.651	63.526	15.183	0.393	0.225	0.823
90	138	62.952	15.93	0.74	0.426	0.671	62.89	15.716	0.668	0.383	0.702
85	170	63.387	16.541	1.202	0.652	0.515	63.599	16.505	1.222	0.663	0.508
80	197	63.222	16.462	0.629	0.372	0.711	63.527	16.491	0.705	0.414	0.679
75	218	63.963	16.226	0.659	0.426	0.67	64.215	16.251	0.728	0.468	0.64
70	231	63.885	16.123	1.129	0.789	0.431	64.208	16.114	1.212	0.843	0.4

Smallest root mean square errors were achieved when plots with the share of coniferous trees over 95% were taken into consideration (Fig. 2). However, this tendency was less evident with aerial image based X-variables. This refers to both types of auxiliary information used independently or together. Decreasing share of coniferous trees within the plot means increasing influence of deciduous trees, affecting the laser pulse reflectance characteristics of the canopy. X-variables were computed based on all laser points or image pixels belonging to the plot, thus our findings are valid regarding pure coniferous stands only. Laser scanning data resulted in lower root mean square errors than the aerial image data for plots with relatively higher share of coniferous forests. If taking into account just the plots with share of coniferous trees 95% and more, the use of laser scanning based X-variables produced root mean square errors approx. 3% less than the use of aerial image based X-variables. Simultaneous use of both types of X-variables resulted in lowest root mean square errors, which were approx. 2% less than the best achieved if laser scanning and aerial image variables used separately.

Best achieved relative root mean square errors mean approx. 17.5% if absolute crown density values are used. There is hardly any application where the plot-wise estimates are used as the final objective. Most likely they are used to provide statistically reliable estimates for some larger areas, such as forest compartments, groups of compartments, some forestry regions, etc. Our experience from the same study area with other forest variables (such as volume, mean height, basal area, etc.) is such that the root mean square error drops twice if the unbiased plot-wise estimates are aggregated to the level of a segment (relatively uniform area unit, generated segmenting laser data based grids, simulating the forest compartments). So, one could expect the compartment-wise estimates of tree crown density with the root mean square errors under 10%.

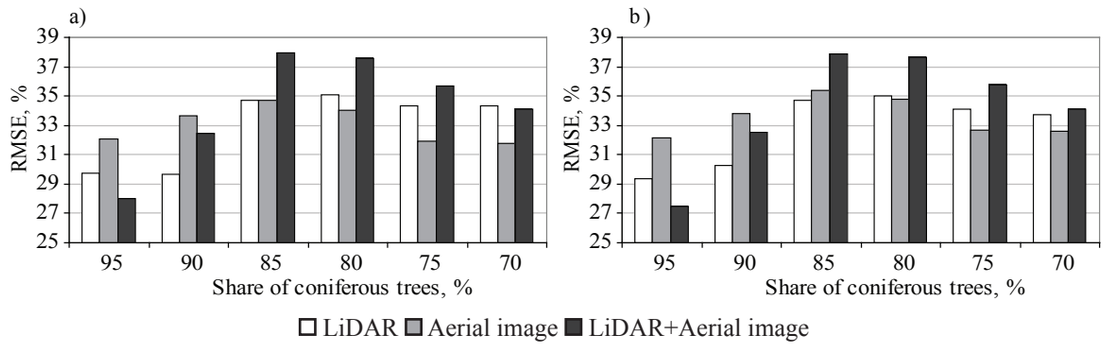

Figure 2. Relative estimation root mean square errors depending on the source of X-variables and the shape of coniferous trees; a) whole stand is taken into consideration, b) only main storey trees are taken into consideration.

Table 2

Relative estimation root mean square errors achieved with reduced list of X-variables

Share of coniferous trees, %	LiDAR variables only		Aerial image variables only		LiDAR and aerial image variables together	
	MSN	kNN	MSN	kNN	MSN	kNN
95	33.44	28.13	31.41	32.46	27.73	28.05
90	34.78	28.37	35.16	32.58	34.91	33.40
85	35.20	32.26	36.87	32.19	33.86	34.23
80	36.22	31.94	33.80	34.16	34.99	35.55
75	34.43	31.49	32.62	33.04	33.56	36.11
70	34.02	32.77	35.68	32.62	35.34	35.62

The main issue in selecting the kNN method as the non-parametric estimation technique is rather complicated operational use of this method. Estimation results are sensitive to the different settings of the kNN (e.g. number of nearest neighbors, power of distance weights, etc.) (Katila et al., 2001). Settings are even being optimized for each particular application case (Tomppo and Halme, 2004). Our own experience previously has been that the deciding factor in success with non-parametric estimators is the quality of auxiliary information first and only then the estimation techniques used (Mozgeris and Jonikavicius, 2008), and there is an increasing interest in using more simple MSN estimator instead of kNN (Mozgeris, 2009). To test the accuracies of MSN estimator with the laser and digital image based X-variables, we reduced the total number of X-variables removing the ones, which had correlation with the plot-wise tree crown density's values under 0.1. There were 43 laser scanning and 10 aerial image related X-variables remaining. The same precondition – results were reasonable for pure (share of coniferous trees is more than 95%) coniferous stands only – remained valid (Table 2). MSN estimator resulted in larger relative root mean square errors than the kNN estimator with laser scanning based X-variables. Using LiDAR and aerial image variables together resulted in lowest root mean square errors, too. It should be noted, that reduced number of laser scanning based X-variables resulted in lower root mean square error with the kNN estimator on the plots with share of coniferous trees above 95%.

Conclusions

The following two conclusions may be drawn from this study:

1. verage coniferous tree crown density was estimated with the root mean square error around 17.5-18% at a sample plot level using the non-parametric estimation methods and 1 pulse per m^2 laser scanning and digital aerial image data as the auxiliary data sources. The estimates were unbiased. Integration of laser scanning based variables with the ones available from digital aerial images resulted in lowest estimation root mean square errors.
2. Laser scanning based variables used as the auxiliary data set independently resulted in better estimation errors than the variables available from digital color infrared images.

For the Future

-We expect the outcomes of this research may contribute to a routine system of forest inventory and health monitoring in Lithuania by remote sensing; however, there are numerous both methodological and technological issues to be solved. We assume that forest health statistics will be most likely just supplementary to the ones describing the growing stock, thus the methods of using laser scanning and aerial images

are much coming here from the field forest inventory related application of remote sensing. The research plans for the nearest future include investigation of single tree detection and identification solutions to evaluate the tree crown density, potentially reducing the impact of factors having influence at the sample plot level. Data available for the test area enable to carry out such study. On the other hand, operational forest inventories in Lithuania require characteristics for forest compartments, so our plot-wise estimation techniques will be tested to get statistically reliable forest health condition attributes compartments-wise.

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DETECTING AND MEASURING INDIVIDUAL TREES WITH LASER SCANNING IN LATVIAN FOREST CONDITIONS

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Abstract. Researching new remote sensing and data processing methods is very important subject in forestry. The objectives of research are to explore methods to determine single tree characteristics using LIDAR and adapt them for Latvian forest conditions. Different algorithms and mathematical relations for automatic calculation of tree species, coordinates, height and diameter at breast height is described. Within the project four different clustering methods for tree identification were evaluated. The first method's construction is based on reflection point count in certain height range. The second and third methods are searching for global and local maximums on height axis of LIDAR data collection. In the fourth method segmentation of aerial photography is done by using the user selected sample data. Tree tops were s discovered by searching similarly colored regions. Field measurements were used for the calibration of LIDAR data and analysis. Sample plots were fitted in the study area with different species composition, age and density. The total number of measured trees in sample plots is 1844. Results show that height can be found mainly for I, II, III craft class trees with average error 2.5%. Stem diameter estimation error of pine is 28%, spruce 17%, birch 4.2% and second storey trees 5.4% using linear equations D = 0.6616*H + 4.6969 (for coniferous trees) and D = 0.7756*H + 3.7132 (for deciduous trees). Dividing trees in classes of coniferous and deciduous can be done by using near infra red photography. The total number of first storey trees identified by LIDAR is 91%, by aerial photographic method 94%.
Key words: Forest inventory, laser scanning, single-tree method, small footprint, aerial photography, data fusion.

Introduction

Traditional forest inventory, which is usually time-consuming and relatively costly, provides the opportunity to develop new remote sensing techniques, which are less labor intensive and less costly. LIDAR (Light Detection and Ranging) technology is now one of the newest and most perspective methods to determine forest stand and individual tree characteristics. Optimal forest inventory method often consists of a variety of data sources that are combined with various methods of remote sensing. Different sensors or methods that encompass certain levels of observation should not be taken as exclusionary alternatives (Korpela et al., 2007). Single-tree remote sensing (STRS) based forest inventory is intended to replace field measurements such as position, species, height, stem diameter and volume. Field data can be collected for LIDAR data correction, but the method's vision is to create it without field visits and measurements (Koetz et al., 2006). However, STRS estimates seem to be prone to bias - e.g. the use of LIDAR often results in an underestimation of tree heights (Korpela et al., 2007) or many trees are not identified (Parker, 2006). This means that for these data calibration is necessary. Field data collection will need determination of the diversity, which cannot be measured from the air, so controlling the gap between the LIDAR data and field measurements.

In studies STRS forest inventory using LIDAR, one of the main problems that the authors mentioned is tree species and tree location accurate determination (Hyyppä et al., 2008), especially in Middle Europe (Diedershagen et al., 2006), since there is a mixture of different deciduous and coniferous trees. As a result, the indication is much harder. The main conclusion is that the usage of LIDAR method to determine forest inventory parameters will never be one hundred per cent correct (Rombouts, 2006 and Onge et al., 2004), especially applying automated tracking methods (Hyyppä et al., 2004). However, compared with traditional inventory methods, laser-based method is faster, more cost efficient, and the results are reliable, but with certain restrictions (Diedershagen et al., 2006).

In Scandinavian countries studies of STRS forest inventory using LIDAR show good results. For example Korpelas research shows that 2% of conifers and 10% deciduous trees location determined inaccurately, 10% of all trees were not identified, 12% of the total stock of wood is inaccurate, species identification accuracy - 93.7%, only 4.7% error in the determination of the tree height (Korpela, 2006). Also, Nicholas Coop research results show that there is a close correlation between the LIDAR data and field measurement data of vertical canopy parameters. As a result, it is possible to successfully obtain quality information on forest indicators (Coops and Hilker, 2007). In Peuhkurinena survey traditional forest inventory data and LIDAR data were compared with harvester data obtained from 22 spruce stands. LIDAR method showed a 17% higher precision of trees diameter of breast height (Peuhkurinen et al., 2008).

Numbers of different methods are used to identify a single tree using STRS. The main criterion for determining choice of method is the specific structure of forest canopy and species diversity. If the area of

construction is more complicated, tree locations and their exact coordinates are difficult to determine. Single-scale template matching has been successfully applied in 2D and 3D treetop estimation of regular stands, where crowns show only moderate variation (Korpela, 2006). In contrast, to determine all the treetops where forest foliage is complex in structure and with a large variation, the most appropriate are the automatic and semi-automatic methods (Korpela et al., 2007).

Practically for all researchers so far it has been difficult to determine the species in mixed forest stands. Automated identification of species with the individual tree method is still problematic, even in cases where access to different types of data (Vauhkonen et al., 2008) is available. Säynäjoki provides significant advantages in use of digital photogrammetric techniques to construct digital surface models. As a key factor to improve species identification results, is the multispectral images admission to the same target from different angles (Säynäjoki et al., 2008). Jari Vauhkonens has published latest results of species identification with the "Alpha-shapes" based approach, using high-density laser data. Later his methods were successfully detected and identified in 98% of the trees (Vauhkonen et al., 2008). This approach is currently being initiated to test the other stands, and the results are still to be published. This could be one of the most promising methods of determination of tree species.

In Latvia, the first attempts to use the LIDAR technology in forestry started in 2007. Technology comes from the Scandinavian countries. With a large number of research and perseverance they are developed and adapted itself suitable data collection and processing methodology, which is capable of providing high quality data acquisition. Unfortunately, one of the biggest problems is that the data collection and processing methods in Latvian conditions work differently, and those methods cannot provide forest inventory data quality requirements. This is mainly due to the large number of tree species and forest diversity in growing conditions, as well as LIDAR technology specifics. The objective of research is to explore methods to determine single tree characteristics such as position, species, height and stem diameter using LIDAR and digital aerial photography in Latvian forest conditions.

Materials and Methods

The study site is a forest in the middle of Latvia in Jelgava district (56º39' N, 23º47' E). The area consists of mixed coniferous and deciduous forest with different age, high density, complex structure, various components and composition. Represented species are pine (*Pinus sylvestris L.*), spruce (*Picea abies (L.) H.Karst*), birch (*Betula pendula Roth*) and aspen (*Populus tremula L.*).

Data were obtained using a specialized aircraft Pilatus PC-6, which is equipped with a positioning and Geomatics technology company Leica Geosystems equipment - a large format digital aerial photography camera and laser scanner. The study area was flown over by plane and scanned at 1000 meters altitude. A LIDAR digital terrain models (DTM) were estimated from leaf-on data from May, 2009 having 5 points per m^2. The image data is RGB (Red, Green, and Blue*)* and NIR *(*Near Infrared*)* spectrum taken the same time.

Field measurements were used for the calibration of LIDAR data and analysis. Seven sample plots were fitted in the study area with different species composition, age and density. Sample plots were established with dimensions 50x50 m (0.25 ha). The total number of measured trees in sample plots is 1844. The first five plots with a total number of sample trees 1207 and the area of 1.25 ha was taken as the basis for calculations, but the 6th and 7th sample plot - for data checks. Field work was to determine tree species, tree coordinates with accuracy of ± 3 cm, mean height in all diameter classes with accuracy of ± 10 cm, and diameter at breast height with accuracy of ± 1 cm. Individual tree coordinates were established by professional surveyors, and result is a topographic map of each sample plot with all trees.

Within the project four different clustering methods for tree identification were evaluated. First method's construction is based on reflection point count in a certain height range. It was made by adopting density based clustering algorithm, which was accompanied by restrictions on the radius determination. The second method was used for LIDAR data set heights of the axis of the global maximum and range limitations. This method worked poorly. First, the LIDAR data set points were read, then divided into quadrants. Afterwards, the maximum points in the upper layer and formed cluster were found, and deleted from the cube. In this way part of points belonging to other trees were lost, and trees were omitted. The third method is searching for local maximums on height axis of LiDAR data collection, but in the fourth method segmentation of aerial photography is done by using the user selected sample data. Results obtained by visual analysis were selected, two best automatic methods for tree identification - local maximum method (Figure 1.) and aerial photo method (Figure 2.) were used.

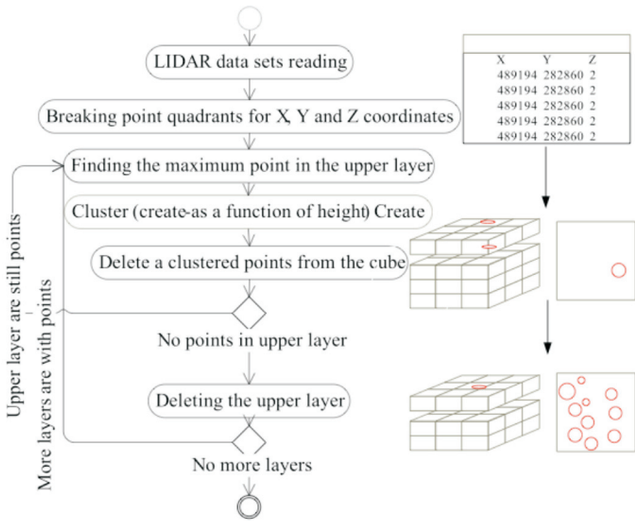

Figure 1. Tree identification algorithm with a local maximum method.

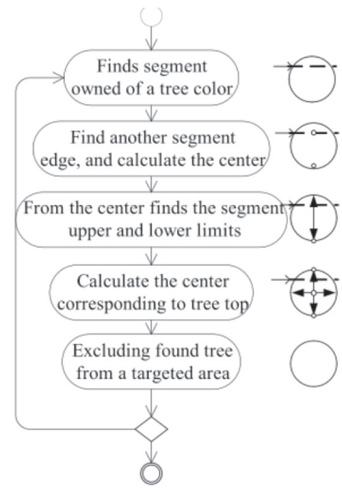

Figure 2. Tree identification algorithm with an aero photo method.

Both algorithms working principles are closely connected with a certain tree crown radius; therefore, they work better in complex structure of forests than methods, which use a fixed radius. The used methods mainly characterized the first storey of forest trees; nevertheless, a part of second storey was identified as well.

The intensity of reflecting absorption rate was analyzed to recognize tree species. Color information from images obtained in the nearest infrared spectrum was used for species identification. From identified trees the pixels of tree were analyzed, and the membership of conifers or deciduous trees was determined. After the color information of identified trees, it was possible to allocate only the conifers or deciduous trees. In addition to above-mentioned method, it is still possible to recognize tree species using crown shape comparison method for LIDAR data, which in this case could not be done because the point density was low. Determination of species composition is one of the most complex processes. For the study, to be able to calculate the single tree characteristics of the species level using LIDAR, and make comparisons, tree species were taken from the plot data.

Tree height is derived by looking at the minimum and maximum signals received by LIDAR. The reflected signal returns at different times depending on the distance. These intervals are taken as the basis for determining the height of the tree. The first signatures are taken to determine the tree crown, but the last signal is used to characterize the land surface. Once the tree location is fixed, a tree height is determined by the algorithm shown in Figure 3.

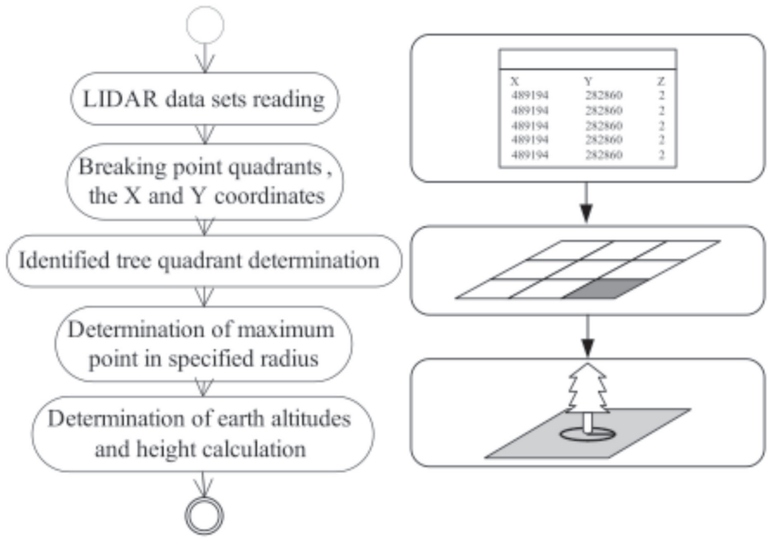

Figure 3. Tree height algorithm.

In the study for estimating tree diameter relation between height and diameter in conifers and deciduous trees was used. Equations that predict diameter in breast height using field data for coniferous (1) and deciduous (2) trees were applied:

$$DBH = 0.6616 \times H + 4.6969 \quad (R^2 = 0.8647) \quad (1)$$

$$DBH = 0.7756 \times H + 3.7132 \quad (R^2 = 0.9137) \quad (2)$$

These relations were created based on 11 223 sample plots established by Latvian State Forest Research Institute "Silava" taken from the period 2005 -2007. To assess the accuracy of the forest inventory parameters the data of tree height, species and number of trees in diameter classes were used. Data for the LIDAR and field methods were recorded and processed in software „Mežaverte" where all forest inventory parameters are determinate.

Results and Discussion

Results of tree detection using aerial photographic method show that 603 of 1207 STRS-trees were unambiguously found, but using local maximum method 881 trees were found. The total number of trees identified by the LIDAR is 73%, but the aerial photographic method, only 50%. The local maximum method partially recognized the second storey trees, which cannot be seen in the aerial photo, because they are obscured by trees on the first storey. If we look at the first storey of the trees, the two methods produce equally good results - with LIDAR -91%, with aero photo -94% of cases - a tree is identified. Here aerial photographic method worked slightly better, because when trees are close together LIDAR combines them. The 43% of the second storey of the trees were identified using LIDAR, but using aerial photographic method only 5% were identified. The calculated coordinates of tree top 86% of cases are located in 3 m limit, but 14% are located more than 3m. Trees vary in crown size, shape and optical properties. Crowns are often interlaced. Occlusion and shading are present, and result in omission errors. These factors affect the treetop positioning and make the identification of STRS-trees difficult. Professor Kocha research shows that automatic segmentation of the tree canopy is working well in conifer stands and rare hardwood stands. The problem is with the small trees and close existing trees identification, as well as high density hardwood stands with homogeneous crown (Kocha et al., 2006).

Maximum height difference between the field measurements and LIDAR measurements of each sample is 2.2 m, the minimum difference -0.2 m. The average height difference between field measurements and LIDAR measurements is 0.27 m (1.2%). Most of trees - 67% LIDAR determined tree heights lower than they were actually with an average error of 1.3m or 4.3%, but the remaining 33% of tree height is determined higher than they were actually with an average error 1.1m or 3.7%. The height of 2/3 trees are definitely lower, it can be explained by the fact that during the laser scanning, transmitted pulses missed the tree top and hit a little below. We can conclude that using the LIDAR system with a low point density per square meter, tree heights will be lower than in systems with high point density. Figure 4., 5., 6. and 7. described average tree height in diameter classes for each species from field measurements and the LIDAR measurements.

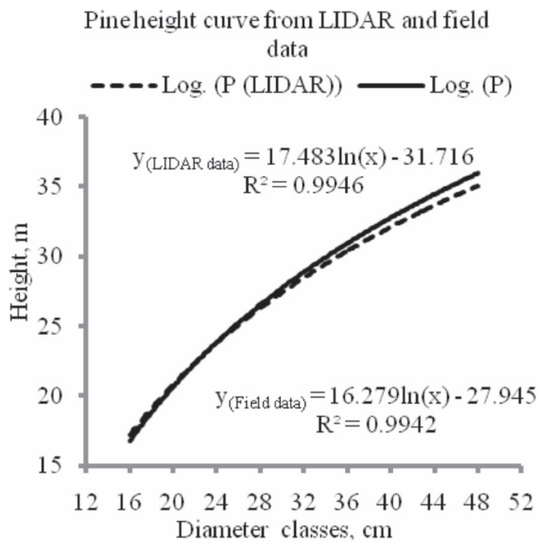

Figure 4. Pine height curve from LIDAR and field data. Curves show average heights in diameter classes.

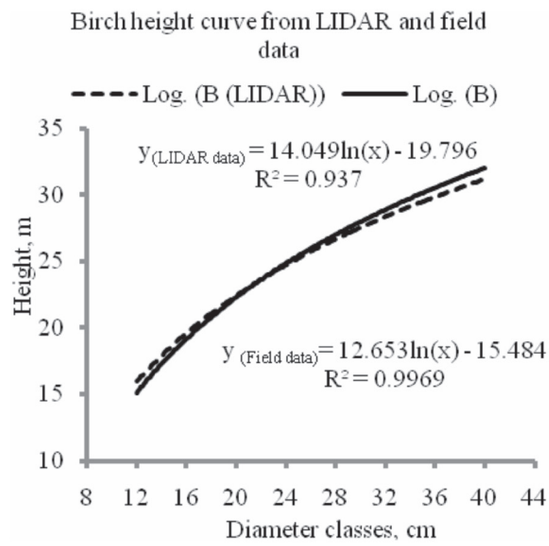

Figure 5. Birch height curve from LIDAR and field data. Curves show average heights in diameter classes.

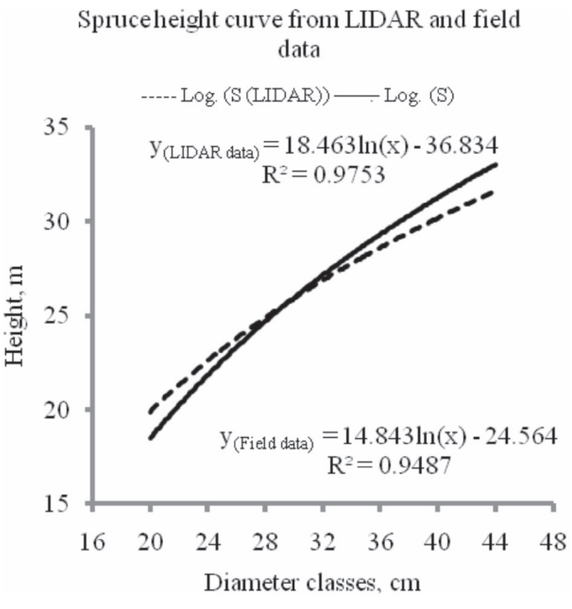

Figure 6. Spruce height curve from LIDAR and field data. Curves show average heights in diameter classes.

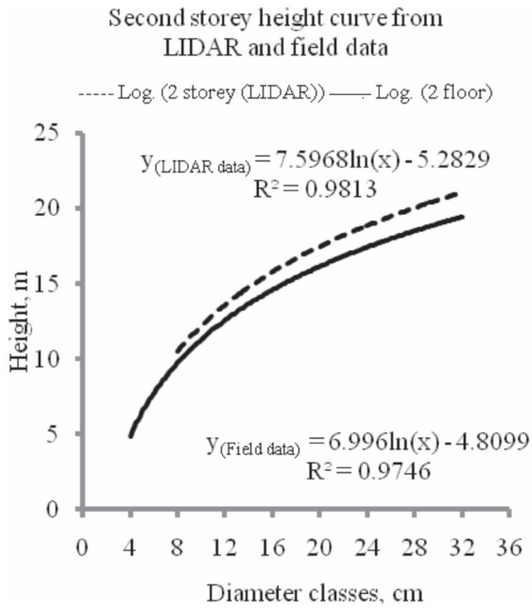

Figure 7. Second storey height curve from LIDAR and field data. Curves show average heights in diameter classes.

Looking at the given height curves, we can see that the first storey of the trees of all species in diameter classes which are greater than 28, the average tree height of the LIDAR data is slightly lower, but in diameter classes which are smaller than 28, the average tree height is set slightly higher. Most apparent correlation is seen in spruce. This is explained by the fact that the spruce crown geometry is triangular and consequently, the LIDAR transmitted pulses often miss the highest tree point. Pine and birch crown geometry is a little flatter; thus, the measurements are more accurate. If we look at the second storey height curve, then the average tree height of all diameter classes from LIDAR data has been determined greater than the average height determined from field measurements. Such relationship would be explained by the fact that during LIDAR data processing, the second storey trees highest point is taken from the first storey canopy lowest points.

In the study, trees were divided into coniferous and deciduous using color information from images obtained in the near infrared spectrum, and it is possible to make high-precision. Results show that 98% of cases classification was correct. But such classification is not applicable for tree characterizing in forest inventory. Recognition of better species by the used study method is not possible to determine, so new studies about individual tree identification methods must be done to resolve these problems. For species recognition it is possible to use tree crown shape comparison method from LIDAR data. Unfortunately, this time it was impossible to do because the LIDAR point density per $1m^2$ was too small. In the future, tree crown shapes and multispectral data analysis can be used for better species recognition.

Results of stem diameter estimation accuracy (Table 1.) show that mean difference between field measurements and LIDAR measurements of pine is 8.2 cm (28%), spruce 5.0 cm (17%), birch 1.8 cm (4.2%) and second storey 1.3 cm (5.2%).

Table 1

Accuracy of stem diameter estimates, cm

Statistical indicators	Pine		Spruce		Birch		Second storey	
	Field data	Estimated	Field data	Estimated	Field data	Estimated	Field data	Estimated
Mean	29.8	21.7	23.3	19.8	26.6	21.9	14.1	15.3
St. Error	0.5	0.2	0.1	0.2	0.1	0.4	0.2	0.1
St. Deviation	8.5	3.0	2.1	2.5	1.6	4.2	4.8	3.0
Minimum	9.3	12.0	2.0	13.3	21.4	11.5	4.9	8.7
Maximum	50.0	29.8	27.9	26.8	30.2	29.3	33.5	21.9
Count	345	345	271	271	123	123	471	471
Confid. Level (95%)	0.902	0.312	0.255	0.302	0.279	0.742	0.434	0.388

Results of stem diameter differences between LIDAR and field data depending on the species can also be seen in Figure 8., 9., 10. and 11.

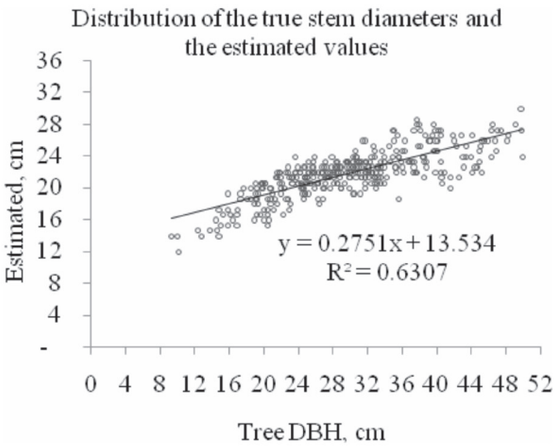

Figure 8. Distribution of the true stem diameters and the estimated values by LIDAR-based method (Pine).

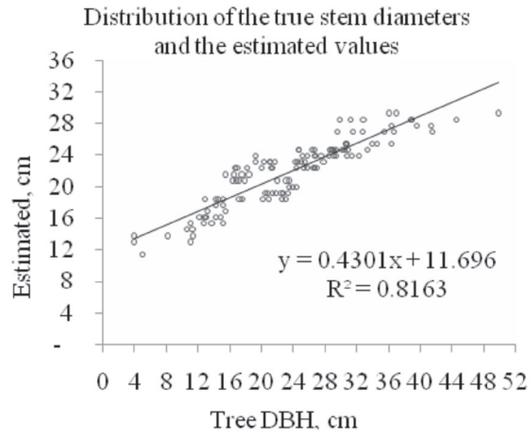

Figure 9. Distribution of the true stem diameters and the estimated values by LIDAR-based method (Birch).

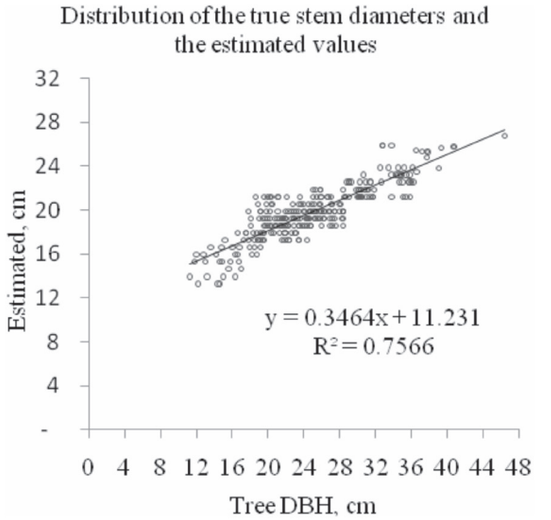

Figure 10. Distribution of the true stem diameters and the estimated values by LIDAR-based method (Spruce).

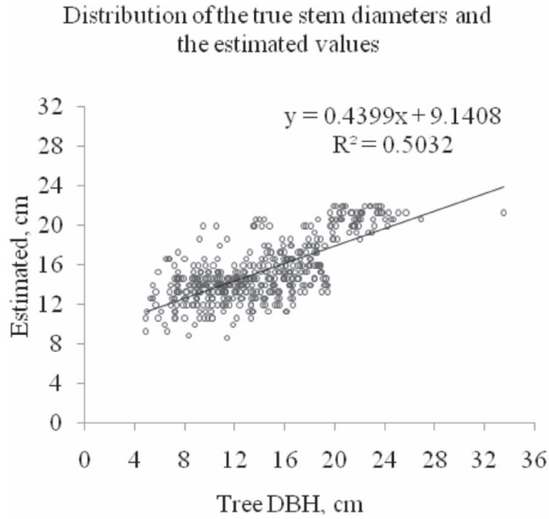

Figure 11. Distribution of the true stem diameters and the estimated values by LIDAR-based method (Second storey).

These errors are explained by the fact that the tree height is not determined with complete accuracy, but the main reason could be inaccurate relationships used for determination of diameter. Estimated diameters of the first storey trees are set 62% lower than in reality, and it shows that tree diameter has a tendency to be smaller. Estimated diameters of the second storey trees are set 97% higher than in reality; the second storey has a tendency to be greater. The tree structure and functioning are closely connected, and it is possible to take account to improve the accuracy of the tree diameter estimation. If STRS method could provide accurate measurements of foliage density and foliage mass (Ilomäki et al., 2003) or crown length (Kantola and Mäkelä, 2004), then the accuracy would be significantly higher. Korpela studies show that using allometric equations, it is possible to identify individual tree diameter with 87% accuracy (Korpela and Tokola, 2006), and using allometric regression models with accuracy 95% (Korpela et al., 2007) results are shown in coniferous stands.

Forest inventory parameters were determined using LIDAR and field measurements in all plots together. Measurements were collected by plots and expressed per hectare. Results of forest inventory data show that average height of stand is predicted with 5% error, the average diameter with a 3% error, basal area -16% error, volume -13% error, density level -20% error and number of trees with 38% error. Mainly errors are explained by omitted trees

Conclusions

Results show that tree height can be found with average mistake 2.5%. Stem diameter estimation error of pine is 28%, spruce - 17%, birch - 4.2% and the second storey trees - 5.2% using linear equations D = 0.6616*H + 4.6969 (for coniferous trees) and D = 0.7756*H + 3.7132 (for deciduous trees). Classes of coniferous and deciduous trees using color information from images obtained in the near infrared spectrum are possible to recognize for 98%. The total number of trees identified by the LIDAR is 73%, but by the aerial photographic method, only for 50%. The total number of the first storey trees identified by LIDAR is 91%, by aerial photographic method - 94%. Latvian forest conditions are difficult for STRS methods mainly of mixed deciduous and coniferous spaces with high level of the second storey trees in one stand. Mostly trees are close together with high density and homogeneous crown. It is one of the main reasons for a large number of trees that are omitted. It is necessary to combine different remote sensing sensors and methods to improve the recognized number of trees. Other way is to perform laser scanning in spring when the forest is less dense, the first storey trees are more transparent and the smaller dimension trees can be recognized. For better species recognition it is possible to use tree crown shape comparison method from LIDAR data, and it means that there is a need for LIDAR data with a higher level of point density per square meter. Or multispectral data analysis or combination of channels can be used for better species recognition. For better DBH estimation allometric equations, from tree crown diameter, length, foliage and density can be obtained.

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IMPACT OF AMELIORATION SYSTEM RENOVATION ON THE GROWTH OF MATURE FOREST STANDS

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Abstract. Both forestry theory and practice have proved that amelioration favours the growth of forest stands significantly. The growing stock increases even 10 times, if the amelioration system is well-functioning. Joint Stock Company "Latvia State Forests" that is managing half of forests in Latvia has started renovation of old amelioration systems aiming to improve the productivity of state owned forests in Latvia. In order to evaluate the impact of the renovation of the old amelioration systems, a tree ring width in 7 pine stands, 5 spruce stands and 3 birch stands was analysed. This study shows that the renovation itself has not affected the productivity of mature pine, spruce and birch significantly 3-4 years after the renovation. Authors have come to a conclusion that the reason for that could be too short observation period or particularities of a stand structure and age.
Key words: amelioration system, mature forest stands, productivity, renovation.

Introduction

The amelioration impact

The hydrotechnical amelioration has been the most effective method for increasing forest productivity during the last century. The average increase of the growing stock has been by 100 m^3 ha^{-1} from 1939 until nowadays (Indriksons and Palejs, 2005; Zālītis, 2006).

Even if the forest soil is rich with diverse mineral nutrients, the forest is unable to use them because of oxygen deficiency, not because of increased groundwater level; and the productivity of forest stands remains very low until amelioration with a purpose to improve the soil aeration is carried out (Буш, 1968; Вомперский, 1968; Залитис, 1968). The amelioration effect can be observed in all parameters of the forest stand. The best "rebirth" of the forest stand can be observed in stands with low initial site index. The best amelioration effect appears in former bogs – the productivity increases 10 times (Odiņš, 1971).

In less fertile site types, the influence of amelioration can become apparent with a delay of 1-3 years and deeper than regular ditching system might be needed (Odiņš et al., 1960).

K. Bušs has stated that productivity of pine and spruce stands after amelioration increases 3-4 times, that of birch stands – 2-3 times, and that of black alder – around 1.5 times. Even aspen and ash can establish high-productive stands (Буш, 1958). From all tree species in Latvia, spruce shows the largest amelioration effect. 85% of trees show increase of diameter during next 5 years after amelioration, but maximal additional increment is reached 10-15 years after amelioration (Столяров and Ананьев, 1986). Even 100 years old spruce and pine stands sometimes react positively on amelioration. On eutrophic drained soils pine stands show high annual increment, but very low stem quality. Considering that, such a stand should be converted to a spruce stand (Буш, 1968).

General overview of ameliorated areas

According to the data of Latvia Ministry of Agriculture, 34% of forests in Latvia are located on drained soils (20% on drained mineral soils and 14% on dried peat soils) (Meža platība, 2009). The forests on drained mineral soils are evenly represented by pine and birch (correspondingly 114 304 and 115 199 ha), but in forests on drained peat soils birch stands are significantly more represented (134 365 ha, pine stands – 104 644 ha). There are apparently more spruce stands on drained mineral soils (20%); on drained peat soils – only 12%.

The largest standing volume is found in forests growing on dry mineral soils - 221 m^3 ha^{-1}, but also the values of growing stock in forests on drained mineral and peat soils is notable - 207 m^3 ha^{-1} on drained mineral soils and 200 m^3 ha^{-1} on drained peat soils (Meža statistika, 2007).

In drained forests with pine as the main tree species the standing volume is 240 m^2 ha^{-1} on drained mineral soils and 235 m^3 ha^{-1} on peat soils), but even black alder stands show average standing volume of 230 m^3 ha^{-1} on drained soils. On both drained soil types spruce shows relatively low standing volume – around 160 m^3 ha^{-1}. The standing volume of birch stands is larger in stands on drained mineral soils - 208 m^3 ha^{-1} and 177 m^3 ha^{-1} on drained peat soils. Grey alder stands can reach 160 m^3 ha^{-1} on drained soils (Meža statistika, 2007).

Nowadays, the area of forests on wet soils equals 1.5 million ha. 700 000 ha of this territory can be regarded as ameliorated or drained (Indriksons and Palejs, 2005; Zālītis, 2006). To describe the effectiveness of amelioration system, a term *amelioration rate* is used. It determines the optimal level of ground water, which ensures the aeration of peat layer in ameliorated area. The fluctuation of ground water below the rate does not influence the growth of forest stands during vegetation period (May-October) (Zālītis, 2006).

The distances between ditches in an amelioration system have to be planned so that optimal amelioration effect is reached in the entire area. Former research shows that guarantee of 100% amelioration rate could be reached if ditches of depth of 3 meters would be distributed evenly over the amelioration area and the maximal distance between them would be 20 meters. In practice, such design of amelioration systems would not be reasonable due to a small number of trees, the growth of which could be improved (Zālītis, 2006).

To keep the amelioration systems functional, they have to be renovated. The period of time between renovations depends on such factors as run-off of water, soil structure of ameliorated areas and vegetation degree. Also the quality of amelioration system itself is of great importance.

Joint stock Company "Latvia State Forests" (JSC "LSF") manages 49% of forests in Latvia. The development of forest infrastructure – forest roads and drainage systems – is important for successful sustainable forest management in a long term.

The aim of research described in this article is to test whether the renovation of amelioration systems causes positive effect on growth of drained forest stands in Latvia. To reach the aim, the following tasks were stated:

1. To analyse the available information about renovated amelioration systems in forests managed by JSC "LSF" and establish sample plots in forest compartments that should be affected by renovation in 2005.
2. To establish methodology for research and collect data about stand productivity affected by melioration (increment cores).
3. To analyse collected data and make conclusions about the impact of the renovation of amelioration systems on the forest stand in 2005.

Materials and Methods

Selection of forest compartments for the analysis

The compartments were selected in order to fulfil the following requirements:

1. The amelioration system around the compartments must be renovated in 2005.
2. The stand should be old enough to show the impact of previous renovation or even establishment of amelioration system. Respectively, stands have to be mature or even over-grown.
3. The compartment has to be located next to the ditch to prove the correlation between the current mean annual volume increment and distance from the ditch.

The establishment of sample plots

After the location of the compartment, the stand structure had to be checked whether the real situation is relevant to the inventory data or thinning had not been performed, because thinning could increase the radial increment more significantly than renovation of amelioration system. Also the quality of amelioration system was described: 1 – good, 2 - medium, 3 - bad. The GPS co-ordinates of the centre of each sample plot were recorded, and the centre was marked with a red band. The distance between the sample plots within every compartment was approximately 50 meters. 2-3 sample plots were established in each com

Table 1

The Distribution of the Sample Plots in Regional Forestries

Regional forestry	Block, compartment	Main tree species	Name of the sampling cluster	Number of sample plots in the cluster	Number of samples in the cluster	Year of renovation of amelioration system	Year of establishment/ previous renovation of amelioration system
Ziemeļlatgales	189., 8.	Pine	A	3	30	2005	1969
Ziemeļlatgales	178., 32.	Pine	B	3	30	2005	1969
Ziemeļlatgales	177., 3.	Spruce	C	2	20	2005	1969
Ziemeļlatgales	2., 8.	Spruce	D	2	20	2005	*
Ziemeļkurzemes	78., 1.	Birch	E	3	30	2005	1974
Ziemeļkurzemes	77., 1.	Birch	F	2	20	2005	1974
Ziemeļkurzemes	96., 8.	Pine	G	3	30	2005	1974
Ziemeļkurzemes	96., 21.	Birch	H	2	20	2005	1974
Ziemeļkurzemes	95., 30.	Pine	I	2	20	2005	1974
Ziemeļkurzemes	95., 24.	Pine	J	2	20	2005	1974
Ziemeļkurzemes	95., 16.	Pine	K	2	20	2005	1974
Vidusdaugavas	329., 15.	Spruce	L	3	30	2005	*
Vidusdaugavas	331., 10.	Spruce	M	2	20	2005	*
Vidusdaugavas	332., 11.	Pine	N	2	20	2005	*
Vidusdaugavas	328., 2.	Spruce	O	2	20	2005	*

* -NO DATA

4 clusters of sample plots were established in Ziemeļlatgale and 4 in Vidusdaugava regional forestries, 7 – in Ziemeļkurzeme regional forestry. 7 clusters were located in pine stands, 5 - in spruce and 3 – in birch stands. Cluster here and later in the text means 2-3 sample plots established within a single compartment.

Data processing

After the specific preparation of increment cores for the measurement of the width of each single year ring (precision 1/100), the following average values were analysed using Mann-Whitney test:

1) the width of the last 3-4 year rings after the renovation of amelioration system in 2005 (4 year rings were used if samples were collected in autumn 2009);
2) the width of 5 year rings before 2005;
3) the width of 10 year rings before 2005;
4) the width of 5 year rings before and after the establishment or previous renovation of amelioration systems;
5) the width of 10 year rings before and after the establishment or previous renovation of amelioration systems.

The year ring width of the last 3-4 years were analysed also within the clusters to prove the correlation between the ring width and distance from the ditch. Possible regional differences were also studied.

Results and Discussion

General description of sample plots

35 sample plots in 15 compartments or clusters were established. General information about sample plots is given in Table 2.

Table 2

General Decription of Sample Plots

Code of sample plot	X-coordinate	Y- coordinate	Condition of the ditch (1- good, 2 – medium, 3 – bad)	Distance form the ditch (1- 0 m, 2 – 50 m, 3 – 150 m)	Mean height H (m)	Basal area G (m^2)
A1	*	*	2	1	23.0	30
A2	*	*	2	2	21.7	28
A3	*	*	2	1	22.6	27
B1	*	*	2	1	24.0	33
B2	*	*	2	2	22.5	31
B3	*	*	2	1	21.8	30
C1	*	*	3	1	25.0	19
C2	*	*	3	2	22.7	16
D1	56°51’08”	27°27’27”	1	1	23.2	12
D2	56°51’08”	27°27’29”	1	2	23.6	8
E1	57°17’39”	21°49’07”	1	1	23.3	12
E2	57°17’37”	21°49’07”	1	2	24.7	9
E3	57°17’36”	21°49’06”	1	3	20.1	9
F1	57°17’34”	21°48’15”	1	1	23.4	14
F2	57°17’33”	21°48’14”	1	2	26.3	11
G1	57°17’14”	21°49’43”	1	1	25.8	14
G2	57°17’13”	21°49’41”	1	2	24.3	14
G3	57°17’13”	21°49’37”	1	3	23.6	12
H1	57°16’60”	21°49’32”	1	1	24.4	14
H2	57°17’01”	21°49’32”	1	2	25.4	8
I1	57°16’57”	21°49’09”	1	1	26.3	14
I2	57°16’59”	21°49’09”	1	2	23.4	17
J1	57°16’54”	21°48’43”	1	1	24.3	20
J2	57°16’55”	21°48’42”	1	2	27.6	21
K1	57°16’59”	21°48’40”	1	1	17.6	10
K2	57°16’60”	21°48’39”	1	2	16.4	14
1	56°22’33”	25°03’38”	2	1	29.7	34
2	56°22’35”	25°03’36”	2	2	25.8	22
3	56°22’36”	25°03’34”	2	3	28.6	25
M1	56°23’14”	25°05’03”	2	1	23.8	18
M2	56°23’13”	25°05’01”	2	2	26.1	23
N1	56°23’18”	25°04’57”	1	1	22.3	8
N2	56°23’20”	25°04’60”	1	2	24.4	16
O1	56°22’26”	25°03’02”	1	1	23.4	14
O2	56°22’26”	25°02’59”	1	2	22.7	14

* - NO DATA

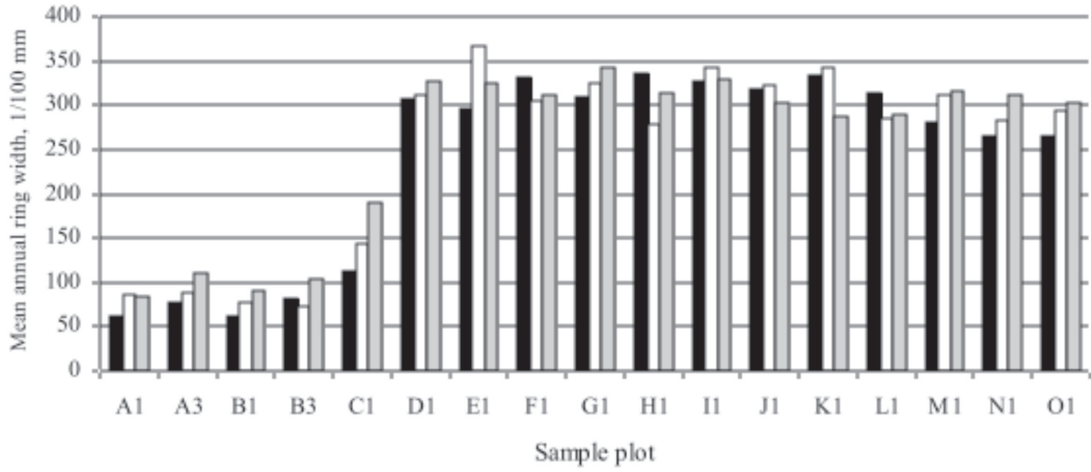

Figure 1. Comparison of a year ring width in sample plots located at the ditches before and after renovation of the amelioration systems.

Legend: ■ - 2006-2008; □ - 2001-2005; □ - 1996-2000

Analysis of the amelioration effect on mean year ring width

To analyse, if the renovation of amelioration system in 2005 has positively affected the stand growth, the comparison of mean width of year rings in sample plots located directly at the ditch was carried out. The width of the last 3 year rings was compared with the width of 5 and 10 year rings before the renovation of the amelioration system. In cases when the year of establishment of the amelioration systems was known, the width of 5 and 10 year rings before and after the amelioration were analysed to prove the effect of the amelioration. Mann-Whitney U test was used for the analysis.

The analysis of width of the last 3 year rings after and 5 (10) year rings before the renovation showed no significant difference; p-values were greater than 0.05 in all cases. Even graphic analysis did not show any notable trends of the year ring width before and after the renovation (Figure 1).

The previous figure shows that the width of the last 3 year rings in some plots (F1, H1, L1) are greater than the width of the year rings before the renovation. It gives a good reason to assume that renovation of the amelioration systems could give a positive effect to the growth of the stand in a longer time period. Another possible scenario for development of the stand after the renovation could be that the growth and annual increment will remain in the same level as before the renovation (Буш, 1958; Bušs, 1960). That could be caused by particularities of a stand structure and other factors, for example, stand age.

An interesting fact is that the comparison of 5 and 10 year ring widths before and after the establishment of the amelioration systems in 1969 and 1974 also does not show significant differences. Only in one birch stand in Ziemeļkurzeme regional forestry a significant difference between widths of 10 year rings before and after amelioration was detected (p-value 0.019) – 2.899 mm before and 3.431 mm after the amelioration. The question, why amelioration has not influenced the growth of analysed stands could possibly be answered by more detailed historical analysis of the forest areas used in this study. It is possible that the year of establishment of the amelioration system is wrong or previous renovation in years 1969 and 1974 was actually carried out. These are questions for further studies.

The analysis of correlation between the mean year ring width, distance of the sample plot from the ditch, condition of the ditch and geographical region

The correlation between mean width of the last 3-4 year rings and the distance of the sample plot from the ditch in all sample plots from all clusters was studied. Mann-Whitney U test shows no significant correlation between these parameters in all 15 clusters (p-value greater than 0.05). Too short observation period after the renovation of the amelioration system could explain such an outcome of the study.

Our study also demonstrates that the condition of the amelioration systems significantly influences the width of the year rings (p-value = 0.000), i.e., there is a positive correlation between condition of the ditch and growth of the stand (Table 3). The average value of the year ring width in the stands next to the ditches with good condition was 3.2±0.67 mm, in stands next to the ditches with medium condition - 2.1±0.11 mm and 1.1±0.68 mm in the stands where the condition of the ditch was described as bad.

Table 3

The Results of One Way ANOVA - Differences of Mean Widths of Year Rings in Stands nNear Ditches of Different Condition

(I) Condition of the ditch	(J) Condition of the ditch	Mean difference (I-J)	Std. error	Sig.	95% confidence level	
					Lower bound	Upper bound
good	medium*	113.178	12.7203	0.000	82.611	143.744
	bad*	210.573	11.7004	0.000	181.443	239.703
medium	good*	-113.178	12.7203	0.000	-143.744	-82.611
	bad*	97.396	14.4280	0.000	62.215	132.576
bad	good*	-210.573	11.7004	0.000	-239.703	-181.443
	medium*	-97.396	14.4280	0.000	-132.576	-62.215

Dependant variable: Mean width of the year rings after the renovation, 1/100 mm
* mean difference is significant at α=0.05

Table 4

The Results of One Way ANOVA - Differences of Mean Widths of Year Rings in Different Regional Forestries

(I) Regional forestry	(J) Regional forestry	Mean difference (I-J)	Std. Error	Sig.	95% confidence level	
					Lower bound	Upper bound
Ziemeļlatgale	Ziemeļkurzeme*	-197.056	11.9503	0.000	-226.434	-167.677
	Vidusdaugava*	-176.544	13.6113	0.000	-210.006	-143.083
Ziemeļkurzeme	Ziemeļlatgale*	197.056	11.9503	0.000	167.677	226.434
	Vidusdaugava	20.511	12.3139	0.251	-9.761	50.784
Vidusdaugava	Ziemeļlatgale*	176.544	13.6113	0.000	143.083	210.006
	Ziemeļkurzeme	-20.511	12.3139	0.251	-50.784	9.761

Dependent variable: Mean width of the year rings after the renovation, 1/100 mm
* mean difference is significant at pie α=0.05

The analysis of possible regional influence on the year ring widths shows that stands located in Ziemeļkurzeme regional forestry demonstrate better growth parameters than stands in other regional forestries (Table 4). The mean values of the year ring width in all forestries included in the study are the following: Ziemeļlatgale regional forestry - 1.2±0.11 mm, Vidusdaugava – 3.01±0.85 mm and Ziemeļkurzeme –3.2±0.72 mm.

Conclusions

1. The analysis of the year ring widths in all 35 sample plots used in this study shows that the renovation of amelioration systems has not improved the growth of the stands significantly.
2. There is a positive correlation between growth of the stand and condition of the ditches.
3. The regional analysis shows that stands in Ziemeļkurzeme regional forestry indicate significantly better volume accumulation since 2005 than stands in the other regional forestries analysed in this study.

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RESEARCH ON GENETIC ASPECTS OF SCOTS PINE RESISTANCE TO ROOT ROT

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Abstract. Scots pine (*Pinus sylvestris*) is Latvia's most economically important tree species. It accounts for 38% of the total forest area of Latvia. One of diseases affecting *P. sylvestris* is root rot caused by the fungus *Heterobasidion annosum*, which causes large economic losses. There is some evidence of Scots pine trees with higher levels of resistance but no absolutely resistant tree clones have been described so far. Many genes encoding peptides and proteins with direct or indirect antifungal activity have been described in various plant species, but only few of them have been studied in conifers. In our study we have utilised various approaches to research genetic aspects of Scots pine resistance to *H. annosum*. Here we present our initial results. Initially, *H. annosum* infection was determined in 300 trees and in a subset of twenty seven trees representing fifteen families (progeny of one mother tree) infection levels were quantitatively characterised. Candidate-genes were selected based on previously published research. Gene copy number variation (CNV) analyses were performed on selected samples. Copy number variation polymorphism was detected for a gene encoding a thaumatin-like protein analogues of which are described as potent antifungal proteins in other plants. As increased gene copy number can lead to increased gene product amounts in cells it is possible that an increased copy number of thaumatin-like protein is beneficial to the pine tree in respect to resistance against *H. annosum* and other pathogens. Further experiments need to be performed to investigate this in more detail.
Key words: pine, resistance, root rot, genetic analysis.

Introduction

Forest is Latvia's biggest natural resource. Forest areas account for 55.9% of the total territory of Latvia. In 37% of all forest stands Scots pine is the dominant species (Anonymous, 2007). *Heterobasidion annosum*, the causative agent for root rot in pine is distributed widely in Latvia (Gaitnieks, personal communication; Korhonen et al., 1998). The economic losses caused by root rot in *Picea abies* are estimated to be hundreds of millions of Euro each year (Asiegbu et al., 2005). There is evidence that Scots pine trees with increased resistance exist in nature but no absolutely resistant tree clone has been described so far. *H. annosum* infects trees in several ways. It can infect stem or root wounds with spores, but it can also spread from one tree to another through root contact. Often spores of *H. annosum* infect stumps of freshly felled trees followed by spread to intact trees via root contacts. To avoid this, trees can be felled in winter when no sporulation occurs or stumps can be treated with chemical or biological agents which prevent stump infection. There are no effective measures for decontamination of a forest site once it had become infected with *H. annosum*. The most popular solution is to plant broad leaf tree species in such sites after clear felling. Removal of stumps and roots from forest soil is expensive and cannot guarantee decontamination because some root fragments may remain in the soil and contain infective *H. annosum* for several decades (Lygis et al., 2004; Stenlid, 1987). The detection of *H. annosum* infection of pine trees is difficult, as the symptoms are not apparent until the latter stages of infection (Asiegbu et al., 2005).

With the development of molecular biology methods a wealth of information about genes encoding plant antimicrobial and antifungal proteins and peptides has been accumulated, and up to now several hundred of them have been described (Broekaert et al., 1997; Selitrennikoff, 2001). Still there is a comparatively small amount of information available about genes and proteins involved in conifer resistance mechanisms. Plant antimicrobial and antifungal proteins show high pathogen growth inhibition *in vitro*, and introducing these genes into plants by genetic modification showed that they are also effective *in vivo* (Feng et al., 2003; Yang et al., 2007).

Copy number variation (CNV) is the duplication of a section of a genome, which may have an impact on levels of gene expression and phenotype. CNV has only recently been described in the human genome, and is more widespread than initially suspected (Redon et al., 2006). No studies about CNV analysis in conifer species have been published to date.

The aim of this research was to detect gene copy number variation (if present) in the thaumatin-like protein gene of *P. sylvestris* using real-time PCR. This would also be a new way how to study genetic aspects of Scots pine resistance to root rot.

Materials and Methods

In this study, we used a PCR-based method to determine the presence of *H. annosum* within individual pine trees growing in a plantation known to be infected by this pathogen (Hantula and Vainio, 2003). Using the same method, a quantitative assessment of *H. annosum* infection was determined for a subset of these trees by analysing multiple samples from one tree. The copy number variation of a fungal resistance candidate gene (thaumatin-like protein – TLP encoding gene) was determined for the subset of trees where quantitative assessment of *H. annosum* was performed. Thaumatin-like proteins from other species have previously been reported as effective against fungi (Pressey, 1997) and the expression level of TLP encoding genes rises in reaction to infection (Adomas et al., 2007).

Plant material

Wood samples from twenty nine year old *P. sylvestris* trees growing in an experimental plantation near Kalsnava were used to perform *H. annosum* detection and infection level determination experiments. Samples were taken from the base of the tree stem. For gene copy number analysis, samples for DNA extraction were taken from tree needles.

DNA extraction

DNA was isolated using Genomic DNA purification kit (Fermentas, cat. no. K0512). Wood samples were cut into small chips, put into 2 ml centrifuge tubes, frozen in liquid nitrogen, homogenised in a ball mill (Retch, model MM400). After that the procedure described in the DNA isolation kit manual was performed with the following modifications: PVP (polyvinylpyrrolidone) was added to the lysis buffer provided in the kit to a final concentration of 4%, centrifugation speed and time in all steps were increased to 13,000 rpm (16,100 g) and 15 minutes. Incubation with RNase A (Fermentas, cat. no. EN0531) was added at the NaCl precipitation step by adding 4 µg of RNase A to each sample together with the NaCl solution. Incubation was carried out at 37 ºC for 30 min.

H. annosum specific PCR

H. annosum specific PCR was performed as described previously (Hantula and Vainio, 2003) except that Taq polymerase (Fermentas, Cat. no. #EP0401) was used instead of hot-start polymerase. DNA concentration was not determined as it wouldn't give information about the content of *H. annosum* DNA in the sample. Instead a PCR reaction utilising *P. sylvestris* specific primers was performed for each sample to ensure the DNA concentration and quality was sufficient for PCR amplification (data not shown). Negative controls for the *H. annosum* detection PCR were performed with water and *P. sylvestris* DNA isolated from needles as template. Negative controls for *P. sylvestris* DNA detection were performed with water instead of DNA.

Thaumatin - like protein gene copy number determination

The *P. taeda* TLP gene sequence is available from GenBank (accession number EF532603). The *P. sylvestris* GAPDH gene (used for endogenous control reactions) sequence is available from GenBank (accession number L07501). Primers for TLP and GAPDH genes were designed using the Applied Biosystems Primer Express 3.0 software.

Twenty seven trees from the plantation at Kalsnava were screened for CNV of the thaumatin-like protein encoding TLP gene. To assess relative TLP copy number, a real-time PCR comparative Ct method was used. The essence of this method is to compare samples to a reference sample and to calculate the difference between them in respect to analysed gene copy number. To normalise results and to achieve higher accuracy several controls are used in this method. First, the reaction master mix contains a fluorescent dye, ROX (5-carboxy-X-rhodamine), and results are normalised using the ROX fluorescence signal strength which should be equal in all samples. This minimises pipetting error effects on the results. Secondly, an endogenous control is used to minimise sample DNA dilution errors. The endogenous control is a control reaction which is performed for each sample with primers that target a gene which copy number should be the same in all samples. In this case *P. sylvestris* glyceraldehyde 3-phosphate dehydrogenase gene (GAPDH) was used as a target for endogenous control reactions. Negative control PCR reactions were performed for both targets with water instead of DNA. Real-time PCR analyses were performed on a StepOnePlus real-time PCR instrument (Applied Biosystems). Data were analysed using StepOne software 2.1 (Applied Biosystems). Maxima™ Sybr Green/ROX qPCR Master Mix (2x) (Fermentas, cat. no. K0221) was used for real-time PCR reaction preparation. Final primer concentration in reaction was 60 *nM* (each primer) See primer sequences in table 1. Reaction volume was 20 µl.

Table 1

Primers used in real-time PCR analyses

Primer name	Primer sequence (starting from 5'end)
TLP-F	CAGTGCCCACAGGCATACAG
TLP-R	CCACCAGGGCAGGTGAAG
GAPDH-F	ACGGTTTTGGTCGAATTGGA
GAPDH-R	CCCCACGAGCTCGATATCAT

The real-time PCR instrument detects the so-called cycle threshold (Ct) value which is further used to compare samples to each other after applying the value to the ΔΔCt equation (equation 1).

$$\Delta\Delta Ct = \Delta Ct_{sample} - \Delta Ct_{reference} \quad (1)$$

In this equation, ΔCt_{sample} is the *Ct* value for any sample normalized to the endogenous control and $\Delta Ct_{reference}$ is the *Ct* value for the reference sample also normalized to the endogenous control. For the ΔΔCt calculation to be valid, the amplification efficiencies of the target and the endogenous reference must be approximately equal. To obtain data about PCR efficiency each sample was used in three different concentrations in both real-time PCR reactions. These concentrations were 0.5 ng/µl, 1 ng/µl and 2 ng/µl (final concentration in reaction mix), all reactions were performed in duplicates. To characterise PCR efficiency Ct values of both PCR reactions for all concentrations were plotted using Microsoft Office Excel software. A linear trend line for Ct values of each reaction was added and the linear equation (equation 2) for each trend line was obtained. Slope values (*m* in the linear equation) of both real-time PCR reactions were compared. A slope value difference of less than 0.1 in PCR efficiency for both reactions for

a sample would be sufficient to consider the results as quantitatively correct.

$$y = mx + b \quad (2)$$

Results and Discussion

Initially, utilising the adapted PCR based method for *H. annosum* DNA detection in wood samples, 300 trees were analysed using one sample per tree in order to obtain initial results about the phytosanitary condition of this plantation regarding *H. annosum* infection. Of the 300 individuals tested, *H. annosum* DNA was detected in 119 trees, while the results were negative in 181 trees.

These results suggest that this plantation is heavily infected with *H. annosum*. In fact, the actual proportion of infected trees could be even higher because in this experiment only one sample was taken from each tree, but *H. annosum* distribution within the stem base is not uniformed.

Therefore, twenty three trees which were found not to contain *H. annosum* DNA and four trees where *H. annosum* DNA was detected in the first experiment were selected to investigate the level of *H. annosum* infection in more detail. Trees found to be uninfected in more detailed examinations could possibly have increased resistance against *H. annosum* infection. The same PCR method was used with the exception that this time five samples were taken from each tree from the perimeter of the stem base with approximately equal spacing between sampling points. As a result, sample trees were divided into infection level groups, for each group a value from zero out of five (0/5) to five out of five (5/5) was assigned, the first number indicating the amount of samples containing *H. annosum* DNA in each sample tree belonging to this group, second number is the amount of samples taken from each tree. Results of this experiment are illustrated in Figure 1. In two cases not all five samples could be analysed (samples Ja15-V-1 and Ja15-V-5).

As shown, the most samples represent result groups where *H. annosum* DNA is detected in less than half of analysed samples, seventeen samples in total. This could be explained by the sample selection (mostly trees where *H. annosum* DNA was not detected in the first experiment).

All of the samples where infection level was quantified were screened for TLP copy number variation using the real-time PCR comparative Ct method. Of twenty seven trees tested, eighteen trees showed a similar copy number of the TLP gene. This group of samples will further be referenced as the group with normal copy number for ease of understanding. Eight trees showed elevated copy number (relative quantity 3.4x-10.5x, average 5.66), and one tree showed highly elevated copy number (relative quantity 23.08) of TLP gene (Table 2). For the ΔΔCt calculation to be valid, the amplification efficiencies of the target and the endogenous reference must be approximately equal. Although we experienced difficulties with this point and the PCR efficiencies for target gene and endogenous control PCR reaction was not sufficiently similar as to give exact quantitative results, using these results, it was possible to divide the analysed individuals into two groups – one with normal TLP gene copy number and the second – with elevated copy number.

The infection level and TLP copy number of individuals is shown in Table 2. Of the 4 individuals where no *H. annosum* DNA was detected, 3 showed an elevated TLP copy number. In addition, when mean value of infection level for those two groups are compared, the group with elevated TLP gene copy number together with the sample with highly elevated copy number shows a lower value (1.22) than the normal copy number group (2.11). However, these

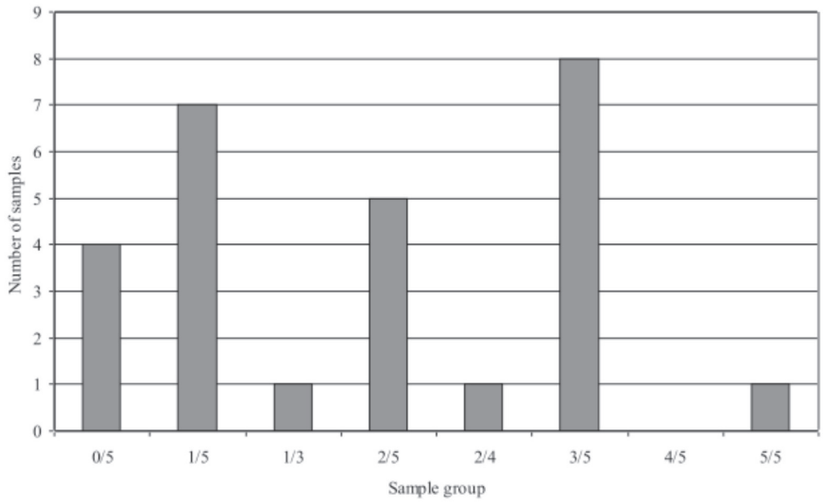

Figure 1. Result summary for infection level determination for individual trees
Annotation: bars represent number of samples in each sample group in the infection level determination experiment. Sample groups are called 0/5, 1/5, 1/3, 2/5, 2/4, 3/5, 4/5, 5/5 where the last digit indicates the number of samples analysed for each tree and the first number – the number of positive samples.

results were obtained from naturally infected pine trees. Further experiments using artificial inoculation are required to allow for the precise determination of the correlation between TLP gene copy number in individual pine trees and their resistance to *H. annosum*. Materials obtained from artificial inoculation experiments are currently being used to investigate TLP gene expression profiles in response to *H. annosum* infection.

Further work needs to be done in order to obtain similar amplification efficiencies for the TLP gene primers and the endogenous control primers, so that the gene copy number can be more accurately quantified. In addition, the length of the amplified fragment analysed is short compared to the total length of the gene. Further experiments analysing a larger amplicon (amplified fragment), covering almost the whole gene would be of greater value because this could assure us that the CNV variation detected in first experiment related to the entire TLP gene, not only a portion of it. Furthermore, we need to take in consideration the fact that CNV has not been studied at all in conifers, and so the extent of this is not known. The experiment should be repeated with alternative endogenous controls in order to confirm these results.

Thaumatin-like proteins are only one part of the plant defence system, and it is important to clarify the effect of increased TLP gene copy number on Scots pine resistance against *H. annosum* and how effective is the thaumatin-like protein itself against *H. annosum*. We are also working on detecting CNV variations for other candidate-genes. Initial results show that differences in gene copy number can be expected to be detected for some of them (data not shown). Not only is the plant reaction after infection important for resistance against pathogens, but also higher levels of antifungal or antimicrobial compounds constitutively found in the plant may influence the resistance to pathogen attack. If an individual with a higher copy number of a resistance gene than in rest of population would be found, it could mean that this individual has higher levels of anti-pathogenic compounds prior to infection (as increased gene copy number could be linked with increased expression and protein levels) making the infection and colonisation of the host a more problematic task for the pathogen. Further investigation in this area is needed to investigate the correlation of gene copy number variation and gene expression levels. The findings that variations in copy number of the TLP coding gene exist between individual trees could be taken into consideration in selection programs for Scots pine for resistance-targeted selection of breeding material.

Table 2

Results of screening for TLP copy number variation

Sample name	Normal copy number	Elevated copy number	Highly elevated copy number	Infection level
Du12-III-4	x			0
Ja15-V-1	x			1/2
Ja15-V-5		x		1/3
Ja15-V-6	x			3/5
Ja18-III-2	x			3/5
Ja21-III-2	x			1/5
Ja21-V-1	x			1
Ja21-V-5	x			1/5
Ja2-III-4	x			2/5
Ja3-II-5		x		3/5
Ja3-III-5			x	0
Ja3-V-1	x			3/5
Ja3-V-3		x		1/5
Ja3-V-5	x			3/5
Ja4-III-2	x			1/5
Je7-III-5		x		0
Lub1-III-1		x		2/5
Ma21-III-3		x		0
Ma6-V-2		x		3/5
Sm3-II-1	x			2/5
Sm3-II-2	x			2/5
Sm3-V-2	x			1/5
Sm6-V-2	x			3/5
Sm9-III-2	x			2/5
Str17-I-1		x		1/5
Str17-I-2	x			3/5
Str17-III-3	x			1/5

Conclusions

A PCR-based method for the detection of *H. annosum* DNA was modified in order to identify and quantify the presence of the pathogen in a naturally infected plantation. Variation in the copy number of the thaumatin-like protein coding gene between individual trees from this plantation was found. The results which we have at the present moment suggest that at least two groups of individuals exist; in individuals of one of these groups the copy number of the TLP gene is at least two times higher. Initial results suggest that some correlation between TLP gene copy number and *H. annosum* resistance may exist although further experiments are required to confirm and to extend these results.

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INFLUENCE OF GROWING CONDITIONS, AGE AND STOCKING DENSITY ON THE DEADWOOD OF PINE FOREST STANDS

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Abstract. The effect of growing conditions, age and stocking density on the amount of deadwood in the pine forest stands of Latvia was analyzed in this research. The material for studies was collected within the framework of the National Forest inventory of Latvia in the period 2004 to 2008. From databases of the National forest inventory the data about 1627 sample plots dominated by pine were selected and analysed. The total amount of deadwood (m^3 ha^{-1}) was compared among five forest edaphical rows - forests on dry mineral soils, forests on wet mineral soils, forests on wet peat soils, forests on drained mineral soils and forests on drained peat soils. Deadwood was also analysed in five age groups – young, middle age, premature, mature and over-mature stands, as well as in six groups of stocking density – small-density, sparse-density, middle-density, high-density, full-density and over-density stands. It was found out, that the growing conditions ($p = 0.000 < \alpha = 0.05$), stand age ($p = 0.000$) and stocking density ($p = 0.000$) have significant influence on the amount of deadwood in pine forest stands. The highest amount of deadwood is in the pine stands on wet mineral soils – 18.6 ± 2.09 m^3 ha^{-1}. With increase of the stand age the amount of deadwood also increases and in the mature pine stands the amount of deadwood is 17.8 ± 1.58 m^3 ha^{-1}. The amount of deadwood in the pine forest stands also increases with stocking density and in the over-density stands it is 21.6 ± 2.50 m^3 ha^{-1}.
Key words: pine forest stands, deadwood, growing conditions, stand age, stocking density.

Introduction

In the forest beside synthesis of organic matter the ongoing process is also mortality of trees. The causes of tree death are various and are categorized in variety of ways. One of the classifications is division into abiotic and biotic factors (Franklin et al., 1987). Abiotic factors are fire, lightning, wind, flooding, drought etc. Biotic factors are age, mechanical imbalance, diseases, insects etc. Tree mortality is also grouped into density-dependent (or growth-dependent) and density-independent (or growth-independent) factors (Franklin et al., 2002; Greenwood and Weisberg, 2008; Ozolincius, 2005). Density-dependent tree mortality is due to the competition among individuals for light, nutrients and water (self-thinning mortality) and density-independent tree mortality is due to other agents. As a result of the influence of these various factors, the deadwood is originating in the forests, primarily in the form of standing dead trees (snags), down wood (logs), pieces of fragmented wood and large branches. This deadwood is considered as an important component of forest biodiversity. Standing and lying deadwood increase a structural diversity within a forest and serve as a habitat for many species of invertebrates, fungi, bryophytes, lichens, small mammals (bats, dormice) and birds (Harmon et al., 1986; Humphrey et al., 2004; Schuck et al., 2004). Deadwood also plays an important role in nutrient cycling and carbon storage (Goodale et al., 2002; Harmon et al., 1986), and it is a significant resource of fuel.

The demand for the information of forest deadwood resources has constantly increased in the last twenty years, both in relation with assessment and preservation of forest biodiversity (Hahn and Christensen, 2004; Schuck et al., 2004), and with assessment of greenhouse gasses emission and sequestration (Woodall et al., 2008). Therefore, in several countries the deadwood survey is also included in the National forest inventory (Roundeux and Sanchez, 2009; Stockland et al., 2004; Woodall et al., 2009). The inventory of deadwood is also used to estimate fuel loads and forest fire risk (Woodall, 2009).

In Latvia, up to now there are no wide researches performed in regard to the amount of deadwood in the forests of commercial tree species. Therefore, the number of publications, which are related to natural mortality, is small and is mainly related to the former regulations for forest estimate survey (Нормативы для таксации…, 1988). Today statistically believable and actual information of deadwood resources in the Latvian forests is provided by the National forest inventory, which was started in 2004 and is performed in the period of five years by covering the territory of Latvia with regular grid of sample plots. Besides determination of traditional forest stand parameters, the volume of deadwood is also estimated in these plots.

According to the National forest inventory data Scots pine (*Pinus sylvestris* L.) is the dominant tree species in Latvia (Jansons, 2009). Pine stands take up 28.8% of the forest total area. Because pine stands are characterized by wide ecological amplitude – from poor sand soils to wet peat soils, the aim of this research is to study the influence of growing conditions, stand age and stocking density on the amount of deadwood in the pine forest stands.

Materials and Methods

The data about the amount of deadwood in Latvian pine forests stands were analysed in this research. The study material was collected within the framework

of the first National forest inventory (NFI) of Latvia from 2004 to 2008. The survey of sample plots and calculations of forest stand parameters is performed in accordance with the approved methodology of the Ministry of Agriculture – 'Methodology of forest statistical inventory and calculations of forest stand secondary parameters'.

According to the aim of the research from the data bases of NFI, the data about 1627 sample plots (SPs) allocated in the period from 2004 to 2008 were selected and analysed. The SPs were selected by such criteria:

1. Kind of sample plots – only permanent plots were selected;
2. Land category – forest;
3. Size of plots – the plots larger than 400 m^2 were selected (sample plots occurring on the boundary of several forest compartments or different land categories are divided into smaller units, i.e. sectors);
4. Proportion of dominant species in the forest stand – such plots were selected, where the proportion of Scots pine is at least 50% of the common growing stock of the first storey;
5. Forest type - only forest types were selected where the Scots pine is aim-species (*Cladinoso-callunosa, Vacciniosa, Myrtillosa, Hylocomiosa, Vaccinioso-sphagnosa, Myrtilloso-sphagnosa, Sphagnosa, Caricoso-phragmitosa, Vacciniosa mel., Myrtillosa mel., Callunosa turf. mel., Vacciniosa turf. mel., Myrtillosa turf. Mel.*) in accordance with the forest typology of Latvia (Bušs, 1981);
6. Age of forest stand - 21-160 years old forest stands were selected;
7. Critical number of trees and critical basal area - in accordance to the Regulations issued by the Cabinet of Ministers of the Republic of Latvia, No. 892 (Noteikumi par koku ciršanu meža zemēs, 2006);
8. Deadwood / live wood volume ratio – only plots were selected where the ratio of deadwood volume to live wood volume is less than 1.0, in order to exclude the SPs where the large disturbances (fire, windthrow, flooding) before measurement were occurred.

In addition to the stands parameters which are included in the databases of NFI, for all single plots the stocking density of the dominant stand was also calculated. It was calculated by summarizing the stocking densities for single tree species. The stocking density for tree species was calculated as ratio of actual basal area for tree species to normal basal area (1).

$$Bs = \frac{Gf}{Gn}, \qquad (1)$$

where:
Bs –stocking density for tree species;
Gf –actual basal area for tree species, $m^2\ ha^{-1}$;
Gn –normal basal area, $m^2\ ha^{-1}$.

$$Gn = a + b \bullet \lg (H + c) \qquad (2)$$

where:
H – mean height for tree species;
a, b, c – coefficients depending on tree species (Нормативы для таксации…, 1988).

The analysis of deadwood volume ($m^3\ ha^{-1}$) was performed in six groups of the stocking density (SD): small-density stands (SD is 0.1-0.3), sparse-density stands (SD is 0.4-0.5), middle-density stands (SD is 0.6-0.7), high-density stands (SD is 0.8-0.9), full-density stands (SD is 1.0) and over-density stands (SD >1.0). The amount of deadwood was also analysed in five age groups – young (21-40 yr), middle age (41-80 yr), premature (81-100 yr), mature (101 -140 yr) and over-mature stands (141-160 yr), and was compared between five edaphical rows of forest types - forests on dry mineral soils, forests on wet mineral soils, forests on wet peat soils, forests on drained mineral soils and forests on drained peat soils.

The Kolmogorov-Smirnov test was used to test the adequacy of empirical distribution to normal distribution. It was ascertained that the amount of deadwood in all the cases, both in sampled population together, and in the groups of stocking density, age groups and forest edaphical rows, did not conform to normal distribution. Therefore, to test the influence of growing conditions, stand age and stocking density on the amount of deadwood in the pine forest stands, from nonparametric methods the Kruskal-Wallis test was used, which are the alternative method to single-factor analysis of variance.

Results and Discussion

Characterisation of pine forest stands

From the analysed sample plots on approximately 25% of the SPs no deadwood was found. On 50% of the SPs the amount of deadwood is not over than 20 $m^3\ ha^{-1}$, but on 15% it is between 20-40 $m^3\ ha^{-1}$ and only on 9% of the SPs the amount of deadwood is greater than 40 $m^3\ ha^{-1}$ (Fig. 1.). Average amount of deadwood in the pine forest stands is $14.4 \pm 0.53\ m^3\ ha^{-1}$. Comparing the mean value with the results from the Swedish National forest inventory, it is evident that the amount of deadwood in the pine forests of Sweden is smaller than in Latvia - 5.8 $m^3\ ha^{-1}$ (Fridman and Walheim, 2000), which can be explained by more intensive forest management in Sweden.

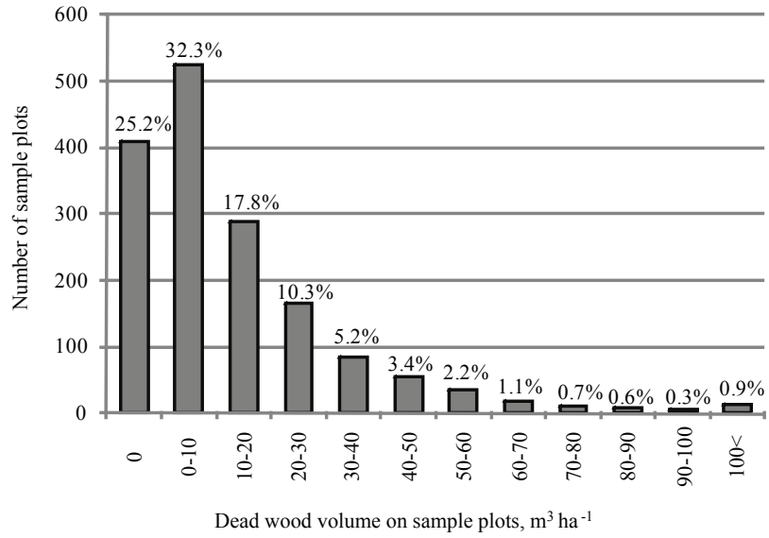

Figure 1. Distribution of dead wood volume plot values (m^3 ha^{-1}).

In the distribution of the analysed SPs among the growing conditions, the highest proportion has to forests on dry mineral soils – 810 SPs or 50% of pine stands. A comparatively large proportion of SPs is also in the forests on drained soils – 479 SPs or 29% of pine stands, from which 16% or 267 SPs are on drained peat soils and 13% or 212 SPs are on drained mineral soils. A little smaller proportion of SPs is in the forests on wet peat soils - 190 SPs or 12% of pine stands, but the smallest proportion of SPs is in the forests on wet mineral soils - 148 SPs or 9% of pine stands.

For its part in the distribution of SPs on age groups, the highest proportion is in middle age stands – 818 SPs or approximately 50% of pine stands, from which 19% are in the age from 41 to 60 yr and 31% are in the age from 61 to 80 years (Fig. 2.). A little smaller proportion is in pre-mature stands – 374 or 23% of pine stands, as well as in mature stands – 294 SPs or 18% of pine stands, from which 12% are in the age from 101 to 120 years and 6% are in the age from 121 to 140 years. The smallest proportion is in young stands and over-mature stands – 110 SPs or 7% and 31 SPs or 2%.

In the distribution of SPs on groups of stocking density, the highest proportion is in middle-density stands and high density stands – 537 SPs or 33% and 465 SPs or 29%, as well as sparse-density stands – 270 SPS or 17%. The proportion of full-density and over-density stands is almost similar – 9% or 154 and 145 SPs. The smallest proportion has small-density stands – 56 SPs or 3%.

Influence of growing conditions on amount of deadwood

Using the Kruskal-Wallis test, it was found out that growing conditions have significant influence on deadwood volume in the pine forest stands (p-value = 0.000 < α = 0.05). The significant deadwood volume differences were detected between forests on wet peat soils and other types of growing conditions (Fig. 3.). In the forests on wet peat soils, the deadwood volume on average is the smallest - 7.2 ± 0.72 ($\bar{x} \pm S.E.$) m^3 ha^{-1}, which can be explained

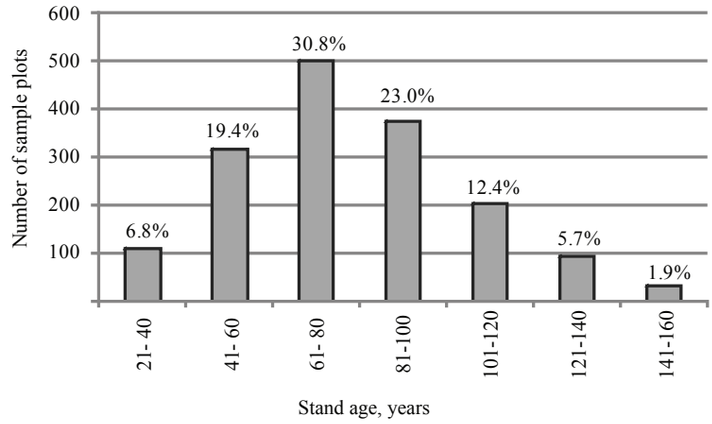

Figure 2. Distribution of sample plots on age classes.

by low productivity of pine stands in this type of growing conditions. In the forests on wet peat soils, the soil aeration and nutrient cycling is weak, leftovers of plants are not decomposing fully but forming accumulations of peat, whose thickness exceeds more than 30 cm and there is no direct contact with mineral soils for tree roots (Bušs, 1981).

On average the highest deadwood volume of pine stands is in the forests on wet mineral soils and drained mineral soils -18.6 ± 2.09 m^3 ha^{-1} and 18.7 ± 1.62 m^3 ha^{-1}, whereas in the forests on dry mineral soils and drained peat soils the amount of deadwood is 13.6 ± 0.78 m^3 ha^{-1} and 16.1 ± 1.18 m^3 ha^{-1}. The greater amount of deadwood in the forests on wet mineral soils can be explained with unstable water and air regime in the soils. In wet summers, the groundwater floods the horizon of tree roots, increments of trees decrease and decomposition rate of plant leftovers is slow. The ongoing process is accumulation of detritus. In dry summers unlike the bogging-up process the ongoing process is the bogging-down process. The groundwater level is low, the decomposition rate of plant leftovers increases and increments of trees rise.

Comparatively large differences of deadwood volume between forests on wet peat soils and forests on drained peat soils and small differences of deadwood volume between forests on wet mineral soils and forests on drained mineral soils, can be explained with that, the drainage has a greater impact on the increase of productivity of pine stands on wet peat soils than on mineral soils.

Influence of stand age on amount of deadwood

The results of Kruskal-Walis test show that the deadwood volume in the pine forest stands depends on stand age (p-value = 0.000 < α=0.05). The significant differences were found between young stands and other age groups (Fig. 4.). In the young stands the deadwood volume on average is the smallest – 4.0 ±0.74 m^3 ha^{-1}, but the highest deadwood volume of pine stands is in the mature stands – 17.8 ± 1.58 m^3 ha^{-1}. In general it is visible, that with increase of the stand age, the accumulation of deadwood in the pine forest stands also increases. Only in the group of over-mature stands the amount of deadwood is smaller than in other age groups – 11.6 ± 2.63 m^3 ha^{-1}, excluding young stands. It can be explained both by the measures of forest management – the lying deadwood probably was removed, and also with a comparatively small number of sample plots in this age group. The amount of deadwood in the middle age and pre-mature stands is 13.6 ±0.69 m^3 ha^{-1} and 16.7 ±1.13 m^3 ha^{-1}.

Comparatively large differences between young stands and middle age stands can be explained by high increase of stand productivity (m^3 ha^{-1}) in the group of middle age stands. For instance, the mean standing volume in the young pine stands is 111.7 m^3 ha^{-1}, but in the middle age stands – 278.4 m^3 ha^{-1} (Lībiete et al., 2009). The current annual volume increment in middle age stands is the highest.

The results from the Swedish National forest inventory have shown that the deadwood volume in the forests of Sweden also increases with the stand age. The lowest and highest average volumes are found in stands younger than 40 years and older than 140 years (Fridman and Walheim, 2000).

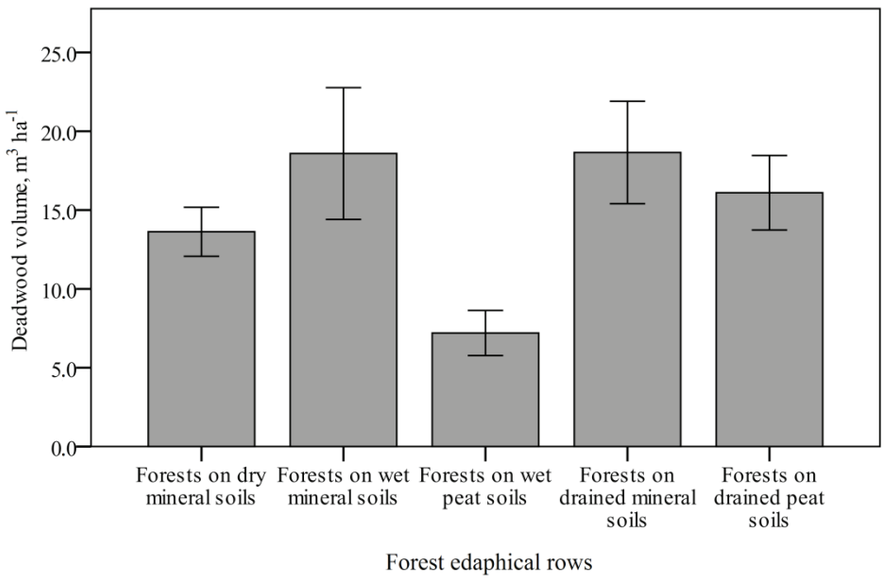

Figure 3. The difference of deadwood volume of pine stands in different growing conditions. Mean values (m^3 ha^{-1}) and 2 standard errors are given.

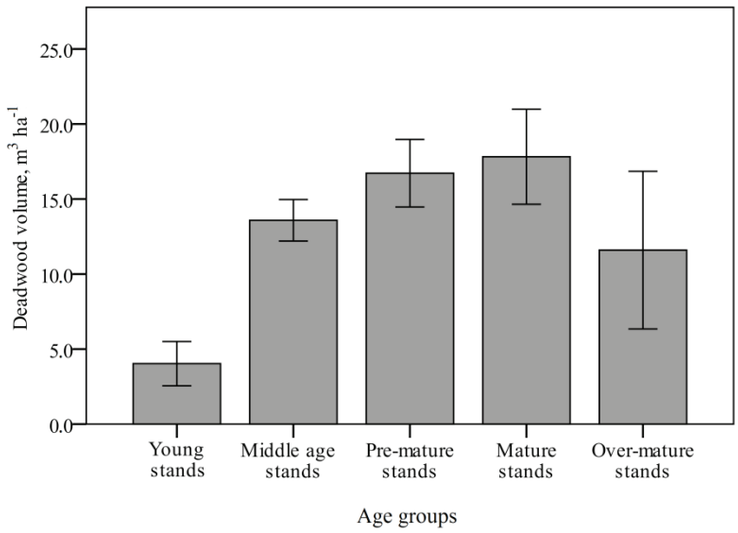

Figure 4. The difference of deadwood volume of pine stands in different age groups. Mean values (m^3 ha^{-1}) and 2 standard errors are given.

Influence of stocking density on amount of deadwood

The effect of stocking density on the deadwood volume in the pine forest stands is also significant (p-value = 0.000 < α = 0.05). The significant differences were detected between full density stands and other groups of stocking density, except the over-density stands, which also significantly differ from other groups of stocking density (Fig. 5.). In the full-density and over density stands amount of deadwood on average is the highest -20.2 ±1.99 m^3 ha^{-1} and 21.6 ±2.50 m^3 ha^{-1}. In such stands the growing space is completely used, the projections of tree crowns fully cover the soil and disallow to grow to any superfluous tree of the given tree species and age.

The smallest volume of deadwood is in the small-density stands –10.5 ±2.18 m^3 ha^{-1}, which does not significantly differ from sparse-density stands – 10.8 ±1.24 m^3 ha^{-1} and other two groups of the stocking density. It can be explained by the effect of disturbances regime. After strong disturbances (wind, insects, diseases) the amount of deadwood increases, but the stocking density decreases. The amount of deadwood in the middle-density and high-density stands is 12.6 ±0.92 m^3 ha^{-1} and 14.8 ±0.77 m^3 ha^{-1}.

In general, it is visible that the amount of deadwood in the pine stands increases with the stocking density, which conforms to the tendency of former data about natural mortality rate (m^3 ha^{-1} in year) of pine stands in Latvia (Нормативы для таксации…, 1988). Natural mortality rate increases with stocking density.

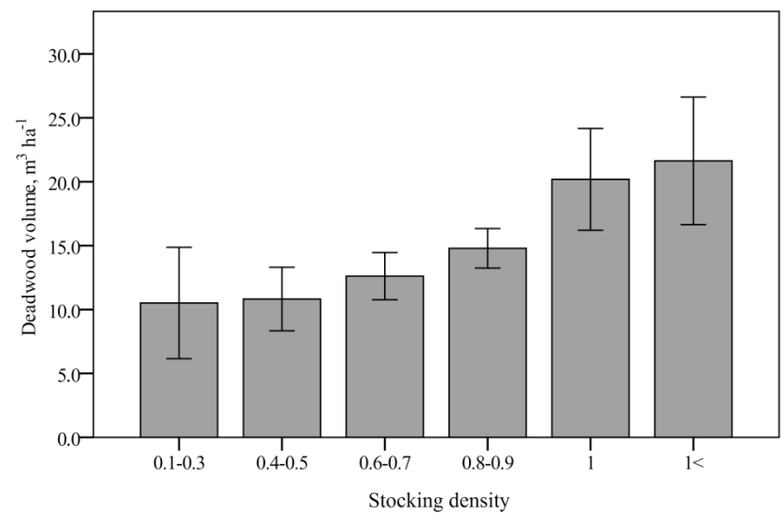

Figure 5. The difference of deadwood volume of pine stands in different groups of stocking density. Mean values (m^3 ha^{-1}) and 2 standard errors are given.

Conclusions

1. The growing conditions, stand age and stocking density have significant influence on the amount of deadwood in the pine forest stands of Latvia.
2. The pine stands on wet peat soils significantly differ from other types of growing conditions. The amount of deadwood in these growing conditions is the smallest, but in the result of drainage the deadwood volume significantly increases.
3. The amount of deadwood in the pine stands significantly increases with the stand age. The highest amount of deadwood is in mature pine stands, but the smallest in young stands.
4. The amount of deadwood in the pine stands also significantly increases with the stocking density. The highest amount of deadwood is in the full density and over-density pine stands, but the smallest in the small-density stands.

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TERRITORIAL ORGANISATION OF GAME MANAGEMENT IN LATVIA

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Abstract. The number of the most important game animals of artiodactyla species (moose, red deer, roe deer, wild boar) has been increasing in Latvia during the last 10 years. At the same time, the number of hunting districts where the census of these game animals is taken is also increasing, whereas the average area of the districts is diminishing. The studies show that at present the area of many hunting districts is notably smaller than the individual territory inhabited by these species. This particular study aims at establishing whether there is a relationship between the area of a hunting district on which a census is taken and the number of animals counted, as well as whether the number of animals is really increasing or the increase in the number is attributed to an increase in the census unit number. It was established that in respect of the four species there is a significant correlation between the area of the hunting district and the density of animals in the district. The number of moose and red deer may be assessed more objectively in the districts with an area exceeding 5,000 hectares. The census of roe deer and wild boars is notably influenced by the area of the territory where the census is taken, but the analysis does not explicitly show whether the number of roe deer and wild boars is under-assessed in large districts or over-assessed in the small ones.
Key words: Artiodactyla, area of a hunting district, census of game animals, density of animals.

Introduction

In Latvia, the rights to hunt are related with property rights; therefore, game management is organised according to the territorial principle. Currently in the world, there are two ways of organising and monitoring game management according to the territorial principle:

1. the system of hunting districts whereby permanent hunting districts are established for long-term management by certain hunter groups;
2. the system of shooting licences whereby in any broader territory the hunting pressure is regulated by the number of the licences issued (Дёжкин, 1983).

It is impossible to draw a strict line between the two systems. Even under the system of hunting districts hunting may be restricted by issuing licences, as is the case with the bag of limited game animals in Latvia. At the same time, the other system may exist in a certain restricted administrative territory that is monitored by respective authorities. Both systems have advantages and disadvantages that demonstrate themselves under certain conditions. For example, in Latvia, the organisation of the system of hunting districts is notably hampered by the fact that property areas are small and the land market is still active (as a result of the change of land owners the signed lease contracts with hunting rights may become ineffective); also, the Latvian laws governing hunting do not contain provisions for approving the external borders of a hunting district. Due to all these factors the borders of hunting districts in Latvia are changing continuously. Even if in Latvia – like in most other European countries – the system of hunting districts has developed historically, in certain time periods and locations there arises the necessity to introduce measures with the features of the licensing system because they are needed to restrict the hunting pressure in a particular hunting district.

The basic task of a hunting district is to regulate hunting pressure within legally established territories in view of the regeneration ability of game resources. In a hunting district, not only hunting, but also the following game management activities are carried out:

- taking census and approving the maximum cull limits of game animal populations;
- summing up the data about the cull, the injured game and the mortality rate apart from the harvest;
- providing supplemental feeding of the game, increasing the carrying capacity of the suitable area;
- improving hunting conditions a.o.

When carrying out these measures, the area and the location of each particular hunting district must be taken into account.

According to the State Forest Service data, the number of hunting districts in Latvia increased from 950 to 1,280 during 2000-2006. The increase was mainly due to division of the existing hunting districts rather than adding new areas where hunting had not been carried out so far because, according to the State Forest Service data, the total hunting area in Latvia has not increased. As a result, the average area of a hunting district is decreasing. Figure 1 shows the profile of hunting districts by area in 2007.

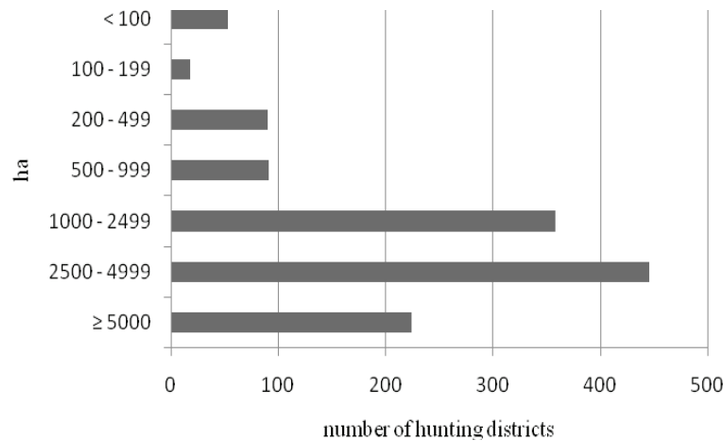

Figure 1. The profile of hunting district by area in 2007.

Both in Europe and throughout the world, there have been comparatively many studies about the areas of the individual territories of mammals (Данилов, 2005; Heikkilä et al., 1996). In accordance with the study by Heikkilä et al. (1996) carried out in the southern part of Finland, the individual territory of a moose *Alces alces L.* may vary depending on the season and in general may be 41.54 km^2. Danilkin (1996) shows that average territory size of roe deer is 1.26 – 2.04 km^2 but according to Fischer et al. (2004) individual territory of wild boar varies between 0.5 – 4.2 km^2. Some results of the studies on size of the individual territories or home ranges are summarized by Jedrzejewski et al. (2004) (see Figure 2).

In Latvia, there have been a few studies about the territorial structure of mammal populations. According to specific studies, a single lynx *Lynx lynx L.* in Latvia inhabits a forest territory of at least 200 km^2 (Ornicāns et al., 2004). The activity centres of otters *Lutra lutra L.* are placed on average after every six kilometres of river bank lines (Ozoliņš, 1999).

The results of these studies uncover that the average areas of individual territories of mammals by large exceed the areas of many hunting districts in Latvia. The studies also show that in small hunting districts, irrespectively of other hunting districts, it is not possible to obtain plausible results about the exact number of animals for such species as a moose and a red deer because the same animals may change their location in a short time.

The aim of this study was to examine applicability of the used reference territories for population census in Latvia from the viewpoint of supposed individual ranges of large game species v.s. size of the existing management areas. Objectives are: (1) to review population densities of four largest game species: a moose, a red deer *Cervus elaphus L.*, a roe deer *Capreolus capreolus L.*, a wild boar *Sus scrofa L.*; (2) to calculate the functional relationship between estimated numbers and size of the reference territories they are reported from; (3) to suggest the threshold area for minimum unbiased census results.

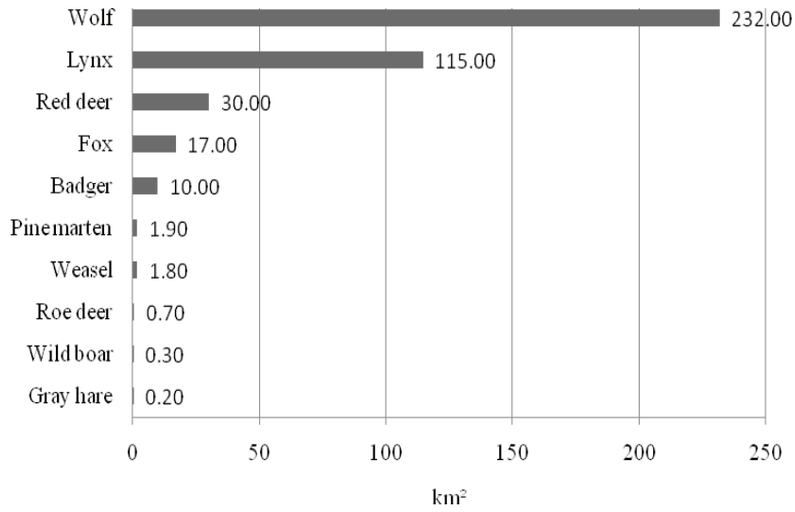

Figure 2. Average areas of the individual territories of some mammal species.

Materials and Methods

The analysis was carried out for four species of limited game: a moose, a red deer, a roe deer and a wild boar, as these are the most important game species in Latvia. The total number of all hunting districts where census was carried out is 1280. For a moose and red deer, this number is slightly smaller because these animals are not present in some hunting districts. The procedure for the census of game animals in Latvia is governed by 23.01.2008. Regulation No. 3 'Methodology for taking the census of game animals and for calculating the maximum cull of the limited game animals' of the Ministry of Agriculture. This study does not analyse whether the census results are accurate or the census complies with the established procedure. On the basis of the official census data about the game animals compiled by the State Forest Service for the hunting season 2006/2007, the density of animals on hunting districts (animals/1000 ha) was compared with the total area of hunting districts. In respect of moose, red deer and wild boars, the density was calculated on the basis of the forest area on the district, but in respect of roe deer – on the basis of the total area of the hunting district. To derive the area of a hunting district at which the above mentioned comparison no longer significantly affects the census results, all data were arranged into five groups: (1) data obtained in hunting districts smaller than 500 ha; (2) 500 – 1,000 ha; (3) 1,000 – 2,500 ha; (4) 2,500 – 5,000 ha; (5) larger than 5,000 ha. The reliability of the results was determined by processing them using MS Excel for Descriptive Statistics (mean values, standard deviation) and for Correlation and Regression Analysis. The significance of the relationship has been assessed according to the Pearson Product Moment Correlation (Fowler et al., 1998). The ranks of variables were examined by the Kruskal-Wallis test (Fowler et al. 1998) using software 'Sigma Stat 3.0 for Windows'. The programme includes Dunn's Test that is used to compare each group versus other. Dunn's test lists the difference of rank means, computes the Q test statistic, and displays whether or not $P < 0.05$ for each group pair. One can conclude from the values of Q that the difference of the two groups being compared is more or less significant.

Results and Discussions

The census of a moose was carried out on the total of 1,032 hunting districts with the overall area of 3,808.939 hectares, of which 2,489.570 hectares was forest. The smallest district, on which the census of a moose was carried out, was only 210 hectares, of which 181 hectares was forest. It is likely that estimates of moose numbers have been biased by the size of reference territories i.e. area of hunting district. On smaller hunting grounds, the moose numbers are more often estimated as comparatively high and probably overestimated as is seen from the distribution pattern of the dots in scatter gram (Fig. 3). Correlation between compared values also appears significant ($P<0.05$) taking into account the large number of variables (Table 1). The multiple comparisons on ranks (Table 2) also show that small hunting districts are considered to be diversely inhabited by moose from large hunting districts. The threshold area between mutually similar moose density groups is somewhere at 2,500-5,000 ha large hunting areas. The districts below and above this size differ significantly in terms of estimate records. The census of a red deer was carried out on 910 hunting districts with the total area of 3,094.826 hectares, of which 2,069.147 hectares was forest. The smallest district, on which the census of a red deer was carried out, was 53 hectares, of which 15 hectares was forest. The number of red deer in the groups of smaller hunting districts has been estimated higher than in the groups of larger ones (Fig. 4). The multiple comparisons on ranks (Table 2) also show that small hunting districts are considered to be diversely inhabited by red deer from large hunting districts. The threshold area between mutually similar red deer density groups is somewhere at 2,500-5,000 ha large hunting areas. The districts below and above this size differ significantly in terms of estimate records. The census of a roe deer was carried out on 1,243 hunting districts with the total area of 4,002.746 hectares, of which 2,582.244 hectares was forest. The smallest district, on which the number of roe deer was attempted to assess, was a forest of 6 hectares. Figure 6 is an obvious proof of higher estimates of roe deer population in smaller hunting districts. There is no significant difference between estimated population density of roe deer in groups 2 and 3 (areas from 500 to 2,500 ha) but between all other groups difference is significant (Table 3). It allows to conclude that more correct estimation of roe deer population size can be done on 500 – 2,500 ha large areas. Taking into account information from the studies confirming the relatively small size of roe deer home range, the number estimates on larger territories might insufficiently cover entire population. The census of a wild boar was carried out on 1,187 hunting districts with the total area of 3,988.535 hectares, of which 2,579.247 hectares was forest. The smallest district, on which the census of wild boar was carried out, was a forest of 4 hectares. Wild boar seems to be most controversial game species for the hunters and hunting administrators, and its population density might be largely impacted by countless other factors than the size of territorial census units, e. g. food availability, uneven distribution of woodlands etc. Therefore, the graph of relationship between area of the hunting districts and population density looks even absurd and determination coefficient for the function studied is extremely low (Fig. 5). There is no significant difference between estimate population density of wild boar in 1 and 2 groups (areas from 1-1,000 ha). Both these groups are significantly different from the others while all the other groups are mutually different (Table 3). Nevertheless, this analysis as

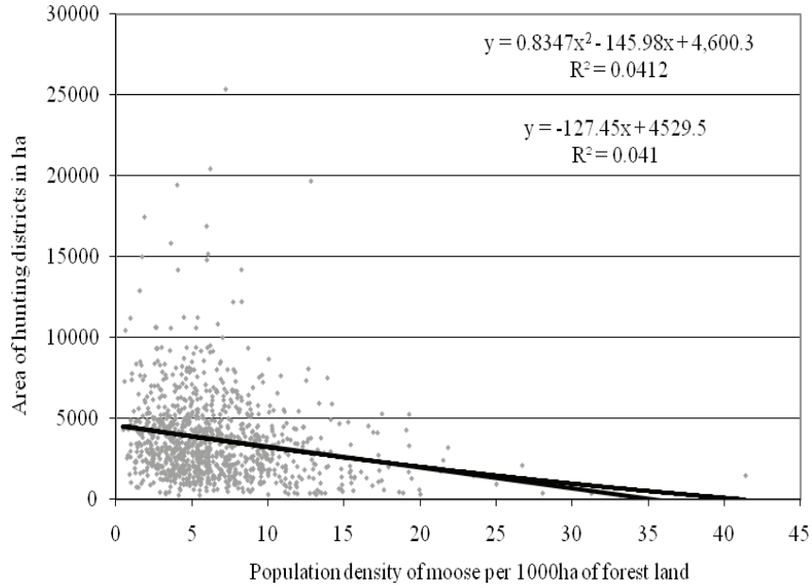

Figure 3. Relationship between area of hunting district and estimated population density of moose.

well as Figure 5 let us conclude that the number of wild boar population might be overestimated in small areas and/or underestimated in large ones. Statistically significant negative or inverse correlation (r = - 0.203; p = 0.05) was uncovered between the area of a hunting district and the number of a moose counted in the census. There was a significant linear relationship in the census of other species – the relationship was the strongest for roe deer (r = -0.347), very similar for red deer and a moose (r = 0.197), but the weakest for wild boar (r = -0.095) for which the relationship was rather non-linear (Table 1).

That relationship shows that the changes in the area on which the census is taken cause the changes in the density of animals in the hunting district.

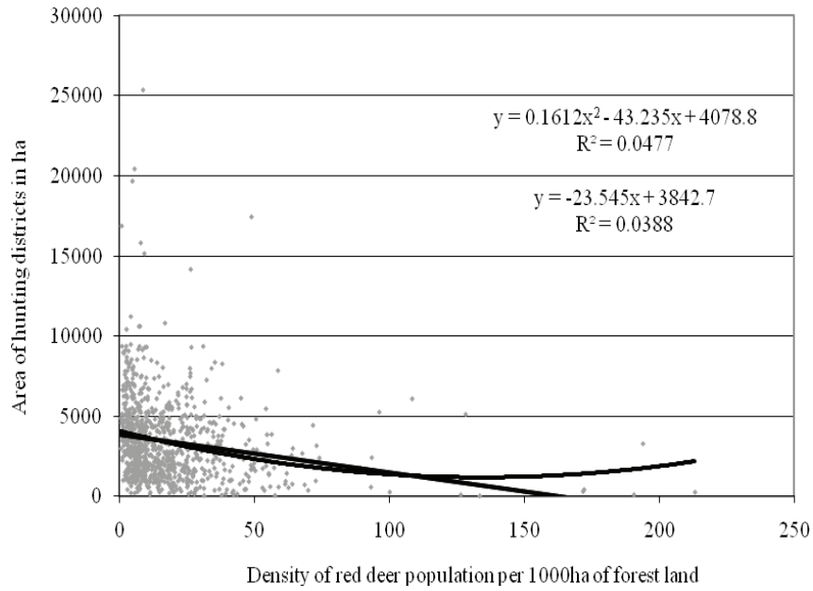

Figure 4. Relationship between area of hunting district and estimated population density of red deer.

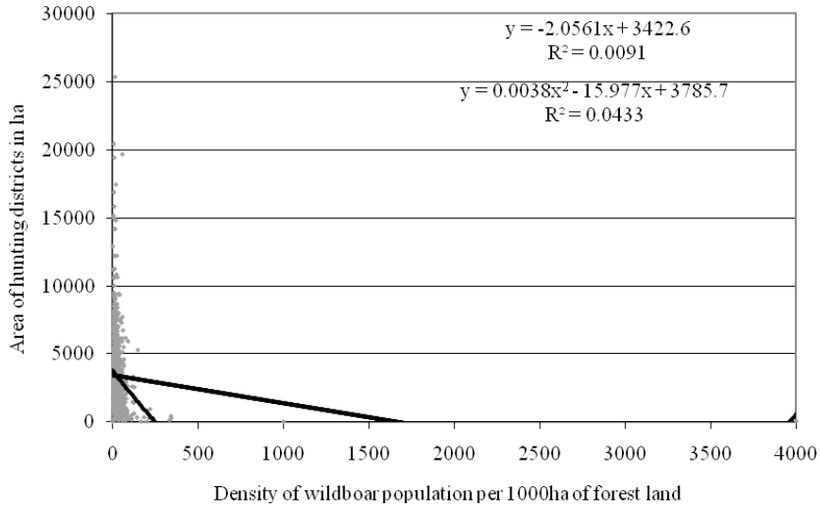

Figure 5. Relationship between area of hunting district and estimated population density of wild boar.

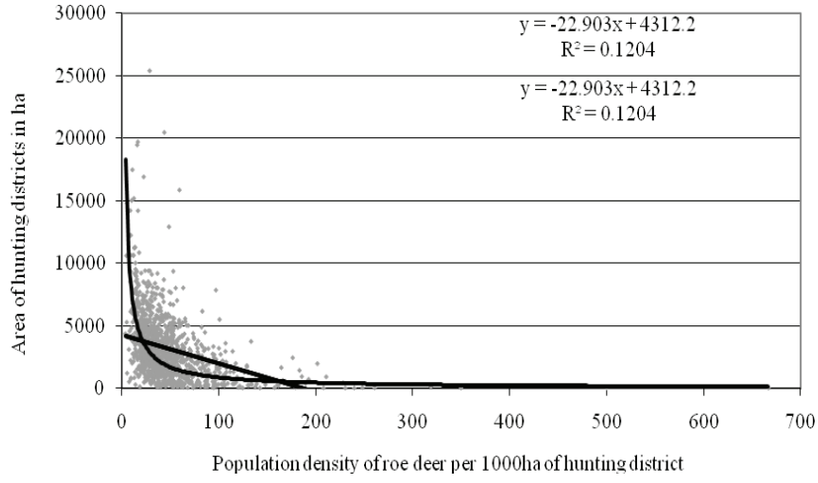

Figure 6. Relationship between area of hunting district and estimated population density of roe deer.

The significance of the relationship was assessed according to the Pearson's r. The analysis made showed that the area of the district affected the census result for roe deer and wild boars within all range of areas. In respect of red deer and moose, this relationship became insignificant if the area of a hunting district reached 5,000 hectares (Table 1).

Table 1

Absolute Values of the Pearson's r for the Relationship Between the Area of a Hunting District and the Counted Animal Density in the District

Area of hunting district (ha)	Moose		Red deer		Roe deer		Wild boar	
	r	n	r	n	r	n	r	n
All	0.203	1,032	0.197	910	0.347	1,243	0.095	1,187

r – correlation coefficient, n – number of districts, * - insignificant correlation

Table 2

Results of All Pair-wise Multiple Comparison Procedures (Dunn's Method) for Moose and Red Deer

Moose			Red deer		
Comparison	Q	P<0.05	Comparison	Q	P<0.05
1 vs 5	5.438	Yes	1 vs 5	7.399	Yes
1 vs 4	5.193	Yes	1 vs 4	6.330	Yes
1 vs 3	3.833	Yes	1 vs 3	4.644	Yes
1 vs 2	2.152	No	1 vs 2	2.237	No
2 vs 5	4.116	Yes	2 vs 5	5.583	Yes
2 vs 4	3.782	Yes	2 vs 4	4.265	Yes
2 vs 3	1.874	No	2 vs 3	2.297	No
3 vs 5	3.846	Yes	3 vs 5	5.141	Yes
3 vs 4	3.461	Yes	3 vs 4	3.241	Yes
4 vs 5	0.907	No	4 vs 5	2.517	No

1: smaller than 500ha; 2: 500-1,000ha; 3: 1,000-2,500ha; 4: 2,500-5,000ha; 5: bigger than 5,000ha

Conclusions

1. Officially reported population densities of the four main game species in hunting districts for the season 2006/2007 are highly varied ranging from single specimen per few thousand of hectares to one individual per hectare, in case of wild boar in particular.
2. The census of a roe deer and wild boar was the least objective. The assessment of the number of these game animals was too notably affected by the area where the census was taken. The analysis made does not explicitly show in which direction the number of roe deer and wild boars was assessed wrongly – as either being too small in large districts, or too large in small districts.
3. The number of moose and red deer may be assessed more objectively in districts with area exceeding 2,500 hectares. Therefore, it is possible that the estimate of the total number of moose and red deer is incorrect due to the fact that the census was taken on districts that are too small for an appropriate procedure.
4. The influence of the size of reference area may be eliminated only by using scientifically motivated census methods and by training and educating the persons taking the census.
5. A management implication from the given analysis is that suggested minimum size for large game census and number estimates should be at least 2,500 ha of woodland.

Table 3

Results of All Pair-wise Multiple Comparison Procedures (Dunn's Method) for Wild Boar and Roe Deer

Wild boar			Roe deer		
Comparison	Q	P<0.05	Comparison	Q	P<0.05
1 vs 5	8.809	Yes	1 vs 5	14.246	Yes
1 vs 4	7.534	Yes	1 vs 4	11.972	Yes
1 vs 3	5.077	Yes	1 vs 3	6.992	Yes
1 vs 2	1.313	No	1 vs 2	3.218	Yes
2 vs 5	7.328	Yes	2 vs 5	9.131	Yes
2 vs 4	5.933	Yes	2 vs 4	6.583	Yes
2 vs 3	3.467	Yes	2 vs 3	2.366	No
3 vs 5	6.053	Yes	3 vs 5	10.091	Yes
3 vs 4	4.000	Yes	3 vs 4	6.783	Yes
4 vs 5	2.821	Yes	4 vs 5	4.628	Yes

1: smaller than 500ha; 2: 500-1,000ha; 3: 1,000-2,500ha; 4: 2,500-5,000ha; 5: bigger than 5,000ha

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TEMPORAL STRUCTURE OF MIDDLE-AGED NORWAY SPRUCE STANDS OF LOW GROWTH POTENTIAL ON DRAINED SITES

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Abstract. Large proportion of 30-50 years old spruce stands originated from overstocked plantations can be characterized by low growth potential and present serious problem to forest managers. Although according to legislation unproductive spruce stands can now be clearcut, possible alternative solution allowing a forest owner to remove only trees with the worst growth potential would be also welcome. To be able to recommend any silvicultural measures, information on the inner structural development of the stands is necessary. The aim of this study was to analyze temporal structure of several spruce stands that have originated from overstocked plantations and now belong to the 2nd (increased risk stands) or 3rd (unpromising stands) growth potential groups. Data for the study from seven sample plots located in Forest Research Station 'Kalsnava' and thinned with different intensity were used. Tree diameter distribution, volume accumulation patterns and tree mortality were analyzed. Correlation between tree diameter and tree diameter difference in the observation period from 2000 to 2006 was weaker than in the observation period from 2006 to 2009, indicating further decline of growth potential. In most cases the major part of the volume was accumulated in the middle of the diameter class distribution. For most of the trees one or both sawlogs were of good or average quality. In order to develop optimal and profitable stand management model, the effect of different thinning methods in spruce stands of low growth potential should be studied in the future.
Key words: Norway spruce, growth potential, stand structure.

Introduction

Norway spruce (*Picea abies* (L.) Karst.) is the second most widespread conifer tree species in Latvia. According to statistics, spruce stands take up 17% of the forest area (Mežaudžu platība, 2010), and total standing volume of spruce equals 100 million m^3 (Mežaudžu krāja, 2010). Spruce timber is also important for Latvian economy: in 2008 total amount of logged spruce timber was more than 1.5 million m^3 (Koku ciršanas apjomi, 2010).

As a result of changes in forest management practices (less dense plantations, well-timed thinnings), the amount of initially sparse, healthy stands is nowadays increasing (Zālītis et al., 2006; Zālītis and Lībiete, 2008; Zālītis and Jansons, 2009). However, a large proportion of presently 30-50 years old spruce stands have originated from overstocked plantations and often have not been properly thinned. These stands present a serious problem to forest owners and managers, as their growth potential is generally low and growth rate stagnation can frequently be observed (Lībiete, 2008; Libiete and Zalitis, 2007; Zālītis and Lībiete, 2005; Zālītis, 2006a). Some years ago principles for identification of unproductive spruce stands were developed (Zālītis, 2004), and now they are also introduced in the legislation (Par koku ciršanu meža zemēs, 2009). After a few steps of simple measurements and calculations the forest owner is now able to declare his spruce stand unproductive and accordingly clearcut it. However, not all forest owners will probably be content with this solution. After the clearcut the stand must be regenerated, either artificially (which is costly) or naturally. The disadvantage of natural regeneration after the clearcut in spruce stands growing mainly on fertile soils is an almost inevitable formation of a broadleaved (birch, aspen or grey alder) stand in the subsequent generation; thus, decreasing the overall quality of forest. An alternative solution would be some type of selective cutting, allowing the forest owner to remove trees with the worst growth potential but maintaining the species composition and substantially improving the quality of the residual stand. Several literature sources confirm that crown thinning (thinning from above) has a strong positive influence on the diameter growth and crown development of the residual stand (Deleuze et al., 1996; Handler and Jakobsen, 1986; Kramer, 1966).

To be able to recommend any alternative silvicultural measures in spruce stands of poor growth potential we must have the information on the inner structural development of such stands. The aim of this study was to analyze the temporal structure of several spruce stands that have originated from overstocked plantations and now correspond to the 2nd or 3rd growth potential groups.

Materials and Methods

Study Area

The experiment was located in Forest Research Station 'Kalsnava' (latitude 56º40´N, longitude 25º51´E). Seven sample plots thinned with different intensity (including 3 unthinned control sample plots) were used for the analysis. All stands were middle-aged: mean age ranged from 42 to 61 years.

Four sample plots (P1-P4) were established in 1986 in 24 years old spruce plantation on drained peat soil (*Myrtillosa turf.mel.*). One sample plot (E1) was established in 1978 in 30 years old naturally regenerated spruce stand on drained mineral soil (*Myrtillosa mel.*). Two sample plots (E3 and E4) were established in 12 years old naturally regenerated birch-spruce stand on drained peat soil (*Myrtillosa turf.mel.*). In 1979, all birches were removed in E4, creating a pure spruce stand. In E3 birch admixture of 10% was retained. Summary about sample plots analyzed in this research is presented in Table 1. All trees in these sample plots are numbered; thus, it is possible to trace the development of every individual tree.

Table 1

Description of the Analyzed Sample Plots

Sample plot		P1	P2	P3	P4	E1	E3	E4
Year of thinning		1988	1988	1988	1988	1982	1979	1979
Number of trees per ha	Before thinning	2700	2900	2100	2400	2400	4960	3640
	After thinning	2700	1600	2100	1500	1060	4960	3100
	In 2006	1300	1350	1250	1320	620	880	920
Mean diameter, cm	Before thinning	13.1	12.7	13.1	14.4	14.7	10.0	10.0
	After thinning	13.1	13.9	13.1	15.0	17.9	10.0	10.0
	In 2006	18.1	18.7	18.3	19.7	27.1	20.8	21.8
Mean height, m	Before thinning	12.5	11.2	12.5	11.8	18.0	10.0	10.0
	After thinning	12.5	12.0	12.5	12.5	19.5	10.0	10.0
	In 2006	17.7	18.2	17.8	18.5	27.1	20.1	20.9
Basal area, m^2 ha^{-1}	Before thinning	29	30	25	30	31	27	28
	After thinning	29	25	25	26	24	27	24
	In 2006	33	37	33	40	36	30	34
Standing volume, m^3 ha^{-1}	Before thinning	179	186	165	190	292	154	169
	After thinning	179	158	165	170	243	154	151
	In 2006	307	344	302	375	481	307	364

In 2000, a sample plot E1 corresponded to the 1st growth potential group (healthy stands) but sample plots P1-P4 - to the 3rd growth potential group (unpromising stands). In 2006, sample plots P1-P4, E3 and E4 corresponded to the 3rd growth potential group (unpromising stands) and sample plot E1 – already to the 2nd growth potential group (increased risk stand) (Zālītis, 2006b).

Measurements and Calculations

For the needs of this research the sample plots P1-P4 and E1 were measured in 2000, 2006 and 2009 but sample plots E3 and E4 – in 2006 and 2009. In total, 524 trees were measured in 2000, 576 trees in 2006 and 507 trees in 2009. The diameter at breast height (accuracy 0.1 cm, in two opposite directions) was recorded for all trees in the sample plots. In every sample plot the height of 13-20 trees with accuracy 0.1 m was also measured and based on these measurements height curves were constructed.

The volume of trees was calculated according to the exact diameter of each individual tree and height from the height curve using the following equation (1). Total standing volume of the sample plot is the sum of individual tree volumes.

$$V = \psi \cdot h^{\alpha} \cdot d^{\beta \cdot \lg h + \varphi} \qquad (1)$$

V – tree volume, m^3
h – tree height, m
d – tree breast height diameter, cm
$\psi, \alpha, \beta, \varphi$ – empirical coefficients (Liepa 1996)

The values of empirical coefficients for spruce:
$\psi = 0.00231$, $\alpha = 0.78193$, $\beta = 0.34175$, $\varphi = 1.18811$

The compliance of data with normal distribution was tested using one-sample Kolmogorov-Smirnov test. Linear regression analysis was performed for time periods from 2000 to 2006 and from 2006 to 2009 in order to test the relation between tree diameter and tree diameter difference between measurement periods.

Results and Discussion

Tree Diameter Distribution

Tree diameter distribution corresponded to normal curve in all sample plots in all measurement periods. (Figure 1) In all cases diameter distribution was characterized by positive skewness, indicating that lesser values prevail. In 2000, the diameters of all sample plots except P2 were characterized by negative kurtosis, pointing to rather high concentration of values in marginal classes. The overall trend remained true also for 2006 and 2009, in 2006 positive kurtosis was detected only in E3 sample plot but in 2009 – in P2 and E3 sample plots. The highest negative kurtosis in all measurement periods was found in P1 sample plot (-1.20 in 2000, -1.00 in 2006 and -0.79 in 2009). This again can be attributed to the management regime. In the unthinned control plots the thinnest trees have remained as suppressed crop, while in some parts of the stand due to natural mortality the competition for some larger trees has decreased, thus favouring trees of marginal diameter classes.

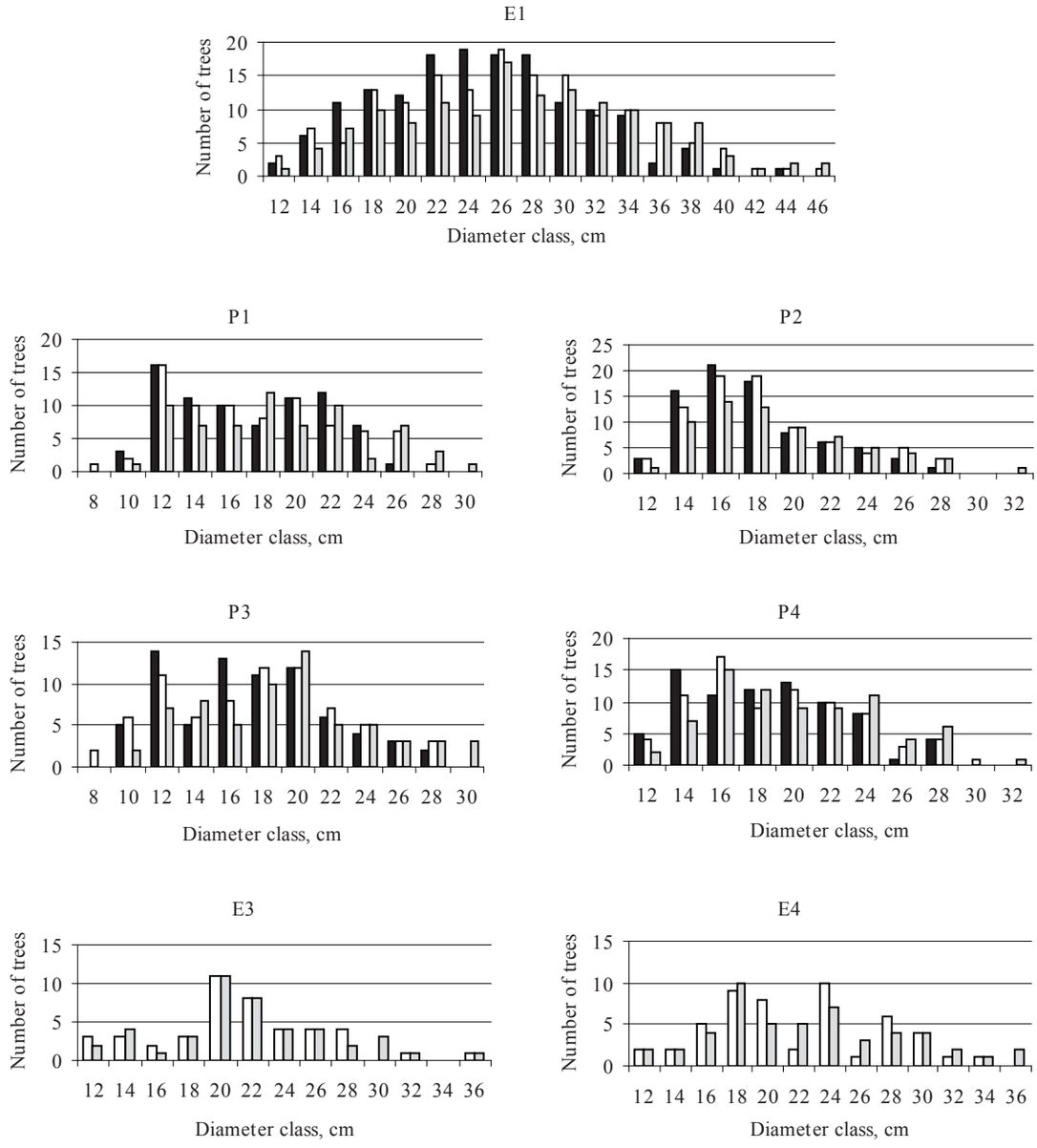


Figure 1. Diameter distribution in sample plots in 2000, 2006 and 2009.
Legend: ■ - 2000; □ - 2006; ▢ - 2009

In a healthy stand the largest trees should also be the ones with the largest diameter increase, but in spruce stands with decreased growth potential an opposite trend can be observed. The results of linear regression analysis confirm this statement. (Table 2)

Table 2

Relation Between Tree Diameter and Tree Diameter Difference – Results of Linear Regression Analysis

Sample plot	Linear correlation coefficient R		Determination coefficient R^2		F value		p-value of F test*	
	2000-2006	2006-2009	2000-2006	2006-2009	2000-2006	2006-2009	2000-2006	2006-2009
E1	0.795	0.507	0.632	0.257	263.020	46.792	0.000	0.000
P1	0.509	0.457	0.259	0.209	26.573	17.162	0.000	0.000
P2	0.443	0.448	0.197	0.201	19.315	16.314	0.000	0.000
P3	0.583	0.459	0.340	0.211	37.594	16.855	0.000	0.000
P4	0.323	0.273	0.105	0.075	8.994	5.972	0.004	0.017
E3	-	0.155	-	0.024	-	1.031	-	0.316
E4	-	0.475	-	0.225	-	14.251	-	0.000

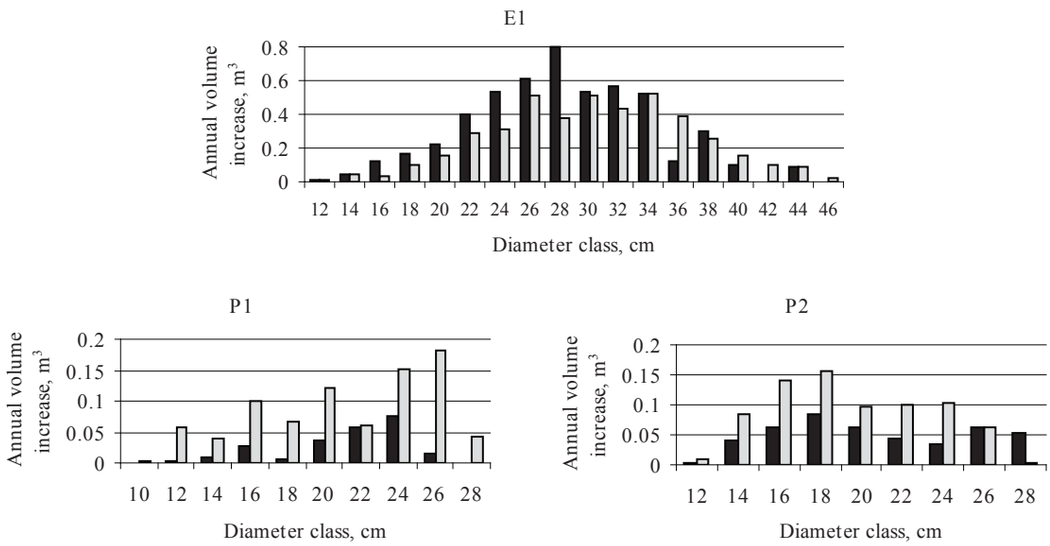
*The impact is significant at $\alpha = 0.05$

Correlation between tree diameter and tree diameter difference was significant in all cases except E3 sample plot in the second test period. The linear relation between the tree diameter D_{2000} and tree diameter difference $D_{2006\text{-}2000}$ was the strongest in E1 sample plot that in 2000 corresponded to the 1st growth potential group (R=0.795). But, as the growth potential of the stand decreased, also the linear relation grew weaker and in the second test period linear correlation coefficient R in E1 sample plot was equal to 0.507. In 2006, this sample plot already corresponded to the 2nd growth potential group. In the second test period linear correlation between tree diameter and diameter difference in sample plots P1-P4 and E4 was weak (R<0.5).

Decrease of growth potential in the initially healthy E1 stand can most likely be explained with the delay of thinning. Thinning was carried out when the stand was already more than 30 years old and the mean height had reached 18.0 m. K. Bušs (1989) has pointed out that well-observable tree differentiation in dense spruce stands starts already in the fourth to sixth year after planting. It is highly likely that pronounced mutual competition at early age has left negative after-effects and affected the volume accumulation rate in the future. The utmost importance of well-timed thinning of young spruce stands is repeatedly stressed also in the foreign literature (Hannelius, 1978; Kairiūkštis and Malinauskas, 2001; Kuliešis and Saladis, 1998; Nilsson 1994).

Growth Dynamics

In order to find out which trees produce most of the standing volume, mean annual volume increment in each 2 cm diameter class for periods 2000-2006 and 2006-2009 was calculated using individual tree volume data. In E1 sample plot most annual volume increase was observed in the middle of diameter class distribution (in diameter classes 22-34 during the first observation period, in diameter classes 26-36 during the second observation period). During the second observation period the annual volume increment in most diameter classes decreased; some increase could be observed only in 36, 40, 42 and 46 cm diameter classes. (Figure 2)



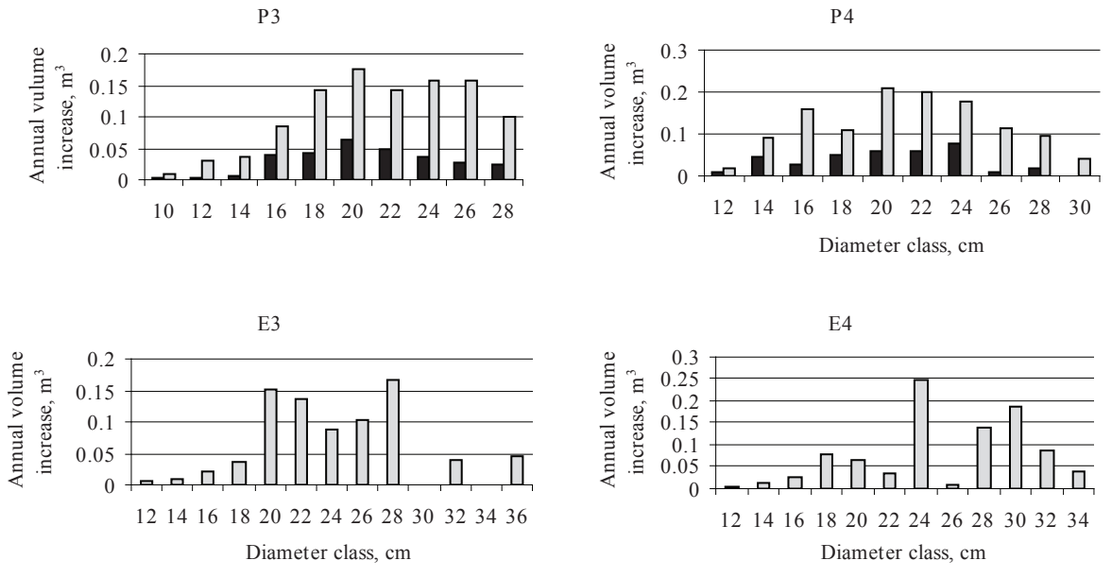


Figure 2. Annual volume increment distribution over diameter classes in sample plots.
Legend: ■ - 2000-2006; □ - 2006-2009

In E1 sample plot the annual volume increment in most diameter classes decreased with the time, but in sample plots P1-P4 exactly the opposite was true; moreover, the increase in the second observation period in most diameter classes was considerable. The reasons for this process presently remain unclear.

During the first observation period, the annual volume increase distribution over diameter classes in the sample plot P1 was irregular, and it remained such also during the second observation period. In the sample plots P2-P4 the annual volume increment during the first observation period was rather evenly distributed over most diameter classes. During the second observation period in the sample plots P2, P4 and E3 the largest mean annual volume increment could be observed in the middle of diameter class distribution, but in P3 and E4 sample plots – in the largest diameter classes. The results indicate that in most cases the major part of the volume is accumulated in the middle of the diameter class distribution, but the situation when most of the volume is accumulated in the largest diameter classes is also possible. Therefore, strict recommendation of crown thinning (thinning from above) in spruce stands with decreased growth potential might possibly lead to elimination of most productive trees of the stand. However, many authors regard thinning from above or thinning from both ends of diameter distribution as preferable in spruce stands (Eriksson, 1994; Haight, 1987; Haight and Monserud, 1990; Pukkala et al., 1998; Solberg and Haight, 1991; Valsta, 1992; Vettenranta and Miina, 1999). The results of several studies indicate that suppressed and co-dominant trees respond better to thinning than dominant trees (Larson, 1969; Pape, 1999). It has also been reported that thinning from above increases thinning revenues and thus can improve the profitability of forest management (Mielikäinen and Hynynen, 2004). This aspect could be of especially large importance for the private forest owners. Another study has showed that for the first thinning in initially dense spruce stands thinning from both ends of diameter distribution would be optimal, but at the second and subsequent thinnings, thinning from above was clearly superior (Cao et al., 2006). Considering our results and information from the literature, we assume that further research regarding this topic is necessary. The impact of different thinning methods on further development of the residual stand should be studied in the future.

Mortality Rate

Only in two sample plots (E1 and P2) the number of dead trees during the second observation period was greater than the number of dead trees during the first observation period. In the sample plots P1, P3 and P4 the opposite was true. (Table 3) Mean diameter of dead trees in the period 2000-2006 ranged from 12.3 cm in P4 sample plot to 16.8 cm in E1 sample plot but in the period 2006-2009 – from 13.0 cm in P3 sample plot to 23.4 cm in E1 sample plot. In both observation periods mean dimensions of dead trees were below mean dimensions of living trees in all sample plots. This corresponds well with statement that smallest, suppressed trees in the stand generally have higher mortality rates (Kobe and Coates, 1997).

The annual mortality rate (calculated from the number of trees) was in all cases higher during the second observation period. From 2000 to 2006 the annual mortality rate ranged from 1% in E1 and P4 sample plots to 3% in P1 and P3 sample plots, but from 2006 to 2009 it ranged from 1% in P4 sample plot to 6% in P2 and E3 sample plots. In all our sample plots the numbers of annual mortality rate were considerably greater than those given in the literature (Fridman and Walheim, 2000; Ranius et al., 2003).

Table 3

Characteristics of Tree Mortality in the Sample Plots

Sample plot		E1	P1	P2	P3	P4	E3	E4
Number of dead trees per plot	2000-2006	11	14	10	14	7	-	-
	2006-2009	18	11	14	10	3	9	4
Mean diameter, cm	2000-2006	16.8	13.6	13.5	12.7	12.3	-	-
	2006-2009	23.4	13.9	17.2	13.0	14.0	13.7	11.8
Mean height, m	2000-2006	19.8	14.5	15.0	14.5	14.3	-	-
	2006-2009	25.9	15.4	17.4	14.8	16.2	15.6	14.5
Basal area, m^2 ha^{-1} per year	2000-2006	0.2	0.6	0.4	0.5	0.2	-	-
	2006-2009	1.1	1.0	1.9	0.8	0.3	0.9	0.3
Volume, m^3 ha^{-1} per year	2000-2006	1.8	5.7	3.5	4.4	2.0	-	-
	2006-2009	15.2	8.7	17.8	7.0	2.4	8.5	2.2

Interesting that in E1 sample plot mean annual volume of dead trees had increased from 1.8 m^3 ha^{-1} per year in the period 2000-2006 to 15.2 m^3 ha^{-1} per year in the period 2006-2009. The increased mortality rate corresponds well with the decrease of growth potential in this sample plot. During the first observation period the highest tree mortality was observed in P1 and P3 sample plots, but in the second observation period tree mortality rate had significantly increased also in the P2 sample plot reaching 17.8 m^3 ha^{-1} per year. During both observation periods tree mortality remained lowest in P4 sample plot.

Conclusions

1. Correlation between tree diameter and tree diameter difference in the second observation period was weaker than in the first observation period, indicating further decline of growth potential.
2. In most cases the major part of the volume was accumulated in the middle of the diameter class distribution, but the situation when most of the volume is accumulated in the largest diameter classes is also possible.

   In order to develop optimal and profitable stand management model, the effect of different thinning methods in spruce stands of low growth potential should be studied in the future.

Acknowledgements

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GREY ALDER FIBREBOARD PROCESSED BY MODIFIED STEAM EXPLOSION UNIT

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Abstract. Experimental self-binding high-density fibreboard is produced of the grey alder (*Alnus incana* L. Moench) steam-exploded fibres without addition of synthetic adhesives. Milled grey alder chips are processed in steam-explosion unit by saturated steam under pressure of 3.2 MPa at temperature of 235 °C for 1 min in a 0.5 l batch reactor. The steam-exploded fibres are pressed at 160 °C temperature under 8 MPa pressure for 10 min in three steps. Properties, such as density, swelling in thickness, water absorption, bending strength, modulus of elasticity at bending, and internal bonding strength of the studied fibreboard samples are reported. Differences between the raw milled chippings and the exploded fibres are observed by scanning electron microscope. The study is focused on modified technical options of the steam-explosion unit supplied with two containers receiving different kinds of the exploded biomass farther used to obtain the hot-pressed boards. The cascade of the receivers is explained in a presently pending patent. The self-binding high-density fibreboard samples show the following properties comparable to commercial products: density of at least 1.35 g cm^{-3}, moisture content of 7.2%, swelling in thickness of 8.1%, water absorption of 3.2%, bending strength of 27 N mm^{-2}, modulus of elasticity of 6,259 N mm^{-2}, and internal bonding of 0.92 N mm^{-2}.
Key words: steam-explosion, wood fibres, self-binding fibreboard, physical-mechanical properties.

Introduction

Wood composite materials, such as particle boards, fibreboards, oriented strand boards, etc., being good materials of self-sufficient strength and form stability for building constructions and furniture are significant products of the wood processing industry around the world. Usually costly synthetic adhesives not always friendly to the environment are used to produce wood composites causing problems at utilization. Costs of synthetic adhesives have a tendency to increase because of the increasing costs of the raw material (petroleum). Therefore, the costs of wood composite materials cannot be stable.

There is increasing interest in rational use of renewable resources. After the invention of Masonite fibres (Boehm, 1930), several industries apply the fibres even nowadays. In 2000, Laemsak and Okuma published a paper on binder-less boards prepared from steam-exploded fibres of oil palm (*Elaeis guineensis* Jacq.) concluding that boards satisfying requirements of the relevant standards can be produced from oil palm fronds without using any binders (Laemsak and Okuma, 2000). In 2002, Velasquez et al. explored the suitability of pre-treated elephant grass (*Miscanthus sinensis*) for manufacturing fibreboards with no synthetic binders. The obtained boards were found to be of very good quality and to satisfy the requirements of the relevant standard specifications (Velasquez et al., 2003). And, finally, binder-less boards were prepared from finely ground powders of kenaf (*Hibiscus cannabinus* L.) core in Japan (Okuda and Sato, 2004). The possibility of production of binder-less board by processing through a flour-mill substituting the steam explosion pre-treatment of the raw material was confirmed. Many other researchers are still working on binder-less or self-binding boards to explore and improve its physical, mechanical, thermal and other properties (Angles et al., 1999; Angles et al., 2001; Mobarak et al., 1982; Shao et al., 2009; Van Dam et al., 2004; Xu et al., 2003). All the studies show a necessity to solve the problems of developing wood composites.

The present study continues to demonstrate the experimental viability of producing high-density fibreboard obtained from local fast-growing species of grey alder without synthetic adhesives. The target of the study was to define and estimate the physical-mechanical properties of the fibreboard samples obtained from the different kinds of the exploded biomass. An earlier report (Gravitis et al., 2008) concerns the possible use of the steam explosion treatment while generating self-adhesive materials from grey alder wood. It was concluded that the obtained self-binding materials have advanced properties and value to the same extent as those available commercially (Tupciauskas et al., 2009).

Materials and Methods

Steam explosion (SE). Grey alder wood chips were cut to obtain chippings of the size in the range of 0.4 – 2 mm. The chippings were air-dried to equilibrium moisture content of about 8%. So prepared, the chippings were submitted to hydro-thermal treatment with saturated steam in a 0.5 l batch reactor at temperature of 235 °C and pressure of 3.2 MPa for 1 minute after which the valve of the SE reactor is opened and the softened chippings are expelled into the receiver unit. The receiver unit consists of two separated and pressurized vessels, the first of which is joined with SE reactor by a vertical pipe and the second is joined to the first by a horizontal pipe. The treatment is controlled so that the exploded fibrous mass would fall in the vessels at proportion 5:4. Approximately one part of the exploded fibres stays in the horizontal pipe joining the vessels. The moist exploded fibrous mass is picked up from the vessels and from the horizontal

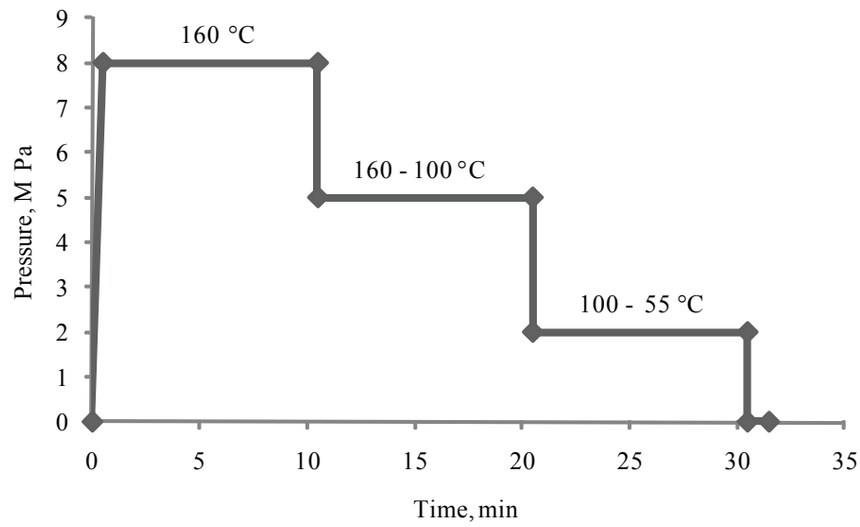

Figure 1. Pressing cycle graph of the self-binding grey alder hardboard.

pipe separately for drying in open air (temperature ~ 20 °C, relative humidity ~ 60%).

Instrumental methods. SE fibrous mass was studied by following instrumental methods. The raw grey alder chippings before and after SE treatment were observed using a Vega TS 5136 scanning electron microscope (SEM) operating at 15 – 20 kV, after sputter coating with gold.

An L&W Fibre Tester analyser was used to determine parameters of the exploded fibres such as length, width, shape factor (the ratio of projected to actual length), coarseness (mass per unit length), ratio of fines (fibres less than 0.2 mm), and aspect ratio (length and width ratio). Testing the fibre parameters only screened fraction equal or less than 0.16 mm was used. The measured fibres were compared with the exploded fibres after a 2 min treatment by SE of a previous study (Abolins et al., 2008). The fibre analysis was carried out in clean water heated to 35 – 40 °C.

Sample pressing. The SE non fractioned air-dried grey alder fibre mass with moisture content of 4–5% was pressed in a "Joos" single-stage hydraulic hot-press. A forming box (inner measurements 200 x 200 mm) was used for the fibre mass pre-pressing by 0.7 MPa in the press. The pressing conditions were set for both bottom and top plate temperature of 160 °C and pressure of 8 MPa during 10 min (Fig. 1). After that, the heating of the press plates was turned off and the cooling was turned on the pressure being reduced to 5 MPa for another 10 min before reducing to 2 MPa for next 10 min. Finally, the pressed sample was left for one minute without pressure for strain relaxation. As shown in Fig. 1, the full pressing time of the board samples was 31.5 min before opening the press and the sample board taken out. By the time the board is removed the temperature of the bottom plate is about 50 °C, and below 70 °C – of the top one. Subsequently, the press was heated again to 160 °C for the next pressing cycle. Three board samples were made from the fibre mass obtained in the first vessel of the SE unit, four samples from the fibres of the second receiver, and one board sample of fibres extracted from the pipe between vessels.

Board testing. After the board samples were cut into various test pieces, density (European Standard EN 323, 1993), thickness swelling (European Standard EN 317, 1993), water absorption, modulus of elasticity and bending strength (European Standard EN 310, 1993), and internal bonding (European Standard EN 319, 1993) properties were determined according to the standards and compared with the European requirements for hardboards (European Standard EN 622-1, 2003 and EN 622-2, 2004). All specimens were conditioned at 21 ± 2 °C temperature and relative air humidity of 65 ± 5% until equilibrium mass, before the testing. The specimens in size 50 x 50 mm were immersed in deionised water for 24 hours, and then thickness and mass measured for determining of thickness swelling and water absorption. Mechanical properties of hot-pressed fibreboard samples were tested by the universal machine for testing material resistance ZWICK/Z100. The bending strength test was carried out by applying a load to the centre of a test piece supported at two points. The test piece size was 50 x 170 mm; the distance between two points 140 mm and the test speed 3 mm min^{-1}. For the determination of resistance to tension perpendicular to the plane of the board (internal bond), the board specimens in size 50 x 50 mm were bonded to hardwood plywood testing block by polyvinyl acetate glue (PVAC) and then after conditioning for at least 72 h tested with test speed of 1.5 mm min^{-1}.

Results and Discussion

The properties of steam exploded fibres. About 10% of the mass is lost during SE even at 1 min treatment. The loss is considered to be a part of the

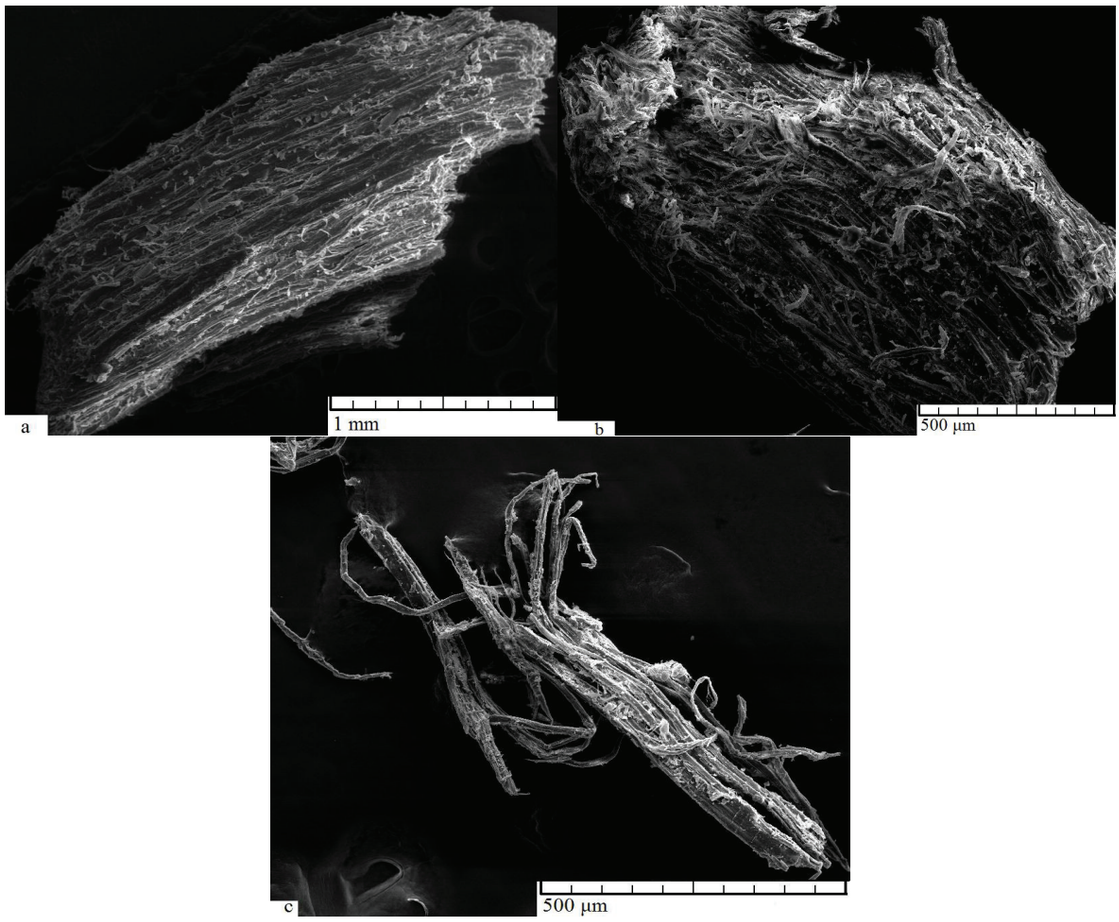

Figure 2. Scanning electron microscope images of grey alder wood.
a – raw chippings; b – fibre bundle after SE from first vessel; c – single fibres after SE from the second vessel.

wood hemicelluloses. At the explosion the chippings are defibrated partially or completely moulding the fibre bundles and the single fibres. There are visual differences (Fig. 2) between the raw chippings (a) and the fibres extracted from the first (b) and the second (c) receivers of the SE unit analysed by SEM. Comparing the raw and the exploded chippings, significant changes are seen in the structure of the material. As detected by SEM, there are more fibre bundles in the exploded mass from the first vessel compared with the mass from the second vessel. This difference is seen comparing images (a) and (b) in Fig. 2. This observation allows suggesting that content of the second vessel of the SE unit comprises the major part of single fibres while the first vessel mainly contains bundles of fibres. Despite the previously expressed viewpoint, the steam exploded fibre mass is not homogeneous enough.

The exploded fibre mass parameters are summarized in Table 1. The different numbers of tested fibres of 14 to 111 thousands correlate with 'fines' (fibres less than 0.2 mm in length). The bigger the number of fibres, the bigger the number of fines in the exploded grey alder mass. On the other hand, the quantity of fines depends on the fraction of testing fibres. As mentioned, the exploded fraction of fibres after 1 min treatment by SE was equal or less than 0.16 mm, not being fractioned after 2 min treatment. The amount of fines is larger in the sample from the second vessel (74.3%, see row 7 in Table 1) suggesting that smaller particles are deposited in the second vessel. This assertion is conformed by SEM results mentioned above. The screening before the test affects both the length and the width of the exploded fibres. Average length of the screened fibres is about 1.6 times shorter: 855 µm (not screened) and 512 µm in the case of screened fibres from the second vessel (see row 4 in Table 1). Different from the length, the average width of the screened fibres is about 1.6 times bigger: 31 µm in the case fibres are not screened and 47 µm in the case of the screened 'pipe fibres' (see row 5 in Table 1). It can be explained by smaller fibre particles having thicker cell walls.

No significant changes were observed in the 'average shape' (the ratio of projected to actual length) of the exploded fibres before and after screening. The shape factor of the exploded grey alder fibres means that if the fibres were fully straightened, they would be by 12 – 15% longer.

Table 1

Steam exploded grey alder fibre parameters

Parameter \ Sample	Screened fibres (0.16 mm sieve)			None screened
	First vessel fibre after 1 min SE	Pipe fibre after 1 min SE	Second vessel fibre after 1 min SE	First vessel fibre after 2 min SE
Number of tested fibres	77,261	81,369	111,806	14,502
Average length, µm	532	565	512	855
Average width, µm	45.5	47	42.5	30.9
Average shape, %	87.8	87.7	88.1	84.8
Fines, %	63.5	56.2	74.3	10.2
Coarseness, µg m^{-1}	499	462	384	339
Aspect ratio	11.7	12	12	27.7

Fibre coarseness often correlates with cell wall thickness. This trend remains in the exploded fibres, too. As supposed, the screened fibres have thicker cell walls; the coarseness of these fibres is bigger due to its greater mass. The coarseness of screened fibres (499 µg m^{-1}) is about 1.5 times larger compared with unscreened fibres (339 µg m^{-1}, see row 8 in Table 1).

The aspect ratio of the screened fibres is even 2.3 times smaller compared with unscreened fibres (see row 9 in Table 1) because of significant changes in the fibres size.

Properties of self-binding fibreboards. In case of one board sample (from the first vessel) the pressing cycle was changed accidentally. At the start (on the first or the second minute) the required pressure was not reached. Thus, the pressing cycle was repeated from the beginning. The event had a significant further effect on some board properties. The moisture content (3.25%), bending strength (45 N mm^{-2}) and modulus of elasticity (9,500 N mm^{-2}) improved significantly compared with other board samples. However, the rest of properties (density, water stability, and internal bonding) of the 'accidental sample' did not show any significant change compared to other board samples. For this reason the tested data of the 'accidental board sample' failed.

Table 2

Grey alder self-binding high-density fibreboard properties according to EN standard

Property / Sample	Density, g cm^{-3}	Moisture content, %	Thickness swelling, %	Water absorption, %	Bending strength, N mm^{-2}	Modulus of elasticity, N mm^{-2}	Internal bond, N mm^{-2}
First vessel	1.399 ±[a] 0.02	7.48 ± 0.1	8.8 ± 0.7	3.54 ± 0.7	27 ± 3	7,151 ± 552	0.94 ± 0.27
Pipe between vessels	1.393 ± 0.01	7.26 ± 0.04	8.1 ± 0.8	3.62 ± 0.03	30 ± 1	7,256 ± 111	0.92 ± 0.12
Second vessel	1.399 ± 0.01	7.38 ± 0.07	8.2 ± 0.8	3.23 ± 0.15	32 ± 4	6,259 ± 774	1.06 ± 0.12
Standard	Type	Ranges of nominal thickness > 5.5 mm					
EN 622-1,2:2004	HB[b]	4 - 9	25	-	25	-	0.5
	HB.H[c]		20	-	30	-	0.6
	HB.E[d]		8	-	32	2,900	0.5
	HB.LA[e]		25	-	30	2,300	0.6

Abbreviations in Table 2: ±[a] – standard deviation; HB[b] – general purpose hardboard for use in dry conditions; HB.H[c] – general purpose hardboards for use in humid conditions; HB.E[d] – general purpose hardboards for use in exterior conditions; HB.LA[e] – load bearing hardboards for use in dry conditions.

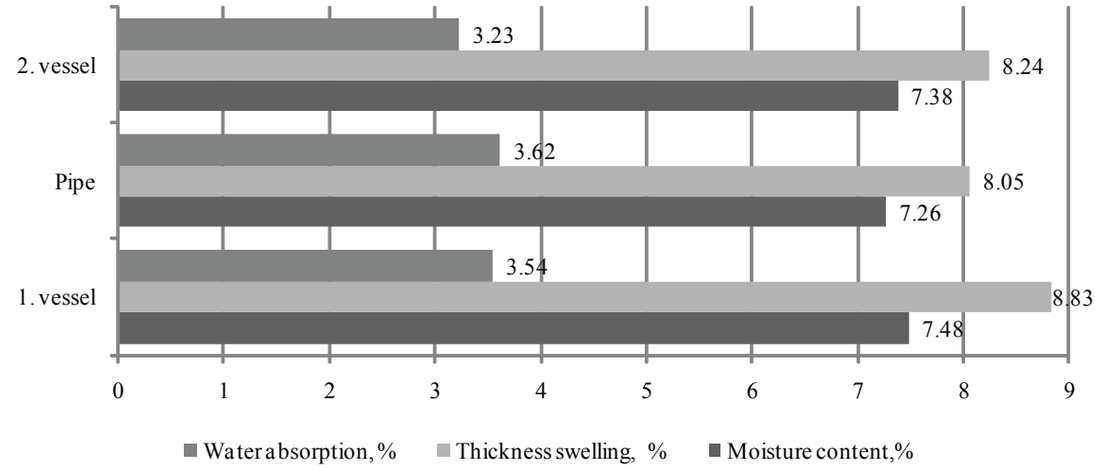

Figure 3. Form stability properties of grey alder self-binding high-density fibreboard samples.

The tested properties of grey alder self-binding high-density fibreboard are summarized in Table 2. The density of board specimens varies in the range of 1.350 – 1.426 g cm^{-3}, while the mean values of the samples (from the first and the second vessels of the SE unit) have no significant difference. High density of boards suggests that the material is homogenous. The obtained maximum density (1.426 g cm^{-3}) of the fibreboard is near to that of wood substance (1.46 – 1.53 g cm^{-3}). This means that the exploded fibres are very capable for self-bonding during the hot pressing.

The moisture content of self-binding fibreboard samples is satisfactory according to the European Standard EN 622-1 (see column 3 in Table 2). It should be noted that dispersity of the moisture content of the obtained fibreboards is very low. It also conforms the suggestion that the material is homogenous.

Water stability of the board samples is characterised by columns 4 and 5 in Table 2. The thickness swelling data range varies from 7.19 to 9.96% among the board specimens, while mean values between samples have no significant difference. The range of water absorption data varies from 2.99 to 5.19%, while mean values, as in the case of the thickness swelling, have not significant difference. The mean values of form stability of the board samples in percents are shown as a column chart in Fig. 3. As seen from Table 1 and Fig. 3, the form stability properties of the board samples vary within one percent limit, suggesting that there are no significant influence of the exploded fibres from different vessels of the SE unit on the form stability properties of the end product.

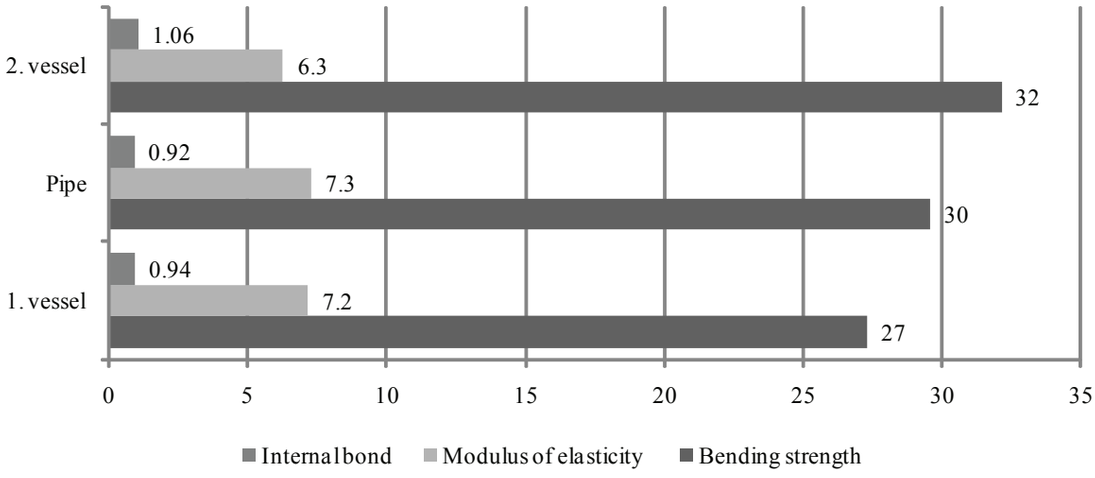

Figure 4. Mechanical properties of grey alder self-binding high-density fibreboard samples.
The internal bond and the bending strength values given in N mm^{-2}; the modulus of elasticity in kN mm^{-2}.

The mechanical properties of the board samples show tolerable results according to the European Standard EN 622-2 (see Table 2). The bending strength values vary from 23.5 to 39.8 (N mm^{-2}) among the board specimens, while the difference between the mean values of the first and the second vessels is 27 and 32 (N mm^{-2}) respectively (see Table 2. and Fig. 4). It should be noted that lower values of bending strength belong to board samples from fibres of the first vessel of the SE unit. As mentioned above, the fibres from the first vessel contain more fibre bundles and fewer fines suggesting that these factors affected the values of bending strength. On the other hand, the board samples made of fibres from the second vessel showed the best results of bending strength. It may have occurred because the fibres from the second vessel contain the largest amount of fines and single fibres. In any case the difference of the bending strength is not statistically significant.

The trend of the modulus of elasticity of the board samples is opposite to that of the bending strength. The range of the modulus of elasticity varies from 5,521 to 7,862 (N mm^{-2}) among the board specimens, while the mean values vary from 6,259 to 7,256 N mm^{-2} (see Table 2 and Fig. 4 where the values are given in kN mm^{-2}). In spite of varying data, there is no significant difference between values of the modulus of elasticity of the self-binding board samples.

An excellent result of internal bonding was obtained with regard to the European Standard (see Table 2). In major number of specimens the rupture occurred near the surfaces between the glued specimen and testing block. This means that the real tensile strength of the fibreboard is higher. The obtained internal bonding strength shows the best result of the detected mechanical properties. The data range of the internal bonding strength varies from 0.67 to 1.31 (N mm^{-2}) among the board specimens while the mean values between the board samples have no significant difference (Fig. 4). A powerful internal bonding strength of the hot-pressed steam exploded fibre mass obtained from either the first or the second vessel without adding any synthetic adhesives.

Conclusions

According to the obtained results of the study the following is concluded:

1. The fibre mass steam exploded after treatment at 235 °C and pressure of 3.2 MPa for one minute is not homogeneous.
2. Screening of the exploded fibre mass through 0.16 mm sieve results in a significant increase of fibres less than 0.2 mm long. The range of the length of screened fibres is 512 – 565 μm, the range of width 42 – 47 μm. Content of fines in screened fibres in the first vessel of the SE unit is 63% while in the second – 74%.
3. The self-binding high-density fibreboard hot-pressed of steam exploded fibres from grey alder at 160 °C and and 8 MPa during 10 min and cooled for other 20 min under decreasing pressure, have the density of at least 1.35 g cm^{-3}, the moisture content of 7.2%, swelling in thickness of 8.1%, water absorption of 3.2%, the bending strength of 27 N mm^{-2}, the modulus of elasticity of 6,259 N mm^{-2}, and the internal bonding of 0.92 N mm^{-2}.
4. The fibreboard samples hot-pressed of steam exploded fibre mass of grey alder without addition of any synthetic adhesive have a powerful internal bonding.
5. The grey alder self-binding high-density fibreboard samples, obtained under the mentioned conditions, are comparable with commercial hardboards.
6. There is no significant difference of the tested properties between the board samples made of the steam exploded mass from either the first or the second vessel of the steam explosion unit; further studies are suggested with only one container receiving the exploded fibre mass.

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RESULTS OF BLACK ALDER (*ALNUS GLUTINOSA* (L.) GAERTN.) IMPROVEMENT IN LATVIA

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Abstract. Black alder wood is suitable for production of high quality veneer and other uses, but doe to its small share in total forest area (3%) in Latvia its genetic improvement have not been a priority. Set of progeny tests, together 22.8 ha, have been established only in the last decade. First of them have reached the age of 8 to 10 years and are suitable for first assessment. Aim of our study was to evaluate potential of improvement of black alder using selection, based on progeny testing. Data from 4 open pollinated progeny tests of phenotypically selected black alder plus trees, located in central part of Latvia, each containing from 15 to 21 families, are evaluated, using breeding value as a criterion. Results reveal that selection of parent trees with the practically possible intensity (10%) yields notable improvement in height at the age of 8 to 10 years: from 10 to 32%. Parent trees of open-pollinated families S9, S14, S16 and 84115 that have superior productivity and above-average quality can be recommended for establishment of second-round seed orchard. Estimates of genetic parameter suggest that selection in black alder trials could be carried out with high accuracy and improvements are possible both in productivity and quality (branch thickness, stem straightness, occurrence of spike knots).
Key words: tree breeding, selection differential.

Introduction

Black alder natural distribution areal includes all European territory as well as parts of North Africa and West Siberia. It has been introduced and spread also in North America (Funk, 1992). Black alder distribution is limited by temperature (at least 6 frost-free months) in north and precipitation (at least 500 mm y^{-1}; optimal 800-860 mm y^{-1}) in south (Kajba and Gračan, 2003). Highest density of stands of this species are located in North-eastern part of Germany, North part of Poland, Baltic states, Byelorussia and Ukraine (Priedītis, 1997). In Latvia black alder is a minor species, that occupies 3% of the area (roughly 78 thousand ha) with the total yield 16.4 mil. m^3 (SFS, 2008). The figure is larger than total black alder stock in Sweden: 9.2 mil. m^3 (Johansson, 1999) and Finland 1.3 mil. m^3 (Kärki, 1999).

Black alder wood is relative easy to process and due to its colour is often used in carpentry products. Average wood density is similar to that of Scots pine: for a pair of samples 0.479 g cm^{-3} and 0.464 g cm^{-3} respectively, for other: 0.503 and 0.520 g cm^{-3} respectively (Malkoçoğlu and Özdemir, 2006). Wood density of this species is slightly increasing by age: at the age of 4 years it is 0.45 - 0.48 g cm^{-3}, at the age of 60 years: 0.49 g cm^{-3} (Chow et al., 1999). In other study increase of wood density from 0.421 (at the age of 5 years) to 0.436 g cm^{-3} (at the age of 11 years) was noted (Klevinska and Bikova, 1999). Described values of wood density and colour ensure the possibility to use black alder wood in veneer industry (Aydin and Colakoglu, 2005). It has been found, that wood properties (module of elasticity, density) of *Alnus* is not tightly correlated with radial increment (width of annual rings) suggesting high possibilities of breeding to simultaneously improve growth and wood quality (Aydin and Colakoglu, 2005; Lei et al., 1997). Wood properties, specifically – ash content – indicate that black alder can be efficiently used as energy-wood (Chow et al., 1999; DeWald and Steiner, 1986, Johansson, 1999).

Due to fast juvenile growth alders can be used as pioneer species, forming shelter to introduce oak or spruce in frost prone sites; due to nitrogen-fixing properties alders can be used in re-cultivation of burned forest or former mining areas (Mangalis, 2004).

Due to rather small share of total forest area, occupied by alder, only few studies of genetic properties and improvement of this species have been carried out. Analysis of 48 provenances from different European regions in common garden experiment revealed, that bud-bust happened simultaneously (largest differences between 2 provenances does not exceed 2 weeks, in majority of cases it happened within 4 days), however time of bud set was strongly correlated with latitude of origin. Length of used vegetation period (time from bud burst to bud-set) for different provenances was from 96 to 155 days and was weakly correlated with total length of annual height increment. It indicates, that differences in growth intensity was the major factor determining differences in height growth (DeWald and Steiner, 1986), therefore selection of faster growing individuals would most likely not lead to increase of frequency of frost damages. Provenance experiments of other *Alnus* species reveal similar trends: length of used vegetation period correlates with latitude of origin and shorter length of it is related to higher intensity of photosynthesis (Benowicz et al., 2000).

Notable differences in frequencies of alleles were found between several populations of black alder, applying molecular markers. However, most of the genetic diversity was distributed within population and only small portion – among populations (Gömöry and Paule, 2002), suggesting possibilities for efficient selection of individual trees with desirable traits.

This conclusion have been supported by results from analysis of 17 black alder populations, consisting from separate open-pollinated families (family – group of progenies of the same mother tree), located in trials in 3 Lithuanian regions (Pliūra and Kundrotas, 2002). It was found, that family and family × trial site interaction have significant influence on tree height and time of development of frost hardiness, but population influence was not significant. Tree diameter was significantly influenced both by family and population. High phenotypic plasticity – genetically determined ability to adapt to different environmental conditions – was found for black alder.

Black alder breeding (improvement) was initiated in Latvia in middle of previous century, partly related to interest on superior growth and wood properties of hybrids between 2 alder species: gray and black alder (Kundziņš and Pīrāgs, 1963). Trees with high productivity and desirable branch and stem quality (phenotype), so called plus-trees, were selected within productive and qualitative stands. In total 70 trees were grafted into seed orchard that supplied (and still supplies) seeds for nurseries. Next period of activities was during last two decades: new set of plus trees were selected and 2 seed orchards established: in 1999 (3.2 ha 53 clones) and in 2003 (4.1 ha 70 clones). In the period between years 2000 and 2008 in total 17 progeny tests, including open pollinated families of more than 200 trees were established with total area 22.8 ha. Oldest of them now have reached the time of first evaluation.

Aim of the study is to evaluate potential of improvement of black alder using selection, based of progeny tests of individual trees.

Materials and Methods

Study material consists of 4 trials, referred by the respective numbers in 'Register of long term scientific experiments' (Baumanis et al., 2006). Trials are established in former arable and fertile, drained forest land (Table 1).

Seed material for experiments No. 71, 72 and 590 has been collected from phenotypically selected plus trees in year 1997 in Jaundziras (Tukuma county, Irlava forest district according to administrative division at seed collection time) and S – Sunākste (Aizkraukles county, Seces forest district).

Height has been measured for every tree in year 2006. Quality was visually assessed in 5 grade scale (experiments No. 72 and 590) or 3 grade scale (experiments No. 71 and 270) in comparison to trees with similar height at the same trial, where 1 – straight stem (sg), thin branches (bg), high overall quality (g), jointly considering previously mentioned traits and spike knots, branch angle, vitality; 5 – stem with more than 2 bends (larger than 5 cm), very thick branches, poor overall quality. Spike knot, double stems and lost tops have been noted in binary scale (yes/no).

Some of the trees have been damaged during early years of growth and regenerating by stump sprouts, forming bush-like structure with 3 and more stems. Notable differences among families in proportions of such trees have not been found, indicating environmental cause of this phenomenon. Therefore such trees have been excluded from further analysis.

Selection differential was calculated as deviation of family mean value form mean of experiment in respective traits. Since selections are made for establishment of seed orchards, in those selected clones contribute to genetic value of seedlings both as mother and father trees, breeding values were calculated as selection differential 2^{-1}.

Probability, that progenies of certain parent tree will have spike knots were estimated based on methodology derived from Roff (2001).

Variance components have been calculated with SAS Proc mixed, using general linear model:

$$y_{irs} = \mu + F_i + B_r + FB(S)_{ir} + FS_{is} + E_{irsk}, \quad (1)$$

where: y_{ir} – trait y measured; μ – overall mean; F_i – the effect of family; B_r – the effect of block; $FB(S)_{ir}$ – interaction among block (within site) and family (plot effect); FS_{is} – interaction between family and site; E_{irk} – the error term. For calculations of single-site estimates site factor is not included.

Open pollinated families were assumed to consist mostly of half-sibs, consequently $4\sigma^2_F$ were considered to be an estimate of the additive genetic variance (σ^2_A).

Table 1

Description of black alder progeny trials used in the study

Trial No.	Trial Locality (county)	Age	Land type	Spacing	Number of families	Number of replications	Survival, %
72	Rembate	10	arable	2×2	21	34*	77
590	Taurene	10	arable	2×2.5	19	28	68
270	Cenu	8	forest	2×2.5	15	40*	86
71	Ukri	10	arable	2×2.5	16	35	86

*average number of replications per family, particular family can be represented in 4 replications more or less than average

Narrow sense heritability (h^2_i) and its standard error as well as half-sib family mean heritability (h^2_{fam}) and its standard error were estimated as described by Falconer and Mackay (1996).

Coefficients of additive genetic variation (cv_a) in percentage were estimated as:

$$cv_a = \frac{100 \cdot \sqrt{4 \cdot \sigma^2_F}}{\mu}, \quad (2)$$

where μ – overall mean; σ^2_F - family variance.

Coefficients of phenotypic variation among family means ($cv_{p\,fam}$) in percentage were estimated as:

$$cv_{pfam} = \frac{100 \cdot \sqrt{\sigma^2_F + \frac{\sigma^2_{FB(S)}}{bs} + \frac{\sigma^2_{FS}}{s} + \frac{\sigma^2_E}{bns}}}{\mu}, \quad (3)$$

where: σ^2_F – family variance; $\sigma^2_{FB(S)}$ – family-block (within site) interaction variance; σ^2_{FS} - family-site interaction variance; σ^2_E – error variance.

Results and Discussion

Breeding values of families in experiments No. 72 and 590 are calculated based on data from trees that are not suppressed: 24 and 25 highest trees per family respectively are used. Notable differences in number of suppressed trees per family have not been found, indicating, that this selection will not bias the results of analysis.

Highest breeding values for trait, characterising productivity of trees – height – in experiment No. 72 have been found for families S15, S5, S8, in experiment 590 – for families J4, J7, S14. Selection of 2 best families that corresponds to intensity 10% (2 out of 21 or 2 out of 19) would guarantee 14% or 10% (respectively) superiority in tree height in comparison to trial average. Part of the difference among the trials could be explained by fact, that mean height of trees in experiment No. 590 exceeds that in experiment No. 72 by 2 m environmental factors

Trial average represents value of progenies of already phenotypically selected plus trees, therefore, if comparison would be made with un-selected material (corresponding to average result in natural regeneration), value of improvement would have been higher. This logical relationship has been proven to be true in assessments of trials of other species (Ståhl and Jansson, 2002). None of the analysed black alder trials contains control-lots of unimproved material, therefore exact calculation of increase in quality or productivity is not possible.

Selection intensity for establishment of second-round (with progeny-tested clones) seed orchard depends on:

1) number of clones needed in seed orchard to ensure genetic diversity: for other wind-pollinated species it is estimated to be 16-25 (Lindgren and Prescher, 2005);

2) number of trees with progeny tests available.

Black alder progeny tests contains slightly more than 200 families, therefore selection intensity 10%, choosing 20 clones for establishment of seed orchard, can be used in praxis and is applied as a criteria for analysis of trials No. 71, 72, 270 and 590.

Establishment of plantation is a costly operation in comparison to natural regeneration. It is economically justified only with higher value of assortments from planted stand in future. Therefore in tree breeding process not only productivity, but also quality needs to be considered.

Notable differences in breeding value for branch quality (bg) have been found in experiment No. 72: it varies from -0.8 to +0.6, average quality grade being 2.6. Variation in breeding values for this trait is lower in experiment No. 590 (from -0.3 to +0.3). Slightly lower variation have been detected for breeding values for stem straightness (from -0.4 to +0.6), slightly higher – for joint quality grade (from -0.7 to +0.7). Variation according to all quality traits is larger in experiment No. 72 indicating possibilities to better detect and exclude inferior families. According to the result in these trial families with highest trees have at least average quality, and only two of them (S15 and S13) needs to be excluded due to poor quality. Especially low quality in both experiments has been found for family J5.

Spine knot or double leaders have been found in 40% of all trees in experiment No. 72 and in 74% in experiment No. 590. Highest possibilities to detect genetically determined differences in binary (yes/no; 1/0) coded traits are in the case, if frequency of trees with the trait (in this case – spike knot) are close to 50%. It is confirmed by the analysis: in experiment No. 72 families S3 and S5 have highest probabilities, that trees will be with spike knots (0.8 and 0.7 respectively); in experiment No. 590 none of the probability estimates exceeds 0.6. Spike knot lowers the value of assortment due to its larger cross-cut area and longer life span than branches and sometimes also causes stem defects. Besides it can indicate lower adaptability to some environmental factors. Therefore trees with high probability that there progenies will have spike knots, cannot be recommended for seed orchards.

Joint analysis of 18 families, represented in both trials (No. 72 and 590) has been carried out, based on data from dominant (10 highest per family in each experiment) trees (Table 2).

Table 2

Breeding values in open pollinated progeny tests of black alder (No.72, 590) at the age of 10 years

Family	Trait				
	h	sg	bg	g	sp
J 2	-7.2	0.1	-0.4	0.3	0.5
J 3	3.5	-0.4	0.0	-0.2	0.5
J 4	2.7	-0.2	-0.4	-0.1	0.5
J 5	-1.8	1.4	0.1	0.7	0.5
J 6	-2.9	-0.5	-0.5	-0.1	0.5
J 7	-6.9	0.0	-0.1	0.1	0.4
S 3	-2.9	0.3	0.0	0.2	0.6
S 5	-2.3	0.2	0.8	0.6	0.6
S 7	0.8	-0.2	0.0	-0.4	0.5
S 8	0.6	0.0	-0.6	-0.4	0.4
S 9	4.3	-0.4	0.2	-0.2	0.5
S 10	-1.6	-0.3	-0.2	-0.2	0.5
S 13	1.6	-0.2	0.4	0.2	0.5
S 14	3.2	-0.2	0.0	-0.3	0.5
S 15	4.6	0.5	0.3	0.2	0.5
S 16	4.2	-0.3	0.1	-0.4	0.5
S 17	-3.5	-0.2	0.4	0.1	0.5
S 33	3.8	0.5	0.0	0.0	0.5
Average:	66.8	1.9	3.0	3.0	0.5

Note: h – height, dm; sg – stem straightness, grades; bg – branch thickness, grades; g – joint quality estimate, grades; sp – probability to have trees with spike knots; average – average values of traits in both experiments jointly.

Joint analysis of data from both experiments confirmed the results listed earlier. Families J3, S9, S14, S16 have high productivity and slightly increased quality and there parent-trees can be recommended for establishment of second-round seed orchard. Families J2, J5, J7, S17 have both low productivity and quality and could not be recommended for further breeding or propagation work.

Experiment No. 71 includes the same set of families as No. 72, but in this trial notably more trees have stem damages: spike knot or sprout from stump was found for 46% of trees, 2 stems or lost leader – for 43% of trees. Observed damages might partly be related to errors in early tending work, when some of the planted trees were cut. It can also be a consequence of substantial frost and/or browsing damages at the first years after establishment of experiment, reflecting genetic differences in some adaptive traits. Later statement is supported by estimates of probability, that tree will have a double stems or spike knot: for particular families it varies from 0.3 to 0.6.

Mean height of trees in the experiment is 4.5 m that is almost by 1m lower than in experiment No. 72. Breeding value of this trait for the best family is 9% compare to trial average. Notable differences have been found in branch quality (±16%), stem straightness (-12 – +27%) and joint quality estimate (-9 – +20%). According to all analysed traits highest quality has been found for families S7 and S9, lowest for families J5 and J7.

Based on data from experiments No. 71, 72 and 590, parent-trees of families S9, S14 and S16 can be recommended for establishment of seed orchard.

Seed production in total in 27 trees from 13 families has been assessed. It indicates, that controlled crossing for recombination of genes from selected trees to obtain seed material for establishment of next breeding cycle is possible at rather early age – just 10 years.

Experiment No. 270 includes different set of families than analysed previously and is located on drained, fertile forest land. Very low number of trees with spike knots (3%) indicates no adaptation problems. However, number of trees with double stems is relative high for such conditions (20%), pointing towards possible mechanical damages during the early cleaning work.

Height of dominant trees (20 highest per family), used for detailed analysis, reaches 4.5 m on average. Selection differential for best family is as high as 32% from the mean in the experiment. If all trees are included in analysis, selection differential reaches 23%. Survival (94%), stem and branch quality for this family (84115) exceeds the average in experiment, indicating a notable potential in use of it in forest regeneration. Differences in quality traits among the families are similar to those, reported in experiment No. 71: for stem quality ±12%, for branch quality ±15%.

Heritability of tree height, based on the data from all trees, is rather low in experiments No. 270: h^2_i=0.13. It is higher in experiments No. 72 and 590, where selection of 25 highest trees per family has been used: h^2_i=0.39. However, both estimates are within the range of values, published for other wind-pollinated species (Haapanen et al., 1997; Stener and Jansson, 2005; Jansons et al., 2008) are more than twice higher than respective standard errors and indicate, that accurate selection can be made in particular black alder experiments. Heritability of quality traits is slightly lower than for height in experiments No. 72 and 590: on average h^2_i=0.21.

Tree height, representing growth traits have higher narrow-sense heritability (h^2_i) estimated across sites than branch traits (Table 3). This trend have been found also in earlier studies both if in single experiments and joint analysis of several experiments (Velling and Tigerstedt, 1984; Merrill and Mohn, 1985; Haapanen et al., 1997). As noticed in several studies, single site heritability values can be up to twice as large as across site estimates, indicating possibilities of serious bias in genetic gain estimates based on results from one test site. High narrow-sense heritability for across-site analysis, especially for tree height, in our study can be related to selection of just 10 highest individuals per family for this analysis.

Table 3

Values of genetic parameters in black alder open-pollinated progeny tests (No.72 and No.590) at the age of 10 years

Trait	Genetic parameter					
	h^2_i	se_i	h^2_f	se_f	cv_a	cv_{pfam}
h	0.63	0.28	0.79	0.35	7.4	4.2
sb	0.48	0.23	0.73	0.35	26.1	15.2
zb	0.24	0.15	0.56	0.35	15.1	10.1
b	0.25	0.16	0.57	0.36	16.6	11.1

Note: h^2_i and se_i – narrow-sense haritability and its standard error; h^2_f un se_f – family haritability and its standard error; cv_a – coefficient of additive genetic variation; cv_{pfam} – coefficient of family mean phenotypic variation; other abbreviations as in Table 2.

Estimates of coefficients of variation indicate a considerable potential of improvement, especially for quality traits, both using selection of best parent-trees (among-families) and selection of best individuals within family.

Experiments, included in our analysis, have reached the age and height, recommended for selection in broadleaved tree species in southern part of Sweden (Stener and Jansson, 2005). Therefore the results of our study can be considered as first selections for second-round seed orchard of black alder in Latvia. Since seeds from such orchard could be used in all territory of Latvia (only one seed zone for black alder is delineated), results must be based on careful testing in different regions and selections, based on results of at least 3 experiments (like No. 71, 72, 590) considered.

Conclusions

1. Selection of parent trees with the practically possible intensity (10%) yields notable improvement in height at the age of 8 to 10 years: from 10 to 32%.
2. Parent trees of open-pollinated families S9, S14, S16 and 84115 that have superior productivity and above-average quality can be recommended for establishment of second-round seed orchard.
3. Estimates of genetic parameter suggests, that selection in black alder trials could be carried out with high accuracy and improvements are possible both in productivity and quality (branch thickness, stem straightness, occurrence of spike knots).

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PROFESSIONALS' ATTITUDE TO NATURALISTIC FOREST LANDSCAPE IN URBAN AREA. RIGA CASE

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Abstract. The urban forest is recognized as a potential to improve the quality of life of urban dwellers and increase the sustainability and ecological stability of the city. However, there is little data on professional attitude to urban naturalistic landscapes. This research studies the attitude of professionals and decision makers to the naturalistic forest landscapes in urban area of Riga city, Latvia, in contrast to more traditional – formal landscape. The survey includes the opinions of territorial planners and environment specialists from Riga municipality and other institutions related to ecological, practical, planning and conservation activities, and private working landscape architects. The statistical analysis and data's empirical distribution showed that professionals in Riga city recognize the values and benefits of naturalistic forest landscape. However, environmental preferences may depend more on affective reactions than on ecologically-based logical operations.
Key words: Naturalistic forest landscape, formal landscape, landscape perception, urban forest, Riga city.

Introduction

During last 30 years there has been a rapid change in attitudes towards nature in the urban environment which has led to an increased interest in and greater appreciation of the value of nature in cities (Özgüner and Kendle, 2006). Chiesura (2004), Prőbstl et al. (2008), Jorgensen et al. (2002) and Sanesi and Chiarello (2006) recognize that the social aspect of city life and importance of the social function of forests – for recreation and nature tourism – is becoming increasing as parameters of sustainable development.

Researches in environmental psychology present that humans emotionally relate to natural elements in positive ways, and people prefer landscapes that they perceive as natural (Yannick et al., 2010). One of the major reasons why people go to the wilder, more natural areas is to escape the daily life of the city (Bell, 2008). However, the numerous studies show that although great majority of people enjoy the diversity in appearance of naturalistic settings, they also want to see evidence of care (Özgüner and Kendle, 2006).

The development of urban forest in Riga city has a long history, and today Riga city is one of the biggest owners of forests in Europe - in inner city there is 5676 ha of woodland. The greatest part of Riga urban forest belongs to the municipality and is accessible for city dwellers. Riga city forests consist of 11 wide massives which are connected with rural forests and some small, isolated forests – the remnants of ancient forest or planted forests (Emsis, 1980). The main tree species is pine (~88.1% in 2008) on the poor sandy soil and is characterized by low tolerance to recreational pressure (Эмсис,1989).

According to Latvia legislation related to management, maintenance and spatial characteristic of urban forest, the timber production and clear – cutting are not allowed and main management activities are applied to the pattern elements of forest and technical aspects of forest management. The difference between maintenance and management of rural and urban forest is not distinguished and there are disagreements between management, functional significance and demands for real use of urban forests. The dynamic of land use and management of green space in Riga city are based on the numerous documents and normatives of Riga city municipality. However, these documents often are in contradiction with legislation and are incomplete.

Lack of sufficient finance, the restrictions of laws and documents about urban forests, as well as lack of investigations about the recreational capacity and demands of inhabitants for recreational use of urban forests in Riga city lead to poor recreational opportunities for inhabitants. The role of the local authorities has been limited in elementary rehabilitation actions and poor maintenance concepts. As a result, the urban forests mostly are perceived as unsafe, inaccessible and consequently have low levels of use (Jorgensen et al., 2002, Wilkinson, 2008). Tyrväinen et al. (2008) argue that in generally the deficiencies of supply of basic infrastructure in urban forests are noted by the new European member states.

The literature review suggests that there are many opportunities for the use of naturalistic landscape design and many benefits to be derived. Still there are little data on professional attitudes to urban naturalistic landscapes available. The research strategy used for the present study is based on the idea that professionals are able to recognize most of the values attached to 'naturalistic forest landscape' (hereafter also NFL) in urban area. The aim of this study is to clarify and measure attitudes towards NFL in urban area in Riga city, thus, highlighting the potential advantages and problems associated with the concept. The research also discerns whether a difference in attitudes exists between some professional groups, and it aims to clarify the style they prefer in their designs.

Materials and Methods

In order to obtain a representative sample of professionals, a stratified sampling method was used in this survey. Considering respondents' involvement

with the design and management of urban landscapes, the territorial planners, specialists of environment, landscape architects and urban forest managers in Riga were identified as sampling units of survey populations. The respondents were chosen in Riga municipality, Agency of Riga Forest, Ministry of Agriculture, Ministry of Environment, Latvia Nature Foundation, State Forest Service and private working landscape architects. The total number of respondents is 63. For realizing of quantitative data all respondents were divided in 3 groups – territorial planners (24 respondents), landscape architects (19 respondents) and managers (20 respondents). The individual questionnaires were conducted in the period 25/01/2010 – 22/02/2010.

To investigate the professionals' attitude to naturalistic forest landscapes, the questionnaire containing 20 statements was prepared. The questionnaire was designed based on the research of Özgűner et al. (2007) about the attitudes of landscape professionals to naturalistic landscapes and formal landscapes of urban areas in the United Kingdom. Contrary to the research in the UK, in the present study the subject of investigation - 'naturalistic landscape' was reduced to 'naturalistic forest landscape' and some statements were replaced.

For better understanding, at the beginning of the questionnaire definitions of FL and NFL and the corresponding pictures were included. Following the definitions, 20 statements about the values and possible benefits attached to NFL in urban areas were made. The valuation scale of 3-points (1- strongly agree, 2 – partly-agree, 3 - disagree) was used for assessing these statements. Direct verbal interaction allowed the interviewer to target specific data collection, to measure the perception of the respondents and to elicit motives behind the answers.

For a successful analysis of statistical data, 3 groups of respondents were joined in two groups (P, planners – 43 respondents; M, managers – 20 respondents). The Statistical Package for Social Science (SPSS V.10) and Microsoft Excel 2007 were used to analyze the collected data. A series of Chi – square (χ^2) tests were applied to determine differences at a 0.1 – 0.01 significance level on attitude statement variables in order to find out the possible differences or similarities between the answers of different groups of respondents. Cronbach's alpha (α) test, used in this survey, revealed that the total scale reliability was high (α = 0.78) for the attitude measurement framework examined, which indicates that the statements were reliable and performed well in capturing the measured construct. The table and charts were used to present descriptive results of Chi – square (χ^2) test (Table 1) and empirical distribution of data (Figure 1, Figure 2).

Results and Discussions

The results of data empirical distribution and statistical analysis on the perception of professionals on the value and benefits of NFL show that most of the statements are generally agreed with by the majority of professionals (Table 1, Figure 1, Figure 2).

Majority of respondents (62%) agreed and 23.8% partly-agreed with the first statement. One of the respondents, for instance, stated that '*Formal landscape is created for a short – term rest, and it should be close to residential areas, but naturalistic forest landscape must be in rural areas as a place for a long – term rest on holidays*' (P-12). The majority of respondents from both groups (70%) agreed with the formulation of the second statement although 10% disagreed, and as one respondent answered: '*Both kinds of landscapes have appropriate elements for environmental education*' (M-10). 80% of respondents confirmed the statement about natural renewal. Surprisingly low agreement in both groups of the respondents (52%) was about the statement on sustainable development strategy with a NFL. The answers show a high rate of partial-agreement – 35%. As one respondent answered, *'The principles of sustainable development in FL are possible to develop successfully too. The FL is not just flowerbeds on the concrete'* (M-14). The analysis showed that 64% of the respondents from both groups agreed with the statement No. 6. To the next statement about community involvement, there are not a common consensus between professionals – strongly agree - 55%, disagree – 17% and partly – agree - 28% of respondents. The answers on the next statement about seasonal changes show - 47% of agreement and 27% of partly-agreement. As one respondent from the managers' group thinks '*An effect of seasonality is more evident in FL by using different flowers and plants*' (M-19). Quite low agreement (50%) is shown by the answers to the next statement about maintenance of NFL. In the answers there was often the following opinion '*NFL occupies wider areas as FL and its maintenance needs special knowledges'* (M-16). The minority of the respondents (28%) agreed that NFL offers a more positive experience than FL. 33% of the respondents partly-agreed with the statement, because '*FL is created with the main target to get positive experience*' (P-16). A similar trend can be seen in the answers about vandalism in NFL. 43% of respondents disagree, because '*In NFL there is a lack of recreational facilities, flowerbeds and other artificial elements and there are less opportunities for vandalism*' (P-23).

Table 1

Results of contingency tables analysis

No.	Statement*	Quality	χ^2	Significance
	Values			
1.	In urban landscape there is a place for naturalistic design	Place	0.182	0.913
2.	NFL are more beneficial to wildlife than FL	Wildlife	2.361	0.307
3.	NFL are more suitable for environmental education than FL	Education	1.601	0.449
4.	Naturalistic style embodies natural renewal more strongly than can be achieved by a FL	Renewal	10.626	0.004***
5.	In most cases, it is easier to formulate a sustainable development strategy with a NFL	Sustainability	4.842	0.088*
6.	In most cases, it is cheaper to manage a NFL than a traditional FL	Costs	4.907	0.085*
7.	It is easier to gain direct participation from the local community in the formation of a NFL	Community	5.127	0.077*
8.	Seasonal changes are more pronounced in NFL than FL	Season	3.689	0.158
9.	In most cases, it is easier to maintain a NFL than a FL	Maintenance	3.219	0.199
10.	NFL offer a more positive experience than FL	Experience	0.007	0.996
11.	NFL are more prone to vandalism than FL	Vandalism	0.173	0.917
	Benefits			
12.	NFL allow people to have more contact with nature than do FL	Contact	7.436	0.024**
13.	NFL allow people to more easily observe how nature works than do FL	Process	0.460	0.794
14.	NFL allow a greater expression of feeling of freedom especially for teenagers	Teenagers	0.659	0.719
15.	NFL are more calming than are FL	Calming	4.439	0.108
16.	NFL allow greater freedom of thought than do FL	Freedom	7.288	0.026**
17.	NFL are more exciting/interesting than are FL	Exciting	1.649	0.438
18.	The general public can easily differentiate between FL and NFL	Differentiate	3.193	0.202
19.	The public often prefer a NFL to a FL	Preference	3.327	0.189
20.	A being in NFL is not safe than are in FL	Safety	0.451	0.798

Designations: * - $p < 0.1$; ** - $p < 0.05$; *** - $p < 0.01$
* - see text for details

The following part of the questionnaire about benefits of NFL included 9 statements. The majority of the respondents (88%) recognized that NFL allows people to have more contact with nature than FL, although, one respondent disagreed: '*The population mostly is not ready for the contact with NFL in Latvia*' (M-20). Very high agreement – 90% of respondents was shown whilst analysing the next statement about natural processes in NFL. The vast majority of the respondents – 82% agreed that NFL allows a greater expression of feeling of freedom especially for teenagers. Only 50% of the respondents in both groups agreed and 40% of respondents' partly – agreed to the statement that NFL provides more serenity than FL. The research of the respondents' comments on the statement revealed that professionals think that it depends on the individual preferences, and the perception and desires of people are very different. Quite similar situation is shown by the answers to the next statement about freedom of thought in NFL – agreed with the statement 54% and partly – agreed – 32%. As answered one respondent '*Although in NFL nothing disturbs the freedom of thoughts; in FL there are more possibilities to release imagination'* (P-22). A substantial difference in answers appears to the next statement that NFL is more exciting/interesting than FL. With the statement agreed – 30%, partly – agreed – 46% and disagreed – 22%. Majority of the respondents - 87% agreed that the general public can easily differentiate between FL and NFL. A low level of agreement (41%) is shown by the answers to the next statement about preference. The analysis of the last statement that being in NFL is safer shows the equal balance between agreement and partly – agreement (together – 48%) and disagreement (52%). One person argued: '*If people have special knowledge about natural processes, it is safe to be in NFL'* (P-24).

Through the series of statistical analysis it became clear that there is no big difference between opinions of two target groups. The greatest significance ($p<0.01$) shows the answer to the statement about natural renewal, the significance $p<0.05$ shows the answers to the statement about freedom of thoughts and about contact with nature. Less-essential significance ($p<0.1$) shows the opinions of representatives in both groups about sustainable development strategy, about costs to manage a NFL and direct participation from the local community (Table 1).

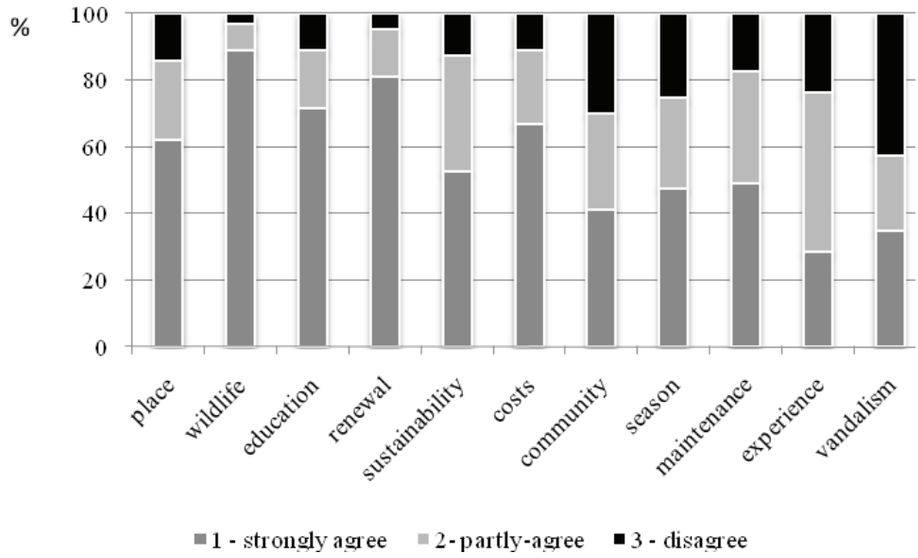

Figure 1. Professionals' perceptions on values of urban naturalistic forest landscapes.

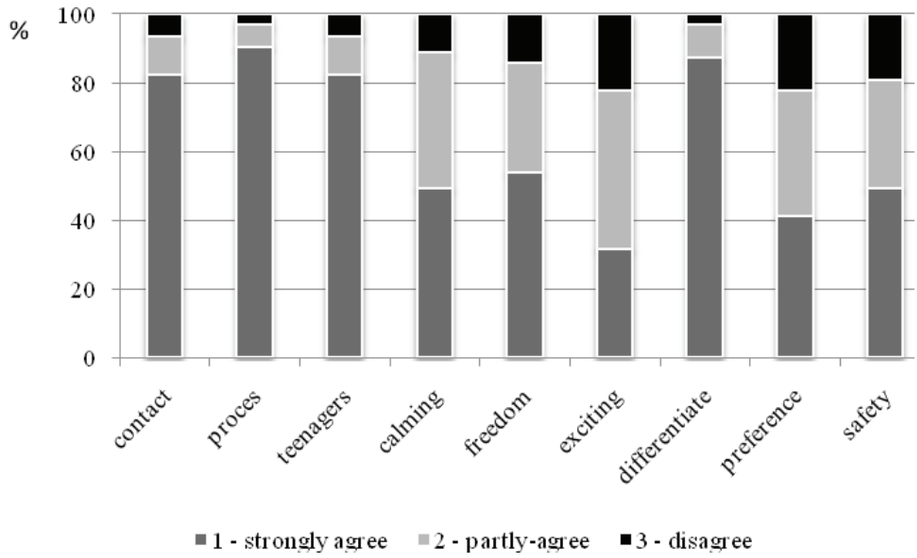

Figure 2. Professionals' perceptions on benefits of urban naturalistic forest landscapes.

The results of the research showed that professionals from groups of managers, planners and landscape architects in Riga city recognize the values and benefits of NFL and confirm supporting the common expectation, obvious in the literature review (Özgüner et al., 2007). This approach suggests that preferences should be strongly influenced by ecological knowledge (Gobster, 1999).

The results of the analysis of the data' empirical distribution show that to the statements which are connected with psychological perception (about experience, serenity, feelings of freedom and excitement, preferences and feelings of safety), the answers show very small differences between 'strongly agree', partly - agree' and 'disagree' (Figure 1). The research has confirmed that landscape evaluation has a close link to important emotion-related psycho-physiological responses, and environmental preferences may depend more on affective reactions than on any knowledge-based logical operations (Tyrväinen et al., 2003).

To the open-ended question for landscape architects about the style they prefer in their designs, the common opinion was that design of naturalistic landscape is not actual in Riga. The comments showed that landscape architects mostly work in a formal style. Obviously the design urban green space in Riga, as in other European countries, mostly is influenced by the 18th century English landscape movement, and a Victorian view of nature as something that has to be

contained, conquered and controlled (Bryant, 2006; Jorgensen et al., 2002).

Conclusion

This research examines professional attitudes to naturalistic forest landscape in Riga city, in contrast to more traditional, formal landscape. The results of the research show that:

1. professionals from both target groups – managers and planners in Riga city recognize the values and benefits of naturalistic forest landscape;
2. there exists a significant difference in attitudes between professional groups of managers and planners to the statements about natural renewal, freedom of thoughts, contact with nature, sustainable development strategy, costs to manage a NFL and direct participation from the local community;
3. there is no significant difference in gradation of answers to the questions about psychological perception of NFL (for instance, about feelings of freedom and excitement, preferences and feelings of safety, etc.);
4. in design of landscape in Riga city the formal style dominantes over the naturalistic style and there is a need for some time to change in perception that would be supported by political decisions and educational programs.

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