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Biotechnical faculty

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CONTENT

80TH ANNIVERSARY OF THE FOUNDING AND FUNCTIONING OF THE BIOTECHNICAL FACULTY, UNIVERSITY OF MONTENEGRO

Scientific and research work in the field of agriculture of Montenegro began in 1937, when a state trial station for southern crops was founded in Bar, by a decision of the Ministry of Agriculture of the Kingdom of Yugoslavia. After the World War II, new institutions were established and former ones reconstructed: the Institute for Agricultural Research in Titograd (1945), the Institute for Livestock Farming in Nikšić and the Institute for Southern Crops and Viticulture in Bar (1947), the Soil Research Station in Bar (1949), the Diagnostic-Veterinary Station in Titograd (1950) and the Fruit Growing Station in Bijelo Polje (1952). Agricultural Institute was founded in 1961 by pooling the abovementioned institutions. The Institute operated under that name by 1997, when, with the inclusion of the forestry sector into a single scientific research institute, it grew into the Biotechnical Institute. Following the launch of agricultural studies for the schoolyear 2005/2006, the Institute was transformed into a faculty, and in 2007 it officially changed its name to the Biotechnical Faculty.

The Biotechnical Faculty, as the oldest scientific and research institution in Montenegro, has achieved outstanding professional and scientific results over the past 80 years and has been the initiator as well as one of the main driving forces of Montenegro's development in all agricultural branches.

Thus far, continuous efforts have been invested into establishing of a sound infrastructure for scientific and technical activities and after the launch of the study program agriculture, special attention has been given to teaching activities.

Having in mind that in the school year 2017/2018 we enrolled the first generation of students in line with the reformed program (3 + 2 + 3), we will certainly continue working on modernization and expansion of the existing premises as well as on improvement of laboratory and other equipment. During practical classes, special emphasis is given to opportunities to apply in practice the knowledge gained at our Faculty.

The Biotechnical Faculty participated in implementation of numerous projects funded by the Ministry of Agriculture and the Ministry of Science of Montenegro as well as by international projects: FP7, Tempus, Erasmus, COST, IPA, HERIC and bilateral projects with faculties from different countries - Austria, Italy, Norway, Russia, China, Croatia, Bosnia and Herzegovina, Serbia and others. The Biotechnical Faculty has had outstanding cooperation with renowned international scientific and research institutions that it is recognized of not only at the University of Montenegro, but even further.

On the occasion of 80th anniversary of the founding and functioning of the Biotechnical Faculty, in this foreword for the new issue of the magazine "Agriculture and Forestry", we wanted to highlight the significance and the role of the Faculty in educational, scientific and expert national public.

Dean of the Biotechnical Faculty

Miomir Jovanović, PhD

80 GODINA OD OSNIVANJA I RADA BIOTEHNIČKOG FAKULTETA UNIVERZITETA CRNE GORE

Naučnoistraživački rad u poljoprivredi Crne Gore utemeljen je 1937. godine, kada je u Baru, odlukom Ministarstva poljoprivrede Kraljevine Jugoslavije, osnovana Državna ogledna stanica za južne kulture. Nakon II svjetskog rata uslijedilo je formiranje novih institucija i obnova ranije postojećih: Zavoda za poljoprivredno istraživanje u Titogradu (1945), Zavoda za stočarstvo u Nikšiću i Zavoda za južne kulture i vinogradarstvo u Baru (1947), Stanica za ispitivanja zemljišta u Baru (1949), Dijagnostičko-veterinarska stanica u Titogradu (1950) i stanica za voćarstvo u Bijelom Polju (1952). Objedinjavanjem pomenutih institucija 1961. godine osnovan je Poljoprivredni institut. Pod tim nazivom funkcionisao je do 1997.godine, kada uključivanjem sektora za šumarstvo u jedinstvenu naučnoistraživačku instituciju prerasta u Biotehnički institut. Pokretanjem studija poljoprivrede, školske 2005/2006. godine, institut prerasta u fakultet, a 2007. godine zvanično mijenja ime u Biotehnički fakultet.

Biotehnički fakultet kao najstarija naučnoistraživačka institucija u Crnoj Gori, u proteklih 80 godina, ostvario je izuzetna stručna i naučna dostignuća i bio inicijator i jedan od glavnih nosioca razvoja Crne Gore u u svim granama poljoprivrede.

U dosadašnjem periodu, permanentno se radilo na stvaranju kvalitetne infrastrukture za bavljenje naučnoistraživačkim i stručnim radom, da bi nakon osnivanja studijskog programa »poljoprivreda« posebnu pažnju posvetili izvođenju nastave.

Imajući u vidu da smo u studijskoj 2017/2018. godini upisali prvu godinu studenata prema reformisanim programima (3+2+3), svakako da ćemo i dalje raditi na osavremenjavanju i proširivanju postojećih prostornih kapaciteta i unapređenju laboratorijske i druge opreme. Tokom praktične nastave, poseban naglasak se stavlja na mogućnosti primjene znanja stečenih na našem Fakultetu u praksi.

Biotehnički fakultet učestvovao je u realizacji brojnih projekata finansiranih od strane Ministarstva poljoprivrede i Ministarstva nauke Crne Gore, kao i međunarodnih projekata: FP7, Tempus, Erasmus, COST, IPA, HERIC i bilateralnih projekata sa fakultetima iz različitih zemalja - Austrije, Italije, Njemačke, Norveške, Rusjie, Kine, Hrvatske, Bosne i Hercegovine, Srbije i dr. Biotehnički fakultet ima izvanrednu saradnju sa renomiranim međunarodnim naučno istraživačkim institucijama, po kojima je prepoznatljiv ne samo Univerzitetu Crne Gore već i šire.

Povodom jubileja 80 godina od osnivanja i rada Biotehničkog fakulteta, željeli smo da i kroz ovaj predgovor novom broju časopisa “Poljoprivreda i šumarstvo” ukažemo na značaj i ulogu Fakulteta u edukativnoj, naučnoj i stručnoj domaćoj i inostranoj javnosti.

Dekan

Dr Miomir Jovanović

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Velibor SPALEVIC, Dragan RADANOVIC, Goran SKATARIC, Paolo BILLI, Goran BAROVIC, Milic CUROVIC, Paul SESTRAS and Abdulvahed KHALEDI DARVISHAN[1]

ECOLOGICAL-ECONOMIC (ECO-ECO) MODELLING IN THE MOUNTAINOUS RIVER BASINS: IMPACT OF LAND COVER CHANGES ON SOIL EROSION

SUMMARY

This research presents recently introduced approach by authors of the Ecological-Economic (Eco-Eco) modelling using the Intensity of Erosion and Outflow (IntErO) model for the assessment of soil erosion intensity and the impacts of different land covers on sediment yield. The Eco-Eco modelling approach has been applied in one of the sub-basins of the River Lim in the Northeast Montenegro: Bijeli Potok River Basin (2.9 km^2). Land use scenarios were simulated in the model aiming to define the optimal scenario of agricultural production. The Ecological (Eco-) modeling demonstrated that the real soil loss under current conditions is 307.16 m^3yr^{-1} (105.91 per km^2). If agricultural production is intensified, introducing the seed potato production on 4.4 ha of the studied river basin, the model calculated a soil loss of 312.96 m^3yr^{-1} as sediment yield (107.91 per km^2). The negative effects (caused with the intensification of agricultural production) were decreased using the measure of afforestation balancing with the identical surface of 3 ha (from meadows to forests). The model calculated that afforestation would result in a decrease of sediment yield to 306.46 m^3yr^{-1} (105.67 per km^2). The Economic (-Eco) modeling revealed that the investment (total cost) of €3,385 per hectare is needed for the establishment of the intensive agricultural – seed potato production. Market value of the final product is calculated to be €8,500 per hectare annually (yield of 17 tons per hectare); Profit €5,115; Net profit: €4,558. As an integral part of Eco-Eco modelling, the investment for the protection of the area with afforestation (implemented through the Government measures).

Farmers should strive to get economic benefits, while respecting at the same time sustainable ecological and in this specific case: river basin - watershed

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Key note paper, presented at the 8th International Agriculture Symposium "AGROSYM 2017", Jahorina, 5-8 October 2017, Bosnia and Herzegovina.

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management. The research results show that the application of the Eco-Eco modelling, by using the IntErO model for studying the effect of soil erosion and possible land use for intensive seed potato production in the selected River Basin provides cost effective solutions for the benefit of the local population.

Keywords: Eco-Eco modelling, IntErO model, Soil erosion, Bijeli Potok River basin, Land cover.

INTRODUCTION

Soil erosion is a geomorphic process that detaches soil particles, rock fragments, soil aggregates and organic matter from its primary location and then transports these to another location by various processes (Poesen, 2017). Soil erosion causes land degradation, water management and hydro-technical problems (Gholami *et al.*, 2014). Mountainous watersheds are affected by natural disasters, above all erosion problems, but also floods, overflows, inundations, landslides and pollution (Tazioli *et al.*, 2015). Soil erosion and sediment yield studies are of great interest in the Mountainous areas, because of their effects on soil thickness and fertility, plant cover, runoff coefficient and flood risk. The problem of soil loss and land degradation, with their huge impact on the environment is a key point for agriculture, ecology, hydrology and hydrogeology studies (Khaledi Darvishan *et al.*, 2017).

Study of soil erosion and sediment yield in the watershed is one of the basic necessities to achieve integrated land management and soil and water conservation (Khaledi Darvishan *et al.*, 2016). Quantification of soil erosion in a watershed is important and one of the basic steps of all studies to encompass lots of environmental problems and to evaluate the amount of sediment moved, transported and deposited in and out of the basin. On the other hand, direct measurements of erosion in a watershed are possible with multi-years measurement of solid transport in the closing-section (Tazioli, 2009).

Sediment load measurements are useful to calibrate soil erosion models (Spalevic, 2011; Sadeghi *et al.*, 2013; Sadeghi *et al.*, 2014; Vujacic *et al.*, 2017) that are after useful tools to evaluate the amount of discharge and erosion in a watershed, especially when hydrometric and discharge data are not available (Behzadfar *et al.*, 2014).

Evaluation of the applicability of soil erosion models to a watershed is not easy, as it is difficult to accurately measure soil erosion in the field (Rawat *et al.*, 2011). In contrast, sediment yield models are easier to apply, because the data for these models can be measured at the watershed outlet (Kinnell, 2010).

Erosion Potential Method – EPM (Gavrilovic, 1972), was in recent times repeatedly applied all over the World (Yousefi *et al.*, 2014) but more frequently in the watersheds of Apennine and in the Balkan Peninsula (Kostadinov *et al.*, 2014; Milevski *et al.*, 2008; Sekularac, 2013; Spalevic *et al.*, 2012; Spalevic *et al.*, 2013; Spalevic *et al.*, 2014; Stefanovic, 2004; Tazioli, 2009; Vujacic *et al.*, 2017; Zorn and Komac, 2008). The method is based on the factors affecting erosion in a catchment; its parameters dependent on the temperature, the mean

annual rainfall, the soil use, the geological properties and some other features of the catchment.

The objective of the present research was ecological-economic modelling by using the IntErO computer graphic model to simulate soil erosion scenarios associated with intensive seed potato production at one of the sub-basins of the River Lim in the Northeast Montenegro: Bijeli Potok River Basin (2.9 km^2). Various land use scenarios were simulated in the model aiming to define the optimal scenario of agricultural production, examining ecological aspects of soil erosion and economic development of areas affected by soil erosion processes as well as proposing measures to overcome these issues.

MATERIAL AND METHODS

Study area. The present study was conducted in a small sub-catchment of the river Lim that is an integral part, in terms of geomorphology and climate, of the natural entity of the Polimlje region in the north eastern part of Montenegro; mountainous, with the presence of deep incised valleys (in Limestone Mountains) but also hilly. Rivers in this region drain to the Black Sea.

The studied basin covers a surface area of 2.9 km^2 encompassing the villages of Brezojevice and Nadgrad. The river Bijeli Potok flows into the Lim river just 0.5 km after the source of the river Lim from the Plavsko Lake; 10 km south of Sekular, settlement of Spalevici; 18 km south-east of Andrijevica, 7 km from Murino (Fig. 1).

The natural length of the main watercourse is 2.7 km. The shortest distance between the fountainhead and the mouth, is 2.3 km. The total length of the main watercourse, with tributaries of I and II class, is 7.9 km (Spalevic *et al.,* 2013).

There are mild slopes around the village Brezojevice and steep slopes in the upper part of the Visitor massive. The average slope gradient in the river basin, Isr, is calculated on 47.39%, indicating that in the river basin prevail very steep slopes. The average river basin altitude (Hsr), the average elevation difference of the river basin (D), the natural length of the main watercourse (Lv) and the shortest distance between the fountainhead and the mouth (Lm) are 1589.89 m, 699.89 m, 2.69 km, and 2.3 km, respectively.

Fieldwork & laboratory analysis. During the field work, using a morphometric methods, various data on intensity and forms of soil erosion, land use, and the measures taken to reduce or mitigate erosion were recorded. Different forms including the shape of the slope, the depth of the erosion base and the density of erosion rills were determined.

Satellite imagery, available from the Google Earth and Google Maps, were used to estimate standard morphometric methods (Spalevic *et al.,* 2014) and analyse the erosion rills density and the depth of the erosion base, to measure specific lengths such as the natural length of the main watercourse, tributaries, the length of the watershed and other physical-geographical characteristics.

The research part related to geology (Zivaljevic, 1989) and soils (Fustic and Djuretic, 2000) is based on previous national geological and pedological studies, who analysed all geological formations and soils of Montenegro.

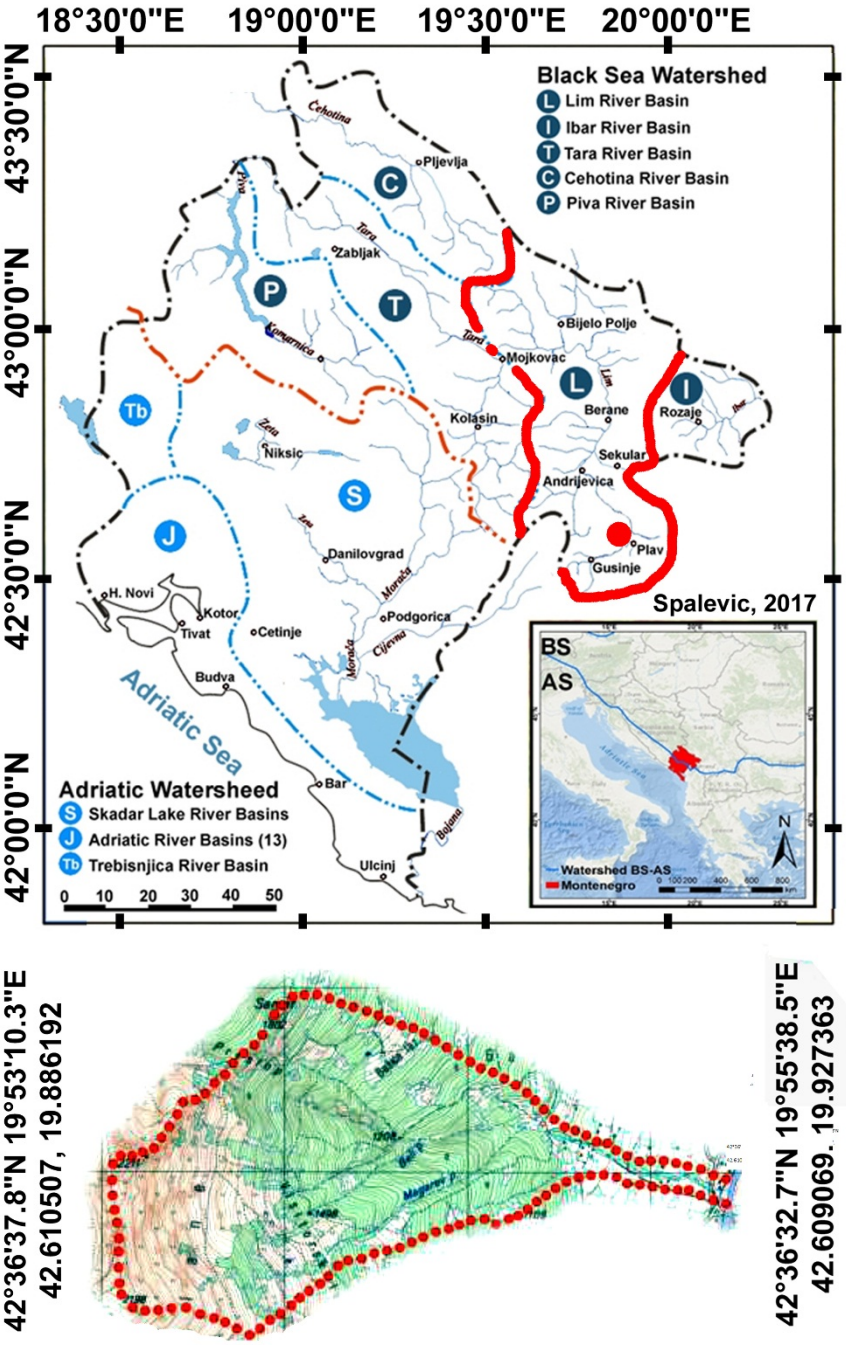

Figure 1. Location of the study area of Bijeli Potok
Polimlje: 43.245703 N, 19.580383 E (North); 42.508046 N, 19.905853 E (South); 43.148092 N, 19.485626 E (West); 42.963960 N, 20.120087 E (East).

Figure 2. View on Visitor Mountain and the studied river basin

Figure 3. Plavsko Lake (907 m.asl.) from the Visitor (2211 m.asl.)

Figure 4. Visitor Lake (1.735 m.asl.; 90 x 70 m.) north-east of the boarder of the Bijeli Potok river basin

Furthermore, some soil samples for chemical and physical analysis were collected. The grain size composition of the soil was determined by the pipette method. The soil samples were air-dried at 105°C sifted through 2 mm sieve and dispersed using sodium pyrophosphate. Total carbonates were determined by the volumetric Scheibler method; the soil reaction (pH in H2O and nKCl) was determined with a potentiometer; the content of the total organic matter was determined by the Kotzman method; easily accessible phosphorous and potassium were determined by the Al-method and the adsorptive complex (y1, S, T, V) was determined by the Kappen method (Spalevic *et al.*, 2017).

IntErO model application. The Intensity of Erosion and Outflow - IntErO (www.agricultforest.ac.me/Spalevic/IntErO) program package (Spalevic, 2011) was used to estimate maximum runoff discharge from the basin and the intensity of soil erosion, with the Erosion Potential Method – EPM (Gavrilovic, 1972) embedded in the algorithm of this computer-graphic method.

The above methodology was used in Bosnia & Herzegovina, Bulgaria, Croatia, Czech Republic, Italy, Iran, Montenegro, Macedonia, Serbia and Slovenia (Kostadinov *et al.*, 2014). In Iran, the IntERO have been successfully used previously in the Regions of Chamgardalan; Kasilian (Amiri, 2010; Zia Abadi & Ahmadi, 2011; Yousefi *et al.*, 2014) and some other sub-catchments of Shirindareh River basin (Behzadfar *et al.*, 2014 and 2015; Barovic *et al.*, 2015; Gholami *et al.*, 2016; Khaledi Darvishan *et al.*, 2017).

In Italy, using the same methodology, Tazioli (2009) found that this model corresponds well concerning annual sediment yield using nuclear probes for suspended-load measurements on Musone and Esino watersheds. Similar studies were applied earlier at the Prescudin catchment in Italy, (Bemporad *et al.*, 1997) recording a minimum deviation between predicted and measured sediment yield values (Spalevic *et al.*, 2017).

At the Bregalnica basin in Macedonia (Milevski *et al.*, 2008), a very good match has been achieved between the results obtained using the EPM method and onsite measurements. It should be highlighted that the EPM/IntErO model considers the total sediment load, whereas most of the measurements conducted in the studies cited take into account suspended load only.

Model verification. Sediment yields were calculated for all the tributaries of the Lim river basins, which include the Bijeli Potok river basin. The model results were then compared with the measurements obtained at the Potpec reservoir. Using the Model the sediment yield was calculated to be 347 273 $m^3 year^{-1}$; while actual geodetically performed measurements were 350 000 $m^3 year^{-1}$. This validates calculations of the results for sediment yield obtained by model. This leads to a conclusion that the model is applicable for the observed area (Spalevic *et al.*, 2016).

Model Parameters Calculation.

Climatic characteristics. The climate is determined by the proximity of one large water area (the Lake of Plav) as well as the Prokletije and Visitor Mountains. Data used in research were provided by the Institute of Hydrometeorology of Montenegro. The studied region has a continental climate, with rainy autumns and springs and cold winters. The absolute air temperatures recorded range from a minimum of -29.8oC up to 35 °C. On the basis of the available data, the average annual air temperature, t0, is 8.1°C; average annual precipitation is 1182.3 mm. Calculated temperature coefficient for this area, T, is 0.95; the torrential rain, hb, is calculated to be 89.4 mm (Spalevic *et al.*, 2017).

Basic data on the area needed for the calculation of soil erosion, intensity, and runoff are presented in Tables l - 6.

Table 1. Monthly precipitation sums in lit/m^2 – Gusinje, Montenegro

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Max	303.7	270.4	235.3	258.9	231.4	162.1	164.5	165.0	328.9	552.7	393.2	398.1
Av	137.9	119.3	104.5	127.6	89.1	73.9	58.6	66.5	90.8	138.5	174.9	163.8
St.d.	104.0	80.0	54.6	56.7	46.8	37.4	39.2	42.7	67.6	117.3	91.5	112.3

Year = 1345.4

Table 2. Monthly precipitation sums in lit/m^2- Plav, Montenegro

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
max	404.0	246.0	166.7	240.0	240.0	246.0	190.0	147.0	214.0	405.0	435.4	310.6
Av	124.1	101.2	89.1	106.4	81.8	69.3	53.9	61.6	85.1	118.7	156.4	134.6
St.d.	100.9	67.6	45.7	55.0	45.7	51.7	39.6	35.5	56.2	93.9	96.7	82.0

Year =1182.3

Table 3. Daily Maximum in lit/m^2 - Plav, Montenegro

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
max	89.4	88.7	76.5	58.8	51.0	48.5	45.7	40.5	40.8	70.0	71.8	65.7
Av	27.7	30.8	29.9	32.1	25.4	18.5	16.7	17.9	25.2	29.7	36.3	34.2
St.d.	25.7	20.7	16.8	12.6	14.8	12.5	10.9	10.0	11.4	18.3	21.7	19.7

Table 4. Monthly average air temperature in ^{O}C - Plav, Montenegro

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
max	1.9	2.9	5.8	9.8	13.6	16.4	20.4	20.0	16.1	11.3	6.3	3.4
min	-5.2	-4.9	-1.9	6.1	10.4	13.2	16.0	15.5	10.4	7.5	-1.3	-3.2
Av	-1.4	-0.4	3.2	7.6	12.4	15.2	17.4	17.1	13.4	9.3	3.2	0.0
St.d.	2.2	2.2	2.3	1.1	1.1	1.0	1.2	1.3	1.5	1.2	2.2	2.0

Table 5. Absolute maximum of air temperature in ^{O}C - Plav, Montenegro

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
max	14.0	18.0	24.0	24.0	28.4	33.0	35.0	35.0	32.0	27.2	22.0	18.8
Av	11.9	12.1	18.1	20.5	25.5	28.6	31.8	31.3	28.0	24.4	17.7	12.7
St.d.	2.1	2.8	3.4	2.4	2.3	2.7	2.0	1.9	2.7	1.8	3.2	2.4

Table 6. Absolute minimum of air temperature in ^{O}C - Plav, Montenegro

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
min	-29.8	-22.2	-18.0	-11.2	-1.6	0.0	0.0	1.0	-1.1	-6.4	-17.0	-21.0
Av	-16.1	-14.1	-9.2	-3.1	0.5	2.3	4.0	3.7	2.3	-3.1	-9.5	-13.6
St.d.	5.2	3.8	4.8	2.9	1.4	1.6	2.0	1.7	2.1	2.0	3.5	4.6

The amount of torrential rain, h_b, is 89.4 mm. The average annual air temperature, t_0, is 8.1°C; average annual precipitation, H_{year}, 1345.4 mm.

The geological structure and soil characteristics of the area. To calculate some inputs of IntErO, the geological data was extracted from the geological map of Montenegro (Zivaljevic, 1989). The area is characterised with sedimentary, magmatic and metamorphic rocks, ranging in age from Palaeozoic to Quaternary. The geological data showed that the structure of the river basin, according to bedrock permeability, is the following: poor water permeability rocks (f0), medium permeable rocks (fpp) and very permeable products from rocks (fp) 5%, 43% and 52%.

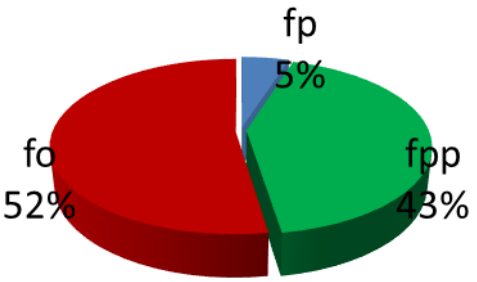

Figure 3. Permeability of the rocks

The coefficient of the region's permeability, S1, was calculated about 0.84.

The fact that soil properties always have an effect on the intensity of erosion has been generally accepted and confirmed by Baver (1959) and Pavicevic (1968). Those studies paid particular attention to the types of soil and their properties, with particular focus on their propensity towards erosion. Pavicevic (1956, 1957), Pavicevic and Tancic (1970), Fustic and Djuretic (2000), Antonovic (2001) and Spalevic (2011) studied the soils of the high mountains in Upper Polimlje.

Going from the inflow of the Bijeli potok past Lim to the surrounding mountainous terrain, the most common soil types are: Alluvial and Alluvial-Deluvial soils, Brown Eutric soils, Brown District Soils (on Sandstones, Granite and Gneiss), Brown Soils on Limestone and Limestone and Dolomite Soils.

The structure of the river basin of Bijeli potok, according to the soil types is presented in figure 4.

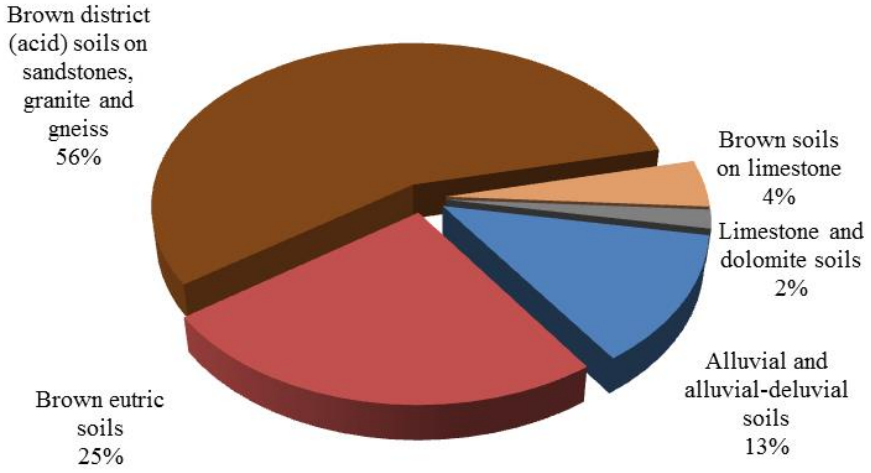

Fig. 4: The structure of Soil types in the studied river basin (Spalevic *et al.*, 2013)

Vegetation and land use. For the purposes of calculating the maximum outflow from the river basin of Bijeli potok (Q_{max}) a vegetative cover (ratio S2: part of the basin covered by forest, the grasses, orchards, as well as the barren land) was analyzed. The studied area is located in Dinaridi Province of the Middle-Southern-East European mountainous biogeographical region. The dominant type of vegetation is forests accounting for more than half of the total vegetation cover. The most important plant communities of the area are in the following classes of vegetation: *Erico-pinetea, Vaccinio-picetea, Arhenanteretea, Festuco brometea, Bromion erecti, Scorzonerion villosa* and *Ulicionae.*

On the vertical profile, according to Spalevic *et al.* (2013), the river basin of Bijeli potok is differentiated from the following forest communities:

1.*Fagetum montanum.* Differentiated into several associations of which the most characteristic is Luzulo - *Fagion moesiacae*.
2.*Abieti - Fagetum moesiacae* Blec and Lak.
3.*Picetum excelsae montanum*
4.*Picetum excelsae subalpinum*, above 1600m.
5.*Fagetum subalpinum* between 1500-1800m (all exposures and geology)
6.*Pinetum heldreichii* between 1500-2000m.
7.*Pinetum peuces:*
   a. *Pinetum peuces montenegrinum* Blec. between 1800-2000 m;
   b. *Pinetum heldreichii-peuces* Lak. between 1700-2000 m;
   c. *Pinetum mughi* above 2000 m.

According to our analysis, the coefficient f_s, (part of the river basin under forests) is 0.50, f_t (grass, meadows, pastures and orchards) is 0.42 and f_g (bare land, plough-land and ground without grass vegetation) is 0.08.

The coefficient of the vegetation cover, S_2, is 0.71. The coefficient of the river basin planning, X_a, is 0.4. Of the total river basin area, related to the river basin structure, well-constituted forests are the most widespread form (35%). Further proportion is as follows: meadows (28%), degraded forests (15%), grassland (9%), bare land (6%), orchards (5%) and arable land - plough-lands (2%). The structure of the river basin of Bijeli Potok, according to the land use is presented in figure 5.

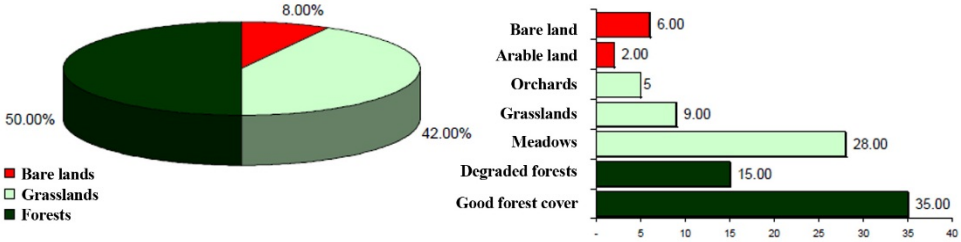

Fig. 5: Land use in the river basin of Bijeli Potok (Spalevic, 2011)

RESULTS AND DISCUSSION

Soil erosion and runoff. The relief of the hilly-mountainous terrain is characterized by steep slopes from which the water runs off and flows quickly, which is favorable for triggering the soil erosion process. The dominant erosion form in this area is surface runoff, but more severe forms of erosion, such as rills, gullies and ravines, occur also.

The erosion causes some places to lose fertile land, and results in sterile alluvial deposits on the fertile soils of the small alluvial terraces close to the main watercourse. Surface erosion has taken place in all the soils on the slopes, with the effect that this erosion is most pronounced on the steep slopes with scarce or denuded vegetation cover (Spalevic, 2011).

The software IntErO was used to process the input data required for calculation of the soil erosion intensity and the maximum outflow.

A part of the report for the river basin of Bijeli Potok is presented in Table 7.

Table 7. Part of the IntErO report (inputs and outputs) for the studied watershed

River basin: /08	1.302132 km	0.047 km^2	0.062 km^2
Input data	1.5839155 km	0.056 km^2	0.103 km^2
=========	1.560719 km	0.072 km^2	0.012 km^2
F = 2.93072 km^2	1.4873385 km	0.108 km^2	h0 = 900 m
O = 8.8377 km	1.46047 km	0.074 km^2	dh = 50 m
Lv = 2.6891 km	1.3958955 km	0.098 km^2	Hmin = 890 m
Lm = 2.30494 km	1.351942 km	0.087 km^2	Hmax = 2211 m
Suma L = 7.9001	1.3419545 km	0.2 km^2	fp = 0.045
km	1.2875715 km	0.116 km^2	fpp = 0.426
Lb = 3.73001 km	1.2`09958 km	0.12 km^2	fo = 0.527
Fv = 1.66941 km^2	1.133764 km	0.215 km^2	fs = 0.5026
Fm = 1.26131 km^2	1.1002825 km	0.144 km^2	ft = 0.422
Liz:	1.0375695 km	0.135 km^2	fg = 0.0754
0.259726 km	0.8958745 km	0.195 km^2	hb = 89.4 mm
0.7709245 km	0.76636 km	0.147 km^2	Up = 100 years
0.7897265 km	0.5009305 km	0.16 km^2	t0 = 8.1 °C
0.7963905 km	0.34068 km	0.131 km^2	H yr = 1182.3 mm
0.8544795 km	0.1650615 km	0.102 km^2	Y = 0.4
0.953394 km	f:	0.141 km^2	Xa = 0.40
1.075335 km	0.019 km^2	0.103 km^2	Fi = 0.38
1.129429 km	0.053 km^2	0.094 km^2	
1.2246205 km	0.061 km^2	0.074 km^2	

Results	K = 1.17	S1 = 0.84	Z = 0.179
A = 0.64	Hsr = 1589.89 m	S2 = 0.71	Wyr = 783.5691 m3 yr-1
m = 0.44	D = 699.89 m	W = 1.1212 m	Ru = 0.392
B = 0.79 km	Isr = 47.39 %	2gDF^½=200 mkm s^{-1}	
a = 0.28	Hleb = 1321 m	Qmax = 87 m3 s^{-1}	G yr = 307.16 m3 yr-1
G = 2.70	Er = 321.37	T = 0.95	G yr = 104.81 m^3 $km^{-2}yr^{-1}$

Potato Production and Afforestation. In the last decades, researchers from all over the world have expressed an increased interest towards studies about economic valuation of natural well preserved areas given that environmental public goods are not always traded on a market. Economists have struggled with this subject for decades and have developed several methods to estimate the economic values of these goods, known as non-market valuation methods (Dumitras *et al.*, 2011).

In the present research recently introduced approach by the same author's team of the Ecological-Economic (Eco-Eco) modeling was used. This approach is highlighting the message that it is possible to get economic benefits with commercial production of seed potato increasing income of farmers, improving at the same time their living standard and quality of life, while respecting at the same time sustainable watershed management.

Establishment of seed potato production. Almost all the farmers from the studied region are producing more or less vegetables for home consumption.

Farmlands at these Mountainous altitudes (range from: Hmin, 890 m at the inflow of the Bijeli Potok to the Lim River; Hmax , 2211 m at Plana) are ideal for producing high quality seed potato given the clean, virus free, low disease conditions. In an environment where no fruit can be cultivated above 1,300 m asl, highland varieties of potato are an important resource for certain households in the study area, on a commercial basis. For the establishment of intensive production it is important to improve land preparation, together with fertilizers and manure usage that will increase yields by one-third (Spalevic *et al.*, 2017).

Intensive crop production / potato production causes environmental damage by increasing the soil erosion intensities, which can be balanced by the introduction of conservation pathways. Soil erosion and its repercussion on the potato farming economic system were calculated. Calculation of seed potato production costs per 1 hectare for the region of Polimlje (Spalevic *et al.*, 2017), where the Bijeli Potok river basin is located is presented in the Table 8.

Table 8. Calculation of seed potato production costs per hectare for the basin

A	**Production operation**	**Operation per ha in €**
1	Ploughing	100
2	Transportation of mineral fertilizers	5
3	Distribution of mineral fertilizers	5
4	Land Planning	50
5	Transport of seed potatoes	10
6	Planting potatoes	50
7	Covering potatoes by soil	20
8	Treatment against weeds	10
9	Treatment of diseases and pests (3 times)	30
10	Extraction of potatoes	100
11	Transporting the extracted potato to the warehouse	120
	Total	**500**
B	**Raw materials**	**Material per ha in €**
1	Potato seed	1200
2	Fertilizer NPK	500
3	Fertilizer KAN	60
4	Herbicides	75
5	Insecticides	100
6	Fungicides	160
8	Bags	150
	Total	**2,245**
C	**Labor**	**Labor per ha in €**
1	Potting of potatoes	20
2	Loading - unloading fertilizers	10
3	Spreading of fertilizers	10
4	Loading - unloading of potatoes	20
5	Seeding of potato	40
7	Weeding weeds	20
8	Treatment against diseases and pests	20
9	Extraction of potatoes	400
10	Loading - unloading of potatoes	100
	Total	**640**
	Total A+B+C	**3,385**

D	Results	Component in €
2	Total cost	3,385
3	Expected yield	17,000 kg ha^{-1}
4	Cost of production	0.199 € kg^{-1}
5	Seed potato market price	0.50 € kg^{-1}
6	Market value of product	8,500 €
7	VAT (7%)	556.07 €
8	Realized profit (6-2)	5,115 €
9	Net profit (8-7)	4,558.93 €

Calculations arranged by the Spalevic *et al.* (2017) from the Table 8 under A, B, C, where the total amount (A+B+C) is € 3,385 were challenged by the calculations of the other producers in Montenegro. The production operations costs, raw material costs and labor costs have been quantified. In agreement with Article 24 of Montenegrin VAT Law, VAT rate for seed potato is 7%; however, in order to not over-complicate the model, the taxation issues did not examine in detail.

For this research the calculations were compared with the production of Jaksic from Zabljak (Durmitor Region, North Montenegro). The total amount realized by Jaksic from Zabljak was € 5,000 and the yield was 18.000 kg ha^{-1}, but in this case 17,000 kg ha^{-1} was calculated. Market value of Jaksic's production was € 9.000 and in the present case € 8,500 was calculated. Profit of Jaksic's production was € 4.000, and our calculation was € 5,115. Net profit of Jaksic's production was € 3.400, but our calculation shown € 4,558.93 of net profit. This led us to the conclusion that our approach was appropriate for the conditions of the Mountainous region of Polimlje on the territory of Montenegro.

The area of intervention of the seed potato production is 2.93 ha, which is 1% of the 293 ha of the total river basin area. The calculated profit per hectare (see table 1d) is €4,558.93, this adds to the total profit of €13,357.66 annually for the area of intervention. This amounts to a total nominal profit of €133,576.60 for the next decade (without taking into account price changes and time value of money), which is the basic time frame for calculation of afforestation costs in this research paper (Table 9).

Calculation of afforestation costs. Calculation of afforestation costs for studied area using the local market prices and current conditions in Montenegro, have been done based on the initial research of Spalevic *et al.* (2017). Calculation of the afforestation costs for studied area has been presented in the table 9.

Table 9. Calculation of afforestation costs for studied area

1	Type of terrain	Pasture & barefoot
2	Conservation measure	Digging with planting
3	Excavation with planting (€/8 work. hours)	15
4	Normative (pieces/8 working hours)	70
5	Number of pieces per ha	2500
6	Price of spruce seedlings (€/piece)	0.18
7	Basic planting per hectare (€)	985.71
8	Filling charges for 2 years (€)	295.71
9	Reforestation costs per hectare (€) – 10 year	1,281.43
10	Reforestation costs per hectare (€) – 1 year	128.14

Reforestation costs per hectare (€) are €128.14 (1 year) and € 1,281.43 (10 years). Net profit after deducting the costs of reforestation for the 10 years period (without taking into count time value of money) is € 133,576.6 - € 1,281.43 = 132,295.22.

The reason for introduction of seed potato production is economic valuation of natural resources at the observed area. Because of the introduction of seed potato production it was necessary to balance out the increased soil erosion at the area. The essence of Eco-Eco modeling is in balancing of economic and ecological effects in dealing with the soil erosion on some selected area of land. Scenarios of land use changes are presented in the following Figure 6, Table 10.

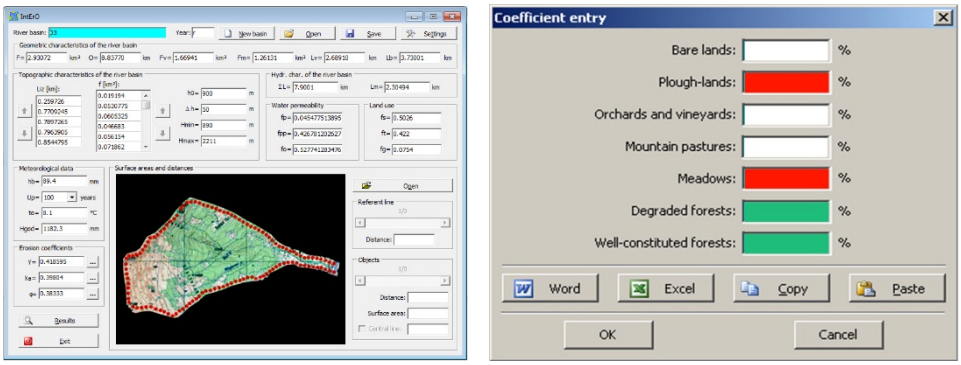

Figure 6. Form of the IntErO model including the sub-form for land use changes

Table 10. Land use changes in the Bijeli Potok river basin

Land use	Symbol	Initial (1)	Potato (2)	Afforestation (3)	Units
Bare lands:	BL	7.03	7.03	7.03	%
Plough-lands:	P	0.50	1.50	1.50	%
Orchards:	O	2.01	2.01	2.01	%
Mountain pastures:	MP	20.10	20.10	20.10	%
Meadows:	M	20.10	19.10	19.10	%
Degraded forests:	DF	15.08	15.08	14.08	%
Well-constituted forests	WF	35.18	35.18	36.18	%
	Suma	100.00	100.00	100.00	%
Land use	**Symbol**	**Initial (1)**	**Potato (2)**	**Afforestation (3)**	**Units**
Bare lands:	BL	20.60	20.60	20.60	ha
Plough-lands:	P	1.47	4.40	4.40	ha
Orchards:	O	5.89	5.89	5.89	ha
Mountain pastures:	MP	58.89	58.89	58.89	ha
Meadows:	M	58.89	55.96	55.96	ha
Degraded forests:	DF	44.18	44.18	41.25	ha
Well-constituted forests	WF	103.08	103.08	106.01	ha
	Suma	293.00	293.00	293.00	ha

Land use	Symbol	Initial (1)	Potato (2)	Afforestation (3)	Units
Bare lands:	BL	0.21	0.21	0.21	km2
Plough-lands:	P	0.01	0.04	0.04	km2
Orchards:	O	0.06	0.06	0.06	km2
Mountain pastures:	MP	0.59	0.59	0.59	km2
Meadows:	M	0.59	0.56	0.56	km2
Degraded forests:	DF	0.44	0.44	0.41	km2
Well-constituted forests	WF	1.03	1.03	1.06	km2
	Suma	2.93	2.93	2.93	km2

The outcomes of model calculation are presented in the following Figures 7-11:

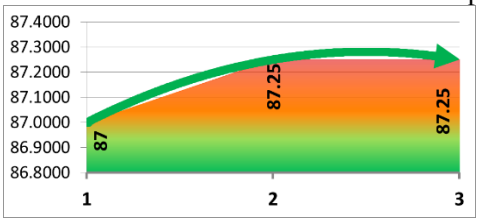

Figure 7. Coeff. of the vegetation cover

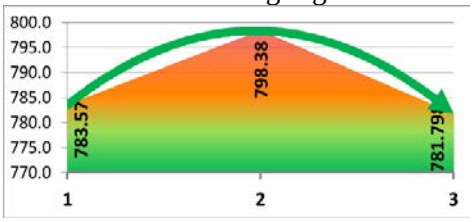

Figure 8. Peak discharge

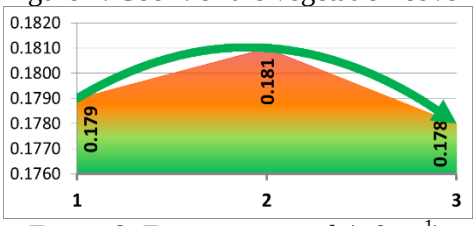

Figure 9. Erosion material ($m^3\ yr^{-1}$)

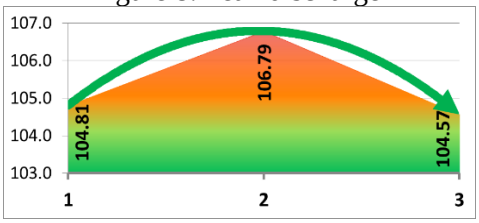

Figure 10. Erosion Coefficient Z

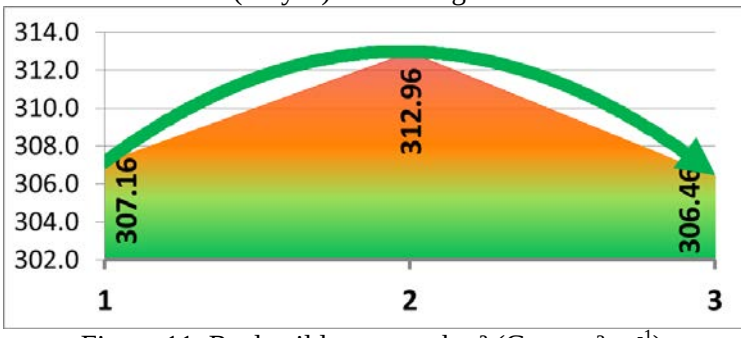

Figure 11. Real soil losses per km^2 (G yr: $m^3\ yr^{-1}$)

The model has taken into account all of the above parameters (27 input data and 22 results) and it was calculated that the real soil loss under current conditions is 307.16 m^3yr^{-1}. If seed potato production is introduced, the model calculated a soil loss of 312.96 m^3yr^{-1} as sediment yield. In order to balance the damage caused by the introduction of seed potato production, the ecological measure of afforestation to reduce soil loss caused by seed potato production has been considered. The model calculated that afforestation would result in a decrease of sediment yield to 306.46 m^3yr^{-1}.

CONCLUSION

Three different land use scenarios were simulated within the IntErO model in order to find the optimal scenario of land use for intensive seed potato production in the Bijeli Potok in Montenegro. It has been concluded that intensive agriculture production may be applied in conjunction with the afforestation without recording additional soil losses in the mountainous river basins.

The effects of afforestation on various variables such as soil physical, chemical and biological characteristics, water infiltration, runoff and soil loss and etc. have been already been proven in previous studies but the interaction effect between afforestation and seed potato production especially in soil loss was studied and proved again in this research at the Bijeli Potok like in a neighbouring watershed of the Velicka Rijeka.

The presented approach of Ecological-Economic (Eco-Eco) modelling using the Intensity of Erosion and Outflow (IntErO) model has potential to become a vital tool to reduce uncertainty for environmental policy decision-making. The team of authors is continuing the further research of this modeling which will use different type of agriculture production.

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***Erika QUENDLER*[1]**

WOMEN'S ROLES IN AUSTRIAN AGRICULTURE

SUMMARY

For ages, family farms have prevailed and are particular to Austria. Since work both on and off the farm and family life are combined, the role of women in agriculture is a complex one. They are mothers, wives, farm managers, partners, labourers, creators of their own branches and employed off farm. Analysing the roles of women in agriculture is more complicated than it may initially appear. Official agricultural statistics give a very limited image. Statistics on women are only available in connection with the ownership, the management and the labour force. Our knowledge of women in agriculture is largely derived from national studies and statistics as well as (periodic) surveys. With this in mind this paper aims to determine the roles performed by female farmers in agriculture according to their activities on and off the farm. Following a literature review, this paper covers an in-depth analysis of women's roles in agriculture based on data from official statistics and select data from a survey done in 2016 throughout Austria. In this respect, this paper contributes to the gender debate in agriculture and to our knowledge of the role of women in agriculture. This paper re-affirms the essential role of women in agriculture and tries to give some suggestions on the need for further research and future perspectives.

Keywords: Agriculture, roles, women, Austria.

INTRODUCTION

In Austria, as in other European countries, farming is a family concern. Women play an important role in family farming. Over the last decades there has been a considerable rise in interest in the roles of women (Björkhaug and Blekesaune, 2008; Gasson 1981; Rossier, 2014; Steinmann, Matasci-Brüngger, 1978). The role of the woman on the farm, the female farmer, is not precisely defined (European Parliament, 2010; Tazza, 1979 in Gasson, 1981). 'Role' means more than the professional title or the sum of the activities of a person within the profession. A woman who works as a female farmer is like an actress who takes on a role with all the related activities, responsibilities and relationships. The question about the roles is very differently answered; the views and ideas are very diverse. (Gasson, 1981) From the tasks, responsibilities and relationships of the female farmer, various roles arise, e.g. wife, housewife, mother, manager, labourer, off-farm worker (Bayerische Landesanstalt für

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Landwirtschaft, 2010a, b; Gasson, 1981; KeyQUEST Marktforschung, 2017; Relf, 2014; Rheinischer LandFrauenverband e.V. and Westfälisch-Lippischer LandFrauenverband e.V., 2016; Rossier and Ressig, 2014). The 'female farmer' is a role with many sub-roles. Analysing the roles of female famers is more complicated than it may initially appear. Official agricultural statistics give a very limited image. They do not reveal the real nature of the contribution female farmers make on the farm. Statistics on women (including female farmers) in agriculture are only available in connection with the ownership, the management and the labour force. Our knowledge of female farmers in Austria is largely derived from national studies and statistics as well as (periodic) surveys. In this paper we draw on the available empirical evidence from official statistics and select data from the survey 'Situation of Female Farmers in Austria 2016' (KeyQUEST Marktforschung, 2017). In light of the renewed recent interest in the roles of female farmers, the aim is to determine the kinds of roles performed by female farmers and how they have changed. Furthermore, this paper contributes to the gender debate in agriculture and to our knowledge of the role of female farmers in agriculture. The remainder of this paper is structured as follows. We first give a view of the literature on female farmers' roles. The subsequent section outlines the methods applied to explore the research questions. The paper continues with the presentation of the roles and closes by drawing conclusions and outlining some prospects for further research.

MATERIAL AND METHODS

The paper was designed to describe the roles of female farmers. A literature review was done. General data (BMLFUW, 2014; Statistik Austria, 2013) were appropriately processed. In order to get a more comprehensive picture also select data, namely questions about ownership, decision making, activities on the farm and off farm employment as well as voluntary engagement were analysed from the survey of female farmers[2] throughout Austria. The agricultural population for the survey was obtained from the Austrian Integrated Administration and Control System (IACS) farm data set 2014 where farm operators had the legal status of natural person or group person (without community pastures and cooperatives). The agricultural population overall was 116,615. For the online survey an email address was a further criterion. The study population for the survey was 36,573 farms. The survey was conducted as an online-questionnaire between the 2 May and the 3 June 2016. 2,200

[2] Since 1976, every ten years, a survey has been conducted on the working and living situation of female farmers in Austria. This initiative is supported by the Association of Austrian female farmers. These regular surveys provide an insight into the life and work situation of the female farmers over the decades. The questionnaire for the survey of female farmers 2016 is based on that of the year 2006 and was divided into five sections. These cover questions on the following topics: A. Everyday life on the farm and household, B. Personal situation, C. Future, D. Relevant statistics on the farm, E. Statistics on the person and the partner. The questionnaire was completed with an open question regarding personal concerns. A total of 70 questions were asked. (KeyQUEST Marktforschung, 2017)

questionnaires from female farmers were used after a quality test (consistency check) for the analysis of the research questions. The response rate was 7% of the net sample. Due to the disproportionate response, a weighting according to the federal states, the age of the manager and the utilised agricultural area was applied. The data thus collected was analysed by using descriptive statistics of Statistical Package for Social Sciences (SPSS).

RESULTS AND DISCUSSION

In this section, we present the findings. We focus on the literature review, the characteristics of female farmers surveyed, the status of the female farmers on the farm and the roles on and off the farm. The comparative analyses of statistics of survey data show what the roles are and, where data from 2006 is available, how the understanding of the roles has changed in the last ten years. Here the results of the year 2016 are presented and, if available, earlier surveys are mentioned.

Literature on female farmers' roles

Decades of research have largely dealt with the roles of women in agriculture (cf. Bayerische Landesanstalt für Landwirtschaft 2010a, b; Björkhaug and Blekesaune, 2008; Boserup 2007; Chaney, Schmink 1976; Gasson 1981; Relf 2014; Rheinischer LandFrauenverband e.V. and Westfälisch-Lippischer LandFrauenverband e.V. 2016; Rossier 2014; Rossier and Ressig 2014; Steinmann and Matasci-Brüngger, 1978; Tinker and Bramsen 1976). According to this research female farmers have a multi-faceted, challenging job profile. They are housewife, owners, farm managers, decision-makers, office workers, labourers/workers, family counsellors, off farm workers and do voluntarily work. Many of their activities are because of their position as wife, mother and/or partner. The interrelated, multi-dimensional aspect of their work, together with their quick adaption to production requirements, and their personal commitment to the family, make it extremely difficult for the family unit to replace them. The female farmers' contribution can hardly be provided by ordinary paid labour. Not only can many farms not afford the additional labour costs, but it is also not possible to find a paid worker, or a combination of paid workers, who can perform the multi-dimensional and interrelated functions of the female farmers. Neither will hired workers use their income to subsidise the farm. Variations in women's participation in work both on and off the farm – as well as other family members – depend on supply and demand factors linked to economic growth and agricultural modernisation as well as the structure of the rural labour market and employment opportunities (Copus et al., 2006). In turn, this needs to be considered in Austrian and European regional policy to ensure stable regional conditions (European Parliament, 2010).

Status of female farmers

The women's status is hardly treated in the debate on family farming. In the following the status of female farmers is discussed by looking at their involvement as owner, manager and decision-maker.

Owner

The majority of female famers (51%) indicated that they are co-owners. In the case of 31% of the interviewees the sole owner is the partner and for 13% it is the female farmer themselves. The remainder (6%) is attributable to the category 'Other', e.g. Banks, two companies. Traditionally the farm transfer is patriarchal. 67% of the farms are transferred to the son (KeyQUEST Marktforschung, 2017). Generally, in the case of marriage with no marriage contract the ownership legally remains with the person who already owns it (pers. comm., Mr. Reinl, Austrian Agricultural Chamber, 2017).

Manager

The Farm Structure Survey (Statistik Austria, 2013) and the IACS data (BMLFUW, 2014) of recent years show an increase in the proportion of women as farm managers. In 2010, 34% of agricultural and forestry holdings were managed by women, compared with 30% in 1999, this share was only 18% in 1980 (BMLFUW, 2014).

Decision-maker

In everyday farm life partnership is gaining in importance. The sole responsibility of the female farmer for company decisions showed a decline in 2016 compared to 2006. In figures, 4% of female farmers make operational decisions alone; in 2006 it was 15%. At the same time, more and more decisions are made together with the partner. On 76% of the farms, this partnership model is being lived and suggests a development that already emerged in 1986 (around 64%). (KeyQUEST Marktforschung, 2017)

New roles – old roles

The life of female farmers is usually very versatile. They practice numerous activities and take on the different roles in the family, on the farm and off the farm.

Roles on the farm

The work on the farm is done by different people: the farmer, the partner and third persons. The graphic illustration in Figure 1 shows the division of the work on an average working day on the basis of the information given by the female farmers. The female farmer has her main focus in the household, parental duties, administration and the diversification of the farm's activities as well as in the garden. Work in the fields, outside work and stable work are mainly in the hands of the partner. Most of the work is divided between the female farmer and the farmer. The answer category 'third person' has a smaller proportion of the work. The workload of the female farmer in everyday work and in family work is very diverse. They have the following roles: housewife and mother, caregiver, self-employed person with garden products, person responsible for an area of operation (diversification), workers (field and outside work, stable work) as well as office worker within the administration. As Figure 1 shows there is a type of work allocation where the man is mainly responsible for the outdoor area – the farm – while the woman is responsible for the interior – the house – as well as for certain areas of the farm work. Over the years the role of the partner has been a

very stable one. The importance of the third person has increased. The activities of female farmers are nowadays done more efficiently than in the past, while more time is spent on off-farm work. This is also confirmed by Rossier and Ressig (2014) and maybe attributed to technical advances.

Roles off the farm

Many female farmers have a role as a working woman, who works off the farm. 37% (2006 22%) of female farmers have this role (KeyQUEST Marktforschung, 2017). Over the last decade the off-farm employment has grown. The situation in Switzerland is similar. According to Rossier (2014), 47% (2002 44%) of female farmers were working off-farm in 2012. Female farmers are not only active on the farm, but also voluntarily engage in associations and organisations. These may be agricultural and/or political ones but also other associations and organisations (not explained in more detail). In 2016, 66% of the female farmers were voluntarily engaged in at least one organisation. In 2006, the figure was 58%, whereas in 1996 only 34% were voluntarily engaged. (KeyQUEST Marktforschung, 2017).

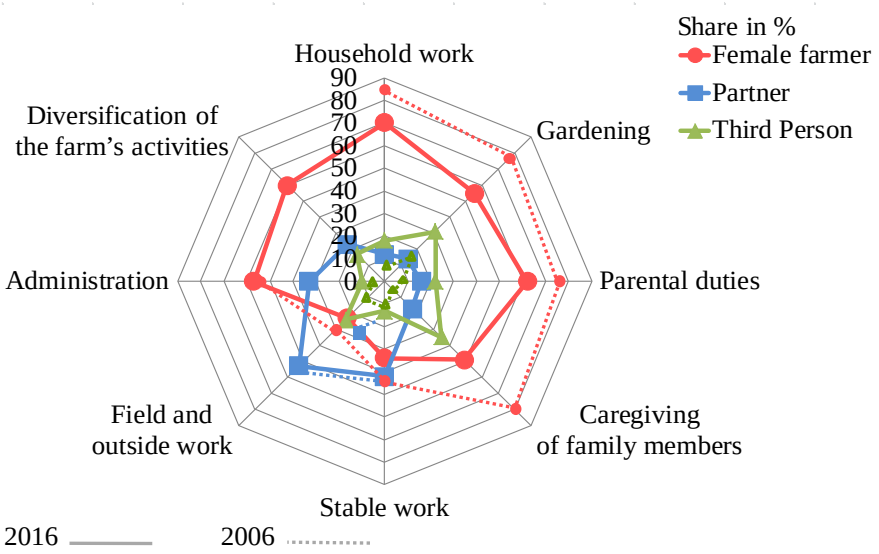

Figure 1. Distribution of work between the female farmer, the partner and third persons in %. (own visualisation according to KeyQUEST Marktforschung, 2017).

CONCLUSIONS

We live in a fast moving world. The roles are very versatile and mingled. Old ones prove themselves and new ones emerge. The female farmers have an important role on the farm, which is characterised by their sub-roles. The following sub-roles currently result from their work on the farm namely: housewife and mother, partner, caregiver, self-provider with garden products, person involved in diversification activities, worker (field and outside work,

stables) and office worker within the administration. The female farmers also take on the role of the decision-maker, manager and owner. Furthermore, they also work off the farm and are engaged in voluntarily activities. This mix creates new opportunities, but also new hurdles. These are due, among other things, to the traditional gender stereotypes. Female farmers must be more ready to grasp their roles clearly in terms of their talents, competences and qualifications and to develop the partnership path further. This results in the following fields of research (i) more information about the reasons behind the change in roles, (ii) greater clarity is needed on the psychological, social, economic and cultural factors determining the specific forms their roles take, (iii) more research into the constraints female farmers face according to the given roles on and off the farm and (iv) proper consideration of the female farmers' roles and the resulting needs within policy activities and development strategies. This all together, when properly applied could encourage more young women to see agriculture as a viable career path.

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PATTERNS OF HERBACEOUS PLANT SPECIES RICHNESS, COMPOSITION AND SOIL PROPERTIES IN AN ORGANIC CULTIVATION "*LEMON VERBENA*" AND ABANDONED AGROECOSYSTEMS OF GREECE

SUMMARY

Aromatic plants constitute a major segment of the flora, which provides raw materials for use in the pharmaceuticals, cosmetics, and drug industries. *Lemon verbena* is one of more than 30 species of aromatic shrubs in the genus *Aloysia* (family Verbenaceae) with high environmental and economic value. More attention is paid in Greece to the *Lemon verbena* essential oil while their effects on the parameters influencing multiple aspects of ecosystem structure (e.g. biodiversity, soil properties etc) are generally overlooked. Hence, this exploratory study aims to evaluate the effects of *Lemon verbena* and neighboring abandoned (10 years) ecosystems on the herbaceous plant species richness and composition, and soil properties. Also, the multiple regression method was used to investigate the relationship between herbaceous plant species richness and soil properties. The study was conducted in April-May 2016 and 2017 of central Greece. In total, 24 species of herbaceous plants (16 plant species in *Lemon verbena* ecosystem and 12 plant species in abandoned ecosystem) were recorded in the study area. The most frequently occurring plant was *Avena sterilis* (Family: Poaceae) in both types of ecosystems. Moreover, there were significant differences ($p<0.05$) between *Lemon verbena* and neighboring abandoned (10 years) ecosystems for any of the soil properties [soil organic matter, pH, $CaCO_3$, P, K and Mg] measured. Furthermore, multiple regression method showed that soil properties had significant effects on herbaceous plant species richness in which soil organic matter, pH, P and K were the most prominent factors influencing species richness in *Lemon verbena* ecosystem.

Keywords: *Aromatic Plant, Species Richness, Environment, Sustainability, Greece*.

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INTRODUCTION

Greece is considered as one of the most biologically diverse countries of the European continent with high plant diversity and endemism. Greece holds 6% of the Mediterranean area and 26% of the Mediterranean flora. Aromatic and medicinal plants are important factors in sustainable development, environmental protection and public health (Solomou et al., 2016).

One of the most important aromatic plants is *Lemon verbena* (*Aloysia triphylla* [L'Hɪrit.]) with important health benefits and ecosystem services. More specifically, *Lemon verbena*, is indigenous to South America (Duarte et al., 2007) and brought to Europe by the Spaniards and now is cultivated in many countries in Latin and Central America,in Southern Europe (Greece, France), in Northern Africa (Algeria, Morocco) China, and Iran. It is used as both a cullinary and a medicinal herb. The genus *Alloysia* belongs to the Verbenaceae family which has many genus and species. *Lemon verbenas*' botanical synonyms include *Aloysia citriodora*, *Aloysia sleumeri* Moldenke, *Aloysia triphylla*(L'Hɪr.) Britton, *Lippia citriodora* (Lam.) Kunth, *Lippia triphylla*(L'Hɪr.) Kuntze, *Verbena citriodora* Cav., *Verbena triphylla*, and *Zapania citriodora* Lam. (Zamorano-Ponce, 2004). It is a medicinal and aromatic shrub about 0.5–2m in height. It is an evergreen perennial plant, a deciduous sub-shrub, and the genus *Alloysia* comprises about 175 genera and 2,300 species. It likes warm moist conditions with plenty of sunlight. When exposed to frost, it becomes deciduous. *Lemon verbena* prefers light, sandy, medium loamy, well-drained acid, neutral, and basic alkaline (pH of 4.5–7.8.) soils (Marta, 2005; Vogel et al., 2002) and has an annual water requirement of 500 to 1,300 mm.

The lemon-scented essential oil from the *Lemon verbena* have been widely studied for its calming, digestive, abdominal-discomfort, lemony flavor properties (Marta, 2005; Vogel et al., 2002; Argyropouloua et al., 2007).

Lemon verbena traditionally has been utilized as a remedy for gastrointestinal and respiratory disorders. In addition, some species have shown antimalarial, antiviral, anti-spasmodic, antibacterial, antioxidant and cytostatic properties (Ragone et al., 2007; Bilia et al., 2008; Funes et al., 2009; Regnier and Combrinck, 2010). The volatile oil yield oil yield (Martvnez et al., 2007; Ragone et al., 2007). Fresh or dried leaves are used in the same way as lemongrass as an ingredient in stews and soups (van Wyk, 2005). Evidence has shown that essential oil and phenolic compounds such as flavonoids are responsible for the related curative properties of lemon verbena (Rosa and Meireles, 2005). *Lemon verbenas'* essential oil obtained by hydrodistillation of the leaves is 0.22–1.00%. The harvesting season, the time of the day, and the particle size present influence on the volatile (Martvnez et al., 2007).

According to the literature more attention is paid in Greece to the *Lemon verbena* essential oil while their effects on the parameters influencing multiple aspects of ecosystem structure (e.g. biodiversity, soil properties etc) are generally overlooked. Hence, this exploratory study aims to evaluate the effects of *Lemon*

verbena and neighboring abandoned (10 years) ecosystems on the herbaceous plant species richness and composition, and soil properties.

MATERIAL AND METHODS

A number of field experiments were conducted in Thessaly plain (Experimental Farm of the University of Thessaly, Velestino, central Greece, 2014) with an altitude 170 m above the sea level (Fig.1). The soil at the site was a deep, moderately fertile, clay loamy soil that was classified as Calcixerollic Xerochrept (USDA, 1975).

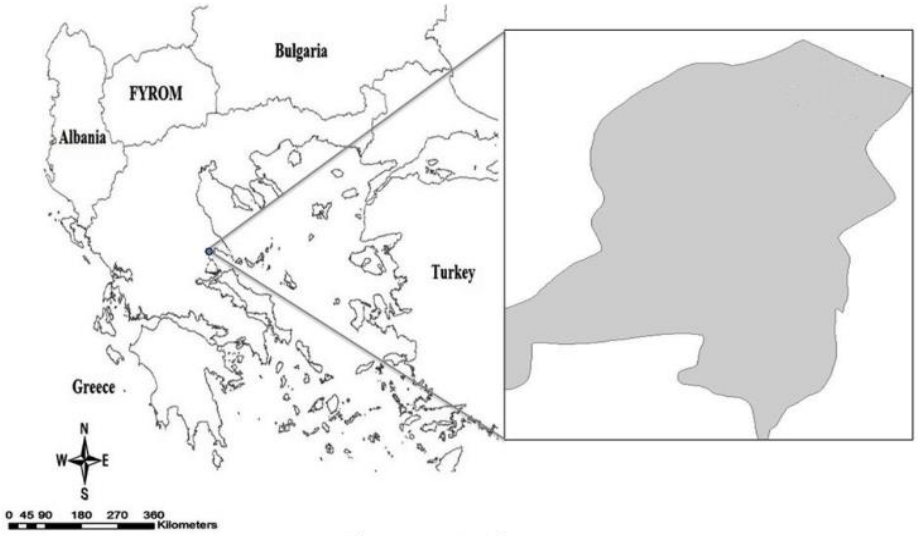

Figure 1. Study area

The climate in Greece is typical Mediterranean climate: mild and rainy winters, relatively warm and dry summers.

Thessaly Prefecture, the largest Greek lowland and the center of the country's agricultural production, is characterized by a more continental climatic character with colder winters and hot summers (Figure 2).

Sampling

The sampling of herbaceous plants was carried out in April-May 2016 and 2017 of central Greece in randomly selected plots of 0.25 m^2, in order to record plant species richness in *Lemon verbena* ecosystem (Cook and Stubbendieck, 1986; Solomou and Sfougaris, 2013). In each plot composite soil samples were taken by the randomized method at a depth of 0–40 cm. Soil organic matter (%) (Nelson and Sommers, 1982), pH (McLean, 1982), CaCO3 (Nelson and Sommers, 1982), texture (clay, silt, sand) (Bouyoukos, 1951), P (Olsen and Sommers, 1982), K and Mg (Thomas, 1982) were measured in each of the soil samples.

Statistical analysis

Relation between plant species richness with soil characteristics was investigated by multivariate analysis. We used the 'multi-regression enter' method that suitable for quantity variables such as species richness (Kalantari, 2002) as a result to create a model. Statistical analyses were performed using the software package IBM SPSS Statistics ver. 19.0 for Windows (SPSS Inc., IBM Company, Chicago, IL, USA 2010).

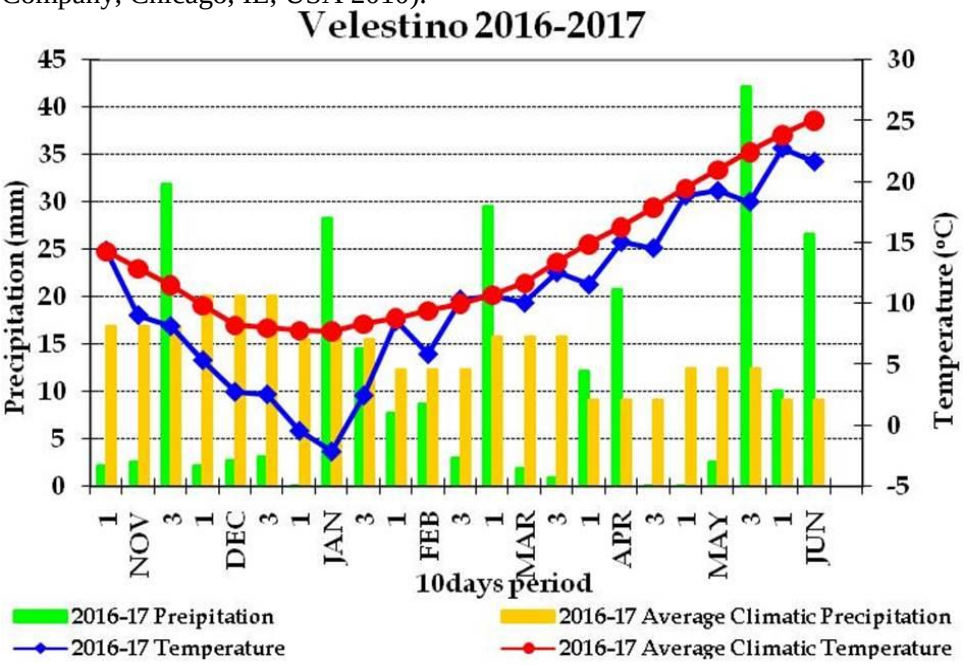

Figure 2. Climate diagram.

RESULTS AND DISCUSSION

Results showed that 24 species of herbaceous plants (16 plant species in Lemon verbena ecosystem and 12 plant species in abandoned ecosystem) were recorded in the study area. The most frequently occurring plant species was *Avena sterilis* (Family: Poaceae) in both types of ecosystems. A possible explanation is that this plant has transferred with seed and dispersal from another areas. *Avena sterilis* is highly invasive in cultivated farms and has probably already invaded many suitable regions of the Greece (Figure 3). According to Cirujeda et al. (2011) management practices, geographical gradients and climatic factors have been found to be the most important factors that explain herbaceous plant species composition and richness.

Figure 3. Distribution of *Avena sterilis* [Geography: **IoI** (Ionian Islands)–**NPi** (North Pindos) **SPi** (Souh Pindos) **Pe** (Peloponnisos) **StE** (Sterea Ellas)–**EC** (East Central Greece) **NC** (North Central Greece) **NE** (North-East Greece) **NAe** (North Aegean islands) **WAe** (West Aegean islands) **Kik** (Kiklades) **KK** (Kriti and Karpathos) **EAe** (Kriti and Karpathos). Greece: (East Aegean islands – present; East Central Greece – absent; Ionian Islands – present; Kiklades – present; Kriti and Karpathos – present; North Aegean islands – present; North Central Greece – present; North Pindos – absent; North-EastGreece – present; Peloponnisos – present; SouthPindos – present; Sterea Ellas – present; West Aegean islands – present)].

Generalized Linear Models (GLM) that came up from the processing of plant species richness data, only, in Lemon verbena ecosystem, showed good to excellent ability to "interpret" the dependent variable (herbaceous plant species richness) (based on adj. R^2). The model showed that soil properties had significant effects on herbaceous plant species richness and interprets 78% of the total variation of herbaceous plant species richness. More specifically, soil organic matter, pH, P and K were the most prominent factors influencing species richness in Lemon verbena ecosystem (Table 1).

Table 1. Generalized Linear Model of herbaceous plant species richness.

F	Adj. R	Likelihood Ratio χ^2	AIC	P	Model
11,26	0,78	18,89	40,42	0,00	$1{,}66+16{,}46(SOM)^{*}+0{,}02(SPH)^{**}$

*SOM: Soil organic matter, **SPH: Soil pH, ***P: Phosphorus, ****K: Potassium.

It is known that ecosystems with different soil nutrients are important for plant growth and community development. Soil parameters like soil organic matter are related to plant species richness. The reason soil organic matter reflects changes in plant species richness is because there is a close relationship between soil organic matter content and plants. Crow (2009) refers that soil organic matter improves the capacity of a soil to hold water and nutrients, and allows their slow release, therefore improving the conditions for plant growth. Also, in sustainable management ecosystems (e.g. natural ecosystem), plant litter and roots are the origin of soil organic matter and higher plant diversity may lead to higher litter diversity, which in turn supports a greater diversity of decomposers and detritivores (Hansen, 2000) that increase soil organic matter content. Our result showed similarity with the study of Grime (1979), where species richness increases linearly with increase in soil pH.

It is noteworthy that soil P and K are very important to plant functions and species richness. The direct effects of P and K on plant species richness probably is due to the application of fertilizers and intensity of land use, as a result in the uptake and storage of (added) nutrients in plants (Grime, 1979; Hautier et al., 2009).

Several studies have found correlations between changes in species richness and a gradient of nutrient availability (e.g. Huston 1980; Tilman 1982). According to a 'humped-back curve' (Grime, 1979; Tilman, 1982) species richness is low at low nutrient levels, increases to a peak at intermediate levels and declines more gradually at high nutrient levels. Similar results have been observed in a several studies (e.g. Tilman, 1982; Vermeer & Berendse, 1983; Janssens et al.,1998; Lichter, 1999).

CONCLUSIONS

Lemon verbena is an ecosystem with very high importance value. A key finding in this study is that *Lemon verbena* ecosystem favors herbaceous plant species richness. Also, soil parameters such as soil organic matter, pH, P and K were the most prominent factors influencing species richness in *Lemon verbena* ecosystem. The higher the species richness of an ecosystem, the higher the probability of maintaining ecosystem functions. Management plans for the biodiversity conservation of aromatic plant ecosystems should therefore focus on the specific site conditions and should take several abiotic and biotic factors into account. Hence, this study will continue into the future.

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Lidiya MISHCHENKO, Alina DUNICH, Ivan MISHCHENKO, Valeriy BERLIZOV, Vera PETRENKOVA, Oksana MOLCHANETS[1]

INFLUENCE OF CLIMATE CHANGES ON WHEAT VIRUSES VARIABILITY IN UKRAINE

SUMMARY

Climate change is closely related to the level of losses from plant diseases because the environment significantly affects plants, pathogens and their vectors. Monitoring of viral infections in agrocenoses is one of the priority measures to preserve the harvest. However, often symptoms of the adaptive response of plants caused by environmental abiotic factors like the symptoms caused by infectious agents. In this regard, there is a problem to identify the reasons of pathological symptoms on the plants, as some of them are extremely important value for technologies of agricultural crops growing. The aim of work was to investigate the variability of composition of viruses' species infecting winter wheat, to study their prevalence in Ukraine under agro-climatic change conditions. The viral monitoring showed that the winter wheat plants cv. Russia and Smuglyanka with the yellowing symptoms on leaves and "purple-yellow" leaves were infected with BYDV. Unlike previous years, WSMV was not detected in this agrocenosis that can be related to the strong drought in autumn and considerable decline of HTC that resulted in limitation of quantity of WSMV vectors. The symptoms of leaf rolling in barley cv. Antigon, leaf yellowing of wheat cv. Ermak and leaves reddening in wheat Donetska-46 were caused by technogenic influence and other abiotic factors. The analysis of temperature indexes that characterize terms of overwintering and vegetation of winter wheat showed that in May (phase of beginning of plants earing) high plus temperatures during the day changed on low temperatures at night. It is necessary to notice that the reason for appearance of symptoms "purple-yellow" and "purple" leaves of winter wheat are changes of carbohydrate balance that arise up as a result of nonspecific reactions of plants to stress caused by virus infection (cv. Russia) or sharp temperature differential (cv. Vasylyna, Podolyanka, Albatros odessky, Myronivska-67, Smuglyanka).

Keywords: WSMV (Wheat streak mosaic virus), BYDV (Barley yellow dwarf virus), climate changes, temperature difference, HTC (hydrothermal coefficient).

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INTRODUCTION

Climate change is closely related to the level of losses from plant diseases because the environment significantly affects plants, pathogens and their vectors. Changes related to global warming affect the occurrence, prevalence, harmfulness of viral plant diseases and impact on the further co-evolution of plants and their pathogens, leading to changes in species composition of the viruses in a particular region, the emergence of differences in the properties of viruses' isolates and appearance of epiphytoties.

About absolute influence of climate changes on plants, phytopathogens and their vectors, and, especially, about direct dependence between density of insects population on the winter wheat crops and its infection with viruses goes in the many scientific works of the last years (Boland et al., 2004; Bourgeois et al., 2004; Garrett et al., 2006; Pangga et al., 2013; Murphy et al., 2013; Jones 2009; Svensson, 2010). The changes associated with global warming (i.e., increased temperatures, changes in the quantity and pattern of precipitation, increased CO2 and ozone levels, drought, etc.) thus, may affect the incidence and severity of plant disease and influence the further co-evolution of plants and their pathogens.

In connection with the expected increase in the air temperature of the Northern Hemisphere, food security will largely depend on how effectively agriculture adapts to the expected climate change, future agro-climatic conditions for growing crops. Therefore, the problem of evaluating the impact of expected climate change on agro-climatic conditions of cultivation, productivity of various crops is very relevant.

Except abiotic factors crop yields are negatively affected by many biotic factors such as fungal, bacterial, viral infections. So, the damage from viral diseases can be up to 70% of the crop and above (Boyko et al., 2004; Mishchenko, 2009). Timely detection of viruses in crops and accurate diagnosis can reduce the risk of losses in the cereal industry of the country.

There are a little number of recent studies on the influences of factors like CO2, elevated temperature and rainfall-related parameters upon a small number of important viruses, eg, PLRV and Potato yellow vein virus (PYVV) (Jones, 2014), BYDV (Nancarrow et al., 2014; Rua et al., 2013), BYMV (Guerret et al., 2016), CMV,PVY, PVX (Del Torro et al., 2015) TYLCV, and TuMV (Chung et al., 2015), and several important their vectors (Gillespie et al., 2012; Ryalls et al., 2015)

It was noted that during extreme weather conditions the emergence of new viruses that cause epiphytotic episodes is occasionally monitored, diagnosed and identified. However, often the manifestation of the adaptive reaction of the plant to the action of environmental factors is similar to the manifestation of pathologies caused by infectious defeat of the plant organism. In 1999, 2001 and 2006 in Poltava, Ivano-Frankivsk, Kyiv regions, and in Khmelnytsky region (2006), we found winter wheat plants with purple flag leaves, which are similar to the symptoms caused with BYDV. It was found that these symptoms are

caused by both viral infections and extreme weather conditions (a sharp drop in the air temperature in the phase of the output of the tube) (Reshetnyk, 2010). An important diagnostic feature of BYDV is that this pathogen causes different symptoms on different cereal crops: on wheat and barley - yellowing of the leaves, and on oats - reddening of leaves in the upper part. It is known that the process of infecting a plant with a virus is largely due to the genetic properties of both organisms. But a significant effect on the course of such process can be caused by external conditions, in particular, abiotic factors. Both the reproduction and the transport of the virus can be limited by the various protective mechanisms that arise in the plant. Recent studies have shown that the number of primary and secondary metabolites (including sugars that fulfill the cryoprotective role) increases and enzymatic activity of winter wheat plants decreases under cold stress (Gaudet et al., 1999; Olenichenko et al., 2008). In this regard, we put forward two hypotheses regarding the reasons of reddening of winter wheat leaves: ecological and infectious. According to the ecological hypothesis, the appearance of red color is caused by cold stress, which plants have undergone in some phase of vegetation. It is known that an enhanced synthesis of anthocyanins in wheat plants appear under the cold effect which manifests in the color of leaves from green to red. In this connection, there is a problem to identify the reasons of the appearance of such pathological symptoms, since some of them are extremely important for the technology of growing crops.

The aim of work was to investigate the variability of composition of viruses' species infecting winter wheat, to study their prevalence in Ukraine under agro-climatic change conditions.

MATERIAL AND METHODS

Monitoring of viruses was carried out on wheat crops of winter cv. Russia, Vasylyna, Smuglyanka, Ukrainka poltavska, Kolomak, Donska napivkarlykova, Albatros odessky, Perlyna Lisostepy etc. (Poltava region), Ermak (Sumy region), cv. Poliska-90 and barley cv. Antigon (Khmelnitsky region), Podolyanka, Kyivska 8, Myronivska 65, Myronivska 68 (Kyiv region), Donetska-46 (Donetsk region, 2008), Podolyanka (Ivano-Frankivsk region).

Identification of the viruses in sap of wheat and barley leaves was performed by DAS-ELISA. Specific antibody against *Wheat streak mosaic virus, Barley yellow dwarf virus*, *Brome mosaic virus* (BMV), *Wheat dwarf virus* (WDV), *Wheat spindle streak mosaic virus* (WSSMV), *Soil-borne cereal mosaic virus* (SbCMV) (Loewe, Germany) were used. Antigen samples were prepared by grinding of leaf tissue in PBS-buffer pH 7.4 in ratio 1:2 (w/V). Leaf samples from healthy plants were also included as negative controls. Positive controls were commercial (Loewe, Germany). The results were recorded on Termo Labsystems Opsis MR reader (USA) with Dynex Revelation Quicklink software at wavelengths of 405 nm. Samples were considered positive when their absorbance values at 405 nm were at least three times higher those of negative controls (Crowther, 1995).

Purification of BYDV was performed using Hammond et al. method in our modification (Hammond *et al*., 1983), WSMV purification – by Mishchenko method (Mishchenko, 2009).

Viral particles morphology was studied by transmission electron microscopy (TEM). Negative staining of virions was performed with the 2% solution of phosphotungstic acid for 2 minutes and studied by electron microscope JEM 1400 (JEOL, Japan).

Statistical analysis of experimental data was carried out according to the parametric criteria of the normal distribution option, the standard deviation of the mean values - according to the generally accepted method.

RESULTS AND DISCUSSION

A survey of winter wheat plants in a field in Poltava, Kyiv, Sumy, Ivano-Frankivsk and Khmelnytsky regions of Ukraine showed yellowing symptoms, streaking or mosaic patterns, “purple-yellow” and "purple" leaves and spiral twisting of the barley leaves (fig. 1-2).

Plant samples with mentioned symptoms were taken for the study. Plants were tested for the presence of viruses which are most harmful and widespread in these regions by ELISA and TEM methods: *Wheat streak mosaic virus, Barley yellow dwarf virus, Brome mosaic virus* (BMV), *Wheat dwarf virus* (WDV), *Wheat spindle streak mosaic virus* (WSSMV), *Soil-borne cereal mosaic virus* (SbCMV).

None of the viruses taken into the study were detect in barley plants cv. Antigon and wheat cv. Vasylyna and Ermak. As in previous years, the reason of these symptoms could be a significant temperature difference.

The results showed that wheat plants cv. Russia and Smuglyanka were infected with BYDV in 2012 (table 1). Unlike previous years, WSMV was not detected under the conditions of the same agrocenosis of the Poltava region.

Figure 1. Viral symptoms on the leaves of winter wheat plants, Poltava region, 2012: a – yellowing on the leaves of wheat cv. Smuglyanka caused by BYDV, 2012; b – streaking and mosaic patterns on the leaves of wheat cv. Smuglyanka caused by WSMV, 2013; c - “purple-yellow” leaves of winter wheat cv. Russia caused by BYDV, 2012.

Figure 2. Symptoms caused by abiotic factors: a - yellowing symptoms on the leaves of winter wheat plants cv. Ermak, Sumy region; b -"purple leaves" of winter wheat cv. Vasylyna, Poltava region; c - spiral twisting of the barley leaves cv. Antigon, Khmelnytsky region

Table1. Detection of viruses in winter wheat plants

Virus	Positive control	Negative control	Wheat				Barley
			Russia	Smuglyanka	Vasylyna	Ermak	Antigon
BYDV	1,603 ±0,110	0,103 ±0,008	1,443 ±0,01	0,938±0,01	0,179±0,005	0,087± 0,002	0,109 ±0,004
WSMV	2,108 ±0,145	0,100 ±0,007	0,090 ±0,001	0,079±0,003	0,082±0,005	0,102 ±0,004	0,102 ±0,007
SbCMV	1,950 ±0,137	0,088 ±0,003	0,105 ±0,007	0,086±0,005	0,093±0,007	0,045± 0,002	0,075 ±0,002
WDV	1,905 ±0,132	0,102 ±0,003	0,092 ±0,005	0,087±0,003	0,094±0,003	0,076 ±0,002	0,108 ±0,006
BMV	1,987 ±0,142	0,069 ±0,002	0,079 ±0,002	0,087±0,005	0,096±0,003	0,079 ±0,003	0,083 ±0,004
WSSMV	1,756 ±0,100	0,105 ±0,005	0,142 ±0,005	0,109±0,007	0,108±0,003	0,091± 0,001	0,118 ±0,003

Indeed, during the analysis of temperature indicators characterizing the conditions of overwintering and the restoration of the winter wheat vegetation, it was found that in May 2012, in the phase of the beginning of earing plants, high positive temperatures in the day changed at low rates at night. Thus, the temperature difference in the Kyiv region in the first decade of the month was 19.2 °C, in the second - 20 ° C and in the third - 23.9 °C. Similar results we obtained earlier for Khmelnitsky and Poltava regions (Reshetnyk, 2010). It should be noted that significant decrease in temperature were registered on the days of the samples selection in the fields:14 th May – on 5 °C, 25th – on 7.2 °C compared to last day. In addition to temperature changes, a lack of humidity and precipitation in this area during the spring also recorded.

Due to the fact that in 2012 we revealed BYDV and did not detect WSMV, in contrast to the previous year, an analysis of meteorological data was carried out. The results for the period 2011-2012 showed that the average monthly temperature in April-May 2012 was higher than in 2011, which could contribute to the propagation of aphids (vectors of BYDV) (table 2).

As seen from Table 2, the air temperature in February 2012 is much lower. Also average monthly temperature at soil surface and minimum soil temperature on tillering node depth of winter crops in 2012 were significantly lower. Minimum soil temperature on tillering node depth of winter crops and perennial grasses was higher than in 2011. Such agro-climatic factors, to our opinion, could assist elimination of WSMV vector - *Aceria tritici*.

Table 2. Meteorological data for 2011-2012 years, Poltava region

Data	Month	2011	2012
Average air monthly temperature , ºC	December	-1.0	- 3.9
	January	-4.9	- 3.5
	February	-4.5	- 12.4
	March	1.5	0.7
	April	10.5	14.1
	May	18.3	20.7
	June	24.1	22.9
	July	21.8	25.4
Maximum soil freezing depth, cm	December	17.0	20.0
	January	14.5	19.0
	February	26.0	43.0
Average monthly temperature at soil surface, ºC	December	- 1.0	-1.0
	January	-3.5	- 2.5
	February	0.5	- 8.4
Minimum soil temperature on tillering node depth of winter crops and perennial grasses (5 cm), ºC	December	- 1.0	-3.5
	January	-3.5	-3.4
	February	-7.5	-10.0

An interesting phenomenon was noted in 2017 on winter wheat and oats. Our previous studies have shown that the reddening of oat leaves is usually a reaction to BYDV infection because oat is the indicator-plant of BYDV (Mishchenko, 2007, 2009; Mishchenko *et al.*, 2010; Reshetnyk, 2010). In 2017 we found winter wheat cv. Smuglyaka and oat plants with leaves yellowing and reddening respectively under optimal agro-technic growing conditions (Poltava region) and droughty spring and summer beginning. But we did not detect any viruses (first of all BYDV as in 2012) in these plants. Only WSMV was detected in single plants which demonstrated classic WSMV symptoms. So, leaves reddening of oats and yellowing of winter wheat are consequence of a significant temperature difference in May. It was registered that air temperature at the night

was -3 °C (May, 11th) and +20...+22 °C - during the day i.e. temperature difference was over 25 °C. We continue to observe the effect of temperature difference (cold stress) on cereals and vectors of plant viruses.

CONCLUSIONS

Thus, the viral monitoring showed that the winter wheat plants cv. Russia and Smuglyanka with the yellowing symptoms on leaves and "purple-yellow" leaves were infected with BYDV. Unlike previous years, in 2012 WSMV was not detected in this agrocenosis that can be related to the strong drought in autumn and considerable decline of HTC that resulted in limitation of quantity of WSMV vectors. The symptoms of leaf rolling in barley cv. Antigon, leaf yellowing of wheat cv. Ermak and leaves reddening in wheat Donetska-46 were caused by technogenic influence and other abiotic factors. The analysis of temperature indexes that characterize terms of overwintering and vegetation of winter wheat showed that in May (phase of beginning of plants earing) high plus temperatures during the day changed on low temperatures at night. It is necessary to notice that the reason for appearance of symptoms "purple-yellow" and "purple" leaves of winter wheat are changes of carbohydrate balance that arise up as a result of nonspecific reactions of plants to stress caused by virus infection (cv. Russia) or sharp temperature differential (cv. Vasylyna, Podolyanka, Albatros odessky, Myronivska-67, Smuglyanka).

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CONTROL OF A PROBLEMATIC WEED *Sorghum halepense* FROM MAIZE CROPS IN ROMANIA

SUMMARY

Romania has one of the highest degrees of infestation with *Sorghum halepense* because this weed is a thermophilic and heliophilous species. It found satisfactory growth and development conditions due to the high fertility of soils, especially under the conditions of substantial underground water intake. Maize is one of the very sensitive crop plants to infestation with *Sorgum halepense* especially in the early stages of development. The only effective way to control this weed is to use selective herbicides for maize crops having aggressive action on this weed. The aim of this study was the control of *Sorghum halepense* from seeds and rhizomes by post-emergence application of nicosulphuron-based herbicides in various doses and different development stages of weeds in maize crop. The experiments were carried out in Afumati farm, Ilfov County, in two years 2015 and 2016, and they were placed in randomized blocks and the observations targeted the degree of effectiveness in controlling weeds and crop selectivity. The herbicides based on nicosulphuron had a good efficacy in controlling *Sorghum halepense* in maize crops. At the dose of 1.0 lha^{-1} this weed was not fully controlled, in conditions of strong infestation. The best results were obtained at the dose of 1.5 lha^{-1} applied in sequential treatments. Also, no phytotoxic symptoms were shown in experimental fields.

Keywords*:* control, herbicide, nicosulphuron, weeds.

INTRODUCTION

Regardless of the historical stage and maize (*Zea mays*) cultivation system, the highest costs in the production process are with weeds control. By its biology nature, corn plants are characterized as lacking the ability to compete with weeds, especially in the early stages of vegetation. Slow growth of corn plants in the first 4-6 weeks after emergence, associated with a low density (4-6 plant/m^2), creates a major advantage in the competition since the beginning in favor of weeds (Ghosheh et al., 1996, Torma et al., 2006). Herbicidation is one of the most important methods for weed control in maize crops. Perennials are difficult to control because they regenerate after application of agro-technical methods and also after application of herbicides. The importance of these weeds (*Sorghum halepens*e, *Elymus repens*) in maize crops increased during last years and it

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requires new control strategies. Weeds by their number, by rapacity for space, water and food, through the presence of diseases and pests common to crop plants, are causing great damage to maize crops. Depending on the degree of weed infestation, the damage varies between 30-80% of the production obtained, and in case of infestation with species *Sorghum halepense* from rhizomes sometimes can reach to total compromise of the crop (Sarpe et al., 1976, Chirila, 2001, Berca 1996, 2004). In this context, the paper presents data on the efficacy of nicosulphuron, sulfonylurea-base herbicides, in control of *Sorgum halepense* an invasive species in maize crops of Romania, aiming to improve agricultural technology and obtaining better crops.

MATERIAL AND METHODS

The experiments were placed at SC Agricola Afumati, Ilfov, Romania, in randomized blocks, in 4 repetitions with plot area of 30 m² on loamy clay soil with a pH of 6.5 and an organic matter content of 2.5%. Each experimental block included an untreated and a standard reference. Nicosulfuron-based herbicides were applied post emergently in a dose of 1.0 and 1.5 lha-1 in one treatment, and in 2 sequential treatments - the first treatment (A) with 0.75 lha-1 when millet was on the stage of four leaves and the second (B) 10 days after the first treatment with 0.75 lha-1 when millet was on the stage of eight leaves. Weed density was assessed in ground % and in plants number on square meter. Weed control (efficacy) was assessed at 10, 28, 42 days after each application in % control comparative with untreated. Also, were performed observations on the weeds present in the experimental plots before treatment, and selectivity - at each date of the efficacy assessments. Determination of segetal flora was performed on a square meter using a metric frame. Statistical preparation of the results was based on the analysis of ARM-9 (P=.05, Student-Newman-Keuls).

RESULTS AND DISCUSSION

Maize, the main cultivated plant in Romanian agriculture, shows a strong and diversified infestation with monocotyledonous and dicotyledonous annual and perennial weeds. The agriculture practiced in recent years has led to changes in the structure and frequency of weeds in maize both quantitatively and especially qualitatively. Grass weed species have become more damaging than dicotyledonous, with an increasing frequency of *Sorghum halepens*e, *Echinochloa crus-galli*, *Setaria* spp., and *Elymus repens*. Romania has one of the highest degrees of infestation with *S. halepense*. *S. halepense* is a thermophilic and heliophilous species that finds satisfactory growth and development conditions in Romania due to the high fertility of soils, especially under the conditions of substantial underground water intake. The large number of seeds (2000-5000 per plant) produced by each plant as well as the extensive rhizome system make this weed to be difficult to combat. The rhizomes grow vertically in depth, reaching 1.5-2.0 m in the soil, which gives them great drought resistance. The rhizome internodes have an average length of 1.3-3.5 cm. From 1 to 10 kg of

rhizomes with 500-2000 buds could be found on an area of 1 m2. Primary rhizomes appear when the sorghum plant forms the 4th leaf. They will generate the secondary rhizomes which will form the aerial part of the plant. Reserve rhizomes appear during flowering period and they will generate, in the very next year, the aerial part of the plant. The rhizome segments resulting from soil work are able to form a new sorghum plant even if they have a single node, especially if it is at a depth of 15 cm (Anghel et al., 1972). The plant height of 1.0-1.5 m allows it to shade the surrounding plants, thus reducing the amount of water and nutrients available to the crop (Monaghan, 1979, Maurer et al., 1987, Camacho et al., 1991, Vitta and Leguizamon, 1991, Chirila, 2001). Maximum sensitivity toward infestation with *S. halepense* manifests itself in the early stages of vegetation. The predominant species identified in the studied locations were: *S. halepense*, but in the experimental field were present also other weed species: *Echinochloa crus-galli* and *Setaria* spp., *Amaranthus retroflexus*, *Convolvulus arvensis*, *Xanthium italicum*, *Solanum nigrum*, but with a low density.

The growth stages majority of *Zea mays* and *Sorghum halepense* at each assessment is presented in table 1.

Table 1. Growth stage of *Zea mays* and *Sorghum halepense*

Plants	BBCH	Description
Zea mays	1st assessment 16	6 leaves unfolded
	2nd assessment 18	8 leaves unfolded
	3rd assessment 39	Flag leaf stage: flag leaf fully unrolled, ligule just visible
	4th assessment 51	Beginning of tassel emergence: tassel detectable at tip of stem
	5th assessment 55	Middle of tassel emergence, middle of tase begins to separate
Sorghum halepense	1st assessment 14	4 leaves unfolded
	2nd assessment 18	8 leaves unfolded
	3rd assessment 48	Constant new development of young plants-vegetative reproductive organs reach final size
	4th assessment 55	First individual flowers visible (still closed)
	5th assessment 67	Flowering finishing: majority of petals fallen or dry

Coverage with species *S. halepense* in the experimental field was high: 45.5% before treatment application 61.3% at 14 days, 66.8% at 28 days and over 70.0% at 42 days after treatment application. In these conditions of weed infestation, a solution for avoiding harvest losses is the post-emergence application of herbicides with the following advantages: removes pre-emergence application of herbicides which under drought conditions becomes useless,

doesn`t require incorporation, reduces the number of treatments and the cost on hectar, low fuel consumption, flexibility in application time depending on degree of weeding. Following the observations we carried out, it has been assessed that this herb's success is due to the plant's subterranean part which is formed by rhizomes and the supplementary roots. During our observations we have identified plants with roots that achieved over 2.50 meters depth in the soil, this allowing indeed a greater tolerance to drought. Rhizomes are another biological feature, as the present lengths of over 25 cm and thickness of over 3 cm, they form more rapidly knots and inter-knots by which *S. halepense* is being multiplied, determining a large number of tillers per plant.

Table 2. Density of weed in maize crop before treatments

Variant	Dose f.p.[1] (l/ha)	Dose a.i.[2] g/ha	Treatment A		Treatment B	
			Density			
			Coupla /m^2	Ground (%)	Coupla /m^2	Ground (%)
untreated	-	-	38.5	45.5	53.3	61.3
nicosulphuron	1.0	60	27.5	33.0	-	-
	1.5	90	28.3	33.8	-	-
	0.75+ 0.75	45+45	25.0	33.0	1.0	1.5.
standard reference	1.5	90	28.3	33.0	0.5	1.0
LSD (P=.05)			5.30	5.99	1.73	2.1
Standard Deviation			3.52	3.99	1.00	1.45

[1]f.p.= Formulated product/ha
[2]a.i.= Active ingredient/ha

Table 3. The efficacy of herbicide in maize crop after 10 days of treatments

Variant	Dose (l/ha)	Appl.	Treatment A		Treatment B	
			Efficacy			
			Dens.[1] (%)	E. [2] (%)	Dens. (%)	E. (%)
untreated	-	-	61.3	-	63.8	-
nicosulphuron	1.0	A	1.5	97.3	-	-
	1.5	A	0.0	100	-	-
	0.75+0.75	A+B	1.0	98.0	0.0	100
standard reference	1.5	A	0.0	100	0.0	100
LSD (P=.05)			1.55	2.87	.	.
Standard Deviation			1.03	1.90	0.00	0.00

[1]Dens. = density (ground %)
[2]E.= efficacy (control %)

The nicosulphuron, sulfonylurea-based herbicide had a good efficacy in control of *S. halepense* weed in maize. At 10 days after treatment A the herbicide had a very good efficacy in control of *S. halepense* 98.0% at a dose of 1.5 Lha^{-1} and 97.3% at 1.0 lha^{-1}, the results being similar to those of the standard reference (Table 3).

Nicosulphuron applied in postemergence is quickly absorbed by weeds mainly through the leaves and roots, is then translocated into the sap stream to the apical meristems, where they cause irreversible disturbances in cell division. The total control of weeds is carried out in a longer period of up to three weeks. The weed ceases to grow immediately after treatment.

Table 4. The efficacy of herbicide in maize crop after 28 days of treatments

Variant	Dose (l/ha)	Appl.	Treatment A		Treatment B	
			Efficacy			
			Dens. (%)	E. (%)	Dens. (%)	E. (%)
untreated	-	-	66.8	-	68.0	-
nicosulphuron	1.0	A	9.0	90.0	-	-
	1.5	A	3.0	97.3	-	-
	0.75+0.75	A+B	0.0	100	0.0	100
standard reference	1.5	A	5.5	94.8	0.0	100
LSD (P=.05)			2.91	3.74	.	.
Standard Deviation			2.01	2.59	0.00	0.00

Table 5. The efficacy in maize crop of herbicides after 42 days of treatments

Treatment name	Dose (l./ha)	Appl. code	Treatment A		Treatment B	
			Dens. (%)	E. (%)	Dens. (%)	E. (%)
Untreated	-	-	71.0	0.0	71.0	0.0
nicosulphuron	1.0	A	29.8	61.3	-	-
	1.5	A	13.5	82.8	-	-
	0.75 + 0.75	A+B	0.0	100	7.5	91.5
Standard reference	1.5	A	9.5	88.8	12.0	85.8
LSD (P=.05)			2.90	3.21	2.90	3.21
Standard Deviation			2.01	2.22	2.01	2.22

Observations shows that the action of the herbicide is influenced by climatic conditions and vegetation at the time of application, absorption and translocation are more intense and faster when weeds have optimal growth conditions (light, heat, water, food) and thus the inhibitory effect is more complete. In order to fully penetrate into the plant, it is necessary that after treatment, the rainfall should not fall for a period of 4-5 hours. Subsequent observations (28 and 42 days after treatments) confirmed the good results of the nicosulphuron herbicide in control of *S. halepense* in maize crops (Tables 3 and 4). At the dose of 1.5 lha^{-1}, the effect of the herbicide was maintained throughout the growing season of maize but at the dose of 1.0 lha^{-1} species *S. halepense* is not entirely controlled such as control rate decreased from 90.0 % at 28 days to 61.3% at 42 days after treatment application. No phytotoxicity symptoms have been shown in the experimental plot. No symptoms of chlorosis, necrosis, leaf deformation, height reduction, distortion and delay at flowering in plots treated with herbicides nicosulphuron. Maize plants managed by its own mechanism to metabolize the active substance and convert it to biologically inactive compounds, so the majority of maize hybrids show no phytotoxicity symptoms. In treated plots, maize plants were more vigorous and taller and culture density was much higher compared to control plot (Figure 1).

Figure 1. Aspects regarding of nicosulphuron efficacy after 42 days of treatment

CONCLUSIONS

The degree of weeds in maize crops was high, the predominant weed species was a perennial weed, S. halepense, with a coverage rate of over 70% at 42 days after treatment application.

Also annual monocotyledonous weed species Echinochloa crus-galli and Setaria spp. were present with low density percentage.

S. halepense is a problem weed for Romanian farmers, which find satisfactory growth and development conditions in Romania due to the high fertility of soils.

The herbicide based on nicosulphuron had a good efficacy in controlling *S.* halepense in maize crops. The best results were obtained at the dose of 1.5 lha^{-1} applied in sequential treatments the first treatment (A) with 0.75 lha^{-1} when the millet would be on four leaves stage, and the second after 10 days after the first treatment (B) with 0.75 lha-1.

At the dose of 1.0 lha-1 *S. halepense* is not entirely controlled, in conditions of strong infestation. No phytotoxicity symptoms have been shown in experimental plots. No symptoms of chlorosis, necrosis, leaf deformation, height reduction, distortion and delay at flowering in plots treated with nicosulphuron.

In treated plots, maize plants were more vigorous and taller and culture density was much higher compared to control plot.

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ISOLATION OF ENTOMOPATHOGENIC BACTERIA FROM LARVAE OF A LEPIDOPTERAN SPECIE; GALLERIA MELLONELLA AND STUDY OF THEIR INSECTICIDAL EFFECT

SUMMARY

Few years ago, considerable progress has been made to explain the disappearance of bee colonies, including correct identification of pests involved and the search for more effective and healthy alternatives to protect them. Indeed, our work is based on the isolation, characterization and identification of entomopathogenic bacterial strains of the genus *Bacillus* from larvae of wax moth *Galleria mellonela* reared in the laboratory, with a preliminary study of the use of these entomopathogenic bacteria on the larvae (L5) of *G. mellonella* under controlled conditions. In fact, 9 bacterial strains of the genus *Bacillus* have been isolated. They are spore forming bacteria, Gram, catalase and oxidase positive and present variable responses to the gelatinase test, lecitinase, caseinase, culture in anaerobiosis and growth at different temperatures (45 ° C., 55 ° C., 65 ° C.). From our study, we also find that the strain S4, probably identified as *Bacillus thuringiensis*, has a better effect on the larvae of *Galleria mellonella*. It caused very remarkable symptoms and mortality rates that vary depending on the strain and bacterial concentration tested and the mode of application. Injection of strain S4 for individuals resulted mortality of 83.33%, 75% and 50%, respectively, after treatment with high, medium and low concentration after only 3 days. The comparative examination of the hemolymph test results shows that the injection of the bacteria into the larvae resulted in a significant increase in hemolymph protein and carbohydrate content as compared to controls.

Keywords: *Galleria mellonella, Bacillus,* isolation, mortality, haemolymph.

INTRODUCTION

Honeybee pests are known to cause significant losses, and to transmit viral pathogens for which therapies remain nonexistent and continue to be challenging to eradicate (Plettner *et al.*, 2017). The greater wax moth, *Galleria mellonella*

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Linnaeus, is a ubiquitous pest of the honeybee, *Apis mellifera*. The damage caused by *G. mellonella* larvae is severe, and is believed to be one of the contributing factors to the decline in honeybee populations. Previously, the pest was Considered a nuisance in honeybee colonies, it is currently widespread, especially in Africa, and the potential of transmitting honeybee viruses has raised legitimate concern.(Kwadha *et al*., 2017). The wax moth was first reported in honeybee colonies of Asian honeybee Apis cerana (Paddock, 1918), but later spread to northern Africa, and other parts of the world (Akratanakul , 1987). Shimanuki (1980) and Williams (1997) later described the pests as ubiquitously distributed everywhere beekeeping is practiced.

Use of chemical and fumigant insecticides to destroy this insect are harmful to bee populations (Calderone, 2000). Certains pose health risks to the handler and lead to residues in hive products such as honey, rendering the product inconsumable (Ritter and Akratanakul 2006). More importantly, they are poisonous to honeybee colonies and non-target species (Ritter and Akratanakul, 2006; Charriere and Imdorf, 1999). Previous researchers have explored various biological agents and bio-products including *Bacillus thuringiensis* Berliner (H-serotype V) (Plettner *al.*, 2017), but evidence for a successful and sustainable biological control agent of *G. mellonela* is still lacking. The objective of the present study is to screen different bacterial strains from the cadavers of the greater wax moth reared on laboratory and testing them against the larva of this insect, in order to explore the toxins of these bacteria.

MATERIAL AND METHODS

Isolation and characterization of bacterial strains

From the larva of the greater wax moth accidentally contaminated in the laboratory during the various manipulations, a few individuals were isolated immediately after their death. These were deposited on the surface of a nutrient agar and then incubated at 32 ° C. 24 hours after incubation, bacterial colonies were obtained around the deposited cadavers. Successive transplants have resulted pure and well-defined colonies. Only colonies with the macroscopic characters of the genus *Bacillus* are taken into consideration.

Preliminary identification of the isolates took place according to the taxonomic characterization proposed by Guiraud (2003). For this, a macroscopic examination of the colonies constitutes a first step which guides the process of characterization of the bacteria. Microscopic examination consists of microscopic observation in the fresh state followed by simple staining with methylene blue, Gram staining and spore staining (Larpen, 1997, Singleton, 2005). For the biochemical characterization, we used the classical identification tests and the API system. Among the tests performed; Simmons citrate test, Voges Proskauer reaction (VP), methyl red reaction (RM), TSI study, growth at 45 ° C, 55 ° C et 65 ° C (Guiraud, 2003). Among the enzymes researched; catalase, oxidase, caseinase, lecithinase, nitrate reductase (NR) (Geraldine *et al.,* 1981; Graden and Luisetti, 1981; Gerard *et al.,* 2003).

Treatment of larvae of the greater wax moth

Fifth-stage healthy larvae were isolated from the rearing, these were treated by introducing 3 different concentrations of the bacterial suspensions, using 2 modes of application, ingestion and injection. The control and treated larvae are fed with honey, pollen and wax and placed in an oven at 30 ° C. In order to study the efficacy of the two bacterial strains selected on the larvae of the greater wax moth, 3 parameters were chosen; Calculation of the corrected mortality, calculation of LT50 (lethal time for 50% of individuals), study of haemolymphatic composition. To study this last parameter, the treatment of larvae was done by injection of a volume of 20µl of the bacterial suspension by individuals, controls received physiological saline. The haemolymph was taken 4h, 6h and 12h after the treatment. Indeed, a volume of 10 µl is taken at the abdominal end of the insect, 5 µl are used for the determination of proteins and 5 µl for the determination of carbohydrates. The protein assay is carried out according to the method of Bradford (1976). The colorimetric method for the determination of carbohydrate by anthrone described by Bachelier and Gavinelli (1966) was used.

Statistical analysis

The significance of the main effects was determined by analysis of variance (ANOVA). The values ($p \leq 0.05$) are considered statistically significant. A correlation matrix is used to describe the degree of relationship between two variables. The software used is the Statistica.

RESULTS AND DISCUSSION

Characterization of bacterial isolates

The isolation of bacterial strains from *G. mellonella* larvae allowed the selection of 9 bacterial strains of the genus *Bacillus*.

Cultural and macroscopic tests: macroscopic examination on solid media (nutritive agar) showed well-isolated colonies.

Table 1. Cultural characteristics of isolates on solid medium

Isolates	Shape	diameter	Color	Opacity	Elevation	Surface	Odor
1	CIB	punctiform	white	opaque	flat	smooth	Ab
2	CRB	punctiform	cream	opaque	flat	smooth	Ab
3	CIB	1.8mm	cream	opaque	flat	smooth	Ab
4	CIB	1.6mm	white	opaque	flat	smooth	Ab
5	CRB	punctiform	white	opaque	flat	smooth	Ab
6	CRB	1.7mm	white	opaque	flat	smooth	Ab
7	CIB	2mm	white	opaque	Convex	Granular	Ab
8	CIB	punctiforme	cream	opaque	Convex	Granular	Ab
9	CRB	1.3mm	white	opaque	flat	smooth	Ab

*CIB : circular with irregular board, CRB **:** circular with regular board, Ab : absence

Their appearance is very variable (table 1); they are opaque, cream or white colored colonies with an average diameter of 1.3 to 2 mm. Some strains have punctiform colonies. The elevation differs from one isolate to the other between flat and convex, some colonies have a granular surface although the majority, their surface is smooth, the shape of the colonies obtained is circular with regular board or not.

The study of the cultural characteristics on liquid medium (nutrient broth) after incubation at 32 ° C shows the presence of a cool at the bottom of the tube for most strains with the appearance of a homogeneous disorder and the presence veils and the ring surface (table 2).

Table 2. Cultivation characteristics on liquid medium

Isolates	rings	sails	homogeneous disorder	heterogeneous disorder	cool
S1	+	+	+	-	+
S2	+	+	+	-	+
S3	+	+	+	-	+
S4	+	-	+	-	+
S5	+	+	+	-	+
S6	+	+	+	-	+
S7	+	+	+	-	+
S8	+	-	+	-	-
S9	+	-	+	-	+

(+) : presence (-) : absence

Microscopic characteristics of the isolates: microscopic observation of the cells after fresh staining, simple staining with methylene blue and Gram staining; has shown that all isolated strains have the shape of a rounded or square rod and are Gram-positive. Malachite green staining indicates that all isolated strains form spores. The spore has an oval shape in a central or terminal position (figure 1).

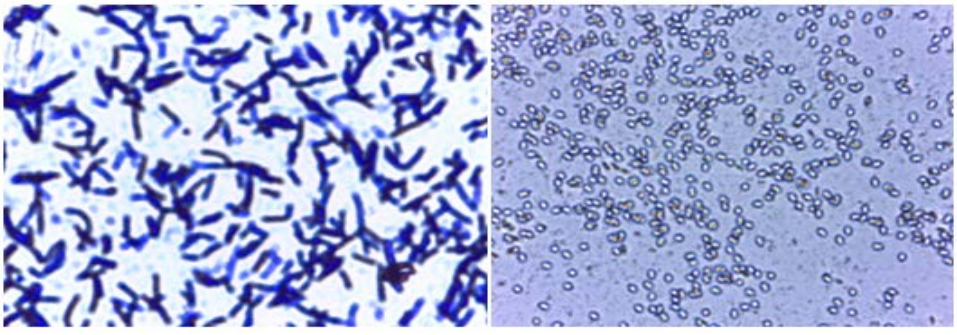

Figure 1: results of observation after Gram staining (right) and staining of spores (left)

Physiological and biochemical characterization: the results of the various biochemical tests carried out (table 3) indicate positive responses by all

the strains for the oxidase, gelatinase, VP, TDA, ONPG, CIT, starch hydrolysis, catalase and caseinase tests , with negative responses for RHA, URA. The responses were variable for NO, MAN, ADH, lecithin, mobility, culture in anaerobiosis and growth at different temperatures (45°C, 55°C, 65°C).

Table 3. Physiological and biochemical characterization of isolated strains

API system	S1	S2	S3	S4	S5	S6	S7	S8	S9
ARA	+	-	+	-	-	-	-	-	+
AMY	+	-	+	-	-	-	-	+	+
MEL	+	-	+	-	-	-	-	-	+
SAC	-	-	-	-	-	-	-	-	+
RHA	-	-	-	-	-	-	-	-	-
SOR	+	+	-	-	+	+	-	-	+
INO	+	-	-	-	-	-	+	+	-
MAN	-	+	+	-	-	-	-	-	+
GLU	-	-	-	-	-	+	-	-	+
GEL	+	+	+	+	+	+	+	+	+
VP	+	+	+	+	+	+	+	+	+
IND	-	-	-	-	-	-	-	-	+
TDA	+	+	+	+	+	+	+	+	+
URE	-	-	-	-	-	-	-	-	-
H2S	-	-	+	+	-	V	V	-	V
CIT	+	+	+	+	+	+	+	+	+
ODC	+	+	+	-	-	+	+	-	-
ADH	+	+	+	-	-	+	+	-	-
ONPG	+	+	+	+	+	+	+	+	+
Other tests									
Gram	+	+	+	+	+	+	+	+	+
Caseine	+	+	+	+	+	+	+	+	+
Lecithine	++ +	+++	-	+++	++	+	+	-	+
Starch hydrolysis	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+
Oxidase	+	+	+	+	+	+	+	+	+
Mobility	+	+	+	+	+	+	-	+	+
RM	+	+	+	+	+	+	+	+	+
Anaerobic culture	+	+	-	+	-	-	+	-	+
Culture at 45°C	+	v	+	-	v	+	+	-	+
Culture at 55°C	-	-		-	-	-	-	-	-
Culture at 65° C	-	-	-	-	-	-	-	-	-

(+): Positive result (-): negative result (v): variable

Several bacteria have been identified as having potential for use in biological control. These entomopathogenic bacteria belong particularly to the Bacillaceae family (Starnes *et al*., 1993). From our study, 9 bacterial strains of the genus *Bacillus* were isolated from the larvae of *G. mellonella*. After purification, the isolates were identified according to their macroscopic, physiological and biochemical characteristics. Guiraud (2003), Geraldine *et al.* (1981) and Singleton (2005), confirm their affiliation to the genus *Bacillus*. According to Brossard and Terry (1984), *Bacillus* species are ubiquitous microorganisms, the majority grow better at 30 ° C up to 37 ° C, many species

are saprophytes of soil, water, air and plants (Claus and Berkeley, 1986). The strains isolated have all the cultural and microscopic traits of the genus *Bacillus* described by Euzeby (2007). These catalase positive bacteria with variable response to the oxidase test (Guiraud, 2003) are mobile by cilia, their spores are ellipsoidal to cylindrical (Cloutier and Cloutier, 1992). Isolation of bacteria from nymphs *Phyllocnistis citrella* revealed the existence of five different bacterial strains. their identification showed that the genus *Bacillus* is the most frequently encountered. The pathogenicity test with the bacteria isolated showed high mortality of larvae of *P. citrella* (Saiah *et al*., 2010).

Evaluation of the effect of bacterial isolates on larvae of *G. Mellonella*

In order to determine the effect of isolated entomopathogenic bacteria on the larvae of *G. mellonella*, two bacterial strains (S3 and S4) were chosen. The individuals treated showed remarkable symptoms (figure 2).

Figure 2. Symptoms observed in individuals treated with bacterial suspensions(A/ appearance deformations in the survivors after the emergence of the butterflies in particular in the wings. B/ blackening and softening of the body just after death due to melanin secretion).

After injection of bacterium *Klebsiella pneumoniae* into the hemolymph of *G. mellonella*, the larvae and hemolymph progressively pigmented during infection indicating the production of melanin by the enzyme phenoloxidase (PO). The activity of this enzyme causes deposition of melanin around bacteria by insect hemocytes, to wrap and isolate it (Insua *et al*., 2013).

Mortality rate: the use of the two strains S3 and S4 against the L5 larvae of *G. mellonella* gave interesting results. For strain S3, 100% mortality was observed after 9 days for the high dose, whereas for the mean and the low dose, the effects were different depending on the mode of application of the treatment. They are more pronounced in injection-treated individuals, with 90% mortality for the mean dose (compared with 66.66% for ingestion) and 62% for the low dose (compared to 33.33% for ingestion) (figure 3).

Injection of S4 strain to *G. mellonela* caused 83.33%, 75% and 50% mortality respectively after treatment with D1, D2 and D3 after 3 days. In larvae treated with ingestion, a mortality rate of 66 % was observed after treatment with D1, 41% for D2 and 25% for D3, after 9 days. Indeed, *Bacillus thurigiensis*, like *Bacillus popilliae, B. alvei, B. larvae, B. lentimorbus* and *B. sphaericus*,

possess the particular property of inducing mortality in certain insects (Joung & Côté, 2001; Lacoursiere & Boisvert, 2004). These latter justify the harmfulness of the *Bacillus* by the interaction of their toxins with specific receptors on the epithelial cells of the digestive system, which causes the death of the insect following the disruption of the osmotic regulation of these cells.

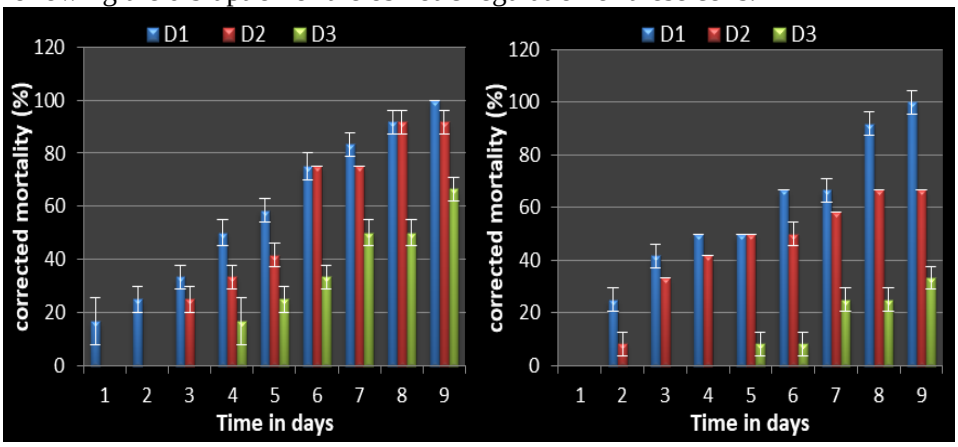

Figure 3: corrected mortality rate in *G. mellonella* larvae treated with strain S3 (left injection, right ingestion) (D1 = 0.9 * 10^8, D2 = 0.3 * 10^8, D3 = 0.16 * 10^8). ANOVA indicate a significant difference in the treated individuels by injection (F = 0,000017) and ingestion (F = 0.000000) compared to controls. Similarly, the correlation matrix test shows that there is a correlation between the mortality factor and the time factor and also between the mortality factor and the dose factor

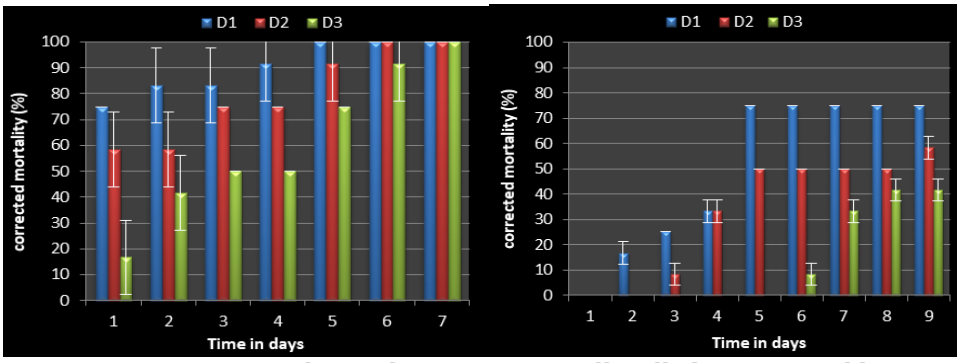

Figure 4: corrected mortality rate in *G. mellonella* larvae treated by strain S4 by injection (left) and ingestion (right). (D1 = 0.9 * 10^8, D2 = 0.3 * 10^8, D3 = 0.16 * 10^8). (A significant difference was demonstrated by the test of variance analysis in the treated individuels by injection (F = 0.000487) and ingestion (F = 0.000000) compared to controls. As for the correlation matrix test, it indicates a correlation between the mortality factor and the time factor and also between the mortality and dose factor).

The present findings are in agreement with El behery *et al.* (2016) who found that rearing the *G. mellonella* on the different ages of beeswax combs with 4% Neem Azal-T/S showed that all the tested larvae died during the first week of treatment.

LT50 : lethal times for 50% of individuals vary depending on the bacteria, the applied doses and the mode of treatment. The larvae of *G. mellonella* treated with S4 have the lowest TL50s, the TL50s increase with the decrease in doses. TL50s are very short for injection mode compared to ingestion.

Table 4. LT50 values recorded in *G. mellonella* larvae treated with strains S3 and S4

Treatment	**Dose**	**Injection**		**Ingestion**	
Strain		S 3	S4	S3	S4
Doses	D1=0,9*108 ufc/ml	0,76 day	0,44 day	4,14 days	3,98 days
	D2= 0,3*108ufc/ml	2,01 days	0,68 day	4,9 days	7,58 days
	D3= 0,16*108ufc/ml	3,69 days	1,18 day	11,69 days	11,11 days

It has been found after treatment of *G. mellonela* with the polyphenols of Bitter Orange (*Citrus Aurantium*) that this extract showed a valuable efficacy against larvae, an LT50 of 2.34 days was obtained after treatment by dose 20µl / ml (Oulebsir-MohandKaci *et al.*, 2016).

Effects of bacteria on the haemolymphatic composition of *G. mellonella* larvae: the hemolymph assay allowed to plot the regression line which allowed to calculate the concentrations of proteins and carbohydrates haemolymphatic in the controls and treated by the strain S4 .

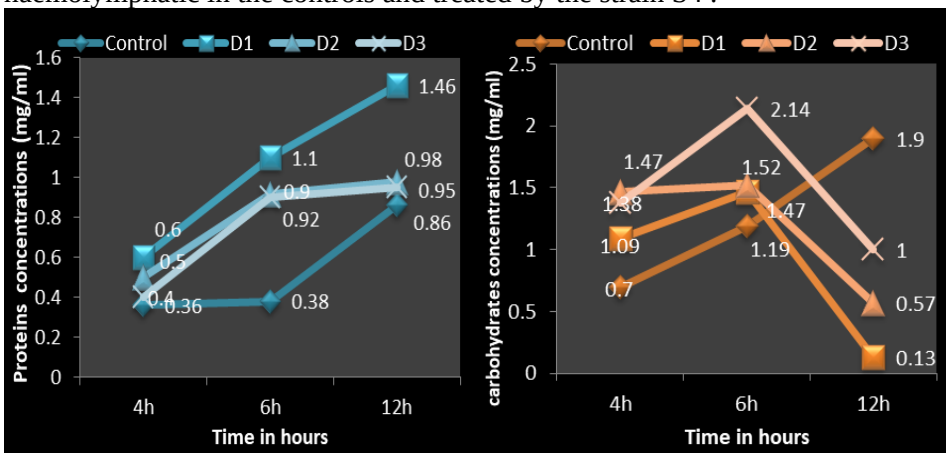

Figure 5: Concentrations (C) of protein and hemolymphatic carbohydrate samples in S4-treated individuals

The injection of the bacterium into the larvae of *G. mellonella*, resulted in a significant increase in the haemolymph protein content compared to the controls, in particular with the high dose. This increase is proportional to the concentration injected. The carbohydrate content also increased in the treats by

the 3 doses compared to the controls and this at 4h and 6h. At 12 h, this concentration was markedly decreased in the treated individuals with a normal evolution in the controls (figure 5).

The results obtained by Oulebsir-Mohand Kaci and Doumandji-Mitiche(2012) show an important decrease of haemolymph protein concentration compared to controls with an increase in carbohydrate concentration, after treatment of larvae of *Locusta migratoria* by *Pseudomonas fluorescens*.

CONCLUSIONS

The isolation of some bacterial strains belonging to the genus *Bacillus* from the dead larvae of *G. mellonella* resulted in the identification of 9 species that differed according to their macroscopic, physiological and biochemical characteristics. These isolates were tested on larvae of the same insect in order to confirm their efficacy and to evaluate their toxicity.

The isolates tested against the L5 larvae of the greater wax moth caused very high mortality rates. Comparative examination of the results of the determination of haemolymph protein and carbohydrate showed a difference between the controls and the treated by the bacteria. Finally, it is clear that isolated bacterial strains represent an interesting step forward in the fight against the greater wax moth because they affect its development and metabolism. It is therefore desirable to study further their ecological and toxicological impacts.

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Roksolana SOVINSKA, Ganna KOROTYEYEVA, Olena ANDRIYCHUK[1]

PROSPECTIVE USE OF BACTERIOPHAGES AS AGENTS TO COMBAT PHYTOPATHOGENIC BACTERIA OF POTATOES

SUMMARY

Plant diseases caused by bacteria are a serious problem in the cultivation and storage of agricultural products. During the last years bacteriophages have attracted increased research interest as a realistic environmentally friendly means for controlling bacterial diseases. The main objective of the study was to characterize the bacteriophages that could be used in the search for and developing the antimicrobial agents based on bacterial viruses. Four isolates of bacteriophages specific to bacteria from Pseudomonas genus were isolated from potato samples with typical symptoms of bacterial disease. These phage isolates differed in terms of the morphology of their negative colonies. Slight variation in the morphology of studied phages was observed using electron microscopy. A group of phages was identified as Podoviridae family of Caudovirales order (icosahedral head without long tail, small size – head diameter 43 ± 1 nm, tail length 1 ± 0,5 nm). To identify host range/specificity of isolated phages, we analyzed the spectrum of lytic activity against 15 strains of phytopathogenic bacteria. Our research revealed that among four phage samples three expressed lytic activity against different strains of phytopathogenic bacteria. The aforementioned data enable us to conclude that these three isolates with broad spectrum of lytic activity can be used as perspective biologic agents in control of bacteriosis. Thereby phages from our collection could be of therapeutic interest, they have the potential to be used in future prospects for phage therapy research.

Keywords: bacteriophage, bacteriosis, biocontrol, potato.

INTRODUCTION

Last decade witnessed more frequent agricultural challenges connected with bacterial diseases exhibiting resistance to chemical agents of control (Campbell, 2006; Hajek, 2004; Rombouts et al., 2016; Jones et al., 2007; Kabeil et al., 2008). Besides, increasing concentration of these compounds may cause damage to plant tissues and lead to more great lesions of plant crops. Significant disadvantage of currently available antimicrobial methods for combating phytopathogenic bacteria is also chemical contamination of crops (Buttimer et al., 2017). Study of bacteriophages aims at their active practical use in agribusiness.

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Bacteriophages have received growing interest for agricultural industry as a realistic environmentally friendly means of controlling bacterial diseases, especially in case of the emergence of resistant pathogens. In addition, study of phages reveals the mechanisms of regulating the number and structure of bacterial populations in different environments allowing improve and fine-tune the strategies used for phage therapy (Grey et al., 2001; Born et al., 2011; Birck et al., 2006). At the same time, the search for lytic bacteriophages with a wide range of hosts (covering not only one type of bacteria strains but different species and even genera of bacteria) is the primary purpose for the creation of high therapeutic phage commercial mixes (Bae et al., 2012).

The main objective of the study was to characterize the bacteriophages with practical potential for the search and developing the antimicrobial agents based on bacterial viruses.

MATERIAL AND METHODS

Samples of potato variety Slavyanka and beet variety Mulatka from Kiev and Chernihiv region with symptoms of bacterial lesions were collected in 2014-2015. In our research we used test bacteria: Pseudomonas syringae pv. 223 tabaci, Pseudomonas savastanoi pv. phaseolicola 4013, Pseudomonas siringae pv. lachrymans 7591 and Pseudomonas fluorescens 8573. Phages were isolated by direct seeding. Pure bacteriophage lines were acquired by 6-times passaging with subsequent accumulation on sensitive bacteria cultivated in commercial nutritional broth with additional aeration at 25ºC. The morphological features of viral particles were studied using electron microscope JEOL-1400 (Japan) (Faidiuk et al., 2015).

RESULTS AND DISCUSSION

Biodiversity of bacteriophages specific to bacterial diseases and development a collection of phages as agents of phage therapy were the areas of research. During the study four isolates of bacteriophages (223, 7591, 4013, and 8573) have been isolated and accumulated from potato samples with signs of bacterial rot. To identify the host range of selected isolates, the spectrum of phages' lytic activity was determined against 15 strains of pathogenic bacteria. Four isolates of selected phages were shown to exhibit lytic activity against different strains and species of pathogenic bacteria. Our research revealed that three (223, 7591, and 8573) out of four phage isolates expressed lytic activity against *Pseudomonas syringae pv. 223 tabaci*, *Pseudomonas syringae pv. lachrymans 7591* and *Pseudomonas fluorescens 8573*. However, 4013 isolate expressed lytic activity against host bacteria *Pseudomonas savastanoi pv. phaseolicola 4013* and against *P. syringae pv. lachrymans*.

It shows that the selected phages possessed a wide range of lytic activity (i.e., polyvalent bacteriophages) and could be used as perspective biologic agents in control of bacterial diseases. Electron microscopy analysis showed that selected phages (223, 7591, 4013, and 8573) differed in colony morphology, virions' size was typical for representatives of *Podoviridae* family of

Caudovirales order (icosahedral head without long tail, small size – head diameter 43 ± 1 nm, tail length 1 ± 0,5 nm) (Fig.1).

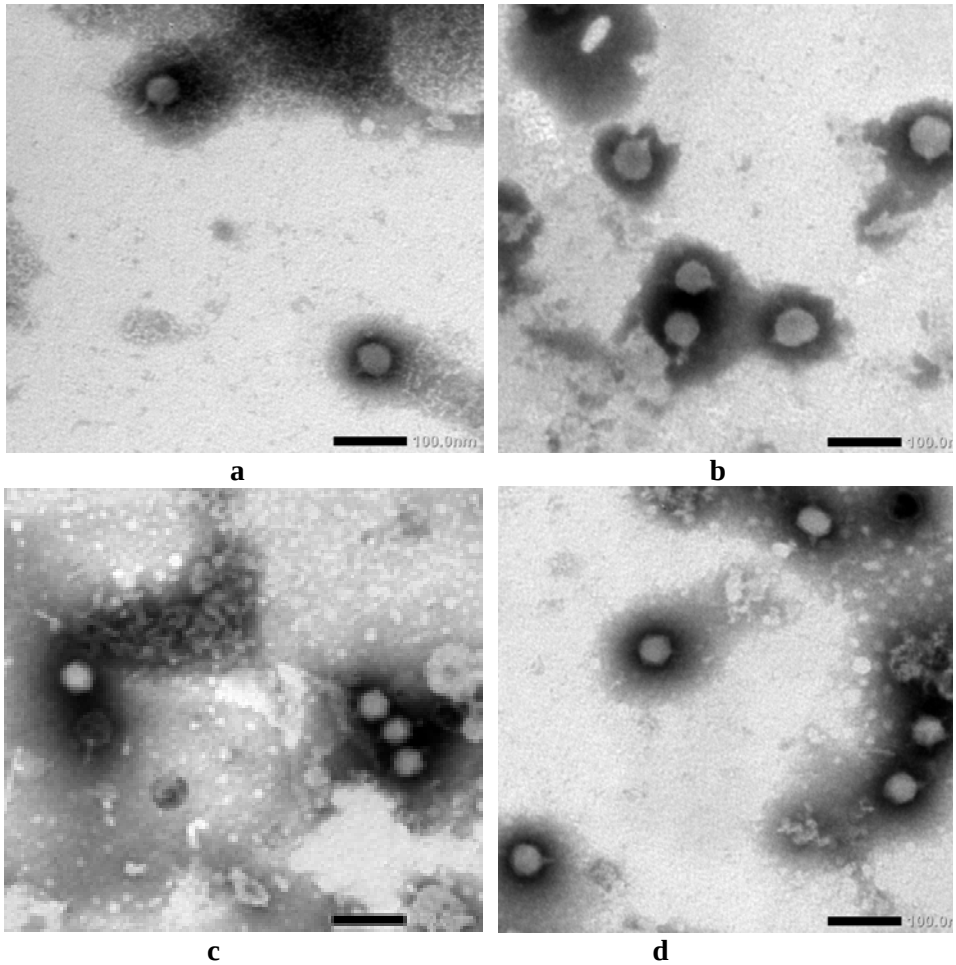

Figure 1. Electron omicroscopy images of phage isolates: a) 223; b) 7591; c) 4013; d) 8573

Practical bacteriophages use to control plant pathogenic bacteria is safe for the environment and human health. Thus, development of an effective preparation based on lytic bacteriophages is the key to successful strategy for control of bacterial plant diseases. There are just a few papers describing use of *Pseudomonas*-specific phages in agriculture. However, representatives of this genus are able to affect a wide range of crops and have serious impact not only on the process of plant cultivation but also the preservation of plant produce during storage. Therefore, we have studied the efficiency of lysis of bacterial population in several samples of important agricultural crops, such as potato and beet.

Samples of potato and beet were treated with a mixture of bacteria *Pseudomonas syringae pv. 223 tabaci*, *Pseudomonas savastanoi pv. phaseolicola 4013*, *Pseudomonas syringae pv. lachrymans 7591* and *Pseudomonas fluorescens 8573*. The first signs of bacterial lesions on the sections developed by the third day, and the maceration of almost all observed sections has occurred by the fifth day (Sovinska et al., 2015).

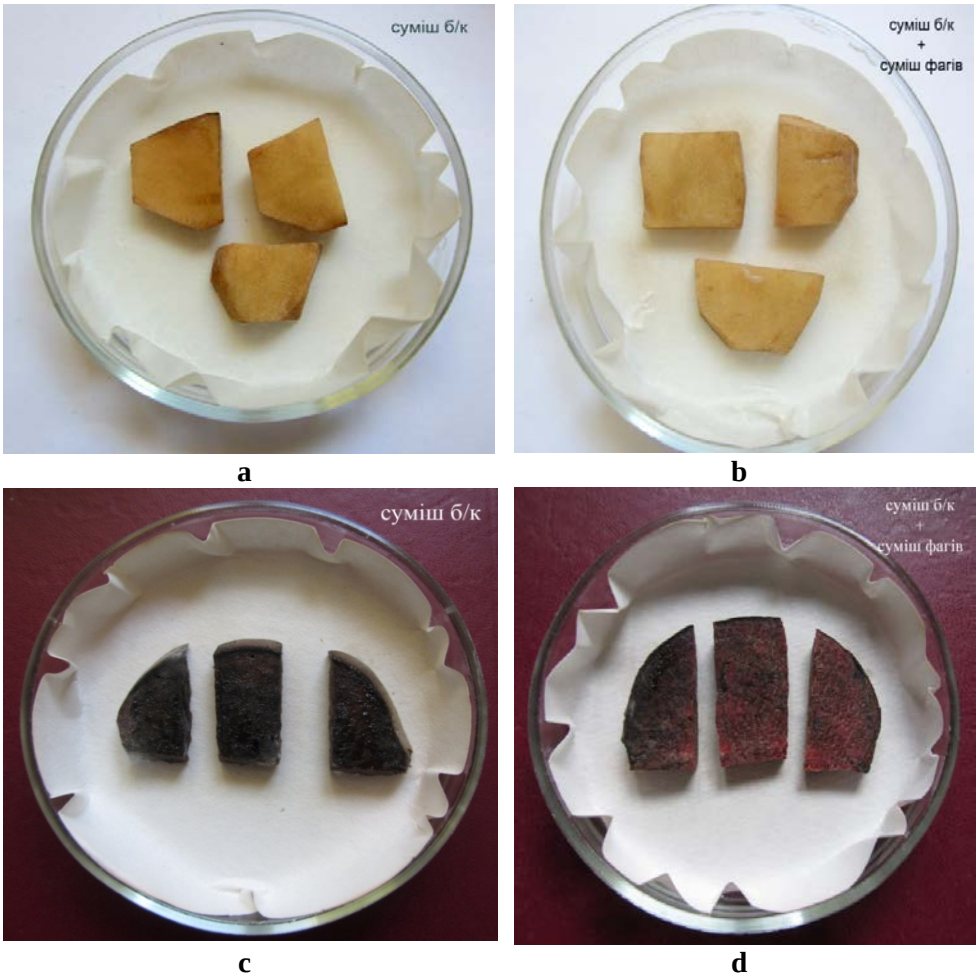

Figure 2. Effectiveness of bacteriophages against bacterial cultures on potato and beet on Petri dish. Plant samples on “a” and “c” were inoculated with bacteria only while plant samples on “b” and “d” were inoculated with bacteria and subsequently with bacteriophage.

There were no active maceration of plant tissue in samples of potatoes and beet post fifth day of simultaneous application of bacterial mixture (*Pseudomonas syringae pv. tabaci 223, Pseudomonas savastanoi pv. phaseolicola 4013, Pseudomonas siringae pv. lachrymans 7591* and *Pseudomonas fluorescens 8573*) and a mixture of four isolates of bacteriophages

(phages 223, 4013, 7591, and 8573). This indicates that tested bacteriophages efficiently lysed the bacterial cells. Further, each separate isolate of bacteriophages was tested on sensitive bacteria. No visible destruction of plant tissue was observed in three to five days' time after treatment of samples with a mixture (bacteria + phage), contrary to samples treated with bacteria only. Data on bacteriophage morphology can be used for the development of pathogenic bacteria biocontrol methods in the environment.

The study presents an effective method of using bacteriophages, which is important for laboratory practice and for creation a commercial bacteriophage mixes for controlling plant pathogenic bacteria. Polyvalent phages can be used in search and for creating antimicrobial agents based on bacterial viruses (Sadunishvili, 2013; Żaczek et al., 2015). The isolated phages may be considered as an effective component in the creation of therapeutic phage mixtures (Rene et al., 2008).

CONCLUSIONS

The resulting collection of phages can be used for the development of antimicrobial agents based on bacterial viruses in agriculture. Use of lytic enzymes of isolated phages for controlling bacterial growth is another perspective approach.

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Cevher İlhan CEVHERİ, Ahmet YILMAZ[1]

RESEARCH ON INVESTIGATION CHARACTERISTICS OF SOME COTTON VARIETIES PRODUCED UNDER ORGANIC AND CONVENTIONAL CONDITIONS

SUMMARY

Cotton is the most important raw material for the textile industry in today's world. Cotton is an industrial crop for which the most chemical fertilizers and pesticides are used. The excess chemicals cause pollution of land and water resources, destruction of biodiversity, and therefore deterioration in human health. One of the most discussed topics in today's world is sustainable agriculture. The biggest obstacle for sustainable agriculture is the use of chemicals. The most important issue when it comes to sustainable agriculture is, undoubtedly, organic agriculture. Organic cotton farming does not use chemical inputs, which is most importance in terms of soil, plant and human health. This study was conducted that four organic and inorganic fertilizers application to two cotton varieties under Harran Plain organic farming and conventional conditions. The aim of this study was to determine that the differences between plant characteristics at cotton varieties produced under organic and conventional production systems, and would be useful in further work and help sustainable agriculture in the future.

Keywords: organic agriculture, conventional agriculture, sustainable agriculture, Biodiversity.

INTRODUCTION

Cotton plant is the most important raw material for the textile sector as well as being a strategical plant supporting oil industry with cottonseed product. In the process of cotton production, in order to increase production yield, some catalyzer products are used. These catalyzer products are chemical fertilizers, agricultural contention products which are used for irrigation and agricultural pest control. These products act a crucial part in growing the plant. However, these products have negative effects on the environment, soil and water sources. Chemicals used in agriculture effects on contamination of the soil. Today, one of the most concerned subjects is the protecting the health of soil, plant and human. These three subjects are considered as an inseperable whole.Today, there are various systems for production in agriculture. These are conventional agriculture, good farming practices, organic agriculture and biodynamic agriculture systems.

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In the production process, every production systems have their own advantages, but the crucial point is being a system that is not harmful for the environment and protects soil and plant health.

Organic Agriculture is a production system that without chemical input, every stage between production and consumption is controlled and certificated. The aims of the organic agriculture are to provide continuance of sustainable agriculture and to protect the health of plants, animals and people by not contaminating the environment. The history of organical agriculture dates back to 20th century. In 20th century, subcjects as environmental awareness, ozone layer depletion and dangers for earth's future became current issues of world's agenda (Anonymous, 2016).

Various data came in view while analyzing the studies in which the effects of fertilizers on yield used in organic and conventional cotton production systems were analyzed. These studies were summed up as following;

Reddy and his friends (2007), in their studies in which they applied urea, fresh poultry manure and poultry manure compost as three seperate nitrogene supply in order to use poultry residual as N supply in 1994 and 1998 reported that there was a significant increase of cotton yield compared to control, they achieve and 1492 kg ha-1 average fibre yield by applying fresh poultry manure to cotton and 1391 kg ha-1 average fibre yield by urea application. Devraj and his friends (2008), in their study which they proceed in 2001 and 2003 in India searched for the effects of 5 and 10 tonnes of farm manure on cotton yield and nutrition usage and stated that plant height, boll weight and boll number per plant in particular were affected dramatically by organic and inorganic supplies. Ali and his friends (2009), in their study consisted of cotton parcels with farm manure, chicken manure, chemical fertilizer and contol stated that farm manure and chicken manure applications increased the palnt height, fruiting branch number and fibre yield. Akyol (2013), in his study on searching for appropriate dosages of liquid animal manure in cotton production reported that liquid animal manure could be used as top fertilizer in cotton production and as a result it could positive effects on yield in particular, plant height, fruiting branch number and boll number. Lopez and his friends (2014), in Mexico, in their organic cotton production study in which they applied 0-40-80-120 tonnes ha-1 cattle manure and 120-60-0 Kg ha-1 stated that the highest seed cotton yield was obtained from 8 ton ha-1 cattle manure dosage. The aims of our study were to analyze and compare the yield components of two cotton types produced in organic and conventional conditions and to recommend the best method to the producers.

MATERIAL AND METHODS

This study was planned and carried out as four replications and with split plot randomized blocks experiement design at the Harran University Akçakale Vocational High School, organic agriculture conditions in 2014 and 2015. The main plots were formed by ST-468 and BA-119 commercial cotton varieties which are computable with the ecology of the region. The sub-parcels were applied NPK(Chemical fertilizer), Cattle fertilizer, Pigeon fertilizer, microbial

fertilizer (*Bacillus subtilis* and *Paenibacillus azotofixans*) and control (no fertilizer) parcels. In the study, the lenght of the parcels was 12 meters, the width of the parcels was 2.8 meters and there was 3 meters gap among the parcels for fertilizer isolation. Planting was done on 5th May 2014 and 28th April on 2015.

In the experiment ST-468 and BA-119 cotton varieties were used. ST-468 is a semi-early Variety. It has a great adaptation capability and it has perfect yield results. It has hairy leaves. It is convenient for mechanical harvesting. BA-119 has earliness and it has medium height. It is adapted to the region and convenient for the mechanical harvesting. The soil of the experiement area was clayish and loamy with 1.36 % of salt, 26.9 % of lime ($CaCo_3$), 1.11% organic material and 7.82 PH of soil reaction. The studies had been carried out as fixed trial format for two years. The soil was plowed 25 centimeters depth after November and the second plow was done by cultivator in March. When the soil was ready, gobble disk array was constructed and soon after Cattle fertilizer and pigeon fertilizer were mixed with the soil. Cattle fertilizer was applied as 2000 kg ha-1, pigeon fertilizer was applied as 1000 kg ha-1, NPK fertilizer was applied as 200 kg ha-1, Cattle fertilizer+microbial fertilizer was applied 1 lt (mf) 100 lt ha water 0.1ha-1, pigeon fertilizer+microbial fertilizer was applied 1 lt (mf) 100 lt ha water 0.1ha-1 and NPK fertilizer was applied as 200 kg ha-1. Organic fertilizers and NPK fertilizer applications were applied in different parcels and different places with isolation distance at the same climate conditions. Hoeing was done six times against weed by manual and mechanic hoe. Drip irrigation was used in the trial and it was done seven times in total. The mixture of soft soap (3 kg per 100 lt water-1) and spirit (600 g 100 lt water-1) was applied against aphid, thrips, white fly and red spider mite. In addition to this application, Neemazal that is produced from Neem tree was applied with the dosage of 300 cc 100 lt water-1 at the chilly times of the day by covering all the plant's surface according to density of pests three times in total (Cevheri and Yılmaz, 2016). One meter was taken out from both sides of the parcels with different organic and NPK fertilizers and two row in the middle were harvested two times manually in the third week of September and in the middle of October. It is seen that between April and October, known as cotton planting season, the average temperature values of July, September and October in 2015 (0.7, 2.3 and 1.8 °C) were relatively higher than 2014 values according to comparison of temperature values of 2014, 2015 and average temperature values. Variance analysis of the data of the yield and the yield components that were acquired from the experiment were processed according to JUMP statistical programme and significant level of them were classified acoording to LSD test.

RESULTS AND DISCUSSION

Plant Height (cm)

According to average data of two years, it is seen in Table 1 that plant height of varieties varies between 85.23 (ST-468) and 87.13 cm (BA-119) , BA-119 variety has a higher value as 87.13 cm; as a result of fertilizer applications average plant height varies between 82.73 (Control) and 93.16 cm (NPK Fertilizer), the highest plant height was obtained from NPK fertilizer application;

according to typex fertilizer interactions the lowest plant height was obtained from BA-119x Control (81.63 cm) and the highest plant height was obtained from ST-468x Pigeon manure application (94.30 cm).

Table 1. Average values of features that are analyzed according to cotton and fertilizer variety used in the experiement and interactions of the variety-fertilizer

R.F.	Fertilizer Applications	Varieties			R.F	Fertilizer Applications	Varieties		
		ST-468	BA-119	Average			ST-468	BA-119	Average
1	1.Cattle fertilizer	84.58	86.87	85.73B	3	1.Cattle fertilizer	4241.10	4615.80	4428.40B
	2.Pigeon Fertilizer	82.95	89.52	86.23B		2.Pigeon Fertilizer	3930.00	4156.20	4045.40B
	3.NPK Fertilizer	94.30	92.02	93.16A		3.NPK Fertilizer	5350.00	5082.50	5216.20A
	4.Cattle Fertilizer+ Microbial Fertilizer	80.35	85.75	83.05B		4.Cattle Fertilizer+ Microbial Fertilizer	3990.40	4159.00	4074.70B
	5. Pigeon Fertilizer+ Microbial Fertilizer	85.36	87.02	86.19B		5. Pigeon Fertilizer+ Microbial Fertilizer	4098.20	4800.80	4449.50B
	6. control	83.83	81.63	82.73B		6. control	3158.40	2504.50	2830.00C
	Average	85.23B	87.13A	86.18		Average	4128.80	4219.80	4174.30
	%CV:10.88 LSD(Variety): 1.40* LSD(Fertilizer): 6.60* LSD(Variety*Fertilizer): n.s.					%CV:19.34 LSD(Variety): n.s. LSD(Fertilizer): 56** LSD(Variety*Fertilizer): n.s.			
2	1.Cattle fertilizer	11.58	12.21	11.89A	4	1.Cattle fertilizer	30.36	33.29	31.82A
	2.Pigeon Fertilizer	11.31	12.72	12.02A		2.Pigeon Fertilizer	25.57	31.66	28.62BC
	3.NPK Fertilizer	11.44	11.65	11.55A		3.NPK Fertilizer	29.54	32.38	30.96AB
	4.Cattle Fertilizer+ Microbial Fertilizer	9.61	11.21	10.41B		4.Cattle Fertilizer+ Microbial Fertilizer	25.98	29.50	27.74C
	5. Pigeon Fertilizer+ Microbial Fertilizer	10.81	12.20	11.50A		5. Pigeon Fertilizer+ Microbial Fertilizer	27.08	26.58	26.83C
	6. control	10.41	11.79	11.10AB		6. control	19.05	19.91	19.48D
	Average	10.86A	11.96B	11.41		Average	26.26B	28.88A	27.57
	%CV:12.97 LSD(Variety): 0.58** LSD(Fertilizer): 1.04* LSD(Variety*Fertilizer): n.s.					%CV:14.71 LSD(Variety): 1.08** LSD(Fertilizer): 2.85** LSD(Variety*Fertilizer): n.s.			

R.F.: Researched Features. there is not any important difference in the level (*): 0.05; (**): 0.01. 1. Plant Height (cm), 2.Sympodial Branch number per plant (number plant^{-1}), 3.Seed cotton yield (kg ha^{-1}), 4.Boll Number Per Plant (number plant^{-1}).

It is determined that there is a difference (0.05) between the types as statistical significance value, and it is found that fertilizer applications have significance effects (0.05) on plant height. There were differrent effects among NPK, organic (Cattle and pigeon manure) fertilizer applications and control parcels (no fertilizer application) on plant height. According to our researches, from the point of variety and fertilizer interreactions there could'nt be found any significant statistical differences among the applications (Table 1).

Our findings are coherent with the finding of Khaliq and his friends (2006), Reddy and his friends (2007), Gunjal and his friends (2009), Kıvılcım and his friends (2010) who stated that when solemly applied organic materials and effectiand microorganisms did not increase the cotton yield and the yield components, but organic materials, effect and microorganisms, mineral NPK and different combinations of these increased the yield and the yield components. Our findings are coherent with the findings of Bondada and his friends (1996), Phipps and his friends (1997), Karademir and his friends (2006), Satyanarayana and Janavade (2006), Devraj and his friends (2008), Ali and his friends (2009), Yolcu (2009), Shah and his friends (2012), Akyol (2013) who stated that appropriate nitrogen dosages increase the plant height. Plant height is a desirable situation in a certain scale. However, under growing of plant height retards the plant physiologically from passing generatiand period from andgetatiand period, so it is an undesirable situation. According to our findings analyzed plant height is in normal values.

Fruiting Branch Number (number plant^{-1})

According to average data of two years from Table 1, it is determined that fruiting branch number of the types varies between 10.86 (ST-468) and 11,96 (BA-119) ; BA-119 has a higher value with 11,96 number plant^{-1}; as a result of fertilizer applications average fruiting branch number varies between 10,41 number plant^{-1} (Cattle fertilizer + microbial fertilizer) and 12.02 number plant^{-1} (pigeon fertilizer), the highest fruiting branch number was obtained from Pigeon Fertilizer application, according to variety and fertilizer interreaction values, there wasn't any difference between the applications as statistical significance and they were in the same group (Table 1). Our findings are coherent with the findings of Ali and his friends (2009) and Akyol(2013).

Seed Cotton Yield (kg ha^{-1})

According to average values from Table 1, It can be said as foolowing; average seed cotton yield of the types varies between 4128.80 kg ha^{-1} (ST-468) and 4219.80 kg ha^{-1} (BA-119); BA-119 types has a higher value 4219.80 kg ha^{-1}; as a result of fertilizer applications average seed cotton yield varies between 2830.00 (Control) and 5216.20 kg ha^{-1} (NPK Fertilizer); the highest seed cotton yield was obtained from NPK fertilizer application; according to varieties fertilizer interreactions values the lowest seed cotton yield value (2504.50 kg ha^{-1}) BA-119 x Control application, the highest seed cotton yield value was obtained from ST-468 x NPK fertilizer (5350 kg ha^{-1}). Any diffecence occured as statistical significance value neither between the types according to seed cotton

yield nor for the varieties fertilizer applications interreactions (Table 1). Fertilizer applications affected the seed cotton yield and the highest value was obtained from chemical fertilizer application (NPK). Pigeon fertilizer+microbial fertilizer and Cattle fertilizer come after NPK fertilizer respectively according to yield values. Our findings are partially or totally coherent with Kumari and his friends (2006) who stated NPK and organic fertilizers application were important effects on cotton yield components, Kısakürek and his friends (2007), who stated conventional production conditions has a higher product increasement compared to organic production, Aydemir (1982) who stated nitrogen increases boll and seed size and fibre yield, Gençer and Oğlakçı(1983) who stated nitrogen increased the seed cotton yield, Bondada and his friends (1996) who stated appropriate nitrogen dosages increase the seed cotton yield of the plant, Phipps and his friends (1997) who stated nitrogen fertilizing increases fibre yield, Anlağan(2001) who stated nitrogen is effectively on plant yield components, Shah and his friends (2012) who stated the highest yield was obtained from the trial by using 50% NPK fertilizer and 50% organic farm fertilizer. In addition our findings are coherent with Reddy and his friends (2007), Ali and his friends (2009) and Lopez and his friends (2014).

Boll Number Per Plant (number plant^{-1})

According to average values of two years from Table 1 it is said that; boll number per plant of the varies between 22.26 (ST-468) and 28.88 number plant^{-1} (BA-119), BA-119 type has a higher value as 28.88 boll number; as a result of fertilizer applications, average boll number per plant varies between 19.48 (Control) and 31.82 a number plant^{-1} (Cattle Fertilizer),The highest number was obtained from Cattle Fertilizer; according to variety and fertilizer interractions values there wasn't a statistically significant difference among the applications and they were in the same group(Table 1). Our findings are totally or partially coherent with the findings of Khaliq and his friends (2006), Kumari and his friends. (2006), Attia and his friends (2008), Devraj and his friends (2008), Gunjal and his friends (2009), Shah and his friends (2012), Ahmed and his friends (2013), Akyol (2013) who stated that when lonely applied organic materials and effective microorganisms didn't increase the yield and the yield components. However application of different combinations of organic materials, effective microorganisms, and mineral NPK increased the yield and the yield components on cotton.

CONLUSIONS

According to our results; under usage of chemicals in conventional conditional result in contamination of the environment, extinction of natural resources and perishing consciousness of sustainable agriculture. As a result of our study, it is determined that the result of usage of NPK fertilizer provides 5216 kg ha^{-1} seed cotton yield, among the varieties the highest seed cotton yield was obtained from BA-119 with 4219.80 kg ha^{-1}.

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INFLUENCE COLLOIDAL SOLUTIONS OF NANOMOLYBDENUM ON THE EFFICIENCY OF SYMBIOTIC NITROGEN FIXATION IN LEGUMES (PEA, CHICKPEA)

SUMMARY

The strategic goal to the solving problem of dietary and fodder protein and restoring fertility of Ukrainian soils is optimal expansion of sowing areas traditional and non-traditional legumes. Creation of materials which are easily assimilated by living creatures and not harmful to the environment is one of the important issues of modern nanotechnologies. The aim of our study was the comparative evaluation of pre-sowing treatment with nanomolybdenum and microbiological preparation for impact on the efficiency of symbiotic nitrogen fixation in pea and chickpea plants. Field studies were conducted in the separated subdivision of the National University of Life and Environmental Sciences of Ukraine «Agronomic Experiment Station» on the typical black soils in the northern part of Forest Steppe of Ukraine. It was study of the influence of biological preparations on the nitrogen fixation capacity of pea (var. Tsarevych, Deviz) and chickpea plants (var. Rozanna, Triumf). The nitrogenase activity of the nodules in the root system of legumes was determined by acetylene-ethylene method. The efficiency of legume-rhizobia symbiosis depends on the number and virulence of symbiotic bacteria, which makes fixation of atmospheric nitrogen. Pre-sowing seeds treatment by strain of microorganisms enhances the quantitative and qualitative increase in the efficiency of the legume-rhizobia symbiosis. Using colloidal solution of molybdenum without seed inoculation also influences the number and diversity of rhizobia in the soil. Number, weight and symbiotic activity of nodules of pea and chickpea plants varied depending on the weather conditions. In the flowering stage the effects from pre-sowing treatment by bacterial inoculants and molybdenum solutions was most notable – the number of nodules was greater at 50-150 % compared with control, using inoculation this figure was higher at 8-9 %.

Keywords: pea, chickpea, nitrogenase activity, colloidal solutions of nanomolybdenum.

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INTRODUCTION

The problem of global agriculture in the past and the beginning this century is the problem of protein production. In deciding this important problem have place increasing plant protein production, the special place belongs to legumes, including peas (Lopatko et al., 2009). According to many researchers, one of the most objective criteria for the best use of chemicals in growing technologies is microbiological testing (Honchar 2013, Priestera et al., 2012). However, according to the researchers of the Institute of Agricultural Microbiology NAAS, considering the close interaction of certain types of microorganisms with cultivated plants and chances of forming close plant-bacterial associations in the soil, only rhysospheric (root microorganisms) can display the system response to certain factors, closest to response of a plant itself (Lin and Xing, 2007; Lopatko, 2012). This perspective is highlighted in the works of many authors of the last century, and in more modern materials of foreign and domestic scholars (Kaplunenko et al., 2008).

We know, that productivity of legumes, is determined by many factors, and oxide plays a primary role to. In order to obtain high yields of crops one should constantly take care of the replenishment of nitrogen in the soil by nitrogen-fixing bacteria (Lopatko et al., 2013). Based on the lack of studies about the impact of mineral fertilization on the formation and activity of symbiotic nodule bacteria, the practical value of this work was established (Frantiichuk et al., 2012, Lopatko et al., 2013b).

The strategic goal to the solving problem of dietary and fodder protein and restoring fertility of Ukrainian soils is optimal expansion of sowing areas traditional and non-traditional legumes. The biological nitrogen accumulate in the soils through fixation from the atmosphere by nodule bacteria upon interaction with plants, secures the increased yield of the major crops, retains the soils fertility and improves ecological condition (Pylypenko and Honchar, 2016).

Traditionally in Ukraine and worldwide the problem of fertilizer and animal feed enrichment with vital micronutrients solve by salts of heavy metals and chelating compounds which on the structure and properties are of small biological meet the needs of plants and animals, and only slightly absorbed by the latter. The result is the accumulation of heavy metals in the environment, ecological condition worst, decreased quality of received food (Taran et al., 2014a).

On applying nutrients Ukraine significantly lags behind Western countries. There is a steady increasing in production and a wider outspread of micronutrients over the last 30-40 years in almost all developed countries. However, there is a reverse process in our country: the production of micronutrients by domestic industry takes place in small quantities, without purpose. Therefore, for our country production of a wide range of micronutrients with balanced content of elements and their application in the production of environmentally friendly products with high content nutrients is an actual problem (Kaiser et al., 2005).

Breeders of our country intensively work to create new varieties of pea and chickpea. Annually is replenished public register varieties of these cultures, so it is important for them to pick up effective strains of rhizobia. As shown by our research and experiments of other researchers at the expense successful selection of macro- and microsymbiotic treatments is possible to increase yield legumes on 0.3-0.5 t / ha without disturbing the ecological balance of the environment (Kalenska et al., 2016). Creation of materials which are easily assimilated by living creatures and not harmful to the environment is one of the important issues of modern nanotechnologies. The colloidal solutions of metals as micronutrients enhance the plant resistance to unfavorable environmental conditions and ensure high yields of food crops (Panyuta et al., 2016).

Nanotechnologies in agriculture involve the use of fertilizing and plant protection preparations latest generation. Colloidal solutions of biogenic metals become widely used to enhance productivity and resistance to abiotic and biotic environmental factors.

The aim of our study was the comparative evaluation of presowing treatment with nanomolybdenum and microbiological preparation for impact on the efficiency of symbiotic nitrogen fixation in pea and chickpea plants.

MATERIAL AND METHODS

Field studies were conducted in the separated subdivision of the National University of Life and Environmental Sciences of Ukraine «Agronomic Experiment Station» on the typical black soils in the northern part of Forest Steppe of Ukraine. It was study the influence of biological preparations on the nitrogen fixation capacity of pea (var. Tsarevych, Deviz) and chickpea plants (var. Rozanna, Triumf). The nitrogenase activity of the nodules in the root system of legumes was determined via acetylene-ethylene method. Using colloidal solutions of nanomolybdenum as micronutrients enhances plant resistance to unfavorable environmental conditions and ensures high yields of food crops due to the active penetration of nanoelements into the plant cells (Lopatko et al., 2013).

Sowing area is 30 m2, accounting area – 25 m2. Repetition – quadruple, accommodation options consistent. Seeding rate amounted 1.2 million of seeds per 1 ha of pea and of the chickpea – 500 thousand seeds / ha. On the day of sowing, the bacterization with ryzohumin suspension was carried out. Suspension in amount of 900 g per1 t of seeds was diluted in 8-10 l of water, and then immediately treated. The rate of colloidal solution of nanoparticles of molybdenum was 1 liter of solution per ton of seed, working solution meets metal concentration of 0.8 mg / l.

The rate of ryzobofit and ST 282 strain in liquid form per hectare was 100 ml; 1 ml contained 6.7 billion nodule bacteria. On the day of sowing, the treatment chemicals were diluted in 1.7 l of water, and then seeds were treated with this solution. During the main cultivation, one had added granular superphosphate (P_2O_5 19.5 %) and potassium salt (K_2O 40.0%) at a rate of 60

kg / ha s. d., Ammonium nitrate (N 34.4 %) 30 kg / ha in the spring (Volkogon, 2006, Volkogon, 2007).

The obtained results were processed by computer program Statistics 6.0.

RESULTS AND DISCUSSION

Pre-sowing treatment of seeds nodule bacteria formation provide the required number of nodules on the roots of plants chickpea. During the growing season in the versions without the use of nodule bacteria inoculated on the roots of plants nodules were not formed, indicating a lack of native soil chickpeas rhizobia that can form nodules on the roots of culture (Shcherbakova et al., 2017).

In experiment indicators number and weight nodules varied depending on investigated factors. Symbiotic activity of chickpea plants largely depend on the climatic conditions of the investigated year. The formation of nodules on the roots of chickpea depends of options on pre-sowing treatment of seeds. The largest number of nodules 18.7-24.6 pcs. / plant and mass 823-976 mg / plant was marked by pre-sowing treatment of seeds with strain ST 282 and CSM.

The use of colloidal solution of molybdenum contributed to increasing nitrogenase activity by 27-28% in variety Rosanna and by 19-20% in variety Triumph. In 2013, the dynamics nitrogenase activity was higher than in previous years. Low temperatures and excessive rainfall that occurred in the flowering period of chickpeas, adversely affected the nodules formation nitrogenase activity.

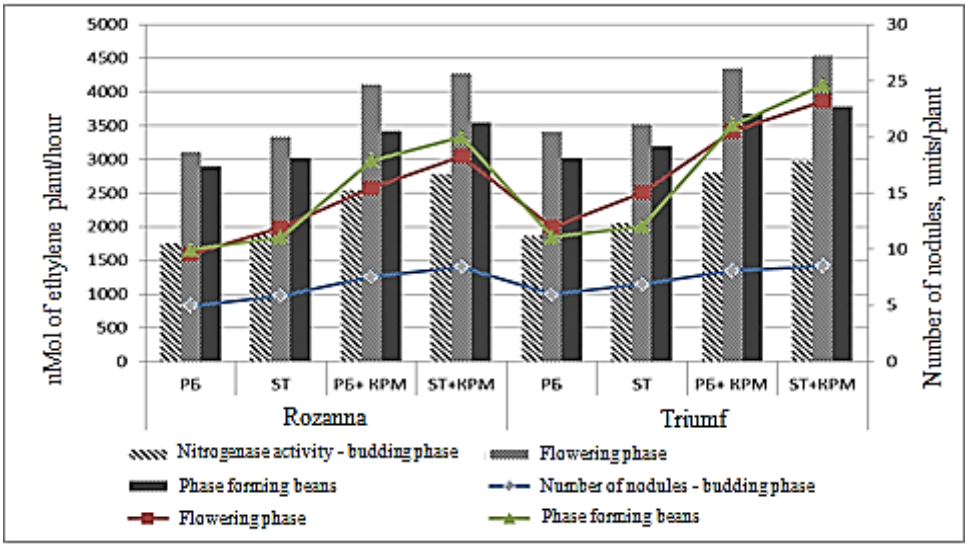
We obtained detailed data on the composition of low molecular weight organic components (organic acids, sugars and amino acids) of chickpea root exudates after inoculation with *M. ciceri* bacteria combined with treatment with CSM. According to our data, the introduction of rhizobia and CSM into the system resulted in changes in the composition of low molecular weight compounds in root exudates (Ryibalkina, 2005, Schwarz et al., 2009).

Qualitative composition and exudation intensity of the major carbon compounds differed in the variants inoculated with *M. ciceri* and treatment with CSM (Fig. 1). Organic acids were the 385 dominant fraction of root exudates in all variants. This fraction, in its turn, was dominated in all variants by succinic and malic acids; on the average, they could represent up to 90% of the total amount of organic acids depending on the variant. Similar data have been obtained in a study of pea, where the amount of malic acid was also high (from 45 to 71%).

However, instead of large amounts of lactic acid characteristic of pea roots (from 49 to 72% of the total amount of organic acids depending on the cultivar) the roots of chickpea produce much succinic acid (Sozer and Kokini, 2009, Taran et al., 2014b, Taran et al., 2016).

Seed inoculation with rysohumin had a positive influence on the formation of symbiotic apparatus of the leafless pea. The number and weight of nodules mg

/100 plants varied during the study (Tab. 1) depending on the use of technological measures, fertilization and varietal characteristics.



Note*: РБ - Inoculation by Ryzobofit; ST - Inoculation by strain ST 282; РБ+ CSM - Treatment CSM + Ryzobofit; ST+ CSM - Treatment CSM + strain ST 282

Figure 1. Number of nodules and nitrogenase activity of chickpea roots, depending of the preplant treatment of seeds options 2014-2016.

Table 1. Symbiotic activity of pea plants nodules depending on fertilizer, foliar application and seed inoculation (average for 2014-2016)

Variant of fertilizing	Stages of plant growth and development					
	BBCH 12-19		BBCH 55-59		BBCH 61-71	
	Deviz	**Tsarevych**	**Deviz**	**Tsarevych**	**Deviz**	**Tsarevych**
C	7.1	8.6	181	19.5	22.2	25.2
PH	17.4	18.6	36.1	38.6	50.6	53.1
ST	10.5	11.2	27.3	29.4	34.3	37.4
CSM	7.2	8.7	27.6	28.5	41.5	44.1
PH+ CSM	18.0	19.6	39.5	41.8	52.4	55.2
ST+ CSM	18.5	19.5	32.4	34.7	54.8	57.0
HIP_{05} for factor «fertilization»					*1.3*	
HIP_{05} for factor «inoculation of seeds»					*0.6*	

Note*: PH - Inoculation by Ryzohumin; ST - Inoculation by strain ST 238; РБ + CSM - Treatment CSM + Ryzohumin; ST+ CSM - Treatment CSM + strain ST 238

Conditions for the formation of symbiotic apparatus of Deviz pea variety were the most favorable while sowing inoculated seeds after presowing treatment of seeds by strain ST 238 and CSM. Thus, the number and weight of nodules were the highest and ranged from 52.4 to 54.8 pc / plant, and from 26.2 to 27.4 g /100 plants.

It had been established, that the varietal characteristics also have an impact on the formation and symbiotic activity of nodule bacteria. After a comparative analysis of the studied varieties we have noted, that Tsarevych variety had formed more nodules and greater weight, respectively.

CONCLUSIONS

The efficiency of legume-rhizobial symbiosis depends on the number and virulence of symbiotic bacteria which makes fixation of atmospheric nitrogen. Presowing seeds treatment by strain of microorganisms enhances the quantitative and qualitative increase in the efficiency of the legume-rhizobiales symbiosis. Using colloidal solution of molybdenum without seed inoculation also influences for number and diversity of rhizobia in the soil. Number, weight and symbiotic activity of nodules of pea and chickpea plants varied depending on the weather conditions. In the flowering stage the effects from presowing treatment by bacterial inoculants and molybdenum solutions was most notable – the number of nodules was greater by 50-150 % compared with control, using inoculation this figure was higher by 8-9 %.

The most favorable conditions for the formation of symbiotic apparatus had been created by the combination of inoculated seed sowing with application of CSM. While applying inoculated and CSM, the formation and performance of the device symbiotic apparatus of pea plants increase. The mentioned rate of mineral nutrition is effective on sowing by seeds, which are not inoculated.

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MAIN CHALLENGES OF RURAL DEVELOPMENT STRATEGIES THROUGH TOURISM AND AGRICULTURE LINKAGES IN MEXICO

SUMMARY

Addressing the model of extensive rural development in Mexico, as well as based on family production units (households) strategies formulation to establish linkages between agriculture and tourism such as Pluriactivity and Pro-poor Tourism, necessarily implies deepening historical analysis of the governmental intervention processes. Added to this, it is crucial to refine its study based on sociological tools which will allow a more complete and comprehensive understanding of the internal dynamics of the Mexican rural family, their trade difficulties within the market and the changeling relation between the city and the countryside. Despite the efforts in the application of rural development strategies and public policies to strengthen the agricultural sector in conjunction with other economic activities such as tourism, the lack of application of participative methodologies in the elaboration of diagnoses focused on the formulation of these, constitutes one of the main causes of the failure of projects such as the insertion of Pro poor tourism initiatives or farming Pluriactivity, due to the incompatibility of these with the economic, historical, social and cultural particularities of the Mexican peasant families. It is in this sense that the present analysis is developed, thus providing relevant data for research, debate and structuring new policies aimed at improving the agricultural sector through tourism.

Keywords: Agriculture, Household pluriactivity, Pro-poor Tourism, Mexico.

INTRODUCTION

Authors such as Escalante et al. (2007), Grammont (2009) and Noreiro et al. (2009) warn that the Mexican countryside faces a decline in agriculture, which means that there is a significant decrease in the contribution of agricultural activities to income generation in rural areas, as well as to an increasing migration and aging of its population. Although this depression does not refer to the disappearance of agricultural activities, it emphasizes the importance of increased income from non-agricultural activities in rural households.

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This situation is due in large measure to the ravages that are experienced as a result of the intensification of the technological process under the development and modernization model that prevailed since the decade of the 1950's. In order to compete under the new commercialization trends, significant extensions of land and strong financial investments are needed. The above, entails local producers out of business which increases unemployment (Timms, 2006), in addition to establish suitable conditions for the entry of foreign products whose costs tend to be lower than those of domestic production.

Faced with these circumstances and policies that promote a greater specialization of productive units, agricultural activities face a stagnation, coupled with the phenomenon of exclusion from the domestic market, with which small and medium producers have to cope and who have gradually opted for abandoning agricultural production, even sell-squander their land, migrate (countryside-city and to the U.S mainly) and move from land owner peasant to wage-laborer. All this describes roughly the main characteristics that are part of the so-called New Rurality, an approach that, without any coincidence, goes into discussion alongside neoliberal policies and globalization circumstances such as rural pluriactivity or multifunctionality (Marsden, 1990; Grammont, 2009; Schneider, 2009; Escalante et al., 2007; Bonnal et al., 2003 Evans and Ilbery, 1993; Sacco dos Anjos, 2001), and the subsequent study of linkages between tourism and agriculture (Rogerson, 2012; Timms, 2006; Torres, 2003, 2002; Torres and Momsen, 2004) and between tourism and poor (Ashley et al., 2001; Gascón, 2011; Ventura-Dias, 2011).

The discussion presented in this paper is based on the analysis of rural development strategies aimed at agriculture strengthening through the establishment of links with one of the main economic activities of the country, tourism. Likewise, there is an urgent need to rethink methodologies such as Pro-Poor Tourism in Mexico, due to the multicultural and heterogeneous nature of the Mexican rural scene.

MATERIAL AND METHODS

This research starts from the concomitant complexity in the unfolding of the reconfiguration of the rural scene in front of the evident civilizational crisis that has been sharpened with the new rules that the system dictates. The spatial, social, economic, technological, environmental and cultural dimensions under which the countryside and the city interact, the design and implementation of public policies and strategies aimed at improving the delicate situation facing agriculture in Mexico, as well as global trends for rural development proposed to reduce the situation of pauperization of peasant families, represent only the tip of the iceberg that such complexity implies.

Although complexity announces the lack of simplicity, its epistemological logos compromises the integrating challenge of connections that become a whole at the time, and not a reductionist way in which the whole is analyzed separately

in its parts, so it is necessary to start from the idea that, "complexity is a problem word and not a solution word " (Morin, 2001).

Under the previous epistemic premise, this document refers to the urgency of using participatory methodological (Selenger, 1997; Chambers, 1994), ethnographic (Guber 2011) and historical-sociological tools for the redesign of global rural development strategies such as agricultural multifunctionality or pluriactivity, synergy between agriculture and tourism, as well as Pro Poor Tourism based on family production units.

Also, an extensive documentary review of cases (national and international) was carried out in which these strategies have been implemented through public policies (Schneider, 2009; Timms, 2006; Rogerson, 2011; Torres, 2002, 2003), whose data were fundamental to the discussion and results.

RESULTS AND DISCUSSION

Since the mid-1940's, tourism in Mexico has represented an important activity of multiple opportunities and dynamism for the country's growth. According to the OECD (2017), tourism in Mexico directly represents 8.5 % of GDP and generates a value above the average of the economy. However, despite the direct and indirect employment generated by tourism, there is widespread criticism of having high external leakages (Telfer and Wall, 1996), besides, important disruptive impacts above local agriculture such as land, labor, water and financial resources competition has been detected (Timms, 2006; Torres, 2003; Momsen, 1998; Telfer, 2000; Rogerson, 2012).

The potential of Mexican tourism to promote inclusive and sustainable growth has a wide margin to be exploited, suggests the OECD (2017), but the sector faces major challenges in terms of competitiveness and sustainability, among which the urgent need to promote public policies that adapt the model of tourist development to potentiate the sector in synergy with other productive sectors related to the tourism value chain.

In this regard, the Mexican government announced on December 15th, 2016, the signing of an agreement between SECTUR (Tourism Secretariat) and SAGARPA (Agriculture, cattle raising, rural development and food Secretariat) that promises to strengthen the participation of national companies as suppliers of goods and services of the tourism sector, new markets for specialty foods and local fresh produce bloom, among other potential positive impacts.

While this initiative represents an important precedent of synergy between Tourism and Agriculture, the current situation of the Mexican rural scene (significant decrease of agriculture, increasing migration, corruption, etc.), implies a challenge per se for the supply of local products to the tourism industry.

According to several researchers (Telfer, 2000; Telfer and Wall, 1996; Momsen, 1998; Torres, 2003; Timms, 2006), main challenges for agriculture and tourism linkages are related to supply or production factors such as informal nature of local farming systems; which generates mistrust on tourism enterprises about quality and quantity supply. This same issue occurs on marketing and

intermediary related factors, where availability and quality of regional transportation, storage and distribution infrastructure fails.

Among this challenging factors, entrenched monopoly and in many cases corrupt marketing networks (Torres, 2003) is a major problem to improve agriculture and tourism linkages benefits for local producers. Despite this, Mexico has important strengths. Based on the pioneer research by Torres (2003) about agriculture and tourism linkages in Mexican Caribbean most important destination, Cancun (sun and sand tourism, characterized by Fordist mass resorts; mainly transnational chains), against all predictions about foreign owned or managed enterprises trend to depend heavily on imports, the level of foreign imports to Cancun hotels are surprisingly low, where most of fresh fruits and vegetables, dairy products, packed and bulk commodity goods are imported from different regions of Mexico thanks to the improved transportation links.

As shown, Mexico has opportunities, nevertheless, local agricultural and tourism linkages which may benefit poorer segments of population (Timms, 2006), has a discouraging future, mainly because of the lack of capital investment and technological assistance access for family farming.

Family production units and Pro-Poor Tourism (PPT)

As well as the context that emerged from the neoliberal policies, globalization and other factors, generated the need to undertake research routes in relation to pluriactivity or synergy efforts between agriculture and tourism; there was also a concern to analyze the links between tourism and poverty. Hence, the evaluation of how tourism has been able or could contribute to the reduction of poverty through “Pro-Poor Tourism” (Ashley et al., 2001; Torres and Momsen, 2004).

However, Pro-Poor Tourism Methodology may not be the way to achieve poverty relief in Latin America due to evidence of ambiguity regarding the concept of "poverty" that may not fit with Mexico’s or other Latin American impoverishment conditions. Added to this, several researchers such as Ventura-Dias (2011) and Ashley et al. (2001) warn that there is no empirical evidence that tourism has succeeded in becoming a positive instrument in the eradication of poverty.

About this, Gascón (2011) states that the PPT methodology discloses contradictions in relation to paradigms such as Food Sovereignty when resorting to transnational tourism capital as an investment partner, a situation that suits the reproduction of the neoliberal economic model, which largely represents the origin of the exclusion, marginalization and impoverishment of peasant families and indigenous communities in countries such as Mexico.

In consideration that Mexico's most representative touristic destinations have a high symbolic load of folklore that inevitably evokes either the pre-Hispanic past or the richness of the cultural diversity of rural Mexico, as well as being located in beautiful natural landscapes, it is not strange that these tourist destinations are located near rural locations. In this sense, Mexican farm household is the main cultural and social structure in rural spaces, that is to say,

countryside families are the most vulnerable people to whom tourism affects, as well as the sector to which agriculture-tourism linkages policies should consider.

For this purpose, it is necessary to recognize the potential of peasant families in the current capitalist economy towards the creation of tourism and agriculture linkages for a better future scenario to poor Mexican peasant families, to deepen the analysis of the internal dynamics of the Mexican rural family and how these interact with the current market mechanisms. There is also a profound need to lead understanding about capital accumulation and its propagation process, the prominence of the State, the increasing complexity and power of the non-agricultural parts of the food chain, as well as to seek conceptual flexibility about assumptions often implied in the analysis of agricultural development (Marsden, 1990).

Therefore, the study of family production units in pluriactivity framework (alternation of agricultural activities and tourism) offers a more adequate way of analyze structural processes in Mexico, a fundamental understanding for the subsequent development of public policies and strategies aimed at halting the decline of conditions of agriculture in the Mexican rural sector, and consequently the living conditions of its marginalized population.

This marginalization and exclusion is exposed by several researchers (Marsden, 1990; Aguilar et al., 2010; Timms, 2006), who highlight evidence regarding the difficulty faced by most rural families in obtaining loans for entrepreneurship or improvement of productive projects because multiple credit agencies set very particular conditions on loans and are selective about which types of farmers receive them. This problem extends even with loan plans granted by the State, and the inflexibility of its operation rules. More worrying the high degree of productive projects that finally fail because of the lack of technical follow-up and the high level of dependence that this type of federal programs generates through one of the main problems of Mexican rural development policies: paternalism.

On the other hand and based on the challenges previously described for rural families, such as the difficulty of competing with specialized agro-industrial companies for lack of access to capital investment, technology and technical assistance, there is a lack of accounting and administrative training, fundamental to any entrepreneurial success.

In order to overcome this type of challenges, and taking into account the potential of family agricultural production units and the development of tourism as a complementary activity, it is important that the incipient policies for synergy between these sectors of the economy in Mexico consider specialized strategies in the promotion of markets directed to the consumption of community tourism, whose projection should be focused on the multifunctional and integral exercise of the agricultural and tourist activity as well as autonomy and self-management of their resources.

Although the creation of cooperatives and the operation of principles of solidarity economy are attractive to form united fronts of local producers to cover

the demand in quantity and quality of accommodation establishments as well as food and beverages, strategies must be anticipated to maintain the unity of these cooperatives and avoid situations of abuse, theft and corruption; unfortunate problems concomitant to the fragile economic situation and the paternalistic Welfare State especially for the rural and peasant sphere.

CONCLUSIONS

It is necessary to consider the particularities of the Latin American and Mexican context in order to overcome challenges that pluriactivity and agriculture and tourism linkages have. Otherwise, exogenous models of economic development based on the diversification of the agricultural and rural sector, may be incompatible.

There is a need for the formulation of economic policy proposals that encourage the integration of rural families into pluriactivity; however, it is necessary not to advance high expectations in this regard, without first elaborating strategies aimed at strengthening traditional forms of production.

It is also urgent to incorporate participatory methodological tools designed for the rural sector in the elaboration of diagnoses for the formulation of productive projects that, through links between agriculture and tourism, strengthen the Mexican countryside and thereby improve the quality of life of the Rural family. That is, to recognize the value of the information that arises from the families themselves: "locals know better".

According to self-experience in projects linking agriculture and tourism in two rural communities on the coast of the State of Oaxaca in Mexico, a careful diagnosis that details economic, environmental, social and cultural aspects is required. Such a diagnosis must consider cultural diversity and otherness as determinant factors, since it opens up opportunities for innovative entrepreneurship, products specialization and new niches opening. The recognition of local families as the main data source and main driver for economic and productive activation is crucial to strengthen the project within the community, otherwise the risk of failure increases greatly. Therefore, the ethnographic work and the application of a participatory rural diagnosis, represent the channels of interlocution between the community, social and economic theory.

In this sense, the undertaking of productive projects of a tourist and agronomic nature through cooperatives or solidarity societies in Protected Natural Areas and/or Native reserves in Mexico, often fail because of the lack of legal commitments by the project members, absence of a realistic business plan, lack of accounting, administrative and technical-operational training (CONANP, 2014).

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REPRODUCTION AND PROPAGATION OF SOME RARE SPECIES OF THE CRIMEAN FLORA

SUMMARY

The problem of conservation of a biological diversity of the biosphere, including its plant component, is currently very relevant and this is especially important for rare and endangered species of plants. Such rare species, which have to be protected, grow in the Crimea are *Arbutus andrachne* L. (Ericaceae), *Pistacia mutica* Fisch et Mey. (Anacardiaceae), *Campanula talievii* Juz.(Campanulaceae), *Fumana thymifolia* (L.) Spachet Webb (Cistaceae) and *Glaucium flavum* Crantz. (Papaveraceae). As the result of the study of a reproductive biology of these species, the types of formation of their generative structures, especially antekology, seed formation and dissemination have been obtained. It is shown that the main limiting factors for an optimal development of the studied species in the conditions of a natural growth are the meteorological factors in critical phase of formation of generative structures, damage caused by large animals as well as an anthropogenic impact (plowing land, construction, industrial pollution, decoration flowers, etc.). However, conservation of the species for the most part provide: stability of formation of viable male and female generative structures; successful processes of pollination and fertilization as the results of a paired development of elements of a flower, structure of insect-pollinator and pollination mechanisms; features of a seed formation and dissemination. An autogamy and vegetative propagation can be considered as reserve means, contributing to the preservation of these species. Thus, the observed variety of tools for effective processes of reproduction of the studied species of plants indicate the potential and reliability of their systems for reproduction and resettlement, and identification of the causes of the decreasing numbers allow us to develop scientifically based methods and optimize the resumption of types (nature reserves, introduction to culture, repatriation, etc.).

Keywords: Rare species of plants, generative structures, antecology, seeds formation, dissemination.

INTRODUCTION

Currently, actively developing industry, agricultural production with the use of various chemical agents, meliorative measures lead to violations of natural landscapes and plant communities, as well as reduce the species diversity of

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plants. Therefore, problem of preserving gene pool of plant world, together with threat of climate warming, presence of ozone holes in a stratospheric layer, acid rain and accumulation of a toxic waste in the ground, is among the global environmental problems. This is especially important for rare and endangered species of plants, number of populations of which and individuals in them gradually decreases. In this regard, one of the primary environmental problems is to find out the reasons for reduction in a number of rare species and to develop scientifically based techniques for optimizing their renewal and reproduction. Due to its origin and geographical location, the Crimea has a unique flora that, according to V.N. Golubev (1996), has about 2.700 species and subspecies, and nearly 750 of them need protection. Therefore, attention of scientists is directed to a comprehensive study of rare and endangered species of the Crimean flora, as evidenced by numerous publications on the ranges of the species studied, the state of their cenopopulations, seed productivity and breeding characteristics (Kosykh, Golubev, 1983; Krainyuk, 1988; Korzhenevsky, Bagrikova, Ryff, Bondareva, 2004; Shevchenko, Kuzmina, Marco, Yaroslavtseva, 2010; Shevchenko, 2014; Shevchenko, Gafarova, 2015; and others). In this aspect, it is very important to know the reproductive biology of rare and endangered species, including the formation of generative structures, antekologii and embryonic development, which is necessary to create a general picture and assess the prospects for natural renewal of the species under study.

MATERIAL AND METHODS

The objects of these studies were 5 species of flowering plants belonging to different families and growing within the natural range in the: *Arbutus andrachne* L. (Ericaceae), *Pistacia mutica* Fisch et Mey. (Anacardiaceae), *Campanula talievii* Juz.(Campanulaceae), *Fumanopsis laevis* (Cav.) Tzvelev (Cistaceae) and *Glaucium flavum* Crantz.(Papaveraceae).

Arbutus andrachne L. is an evergreen multi-stem tree up to 12 m high. The bark is thin, smooth, dark coral in color, in June-July it cracks and flakes, revealing a young green bark, which by the end of the summer acquires a coral-red color. Leaves are leathery, glossy, ovate-oblong, entire. Tertiary relic, in the Southern Coast of the Crimea grows in the form of small groves or individual trees on dry stony and rocky slopes. In accordance with the classification of V.N. Golubev (1996), according to ecomorphological features this species can be characterized as lithophyte, mesoxerophyte, heliophyte and glycophyte.*Pistacia mutica* Fisch et Mey. - deciduous, often multi-barreled tree up to 15 m high. Leaves pinnate, consist of 3-7 leaves of elliptic form. The species is characterized as hemikserophyte, aeropedophyte, heliophyte, glycophyte. According to E.L. Kordyum and G.I. Glushchenko (1976), *Pistacia mutica* refers to monoecious, dioecious polygamous plants. Most of the Crimean populations of *P. mutica* are represented by dioecious individuals. It grows singly, in small groups or communities in large areas. *Campanula talievii* Juz. - it is a polycarpic, herbaceous, half-rooted plant up to 25-30 cm tall. According to V.N. Golubev

(1996), this is the endeme of the Crimea. Generative shoots are pubescent, numerous. Flowers are also numerous. Above-ground shoots are represented by rosettes of lanceolate leaves. An underground shoot is branched, rod-shaped, fusiform-shaped, light-white in color. The species can be characterized as aeropedophyte and calfyte with a deep rod-root system.

Fumanopsis laevis (Cav.) Tzvelev (syn. *Fumana thymifolia* (L.) Spachet Webb) this is an evergreen Mediterranean summer-winter-green dwarf shrub of loose form, 25-40 cm high. The stem is erect, branchy, with pubescent and protruding branches. The species is characterized as euxerophyte, heliophyte, glycophyte. According to N.I. Rubtsov and G.A. Kupatadze (1978), the only place where the species grows in the Crimea is the southeastern gravelly slope of Mount Koshka (Simeiz village). It grows on dry open rocky and gravelly limestone slopes, among juniper woodlands.

Glaucium flavum Crantz – it is a biennial or perennial bluish-green herbaceous plant with a height of 20-50 cm. The stem leaves are lyrate-pinnately divided, up to 30 cm long. It grows in the littoral belt on seaside sand, shell rock, pebble, less often on seaside limestone rocks, stony-gravelly slopes, clayey and marly cliffs. Xeromesophyte, heliophyte, psammopetrophyte. This species is resistant to sea spray, falling asleep with sand and gravel.

The material for embryological studies was fixed with a Carnoy solution (6: 3: 1) and Chamberlain fixative (ethyl alcohol 70% -90 parts: formalin 40% -5 parts: glacial acetic acid-5 parts). In the preparation of permanent preparations, conventional methods were used (Romeys, 1954; Pausheva, 1990). Sections of 10-12 μm in thickness were obtained using a semi-automatic rotary microtome RMD-3000. The preparations were stained with methylgrunpironin with an Alcyan blue tint (Shevchenko, Ruguzov, Efremova, 1986; Shevchenko, Chebotar, 1992) and Heidenhain hematoxylin with Alcyan blue tint (Zhinkina and Voronova, 2000). The analysis of the preparations was carried out with the help of the Enamed 2 microscope of Carl Zeiss (Jena), photographs were taken with a digital camera A 550. Anecological observations were carried out under conditions of natural growth of the species under study.

RESULTS AND DISCUSSION

In the Crimea, the species studied bloom in the spring - early summer. Thus, *A. andrachne* blooms in April-May at an average daily temperature of +7 - + 10 ° C and can be attributed to the group of medium-spring-flowering plants. Its flowers are bisexual, collected in racemose inflorescences. Androecium is represented by 10 stamens, anthers of which are free, quadrilocular, are opened, like many other representatives of the subfamily *Vaccinioidae* (Yakobson, Terekhin, 1983), with apical pores. The wall microsporangium develops centripetally, and formed consists of the epidermis, endothecium, 3 middle layers and tapetum. The wall of the mature anther is represented by the epidermis, the cells of which are filled with tannin, the fibrous endothecium and the tapetal glume with orbiculs. Mature pollen grains are meridional-3-furrow-aperture,

remain collected in tetrads, 3-cell, consisting of a vegetative cell and two spermiums. Ovary *A. andrachne* upper, 5-locular, ovule anatropic, unitegmal. The embryo sac is monosporous, formed by Polygonum-type from the halazal megaspore. The mature embryo sac is 8-nuclear, 7-cell, elongated with a somewhat enlarged micropylar part (Figure 1).

P. mutica also blossoms in April-May, according to the flowering time can be attributed to the late-spring-flowering plants. Flowers unisexual, male are represented by free stamens, anthers are 4-locular, dithecal, are opened with longitudinal slits. The wall microsporangium develops centripetally, and formed consists of the epidermis, endothecium, 1-3 middle layers and tapetum. The wall of a mature anther is represented by epidermis and fibrous endotecium, sometimes there are remnants of tapetum. Mature pollen grains are single, 2-celled, consisting of vegetative and generative cells. Ovary *P. mutica* upper, ovule crassinucellate, unitegmal, anatropic. However, as the seed develops, the ovules change their position and become campylotropic. The embryo sac is formed from the chalazal megaspore by the Polygonum-type. The mature embryonic sac is large, rounded, 8-nuclear, 7-celled. As the endosperm and embryo develop, it increases and lengthens (Figure 2).

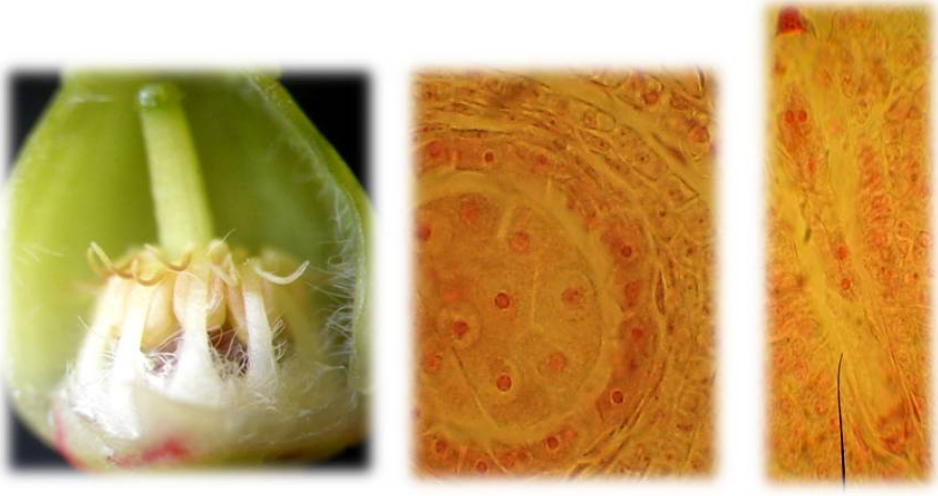

Figure 1. Fragments of *A. andrachne* generative structures

The flowering of *C. talievii* is much longer and lasts from May to August-September. Androecium represented by 5 stamens. The stamens are straight and equal, attached to the nectarean disk, located symmetrically. Stamen filaments have enlarged bases, which, when closed, formed a dome with an opening at the top. The link continues the stamen thread. Anthers are 4-locular, 2-thecal. The wall microsporangium is formed centrifugally. The formed wall of microsporangium consists of epidermis, endothecium, one middle layer and secretory tapetum. The wall of a mature anther consists of squashed cells of the

epidermis, which is covered with cuticle, and endothecium with fibrous thickenings.

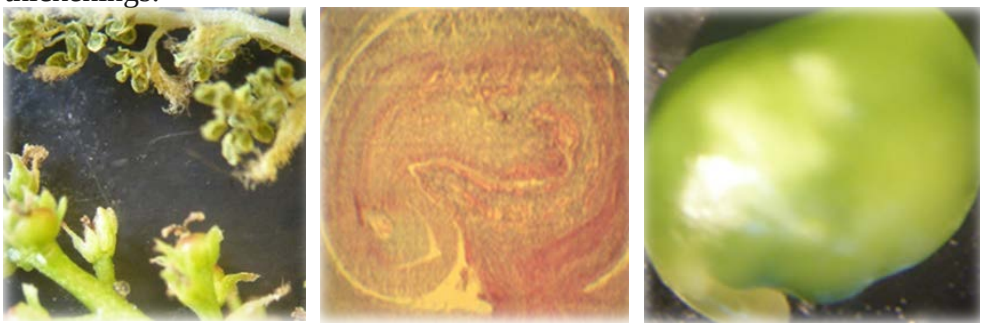

Figure 2. Some elements of *P. mutica* reproductive sphere

Endothecium is often two-row, from the side of the connective it can be two- and three-rowed. The anther is opened introrzno with the help of a longitudinal slit. Mature pollen grains 3-furrow-poral, 3-cell, spermiogenic division often passes on the stigma of the pistil.Ovary of*C. talievii* is lower, ovule is anatropic, medionucellate, unitegmal. The embryonic sac, like many other representatives of the Campanulaceae family (Korobova, Zhinkina, 1987; Zhinkina, 1995), is formed in accordance with Polygonum-type. It is 7-celled, 8-nucleus, elongated, surrounded by tabular cells of the integumental tapetum, reaching the base of the egg apparatus (Figure 3).

Figure 3. Embryo sac and fruits with seeds of *C. talievii*

F. laevis blooms for a long time, from the end of April to July. Androecium is represented by numerous stamens, located in circles. The stamens of the outer circle are sterile (without anther). Anthers 2-thecal, 4-locular, sometimes two-locular. The wall microsporangium develops centrifugally,tapetum is a derivative of the internal parietal layer.

A fully formed wall of microsporangium consists of the epidermis, endothecium, one middle layer and tapetum (Figure 4). The wall of a mature anther consists of the epidermis and endotecium, there is a tapetal glume. Mature pollen grains are two-celled, three-pore. Ovary of F. laevis upper, 3-locular, ovule ortorophic, bitegmal, crassinucellate. The embryo sac is formed by Polygonum-type, 7-cell, 8-nuclear, contains a well-differentiated egg apparatus, the polar nuclei merge before or during fertilization. Antipodes are represented by three cells that can form an antipodal complex.

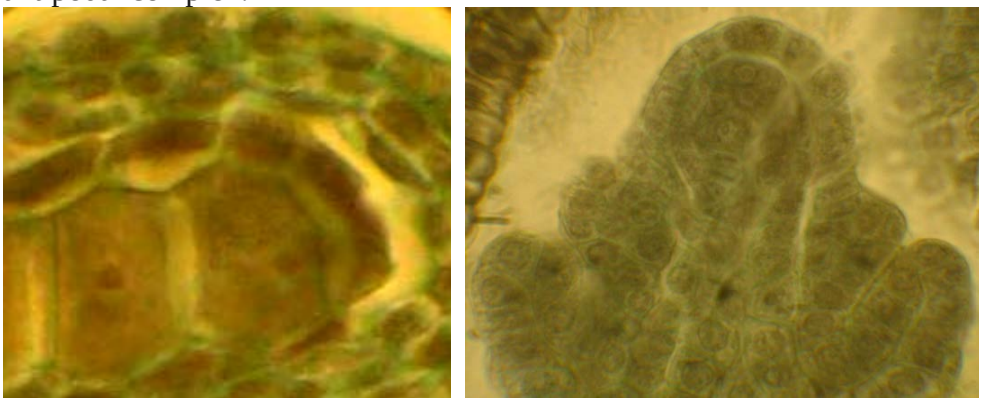

Figure 4. Some stage form of *F. laevis* generative structures

G. flavum blooms for a long time, and it is characterized by the stretching of the phenophases in time and their overlapping. Androecium is represented by numerous free stamens. Anthers 4-locular, 2-thecal.The wall microsporangium develops centripetally, and formed consists of the epidermis, endothecium, 2 middle layers and tapetum, which is a derivative of the secondary parietal layer. The wall of a mature anther consists of the flattened cells of the epidermis, fibrous endothecium and the remains of the middle layer. Mature pollen grains are 2-celled. The ovule of *G. flavum* can be described as camphilotropic, crassinucellate, bitegmal. Embryo sacs Polygonum-type, 7-celled. The egg apparatus is represented by two pear-shaped synergids and an egg. The polar nuclei are located in the central zone of the central cell, closer to the antipodes than to the egg cell. Antipodes are large, occupy more than a third of the embryo sac, often greatly expand, forming an antipodal complex and performing the function of haustorium.

One of the most important stages of the reproductive cycle is pollination, the effectiveness of which depends on the subsequent processes of fertilization and seed formation. Of the species represented, only *P. mutica* is an anemophilous plant, and the rest are entomophiles. However, the processes of pollination are provided by different devices.

Thus, in *A. andrachne* the base of the filamentous filament is strongly pubescent, the anther has excrescence, and the pollen grains have the viscin

filaments (see Figure 1) . All this contributes to the success of pollination. In *C. talievii* the device for pollination is the thickening of stamen filaments at their base. The attraction of insects in *F. laevis* and *G. flavum* is bright yellow flowers, movements of stamens. At the end of flowering flowers in the case of lack of allogamy, they can also have autogamy. A similar phenomenon is observed, for example, in some other species of the genus Fumana (Güemes, Boscaiu, 2001; Carrio et al., 2008).

The presence of viable male and female generative elements, effective pollination provides the subsequent processes of fertilization and the formation of fruits and seeds. Fruit in *A. andrachne* wrinkled orange berry, in *P. mutica* - small turquoise dry turtle, in *F. laevis* the fruit is spherical, trihedral skinny capsule, opening with three valves, in *G. flavum* the fruit is elongated (up to 25 cm) pod. *C. talievii* fruit is a drooping, trilocular, multi-seeded capsule with three rigid bases at the base, through which seeds are scattered.

Seed productivity in *F. laevis*, *G. flavum* and *C. talievii* is quite high, in *A. andrachne* the real seed productivity is only 2-3%, which is a consequence of inbred depression. In *P. mutica*, up to 80-90% of the embryos are formed, which, however, are eaten by the pistachio semeid in the development process, and there is no normal seed in this species.

Dissemination of *C. talievii* and *G. flavum* is provided by ballistochoria (ballistoanemochory and ballistozoochory), as well as epizoochory (with the direct participation of animals). After rash from the capsule, light and small seeds can be carried for considerable distances by gusts of wind (anemochory). The process of dissemination in A. andrachne and *P. mutica* is promoted by birds (ornithochory), the seeds of *F. laevis* are spread with the help of ants (myrmecochory).

CONCLUSIONS

It should be noted, that the main limiting factors in the reproduction of these species are possible extremely low air temperatures, leading to anomalous development of generative structures, the absence of pollinator insects during the flowering period, weak competitiveness, negative anthropogenic impact (trampling, bouquets, Development of territories), damage by animals. However, the formation of effective viable male and female generative structures in the studied species, effective pollination, fertilization and seed formation processes, successful dissemination show that these species have a reliable multiplicative and reproductive system, as well as the potential for species dispersal and colonization of new neighbouring erritories.

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FORAGE AND GRAIN PRODUCTION DYNAMICS OF *TRITICALE* SOWN ON DIFFERENT DATES UNDER IRRIGATED CONDITIONS

SUMMARY

To study the forage and grain production dynamics of *triticale*, a trial was c arried out having four genotypes planted on three different dates under irrigated conditions at Dera Ismail Khan, Pakistan (31°49'53"N 70°54'7"E). All the four genotypes were of different pedigree/parentage background. Trial was laid out in split-plot design with genotypes in main plots while dates of sowing in sub-plots. The trial was replicated three times. First date of sowing started with planting the genotypes on Oct. 25 (T_1) followed by Nov. 15 (T_2), and Dec. 05 (T_3). All the genotypes planted at each date were harvested 50 days after planting. The results showed that the earliest planting produced significantly the highest green forage yield, maximum plant height and grain spike^{-1}. However, grain yield remained similar statistically when planted the *Triticale* on different dates. Comparing the results of four genotypes studied, it was observed that each genotype behaved significantly different for green forage yield, days to 50% heading, plant height, tiller m^{-2}, grains/spike, 1000-grain weight and bio-mass yield. Genotypes 3 and 4 produced maximum green forage yield i.e. 3.70 and 3.65 t ha^{-1}, respectively, however differences among the four genotypes for grain yield were found non-significant statistically. Net Photosynthesis Rate and Chlorophyll content recorded for all genotypes and dates of sowing remained similar statistically. The highest Benefit-Cost ratio (3.23) was calculated for gynotyp-4 when planted on the earliest dates i.e. Oct-05. It was, however, observed that net benefit decreased with delay in each planting date irrespective of the genotypes.

Keywords: *Triticale*, Sowing Dates, Genotypes, Forage Yield, Grain Yield.

INTRODUCTION

Triticale (*Triticosecale*) is a self-pollinated cereal crop belongs to the family *Gramineae* or *Poaceae* and is similar in appearance with wheat. It is a crop having maximum protein and is used for grain as well as forage purposes in many parts of the world. Wojtkowiak *et al*. (2015) reported that the highest grain and protein yield of Milewo variety spring triticale was obtained after the

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application of nitrogen fertilization at the rate of 120 kg ha^{-1}. In Pakistan it is normally cultivated in cool and dry rabi season in some districts of KPK (from November to March) when small growers face severe shortage of hay in the month of November to March, usually obtain 6 to15 t/ha green fodder of triticale. Green triticale fodder contains approximately 25% crude protein, grains and straw are extremely nutritive feedstuffs (Ahmed and Meisner, 2002). It has potential to give maximum yield under drought condition as compared to wheat crop due to it's well develop root system. Thus the use of triticale for dual purpose was known as an exhilarating new choice for green fodder and grain production (Ahmed and Meisner, 2002). Triticale produces more fresh fodder, approximately 20%, than wheat and is rich in quality than rye or wheat (Koch and Paisley, 2004). Acar *et al.* (2011) observed that the assessment of total yield obtained at the end of the experiment showed that the highest hay yield was realized at the milk dough stage with 500 or 650 seed m^{-2} of triticale. It is used in the preparation of alcoholic drinks and other bakery products. The only disadvantage of the triticale is that its floor could not be made in to bread alone because of lower gluten content in it. It can be used as sustainable agriculture crop by rotating it with vegetable and other cereal crops. Triticale is a long day crop and produces maximum tillers and other yield components when it is planted on optimum sowing date. Tillering in triticale increases when temperature rises and days become long (Wladyslaw and Bogdan, 2012). Late sowing of winter cereal never allows plant to produce maximum number of tillers. Normal planting of triticale like wheat crop gives high grain yield due to the availability of long photo period and optimum grain filling duration. Both the varieties and sowing time significantly affect the yield and yield parameters of triticale. On the other hand, late planting not only reduces forage yield but the grain yield as well. Triticale varieties were planted on various sowing dates on two different seasons due to delaying in both season grain yields was adversely affected. Early planted (Nov. 15- December-01) showed more hay yield and late planted (from Nov. 15 to Dec. 15), which affected grain yield up to 22.9% - 46.07% respectively (El-Metwally *et al.*, 2012). Being a new crop (in Dera Ismail Khan, KPK. Pakistan), no work has been done on triticale agronomic management for fodder or grain production. Therefore, the present study is being conducted to evaluate the various genotypes production potential under different sowing dates under agro-ecological condition of D.I. Khan.

MATERIAL AND METHODS

The experiment was conducted in a randomized complete block design with split plot arrangement having three replications during 2013-2014 in Pakistan. The net plot size was 1.8 m x 5 m (9 m^2) with six rows, 5 m long and 30 cm apart. The main plots consist of three sowing dates while the triticale genotypes were assigned to sub-plot. Total six (6) numbers of irrigations were applied by canal water throughout the life of experimental period. All the culture practices and recommended doses of NPK were applied accordingly. The 100 kg

ha^{-1} seed rate was used for sowing of experiment. Sowing dates were: Oct. 25, Nov. 15, and Dec. 05. Four genotypes tested were as follow with given pedigree record.

Table: Factor B (Genotypes) sub- plot

Triticale Line	Pedigree/Parantage
Genotype 1	POLLMER_1.2//ANOAS_5/STIER_13/4/GAUR_2/HARE_3//JLO97/CIVET/3/ ARDI_1/TOPO 1419//ERIZO_9 CTSS00B00127S-0M-9Y-010M-1Y-2M-0Y
Genotype 2	POLLMER_2.2.1*2//FARAS/CMH84.4414 CTSS99B00990F-0TOPY-0M-2Y-2M-1Y-1M-0Y
Genotype 3	ALPACA_1/3/ZEBRA 31/CIVET//URON_ 5/7/CIN/PI//PATO/3/BGL/4/DRIRA/5/ DLF99/3/M2A/SNP//BGL/4/TESMO_1 /6/FAHAD_1/8 /GAUR_3/ANOAS_2//BANT_1CTSS01Y00781T-0TOPB-7Y-010M-7Y-10M-0Y
Genotype 4	DAHBI/3/FAHAD_8-2*2//PTR/PND-T/7/LIRON_2/5/DISB5/3/SPHD/PVN// YOGUI_6/4/KER_3/6/BULL_10/MANATI_1 CTSS02Y00771S-040Y-5Y-3M-0Y

Data were collected for forage yield (t ha^{-1}), chlorophyll contents (µ g cm^{-2}), net photosynthesis rate (µ mole m^{-2} sec^{-1}), number of tillers (m^{-2}), number of grains ($spike^{-1}$), 1000- grain weight (g), biological yield (t ha^{-1}), grain yield (t ha^{-1}) and harvest index (%). The data were analyzed statistically by using the analysis of variance technique and subsequently least significance test (LSD) were applied for comparing the treatment means using computer software (Statistix version 9).

RESULTS AND DISCUSSION

The data presented in Table 3 regarding forage yield indicated that sowing dates, triticale genotypes/lines and their interaction showed significant variation among the treatment means. Data regarding interaction clearly indicated that line 3 produced maximum (7.29 t ha^{-1}) green forage yield on D1 (Oct. 25) followed by (6.70 t ha^{-1}) in line 4 on the same date D1, where as the lowest (1.33 t/ha^{-1}) green forage yield was noted in line 3 when planted on D3 (Dec. 05).The lowest green forage yield in the late sowing dates may be due to less vegetative growth period from sowing, that resulted in lower canopy development of the triticale lines. The higher green forage yield among the genotypes/lines may be due to the genetic potential for rapid growth during the early stages which got maximum time for vegetative growth.

Four different lines of *Triticale* showed significant differences for various yield and yield contributing parameters (Table 1). Line-3 and Line-4 produced maximum forage yield of 3.67 and 3.65 t ha^{-1}. Chlorophyll content remained same for all the lines tested, however, Line-1 found to be more efficient than other showing maximum chlorophyll content of 52.19. Similarly net

photosynthesis rate was recorded the same for all lines. Line-1 significantly took maximum number of days (106) in producing 50% heading as compared to all other lines which took statistically similar number of days to 50% heading. Line-2 produced the tallest plants with height of 113.13 cm while all other lines had statistically similar plant height. Maximum numbers of tillers were produced by line-3 and line-4 i.e. 247.36 and 255.52 tillers m^{-2}. The highest number of grains $spike^{-1}$ was produced by line-1 (60.56) while minimum grain spike were produced by line-3 (48.56 grains $spike^{-1}$). Line-1 and Line-2 produced highest 1000-grain weight with 44.91 and 45.48 g, respectively. Biological yield remained similar statistically for all lines tested (Table-1). Grain yield was also found similar statistically for all lines. Harvest Index was maximum (41.69%) for line-4 and minimum for line-3 (36.55%). Similar biological yield, grain yield in all the lines test might be due to start of rains (favorable environment) during the final grain filling stage, which prolonged to more than a month, in which the late planted lines recovered their photosynthates which otherwise might be much lower if the season remained normal (dry and hot).

Table 2. Yield and other parameters as influenced by three sowing dates

Parameters	Date of sowing		
	Oct-25	**Nov-15**	**Dec-05**
Forage Yield (t ha^{-1})	6.3609 a	2.2593 b	1.4077 c
Chlorophyll content	49.783ab	46.750b	53.175a
Net Photosynthesis Rate (Pn)	54.083	36.683	25.283
Days to 50% heading	110.92 a	102.17 b	99.50 b
Plant height (cm)	117.74 a	121.13 a	109.60 b
Tillers (m^{-2})	224.91	228.33	227.89
grains ($spike^{-1}$)	56.750 a	50.833 b	53.167 b
1000- grain weight (g)	44.943	43.829	41.293
Biological yield (t ha^{-1})	14.544 a	15.099 a	11.342 b
Grain yield (t ha^{-1})	5.6813	5.0405	4.9584
Harvest Index (%)	39.068ab	33.641 b	44.238 a

Means followed by different letters in respective column are significant ($P<0.05$)

Interaction between the two factors was found non-significant for all the parameters studied except forage yield (Table 3). Maximum forage yield of 7.29 t ha^{-1} was obtained from D1L3 followed by D1L4 (6.70 t ha^{-1}), D1L2 (6.16 t ha^{-1}) and D1L1 (5.28 t ha^{-1}). The plausible reason for higher green forage yield in early planting might be due to more available time for vegetative growth and development which accumulated more food and attained maximum height and gave the maximum forage yield as compared to late planted lines which remained smaller in height due to low temperature during the month of December and January.

Economic Analysis

Economic analysis of the data regarding different triticale genotypes sown at various dates has been presented in Table 4. The highest net income of Rs.

155800 was received from the sale of green fodder (GF) and grain yield (GY) of triticale in treatment T4 (D1L4), followed by T1(D1L1), T2(D1L2) and T3(D1L3) with Rs. 132,200/-, 122,150/- and 117, 950/-, respectively. The least net income of Rs. 71,700/- was recorded in treatment T9 (D3L1). The data regarding benefit cost ratio (BCR), the maximum BCR of 3.23 was recorded in T4 (D1L4) followed by T1(D1L1), T2 (D1L2) and T3 (D1L3) 2.8 ,2.75 and 2.69 respectively, while the least BCR of 2.02 was recorded in T9 (D3L1).

Table 3. Interactive effect of sowing dates and *Triticale* lines for yield and yield parameters

Treatments	Forage Yield t ha^{-1}	Chlorophyll content	Net Photosyn. Rate (Pn)	Days to 50% head.	Plant height (cm)	tillers (m^{-2})	grain (spike^{-1})	1000-grain weight (g)	Biolog. yield (t ha^{-1})	Grain yield (t ha^{-1})	Harv. Index (%)
D1L1	5.28 d	48.47	35.77	111.00	116.27	183.55	68.00	46.57	15.19	5.87	38.45
D2L1	1.41 g	54.47	43.80	105.00	116.73	197.77	58.33	44.79	17.02	5.14	23.23
D3L1	1.41 g	53.63	20.67	103.00	106.40	186.89	55.33	43.38	10.54	4.49	43.05
D1L2	6.16 c	51.63	51.37	112.00	124.21	213.65	54.00	46.79	13.93	5.39	39.25
D2L2	2.33 f	45.67	49.67	103.33	128.43	227.66	51.67	46.80	15.63	5.49	35.13
D3L2	1.48 g	54.73	30.97	98.00	113.98	206.33	53.67	42.75	10.30	4.70	46.96
D1L3	7.29 a	48.60	58.00	111.67	116.37	244.99	50.33	41.14	13.62	5.05	37.25
D2L3	2.47f	39.03	28.17	99.00	119.22	245.66	43.67	41.14	14.42	4.44	30.69
D3L3	1.33 g	54.03	29.27	98.33	109.36	251.44	51.67	40.11	12.48	5.21	41.72
D1L4	6.70 b	50.43	71.20	109.00	114.13	257.44	54.67	45.28	15.43	6.41	11.32
D2L4	2.83 e	47.83	25.10	101.33	120.13	242.22	49.67	42.59	13.33	5.10	38.52
D3L4	1.41 g	50.30	20.23	98.67	108.67	266.89	52.00	38.93	12.05	5.43	45.22

Means followed by different letters in respective column are significant (P<0.05)

Table 4. Benefit Cost Ratio (BCR) of triticale grain and green fodder yield as affected by various sowing dates

Treatment	Cost of Production	GFY	Grain Yield	Income (Rs:)		Total Income	Net Income	BCR
				Green fodder	Grain yield			
T1(D1V1)	70,000	5.28	5.86	26400	175,800	202,200	132,200	2.89
T2 (D1V2)	70,000	6.15	5.38	30750	161,400	192,150	122,150	2.75
T3(D1V3)	70,000	7.29	5.05	36450	151,500	187,950	117,950	2.69
T4 (D1V4)	70,000	6.7	6.41	33500	192,300	225,800	155,800	3.23
T5 (D2V1)	70,000	1.41	5.14	7050	154,200	161,250	91,250	2.30
T6 (D2V2)	70,000	2.32	5.48	11600	164,400	176,000	106,000	2.51
T7 (D2V3)	70,000	2.46	4.43	12300	132,900	145,200	75,200	2.07
T8 (D2V4)	70,000	2.83	5.09	14150	152,700	166,850	96,850	2.38
T9 (D3V1)	70,000	1.4	4.49	7000	134,700	141,700	71,700	2.02
T10 (D3V2)	70,000	1.48	4.7	7400	141,000	148,400	78,400	2.12
T11 (D3V3)	70,000	1.33	5.2	6650	156,000	162,650	92,650	2.32
T12 (D3V4)	70,000	1.4	5.4	7000	162,000	169,000	99,000	2.41

Market price for green fodder/kg = Rs: 5/- (Pakistani rupees)
Market price for grains/kg = Rs: 30/- (Pakistani rupees)

CONCLUSIONS

It can be inferred from the data in Table 4 that the late planted treatments (D2 and D3) gave lower yield of green fodder thus resulted in low net return and BCR, respectively. Hence it is recommended that the treatments (T4, T1, T2, and T3) with reasonable BCRs are the most suitable combinations of early sowing dates (Oct. 25) for excellent fodder and grain yield.

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RESPONSE OF DURUM WHEAT TO NITROGEN FERTILIZATION RATES

SUMMARY

The response of new Bulgarian durum wheat variety Predel to nitrogen fertilization was studied in a field fertilizing experiment with cotton – durum wheat crop rotation for the period 2008 – 2014, at the Institute of Field Crops – Chirpan, Bulgaria under rainy conditions. The studied nitrogen rates were 0; 60; 120 and 180 kg N.ha-1. The experimental design consisted of randomized block design with four replications. A tendency was found that nitrogen fertilization increased grain yield. The nitrogen rates N120 and N180 proved increased grain protein yield with 49.6 and 48.7 % compared to the control. The rate N180 decreased the harvest index. The highest agronomic efficiency of nitrogen for grain and grain protein were obtained with moderate rate N120 average for the period. Nitrogen fertilization decreased partially the factor of nitrogen productivity from 68 kg.kg-1 at rate N60 to 25 kg.kg-1 at rate N180. The highest concentration of grain protein - 15.97 % and vitreousness of the grain - 75.84 were obtained after applying N180. The content of wet and dry gluten slightly depended on nitrogen fertilization. A strong positive correlation was established between nitrogen fertilization and grain+straw yield (r=0.726**), grain protein concentration (r=0.862**), grain protein yield (r=0.635**) and vitreousness of the wheat grain (r=0.856**).

Keywords: Nitrogen rates, Durum wheat, Grain quality, Efficiency.

INTRODUCTION

Nitrogen fertilization is a major factor for high yields and grain quality of durum wheat (Panayotova, 2010; Ricciardi, 2001). Nitrogen has a strong influence on growth, but its impact on yield, quality and dry matter formation depends on the growing conditions. In meteorological terms, the favorable years strongly manifest the effect of higher nitrogen rate (Panayotova and Dechev, 2002; Rharrabti et al., 2003). According to Panayotova and Dechev (2003) the coefficient of yield variation of durum wheat from year to year is higher after fertilization with higher norms (N14P6). A number of studies (Hawkesford, 2012; López-Bellido and López-Bellido, 2001; Mohammadi and Amri, 2009; Panayotova, 1998) have established the fertilizing effectiveness of varieties with different genetic traits under different soil fertility was established. Durum wheat

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requires optimum fertilizer rates, consistent with the conditions of the area and the specifics of the field to increase the grain quality (Panayotova and Gorbanov, 1999). The main requirement for good quality of the grain is for the plant to obtain the optimal nitrogen amount during vegetation (Fixen, 2009). Nitrogen fertilization increases many of the quality indicators of durum wheat - crude protein content, wet and dry gluten, weight of 1000 grains, cooking properties of the pasta products (Panayotova and Gorbanov, 1999). The interaction between environmental conditions and nitrogen rates has a significant impact on grain quality (Sanjeev et al., 2000). According to Schulthess et al. (1993) the nitrogen content in the grain correlates significantly with the growing conditions. The yield is highest and of best quality when at the end of the grain filling stage begins slow drying and the temperature is raised gradually. The sharp temperature rise, coupled with rapid drought, leads to worse quality (Panayotova and Valkova, 2010).

The aim of the present study was to analyze the relationships between the main grain indicators of productivity and quality and the nitrogen fertilization rates for durum wheat Predel cultivar grown in southern Bulgaria over years of different meteorological conditions.

MATERIAL AND METHODS

The response of the new Bulgarian durum wheat cultivar Predel to nitrogen fertilization rates was studied in a field fertilizing experiment with cotton – durum wheat crop rotation during the period 2008 – 2014, at the Institute of Field Crops – Chirpan, Bulgaria, under rainy conditions. The experimental design consisted of randomized block design with four replications. The harvested size of the plots was 10 m^2. The studied fertilization rates were as follows: 0; 60; 120 and 180 kg $N.ha^{-1}$. Nitrogen fertilization in the form of NH_4NO_3 was applied before sowing (1/3 of the rate) and at early spring (2/3 of the rate). The phosphorus fertilization (P_{80}) was done before sowing in the form of triple superphosphate. The precursor crop was cotton fertilized by N_{80}. The soil type of the experimental field was *Pellic vertisols* (FAO), generally referred to as the so called Mediterranean chernozems. The soil type is one of the most generous and widely spread and significant in Bulgaria. It is suitable for growing most of the field crops and has a potential for high yield. The main parent materials were pliozen clay deposits. It has a high-powered humus horizon (70–80 cm), with a compact zone of the profile (united horizon). By humus content it belongs to the mean humus soils. It characterizes with high humidity capacity, caused by the high percentage of clay minerals, with clay soil texture, small water-permeability, bulk density of the arable soil layer - 1.2-1.3 $g.cm^{-3}$, with specific gravity 2.4-2.6 and low total porosity, neutral soil reaction and high cation exchange capacity (CEC) - 35-46 meq per 100 g soil, with a high degree of bases saturation (93.4-100.0 %), with total N in the arable layer ranging within 0.095-0.14 % and low content of total phosphorus (0.05-0.11 %), poor to medium supplied with hydrolyzed nitrogen, poorly supplied with available

phosphorus and well-supplied with available potassium. Regarding the temperature during durum wheat vegetation, two of the experimental years were characterized as very warm, two as hot and two with values close to the multi-annual rate. In terms of precipitation one of the experimental years was wet, two years were dry, but in the three harvest years the rainfall values ware close to the norm. The productivity of durum wheat was determined by grain yield ($kg.ha^{-1}$), grain+straw yield ($kg.ha^{-1}$), grain protein yield ($kg.ha^{-1}$) and harvest index. The protein content in the grain (%) was determined by the Keldahl method (N, % x 5.7) according to BSS ISO 1871, and wet gluten content (%) - according to BSS 13375 by means of an automatic gluten-washable apparatus. The vitreousness of the grain (%) was determined by cutting the grains with pharynotom of Heinsdorf (standard ICC 129).

The main nitrogen use efficiency indicators of agronomic efficiency and partial factor productivity were used to assess the durum wheat response to nitrogen (Dobermann, 2007). agronomic efficiency (AE) and partial factor productivity (PFP) were calculated on dry weight basis using the following formulas: $AE = (Y-Y_0)/F$ ($kg.kg^{-1}$); and $PFP = Y/F$ ($kg.kg^{-1}$), where Y and Y_0 were grain or grain protein yields from fertilized treatments and unfertilized control, respectively, and F - amount of N fertilizer applied ($kg.ha^{-1}$). The data were statistically analysed with the ANOVA procedure within the SPSS statistical program and Duncan's multiple range test (P = 0.05) to find significant differences among means.

RESULTS AND DISCUSSION

A positive effect of nitrogen fertilization at rates 0 to 180 $kg.ha^{-1}$ on grain yield of the Predel cultivar was found but the differences were not mathematically proven (Table 1). In years with different hydro-thermal conditions without nitrogen fertilization were obtained strongly variable yields - from 2790 to 4250 $kg.ha^{-1}$. With high nitrogen rate N_{180} the lowest average yield was 3850 $kg.ha^{-1}$ and the highest registered grain yield was 5380 $kg.ha^{-1}$. These results confirmed that the nitrogen effect on grain yields of durum wheat was strongly dependent on the weather conditions during durum wheat vegetation (Modhej et al., 2008).

The total grain+straw yield increased significantly with the increase of nitrogen fertilization. It was highest at the rate of N_{180}, exceeding the unfertilized control with 46.3 %. Application of fertilizer rate N_{180} was proven to decrease the harvest yield index - with 15.3 % compared to the unfertilized plants. The low N_{60} and moderate N_{120} nitrogen rates had no significant effect on the harvest yield index and its values were close to the control. The yield of grain protein during the study period varied over a wide range - from 346 to 905 $kg.ha^{-1}$. Application of moderate and high nitrogen rates demonstrated proven increase of the grain protein yield with 49.6 and 48.9 %, respectively, compared to the variant without nitrogen fertilization. The results confirmed that the nitrogen

fertilization is a factor that is of major importance for the protein concentration in the grain (Panayotova and Dechev, 2002).

Table 1. Productivity of durum wheat as dependent on nitrogen rates (kg.ha^{-1})

Nitrogen rates	**N_0**	**N_{60}**	**N_{120}**	**N_{180}**
Grain yield	3658^{ns} ± 659	4068 ± 724	4745 ± 925	4510 ± 636
% to N_0	100	111.2	129.7	123.3
Grain+straw yield	9540^{c} ± 1295	10883^{bc} ± 1527	12728^{ab} ± 2024	13958^{a} ± 2328
% to N_0	100	114.1	133.4	146.3
Harvest index	0.383^{a} ± 0.03(	0.372^{a} ± 0.022	0.372^{a} ± 0.034	0.324^{b} ± 0.009
% to N_0	100	97.3	97.1	84.7
Grain protein yield	486^{b} ± 107	554^{ab} ± 113	727^{a} ± 168	723^{a} ± 132
% to N_0	100	113.9	149.6	148.7

Average for the period the cultivar Predel formed grain with crude protein content of 13.4 - 13.55 % without fertilization and after a low nitrogen rate of 6 kg N.ha^{-1} (Table 2). The use of N_{120} and N_{180} showed an increase in protein concentration in the grain compared to the non-fertilized plants. The protein content of the grain after moderate and high nitrogen fertilization reached 15.20 - 15.97 % ensuring a high biological value of the grain and obtaining high-quality pasta.

Table 2. Grain quality of durum wheat as dependent on nitrogen rates

Quality parameters	**N_0**	**N_{60}**	**N_{120}**	**N_{180}**
Grain protein concentration	13.40^{b} ± 0.90	13.55^{b} ± 0.57	15.20^{a} ± 0.80	15.97^{a} ± 0.78
% to N_0	100	101.1	113.5	119.2
Wet gluten content, %	25.82^{ns} ± 5.90	28.04 ± 5.43	30.32 ± 4.40	32.41 ± 3.38
% to N_0	100	108.6	117.4	125.5
Dry gluten content, %	9.34^{ns} ± 1.97	10.07 ± 2.03	11.10 ± 1.59	11.93 ± 1.42
% to N_0	100	107.8	118.8	127.7
Vitreousness of the grain,%	56.93^{c} ± 6.02	60.24^{cb} ± 5.7	67.45^{b} ± 0.68	75.84^{a} ± 4.52
% to N_0	100	105.8	118.5	133.2

The standard requires that the content of wet gluten in the grain of strong and durum wheat is over 28 %, and dry gluten - over 10 %. The applied rates of nitrogen fertilization increased the content of wet and dry gluten in the grain of cultivar Predel and it met the quality requirements for wet and dry gluten content, unlike the unfertilized. Grain vitreousness is an important indicator for grain structure with proven impact on yield and quantity of the semolina. The high quality durum wheat has vitreousness of over 75-80 %. Grain of high quality in terms of vitreousness was obtained at fertilization with N_{180} - 75.84 % average for the period. The effect of the low rate N_{60} on grain vitreousness was not proven compared to the untreated variant.

Table 3. Agronomic efficiency and partial factor productivity of nitrogen in durum wheat, average for the period 2008-2014 ($kg.kg^{-1}$)

Nitrogen rates	N_{60}	N_{120}	N_{180}
AE-N for grain	$6.8^{ns} \pm 2.7$	9.1 ± 3.0	4.7 ± 2.7
% to N_0	100	133.8	69.1
AE-N for grain protein	$1.2^{ns} \pm 0.4$	1.9 ± 0.6	1.3 ± 0.4
% to N_0	100	158.3	108.3
PFP-N for grain	$68^{a} \pm 12$	$40^{b} \pm 8$	$25^{c} \pm 4$
% to N_0	100	58.8	36.8
PFP-N for grain protein	$9.5^{a} \pm 1.9$	$6.0^{b} \pm 1.4$	$4.0^{b} \pm 0.7$
% to N_0	100	63.2	42.1

The index of agronomic efficiency characterizes the ability of plants to increase grain yield in response to nitrogen fertilization (Craswell and Gowdin, 1984; Novoa and Loomis, 1981) and for wheat depends most heavily on nitrogen fertilization and climatic conditions (Delogua et al., 1998). The results indicated lack of proven differences in nitrogen agronomic efficiency for grain yield and grain protein, average for the period (Table 3). The values of nitrogen agronomic efficiency for grain yield ranged from 0.9 to 12.5 $kg.kg^{-1}$. The highest AE-N for grain - 9.1 $kg.kg^{-1}$ was established for moderate fertilization with N_{120}. The high nitrogen rate N_{180} led to decrease in the additional grain yield per one kilogram of nitrogen fertilizer, 31.9 % over the fertilizing rate N_{60}. The agronomic efficiency of nitrogen for grain protein - 1.2-1.9 $kg.kg^{-1}$ shifted slightly and decreased with the increase of the fertilizing rate over N_{120}. The partial factor productivity of nitrogen represents the kg of grain or grain protein harvested per kg of N fertilizer applied. It can be used as an index of total economic outputs relative to the use of all N sources (soil N and applied fertilizer). Typical levels of PFP for cereal crops are 40-80 units (Dobermann, 2007). The obtained values of the PFP reduced with the increase of the applied amount of nitrogen. The average values of PFP-N for grain yield and for grain protein for cultivar Predel fertilized at rate N_{60} were 68 and 9.5 $kg.kg^{-1}$, respectively. Application of three times more nitrogen fertilizer decreased PFP-N for grain and PFP-N for grain protein with 63.2 and 57.9 %, respectively.

A strong positive correlation was established between nitrogen fertilization and grain+straw yield (r = 0.726**), grain protein concentration (r = 0.862**), grain protein yield (r = 0.635**) and vitreousness of the grain (r= 0.856**) for durum wheat variety Predel (Table 4).

Regression analysis for the dependencies between the resulting parameters (grain yield, grain+straw yield, grain protein yield, grain protein concentration, wet gluten content, dry gluten content and vitreousness of the grain) and the factor nitrogen fertilization on durum wheat cultivar Predel indicated that correlations were represented by equations of the second degree (Table 5).

Table 4. Correlations between nitrogen fertilization, productivity and grain quality of durum wheat

Parameters	Nitrogen fertilization	Grain yield	Grai+straw yield	Grain protein yield	Grain protein, %
Wet gluten content	0.504*	-0.136	0.001	0.054	0.372
Dry gluten content	0.542*	0.037	0.145	0.221	0.492
Vitreousness of the grain	0.856**	0.123	0.435	0.328	0.667**
Grain protein concentration	0.862**	0.741**	0.852**	0.874*	
Grain protein yield	0.635**	0.970**	0.927**		
Grain+straw yield	0.726**	0.875**			
Grain yield	0.471				

Table 5. Regression models for dependence of productivity and grain quality (y) on nitrogen fertilization (x) for durum wheat

Relationships y /x	Equation	R^2
Grain yield	$y = 3598 + 13.4x - 0.0448x^2$	0.267
Grain+straw yield	$y = 9484 + 26.6x - 0.0078x^2$	0.528
Grain protein yield	$y = 471 + 2.4 - 0.0051x^2$	0.416
Grain protein concentration	$y = 13.2 + 0.0094x + 0.005x^2$	0.753
Wet gluten content	$y = 25.8 + 0.0385x + 0.002x^2$	0.254
Dry gluten content	$y = 9.3 + 0.0134x + 0.0001x^2$	0.291
Vitreousness of the grain	$y = 56.8 + 0.0431x + 0.0004x^2$	0.756

High values of coefficients of determination ($R^2 > 0.750$) were found for the grain protein concentration and grain vitreousness in dependence of nitrogen fertilization. The regression model indicated that the values of grain protein concentration, wet gluten content, dry gluten content and grain vitreousness significantly increased with the increase of applied nitrogen fertilization up to rate N_{180}.

CONCLUSIONS

The nitrogen rates N_{120} and N_{180} applied to durum wheat proved increased grain protein yield with 49.6 and 48.7 % compared to the control. The rate N_{180} decreased the harvest index. The highest agronomic efficiency of nitrogen for grain and grain protein were obtained with moderate rate N_{120} average for the experimental period. Nitrogen fertilization decreased partial factor productivity of nitrogen from 68 $kg.kg^{-1}$ at rate N_{60} to 25 $kg.kg^{-1}$ at rate N_{180}. The highest concentration of grain protein - 15.97 % and vitreousness of the grain - 75.84 were obtained after applying N_{180}. The content of wet and dry gluten slightly depended on nitrogen fertilization.

A strong positive correlation was established between nitrogen fertilization and grain+straw yield (r=0.726**), grain protein concentration (r=0.862**), grain protein yield (r=0.635**) and vitreousness of the wheat grain (r=0.856**).

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ESTIMATION OF CALCIUM CARBONATE IN ANTHROPOGENIC SOILS ON FLYSCH DEPOSITS FROM DALMATIA (CROATIA) USING VIS-NIR SPECTROSCOPY

SUMMARY

This study aimed to evaluate the ability to use Vis-NIR spectroscopy to predict $CaCO_3$ in the soil and to determine the contribution of the spectral ranges and wavelengths to the prediction. A total of 180 topsoil samples (0-25 cm) of anthropogenic soils derived from Flysch deposits in Dalmatia (Croatia) were analyzed for $CaCO_3$ and scanned in the laboratory with an ASD FieldSpec spectroradiometer (350-2500 nm). The partial last square regression (PLSR) with leave-one-out cross-validation method was used for calibrating the Vis-NIR spectra and $CaCO_3$ measured in the laboratory. The $CaCO_3$ content in investigated soils varies within a very wide range from 186.0 to 894.7 g kg^{-1} and has a high an average value of 547.2 g kg^{-1} and normal - near symmetrical frequency distribution. Prediction parameters, the coefficient of determination (R^2), the ratio of performance to deviation (RPD) and the range error ratio (RER) were 0.86, 2.42 and 11.4, respectively indicating that created PLSR model was able to predict $CaCO_3$ content in soil with moderately successful accuracy. The prediction error of the $CaCO_3$ measured as the root mean square error of prediction (RMSEP) was 57.9 g kg^{-1}. These results suggest that Vis-NIR spectroscopy in combination with PLSR is acceptable as a rapid method for quality control (screening) of the $CaCO_3$ content in investigated soils.

Keywords: $CaCO_3$, PLSR, RPD, soil, Vis-NIR

RESULTS AND DISCUSSION

During the last two decades many researchers have demonstrated that Vis-NIR diffuse reflectance spectroscopy (DRS) is capable of providing low cost, fast and reliable tool for prediction of different soil properties compared to laboratory analyzes (Ben-Dor and Banin, 1995; Viscarra Rossel *et al*. 2006a; Volkan Bilgili *et al*. 2010; Stevens *et al*. 2013). The carbonates as one of the essential soil properties were often estimated using DRS (Ben-Dor and Banin, 1995, Canasveras *et al*. 2012; Gomez *et al*. 2012 and 2013; Gras *et al*. 2014; Leone *et al*. 2012; Summers *et al*. 2011 Volkan Bilgili *et al*. 2010 and Khayamim *et al*. 2015). The above-mentioned researchers have shown substantial differences in parameters of prediction accuracy (the coefficient of determination (R^2) and the ratio of performance to deviation (RPD) that varied between 0.64-0.99 and 1.74-

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8.6, respectively. The Vis–NIR technique was not as precise as conventional chemical analyses but provides an opportunity to analyse a large number of samples in a short time. $CaCO_3$ significantly influences the reflectance characteristics of a soil and has spectral activity in the NIR spectral region (700-2500 nm). The strongest diagnostic vibrational absorptions are at 2300-2350 nm and other three weaker bands occur near 2120-2160 nm, 1997-2000 nm and 1850-1870 nm (Clark, 1999). The soil spectrum characterizes complex absorption patterns with a large number of predictor variables that are highly collinear, and therefore analyses of diffuse reflectance spectra require the use of multivariate calibrations (Martens and Naes, 1989).The most common calibration method for analyses of $CaCO_3$ (and soil) spectra is partial last square regression (PLSR), developed by Wold *et al*. (2001). A calcium carbonate is the most common carbonate polymorph in soil particularly abundant under semi-arid and dry subhumid conditions (Khayamim *et a*l. 2015). The Ca carbonate has a marked influence on soil chemical properties, eg. pH, cation and anion retention. Ca carbonate surfaces specially interact with phosphate anion, although $CaCO_3$ also controls Ca concentration in soil solution and in the soil exchange complex (Braschi *et al*. 2003). The high content of carbonates increases pH and favours the formation of HCO_3^- ions that lead to disturbance in the availability of some plant nutrition and various chlorosis eg. iron (Ksouri *et al*. 2005). The presence of free calcium carbonate in calcareous soils ensures a very high soil buffer capacity (Bache, 1984). The carbonates interact with soil organic matter (SOM) in aggregate formation and stabilization processes and can thus also contribute to SOM stabilization (Virto *et al*. 2011). Soils derived from Flysch deposits contain a high $CaCO_3$ content that varied in a wide range (Miloš and Maleš, 1998). Rapid, nondestructive, inexpensive and accurate determination of carbonate content in these soils could be very useful for planning of agricultural production. The aim of this work was to estimate the ability of Vis-NIR diffuse reflectance spectroscopy in combination with PLSR for the prediction of $CaCO_3$ content in surface horizon of anthropogenic terraced soils derived from Flysch deposits and to determine the contribution of the spectral ranges and wavelengths to the prediction.

MATERIAL AND METHODS

Study area and soil data

The study area is situated in the central part of the Adriatic coastal area of Croatia near the city of the Split wider region, centred around 43°32′ N; 16°29′ E. This coastal region has a Mediterranean climate characterized by hot summers and mild, moderately rainy winters classified as Csa. The mean annual air temperature of the Split for the period between 1981 and 2010 was 15.9 °C, the mean annual precipitation for the same time was 1052 mm. Geologically, this area was built of Eocene Flysch marls, sandstones and siltstones with lenses of calcirudites and calcarenites (Marinčić *et al*. 1971; Marinčić *et al*. 1976). These sediments characterized a high proportion and wide range of carbonate

component. According to Miščević and Vlastelica (2014) and Vlastelica (2015), $CaCO_3$ varies in the range of 42% to 79% and 32% to 89%, respectively. Water impermeable geological base and sloping terrain, mainly between 10-30% make this area vulnerable to the erosion. So, terracing is basic measures to the soil protection. Investigated soils are rich in carbonates, have alkaline reaction, very low to medium humus content and silty loam texture (Miloš and Maleš, 1998). According to the World Reference Base for Soil Resources (IUSS Working Group WRB, 2014) investigated soils we classified as Terric Anthrosols (Calcaric, Siltic/Loamic, Escalic). Current agricultural production is characterized by the small, mixed and dislocated parcels of the olive groves, vineyards, Mediterranean orchards and abandoned terraced soil. For PLSR predictions we used laboratory and spectral measurement of the $CaCO_3$ content in a total of 180 top-soil samples selected from a Soil spectral library of Dalmatia, Croatia described by Miloš (2013). The $CaCO_3$ content was analysed using Scheibler calcimeter (JDPZ, 1966).

Spectra measurements, data pre-processing and selection of the optimal PLSR model

The spectra measurements of air-dried and sieved (2mm) soil samples were obtained in a laboratory using a portable TerraSpec 4 Hi-Res Mineral Spectrometer with a wavelength range of 350-2500 nm that were recorded output on a 1 nm interval. The correction with a standardized white Spectralon® panel (Analytical Spectral Devices, Boulder, CO, USA) with 100% reflectance was made prior to the first scan and after every ten samples. The PLSR model was optimized by spectral data pre-processing treatments that included (i) a wavelength reduction to 5 nm for the whole region 350–2500 nm using Savitzky-Golay smoothing algorithm and (ii) first-order derivative algorithm with a second order polynomial fit (Savitzky and Golay, 1964). Furthermore, to eliminate the noise at edges of each spectrum the spectral range of the soil spectra was reduced to 400 - 2490 nm range. The PLS regression with leave-one-out cross-validation method (Martens and Næs, 1989; Wold *et al.* (2001) was used for calibrating the spectra and $CaCO_3$ content measured in the laboratory. The optimum number of factors in the PLSR model was obtained using leave-one-out cross-validation method (Efron and Tibshirani, 1994).

Model Performance Evaluation

The performance of the PLSR models was evaluated based on four parameters: first, the root mean square error of prediction (RMSEP); second, the ratio of performance to deviation (RPD); third, the range error ratio (RER) and fourth, the coefficient of determination (R^2). RMSEP is the average prediction error of the validation samples around the regression line. RMSEP is defined as the square root of the average of squared differences between predicted and measured Y values of the validation samples (Equation 1).

$$RMSEP = \sqrt{\sum_{i=1}^{N} \frac{(\hat{y}_i - y_i)^2}{N}} \qquad \text{Eq. (1)}$$

where y_i and $\hat{y}_i$ are the measured and predicted values of sample *i*, respectively, and *N* is the number of samples.

The RPD is defined Williams (1987) as the ratio between the reference data standard deviation (SD) and the standard error of the prediction (SEP) given with Equation (2):

$$RPD = \frac{SDv}{SEP} \qquad \text{Eq.(2)}$$

where SDv is the standard deviation of the validation dataset. The standard error of prediction (SEP) is the standard deviation of differences between the reference values and the predicted values in the validation set (Equation 3). The SEP is the RMSEP corrected for bias (Equation 3). Bias is the average value of the difference between predicted and measured values (Equation 4).

$$SEP = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\left(\hat{y}_i - y_i - Bias\right)^2} \qquad \text{Eq. (3)}$$

$$Bias = \frac{1}{N}\sum_{i=1}^{N}\left(\hat{y}_i - y_i\right) \qquad \text{Eq. (4)}$$

The range error ratio (RER, Equation 5) is the ratio of the difference between the largest and smallest values observed in the reference data set and the SEP (Starr *et al*. 1981).

$$RER = \frac{Max - Min}{SEP} \qquad \text{(Eq.5)}$$

where Max and Min are the maximum and the minimum values in the reference dataset.

Classification of prediction success is according to the thresholds given by Malley *et al*. (2004) which are tabulated in Table 1.

Table 1. Guidelines for evaluating calibrations performance criteria in soil analyses according to Malley et al. (2004)

Degree of prediction success	**R^2**	**RPD**	**RER**
Excellent	>0.95	>4	>20
Successful	0.90–0.95	3–4	15–20
Moderately successful	0.80–0.90	2.25–3	10–15
Moderately useful	0.70–0.80	1.75–2.25	8–10

RESULTS AND DISCUSSION

Soil and Spectral Properties

Table 2 shows the descriptive statistics of the carbonate content ($CaCO_3$) analysed using conventional laboratory method analysis (reference dataset) and their calibrated and cross-validated PLSR predictions for the 180 soil samples. The $CaCO_3$ content for the whole dataset varies within a very wide range from 186.0 to 894.7 g kg^{-1}. A high an average values of $CaCO_3$ (547.2 g kg^{-1}) shows

that the analyzed soils are rich in carbonates. The skewness value for $CaCO_3$ reference data set of 0.09 and it graphically displays shows normal and near symmetrical distribution (Table 2; Figure 1).

Table 2. Statistical description of the $CaCO_3$ content (g kg^{-1}) for the reference, calibration and validation data-sets for 180 soil samples

Data-set	Mean	Max	Min	Range	Std Dev.	Skewness
Reference	547.2	894.7	186.0	708.7	142.54	0.09
Calibration	547.2	927.1	242.6	684.5	141.6	0.64
Validation	547.4	938.6	248.5	690.1	140.4	0.67

Figure 2. shows the mean raw soil spectra (Figure 2a) and mean first-derivative (Figure 2b) equivalents for 180 soil samples in this study. The mean raw soil spectra (Figure 2a) is characterized with reflectance increasing with increasing wavelength in the visible range (400–700 nm) and without sharp peaks that can be directly associated with specific constituents. Absorptions in the visible range are associated with Fe minerals (eg. haematite and goethite; Sherman and Waite, 1985).

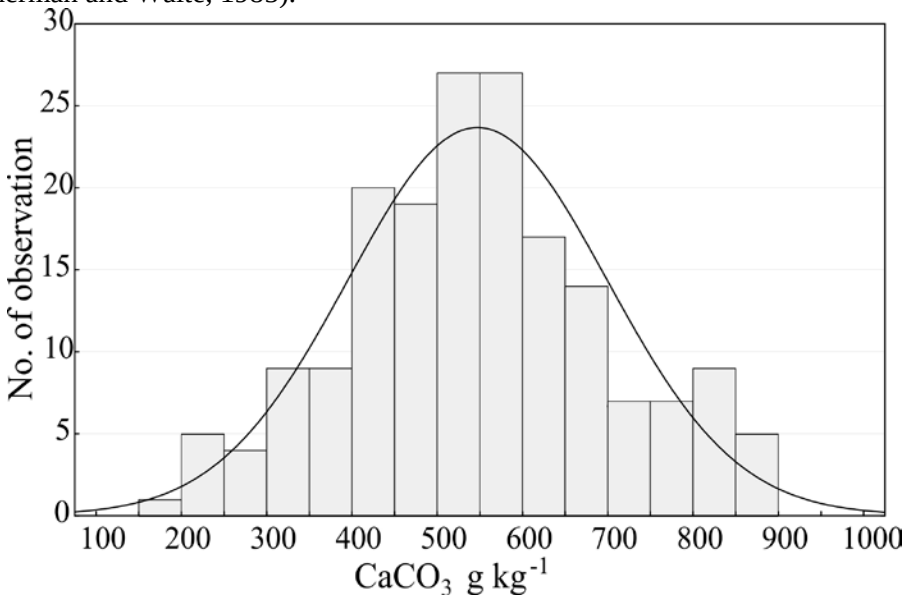

Figure 1. Histogram of $CaCO_3$ content in soil

In the visible range, the mean first-derivative reflectance spectra (Figure 2b) shows adsorption peak around 465 nm and a weak concave shape at the wavelengths around 565-665 nm. They indicate the presence of the chromophorous constituents mainly, Fe oxides and darkness of the organic constituents (Ben-Dor *et al.* 1999).

The mean raw and first derivative spectra (Figure 2a and b) show strong water and OH^- absorption in the NIR near 1400 and 1900 nm (Ben-Dor and Banin, 1995; Clark, 1999). Figure 2b shows characteristic carbonate band with an absorption peak of calcite at 2335 nm as a result of the vibrational combinations and overtones of the CO_3. According to Clark (1999) carbonates have a strong diagnostic vibrational absorption band at 2300 to 2350 nm and three weaker bands occur near 2120 to 2160 nm, 1970-2000 nm and 1850-1870 nm. Figure 2b shows a few other prominent absorption peaks between 2200-2300 nm and around 2440 nm. This is due to metal-OH combination indicating vibrational stretching of H-O-H and OH^- ions in secondary clay minerals (Clark 1999; Viscarra Rossel *et al.* 2006b). These absorptions indicate the presence and the combined effect of secondary minerals such as smectite, illite and vermiculite (Viscarra Rossel *et al.* 2006b).

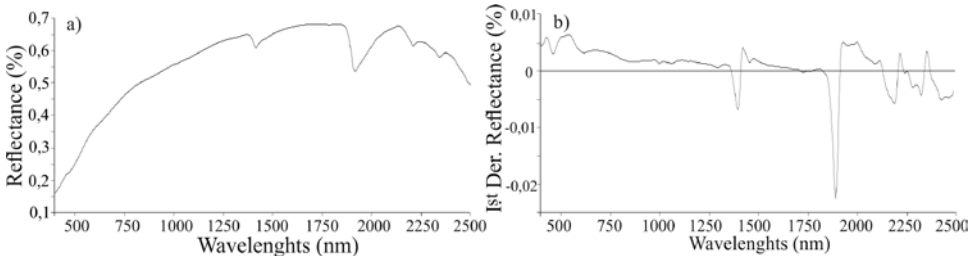

Figure 2. The mean raw spectra (a) and mean first derivative equivalent (b) for all of 180 soil samples of the study

Performance of calibration and validation models

Table 3 shows the calibration and cross-validation results of the PLS regression models for the $CaCO_3$ content. The prediction error of the $CaCO_3$ measured as RMSEP was 57.9 g kg^{-1} (Table 3). It can be considered that 2 times the RMSEP represents about 95% confidence interval of the test set mean. So for that confidence limit, there is a 95% chance that the mean value of the $CaCO_3$ predicted model lies between 431.4 and 663.0 g kg^{-1}. The most commonly used parameters for evaluation of prediction accuracy of the $CaCO_3$ model (R^2, RPD and RER; Table 3) indicated moderately successful prediction according to thresholds given by Malley *et al*. (2004).

The predicted parameters (R^2, RPD and RER) in the combination with a high value of standard prediction error, measured as RMSEP (Table 3) suggest that the created model is suitable as a quality control method (screening) of the $CaCO_3$ content in investigated soil.

Our results show better prediction accuracy of $CaCO_3$ content compared to study of Volkan Bilgili *et al.* (2010) that reported R^2 0.71, RPD 1.84 and RER 11.02 with a significantly narrower range of carbonates (25.7-98.7 g kg^{-1} $CaCO_3$) and lower mean value of 55.1 g kg^{-1} $CaCO_3$. Leone *at al*. (2012) also achieved a lower prediction accuracy of PLSR model compared to our results (R^2 0.79 and RPD 2.07) with carbonates ranging from 0.0 to 636.0 g kg^{-1} $CaCO_3$ and mean

value of 70.9 g kg^{-1} $CaCO_3$. Gomez *et al*. (2013) obtained R^2 0.71 and RPD 1.89 with carbonate range of 0.5-375 g kg^{-1} $CaCO_3$ and mean value of 65 g kg^{-1} $CaCO_3$, that also show lower prediction accuracy compared to our model. Some researchers reported even lower values of validation parameters e.g. Summers *et al.* (2011) R^2 0.69 and RPD 2.1 and Khayamim *et al.* (2015) R^2 0.58.

Table 3. Calibration and validation results of the $CaCO_3$ (g kg^{-1}) model diagnostic

Calibration		Validation					
RMSEC	R^2	RMSEP	Bias	SEP	R^2	RPD	RER
54.9	0.90	57.9	0.2	58.1	0.86	2.42	11.9

However, some authors obtained better validation parameters of $CaCO_3$ prediction models compared to ours. For example, Canasveras *et al*. (2012) achieved R^2 0.93 and RPD 3.5 with carbonate content variation from 20-969 g kg^{-1} $CaCO_3$ and a mean value of 559 g kg^{-1} $CaCO_3$. Carmon and Ben Dor (2017) reported R^2 of 0.94 for range of 0.0 to 74.27 % $CaCO_3$, while Gras *et al*. (2014) obtained even higher R^2 of 0.99 and RPD 8.6 for data set with carbonate range of 0.0-84.9 g/100 g of soil and mean value of 16.1 g/100 g of soil. The possible factors of the relatively large differences in the accuracy of the $CaCO_3$ content estimation are related mainly to nature of soil as a very complex mix of the mineral and organic compounds, parent material and calibration methods.

Importance of the spectral ranges and wavelengths

Figure 3 illustrates the importance, measured with regression coefficients, of each wavelength to the prediction model of $CaCO_3$ content.

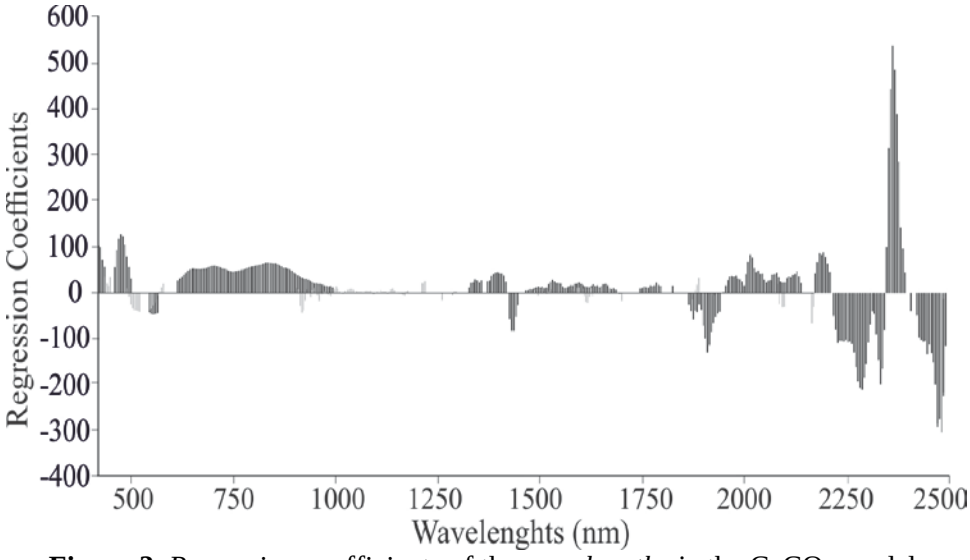

Figure 3. *R*egression coefficients of the *wavelengths in* the $CaCO_3$ model.

The highest values of the regression coefficients had wavelengths in the NIR spectral range at 2325 nm to 2365 nm with peak at 2340 nm. This is stated in accordance with previously established spectral activity for calcite in the NIR spectral range (700-2500 nm) with the strongest diagnostic vibrational absorptions at 2300-2350 nm (Clark 1999).

The wavelengths retained as a significant ($p < 0.01$) are marked in black. Furthermore, Figure 3 shows a high regression coefficient (contribution to the model) of the wavelengths between 2430 nm to 2470 nm and between 2215 nm to 2285 nm. The high values of regression coefficients of these absorptions can be related to the presence of secondary clay minerals (Clark 1999; Viscarra Rossel *et al.* 2006b). In visible range, the most significant wavelengths were obtained between 455 and 475 nm, that can be related to the presence of the Fe oxides and organic constituents (Ben-Dor *et al*. 1999).

CONCLUSION

This study showed that:

- the $CaCO_3$ content in anthropogenic soils derived from Flysch deposits varied within a very wide range (186.0 to 894.7 g kg^{-1}) with a mean value of 547.2 g kg^{-1} and showed normal near symmetrical frequency distribution

-the PLSR model for quantitative prediction of $CaCO_3$ content in investigated soils with R^2 0.86, RPD 2.42 and RER 11.9 was moderately successful

-the created model is suitable as a quality control (screening) of the $CaCO_3$ content in terraced soil derived from Flysch deposits

-the largest contribution to the $CaCO_3$ prediction model gives wavelengths indicating the spectral activity of calcite and clay minerals.

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CHARACTERISTICS OF THE MONTENEGRIN ROSE WINE

SUMMARY

Rose wine production and its consumption in Montenegro have become more intense in recent years. This paper presents results of rose wine quality analyses in vintages 2014, 2015 and 2016 with the aim to perform characterization of these wines in Montenegro. Chemical and polyphenolic composition of wine were analysed, as well as the influence of vintage on wine quality. The composition of grape varieties used for producing wines was examined, as well.

When it comes to composition by grape variety, the analysis showed that most producers use international varieties for production of rose wines: Cabernet Sauvignon (the most frequent), Grenache, Marselan and Cabernet Franc. One producer uses exclusively the indigenous variety Vranac, and one uses a coupage of Vranac and Grenache for production of rose wines.

Chemical analyses of rose wines in the reference three-year period have shown that most parameters varied significantly, apart from density and volatile acids that were stable. The average parameters of the chemical composition of rose wine are as follows: alcohol 13.1vol%, total extracts 22.9 g/l, total acidity, 6.3 g/l, pH 3.28, the volatile acidity of 0.5g/l, and total SO2 of 109.4 mg/l. The chemical composition of the tested wines was significantly affected by the vintage. Namely, in 2014, wines had lower alcohol content, a higher total acid content and a lower pH value, while in 2015 and in 2016, alcohol content was higher, the total acid content was lower and consistent for these two vintages, while pH value was higher.

The results obtained from the investigation of the polyphenol composition showed that the total phenol content, the phenol index and the anthocyanin content varies statistically significantly among the examined wines and the average values amounted to 267 mg/l, 12.9 and 37.6 mg/l respectively.

Keywords: chemical composition, phenols composition, vintage

INTRODUCTION

The rose wine production has a growing trend and accounted for 10% of world wine production in 2011. The increase in rose wine consumption is recorded on the global wine market, especially in the United States and in France

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(Blot and Couderc, 2013). In the last decade, Montenegro experienced an increase in the number of producers and the number of wine types produced. According to the available data, in 2012, the total production of rose wines amounted to 3.3% of the total production of wine (Pajović et al. 2016). However, over the last few years, there has been a significant increase in the production of rose wines and in their consumption in Montenegro.

The composition of rose wine (chemical, polyphenolic and aromatic composition) depends on rose wines production technology, as well as on the composition of the grape variety used for wine production. Among the most important wine grape varieties, the most suitable for production of quality wine is an international variety – Cabernet Sauvignon. It is suitable for the production of rose wine as monovarietal grape with typical pink colour, good acidity and lingering note of rose. However, each country should find its own style in production of rose wines that should be mostly based on coupage of some autochthonous varieties, or on their mix with some international varieties.

In the past, production of rose wines was based on only one type of rose and that was "Montenegrin quality wine Rose". Its characteristics were described by Pejovic (1987). He stated that this wine belonged to the light, fresh wines (lower alcohol content); it had balanced and harmonious taste and its colour tone was somewhat more intense - adjusted to the taste of that time consumers. Production of this rose was mosltly based on indigenous Montenegrin varieties. Recent researches of rose wines in Montenegro, however, indicate that rose wine have a higher content of alcohol and total acids, and more intense aromatic properties with noted differences in style among producers (Pajović et al. 2016).

Since the production and consumption of rose wines in Montenegro is becoming more intense, with this study we wanted to investigate the quality of a number of rose wines in vintages 2014, 2015 and 2016. In this study, the chemical composition of wine, the impact of the harvest on wine quality and polyphenolic composition of Montenegrin rose wines were analysed, while the special attention is given to composition of grape varieties used for producing rose wines, with the aim to perform characterization of rose wine produced in Montenegro.

MATERIAL AND METHOD

This paper analyses eight rose wines from seven different wine producers during the three vintages - 2014, 2015 and 2016. Seven dry and one semi-sweet rose wine were examined. Table 1 presents data on wine labels, wine names, names of wineries/producers, composition of grape varieties used for producing wines, locality/ sub-region /region from which the grapes come from.

Material

Table 1 shows that the majority of producers use international varieties such as: Cabernet Sauvignon (the most frequent), Grenache, Marselan, Cabernet Franc and Sangiovesse. Only one producer uses solely the indigenous grape

variety of Vranac, while "13.jul - Plantaže" predominantly use the coupage of Vranac and Grenache.

As regards the origin of the raw materials, Table 1 shows that the grapes used for wine production come from Montenegrin region of Skadar Lake, mostly from Sub-region Podgorica (3, 4, 6); Sub-region Piperi (1 and 2); Sub-region Katunski (7); Sub-region Crmnica (8) and only one producer comes from Montenegrin Costal region and that is from the Sub-region of Boka Kotorska (5).

Table 1: Data about analysed Rose wines

Nb	Wine	Winery/ Producer	Grape varieties used for producing wines	Locality/ Subregion/ Region
1.	Arhonto Rose	Krgović	Cabernet Sauvignon, Sangiovesse	Rogami, Podgorica Sub-region Piperi, Montenegrin region of Skadar Lake
2.	Zenta-Rose	Vučinić	Cabernet Sauvignon	Rogami, Podgorica Sub-region Piperi, Montenegrin region of Skadar Lake
3.	Crnogorski rose	13. jul Plantaže	Vranac, Grenache	Ćemovsko polje, Podgorica Sub-region Podgorica, Montenegrin region of Skadar Lake
4.	Moje vino roze	13. jul Plantaže	Vranac, Grenache	Ćemovsko polje, Podgorica Sub-region Podgorica, Montenegrin region of Skadar Lake
5.	Savina Rose	Castel Savina	Grenache	Meljine, Herceg Novi, Sub-region Boka kotorska, Montenegrin Costal region
6.	Monte-Grande rose	Monte Grande	Cabernet Sauvignon	Zeta, Podgorica Sub-region Podgorica, Montenegrin region of Skadar Lake
7.	Harmonia	Ravil	Vranac	Zagarač, Danilovgrad, Sub-region Katunski, Montenegrin region of Skadar Lake
8.	Buk rose	Winery Buk	Marselan, Cabernet Sauvignon, Cabernet. franc	Crmnica, Bar, Subregion Crmnica, Montenegrin region of Skadar Lake

Method of work

Analyses were carried out in oenological laboratories of Biotechnical Faculty. Wines were the representative samples.

Physicochemical analyses

The following parameters of physicochemical composition of wine were tested: density, alcohol, extract, total acids, pH, volatile acids and residual sugar

in wine. The analyses were performed in accordance with compendium of international methods of wine and must analyses (OIV, 2011).

Spectrophotometrical analyses

The analyses of the flavonoids were done by spectrophotometric methods (Di Stefano at al. 1989) carried out under optimized conditions for red wine analysis (Rigo at al. 2000) with the use of spectrophotometer Varian Cary 100 Bio UV-Visible (Bio Tech, Maryland, United States). Total Phenols were assessed by the method of Folin-Ciocalteu. Concentrations were determined by means of a calibration curve as (+)-catechin in mg/kg of grape or mg/L of wine.

Total anthocyanins were determined on the basis of maximal absorbance in the visible range (536-542 nm). They were quantified in mg kg-1 FW by assuming an average absorbance of the mixture of anthocyanins extracted from grape Cabernet Sauvignon (average MW =500 Da, ε = 18800 M-1 cm-1 in 70:30:1 ethanol:water:HCl solution).

Index of 280 is method for determination of total phenols based on direct reading of absorbance μ=280nm after diluting wine samples with water 1:10 (Ribereau-Gayon et al. 1982).

Data analysis

In order to establish the significance of differences between examined wines and their interaction for each studied parameter, a two factorial analysis of variance (ANOVA) was applied. For those parameters and factors where significant differences were detected, additionally an LSD test was applied to the significance level of $p<0.05$. Analysis of the experimental data was performed using the statistical package IBM SPSS Statistics 20.

RESULTS AND DISCUSSION

Chemical composition of red wines

Table 2 shows the chemical composition of the tested wines (1-8), per vintage (2014, 2015 and 2016), as well as the three-year average values of the analysed parameters.

Table 2 shows that density of rose wines, in a three-year period, had similar values without significant statistical difference, ranging in the expected frame of 0.9900 to 0.9920 for dry wines, while the density of semi-sweet wine was 0.9970. The average content of alcohol in a three-year period significantly varied, and in the majority of wines (six out of eight) amounted to about 13 vol%, that is from 13.1 vol% (wine 5) to 13.9 vol% (wine 1). The extract content in rose wines also varied from 19.2 g/l (wine7) to 24.3g/l (wine 2) for dry wines.

The parameters which define the acidity condition of wine - total acid content and pH value significantly varied among the tested wines, observed as a three-year average.

Table 2: Chemical composition of Montenegrin rose wines (1-8) in three vintage and averages values of parameters (mean values ±SD)

		Density	Alc. vol%	Total extracts g/l	Total acidity g/l	pH	Volatile acidity g/l	Total SO_2 mg/l	Residual sugar g/l
1	2014	0.9900	13.6	19.8	6.0	3.33	0.6	169.7	1.9
	2015	0.9900	14.2	24.2	6.0	3.26	0.6	120.0	2.4
	2016	0.9900	13.9	20.9	5.0	3.42	0.6	140.9	2.6
	mean	**0.9900^{AC}**	**13.9±0.3^{A}**	**21.6±2.3^{A}**	**5.6±0.6^{A}**	**3.36±0.08^{A}**	**0.6±0.1^{AB}**	**143.5±27.1^{A}**	**2.3±0.6^{A}**
2	2014	0.9945	12.6	36.1	7.1	3.25	0.7	97.3	3.5
	2015	0.9920	14.0	26.3	6.2	3.28	0.7	126.7	1.7
	2016	0.9900	13.5	19.6	6.8	2.42	0.7	103.4	1.6
	mean	**0.9920^{B}**	**13.4±0.7^{BC}**	**24.3±3.7^{A}**	**6.7±0.5^{B}**	**3.31±0.13^{AB}**	**0.7±0.1^{B}**	**109.1±14.7^{B}**	**2.2±0.9^{A}**
3	2014	0.9920	12.5	21.6	6.8	3.01	0.4	106.8	2.8
	2015	0.9890	12.9	15.4	6.1	3.30	0.4	85.7	2.1
	2016	0.9924	12.5	22.9	5.9	3.16	0.3	55.7	2.1
	mean	**0.9910^{AB}**	**12.6±0.3^{D}**	**20.0±3.5^{A}**	**6.3±0.5^{C}**	**3.16±0.18^{CD}**	**0.4±0.1^{C}**	**82.7±23.5^{C}**	**2.3±0.5^{A}**
4	2014	0.9970	11.0	30.2	6.7	3.30	0.5	193.8	10.0
	2015	0.9970	11.6	29.4	5.6	3.67	0.8	181.0	19.1
	2016	0.9960	11.8	39.3	4.5	3.68	0.4	82.2	15.0
	mean	**0.9970^{C}**	**11.5±0.4^{E}**	**33.0±5.5^{D}**	**5.6±1.0^{A}**	**3.55±0.22^{E}**	**0.5±0.3^{AB}**	**152.4±54.1^{A}**	**14.6±4.3^{B}**
5	2014	0.9915	12.3	19.8	7.1	3.07	0.6	118.8	2.6
	2015	0.9900	13.5	19.6	6.8	3.09	0.5	85.3	2.8
	2016	0.9915	13.5	23.5	6.6	3.06	0.4	87.9	2.6
	mean	**0.9910^{AB}**	**13.1±0.6^{B}**	**21.0±2.1^{AC}**	**6.8±0.3^{B}**	**3.07±0.05^{D}**	**0.5±0.1^{AC}**	**97.3±16.3^{D}**	**2.6±0.4^{AC}**
6	2014	0.9920	12.9	22.9	6.6	3.26	0.8	103.1	2.9
	2015	0.9910	13.7	22.9	6.2	3.35	0.7	86.6	2.6
	2016	0.9910	13.7	22.7	6.4	3.07	0.5	109.9	2.0
	mean	**0.9910^{AB}**	**13.4±0.5^{C}**	**22.8±5.6^{AB}**	**6.4±0.3^{C}**	**3.22±0.15^{B}**	**0.7±0.2^{B}**	**99.9±12.8^{BD}**	**2.5±0.5^{AC}**
7	2014	0.9910	12.9	20.1	7.0	3.30	0.6	103.4	3.5
	2015	0.9900	13.4	19.3	6.6	3.41	0.5	104.7	3.4
	2016	0.9910	13.7	18.3	6.6	3.47	0.6	98.3	3.0
	mean	**0.9910^{AB}**	**13.3±0.5^{BC}**	**19.2±1.1^{CB}**	**6.7±0.2^{B}**	**3.39±0.10^{A}**	**0.5±0.1^{B}**	**102±4.1^{BD}**	**3.2±0.5^{C}**
8	2014	0.9900	12.6	20.1	6.3	3.13	0.6	86.6	2.6
	2015	0.9900	14.1	21.4	5.7	3.23	0.5	86.6	2.7
	2016	0.9910	13.40	21.90	6.15	3.19	0.4	91.8	2.3
	mean	**0.9900^{A}**	**13.4±0.7^{BC}**	**21.3±1.3^{AC}**	**6.1±0.4^{C}**	**3.18±0.08^{C}**	**0.5±0.1^{AC}**	**88.3±5.8^{C}**	**2.5±0.3^{AC}**

Different capital subscript letters indicate significantly different means ($p < 0.05$) for average values of three years examined wines

The values ranged from 5.6 g/l and 3.55 (wine 4) to 6.8 g/l and 3.07 (wine 5). The volatile acids were low and uniform among the analysed wines. The three-year average value ranged from 0.4 g/l (wine 4) to 0.7 g/l (wine 6). The residual content of sugar differs statistically significantly and for most wines it was about 2 g/l for a three-year period, except for a semi-sweet wine 4, where the

values were expectedly higher. The greatest variation among the studied parameters of chemical composition was recorded in the total contents of the total SO_2 in wines. Namely, it ranged from 82.7 mg/l (wine 3) to 143.5 mg/l (wine 1) for dry wines, while a slightly higher value was recorded in a semi-sweet wine - 152.4 g/l.

Regarding the vintage, differences were found between the tested wines and they will be presented in the next chapter.

The influence of vintage on the chemical composition of the examined rose wines

The vintage examined in our study differ in weather conditions, which was reflected in the chemical composition of must, and therefore of wine. Popović et al., (2017) concluded that 2014 was unfavourable for grapes ripening due to heavy rainfall during the growing season which affected the chemical composition of must - acid content was significantly higher, while the sugar content was lower. On the other hand, the vintage 2015 and vintage 2016 had much favourable conditions for grapes maturing - higher mean annual and mean vegetation temperatures resulting in better chemical composition of must and wine.

Table 3 shows the average chemical composition of the examined wines in three tested vintage years: 2014, 2015 and 2016.

Table 3: Influence of vintage on the chemical composition of Montenegrin Rose wines (mean values ±SD)

	Density	**Alcohol vol%**	**Total extracts g/l**	**Total acidity g/l**	**pH**	**Volatile acidity g/l**	**Total SO_2 mg/l**	**Residual sugar g/l**
2014	0.9921^{A}	12.6 ± 0.7^{A}	22.7±4.7	6.9 ± 0.4^{A}	3.20 ± 0.13^{A}	0.6 ± 0.1^{A}	122.4 ± 37.7^{A}	3.7 ± 2.5^{AC}
2015	0.9911^{B}	13.4 ± 0.8^{B}	22.3±5.4	6.1 ± 0.5^{B}	3.32 ± 0.18^{B}	0.6 ± 0.1^{A}	109.6 ± 32.8^{B}	4.6 ± 5.6^{B}
2016	0.9916^{AB}	13.2 ± 0.7^{C}	23.6±6.4	6.0 ± 0.8^{B}	3.30 ± 0.24^{B}	0.5 ± 0.2^{B}	96.3 ± 24.6^{C}	3.9 ± 4.3^{C}

Different capital subscript letters indicate significantly different means ($p < 0.05$) for average values of examined wines among vintages

Table 3 shows that the density was lowest in 2015 and significantly different from the values found in the vintages 2015 and 2016. Alcohol content was significantly different in different vintages.The lowest alcohol content was recorded in 2014 and amounted to 12.6 vol%, while in the vintages 2015 and 2016, it was 13.4 vol% and 13.2 vol% respectively. The content of total extract had uniform values and did not differ in different vintages. The total acid content was highest in 2014 - 6.9 g/l and it was statistically significantly different from the values found in the vintages 2015 and 2016, where the content was lower and it was 6.1 g/l and 6.0 g/l. The pH value of the wine was also statistically significantly different in 2014. As expected, the value amounted to 3.20 and it was lower than the values recorded in other two vintages where there was no difference (3.30 and 3.32). Volatile acid was lowest in the wines produced in 2016 and it was 0.5 g/l, which is statistically significantly different from the values found in the vintages 2014 and 2015 reaching the value of 0.6 g/l. The

contents of total SO_2 were significantly different in different vintages. The highest value was recorded in 2014 - 122.4 mg/l; slightly lower value was found in 2015.- 109.6 mg/l, while the lowest value was recorded in 2016 and it amounted to 96.3 mg/l.

The foregoing data on the average chemical composition of wine are significantly distinct from the data provided by Pejović, (1987) - 11.5% by volume of alcohol, the total acid 5.3 g/l, who analysed the five-year average of rose wines in Montenegro. The reason for this is mainly a change in a style of the rose wines production. Pajović et al., (2016) report the following findings: average alcohol content of 13.3 vol%, total acid content of 6.0 g/l and a pH value of 3.20 for three vines observing them as a five-year and seven-year average. Our results, especially for the vintages 2015 and 2016, are completely compatible with these values, confirming the fact that in recent years a change of style in the production of rose wines is moving towards higher alcohol content and higher acid content.

Polyphenolic composition of the examined rose wines

The paper also examined the content of phenolic compounds in rose wines and the content of total polyphenols and anthocyanins in vintage 2016. The results are shown in table 4.

Table 4: Content of phenols in Montnegrian rose wines

	Total phenols (mg/l)	**Index 280**	**Total anthocyanins (mg/l)**
1	265.1 ±8.2 ^{A}	10.2±0.1 ^{A}	39.4±5.8 ^{A}
2	313.1±50.9 ^{C}	10.5±0.1 ^{AB}	40.6±4.3 ^{A}
3	209.4±18.0 ^{B}	8.9±0.6 ^{C}	22.3±2.1 ^{B}
4	356.6±13.7 ^{D}	33.2±1.1 ^{E}	24.1±3.1 ^{B}
5	200.5±17.2 ^{B}	8.8±0.2 ^{C}	40.1±0.3 ^{AD}
6	213.1±9.3 ^{B}	8.6±0.1 ^{C}	38.4±0.5 ^{A}
7	289.6±8.2 ^{AC}	11.0±0.3 ^{B}	50.5±1.6 ^{C}
8	291.2±8.6 ^{AC}	12.0±0.2 ^{D}	45.3±0.4 ^{D}
mean	**267.3±53.4**	**12.9±7.9**	**37.6±9.6**

Different capital subscript letters indicate significantly different means ($p < 0.05$)

The total content of polyphenols statistically quite varied among the examined wines and ranged from 200.5 mg/l (5 wine) to 356.6 mg/l (wine 4), while the average value amounted to 267.3 mg/l. The values obtained in our study are significantly lower than the value of 1304 mg/l reported by Minussi et al. (2003) for the rose wine made by coupage. These values are also lower than the one cited by Paixero et al. (2007) for a rose wine Tinta Negra Mole - 665 mg/l, but they are compatible with values specified by Zhu et al. (2012) for rose wine of north American *V. labrusca* Catawba, which amounted to 368.83 mg/l. As expected, the values of total phenols in the examined rose wines are lower than the values in red wines from Montenegrin region which ranged from 890 to 1600 mg/l in the vintage 2011 and 2012 (Pajović et al. 2014).

Phenol index values were low, ranging from 8.6 to 33.2, which was in proportion to total phenol content in each tested wine, determined by Folin-Ciocalteu method.

The content of anthocyanin in the examined Montenegrin wines ranged from 22.3 mg/l (wine 3) to 50.5 mg/l (7 wine), while the average value was 37.6 mg/l. The observed values are in accordance with the values of 36.1-53.2 mg/l reported by Suriano et al.2015 for the young rose wine Bambino Nero.

CONCLUSION

This paper presents an analysis of the following rose wines: "Arhonto Rose", "Zenta Rose", "Crnogorski rose", "Moje vino rose", "Savina Rose", "Monte-Grande Rose", "Harmonia" and "Buk Rose". All tested wines are dry, except one which is semi-sweet. The grape for production of these wines comes from Montenegrin region of Skadar Lake, mainly from Subregion Podgorica -3; Subregion Piperi - 2; Subregion Katunski -1; Subregion Crmnica -1, while one producer comes from Montenegrin Costal region and it is Subregion Boka Kotorska.

The results showed that the majority of producers use international varieties for production of rose wines: Cabernet Sauvignon (the most frequent), Grenache, Marselan, Cabernet. Cabernet Sauvignon (the most frequent), Grenache, Marselan, Cabernet Franc. One producer uses exclusively the indigenous variety Vranac, and only one uses a coupage of Vranac and Grenache for production of rose wines.

Chemical analysis of rose wine on a three-year average showed the following parameters: density of wine was balanced; the alcohol content significantly varied among wines, but averaged 13.1 vol%. The total acid content and pH values also significantly varied and they were on average 6.3 g/l and 3.28 respectively; extract content also varied and averaged 22.9 g/l; volatile acids were equal to the average value of 0.5 g/l; SO2 varied significantly, and it was 109.4 g/l, on average.

The chemical composition of the examined wines was influenced significantly by the vintage. Namely, in 2014, wines had a lower alcohol content, higher total acid content and lower pH value, while in 2015 and in 2016, alcohol content was higher, the total acid content was lower and consistent for these two vintages, while, proportionally to these values, pH value was higher.

The results of the investigation of the polyphenol composition showed that the total phenol content, the phenol index and the anthocyanins content varies statistically significantly among the tested wines and the average values are 267 mg/l, 12.9 and 37.6 mg/l respectively.

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THE POPLAR SAPLINGS SURVIVAL IN RECLAIMED MINELAND DEPENDING ON CLONE AND ROOT TREATMENT

SUMMARY

The genus *Populus L.* is one of the promising energy plants for growing on marginal lands. To assess its potential under steppe conditions, growth morphological parameters of 9 hybrid poplar clones grown over two years on a mix of loess-like loam and red-brown clay have been studied. In the first year, the degree of the sapling survival and the intensity of development were explored. At the end of the year, two clones which showed the best results (Ijzer-5 and Robusta), were selected for further breeding and rearing. Researches of the second year were devoted to the effect of biological agents on the survival and growth of these two clones. The treatments with vermicomposting extract (VCE), trichodermin, mycorrhiza and mixture of these agents were applied. The experiment with poplar clones Ijzer-5 showed a positive effect of all bioagents on the growth morphological parameters. The increase of the length and diameter of an annual shoots, leaf area and total assimilation surface from 10 to 38% was revealed. The treatment with vermicomposting extract gave the best result. For clones Robusta, only three agents out of four had a positive effect. The rise in morphological parameters was at the level of 9-55%. The best results were noted in the experiment with a mixture of agents. Treatment with trichodermin caused an inhibitory action on most of the growth characteristics of the clone Robusta.

To obtain the wood thermal stability information a comparative thermogravimetric analysis of poplar wood samples grown on different soil types was carried out. A larger value of DTG at all stages was observed in a sample of poplar grown on sod podzolic soil. The difference in the value of the rate of mass loss is explained by the larger content of humus in sod podzolic soils, than in the phytomeliorated mix of rocks.

Keywords: poplar, reclaimed mineland, survival, growth parameters, biological agents, thermogravimetry.

INTRODUCTION

In the modern world the issue of renewable energy production is becoming more urgent. By this time, many agricultural plants are successfully grown as bioenergy crops. However, to obtain high yields of agricultural plants sufficiently

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Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

fertile soils are needed, the stock of which is limited. Therefore, the most expedient is to search for bioenergy species that can successfully grow on marginal areas and produce sustained yields.

The genus *Populus* L. can be attributed to such plants. This is a fast-growing woody energy plant, which makes it possible to create high-productive plantations with a long service life. The energy poplar is predominately grown by two technologies: short rotation system and medium rotation system (Bouriaud *et al.*, 2015; Cizcova *et al.*, 2010). Such short- and medium rotation coppice systems the first harvests take place after 4-5 years of cultivation and subsequently every 2-3 year intervals. The poplar productivity on fertile soils can reach 20 t/ha. The life time of energy poplar plantation is 15-20 years. (Klasnja *et al*., 2012; Karacic *et al.*, 2006). There are poplar unpretentiousness data to the soil fertility and the possibility of its cultivation in marginal lands, although the biomass productivity in these cases is much less - from 6 to 11.5 t / ha. Nevertheless, it is noted that irrigation and fertilization contribute to yield increase. (Stolarski *et al.*, 2014; Benetka *et al.*, 2007). The genetic component of plants has a great importance in determining their adaptive potential, and success of growing in specific conditions. (Kutsokon *et al.*, 2014; Panacci *et al.*, 2009; Labrecque and Teodorescu, 2005). At the same time, demonstrable indicators of promising poplar clones can be growth morphological parameters, the assimilation surface area, the photosynthesis rate and overall productivity (Ma *et al.*, 2015; Andrasev and Roncevic, 2008; Marron *et al.*, 2007). In Ukraine, by this time there is a successful experience of hybrid poplar cultivating for bioenergy purposes under conditions of the Polissya and Forest-Steppe region (Shylin, 2016; Odarchenko and Maurer, 2016; Geletukha *et al.*, 2014). The availability issue of growing this crop in the steppe zone is still open and requires a thorough consideration.

Controlled pyrolysis of woody biomass is a major means for the production of gaseous or solid fuel or a source of raw materials for chemical synthesis. Thermal stability of wood is an important characteristic to the understanding this process (Slopiecka *et al.* 2011; Zhang *et al*, 2010). The matter of structural soil component influence on this parameter has been poorly studied and needs to be investigated.

The objective of this study was to estimate in the micro-field experiments the poplar saplings survival degree depending on clone and root treatment.

MATERIAL AND METHODS

This research was carried out under Ukraine steppe zone conditions in the land reclamation station of DSAEU (Dnipropetrovsk State Agrarian and Economic University) in the Pokrov city (south of Ukraine) for two years (2016-2017). At the first stage all 9 poplar clones saplings were grown in the sod podzolic soil at the Boyarska Forest Research Station of NULES (National University of Life and Environmental Sciences) situated in the north of Ukraine.

In the spring of 2016, cuttings of 9 hybrid poplar clones have been planted on experimental plots (Table 1).

Table 1: Objects and their parentage

Clonal names	**Parentage**	**Sex**
Blanc du Poitou	Populus × euroamericana (Dode) Guinier	M
Dorskamp	Populus × euroamericana	M
Ghoy	Populus deltoides Bartr. Ex Marsh × Populus nigra L.	M
Marilandica	Populus × euroamericana	F
Robusta	Populus nigra var. plantierensis × Populus deltoides ssp. angulata Henry	M
Heidemij	Populus × euroamericana	M
Ijzer-5	Populus × euroamericana	M
Tardif de Champagne	Populus × euroamericana	M
Vereecken	Populus nigra	M

In the first year, the survival degree of the saplings and their development intensity were studied. At the end of the year, the clones that showed the best results were selected for further reproduction and cultivation. Researches of the second year were devoted to the effect of biological agents on the survival and growth of the clones, which was picked out last year. The plot substrate in reclaimed mineland was a mixture of loess-like loam and red-brown clay, which had passed through a long-term phytomelioration stage. The humus content in the substrate is about 1.5% (Kharytonov, 2007). The ratio of humic and fulvic acids was 0.2-0.5, which indicates a weak humus accumulation and active destruction of the soil mineral part. The main minerals of rocks silty fraction consist of feldspar, calcite, hydro mica, montmorillonite, chlorite and kaolinite. The near reserve of mobile phosphorus is represented by its medium-accessible forms (Kharytonov and Resio Espejo, 2013). In the spring of 2017, four variants of the experiment were laid: treatment with vermicomposting extract (VCE), trichodermin, mycorrhiza and mixture of these three agents. The choice of these agents was justified by their role in improving the soil nutrition regime (Kharytonov et al., 2009). Before planting in the substrate, the saplings were soaked, and after planting, they were irrigated with an aqueous solution of VCE in a ratio of 1: 100. In the same way and in identical ratio the trichodermin preparation was used. In the variant with mycorrhiza, the roots of the saplings before planting were dipped into a suspension. The dilution was 1 g for two saplings. Growth indicators were assessed by morphometric parameters. The plant height and length of the annual shoots were measured by a tape measure, the shoot diameter by a caliper. The leaf area was determined by scanned image using a computer program AreaS 2.1. The dry weight of the leaf and the leaf mass per area (LMA) were identified as well.

To obtain the wood thermal stability information a comparative thermogravimetric analysis of poplar wood samples grown on different soil types was carried out. The analysis was performed using the derivatograph Q-1500D of the "F. Paulik-J. Paulik-L. Erdey" system. Differential mass loss and heating effects were recorded. The results of the measurements were processed with the software package supplied with the device. Samples of annual wood were analyzed dynamically at a heating rate of 10 ° C / min in an air atmosphere. The mass of samples is 100 mg. The reference substance was aluminum oxide. The received data were analyzed statistically using the software package StatGraphics Plus5 with all tests of significance being made at a type 1 error rate of 5%.

RESULTS AND DISCUSSION

The survival percentage of 9 poplar clone saplings planted in 2016 was very different between varieties (Figure 1).

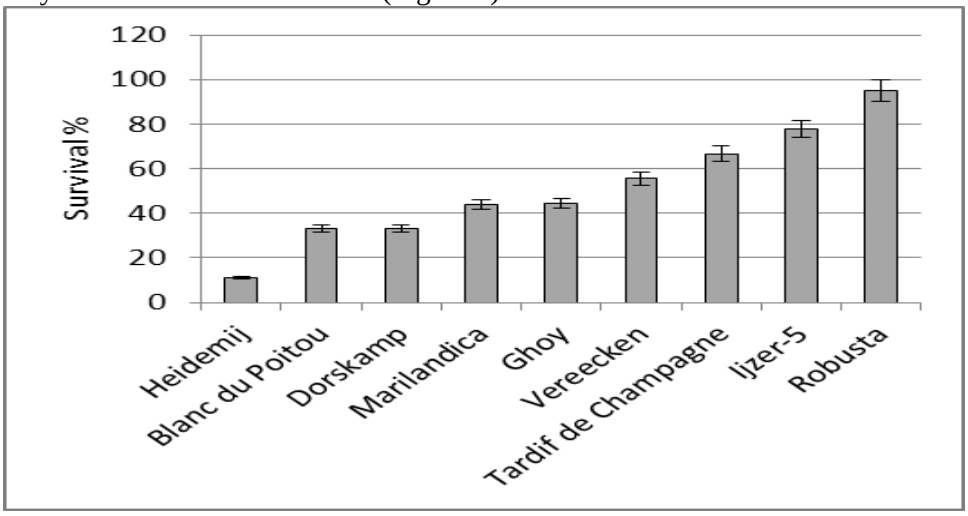

Figure 1. Survival of poplar clones on marginal soils, %.

The worst indicators were observed for the clone Heidemij - only 11%. Clones Tardif de Champagne, Ijzer-5 and Robusta showed a high level of survival (70, 80 and 95%, respectively). For the rest clones, this index varied in the range of 33-55%. During the growing season processes of plant growth and development passed in the best way for the clones Ijzer-5 and Robusta (Figure 2). By the end of the year the average height of these plants was 80-93 cm, and some specimens reached 170 cm. Clone Dorskamp also showed good growth rates, but bad survivability does not give grounds for the expediency of its further cultivation on marginal soils. The clone Tardif de Champagne, despite the good sapling survival, showed a low growth rate and therefore also lacks a good potential. Thus, according to the results obtained in the first year of cultivation, two clones - Ijzer-5 and Robusta - were evaluated as the most promising and selected for further research. Researches of the second year were devoted to the

effect of biological agents on the survival and growth of these two clones. It was revealed that the sapling survival rate of both clones in the control, experiments with vermicomposting extract, mycorrhiza and a mixture of agents was practically the same and amounted to 87-93%.

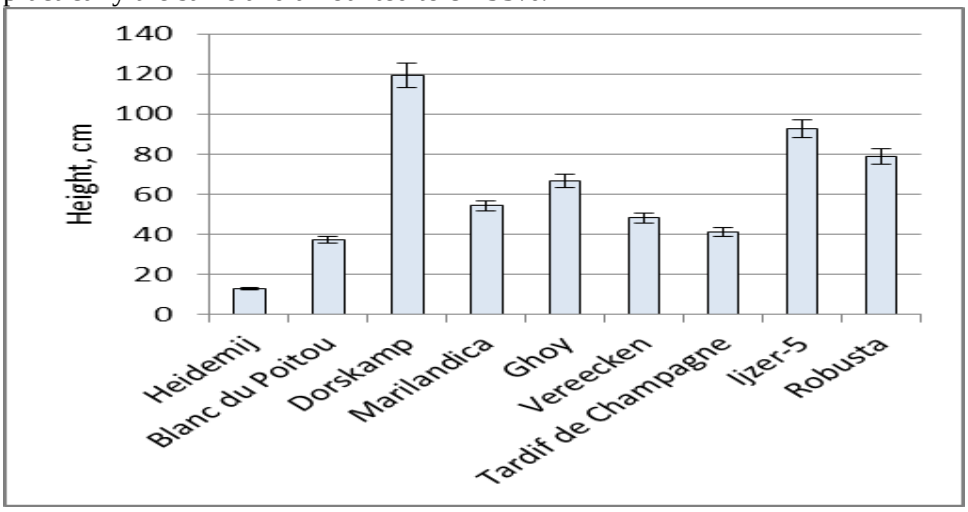

Figure 2. The height of poplar clones at the end of the first year grown on reclaimed mineland

Treatment with trichodermin had a supressive action, as a result of which the survival of clones Ijzer-5 was 73%, and of clones Robusta was even lower - 66.7%. The year 2017 was more drought-ridden than the year 2016. The total amount of precipitation for March-October was only 260 mm against 383 mm in 2016. This affected the experimental plants. Indeed, growth rates were lower than in the previous season. For example, the length of an annual shoots did not overtopped 60 cm. Treatment of clones Ijzer-5 with biopreparations promoted growth acceleration of all experimental specimens by 10-19%. The clone Robusta responded to the influence of biopreparations by growth intensification from 8.5 to 46%. Only treatment with trichodermin had no effect on this parameter (Figure 3).

Measurements of the annual shoot diameter showed that in the clone Ijzer-5 it is 22-30% higher than in the clone Robusta in the control plot and experiments with vermicomposting extract, mycorrhiza and trichodermin, and 4.5% less in the experiment with the mixture of agents. Treatment with biopreparations stimulated the activity of annual shoot lateral meristems in all experimental variants in both clones. The treatment with vermicomposting extract gave the best result for the clone Ijzer-5, and for clone Robusta in the experiment with a mixture of agents (Figure 4).

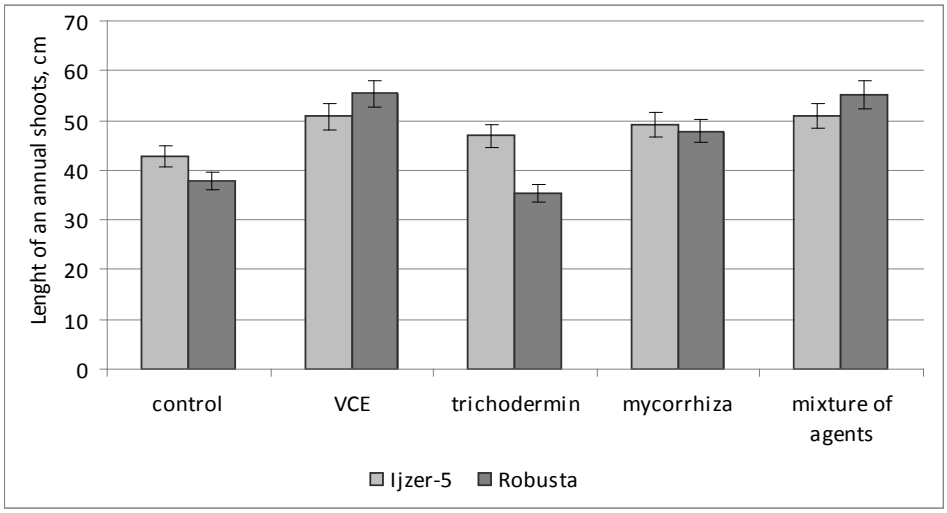

Figure 3. The length of an annual shoots of poplar clones grown on reclaimed mineland in 2017

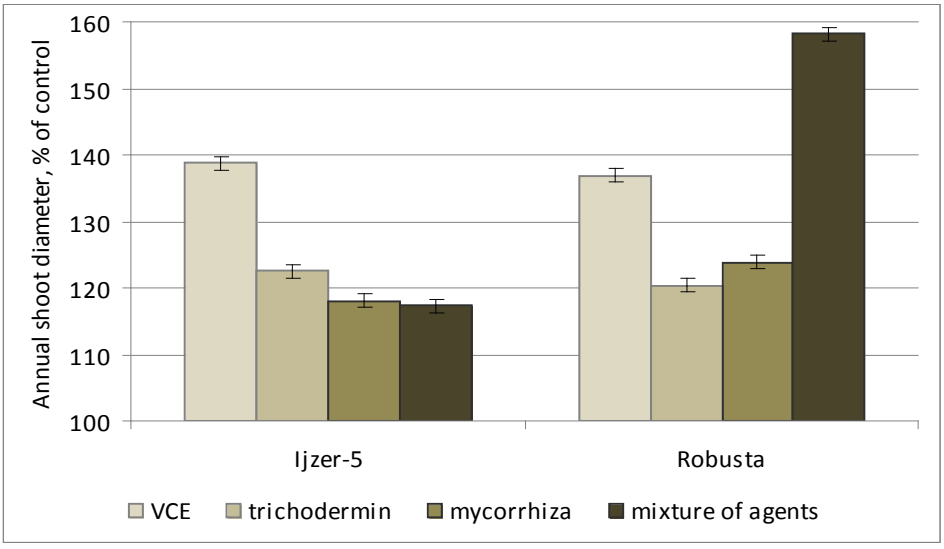

Figure 4. Diameter of annual shoots of poplar clones grown on reclaimed mineland in 2017, % of control

For clone Ijzer-5 an increase of the leaf area in all experiment variants was observed from 19.5 to 38%. The treatment with trichodermin had the greatest impact. In clone Robusta, quite the contrary, trichodermin caused a decrease in leaf area by 25% compared to the control. In other variants, an increase of this parameter was noted, but less intense than for clone Ijzer-5, only by 13.5-20.5% (Figure 5). At the same time, clone Robusta showed a higher

degree of increase of the total assimilation surface area (from 20 to 55%) compared to clone Ijzer-5 (from 20 to 32%). Treatment with trichodermin was an exception. This agent had a stimulating effect only on the clone Ijzer-5 plants, whereas in the clone Robusta the total assimilation surface area decreased by 52% compared to the control. One of the attribute among the leaf structural organization is the leaf mass per area (LMA), which is the ratio between leaf dry mass and leaf area. LMA is closely correlated with the photosynthesis intensity, potential growth rates and ecological flexibility (Pugliell *et al.*, 2015; Poorter *et al.*, 2009; Wright *et al.*, 2002). Factors such as light, CO_2 concentration, water supply and mineral nutrition can significantly influence the LMA. However, since in this study these factors were the same in all variants of the experiment, it is possible to trace the impact of biopreparations on the LMA.

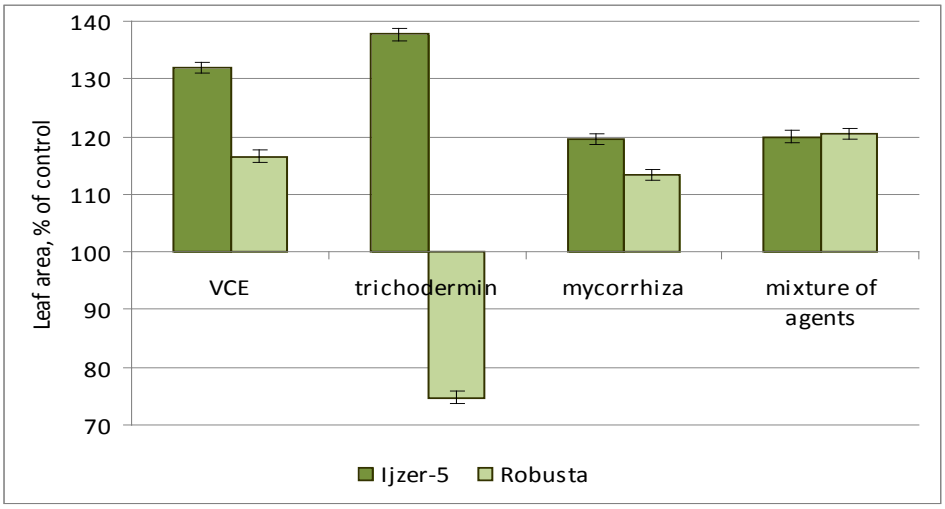

Figure 5. Change in the leaf area of poplar clones under the influence of biopreparations, % of control.

Comparison of control samples showed that LMA of the clone Ijzer-5 was 18.5% more than those of clone Robusta. Treatment with biopreparations led to an increase in this parameter for both clones in all experiment variants. However, it was insignificant for clone Ijzer-5 (6-13%), and stronger for clone Robusta (from 17 to 48%). There is a positive linear correlation between the LMA value and the leaf dry mass (Figure 6). Increase in leaf dry mass lead to increase in LMA. It can be assumed that the growth of LMA is due to the increase in mesophyll volume by formation of more quantity of structural and functional photosynthesis elements. Thus, in plants that have undergone treatment with biopreparations, photosynthetic processes are more intense. This is indirectly confirmed by their more vigorous growth and development.

The comparative thermogravimetric analysis of wood samples of poplar clones Ijzer-5, grown on plant meliorated mix of rocks and sod podzolic soil was

carried out. Two curves obtained due to differential thermogravimetry (DTG) estimation are shown in the figure 7.

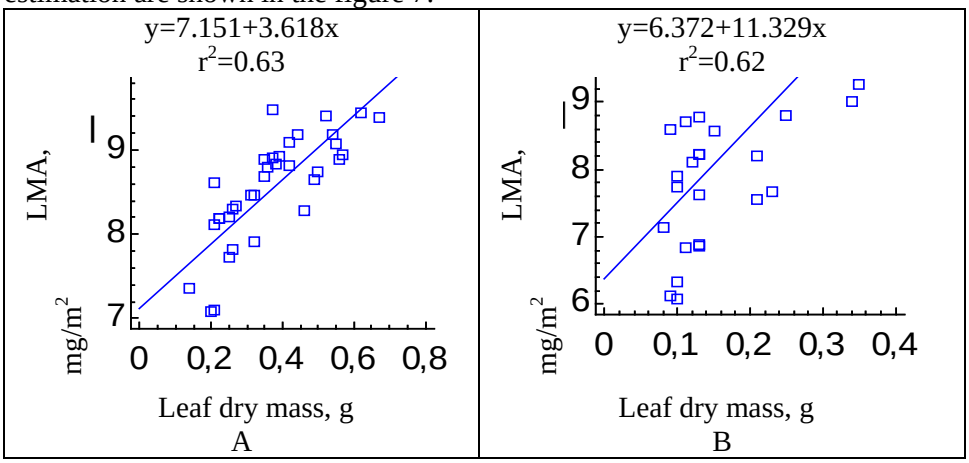

Figure 6. Relationship between LMA and leaf dry mass. Lineare regression has been calculated for the clones Ijzer-5 (A) and Robusta (B), $p \leq 0.01$

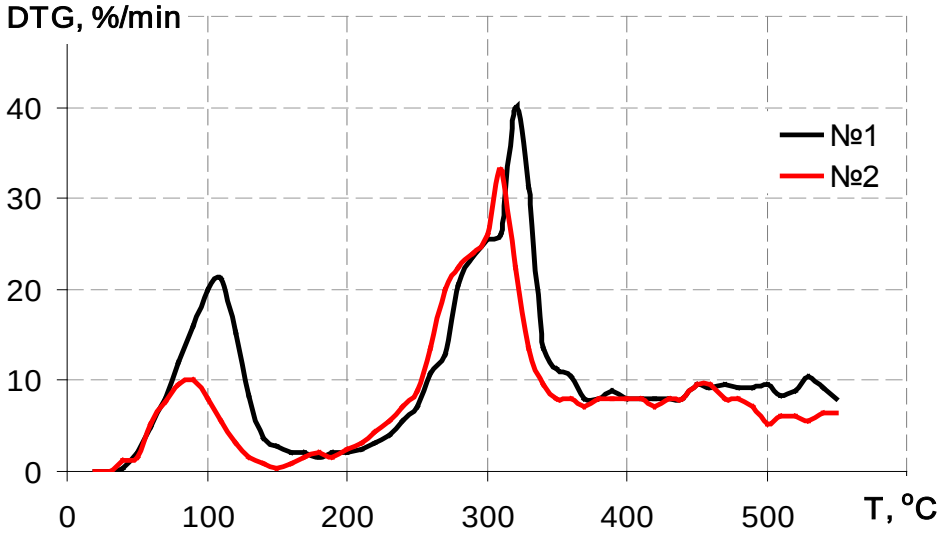

Figure 7. The results of thermogravimetric analysis of wood samples of poplar clone Ijzer-5, grown on sod podzolic soil and reclaimed mineland.
(№1 – sod podzolic soil, №2 - reclaimed mineland)

It is known that thermal decomposition of wood takes place in several stages. At the first stage of heating the wood, extraction of moisture, water adsorption and removal of volatile components take place. Further mass loss is related to hemicellulose, cellulose and lignin decomposition. At the last stage, the thermal destruction of lignin is completed and the combustion of coal formed during the thermal decomposition takes place (Loskutov *et al*, 2015; Muller-

Hagedorn *et al.*, 2003). In this study, it is distinguished three temperature ranges for the combustion of woody biomass of the poplar clones Ijzer-5 as well . The first stage of thermal destruction proceeds in a range from approximately 40°C to 150°C. The second interval is in the temperature range from 200°C to 300°C. The third stage is characterized by the highest combustion rate in the range from 300°C to 350°C. A larger value of DTG at all stages was observed in a sample of poplar grown on sod podzolic soil (1.2 times). Apparently, the difference in the value of the rate of mass loss is explained by the larger content of humus in sod podzolic soils, in comparison with the phytomeliorated mix of rocks.

CONCLUSIONS

Thus, marginal soils, together with low water supply under the steppe zone conditions, require searching for poplar clones which can to ensure high growth potential and plant biomass productivity. As a result of the research, only two clones proved to be promising for growing in such conditions. The experiment with poplar clone Ijzer-5 showed a positive effect of all bioagents on the growth morphological parameters. The treatment with vermicomposting extract gave the best result. For clone Robusta the best results were noted in the experiment with a mixture of agents. Treatment with trichodermin caused an inhibitory action on most of the growth parameters. And so there do not appear to be sufficient reason to use this preparation as individual agent for growing poplar clone Robusta. Nevertheless, in a mixture with vermicomposting extract and mycorrhiza, it has a significant positive effect.

The comparative thermogravimetric analysis of wood samples of poplar clone Ijzer-5 showed that DTG indexes were larger in poplars grown on sod podzolic soil. The difference in the value of the rate of mass loss is explained by the larger content of humus in sod podzolic soils, than in the phytomeliorated mix of rocks.

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MODELING OF SOIL CARBON STORAGE CAPACITY USING FARM MANAGEMENT FACTORS IN DRYLANDS

SUMMARY

The Carbon Storage Capacity (CSC) of land ecosystems is considered as the criteria for dry land sustainability assessment through analyzing of its management factors. We studied the commonly used management systems and their affected factors to model soil carbon storage in Sarfirooz-abad watershed, Kermanshah province, IRAN. GIS layers of slope degree, slope direction and elevation created, combined and used as the homogeneous map units. Three field management systems of tillage, crop rotation and residual management were defined and their relevant indicators were quantified. The systematic random method was used for soil sampling and 95 composite soil samples were taken from 0-30cm soil depth. In the laboratory, bulk density, texture and organic carbon content of soils was determined and soil organic carbon storage stocks was calculated. To estimate and model the carbon storage capacity and factors affecting, it was studied by stepwise regression, factor analysis and clustering. Results showed that using cluster analysis by seven variables of 15 variables has the significant relation with CSC with a correlation coefficient of 0.724 including plow index, cereals in crop rotation, stubble burning, animal fertilizer, crop rotation, winter fallow and plow direction. The cluster model efficiency of 0.46 was obtained that could predict about 52% of the CSC variability. The crop rotation and tillage were the variables of precise agricultural systems management that is undoubtedly important in CSC of dry lands.

Keywords: cluster analyzing, crop rotation, tillage, Organic carbon, crop residue.

INTRODUCTION

Soils as a part of terrestrial ecosystems contain the largest stock of organic carbon. Sustainable management of soil and in particular soil organic carbon (SOC) that was supported plant productivity (Hoyle *et al*. 2011). Soils hold approximately 75% of the C stored on land and thus plays a large role in the global C cycle (Swift, 2001). Soils of the semi-arid and arid areas constitute a

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Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

third of the global land area and are used for agricultural production (Harrison and Pearce 2000). Dry land in arid and arid areas was characterized by a low ratio of mean annual precipitation to potential evapo-transpiration and cover about 41 % of the surface of the Earth (Lal, 2004). The soils of these areas have an inherent low stock of organic carbon (C) due to climatic limitations. Soil carbon in dry land areas is of crucial importance to maintain soil quality and productivity. Soil mismanagement has led to a significant loss of carbon in these areas (Plaza-Bonilla1 et al. 2015). Agronomic choices such as crop or pasture selection, fertilizer application rates, net organic matter removal (e.g. burning), management of the soil (e.g. tillage practices), and removal of soil constraints to plant growth (e.g. liming to increase soil pH) alter plant biomass production, contributing to whether or not the actual SOC storage is as high as the attainable SOC capacity (Hoyle *et al*. 2011). The data on crop management and land-use could well be used to determine carbon storage capacity (Sayyadian et al., 2007). Crop management practices providing opportunities to reduce or increase the concentration of atmospheric carbon dioxide which store additional carbon through vegetation biomass and soil organic matter. Increasing organic matter in the soil can increase soil fertility, porosity, permeability and soil and water conservation (Dadgar, 2012). Conservation agriculture can affect the quantity of soil organic carbon by relying on management elements such as conservation tillage, residue management and crop rotation systems effectively (Parvizi, 2010). The most important determining factor for the organic carbon variability that can be controlled is farm management practices. Some of those include the change of forest and grassland to agriculture, use of equipment and intensity of tillage, fallow and crop rotation, crop residue management, use of green fertilizers and manure (Maia et al., 2010). Results of Franzluebbers (2010) showed soil organic carbon storage can be relatively high by conservation tillage. Organic carbon storage in soils creates a nutrient-rich environment for plant propagation. Conservation tillage, plant residue and animal manure on rangelands are the effective methods to increase soil organic carbon storage. Lopez et al (2011) conducted that no-till farming can be recommended as an alternative to conventional traditional tillage farming to increase organic carbon of the rainfed grain-producing areas soils. No-till system can also cause an increase of 20% of soil organic carbon storage compared to the traditional tillage system. Brown et al. (2012) found soil organic carbon change placed at the soil depth of 0-30 cm and introduced the soil depth of less than 30 cm to identify the impact of management on the surface and subsurface soil organic carbon. The Sombrero et al (2012) conducted that lands with minimal or no agricultural practices can enhance soil structure and increase the carbon storage in agricultural soils. The impact of these methods depends on soil and crop type, and farm management system. The dynamics of soil organic carbon reserves were studied by Yu et al. (2012) that was found soil organic carbon stocks on the beach, wetlands, agricultural lands and saline lands that were most affected by the human activities, can vary greatly. The results showed that human activities is

a key factor for change and storage of the soil organic carbon. The tillage system and crop residue management is required for crop production and maintaining soil fertility. In this regard, Hou et al. (2012) assessed the various effects of crop residue management and tillage on soil organic carbon and concluded that the conservation tillage is suitable method for maintenance of soil fertility and crop productivity through crop residue increasing. Results of Blanco- Canqui (2013) showed potential ways to compensate for the loss of soil organic carbon through the plant residue removal and lead to increase the soil organic carbon storage by crop management, tillage, fertilizer and compost. Given the importance of carbon storage in the soil, most studies have been done on a global level limited to compare the effects of one or two management action focused on carbon storage capacity. Also, a little research was conducted on methods of quantitative analysis and an effective way to evaluate and modeled appropriate field management factors to estimate soil organic carbon. Therefore, a comprehensive study on the status of management actions that govern the land areas and in real terms is not addressed. Therefore, the aim of this study was to identify management factors and prioritize effects of these factors for the quantity of soil organic carbon storage in dry farming areas at the traditional field management condition.

MATERIALS AND METHODS

Study Area

The study area, Sarfirooz-abad catchment, with surface area of 14000 ha is located in Kermanshah, IRAN. The geographic coordinates are 47° 04' 25" to 47° 22' 18" E and from 34° 00' 38" to 34° 09' 31" N (figure 1).

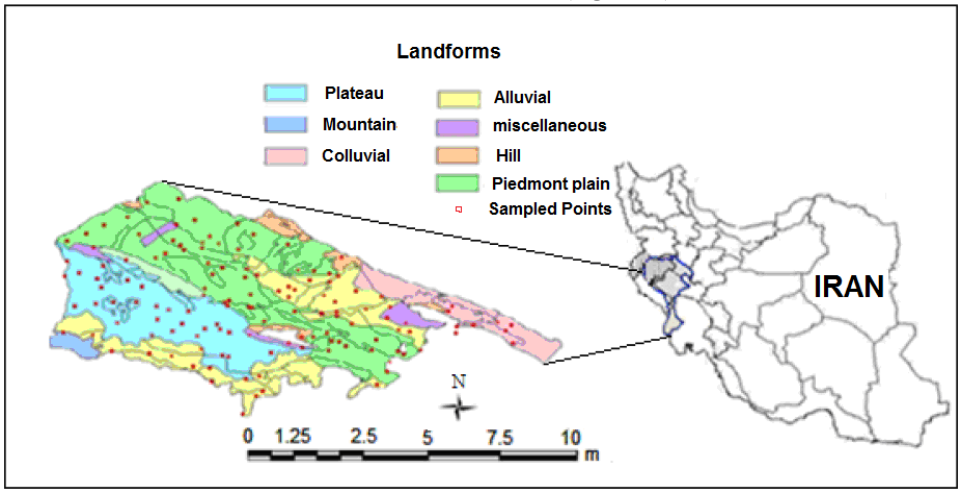

Figure 1. Location of study area (sar-firooz-abad catchment)

The average elevation is 1666 meters above sea level, mean annual temperature 8.86 ° C and mean annual rainfall 590 mm. Slope is mainly 0-5% and altitude ranges from 1500- 1800 m above sea level. The soils classified as

Inceptisols (Typic Calcixerepts, Loamy, mixed, mesic) according to the USDA Soil Taxonomy lies on Piedmont plain and Plateau. Conventional crop rotation in area is: winter fallow, Pease, Wheat and Barley planted rainfed.

Research Methodology

According to the agricultural land management practices in the study area (tillage, crop rotation and crop residue management) that could have a decisive effect on the amount of soil organic carbon storage, 15 single or combined variables based on their nature, extent and interactions identified as table 1.

Table 1. Main field management groups and related variables

	Management practices	**Variables**
1	Crop rotation	Farm size
2		Fertilization
3		Legume in rotation
4		Cereals in rotation
5		Crop rotation
6	Crop Residue	Pasture feeding
7		Hay harvesting
8		Hay burning
9		Livestock density
10		Residual management
11	Tillage	Soil erosion
12		Plow index
13		Mechanization index
14		Plow direction
15		tillage

With respect to the 3 management systems, the study area was delineated to 10*10 meters quadrates. The systematic random sampling (Yu et al., 2012) was performed in each quadrate. Soil samples from the 4 corners of the quadrates and their center were taken from depth of 0-30 cm (Stöckle et al, 2012) and mixed as a composite sample. Totally 95 composite soil samples are placed into the plastic bags and transferred to the laboratory. In the laboratory samples air dried, crushed lumps, separated the roots, rock and other impurities and passed from the sieve 0.5 and 2 mm (Mesh 20). Soil bulk density (gr/cm3) was measured by Clod method and soil organic carbon by Walkley-Black method (Black et al, 1965). The amount of soil carbon storage (ton/ha) calculated as relation 1 (Nieto et al., 2013).

$$CS = 10000 * SOC\% * Bd * d \quad (1)$$

Where, CS: Carbon storage (ton/ha), SOC: Soil organic carbon (%), Bd: bulk density (ton/m3), D: Soil sampling depth (m)

Descriptive statistics of the data including average values, maximum, minimum, and standard deviation were calculated. Relationships between management factors and soil carbon storage were investigated using the Pearson correlation method. To remove of the variables that had not a significant effect on the soil carbon storage, stepwise regression was applied. The investigated management factors were soil erosion, farm size, fertilizing manure, legumes and cereals in rotation sequence, winter fallow, crop rotation pattern, animal feeding, hay harvesting and burning, livestock density, residue management, mechanization energy index, plow direction and tillage. The multivariate regression, factor analysis and cluster methods were used to evaluate the effect of the management factors on the soil carbon storage and its estimation modeling

RESULTS AND DISCUSSION

Summary of statistical indicators of the farm management and soil factors are as table 2.

Table 2. Some statistical indicators of the soil organic carbon and related management variables

	Variable	Min	Max	Average	Standard deviation
1	CS (t ha $^{-1)}$	9.65	75.40	31.196	12.490
2	Er.	0.00	3.00	0.801	0.927
3	O h (ha)	0.40	4.00	1.716	0.908
4	Mn.	0.00	1.00	0.134	0.342
5	leg.F	0.00	1.00	0.411	0.173
6	Cer.F	0.00	1.00	0.547	0.192
7	Fw	0.00	1.00	0.702	0.458
8	R.Scn.	1.00	7.00	4.411	1.612
9	Pas.	0.00	1.00	0.148	0.357
10	SH	0.00	70.00	41.631	14.693
11	Burn.	0.00	1.00	0.546	0.499
12	D.dens.	0.35	6.40	1.564	1.358
13	S.Scn.	1.00	9.00	6.721	2.098
14	Energy	0.00	2240.00	1440.255	448.630
15	T_{index}	0.00	1.00	0.759	0.250
16	Pl.dir.	0.00	1.00	0.439	0.498
17	Till.Scn.	1.00	6.00	2.546	1.523

CS: carbon storage (t/ha), Er: soil erosion, O (h): farm size, Mn.: livestock Fertilization, Leg.F: legume in crop rotation, Cer.F: cereals in crop rotation , Fw: Winter fallow, R.Scn.: crop rotation pattern, Pas: animal feeding, SH: residue removal, Burn: stubble burning, D.dens: livestock density, S.Scn.: Residue Management, Energy: mechanization Energy index (MJ/ha/y), Tindex: tillage index , P.dir.: plow direction, Till.Scn: tillage.

According to the table 2 average stock of soil organic carbon stored per unit area is about 31.196 ton/ha. Average soil erosion at the sampling points was less than one. However, in most parts of the catchment, the surface crust formation is evident at the farms. Plant residue removal at the sampling sites

varies between 0 to 70% with a weighted average of 40%. Calculated linear correlation coefficients between management agents and soil organic carbon are shown in Table 3.

Table 3. Correlation matrix of farm management variables

	CS(t ha $^{-1)}$	Er.	O (h)	Mn.	leg.F	Cer.F	Fw	R.Scn.	Pas.
CS (t ha $^{-1)}$	1.000	-0.105	-0.182	0.424	0.381	-0.549	0.169	-0.436	0.171
Er.		1.000	-0.365	-0.253	0.057	0.127	0.046	0.108	-0.016
O (h)			1.000	0.051	-0.170	0.239	-0.030	0.063	-0.158
Mn.				1.000	0.169	-0.297	-0.062	-0.635	0.182
leg.F					1.000	-0.493	0.453	-0.546	-0.042
Cer.F						1.000	-0.157	0.381	-0.231
Fw							1.000	-0.657	-0.122
R.Scn.								1.000	-0.055
Pas.									1.000
SH									
Burn.									
D.dens.									
S.Scn.									
Energy									
T $_{index}$									
Pl.dir.									
Till.Scn.									

	SH	Burn.	D.dens.	S.Scn.	Energy	T $_{index}$	Pl.dir.	Till.Scn.
CS (t ha $^{-1)}$	-0.260	0.360	0.081	-0.424	-0.507	-0.580	-0.181	0.454
Er.	0.223	0.073	-0.285	0.083	-0.029	0.071	-0.243	0.180
O (h)	-0.037	-0.185	0.051	0.174	0.189	0.179	0.226	-0.301
Mn.	-0.360	-0.068	0.127	-0.293	-0.352	-0.354	0.231	0.270
leg.F	0.117	0.224	0.014	-0.219	-0.261	0.318	-0.210	0.278
Cer.F	0.373	-0.264	-0.080	0.511	0.723	0.750	0.071	-0.590
Fw	-0.001	0.156	-0.118	0.055	0.023	0.065	-0.114	-0.090
R.Scn.	0.193	-0.142	-0.034	0.248	0.343	0.322	-0.043	-0.220
Pas.	-0.343	0.011	-0.064	-0.381	-0.379	-0.340	0.104	0.218
SH	1.000	-0.180	-0.088	0.496	0.392	0.425	-0.099	-0.259
Burn.		1.000	-0.076	-0.594	-0.269	-0.372	-0.223	0.373
D.dens.			1.000	-0.096	-0.038	-0.096	0.064	-0.083
S.Scn.				1.000	0.533	0.661	0.065	-0.545
Energy					1.000	0.885	-0.054	-0.792
T $_{index}$						1.000	039/0	-0.755
Pl.dir.							1.000	-0.159
								1.000

As table 3, there are a significant and negative relation between soil carbon storage with the farm size and plow direction at (α=5%). The relation between soil carbon storage with the Cereals in crop rotation, crop rotation pattern,

residue removal, residue management, energy index and plowing index were significant and negative, and with the fertilization, legume in crop rotation, stubble burning and tillage there were a significant and positive relationship at the level (α=1%).

To find out the most significant and effective factors on the soil organic carbon storage the following steps were employed.

Stepwise regression

Results of Stepwise regression application to predict carbon storage (table 3) showed that in the first step only the factor of cereals in crop rotation has a significant relation (R^2=0.34) with soil carbon storage. In the later step, crop rotation was added to the first model and the correlation coefficient increased to 0.41. In the third and fourth steps by adding winter fallow and stubble burning the correlation coefficients increased to 0.45 and 0.49 respectively. The final model was obtained by adding the factor of plow direction that could explain 0.52% of the soil carbon storage variety with 5 factors (equation 2).

$$CS = 5.91 - 0.298CerF - 0.051R.Scn - 0.108Fw + 0.064Burn - 0.044P.dir \quad (2)$$

Where:

CS: Organic carbon storage, *Cer.F*: Cereals in crop rotation, *R.Scn*: Crop rotation, *Fw*: Winter fallow, *Burn*: Stubble burning and *P.dir*: plow direction.

Table 4. Stepwise regression modeling of the soil carbon storage

Stepwise regression procedures	Factors	R	R^2
1	Cer.F	0.580	0.337
2	Cer.F + R.SCN	0.642	0.412
3	Cer.F + R.SCN + Fw	0.667	0.446
4	Cer.F + R.SCN + Fw + Burn	0.702	0.493
5	Cer.F + R.SCN + Fw + Burn + P.dir	0.714	0.517

Factor analysis

This model was used to more data reduction and limiting number of factors that could explain the most primary variables. Figure 2 shows changes of the specific value (the value of the total variance estimated by a special agent) has descending trend. According to the figure 2, five axes can be as important issues that have the most significant role to explain the variance of the data with the Eigen-value above one and explain about 71% of the variability.

According to the table 4, the first axis consists of cereals in crop rotation, the energy and tillage index as effective variables. The second axis includes the crop rotation, winter fallow and legumes in crop rotation. The third axis involves the residues removal, animal feeding and fertilization. Axis fourth consists of farm size, livestock density and finally axis fifth involves residue management and burning stubble.

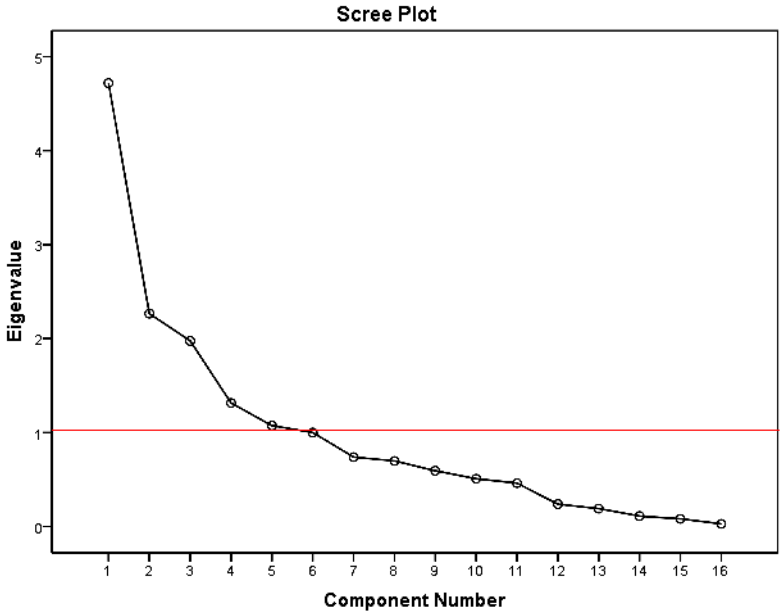

Figure 2. Number of distinct aspects of variables based on hidden roots

Table 4. Factor analysis of management variables

variables	axes				
	1	2	3	4	5
Er	0.009	-0.069	0.093	0.421	0.056
Oh	0.332	-0.034	-0.042	-0.630	-0.035
Mn	-0.153	0.233	0.182	-0.141	0.263
Leg.F	-0.369	0.738	0.191	0.111	-0.001
Cer.F	0.764	**-0.878**	0.163	0.041	0.103
Fw	0.174	0.355	0.008	0.071	-0.117
R.Scn	0.237	-0.840	**-0.757**	0.106	0.027
Pas.	-0.280	-0.146	-0.683	0.131	-0.034
SH	0.272	0.007	0.656	0.283	0.339
Burn.	-0.242	0.125	0.124	0.105	0.262
S.Scn	0.571	-0.017	0.237	0.077	0.646
Energy	0.858	-0.094	0.277	-0.052	0.096
Tindex	**-0.872**	-0.086	0.216	0.032	0.238
P.dir	0.054	-0.093	-0.327	**-0.866**	-0.437
T.Scn	-0.844	--0.013	-0.038	0.217	-0.170
D.dens	-0.355	-0.105	0.347	0.565	0.190

Based on the figure 2 and Table 4, preliminary variables of each axis applied in the regression model with four variables could explain about 48% of the carbon storage variation (equation 3). These four variables are prioritized as

tillage index, cereals in crop rotation, rotation pattern and plow direction with correlation coefficient of 0.697.

$$CS = 5.864 - 0.225Tindex - 0.153Cer.F - 0.029R.Scn - 0.043P.dir \quad (3)$$

Where:

CS: Organic carbon storage, *Tindex*: Tillage index, *Cer.F*: Cereals in crop rotation, *R.Scn*: Crop rotation *P.dir*. Plow direction.

Cluster analysis

Cluster Analysis was used after factor analysis at specified homogenous levels and the factors that have more coordination and assimilation in clusters categories and thus should be employed after continuation of factor analysis. The results of cluster analysis are shown in Figure 3 that involves a summary of the similarities of the variables.

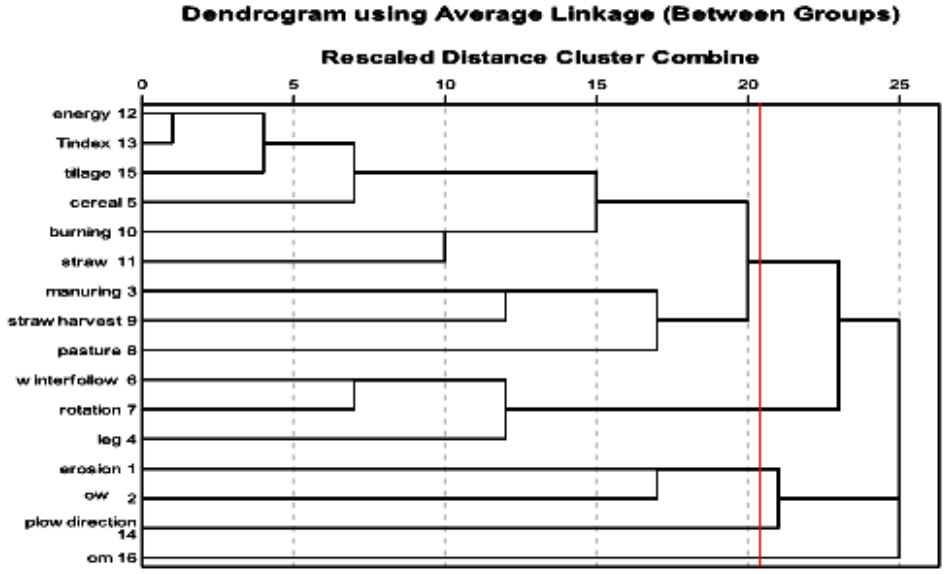

Figure 3. The cluster dendrogram for class variables of management

According to the dendrogram obtained, evaluated indicators can be classified into 5 clusters. The first cluster from below includes farm size, the second cluster includes plow direction and the third cluster involves fertilizing and erosion. The forth cluster involves rotation and winter fallow, and remained variables placed into the fifth cluster. So, applied multivariate model could explain about 52% of the variability of carbon storage by 7 variables.

$$CS = 5.863 - 0.11Tindex - 0.19Cer.F + 0.06Burn + 0.005AM - 0.04RScn - 0.07Fw - 0.04Pdir \quad (4)$$

Where:

CS: Organic carbon storage, *Tindex*: Tillage index, *Cer.F*: Cereals in crop rotation, *Burn*: Stubble burning, *AM*: Animal Manure, *R.Scn:* . Crop rotation, *Fw*: Winter fallow and *P.dir*: Plow direction.

Model evaluation

In Table 5 the results of the stepwise were showed that the step-by-step regression can estimate soil carbon storage by limiting the input variables with an average error of about 2.16%. The model efficiency calculated about 0.45.

Table 5. Criteria evaluated three models

Model	R	R^2	% RMSE	MBE	EF
Stepwise	0.714	0.517	2.160	0.005	0.448
Factor analysis	0.697	0.485	2.162	0.004	0.447
Cluster analysis	0.724	0.524	2.137	0.016	0.460

Results of the factor analysis showed that the average estimation error was about 2.16%. Efficiency of the model implies that 44% of the variation in the observed values can be described by the model. Evaluation of the cluster analysis showed that the MBE of the model was about 0.016 and the average estimation error of the linear model was about 2.14%. The efficiency coefficient of the obtained model implies that 46% of the variation in the observed values can be described with a correlation coefficient of 0.724.

Results indicated that there are a significant correlation between carbon storage and crop rotation pattern variables and its components like sequences of crops in rotation, animal manure, crop rotation pattern, winter fallow, and tillage component like plowing index and plow direction are effective more than others. Carbon storage has a significant correlation with residue removal and its component the stubble burning. This finding is according to the Blanco- Canqui et al (2009) results. Residue removal is more effective on reducing soil organic carbon. It can also point out to the results of Huang and colleagues (2013) that showed excessive removal of crop residue reduce soil organic carbon storage. The Blanco- Canqui (2013) showed that the management practices including tillage and animal fertilizer were corresponded with the soil organic carbon storage increasing.

Sombrero and et al (2012) showed crop rotation has not a significant effect on the amount of soil organic carbon. In addition, the lands with the territory of the legume residues returned to the soil, has higher organic carbon. Huang et al (2013) conducted that soil carbon stocks in the 0-10 cm of soil depth has not a significant difference with stubble burning and residue removal at the level of 5% because stubble burning decreases of organic residues and residue removal leads to biomass and soil respiration reduction.

The results of this study have indicated that the use of cluster analysis for the projected carbon storage (Breuer, 2012) using the farm management variables, improve the ability to predict multivariate linear model. Blanco-Canqui and et al (2009) reported that soil organic carbon storage linearly decreased by harvesting corn residue regardless of the soil tillage and farming system. Almagro et al (2013) has also used linear regression and stepwise model

in a Mediterranean ecosystem to determine the effect of vegetation type on carbon storage.

Our results showed that using cluster analysis model by insertion of 7 variables from a total of 16 variables that are prioritized as plow index, cereals in rotation, stubble burning, animal manure, rotation pattern, winter fallow and plow direction has a correlation coefficient 0.724 and explained about 52% of the of carbon storage variability. This finding corresponds with Parvizi (2010) partly due to 3 variables of tillage, stubble burning and fertilization.

The results of the cluster analysis showed that this model is low estimates at the high carbon storage levels (MBE= -0.016). The average estimation error of prediction using linear model was about 1.2%. The results of Wang et al (2009) showed that land use management and of soil type have the greatest impact on spatial variability of soil organic carbon.

Soil organic carbon storage changes in the agricultural land uses was affected mainly by management factors based on obtained Correlation coefficients. Cluster analysis of all the management variables showed that the plowing index, the sequence of cereals in rotation, stubble burning, fertilizing manure, periodic pattern, to plow the fallow winter were the most effective factors on the carbon storage changes.

According to the results of the three methods, cluster analysis has more precise estimate for carbon storage in soils. Among the factors that determine carbon storage changes in the agriculture land uses, the main contribution to belong to management factors can be soil tillage (plowing index and soil), rotation (succession of crops in rotation, animal manure, rotation pattern, fallow winter) and management of crop residue (stubble burning).

CONCLUSION

The impact and importance of various management factors on soil carbon storage is different. According to the results, the well-known effects of management on soil carbon storage variability implied that experts need to review the approach and attitude towards the sustainable utilization of land resources in agriculture land uses. Therefore, it is suggested that improved management systems of the different regions is a positive step in reducing atmospheric carbon density and thus storage of more carbon in the soil and also to precise land use management approach to sustainable agriculture and maintain soil carbon are highly recommended.

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PRODUCTIVITY OF TRANSPORT AGGREGATES IN HARVESTING CORN GRAINS

SUMMARY

Transport in plant production includes the entire transport of products and raw material from the place of production to the storehouse. Participation of transport in plant production relative to the total volume of mechanized works ranges from 35-50%, which significantly affects the total production costs. Determining the optimal organization of transport, the formation of transport aggregate and transport means can affect the increase in the economy of production. The paper presents the results of research of transport aggregate and transport means for harvesting corn grains at the distance of: 1km, 10km, 15km. Based on the data processed, the most efficient transport aggregate is determined.

The results of the research of transport aggregates (A, B, C) show that they have made significant differences in the quantity of transported corn grain at different operating speeds.

At a distance of 1km, the transport aggregate (A) transported 150.4 tonnes of corn grains, with an average operating speed of 11.2 $km.h^{-1}$, the transport aggregate (B) transported 104.58 tonnes of corn grains with an average operating speed from 7.41 $km.h^{-1}$ and transport aggregate (C) transported 103.65 tonnes of corn grains with an average speed of 14.69 $km.h^{-1}$.

At a distance of 10 km, the transport aggregate (A) transported 27.64 tonnes of corn grains, with an average operating speed of 12.78 $km.h^{-1}$, the transport aggregate (B) transported 72.50 tonnes with the average operating speed of 10.75 $km.h^{-1}$ and the transport aggregate (C) transported 187.50 tonnes of corn grains with an average operating speed of 23.25 $km.h^{-1}$.

At a distance of 15 km, the transport aggregate (A) transported 19.45 tonnes with an average operating speed of 17.00 $km.h^{-1}$, the transport aggregate (B) transported 56.68 tonnes with an average operating speed of 15 $km.h^{-1}$and the transport aggregate (C) transported 143.5 tonnes of corn grain with an average operating speed of 30.50 $km.h^{-1}$.

Keywords: aggregate, corn, transport, effectiveness, efficiency.

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INTRODUCTION

Transport in agriculture is an integral part of the production process from which organization the economy of production depends. Modern, intensive agricultural production as well as transport of raw materials and finished products have caused application of modern transport aggregates and transport means. The organization of transport and the formation of a transport aggregate depends on : types of products, available transport means, road network, weather conditions, productivity of harvesting and harvesting machines and warehouses. (Novaković and Đević 1999).

The share of transport in plant production in relation to the total volume of mechanized works ranges from 35% -50% (Stanimirović *et al* 2008). For these reasons, transport in agriculture, with the complete organization and synchronization of transport means, significantly affects the productivity and cost-effectiveness of production. Planning and organization of transport is difficult due to the characteristics of agricultural production and the type of products and raw materials. According to research (Čuljt 1988) in agricultural production about 80% of product transport takes place (five months) from June to November.

The design of transport means for harvesting and proper selection depends on: the number of available combines, harvesting capacity, capacity of the bunker, and the distance of the production area to the warehouse. (Tatomirović and Velimirović 1977)

MATERIAL AND METHOD

Research on transport aggregates was carried out in 2014 in the area Dervente Republika Srpska.

The research methodology is based on the analysis of the influence of the transport aggregate structure and the transport medium depending on: the distance of the production parcel, the structure of the transport aggregate and the transport means, the transport efficiency, the volume and capacity of the vehicle, the aggregate movement speed, the loading time, the transport time, the unloading time, the duration of the transport cycle, the coefficient of time shift utilization (τsm) and the efficiency of the transport aggregate and the transport means depending on the distance of the production parcel to the warehouse.

The harvesting of hybrid corn in the grains, ripening group 600, with a humidity of 30-34%, is intended for storage of silage in grain. Grain corn harvest

Based on work programs and established methods of work, organization of the harvest was based on the number of combines and their productivity. Based on the mentioned characteristics, the number and capacity of transport means was determined.

Corn grain transport was carried out by three transport aggregates, which were complemented in terms of manipulation of harvesters on the plot and during discharge. Based on the results of the research, the parameters as well as the mathematical data processing were summarized. In Table 1. the average results

of the corn grain transport from different distances, the total cycle time, the number of cycles, and the timing shift coefficient (τ_{sm}) for each transport unit are given.

The productivity of the transport aggregate depends on the proper aggregation of the tractor with the trailer and the organization of transport.The traction force of the tractor is greater than the overall resistance of the transport aggregate. (Mitrović *et al* 2014)

$$\mathbf{F_t > R_{or}}$$

F_t – traction force of tractor,

R_{or} – overall resistance of the transport aggregate.

The total resistance of the transport aggregate on a flat terrain depends: the total weight of the aggregates, the characteristics of the tractor, the characteristics of the empty trailer, the load and the conditions of the road surface.

$$\mathbf{R_{or} = G_p \cdot f_t \cdot G_{ft} \cdot f_{tr}}$$

G_t – tractor mass,

G_{ft} – mass of full trailer and cargo,

f_{tr} – trailer resistance coefficient,

f_t – coefficient of resistance of tractor movement.

The number of trailers that the tractor can pull can be determined based on the equation:

$$\mathbf{n = F_p / R_{or}}$$

F_p – traction force of tractor

R_{or} – overall resistance of the transport aggregate

When assembling the tractor aggregate (tractor + trailer), the ratio of the total weight of the trailer and the weight of the tractor is important:

$$\mathbf{f_a = G_{ft} / G_t}$$

f_a – aggregation coefficient

G_p – mass of full trailer and cargo

G_t – tractor mass

The productivity of the transport aggregate can be determined on the basis of the equation:

$$\mathbf{W_{ta} = G_{car} \cdot n_{cy}}$$

W_{ta} – productivity of the transport aggregate

G_{car} – cargo mass

n_{cy} – number of cycles of transport aggregate.

$$\mathbf{n_{cy} = T_{gt} \cdot C_{ut} / t_{cy}}$$

T_{gt} – total working time per day (gross time)

C_{ut} – coefficient of time use

t_{cy} – the duration of one cycle

The duration of one cycle of transport aggregate (t_{cy}) depends on: trailer load, trailer number, trailer loading time, transport time, type of substrate, unloading time and work organization**,** (Mitrović et al. 2014).

The duration of one cycle of the transport aggregate can be determined on the basis of the equation:

$$\mathbf{t_{cy} = t_{lo} + t_{dis} + t_{tft} + t_{tet} + t_{ch}}$$

t_{lo} – loading time,

t_{dis} – discharge time (unloading),

t_{tet} – the time of empty trailer transport

t_{tft} – the time of transport full trailers

t_{ch} – checking time.

The required number of transport aggregates, in the transport of certain products and materials,depends primarily on the volume of transport and productivity (performance) of the machine transport unit, and it is determined on the basis of the equation:

$$\mathbf{n_{ta} = Q_{uk} / W_t}$$

n_{ta} – number of transport aggregate

W_t – productivity of the transport aggregate

Q_{vt} – total volume of transport

$$\mathbf{n_{ta} = Q_{uk} / G_t \cdot n_{cy}}$$

G_t – mass of tractor

n_{cy} – the duration of one cycle.

Coefficient of time shift utilization (τ_{sm}) for achieving technological useful work is determined based on the equation:

$$\boldsymbol{\tau_{sm} = t_{uk} / T_s}$$

τ_{sm} – coefficient of time shift utilization

t_{cy}– total transport cycle duration

T_s – time of shift

Depending on the conditions and complexity of the technological process, the coefficient of time shift utilization is different. Under the test conditions, the minimum coefficient is 0.34 for the transport aggregate (C) at a distance of 1 km, while for the transport aggregate (C) the maximum is 1.0 at a distance of 10 km. At a distance of 1km, the resulting coefficient for the transport aggregate (A) was 0.72, for transport aggregate (B) was 0.44 and for transport aggregate (C) was 0.34.

At a distance of 10 km, the resulting coefficient for the transport aggregate (A) was 0.62, for transport aggregate (B) was 0.96 and for transport aggregate was (C) 1.0.

At a distance of 15 km, the resulting coefficient for the transport aggregate (A) was 0.54, for transport aggregate (B) was 0.87 and for transport aggregate (C) was 0.98.

The coefficient of time shift utilization was significantly influenced by the distance of the production plot from the economic yard, which can be concluded that the transport aggregate (A) at the distance of the production area of 1km achieved the highest coefficient.

Considering the conditions and complexity of the technological process, as well as congestion due to technical, organizational and personal character, the value of the coefficient of time shift utilization in the transport aggregate (A) was 0.72, which was characterized by the mobility and the possibility of accessing combines on the plot.

The transport cycle consists of empty driving, loading, freight and unloading. The duration of each operation is required by the time dimension (Marković, D., 1997) and can be determined on the basis of the equation:

$$t_{uk} = t_o + t_u + t_p + t_i \qquad (h)$$

RESULTS AND DISCUSSION

Observing the number of cycles performed for each transport aggregate, from different distances, the result was that the transport aggregate (A) at a distance of 1km, performed 20 cycles, the transport aggregate (B) 7 cycles and the transport aggregate (C) 5 cycles.

At a distance of 10 km the transport aggregate (A) has performed 4 cycles, transport aggregate (B) 5 cycles and transport aggregate (C) 9 cycles.

At a distance of 15 km, the transport aggregate (A) has performed 3 cycles, transport aggregate (B) 4 cycles and transport aggregate (C) 7 cycles. On smaller distances, the more mobile transport aggregate (A) performed several cycles more in contrast to the vehicle (C), and in the case of a larger distance, the situation is inverted.

The carrying capacity of the transport unit, the number and structure of transport means has been affected by the duration of the transport cycle. The loading time (t_u) can be achieved by applying a self - loading trailer with a bunker that can monitor the work of the harvester and harvested grain unloading in the means of transport at the end of the parcel

Driving with load (t_l), depends on transport speed with load, distance and road network status.

Time of unloading (t_{ul}) appears as inevitability in the duration of the transport cycle, and depends on the method of discharging the transport means.

Table 2. shows the quantities of transported corn grains, from which it can be concluded that transport aggregates A, B, C transported different quantities depending on the distance of the parcels.

The transport aggregate (A), at a distance of **1 km**, transported 150.4 tonnes or 42.0% of the total 358.6 tonnes of transported corn grains. The

transport aggregate (B) transported 104.58 tonnes or 29.2% of the total, and the transit aggregate (C) transported 103.65 t or 28.9%.

Table 1. Average results: duration of the cycle, number of cycles and coefficient of time shift utilization (τ_{sm})

Transport aggregate	Duration of the transport cycle (*min*)					τ_{sm}	Number of transport cycles
	t_o	t_u	t_p	t_i	t_{uk}		
distance 1 *km*							
A	4,40	8,36	5,40	3,41	21,57	0,72	20
B	5,10	18,12	8,10	6,10	37,42	0,44	7
C	3,45	28,14	4,08	4,48	40,16	0,34	5
distance 10 *km*							
A	33,44	8,36	47,00	3,07	91,87	0,62	4
B	35,00	18,12	56,00	6,06	115,18	0,96	5
C	15,34	28,10	26,00	4,10	73,54	1,11	9
distance 15 *km*							
A	44,00	8,38	52,00	3,03	107,41	0,54	3
B	47,33	18,13	60,00	6,07	131,53	0,87	4
C	22,00	28,13	30,00	4,10	84,23	0,98	7

Table 2. Amount of transported corn per transport means

Transport aggregate	Number of transport cycles	Amount of transported corn				Percentage of participation in transport (Pup)
		By cycle (*t*)	*t*	*tkm*	*tkm/h*	
distance 1 *km*						
A	20	7,52	150,40	150,40	15,04	42,00
B	7	14,94	104,58	104,58	10,46	29,20
C	5	20,73	103,65	103,65	10,37	28,90
in total			358,63			
distance 10 *km*						
A	4	6,91	27,64	276,40	27,64	9,62
B	5	14,50	72,50	725,00	72,50	25,20
C	9	20,83	187,50	1875,00	187,50	65,25
in total			287,34			
distance 15 *km*						
A	3	6,48	19,45	291,75	29,18	8,86
B	4	14,17	56,68	850,20	85,02	25,81
C	7	20,50	143,50	2152,50	215,25	65,34
in total			219,63			

At a distance of 10 km, the transport aggregate (A) transported 27.64 tonnes or 9.62% of the total 287.34 tonnes of transported corn grains, transport aggregate (B) transported 72.5 tonnes or 25.2% of the total transported corn grains and the transport aggregate (C) transported 187.5 tonnes or 65.25% of total transported corn grains.

At a distance of 15 km, the transport aggregate (A) transported 19.45 tonnes or 8.86% of the total 219.63 tonnes of transported corn grains, the transport aggregate (B) transported 56.68 tonnes or 25.81% of total, and the transport aggregate (C) transported 143.50 tonnes or 65.34% of total transported corn grains.

The research results show that the transport aggregate (A) is more mobile than other two aggregates and at a smaller distance, transport aggregate (A) transported a larger amount of corn grains than the transport aggregate (C).

Comparing the results (Table 2) the transport aggregates efficiency is directly proportional to the increase of aggregates capacity and distance plots of the economic yard. If the productivity is expressed in (tkm/h), it is reversely proportional if the productivity is expressed in (t/h).

CONCLUSION

Presented data show that the problem of transport in agriculture is very complex and that in the current conditions of agricultural production with higher yields it becomes a brake on its further development, if it is not equipped with modern means of transportation.

At greater distances, transport with the truck in relation to the transport with tractor (tractor + trailer) has a greater advantage. At a distance of 15 km, the truck transported 65.34% while the tractor (tractor + trailer) transported 8.86% of the total quantity, which is 7.36 times more.

At smaller distances, productivity (t/h) is important because it is growing, while at larger distances it is decreasing.

The structure of the transport aggregate has shown that the transport aggregate (A) at a distance of 1km has priority over other transport aggregates for easier manipulation on the plot.

Transport means such as a container or a specialized trailer represent a better conceptual, technical and technological solution that affects transport efficiency and cost-effectiveness.

Solving the rationalization of transport, especially internal in agricultural production, is not enough to solve in the field, but it should be addressed in the warehouse and later in the production, packaging and transport to consumers.

Since transport in agriculture is specific, it is necessary to abide by the directive of the European Parliament and the Council of Europe on transport in agriculture. The influential transport factors are: the structure of the transport aggregate, the road network, the transport distances, the volume and load capacity of the transport meeans, the movement speed, the time of loading and unloading of the product or repro materials.

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SENSITIVITY OF AGRI-FOOD SECTOR TO THE FINANCIAL CRISIS: EMPIRICAL EVIDENCE FROM A BALKAN COUNTRY

SUMMARY

In post-communist economies, agri-food sector can suffer financial instability in periods of external shocks, such as the global financial crisis of 2008–2010. By applying two different approaches, authors examine the financial response of the listed and unlisted Macedonian agri-food companies to the crisis. The results show that the crisis had no great effects over the Macedonian agri-food companies, except for the reduction of the production volume of the unlisted companies and the decreased investment activities of the listed companies. Finally, this paper suggests an understanding of the financial behaviour of the agri-food companies when faced with systematic risk factors, and sets grounds for further researches in the context of other post-communist economies.

Keywords: Systematic risk, financial behavior, listed and non-listed companies, Republic of Macedonia

INTRODUCTION

Agri-food companies in post-communist economies suffer financial distress in turbulent economic, political and social environments. The most recent external economic shock was the global financial crisis (2008–2010), probably posing significant challenges to the most of the agri-food sectors in the Balkans, such as the Republic of Macedonia.

A number of Macedonian authors have studied the effects of the global crisis on the Macedonian listed companies; however none have portrayed the response of the agri-food companies to this shock. Moreover, there are no empirical evidences on the financial sensitivity of unlisted Macedonian agri-food companies to the financial crisis. In fact, the agri-food sector is predominantly composed of micro, small and medium-sized companies, due to decentralization of the country with the transition process (Simonovska et al., 2014). These are not listed on the stock exchange market, but they play a major role in the Macedonian agri-food sector in terms of overall business activity and especially employment.

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Even a decade after the crisis, the issue of sensitivity of the Macedonian agri-food sector to the global financial crisis remains unclear. Thus, the aim of this study is to examine the financial response of the Macedonian agri-food companies to the financial crisis (2008-2010).

Considering that some of the agri-food companies are stock companies and some are limited liability companies, two objectives underline this research: (1) examining the response of listed Macedonian agri-food companies to the market risk before, during and after the crisis period, and (2) examining if a change has occurred in financial performance of unlisted Macedonian agri-food companies due to the crisis. Since the wine sub-sector is being one of the most prospective agri-food industries in the country, it will represent the case for the unlisted agri-food companies.

The results of this analysis contribute to a better understanding of the financial flexibility of the agri-food companies when faced with systematic risk factors, and set grounds for further national strategic decisions in providing sustainability and development of the agri-food industry. The agricultural sector and the adjacent food industry are very important economic segment of the country, contributing with around 16% to the national GDP (SSO, 2011). Almost 435,500 people, out of a population of 2 million, make whole or part of their income from agri-business activities (EIB, 2016). Finally, the results and analysis may be used as a guideline for related researches in the context of other post-communist economies.

The following section provides description of the materials and methods. The next section presents the results. First, insight in the macroeconomic condition of the country is provided, and second, the underlying results are presented in respect to the objectives. Finally, conclusions are drawn, followed by a short discussion.

MATERIAL AND METHODS

Since the aim of this study is two-fold, two different approaches are applied. First, the level of response of listed companies is assessed by estimation of the systematic risk that each agri-food company faced during this period. The systematic risk (also known as portfolio risk, market risk, or non-diversifiable risk) is the risk that cannot be controlled by company's management, unlike the unsystematic risk that is diversifiable, unique or firm specific risk (Fama and French, 2004). Second, the response of companies with limited liabilities to systematic risk factors is explained through hypotheses testing of the changes in the financial behaviour of unlisted companies (wineries), under the assumption that their financial performance is at certain extent affected by the crisis. Both methods are explained below in a consecutive order.

Measuring the level of systematic risk of listed companies

Beta coefficient (β) measures the sensitivity of stock returns in relation to market returns, which is strongly influenced by the state of the economy. The market model is not based on investment behaviour assumptions, but is examined

as a linear relationship between stock returns and market returns. It has traditionally been estimated by employing a market regression model, thus measuring the level of systematic risk that arises from general factors such as political influences, economic crises, wars, and natural catastrophes, all of which affect every economic entity. In other words, the degree of systematic risk depends on the degree to which a company's revenues are determined by the macroeconomic factors that cannot be controlled by its management.

The model and its interpretation

Risk, as approached herein, equals the variance of historical rates of return in relation to the average rate of return (Hotvedt and Tedder, 1978). In the standard financial literature, the beta value is derived from the CAPM (Capital Asset Pricing Model) or the Sharpe-Lintner-Black model (Sharpe, 1964; Lintner, 1965; Black, 1972). It is expressed as in the following equation (1):

$$\beta_j = \frac{\sigma_j \rho_{jM}}{\sigma_M} \tag{1}$$

Where $\sigma_j \rho_{jM}$ is the systematic risk of company j, and σ_M is the total market risk. Hence, the beta value of company j's shares is an index of the amount of the company's systematic risk relative to the risk of the market portfolio.

To derive β_i, we constructed a monthly return series for both the stock and the market index, as suggested by Shalit and Yitzhaki (2002). We then used standard regression formula for each agri-food company in the sample. The main equation (2) is:

$$R_i = a_i + \beta_i R_m \tag{2}$$

Where R_i is the return on a stock; a_i is the component of security *i*'s return that is independent of market performance – a random variable; R_m is the rate of return on the market index – a random variable, and β_i is a constant that measures the expected change in R_i given a change in R_m.

Alternatively, Hotvedt and Tedder (1978) define the intercept α_i as the rate of return of an asset given a stationary market, and β_i as a measure of the volatility of the rate of return of an asset in relation to the rate of return of the market, i.e., it is reflected by the slope of the regression line.

This equation divides the return on a stock into two components: one component is related to the market and is referred to as β_i (the part of the return that is sensitive to market movements), while the other is independent of the market (company specific) and is referred to as α_i (the part of the return that is insensitive to the market returns)

Sample selection of listed companies

The study focuses on the period from June 2007 to June 2010, the time of the financial crisis. Shorter time series give more accurate results for this kind of research; Bradfield (2003) considered that estimates based on many years of historical data may be of little relevance, because the nature of the business risks taken by companies may have changed significantly over a long period.

The sample consists of Macedonian agri-food companies that are listed on the Macedonian stock exchange. Since the Macedonian stock exchange, being an emerging market, is volatile, it was difficult to extract larger sample with continuous series during the estimation period. Some of the listed Macedonian agri-food companies were considered as outliers and were excluded from the analysis. Therefore, the sample consists of the three largest food processing companies and the three largest beverage industries in the country. In addition, two large companies engaged in agri-food processing and trading activities were included in the sample, having been consistently listed on the stock exchange during the whole estimation period. All these companies are dominant in the Macedonian agri-food sector.

Hypotheses testing of the financial behaviour of unlisted companies

The wineries' profitability, liquidity, activity and debt situation is assessed and hypotheses are tested so to observe the change in their financial behaviour, which was affected by the crisis.

Hypotheses setting and statistical tools

The main *alternative* hypothesis is set as follows: 'There is a significant difference in the financial performance of the wineries (more precisely, in their profitability, liquidity, activity and debt condition) before and after the crisis period'. The rejection of the *null* hypothesis confirms an existing change in the financial behaviour of the wineries. This hypothesis is tested through sub-hypotheses testing of the entire set of financial performance indicators (profitability, liquidity, activity, and debt ratios). For this reason, we observed the same sample of wineries for each financial performance indicator during two time periods, i.e. for the year 2008, which determines the pre-crisis period, and for the year 2010 that represents the post-crisis period (the 2009 is the crisis period since the major macroeconomic effects from the crisis were reflected during this year, confirmed with data presented in Table 1).

We conducted the paired *t*-test in Stata 12.0 (StataCorp, 2011) to examine the null hypothesis that there is no difference between the financial condition of the wineries before and after the crisis period. The paired *t*-test is used to compare two population means of two samples in which observations in one sample can be paired with observations in the other sample (Boslaugh, 2012). For those variables with too many outliers, the nonparametric Wilcoxon matched-pairs-ranks test was run, again in Stata 12.0. With this, we tested the equality of matched pairs of observations. We have decided upon this approach due the small sample size.

The paired *t*-test was conducted for the gross profit margin – GPM, the asset turnover ratio – AT, the days in inventory – DI, the inventory turnover ratio – IT, and the debt-to-assets ratio – DTER. Those variables that were not normally distributed were not tested with the paired *t*-test, but with the nonparametric Wilcoxon matched-pairs-rank test (such as, the net profit margin – NPM; the return on assets – ROA; the return on equity – ROE; both liquidity ratios – CLR and QLR; the receivables turnover ratio – RT, and for the debt-to-equity ratio – DTER).

Sample selection of unlisted companies

The sample consists of not listed Macedonian wineries, legally registered as a limited liability companies. The sample was randomly selected and it represents a major part of small and medium sized wineries.

The official financial records on wineries, i.e. the balance sheets and income statements, are provided by the Central Register of the Republic of Macedonia (CRM, 2012). The data are grouped in a panel-database with a total of 45 observations, including nine wineries analysed during the period of five years (2006–2010). The small sample size hindered application of advanced econometric methods of panel data analysis. However, it does not limit application of traditional hypothesis tests.

RESULTS AND DISCUSSION

Macroeconomic overview of the Macedonian economy

The financial crisis first struck the United States of America in 2007, encompassing only one region and one specific financial market at the time. The crisis expanded internationally during 2008 and caused a deceleration in global economic growth (NBRM, 2009). Although the Macedonian economy was not directly affected by the crisis, the country indirectly felt its negative effects, mainly during the last quarter of 2008 and the beginning of 2009. This came as a result from the crisis induced in neighbouring countries, which experienced a decrease in economic activity and consumption (NBRM, 2010). Since Macedonia and its neighbours are traditional trading partners, this situation caused a decrease in export consumption and deterioration in the international position of Macedonian companies. Several macroeconomic indicators confirm the halt in economic performance during 2009 (Table 1).

The crisis that evolved in the economies of Macedonia's trading partners evidently discouraged investment in its private sector. Faced with an insecure export market, investors were not inclined to risk their money. At the same time, bank credit suffered appallingly low annual growth (only 3.5%), a decline of 30.9 percentage points since 2008. However, the banking sector has remained stable during the global financial crisis and the overall negative effects of the crisis were decreased volumes of trade, foreign direct investments and remittances (Rahkola *et al*., 2009).

In the financial sector, the negative effects of the crisis were most visible in the Macedonian stock market (MSE). Macedonia is an emerging economy and

established the Macedonian Stock Exchange (MSE) four years after gaining its independence, in 1995 (MSE, 2010). The first index (MBI) was introduced in 2001. Since then, the country has gradually introduced market indices weighted with market capitalization. In 2005, the index MBI10 was introduced, which is a price index weighted with market capitalization and refers to companies quoted on the official market. As such, it is suitable for assessment of systematic risk (Bradfield, 2003). In 2007, MSE started to calculate a new index of publicly held companies – MBID, i.e., for companies quoted on the regular market.

Table 1. Macroeconomic indicators of RM, 2005–2009 (www, NBRM, 2009)

Macroeconomic indicator	**Units**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**
GDP annual real growth rate	%	4.7	5.1	6.5	5.5	-0.4	3.4
Inflation, end of period (yearly basis)	%	1.2	2.9	6.1	4.1	-1.6	3.0
Unemployment rate	%	37.3	36.0	34.9	33.8	32.2	32.1
Bank credits in private sector (yearly rate of change)	%	21.0	30.5	39.2	34.4	3.5	7.1
Weighted average active interest rates	%	12.1	11.3	10.2	9.7	10.1	9.5
Exchange rate (1 EUR=)	MKD	61.30	61.19	61.18	61.27	61.27	61.51
Trade balance	mil. EUR	-961.1	-1062.2	-1356.5	-1966.9	-1699.8	-1602.2

MSE experienced a sharp decline in both prices and trading volumes (Filipovski, 2008), starting in October 2007, continuing throughout 2008, and reaching its lowest point at the beginning of 2009 (MSE, 2010) (Figure 1). Evidently, the MSE followed developments in the global economy during the crisis period.

In summary, three main factors emerged in this period: (1) the MSE has been to a large extent dependent on the liquidity provided by foreign portfolio investments, (2) the policy of strengthening credit conditions has lowered the available capital for investment in the stock market; and (3) investors did not react to the government's anti-crisis measures.

The responsiveness of agri-food companies to systematic risk

The Macedonian Stock Exchange (MSE) experienced a sharp decline during the financial crisis period, starting in the end of 2007 and reaching its lowest point at the start of 2009. Thus, we portray the response of the listed Macedonian agri-food companies to systematic risk before, during and after the crisis period in a single-country context.

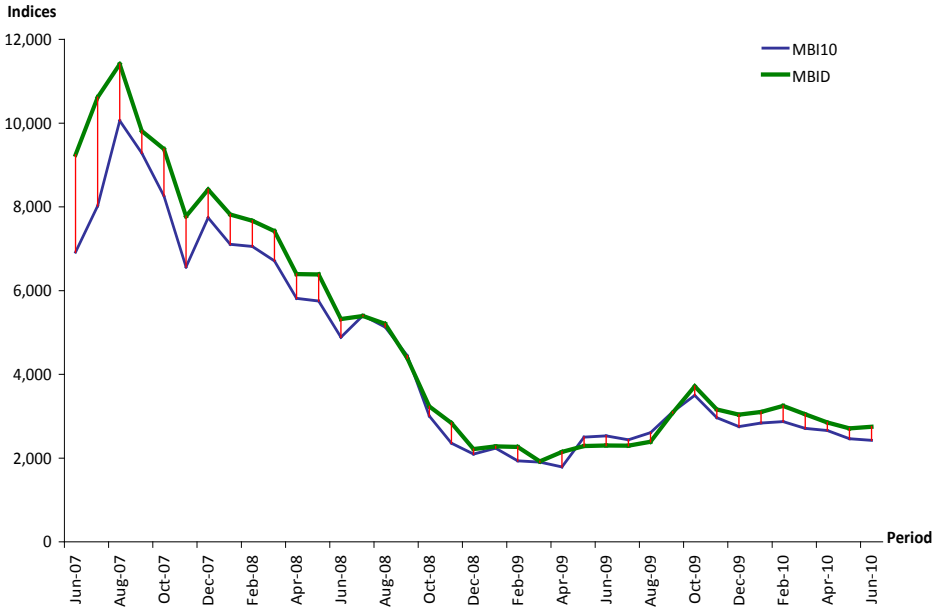

Figure 1. Macedonian stock exchange development, 2007–2010 (www, mse, 2010)

To depict the response of the Macedonian listed agri-food companies in relation to the market during the period of the financial crisis, we used a financial approach to evaluate the level of systematic risk. The theory suggests that the level of the systematic risk depends on macroeconomic factors including crises; therefore, we chose this approach as applicable and relevant to this type of analysis. This model was developed by Sharpe (1964), Black (1965), Lintner (1965), Treynor (1965) and Mossin (1966). Beta coefficient has been widely used by many authors in the financial literature, among them Pogue and Solnik (1974), Blume (1975), Roll (1977), Basu (1977), Banz (1981), Brown *et al.* (1983), Eubank and Zumwalt (1979), Fama and French (1992), Corhay (1992), Bruner *et al.* (1996), Arsov (2008), and Kapusuzoğlu (2008). They all consider that a correlation between security returns is a common response to market changes. For this reason, beta has been and still is considered a useful measure to relate the return on stocks to the return on the stock market index. Accordingly, we emphasized the value of β as a core contributor to fulfilling this aim, while not neglecting the other regression components.

In order to interpret beta, Ross *et al.* (2008) give the following example: 'If beta of a company is 1.5, means that the returns of this company are magnified 1.5 times over those of the market'. Thus beta expresses a positive value, which means that there is a positive correlation between the company's shares and the market; so if the market return moves up by 1, the return on the company's

shares will move by 1.5. On the other hand, if beta is negative, then the correlation is negative, and the return on the market will go in an opposite direction from the return on shares.

Lumby and Jones (2003) explained beta in terms of a share's risk premium, that is, the excess return that must be paid to compensate for the uncertainty of the return's being achieved (Vessey *et al.*, 2006). Following their reasoning, we have set the following 'benchmarks' of companies' beta: $\beta>1$ if movements in the share's risk premium are likely to be greater than movements in the market portfolio's risk premium, $\beta\approx1$ if the movements in the shares' risk premium tend to be the same as movements in the market portfolio's risk premium, and $\beta<1$ if the share's risk premium under-responds to movements in the market portfolio's risk premium.

To calculate the return on the official market, we used the Macedonian stock exchange index MBI10 in a regression relationship with the security returns of the companies listed on this market (Appendix 1). The return on the regular stock exchange was calculated using the index of publicly held companies – MBID (Appendix 2).

The results for both exchange markets are summarized in Table 2. The value of the beta coefficient showed high variations, ranging from 0.26 for Co. 6 to 1.79 for Co. 1, indicating different levels of response by individual companies to market movement during the crisis period. Only one company (Co. 1) over-responded to market movements during this time. This company produces bread and pastry, for which demands is inelastic. On the other hand, demand for the products of the company with the lowest beta (Co.6), mainly confectionery, is more elastic. In the case of Co. 3 and Co. 8, the risk premium moved in parallel with the market portfolio's risk premium; thus they did not achieve excess returns during the crisis in relation to the non-diversifiable risk measured by beta. These companies differ in their core activity, the first being mainly engaged in agri-food trade and retail, the second in the beverage industry. The remaining companies moved more slowly than the market; thus their low level of beta contributed to low stock returns during the crisis period. The beta indicator is suited for analysis of individual cases and comparison between cases, but cannot yield a general conclusion for the whole sector.

The alpha coefficients indicated different levels of non-systematic risk in the market, ranging from -0.01 for Co. 6 to 0.16 for Co. 1. This indicator represents the average return on shares when the market on average does not move; thus investors endeavour to minimize this risk through optimum diversification, which is one of the basic objectives of portfolio management (Kapusuzoğlu, 2008). However, as negative alpha reduces the level of stock returns, buying shares in a company with negative alpha could be considered as a bad investment and could impel shareholders to sell the shares they own (Lumby and Jones, 2003). This situation occurred in four cases (Co. 4, 6, 7, and 8) during the crisis period.

Table 2. Summary of results from the regression estimates on the level of systematic risk

Case	β-value	α-value	R^2
Co.1 (food processing: bread and pastry)	1.7927	0.1612	0.0447
Co.2 (agriculture production and feed processing)	0.7754	0.0115	0.3403
Co.3 (retail and trade in agri-food industry)	0.9489	0.0258	0.3743
Co.4 (processing and wholesale: feed, flour, pastry)	0.4726	-0.0078	0.2426
Co.5 (processing and trade: wine and beverages)	0.5968	0.0291	0.2581
Co.6 (agri-food processing and confectionery industry)	0.2575	-0.0136	0.2234
Co.7 (wine production and local tourism)	0.4886	-0.0295	0.2101
Co.8 (beverage industry: beer and soft drinks)	0.9110	-0.0061	0.4316

The coefficient of determination (R^2), being a measure of the percentage of total risk accounted for by the non-diversifiable, systematic risk, ranged from 0.04 for Co. 1 to 0.43 for Co.8. The share of the systematic risk within the total risk is usually found to be lower than the share of the diversifiable unsystematic risk (Hotvedt and Tedder, 1978; Bradfield, 2003), as revealed in the case of the Macedonian agri-food companies. It is important to stress that in this type of analysis there is no direct relation between beta and R^2; hence, high beta does not necessarily produce a high coefficient of determination.

The financial flexibility of the wine sector to economic crisis

The global financial crisis posed significant challenges to the wine industry, considering that wine is an elastic product. For instance, the global financial crisis affected the Greek consumption of wine that went down by 5–20% (Palate Press, 2010). The micro, small and medium sized wineries in emerging countries are more vulnerable to external shocks since their small size and limited resources constrain their financial flexibility in uncertain economic conditions (Skorvagova and Pasztorova, 2014). Understanding the level of their financial stability during some volatile times may contribute to limit the risk of bankruptcy in future uncertainties.

Since the Macedonian agri-food sector was faced with significant challenges due to economic crisis, this section attempts to provide an assessment of the financial performance of Macedonian wineries during the period of 2005–2010, capturing the period before, during and after the crisis (Appendix 3).

The financial performance of wineries is observed through a set of financial indicators. Profitability ratios show how efficient is the company in its operation in relative terms, and higher ratio indicates higher profitability (Arsov, 2008). The gross profit margin (GPM) calculates the share of operating profit to revenue sales. The net profit margin (NPM) represents the share of net profits from revenue sales. If the gross is higher than the net profit margin, indicates that non-operating expenditures or the tax rates have increased for the company (Simonovska, 2014). The return on assets (ROA) measures the rate of return on assets employed by a company and shows how profitably the company is using

its assets. The return on equity (ROE) shows the share of net profit attributable to equity owners for each unit of capital invested in the company. In favourable credit conditions, the amount of ROE should be greater than ROA (Simonovska *et al.*, 2014).

Liquidity ratios show the ability of the company to timely payback its liabilities and it is provided by owning liquid assets or possessing the capacity to borrow additional funds (Barry and Ellinger, 2012). The difference between the current ratio (CLR) and the quick liquidity ratio (QLR) is that the later ratio excludes inventory from current assets, as being low liquid asset (Simonovska *et al.*, 2014).

Activity ratios show how efficiently assets are used, and the higher the ratio is, the more efficiently the assets are used, with an exception of the average payment period (APP) and the days in inventory (DI), where shorter period is related to a greater efficiency (Hunger and Wheelen, 2009). The others in this category are: (1) The asset turnover ratio (AT), (2) The receivables turnover ratio (RT), and (3) The inventory turnover ratio (IT).

Debt ratios show the level of debt that companies have and their capability to service that debt (Huzjan *et al*., 2015). The debt-to-assets ratio, or total debt ratio (DR) shows the proportion of total assets financed by external sources of capital, and the debt-to-equity ratio (DTER), the proportion between external and internal sources of capital (Simonovska and Gjosevski, 2016).

Generally, the wineries do not represent liquid companies due to the large share of inventories which is within the nature of the wine industry. However, wineries manage to collect their receivables in a relatively short period. In the analysed period, there was a change in the tax policy, as well as changes in the use of non-operating assets, resulting in increased non-operating expenses.

Nonetheless, wineries are profitable, but they operate with low profit margins in the price formation. Their strategy to increase profitability is to hold large turnover of assets. This strategy mainly occurs due to the large production of bulk wine and the low degree of wine differentiation as bottled. Those wineries that work with high profit margins and value-added production, have the capacity to absorb the economic turbulences through their price flexibility. The wineries are highly dependent on debt to finance investments and working capital; however they do not face high financial risk by holding more assets than debt.

Previous studies (Simonovska, 2014; Simonovska *et al*., 2014; Huzjan *et al*., 2015; Georgiev *et al*., 2015; Simonovska and Gjosevski, 2016) analysed the financial condition of the agri-food sector, some including the wine industry in the country. For instance, Simonovska *et al*. (2014) observed the financial condition of agricultural companies and econometrically tested their capital structure strategies in increasing profitability, defining a typical farm company that is a low-levered, relying on assets rather than debt, diversifying production with small inventories, operating at high capital intensity and able to cover current liabilities. Specifically for the wine sector in the country, Huzjan *et al*.

(2015) determined the financial condition of the wineries during the period of 2008–2013 and found out that wineries did not represent liquid companies due to large share of inventories, but they were able to collect receivables in a short period, frequently used debt financing, and operated with low margins and large assets' turnover.

However, none of these previous researches did not provide an understanding of the financial behaviour of the wineries during their exposure to external shocks. Thus, herein, the wineries' profitability, liquidity, activity and debt situation is assessed and hypotheses are tested with the paired *t*-test and nonparametric Wilcoxon so to observe the change in their financial behaviour affected by the crisis. The results presented in Table 3, intend to explain the observed change in the financial condition of the wineries due to the economic crisis.

Before we conducted the paired *t*-test, we have tested the relevant assumptions according to the statistical literature (Risteski and Tevdovski, 2008). The first assumption is about the type of variables included, which should be continuous, i.e. interval or ratio data. The second assumption considers that the dependent variable consists of either two categorical groups, two 'related groups' or two 'matched pairs'. The third assumption is that, there should be no significant outliers in the differences between the two related groups since they can affect the statistical significance of the test. The final assumption is about normality of the distribution of the differences in the dependent variable between the two related groups, which was tested with Shapiro-Wilk test in Stata 12.0 (StataCorp, 2011) (Appendix 4).

If any of these four assumptions is not met, the data cannot be analysed with the paired *t*-test. The first two assumptions cannot be statistically tested, hence we used subjective norms to decide whether they are met. They are related to study design and choice of variables. The data are financial ratios, thus the first assumption is satisfied. The assumption that presumes the sample has no significant outliers in the differences between the two related groups is tested with the Q-Q plot. The observed outliers were removed so to meet the third assumption. Otherwise, we run the nonparametric Wilcoxon matched-pairs-ranks test so to examine the equality of matched pairs of observations for those variables with too many outliers.

The results from the paired t-test and the Wilcoxon signed-rank test revealed different outcome for a different financial performance ratio (Table 3).

The least differences are observed among the profitability indicators. In fact, the gross profit margin remains almost unchanged before (0.14 ± 0.21) and after the financial crisis (0.14 ± 0.17), and this minor increase in the ratio of 0.004 is not statistically significant (95% CI, -0.12154 to 0.12998), $t(7)=0.0792$, $p>.05$. However, the average net profit margin increased after the crisis period (0.22 ± 0.30), and there are more variations between wineries in the post-crisis period rather than in the pre-crisis period (0.08 ± 0.10). In addition, the results from the Wilcoxon signed-rank test also seem to indicate an increase in the net-

profit margin after the crisis period compared with that before the crisis (average rank of 30 vs. average rank of 15), but the observed difference is not statistically significant. Similar observations are recorded for the return on assets (ROA) and the return on equity (ROE). The average ROA slightly increased after the crisis period (0.07 ± 0.07), compared with that of the pre-crisis period (0.05 ± 0.06); but the Wilcoxon signed-rank test shows an insignificant increase of the ROA after the crisis period (an average rank of 19 vs. average rank of 17). There was an increase in the ROE as well after the crisis period (0.13 ± 0.13) compared with that before the financial crisis (0.08 ± 0.10), but according to Wilcoxon signed-rank test, this increase in profitability (from an average rank of 16 to an average rank of 29) is statistically insignificant. Thus, for all profitability ratios, we can accept the null hypothesis that there is no observed change in the profitability of the wineries before and after the financial crisis.

Table 3. Descriptive statistics, paired *t*-test and Wilcoxon signed-rank results for each financial indicator of the wineries

Group of indicators		**2008=Pre-crisis period**		**2010=Post-crisis period**			**Paired *t*-test**			**Wilcoxon signed-rank test**	
		M	SD	M	SD	n	95% CI for Mean Difference	T	df	Adjusted variance	Z
Profitability indicators	GPM	0.14	0.21	0.14	0.17	8	-0.12; 0.13	0.08	7	-	-
	NPM	0.08	0.10	0.22	0.30	9	-	-	-	71.3	0.89
	ROA	0.05	0.06	0.07	0.07	8	-	-	-	51.0	0.14
	ROE	0.08	0.10	0.13	0.13	9	-	-	-	71.3	0.77
Liquidity indicators	CLR	1.58	1.23	2.82	2.75	8	-	-	-	51.0	1.12
	QLR	0.69	0.71	1.62	2.32	9	-	-	-	71.3	0.77
Activity indicators	AT	0.36	0.21	0.25	0.13	8	-0.22; -0.02	-2.75*	7	-	-
	RT	2.85	2.90	1.74	0.79	8	-	-	-	51.0	-0.56
	APP	189.0	114	237.7	145.3	8	-	-	-	71.3	0.53
	DI	218.5	136	344.5	214.7	7	-31.23; 283.3	1.96	6	-	-
	IT	1.31	0.90	0.86	0.55	8	-1.09; 0.18	-1.68	7	-	-
Debt indicators	DR	0.57	0.25	0.46	0.18	8	-0.29; -0.07	-1.45	7	-	-
	DTER	2.22	1.93	1.14	1.00	8	-	-	-	51.0	-1.54

* $p < .05$, the null hypothesis of that no change was observed before and after the crisis is rejected.

Macedonian wineries increased their liquidity during the post-crisis period. In fact, if the inventory is not accounted in the total assets, the wineries seem to be non-liquid before the crisis (0.69 ± 0.71), but liquid after the crisis (1.62 ± 2.32). However, the Wilcoxon signed-rank test shows that this increase in both current and quick liquidity ratios is not statistically significant. The observed increase may be due to the large increase in the liquidity of certain wineries, while others are not liquid. In general, for the both liquidity ratios, we can accept the null hypothesis that there is no change observed in the liquidity of wineries before and after the financial crisis.

In regards to activity performance of the wineries, a different situation is observed. The paired *t*-test run for eight wineries shows that the turnover of assets after the financial crisis slightly decreased as opposed to the pre-crisis period (from 0.36 ± 0.21 in 2008 to 0.25 ± 0.13 in 2010), and this decrease of -0.11586 is statistically significant (95% CI, -0.21544 to -0.01630), $t(7)=-2.7518$, $p<.05$. Thus we can reject the null hypothesis that no change was observed in the assets turnover before and after the crisis.

A decrease is observed in other turnover indicators during the post-crisis period, such as the receivables and the inventory turnover ratios, which is not statistically significant. Quite the opposite situation is observed for the average payment period and the days in inventory ratios that increased after the crisis period, however, this change is not statistically significant. Thus, for all activity ratios except for the assets turnover, we can accept the null hypothesis that no change was observed in the activity performance of the wineries before and after the crisis period.

The wineries decreased their level of debt after the financial crisis period; the debt-to-assets ratio decreased from 0.57±0.25 to 0.46±0.18, and the debt-to-equity ratio from 2.22±1.93 to 1.14±1.00. However, there are no statistically significant evidences for the difference between these increases. Thus, we can accept the null hypothesis that no change was observed in the debt structure of the wineries before and after the crisis period.

CONCLUSIONS

The global financial crisis posed significant challenges to the agri-food sector in other post-communist economies, but our results show that the Macedonian agri-food sector was quite stable, and generally flexible to cope with the crisis effects. The listed Macedonian agri-food companies were less responsive to movements in the market exhibiting low stock returns, and the unlisted agri-food companies were not financially vulnerable due to crisis. In fact, there was not a statistically significant change in the financial performance of the Macedonian wineries before and after the crisis, except that they decreased their volume of production.

The Macedonian economy was not heavily affected by the crisis so this could be a reasonable explanation on the observed financial stability of the agri-food sector. However, investments were hindered during this period. The agri-food companies did not take excessive risks during the crisis period. Revisiting portfolio theory by Elton et al. (2007) which asserts that investors can expect to receive a return only for holding systematic risk, and given that the majority of cases in the sample had lower returns than the market in the crisis period, it may be deduced that the companies have felt uncertainty about investing. Their unwillingness to invest during a period of market instability is reasonable, because greater risk is not only associated with a greater return, but also with a greater loss.

A decade after the economic crisis of 2008–2010, economic growth remains below the potential of the Republic of Macedonia. Trade is the largest activity and is one of the main drivers of economic growth, however the country has high trade deficit. Despite the substantial foreign trade deficits, the inflation rate has consistently been low. The country has been successful in attracting some FDIs, but backward linkages to drive the development of the agri-food sector are missing.

Further economic reform and development are hindered by a certain level of exhaustion in the EU integration process and national political instability since early 2015. For instance, innovations in the agri-food sector remain low. There are no innovative financing models that relate to the agri-food sector demand. Loans continue to account for the largest part of the formal capital structure in the agri-food sector. Marketing strategies are poorly reached and executed. Agri-food companies need to differentiate themselves in order to sustain profitable. Most export volume is produced by large agri-food companies that add value to production. Increasing branding and marketing differentiation may be a good competitive strategy. Due to their small size, the agri-food companies have difficulty competing in export markets because of inefficiencies and high costs related to customs, logistics, and trade infrastructure. In addition, producer prices are relatively high in comparison with the European Union's (EU) (Erjavec and Salputra, 2012). One way for these companies to reach an economic convergence with the rest of the EU is to introduce innovations. However, they do not invest sufficiently in distinguished quality products and brand equity.

It is evident that many past and future challenges hinder the Macedonian economic growth. Considering that the agri-food sector is a very important part of the Macedonian economy, its development may secure the overall national economy. Since investors expect to receive a greater return only for holding systematic risk, understanding the sensitivity of agri-food companies during fragile periods, such it is the financial crisis, creates basis for identification of future strategic decisions for this sector.

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URBAN COMMUNITY AND ALLOTMENT GARDENS: RESEARCH TRENDS AND A LOOK AHEAD

SUMMARY

Growing concerns about rapid urbanization and food insecurity in cities worldwide have renewed interest of academic community in urban gardening and urban agriculture. Urban gardens, including community and allotment gardens, are places of local food production that bring many other benefits to local communities, such as improved health, social networking and cohesion, community development and citizen activation. The paper attempts to comprehensively review the extensive body of literature on urban gardens by looking into geographic locations, disciplines and methods applied in each of the examined papers. The aim of the review is to identify gaps in existing knowledge and to suggest research directions for the future. A total of 27 academic articles in English published between 2000 and 2017 have been reviewed. The results of the data analysis point to uneven geographic distribution of research papers on urban gardening, having large body of literature coping with the North American and Western European context. Fields of research are diverse and fall into categories of planning and social studies (37%), health and community development (26%), politics and citizen participation (26%), and food production (11%). The findings recommend future more rigorous research on quantities of food production, effects of urban gardening on health and nutrition, and social aspects of community and allotment gardens. There is also a need to expand geographic scope of the research beyond the cities in developed countries in order to enrich our understanding of urban gardening in various contexts.

Keywords: community gardens, allotment gardens, local food production, literature review.

INTRODUCTION

There is a growing interest of scientific and academic community in political, social, economic and ecological aspects of urban gardening. Relevant papers are published in scientific journals ranging from geography and planning to health (Guitart et al., 2012). Many authors relate the expansion of interest in urban gardening to rapid urbanization and food insecurity issues worldwide (Guitart et al., 2012; Turner & Henryks, 2012; Eigenbrod & Gruda, 2015).

Urban gardens are form of urban agriculture. Depending on the local context, management methods and available land they can be organized as community gardens, allotment gardens, home gardens, gardens within schools

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and kindergartens, hospitals, prisons, gardens on abandoned public land (Turner & Henryks, 2012). Such open green urban spaces, planned for urban agriculture, are multifunctional areas that support ecological functions of the urban system, food production, provide opportunities for recreation, environmental education, and spiritual development (Langemeyer et al., 2016).

Drescher et al. (2006) distinguish three categories of urban gardens: home gardens, allotment gardens and community gardens. Home gardens, also referred to as domestic gardens, are typically located near the place of residence and are cultivated by individuals or households. Allotment gardens consist of separate parcels of land assigned to individuals or households for personal use (Drescher et al., 2006). The parcel size in German allotments ranges from 200 to 400 m² and often includes a shed for tools and shelter (Holmer & Drescher, 2005). Plots are cultivated individually, but gardeners are organized in allotment associations which are in charge of the land lease and setting up of the common rules for the management and functioning of the gardens. Gardeners pay small membership fee to the association (Holmer & Drescher, 2005).

There are many similarities between allotment and community gardens, though community gardens are more diverse in terms of design, organization and management (Turner & Henryks, 2012). Community gardens are cultivated collectively by a group of individuals or households who produce fresh vegetables and fruits primarily for self-consumption (Drescher et al., 2006; Wang et al., 2014; Martin et al., 2017). Glover (2003) defines community gardens as organized initiatives whereby parts of land are used for the production of food or flowers in the urban environment for the individual or collective benefit, while members share resources, such as space, tools, water (cited in Pitt, 2013). Some authors point out that gardens are community spaces that are used and managed jointly by members of the local communities (Guitart et al., 2012, Turner & Henryks, 2012). Although community gardens may contain individual plots that are used privately, the focus is on the collectively shared space. This way community gardens are more public and democratic places than allotments (Firth et al., 2011 cited in Pitt, 2013; Bell et al., 2016), in which “community spirit” is recreated through collective effort and investment in the shared space (Teig et al., 2009). Community gardens are often associated with temporary use of the urban land which has not yet been brought to its final use (Bell et al., 2016).

Historical development of allotment and community gardens is closely linked to the poor quality of living and food insecurity in the times of crises. Origins of the allotment gardens in Europe are traced back to the mid-1800s, whereas in the USA those gardens emerged during the period of industrialization that brought large numbers of people into cities to work and live in poor conditions (Turner & Henryks, 2012). Community and allotment gardens have played significant roles in ensuring food security in times of economic and humanitarian crisis (Turner & Henryks, 2012; Bell & Keshavarz, 2016). Victory Gardens provided 40% of the fresh vegetables consumed in the USA during the World War II. The number of the gardens was estimated to 20 million (Turner & Henryks, 2012). Similar reaction to crisis occurred during the Great Depression

and in the 1970s Oil Crisis in the USA (Turner & Henryks, 2012). Bell et al. (2016) remind us that a similar economic crisis is taking place in several European countries and that it is no coincidence that various types of urban gardens have been emerging in Greece, Spain, Portugal and Cyprus since 2008.

Given the re-emerging importance of community and allotment gardens in the contemporary city and the growing academic interest for the topic, this paper attempts to comprehensively review the existing literature on urban gardens. There have been a few important literature reviews on the topics of urban gardens and urban food production so far (Guitart et al., 2012; Eigenbrod & Gruda, 2015; Poulsen et al., 2015). The aim of this research is to extend the knowledge that has previously been systematized by specifically looking into: 1) fields of research, 2) locations of the gardens investigated in the research, 3) methods applied, 4) motivations of the gardeners, 5) benefits of the gardens, and 6) constraints on urban garden development. The purpose of the review is to identify gaps in existing knowledge and to suggest future research directions in reference to variables assessed in the review.

MATERIALS AND METHODS

The paper presents a review of those academic articles on community and allotment gardens published in English between 2000 and 2017 that have been identified and selected via searches of electronic database of Science Direct. Search of articles included combination of primary keywords “community gardens” and “allotment gardens” with secondary keywords “food production”, “urban gardening”, “motivations”. Only articles that contained keywords “community garden” or “allotment garden” in the title were selected for the review. Furthermore, the search was extended by including relevant referenced literature from the selected articles. The review excluded articles on gardens within schools, kindergartens, hospitals, prisons. It also excluded papers focusing on ecological aspects of urban gardens as this was beyond the scope of the research. The paper focuses on social, institutional and economic aspects of urban gardening.

A total of 27 research papers were found and reviewed (Table 1). Relevant data were collected and systematically organized in tables. The author performed descriptive statistics to analyze geographic and disciplinary scope of the selected articles, methods applied, gardeners’ motivations, benefits and constraints on urban garden development.

For each article, the author identified and recorded the following information: name of the journal in which the article was published, names of the authors and the year of publication, research discipline and general focus of the research, location of the gardens and methods used in the research (Table 1). Other relevant information collected from the reviewed articles included motivations for gardening, benefits and constraints on development of community and allotment gardens, knowledge gap in the existing literature and possible directions for the future research.

RESULTS AND DISCUSSION

The focus of the research is on the social and economic aspects of community and allotment gardens, including several major research directions: food security and food production, health and nutrition, community development and citizen participation, institutional framework and garden governance.

The largest part of the examined articles refers to urban gardening in the USA cities (52%). Interestingly, 36% of those studies is performed in the low income neighborhoods in the post-industrial cities of New Orleans, Baltimore, Flint, Milwaukee, with disadvantaged communities involved in urban gardening. Research articles about urban gardens in Europe make 37% of the reviewed papers. There is one research paper from Australia and one from Israel.

This review confirms the findings of some authors that literature on community and allotment gardens is geographically limited (Guitart et al., 2012; Spilková & Vágner, 2016) with papers from the USA, Western Europe, Canada and Australia being the most numerous (Borčić et al., 2016). What also becomes evident via analysis of the geographic distribution of the studies is the lack of scientific articles from developing countries. The fact that the context of developing countries is missing from the articles that have been identified and reviewed in this paper may imply limitations of the terminology used in the search of the literature database. “Community gardens” and “allotment gardens” are expressions that typically apply in the context of the North American and Western European cities. In research papers on urban gardening in developing countries there is a preference for the term of “urban agriculture” to characterize similar initiatives (Ernwein, 2014). This may also be linked with the distinction often made between the character of urban gardens in developing and developed countries. In developing countries, urban gardens play significant role in nutrition and food security of urban population, whereby poverty and basic existential needs are main drivers of urban agriculture (Holmer & Drescher, 2005; Eigenbrod & Gruda, 2015; Poulsen et al., 2015). Community and allotment gardens in developed countries, apart from food production, may take on an important role in providing recreational opportunities and educational functions, as well as providing place for social interaction and cohesion (Dubbeling et al., 2010, cited in Eigenbrod & Gruda, 2015).

A variety of disciplines cope with the subject of community and allotment gardens. The reviewed articles are clustered in four groups based on the topic of research and the disciplinary focus of the journals in which papers are published (Table 1).

Most of the selected articles could broadly be placed within the domain of planning and social studies (37%). These studies include research of gardeners’ motivations and benefits of gardening (Middle et al., 2014; Church et al., 2015; Da Silva et al., 2016), food security (Corrigan, 2011; Wang et al., 2014), education (Bendt et al., 2013), aesthetics and beautification of green areas (Morckel, 2015), and garden management strategies (Drake & Lawson, 2015). They provide possible implications for urban planning and policy based on the examined social and spatial criteria.

Articles that cope with community gardens within domain of politics and citizen participation make 26% of the examined papers. Some of the issues that are addressed in these articles are importance of social networking in grassroots garden development (Ghose & Pettygrove, 2014), placemaking and civic engagement (Filkobski et al., 2016; Passidomo, 2016), meanings and functions of gardens in historically different social and political settings (Borčić et al., 2016), top-down and bottom-up garden governance (Fox-Kämper et al., 2017).

An important number of authors have examined the role of community gardens in health promotion and community development (26%). These articles investigate influence of community gardens on healthy lifestyles (Teig et al., 2009; Castro et al., 2013), therapeutic effects of urban gardening (Hale et al., 2011; Pitt, 2014), fruit and vegetable intake among urban gardeners (Alaimo et al., 2008; McCormack et al., 2010).

Only a few authors have investigated and quantified the amount of food produced in community and allotment gardens (Algert et al., 2014; Pourias et al., 2015; Martin et al., 2017). Most of the selected papers have applied qualitative research methods using in-depth interviews and observations as main research techniques. Eight papers used quantitative data. Only three articles combined quantitative and qualitative data in the research (Table 1).

Table 1: Collected data on reviewed papers

	Authors (year)	**Journal**	**Location**	**Field of research**	**Method**
1	Armstrong, D., (2000)	Health and Place	New York, USA	health and community development	qual
2	Ghose, R., & Pettygrove, M. (2014)	Geoforum	Milwaukee, USA	politics and citizen participation	qual
3	Wang et al. (2014)	Applied Geography	Edmonton, Canada	planning and social studies	quant
4	Da Silva et al. (2016)	Urban Forestry & Urban Greening	Vila Nova de Gaia, Portugal	planning and social studies	qual
5	Bendt et al. (2013)	Landscape and Urban Planning	Berlin, Germany	planning and social studies	qual
6	Teig et al. (2009)	Health and Place	Denver, USA	health and community development	qual
7	Passidomo, C. (2016)	Urban Forestry & Urban Greening	New Orleans, USA	politics and citizen participation	qual
8	Martin et al. (2017)	Appetite	Marseille, France	food production	qual & quant
9	Filkobski et al. (2016)	Urban Forestry & Urban Greening	Israel	politics and citizen participation	qual
10	Morckel, V. (2015)	Urban Forestry & Urban Greening	Columbus, USA	planning and social studies	quant
11	Hale et al. (2011)	Social Science & Medicine	Denver, USA	planning and social studies	qual
12	Borčić et al. (2016)	Geoforum	Zagreb, Croatia	politics and citizen participation	qual
13	Ernwein, M. (2014)	Geoforum	Geneva, Switzerland	politics and citizen participation	qual

14	Alaimo et al. (2008)	Journal of Nutrition Education and Behavior	Michigan, USA	health and community development	quant
15	Castro et al. (2013)	American Journal of Preventive Medicine	USA	health and community development	quant
16	Corrigan, M. P. (2011)	Applied Geography	Baltimore, USA	planning and social studies	qual
17	Church et al. (2015)	Ecological Economics	Europe	planning and social studies	quant
18	Middle et al. (2014)	Urban Forestry & Urban Greening	Perth, Australia	planning and social studies	qual
19	Pudup, M. B. (2008)	Geoforum	San Francisko, USA	politics and citizen participation	qual
20	Pourias et al. (2015)	Journal of Agriculture, Food Systems, and Community Development	Paris, France Montreal, Canada	food production	qual & quant
21	Drake, L. & Lawson, L. J. (2015)	Agriculture and Human Values	USA and Canada	planning and social studies	qual & quant
22	McCormack et al. (2010)	Journal of the American Dietetic Association	USA	health and community development	qual
23	Krusky et al. (2015)	Landscape and Urban Planning	Michigan, USA	planning and social studies	quant
24	Spilková, J. & Vágner, J. (2016)	Land Use Policy	Prague, Czechia	planning and social studies	quant
25	Pitt, H. (2014)	Health &Place	Wales, UK	health and community development	qual
26	Fox-Kämper et al. (2017)	Landscape and Urban Planning	developed countries	politics and citizen participation	qual
27	Algert et al. (2014)	Journal of the Academy of Nutrition and Dietetics	San Jose, USA	food production	quant

In several articles authors have investigated gardeners' motivations and have identified benefits of gardening. Some of the most mentioned reasons for gardening are access to fresh and organic food (Wang et al., 2014; Da Silva et al., 2016), supplementing family budget especially in times of economic hardship (Church et al., 2015; Da Silva et al., 2016), enjoying nature (Wang et al., 2014; Martin et al, 2017), health (Wang et al., 2014), social networking (Teig et al., 2009; Martin et al., 2017), pleasure and enjoyment (Martin et al., 2017), relaxation and stress relief (Corrigan, 2011; Pitt, 2014; Filkobski et al., 2016), education (Da Silva et al., 2016), physical exercise (Da Silva et al., 2016), connecting with the soil and watching plants grow (Martin et al., 2017). Benefits of gardening often correspond to reasons why people garden. Urban gardens are important for health promotion through increased physical activity, improved

nutrition and mental health (Armstrong, 2000; Teig et al., 2009), for community development and citizen activation (Armstrong, 2000; Borčić et al., 2016; Passidomo, 2016), for recreation (Ghose & Pettygrove, 2014), and food production (Church et al., 2015; Pourias et al., 2015). Most of the examined articles address the constraints on development of community and allotment gardens. At the institutional scale, major challenges are access to land and land tenure (Armstrong, 2000) and lack of official documents and strategies that integrate gardens into urban planning (Borčić et al., 2016). At the level of garden governance and day-to-day management, the most common challenges are funding, supply of resources including water, compost and manpower and discontinuity in participation (Drake & Lawson, 2015; Filkobski et al., 2016).

Finally, nearly all of the selected articles have given recommendations for future research directions to expand the knowledge related to their field of study. Identified gaps in literature include research on integration of community and allotment gardens into formal green space planning (Middle et al., 2014), quantity of food productions and assessment of household cost savings realized through the practice of gardening (Algert et al., 2014; Pourias et al., 2015), qualitative and quantitative research on day-to-day garden management (Drake & Lawson, 2015), effects of urban gardens on public health (Armstrong, 2000; Hale et al., 2011), gardeners' motivations prior engagement in gardening and motivations arising from the experience of gardening (Da Silva et al., 2016).

CONCLUSIONS

Urban community and allotment gardens are becoming increasingly present in cities of industrialized countries. They have received a great deal of attention in the research of various disciplines. The purpose of this paper was to review the existing literature, to categorize the selected articles according to the geographic scope, discipline and method applied in the research and to identify gaps in the knowledge.

The author acknowledges the limitations of the methods used in the review. Namely, selection of only one database for literature searches as well as limited use of search keywords increase the chances of excluding some of the relevant papers from the research and may lead to reduced validity of conclusions. Adding other relevant keywords in the search of database would probably lead to inclusion of research papers from developing countries. The author has tried to tackle these shortcomings by including relevant referenced literature from the selected articles into discussion of the results to verify the findings.

The review has confirmed that there is a vast body of literature on community gardens in the post-industrialized cities in the USA, as previously reported by Guitart et al. (2012) in their important review on urban community gardens. To increase our knowledge of benefits of urban gardening and constraints on development of community and allotment gardens, the future research should geographically expand beyond the context of developed

countries. This would enrich our understanding of social, economic and political aspects of gardening.

Further research is recommended on the methods of integration of urban gardens into official planning strategies. Urban planning and decision making depends on systematic, consistent and valid data. This is why reliable and methodical knowledge of food production, health and social impacts of urban gardening is needed in the future.

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USE OF MPSIAC AND EPM TO ESTIMATE SEDIMENT YIELD AND EROSION - A CASE STUDY OF A WATERSHED OF THE SECOND URBAN PHASE, MASHHAD, KHORASAN PROVINCE

SUMMARY

The study area of Mashhad-Chenaran, measuring 223989.7 acres, is the largest and most important subbasin of Kashafrood. This area consists mostly of mountains and plains with variable slopes. The study area is an uneven land type and thus prone to soil erosion. Various practical methods have been developed to study soil erosion both qualitatively and quantitatively, but most of them do not accurately process information regarding soil erosion. Therefore, it is essential to confirm the credibility of these methods by investigating the results yielded by examinations compared with measured quantities taken from watersheds of Iran. The importance of the practical role of soil maps in evaluating erosion and sedimentation must also be considered. In this study, both MPSIAC and EPM were used to estimate erosion and sediment yield. Sediment measuring stations showed a rate of 2.74 t/ha per year; however, the MPSIAC model showed a rate of 1.56 t/ha per year and the EPM model showed a rate of 5.73 t/ha per year. Both the EPM and MPSIAC models were created in countries with climates and geology attributes that differ from those of Iran. Hence, the coefficients and factors affecting erosion do not correspond precisely to the conditions in Iran.

Keywords: Erosion, Gavrilovic method (EPM), MPSIAC, Qualitatively, Quantitatively, Sediment.

INTRODUCTION

The role of erosion and sediment yield in reducing soil fertility; soil waste; the filling of reservoirs; the obstruction of irrigation channels, streams, and rivers and the worsening of their states; and the contamination of downstream waters is undeniable. To prevent and reduce these consequences, soil, watershed, and sediment control measures are essential (Hakimkhani, 2002).

Today in most developing countries, population growth, the imbalance in the ratio of livestock to pastures, overuse of pastures and forests, and the unprincipled exploitation of forests, pastures, and farms have resulted in

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irreparable negative environmental, economic, and social consequences. Facts and figures attest to the critical state of Iran's water for reasons mentioned above. There has been a significant increase in the quantity and severity of flood and soil erosion in Iran since 1952 (Arekhi and Nazari, 2008; Ghodoosi, 2002).

To implement protection programs, determine measures to remedy problems in erosion and sediment control, and calculate and design the capacity of dams in reservoir construction, the annual mass of sediment yield in a watershed must be evaluated. If the statistics on the water flow rate and sediment yield of a watershed are available, the annual sediment yield can be calculated through statistical methods. The lack of such information about most of Iran's watersheds, however, calls for the implementation of suitable practical methods for estimating soil erosion and sediment yield (Refahi, 1999). The purpose of identifying soil hydrologic units is to determine runoff and probability of flooding in an area (Ahmadi, 2009).

The difficulties inherent in soil protection and sediment yield prevention, the lack of suitable equipment to estimate sedimentation, the incompatible statistics about most watersheds, and the adaptive methods implemented in Iran have resulted in a lack of appropriate, logical, and expected results in erosion and detriment evaluation. The MPSIAC and EPM models are two practical methods for evaluating erosion and sedimentation that have caught the attention of Iranian researchers. However, there have been no definite results about the accuracy of these models, only contradictory ones (Malekian, 2012).

Choosing a suitable model for determining erosion and sedimentation and calculating and drawing maps will yield important information that can be used in the management of renewable natural resources, soil conservation measures, dam designing, channel reservoirs, and land use projects. The soil erosion phenomenon is related to natural factors (morphology, soil type, climate, vegetation in the area, and human activities) (Hessel and Jetten, 2007; Vrieling et al., 2009). These factors include plowing, overgrazing, unsystematic farming, general overuse, and unprincipled management of the lands (Barovic et al., 2015).

According to the findings of some researchers (Tazioli, 2009; Tazioli et al., 2015), it is essential to have the effective load of sediment for several consecutive years to calibrate the erosion potential model. Modeling is a stable useful tool for estimating the erosion and discharge in a watershed with no available hydrometric information. Mathematical methods have been developed to predict erosion and sedimentation (Tazioli et al., 2015).

In a study of sedimentation in watersheds, Devenet et al. (2005) found that topographic data and satellite images in addition to prediction models are needed to achieve a relatively accurate determination of sediment yield and more executable results. Tangestani (2006) compared maps of sediment yield estimated by EPM and MPSIAC models and concluded that the MPSIAC model is more accurate.

To calculate the potential sediment entering Breggia-Greggio delta system in Italy, Fanetti and Vezzoli (2007) made numerical and practical estimations in their study area. Tazioli (2009) used EPM to estimate the sedimentation in a semi-arid area and believed that the EPM method in GIS yielded better results than MPSIAC. Milevski (2008) studied the risks of soil erosion in the Bregalinica watershed in Macedonia using satellite images, EPM, and GIS and concluded that GIS is a very valuable tool for predicting the potential risks of soil erosion.

On studies done on the Tangkonesht watershed in northern Kermanshah state, which has a humid climate and vegetation cover varying from 25% to 55%, Rastgou et al. (2006) achieved more accurate results using the MPSIAC method than with EPM.

Mohseni et al. (2011) studied the accuracy of EPM, MPSIAC, Geomorphology, and Hydrophysic models in estimating erosion and sediment yield at the Kasilian watershed in Mazandaran state. They found that the Geomorphology model was more suitable than the other models. The results of studies done by Moradi et al. (2011) on the Pourahmadi watershed in Hormozgan state showed that in some subbasins, the estimated erosion in high erosion areas was lower compared to the MPSIAC model.

Considering that the EPM method is implemented to estimate erosion potential in a watershed, the results achieved using this method in high erosion areas are less accurate (Moradi et al., 2011). Researchers such as Ahmadi et al. (2011), Amiri (2010), and Khodabakhsh et al. (2010) unanimously agree on their findings about the comparison between EPM and MPSIAC models.

By estimating sediment yield in the Forg watershed in southern Khorasan using the MPSIAC model and a modulation of GIS tools, Malekian et al. (2012) found that the results were significantly accurate. Overall, findings from research done inside and outside Iran has shown that a combination of traditional methods and modern tools like GIS and RS are able to satisfy requirements considering the lack of statistical information about most of Iran's watersheds.

Blinkov and Kostadinov (2010) evaluated the applicability of various erosion risk assessment methods for engineering purposes. Factors taken into consideration depended on scale, various erosion tasks, and various sector needs. The erosion potential method (EPM) was, according to them, most suitable on assessing catchment level for the watershed management needs in this region. It was created, developed, and calibrated in Yugoslavia (Gavrilovic, 1988).

This methodology is currently used in Bosnia and Herzegovina, Bulgaria, Croatia, the Czech Republic, Italy, Iran, Montenegro, Macedonia, Serbia, and Slovenia (Spalevic, 2014b; Kostadinov et al., 2014). The use of this methodology in research on runoff and the intensity of soil erosion has been demonstrated in Montenegro, specifically in the region of Polimlje (Spalevic et al., 2014a, 2014b, 2013a, 2013b, 2013c, 2013d, 2013e, 2013f, 2013g, 2012, 2011, 2008, 2007, 2004, 2003, 2001, 2000a, 2000b, 1999a, and 1999b; Fustic and Spalevic, 2000).

The EPM is distinguished by its high degree of reliability in calculating sediment yield as well as reservoir sedimentation (Ristic et al., 2011).

MATERIALS AND METHODS

The study area (Fig. 1) of Mashhad-Chenaran is the biggest and the most important subbasin of Kashaf Rood. It extends 223989.7 acres, with geographical coordinates of $58° - 22'$ to $60° - 7'$ eastern longitude and 36 to $37° - 5'$ northern longitude.

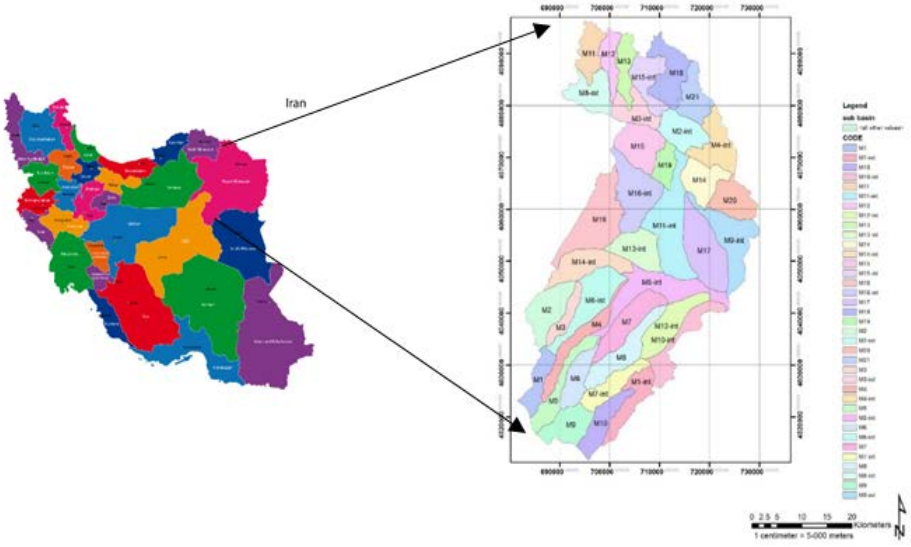

Figure 1: Study Area: Mashhad watershed phase II and intended area location in map of Iran

Mashhad-Chenaran is a relatively steep plain located between the Hezar-Masjed and Binaloud mountains. This plain is rectangular, 120 km in length and 28 km in width, and extends from northeast to southeast between the Hezar-Masjed and Binaloud mountains. The most important population centers of this plain are Mashhad, Chenaran, Shandiz, and Torghabeh. The population density is higher in the southern half of the plain.

Identifying soil hydrologic groups helps in estimating the runoff and flooding potential in an area. To calculate and identify soil hydrologic groups, the following measurements should be performed: soil gravel mass, depth, pores, texture and structure, and type and depth of the limiter layer. The influential factors affecting influx and speed of water in the hydrologic groups were determined based on the USA's soil conservation standards and then were divided into four hydrologic units.

The study area of Mashhad Urban Phase II was separated into 4 hydrologic units and 36 subbasins (Fig. 1, Table 3). Based on the results of recourse assessment and land ability, the area contains 7 main land types: mountains, hills,

plateaus, upper terraces, piedmont plains, flood plains, gravel debris and fan-shaped alluvial gravel, a miscellaneous type, and composite and non-arable lands. Paleozoic formations such as Lalun and Mila can be found in this area, and the middle section includes a quaternary deposit. Diversity of formations is one of the important factors for the constitution of different soil types and different geomorphological forms; hence, it rightfully has an effect on soil erosion and sedimentation.

The rainfall regime in this area is categorized as Mediterranean. The dry season coincides with summer, and the rainy season coincides with the cold season. Due to its low vegetation, this land is threatened by considerable erosion. Average rainfall amounts at the Mashhad station is 250 millimeters. Furthermore, snow in winter is one of the effective factors in reducing soil erosion.

Soil erosion and sedimentation in this study were determined by implementing a modified version of the Pacific Southwest Interagency Committee (PSIAC) (1968), about the evaluation of sediment yield in the southwestern USA that has an arid to semi-arid climate (Hadley, 1984). This model takes into consideration nine factors in erosion and sediment yield (Table 1): surface geology, soil, climate, runoff, topography, ground cover, land use, upland erosion, and channel erosion; because of this, it is widely used in Iran (Refahi, 1999).

Table1: The effective factors and their point's calculation formula in MPSIAC model

The effective factors	**The points calculation formula**	**Explanation Parameter**
Geology	$Y_1=X_1$	X1: stone sensitive point
Soil	$Y_2=16.6K$	K : erodibility factor in USLE
Climate	$Y_3=0.2X_3$	X3 : precipitation intensity with 2 year interval return
Water runoff	$Y4=0.006R+10Q_p$	R : annual runoff depth (mm), Q_p : annual specific discharge (CmS/km^2)
Topography	$Y_5=0.33S$	S : average watershed slope (%)
Land vegetation cover	$Y_6=0.2X_6$	X6 : bare soil (%)
Land use	$Y_7=20-0.2X_7$	X7 : canopy cover (%)
Surface soil erosion	$Y_8=0.25X_8$	X8 : points summation in BLM model
Gully erosion	$Y_9=0.16X_9$	X9 : point of Gully erosion in BLM model
$\mathbf{R= Y_1+Y_2+Y_3+Y_4+Y_5+Y_6+Y_7+Y_7+Y_8+Y_9}$		

The Erosion Potential Method (EPM) was presented in the former Yugoslavia to determine the percentage of soil erosion. This model was introduced in 1988 at an international conference in China. Results showed that by applying EPM, not only could erosion levels in a watershed, but also the

quantity of sediment produced in the subbasin and comparative parts of the land could be determined. Factors affecting soil erosion in this model were: slope, lithology, erosion faces, and land use (Gavrilovic, 1988).

The steps to drawing a map are as follows (Figs. 2 and 3):

With EPM, four characteristics were investigated: the coefficient of watershed erosion, land use coefficient, rock and soil erosion sensitivity coefficient, and the average slope of the land area in units or in networks of the map. Considering that there are several effective layers in the EPM and MPSIAC models that are used to achieve erosion intensity and sediment yield maps, the first step is to overlap these layers. Then, by merging the data, the erosion state of the study area can be determined.

Georeferencing information layers; Matching watershed and subbasin boundaries in different layers;polygoning target units; Converting polygons to raster layers; Super positioning and calculating on raster layers (9 MPSIAC factors (Table 1) and the factors affecting EPM); Obtaining maps of erosion intensity based on the weight of each layer.

RESULTS AND DISCUSSION

The efficiency of the EPM and MPSIAC methods in estimating the soil erosion and sediment yield of phase two of the Mashhad urban watershed was compared. The findings presented in Tables 2 and 3 show that the sediment and erosion estimations done by both models are significantly different from the erosion reported by the hydrometric station.

Table 2: Erosion and sediment yeild of Mashhad second urban phase watershed

Area (Hectare)	Sediment (ton/ha.year)			Erosion (ton/ha.year)	
	Hydrometric station	MPSIAC	EPM	MPSIAC	EPM
223989.7	2.74	1.56	5.73	3.19	9.45

Results of the one sample test also showed that the estimations of both models have noteworthy differences with reports from the hydrometric station. By taking into consideration the incompatible statistics and database in some stations, the sediment rate estimated by the MPSIAC method was determined to be more accurate than that of EPM. The comparison of the estimation by EPM and the actual erosion calculated at the location indicated that EPM overestimates the value of erosion compared to the MPSIAC method (Tables 2 and 3). In the MPSIAC model, more factors are taken into consideration for estimating erosion (Table 1). The values estimated by the MPSIAC model in all subbasins were a great deal smaller compared with the values estimated by the EMP model (Table 2).

Table 3: Erosion and sediment yeild of Mashhad second urban phase watershed

Sub basin	Area (m^2)	Erosion (ton/ha.year)	
		MPSIAC	EPM
M1-int	48811001.09	2.98	7.64
M2-int	53220952.54	7.36	18.80
M3-int	41687233.37	4.36	17.42
M4-int	61814704.09	7.72	9.78
M5-int	95548948.09	1.70	2.16
M1	38031119.77	4.27	12.05
M2	64490828.47	1.80	8.53
M3	27175854.66	2.00	7.16
M6-int	78248369.32	1.51	4.29
M4	51449433.58	2.78	9.28
M5	49677914.63	3.96	12.92
M6	45456422.64	3.70	7.72
M7	66903109.61	1.56	9.02
M8	68146942.07	2.53	9.62
M9	52994801.19	4.62	12.32
M7-int	45305655.07	3.19	10.51
M10	48886384.88	4.10	7.16
M11	41008779.30	4.81	13.20
M8-int	53107876.87	5.55	18.90
M12	34111162.93	5.84	14.70
M13	36862671.10	5.72	16.44
M20	53145568.76	3.48	27.50
M15	65433125.79	4.85	24.56
M9-int	85560596.51	2.98	8.03
M16	111869537.64	1.44	4.55
M10-int	53183260.65	1.95	7.38
M11-int	120576364.86	2.73	5.55
M17	100298126.57	1.74	2.88
M12-int	64415444.69	1.42	4.95
M13-int	57857055.35	1.36	1.47
M14-int	66903109.61	1.41	3.58
M21	48622541.63	3.64	4.92
M15-int	50017141.66	4.25	13.10
M18	67355412.32	3.97	9.29
M16-int	90121315.53	2.23	11.14
M14	52806341.72	6.38	10.43
M19	29022757.41	3.90	10.62

One reason for this significant difference is that EMP predicts the erosion potential of the watershed, and in locations with a high degree of erosion, the accuracy of the model decreases.

When choosing a model, it is important to pay attention to its origins. As previously mentioned, the EPM model was first designed and applied in the former Yugoslavia. Since this method is widely used in erosion and sediment yield estimation in Iran, there must be a high level of similarity between the study area and the location for which the model was designed in order for the model to achieve an accurate estimation. Therefore, if the climate of the study area is different from Yugoslavia, the coefficients must be calibrated based on the climatic differences between the study area and the original location.

The findings of the present study agree with those of studies done by Abedini et al. (2013), Ahmadi et al. (2011), Amiri (2010), and Khodabakhsh et al. (2010) in Iran. The application of GIS and RS would not only reduce the expenses and time compared to traditional methods, it would also increase accuracy in estimating erosion. Therefore, the use of up-to-date satellite images will help increase the accuracy of estimations by a great deal. To use the EPM model under different climatic conditions, it is recommended that the model be calibrated by the climate coefficients of the climate in the study area. In addition, by changing the work-units and making them more homogenous, the results will be more accurate in studying the erosion maps in both models (EPM and MPSIAC).

While choosing a method, it is very important to place significance on its place of origin. Studies done on soil erosion and sediment yield estimation in Iran have worked particularly with the EPM model which, as mentioned before, was designed and demonstrated first in the former Yugoslavia. The former Yugoslavia has been divided into the countries of Bosnia and Herzegovina, Macedonia, Croatia, Montenegro, Serbia, and Slovenia on the west of the Balkan Peninsula, and the area has completely different climatic conditions and vegetation from the study area in the present research.

In the study area, the precipitation follows the Mediterranean regime; summer is the dry season, and rainfall occurs during winter. About 40% of rainfall happens during one month of winter. The temperature of the area is influenced by the air masses and height of the area. The average annual temperature of the Mashhad Urban Watershed Phase II is 10.7 °C and fluctuates between -0.7 °C and 21.7 °C during the year. The average humidity ranges from 51.6% to 37.9% in July and reaches 66.9% in February.

Maximum wind speeds at the synoptic stations of Golmakan and Mashhad are 74 and 45 knots, respectively, and the wind direction is 290° north and 310° north, respectively. The average evapotranspiration calculated at the Zoshk evaporative station using the Penman method is 1356.2 ml. Rainfall in the whole basin equals 305.2 ml. According to hyetograph curves, the humid months are from mid-November to mid-May, and the rest of the year is dry. The climate of

the region is identified as arid/semi-arid to semi-humid by the Domarten method and semi-cold arid to cold arid by the Emberger method.

The dominant vegetation in the research area consists mostly of thorny Astralagus and thorny/unthorny perennial herbaceous bushes. Due to unchanging growth conditions and the lack of diversity of species in the area, a total of 9 vegetation types were found, 3 of which were scattered in the Binaloud heights and the rest in the Hezar-Masjed heights. The Binaloud heights include numerous slopes and valleys within which agriculture and horticulture are common activities. In higher elevations, due to the construction of multiple roads, the access routes of the nomadic tribes to the heights have been either destroyed or covered in vegetation with little to no palatability value.

Unlike the Binaloud heights, there are fewer villages in the Hezar-Masjed heights located in Mashhad Urban Watershed Phase II, and environmental degradation in this area is more significant. The slopes are covered mostly with rocks and sparse vegetation. A relatively deep valley leads the watershed of this area to Mashhad Urban Watershed Phase II, and except for the Moqmenj river valley which is covered in farms, apple orchards, and other agricultural products, vegetation is sparse; lands are even degraded or covered with invasive or inedible plants.

The upper elevations of the Hezar-Masjed heights are generally covered by Astragalus meschedensis, Artemisia kopetdaghensis, Rosa persica, Cousinia adenostegia, Acantholimon erinaceum, Artemisia scoparia, Acanthophyllum sordidum, Acanthophyllum spinosum, and Acantholimon scirpinum. The countries of the former Yugoslavia, despite slight differences, all generally include dense forest vegetation, vast lush meadows, and forests even in the mountainous areas.

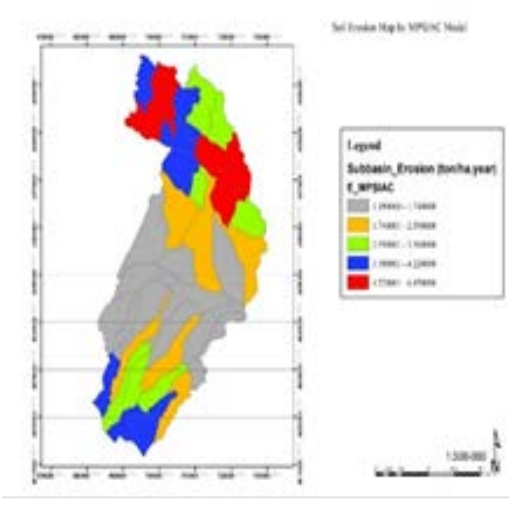

Figure 2: Soil erosion map using (EPM)

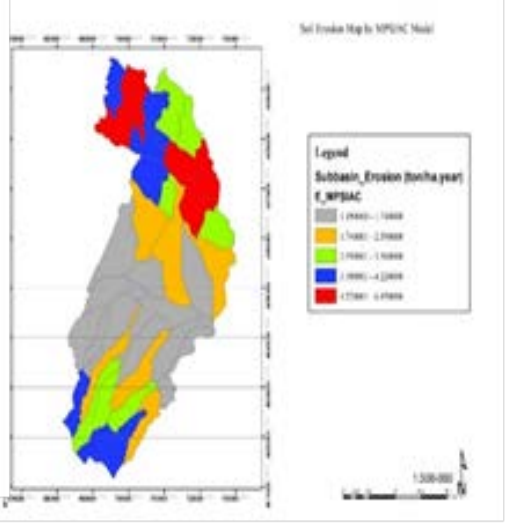

Figure 3: Soil erosion map using MPSIAC model

CONCLUSIONS

As mentioned before, the vast differences between the climatic conditions and vegetation in the study area and the place of origin of the Gavrilovic method (the former Yugoslavia) can explain the Very high estimations of the model in soil erosion and sediment yield estimation in the study area. Based on the findings of this study, models do not have the same results in all lands. Therefore, for selecting a model, there should be the most similarity between the model domain area and the study area. The results showed that the estimates in the Gavrilovich model are higher; it is likely that large rocky outgrowths in a large part of the research area are one of the reasons for the high estimates, which further explains the choice; More research is needed, such as selecting more homogeneous units than hydrological units.

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PHYSIOLOGICAL AND MORPFOLOGICAL CHARACTERISTICS OF ONE-YEAR OLD SEEDLINGS OF COMMERCIAL MEDLAR CULTIVARS *(Mespilus germanica L.)* IN THE REGION OF NORTH MONTENEGRO

SUMMARY

The paper was written based on a study conducted in a nursery in the north of Montenegro during three successive years. The study had helped author of this paper to examine the possibility of producing one-year old seedlings of commercial medlar cultivars (*Mespilus germanica* L.) grafted on quince Ba 29 and wild pear generative rootstocks (*Pyrus communis* L.) during the first year after bud grafting. The aim of this study is to determine morphological characteristics, compatibility of commercial medlar seedling cultivars (Domestic medlar, Pomoravka - seedless medlar genotype, Royal medlar, Medlar without seeds) with the quince Ba 29 and wild pear generative rootstocks and water attaining capability of the leaves in one-year old seedlings of commercial medlar cultivars.

The results of this study also showed that the water attaining capability of the leaves in one-year old seedlings of commercial medlar cultivars *(Mespilus germanica L.)* as an indicator of their resistance to drought was genetic characteristics of the cultivars.

Keywords: Medlar *(Mespilus germanica L.)*, morphological characteristics, one-year old seedlings, resistance to drought, water attaining capability.

INTRODUCTION

Medlar (*Mespilus germanica L.*) belongs to Rosaceae family, and it is called ‘Döngel’ or ‘Beşbıyık’ in Turkey, ‘Ezgil’ in Azerbaijan, ‘Bushmala’ in Georgia and ‘German’ or ‘Germanic Medlar’ in the most of European countries (Anonim.2009.). Its tree is generally 3-5 m tall, but it may reach nearly 8 meters. It is self-fertile and long-lived tree. It lives approximately 30-50 years. There are 100 year old trees in UK as well. The flowers are white-pink and hermaphrodite. Flower buds are formed in May-June, and each bud has one flower. Fruit shape may be variable. Fruits are uneatable during tree maturity stage because of tannin content. It becomes eatable when the skin color becomes chocolate brown. Medlar contains organic acids, sugars, pectin, vitamin C, and small amounts of vitamin A (Korbanova *et al.* 1998.).

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Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

The commercial cultivars of medlar are reproduced by grafting. The best results are achieved by grafting on a sleeping bud. As good rootstocks for grafting, they showed: generative rootstock of medlar (*Mespilus germanica L*) ,sorb apple (*Sorbus domestica L*), a whitethorn (*Crataegus sp*.), waild pear (*Pyrus communis L*) and vegetative rootstock of pear and quince (Quince A, Quince C and Ba 29).

The rapid developments fruit tree nursery technology and rootstock research and introduction of new clonally propagated rootstocks opened in new area in fruit science (Ecisli *et al.* 2006). For this reason more recently modern orchards with different modern training systems to start establish with use of clonal quince (*Cydonia oblonga* L.) rootstocks such as Quince A, Quince C and Ba 29 in Europe. These clonal rootstocks with dwarfing characteristics well reported to increase precocity and fruit quality, especially in the high intensity modern orchard sand thus gained more importance (Lewko *et al.* 2007).

The selection of clonal quince (*C. oblonga*), such as Quince A (MA), Quince C (MC) and BA 29 in Europe, or of clonal *Pyrus communis* L., such as 'Old Home' × 'Farmingdale' (OHF) in the USA or in South Africa, as substitutes for valid pear seedling rootstock, have clearly improved the precocity, productivity and quality of some European cultivars (Ikinci *et al.* 2014).

From year to year, fruit production in Montenegro is more and more difficult without watering. The fertile buds production is extremely reduced in the drought conditions. Since these buds should bring the yield in the next year, dry year indicates low yields in the next one too.

In Dinaric mountains annual rainfall varies from 1.000 l/m² to 5.500 mm/m² in Crkvice (Montenegro) what is the European maximum. Though these regions abound in precipitation, the lack of water appears in the summer period because of porous soils and in some summers because of drought. Traditionally the people who live there collect rain from the roofs. The rainfall potential could be used in more economical way with building accumulations with concrete walls or by finding and protecting autochthonous varieties. In the most fertile valley in Bijelo Polje (Nedakusko-Rasovska ravnica), vegetable production depends on watering. Water capacity of the leaves of plum autochthonous varieties was tested as an indicator of their resistance to drought in Bjelo Polje, Montenegro (Šebek, 2016).

The development of drought resistant cultivars or lines of crops through selection and breeding is of considerable economic value for increasing crop production in areas with low participation or without any proper irrigation system (Subbarao *et al*. 2005). However, availability of genetic variation at intra – varietal level is of prime importance for selection and breeding for enhanced resistance to any stress (Serraj *et al*. 2005). In order to develop drought tolerant cultivars, it is imperative to develop efficient screening and suitable selection criteria. Various agronomic, physiological and biochemical selection criteria for drought tolerance are being used to select drought tolerant plants, such as seed yield, harvest index, shoot fresh and dry weight, leaf water potential, osmotic

adjustment, accumulation of compatible solutes, water use efficiency, stomatal conductance, chlorophyll fluorescence (Araus *et al*. 2002; Richards *et al*. 2002; Flexas *et al*. 2004; Ashraf and Foolad 2007; Tambussi *et al*. 2007).

Development of drought tolerance for a plant is the result of overall expression of many adaptive traits in a specific environment. The adaptive traits can be physiological and morphological, such as: selection of rootstocks resistant to drought, selection of autochthonous cultivars with greater water attaining capabilities, selection of cultivars tolerant to dry conditions, etc. Since many adaptive traits are effective only for certain aspects of drought tolerance and over a limited range of drought stress, there is no single trait that breeders can use to improve productivity of a given crop in a dry environment. In this context, Subbarao *et al*. (2005) suggested that those traits, whether physiological or morphological, that contribute to check water loss through transpiration, and enhance water use efficiency and yield are traits of interest. However, priority should be given to those traits that will maintain or increase yield stability in addition to overall yield, because traits for higher yield may in fact decrease yield stability (e.g. longer growth period). Thus, in order to improve crop productivity under drought stress conditions, selection of cultivars with short life span (drought escape), incorporation of traits responsible for well – developed root system, high stomatal resistance, high water use efficiency (drought avoidance) represent the traits responsible for increasing and stabilizing yield during drought stress period (drought tolerance).

Drought stress is highly variable in its timing, duration and severity, and this result in high environmental variation and G×E variation (Witcombe *et al*. 2005).

Many studies interactions rootstock - scion show that the rootstock controls the overall growth, while scions affects the number and type of shoots (Ferree *et al*., 2001a, b) as well as the number of buds that will become a flower (Hirst and Ferree, 1995).

Scion has a greater impact on the rootstock at a monthly growth rate of trees (Tworkoski *et al*., 2007). Dwarfing apple rootstocks M9 combined with different scions consistently has the lowest, and seedling rootstocks of *Malus sylvestris* has the highest vegetative growth and tree trunk corpulence (Tworkoski *et al*., 2007). Although the rootstock is used to control the size of the tree, the mechanism that is responsible and which is closely related to the action of the growth of the tree is still unclear (Atkinson *et al*., 2001). The main difficulty in determining the influence of the rootstocks are connected with the fact that these cumulative effects of variations in the development of seedlings overlap from year to year (Barritt *et al*., 1995; Ferree *et al*., 1995).

Nursery material of high quality is the basic of intensive fruit growing (Baryla and Kaplan 2006). Namely, modern pear orchards are planted at at 2000 - 5000 trees per ha $^{-1}$ on under Hight Density Planting (HDP), if it is grafted on dwarf or semi-dwarf quince rootstocks, yielding at least 40-50 t per ha^{-1} (Wertheim, 2002). Intensive pear orchards are based on the concept of high

density planting, training systems of low tree height and high productivity on the basis of the unit area (ha or m²). However, quince is graft incompatible with some of the major pear cultivars such as Bartlett (Tukey, 1978; Hartmann et al., 1997). Vegetative rootstocks that are used for production of pear , quince and medlar seedlings are Anžerska quince MA and Provencal Ba 29 (Stančević et al., 1993). There are other Quince rootstocks in use such as 'Adams', 'Ba29' and the more recently introduced 'EMH' and 'Eline' and it must be assumed for the sake of caution that the incompatibilities mentioned here are probably expressed in some degree in all such Quince rootstock forms. This paper uses practical experience from the conducted study and also literature related to the compatibility of commercial cultivars of medlar with quince rootstocks clone Ba 29 and wild pear generative rootstocks (*Pyrus communis L*).

Quince is graft incompatible with some of the major pear cultivars such as Bartlett (Tukey, 1978; Hartmann et al., 1997). The study showed that the incompatibility of pear seedling sorts with the quince Ba 29 and wild pear seedlings expresses itself in several ways. Firstly, a poor 'bud' or 'graft' that was taken from the nursery would be evidently of not sufficient quality; secondly, even if growing is successful in the nursery, very often 'lifted' breakages (brittle unions) could occur between the rootstock and the scion; and thirdly, 'delayed' incompatibility may occur on a place where the union between the rootstock and the scion breaks suddenly in later years. The last process is unpredictable and can often happen when there is a heavy crop load assisted by strong autumnal winds.

MATERIALS AND METHODS

The material of this study were commercial cultivars of medlar: 'Domestic medlar', ' Pomoravka - seedless medlar genotype', 'Rojal medlar', and 'Medlar without seed'. These commercial cultivars of medlar are grafted on vegetative rootstocks quince Ba-29. Comparative or control graft was performed on generative rootstock of wild pear (*Pyrus communis L*).

Seedless medlar genotype Pomoravka was found in Pomoravlje, in the close vicinity of Svilajnac (Serbia), 1994. It was grafted and transferred at three locations so as to be protected from deterioration. Apart from being used fresh, its fruit is very interesting for processing industry for making pastes, jelly, mash, liqueur etc., this genotype can be beneficial in the breeding aimed at development of seedless medlar cultivars of satisfactory fruit size. This genotype has a relatively small fruit (8.2 g) but high stone flesh ratio (96.5%) and it is highly qualitative especially when it is overripe. Due to its high using values this genotype should be more propagated especially in view of the fact that the presence of pathogen *Erwinia amylovora* has not been detected on any young tree of this medlar genotype (Nikolić,2005).

The experiment was conducted in the village Njegnjevo in the period from the year 2009 to the year 2012. The nursery was located at Njegnjevo near Bijelo Polje (43º05'N; 19 º05'E), North Montenegro. This is mainly an upland area, with an average altitude of about 320 m, characterized by temperate continental

climate. The nursery soil was typically eutric land on alluvial and colluvial deposits, mildly acid (a pH of 5.41 in the topsoil), with a moderate organic matter (3.88%) and a very low Ntot content (0.18%), the values thereof gradually decreasing with the depth (data not show). The contents of available P2O5 and K2O in the 0-30 cm soil depth were 6,7mg•100g-¹ and 14,07mg•100g-¹, respectively. Fertilization treatments included applications of mineral nitrogen fertilizers at the rate of 80 kg N•ha-¹ prior to growing season and following the cutting of the rootstock above the graft union, i.e. towards the end of March in three seasons.

During the year of 2009, the vegetative and generative rootstocks were cultivated. Seeds of wild pear were collected the year before from local trees, they were cleaned of flesh, dried and stratified in wet sand during the winter of 2008/2009. Wild pear seedlings were cultivated in 2009. The same procedure of producing generative and vegetative rootstocks was repeated two times more in order to have results from three different years. We already knew that generative rootstocks have diverse genetic characteristics but we included them in this project in order to compare them to the vegetative rootstocks. What we are hoping to accomplish in this project is to determine the compatibility between vegetative rootstocks (Ba-29) and commercial cultivars of medlar. In Montenegro, commercial cultivars of medlar were only grafted on generative rootstocks. This fact prevents raising of medlar orchards with intensive production. Budding of sleeping buds was conducted in the autumn (late August) during the years of 2009, 2010 and 2011. Due to poor production results there were other graftings that took place in the spring of years 2010, 2011 and 2012, in which the method of 'English linking' was used. Grafting height is 10 cm from the root collar of wild pear generative rootstock or vegetative rootstock for medlar (quince Ba-29). Scions for grafting were collected in the spring before the abrupt movement of buds and stored in the basement until the proper grafting conditions. Acceptance of grafting was monitored during three years of production. The study (2010 -2012) includes those morphometric characteristics of plants that are used as basic parameters for their classification according to outward, phenotypic characteristics. Determination of seedling growth indicators was done with a sample of of 80 rootstocks. One-year seedling height was measured with a meter. The diameter of seedlings on 3 cm from the seedling grafting point was measured with a micrometer of 0.01 mm precision. The dynamic of evolution of the one-year seedlings (seedling height and thickness) was followed during the growing season treatments: June, July , August and September. The results were analyzed using one-way analysis of variance (statistical program Systat 11) where the middle of treatment compared to the LSD test.

Examples of leaves for analysis were taken when it was dry weather: three times a year – at the end of June, the end of July and the end of August. The dynamics of leaf dehydration per measured interval was determined by method of Eremeev 1964 (cit. according to Šebek,2016). Eremeev's method

(Šebek,2016) is relevant for determination of water attaining capability of leaves. Loss of water at the time of transpiration will be monitored by measuring of the weight of cut leaves. (Slavik,1974 cit. according to Šebek,2016). Level of regained hydration will be monitored after 12h and 16h from cutting the leaves from one-years seedlings of commercial medlar cultivars. The loss of water due to transpiration followed by measuring the weight of leaves (Šebek,2016) The dynamics of leaf dehydration was measured in order to obtain initial resistance rate of commercial medlar cultivars towards drought conditions. The dynamics of leaf dehydration depends on the thickness of leaf cuticle and leaf average size. The results were analyzed using one-way analysis of variance (statistical program Systat 11) where the treatment was compared to the LSD test.

RESULTS AND DISCUSSION

The average acceptance of autumn grafting process in the shape of the 'T' letter of comercial medlar cultivars with quince clone BA 29 seedlings (vegetative rootstocks) had been with the following percent of success: 73.30% ('Domestic medlar'); 90.30% (' Pomoravka - seedless medlar genotype '); 51.67% ('Royal medlar') and 44.00% ('Medlar without seeds').The acceptance of autumn grafting process in the shape of the 'T' letter of comercial medlar cultivars with wild pear seedlings (generative rootstocks) had been with the following percent of success: 89.30% ('Domestic medlar'); 84.00% (' Pomoravka - seedless medlar genotype '); 96.70% ('Royal medlar')and 75.00% ('Medlar without seeds').Due to the results of the grafting process in the shape of the 'T' letter there was a need for repetition of the grafting process (next spring: English linking) for defining causes of low acceptance of seedlings regarding individual sorts and for increasing of production results. When the grafting process was repeated, satisfied results from the aspect of plantation production profitability had been achieved. Achieved percent after the repetition of the grafting process for comercial medlar cultivars with Ba 29 seedlings was: 99.30% ('Domestic medlar''); 99.00% (' Pomoravka - seedless medlar genotype '); 93.67% ('Royal medlar') and 85.67% (' 'Medlar without seeds' ').Achieved percent after the repetition of the grafting process for comercial medlar cultivars with wild pear seedlings (Pyrus communis L) was: 96.30% ('Domestic medlar'); 88.70% (' Pomoravka - seedless medlar genotype '); 99.3% ('Royal medlar'); and 99.00% ('Medlar without seeds'). Growth dynamic of one-year-old seedlings (height and corpulence of the seedlings) was monitored during vegetation in time treatments: June, July and August. Values of the monitored parameters (height and corpulence of the seedlings) showed differences in average values and seedling growth dynamic. By analyzing the data for medlar cultivar 'Domestic medlar'' (rootstocks is quince Ba 29), average height of the seedling in June was 33 cm. In the month of the July average height was 58 cm. For August average height was 94 cm. The average corpulence of the seedlings, 10 cm from the grafting spot, of the same sort was 3.20 mm in June. In July, data for the average corpulence was 5.45 mm. In august, corpulence was 9.05 mm. Parallel data for

medlar sort ʻ Pomoravka - seedless medlar genotype ʼ (rootstocks is quince Ba 29), of studied parameters in three different time treatments were following: 36 cm; 61 cm; 98 cm; (height) and 3.30 mm; 6.25 mm; 9.55 mm (corpulence). Parallel data for pears sort ʻRoyal medlarʼ of studied parameters in three different time treatments were following: 41 cm; 65 cm; 113 cm; (height) and 3,80 mm; 7.05 mm; 10.30 mm (corpulence). Parallel data for pears sort ʻMedlar without seedsʼ of studied parameters in three different time treatments were following: 43 cm; 73.5 cm; 120 cm; (height) and 3.55 mm; 7.80 mm; 11.05 mm (corpulence). Based on the data in Table 1, the highest average tree height (163.2 cm) had the variety ʻMedlar without seedsʼ grafted on the rootstock quince Ba 29. Based on LSD values, we can note that the height of the seedling in interaction between ʻ ʻMedlar without seedsʼʼ and the rootstock quince Ba 29 was significantly higher compared to other seedling (interactions) height. The corpulence of the seedlings was the greatest in the variety ʻ Pomoravka - seedless medlar genotype medlarʼ grafted on the rootstock quince Ba 29 (21.80 mm), which is statistically significantly higher than all other. The results showed that the low amount of successfully grafted seedlings after the autumn grafting on the rootstock quince Ba 29 of cultivars ʻMedlar without seedsʼ (41%) and ʻRoyal medlarʼ (51.67%) can be significantly improved in next spring grafting (85.67% ʻMedlar without seedsʼ and 93.67% ʻRoyal medlarʼ). The same effect of improving was evidenced in grafting of the variety ʻ Domestic medlarʼ, the percentage of successfully grafted seedlings was increased from 73.30% to 99.30%. The lowest effect of improving was evidenced in grafting of the varieties ʻMedlar without seedsʼ.

Results of autumn grafting of comercial medlar cultivars ʻDomestic medlarʼ(89.3%) and ʻRoyal medlarʼ (96.7%) on the generative rootstock (Pyrus communis L.) show that they have higher percentage of successfully grafted seedlings than varieties ʻ Pomoravka - seedless medlar genotypeʼ (84%) and ʻʻMedlar without seedsʼ (75%). Because of the re-grafting method in spring we had higher amount of successfully grafted seedlings on both generative and vegetative rootstocks.

Based on the data in Table 4 the highest average tree height (183 cm) had the variety ʻ Medlar without seedsʼ grafted on the generative rootstock (Pyrus communis L.). Based on LSD values we can note that the height of the seedling in interaction between ʻMedlar without seedsʼ and the generative rootstock (Pyrus communis L.) was significantly higher compared to other seedling (interactions) height. Seedlings of all studied comercial medlar cultivars grafted on the generative rootstock (Pyrus communis L.) had significantly higher height than any seedlings grafted on the vegetative rootstock (ʻquince Ba -29ʼ).

Out of the studies autochthonous pears cultivars, the highest water attaining capability had the leaves of cultivar ʻ Pomoravka - seedless medlar genotype ʼ(Table 5). Over the monitored time interval (8 hours upon sample taking), leaves taken from the annual twigs of the studied cultivars (one-year old seedlings) lost on average 35.50% of water. The lowest level of the stated capability was recorded with the leaves of cultivar ʻMedlar without seedsʼ

(40.44%). Over the monitored time interval (8 hours upon sample taking), leaves taken from the annual twigs of the control (one-year old rootstocks Pyrus communis L) lost on average 35.08% of water.

Table 1. Achieved percent of the grafting process in autumn and the percent of the re-grafting proces in spring (rootstocks is quince Ba-29)

Cultivar/ rootstocks	Percent of the grafting process (%) (autumn: „T“letter)				Percent of the re-grafting proces in spring (%) (spring: English linking)			
	2009	2010	2011	X	2010	2011	2012	X
Domestic medlar/Ba 29	68	77	75	73.3dc	98	100	100	99.3a
Pomoravka - seedless medlar genotype /Ba 29	90	87	94	90.3a	99	100	98	99a
Royal medlar/ Ba 29	50	53	52	51.67h	91	94	96	93.67b
Medlar without seeds/ Ba 29	37	44	42	41ij	82	90	85	85.67c
LSD 0.05				4.6				3.4
LSD 0.01				6.8				5.5

Table 2. Height and corpulence of one-year-old seedlings of medlar and achieved percent of the grafting process (rootstocks is quince Ba-29)

Cultivar/ rootstocks	Height of the seedlings (cm) (average)				Corpulence of the seedlings (mm) (average)			
	June	July	August	Sept.	June	July	August	Sept.
Domestic medlar/Ba 29	33	58	94	151.3cb	3.2	5.45	9.05	17.6c
Pomoravka - seedless medlar genotype /Ba 29	36	61	98	149.8c	3.3	6.25	9.55	21.8a
Royal medlar/ Ba 29	41	65	113	151.6cb	3.8	7.05	10.3	19b
Medlar without seeds/ Ba 29	43	73.5	120	163.2ab	3.55	7.8	11.05	18.8bc
LSD 0.05				9.3				1.6
LSD 0.01				10.3				1.9

Out of the studied autochthonous plum cultivars, the highest water attaining capability had the leaves of cultivar ʻCrvena rankaʼ (Šebek,2016). Over the monitored time interval (8 hours upon sample taking), leaves taken from the annual twigs of the studied cultivars (one-year old seedlings) lost on average 33,54% of water. The lowest level of the stated capability was recorded with the leaves of cultivar ʻObični piskavacʼ (41.74%). Out of the studied water attaining capability of leaves in autochthonous apple cultivars (Šebek, 2004), the highest water attaining capability had the leaves of cultivar ʻPašinkaʼ. Over the monitored time interval (8 hours upon sample taking), leaves taken from the annual twigs of the studied cultivars (in situ) lost on average 38.09% of water.

The lowest level of stated capability was recorded with the leaves of cultivar ʻArapkaʼ (40.64%). In terms of the selected wild apples (Šebek, 2004), the highest level of water attaining capability was registered in the leaves of type 2 (32,44%). Leaves taken from the annual twigs out of the studied selected types (in situ) lost on average level (36,61 %) showed the leaves of type 6.

Table 3. Achieved percent of the grafting process in autumn and the percent of the re-grafting proces in spring (generative rootstocks is *Pyrus communis L.*)

Cultivar/ rootstocks	Percent of the grafting process (%) (autumn: „T“letter)				Percent of the re-grafting proces in spring (%) (spring: English linking)			
	2009	2010	2011	X	2010	2011	2012	X
Domestic medlar/ *P.communis*	92	86	90	89.3b	98	91	100	96.3a
Pomoravka – seedless medlar genotype / *P.communis*	83	82	87	84c	90	86	90	88.7c
Royal medlar/ *P.communis*	92	98	100	96.7a	98	100	100	99.3a
Medlar without seeds/ *P.communis*	70	77	78	75e	99	98	100	99a
LSD 0.05				4.6				3.4
LSD 0.01				6.8				5.5

CONCLUSION

The research was conducted on 4 different medlar cultivars and that allowed us to obtain important morphological and physiological traits.

1. Seedlings of all studied comercial cultivars of medlar grafted on the generative rootstock (*Pyrus communis L.*) had significantly higher height than seedling grafted on the vegetative rootstock (‘quince Ba-29’).

2. We also have significant difference between the diameters of seedlings. Those differences are the consequences of lower verdure in vegetative rootstocks than in generative rootstocks.

3. The most important result of our research is the fact that we determined the compatibility between researched comercial cultivars of medlar and vegetative rootstocks (ʻquince Ba -29’).

4. The method of re-grafting in spring is very useful because we had higher amount of successfully grafted seedlings on both generative and vegetative rootstocks after re-grafting.

5. Production of seedling material of comercial cultivars of medlar with vegetative rootstocks ‘Quince Ba -29’ will be enormous contribution for product of our ecological environment .

6. The results of this research show that the plant height, stem corpulence one-year old seedlings are characteristics of comercial cultivars of medlar, from which rapid growth and uniformity of scions depend.

7. The highest water attaining capability had the leaves of cultivar ' Pomoravka - seedless medlar genotype '. The lowest level of the stated capability was recorded with the leaves of cultivar ' Medlar without seeds'.

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THE *POST-HOC* POWER ANALYSIS OF FOREST PRODUCTIVITY ATTRIBUTES IN EXPERIMENTAL STUDY IN CENTRAL BOSNIA

SUMMARY

Forest productive attributes changes over time in native forests has been recognized as crucial challenge for management of uneven aged mixed forests in Bosnia and Herzegovina since middle of the last century. Experimental study has been carried out on set of experimental plots established in mixed stands on mountain Igman in central Bosnia. The most important forest productivity attributes changes based on repeated measures have been monitored over time. The aim of this research was to conduct the *post-hoc* power analysis for monitored forest attributes: basal area per ha (BA), growing stock per ha (GS) and current annual increment per ha (CAI_v). Here are used repeated measures conduced on the 10 experimental plots in two types of mixed stands: fir-spruce and fir-spruce-beech plots (five plots per each type) measured in five (BA and GS) and four (CAI_v) occasions in periods between 10–20 years. Analyses of variance (ANOVA) within and within-between repeated measures were applied and power analysis was performed. ANOVA within forest type over time showed highly significant differences for all attributes ($\alpha = 0.05$, $p < 0.001$). Here, power analysis for comparison of stand attributes resulted in observed high power values ranged from 82% to 99% (very low risk of Type II errors). Then, ANOVA between two forest types over time showed different significances for forest attributes ($\alpha = 0.05$, $p_{BA} = 0.25$, $p_{GS} = 0.23$ and $p_{CAIv} = 0.02$). The risks of Type II errors were high for BA and GS (from 66% to 72%) while conclusions for CAI_v could be accepted with very low risk (4%). So, the *post-hoc* power analysis of comparisons of stand attributes between forests types found low power for BA (28%) and GS (34%) and high power for CAI_v (96%). These findings confirm importance of proper forest species composition planning in mixed stands related to highest wood productivity and other forest characteristics as biodiversity.

Keywords: forest productivity, uneven aged mixed forest stands, experimental study, repeated measures, power analysis

INTRODUCTION

The native uneven aged mixed forests are the most productive and the most important forests in Bosnia and Herzegovina covering about 30% of

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forested area. The dominant native mixed beech (*Fagus sylvatica* L.), fir (*Abies alba* Mill.) and Norway spruce (*Picea abies* (L.) H. Karst.) forests in different tree species mixtures have both ecological and productivity importance (Matić 1959, Kotar 2005). Their complex structural and productivity characteristics and dynamic changes have been analyzed in many researches (Bozalo 1980, Bončina 1994, Bončina and Devjak 2002, Bončina 2011, Kotar 2002, 2003, Dukić and Maunaga 2008, Diaci *et al.* 2008, Lojo 2013, Ibrahimspahić 2013, Motta *et al.* 2014).

The experimental research related to proper silvicultural treatments in those forests in Bosnia and Herzegovina (BiH) started at the middle of last century when series of permanent experimental plots were established in central Bosnia (Matić 1959). The strategic goal was to develop and maintain uneven aged mixed multilayer forests using selection cutting based on the positive selection principle aiming to create optimal stand structures that would support high wood production permanently.

Experimental plots were distributed randomly in two forest types: mixed fir and spruce forest (FS) and beech, fir with spruce forest (BFS). Measurements of forest attributes were conducted in several occasions with time span of 10 to 20 years reporting the most important forest productivity attributes: number trees per ha (N), number of ingrowths' trees per ha ($N_{ingrowth}$), number cut trees per ha (N_{cut}), basal area per ha (BA), growing stock per ha (GS) and current annual increment per ha (CAI_v). The main research questions were related to influences of stand structural changes affected by selection cutting in interaction with time on the most important forest productivity attribute (CAI_v). Experimental results related to stated research questions for successive occasions were reported (Drinić 1974, 1976, Pavlič 1987). The last occasion refers to measurements conducted in period 2006–2008. Then all long-term experiment data are summarized, analyzed and reported (Ibrahimspahić 2013).

Ibrahimspahić (2013) analyzed forest attributes mean differences applying univariate analysis of variance (ANOVA) as between forest types so between occasions. Conclusion related to stated null hypotheses that effect size (ES) is zero, was based on statistical significance of obtained *p*-value (the probability of Type I error). In the case of ANOVA, ES is the difference between means related to forest type and the means within occasions. Usually the ES is low in most monitoring studies so likelihood that a statistical test will detect a significant ES, if it exists, remains low.

Power is the probability of getting a statistically significant difference when a real treatment difference exists (Nemec 1991). Power analysis enables to calculate power in relation with experimental design, sample size, ES, significance level α and the variability of data (Foster 2001, Di Stefano, 2001). South and VanderSchaaf (2006) proposed a "*hybrid*" power analysis that could be useful to discuss reason for non-significant result: no effect or not enough replication to produce a small enough error term. Foster (2001) demonstrated application of power analysis related to forest monitoring program.

The objective of this research was to evaluate dynamic changes of forest productivity attributes between two forest types in interaction with successive occasions in long-term experimental research established in native uneven aged mixed multilayer forest and to perform power analysis. Following research aims were stated:

- to compare and evaluate dynamic changes of forest productivity attributes over the different occasions (main effect for occasions), between two forest types in terms of their influences on forest productivity attributes (main effect for factor) and over occasions for two forest types (interaction effect);
- to compare and evaluate magnitude of effect sizes on forest productivity attributes influenced by two different forest types;
- to identify minimal detectable effect (MDE) to obtain statistically significant result between forest types assuming power of 0.8 with this experimental design;
- to perform power analysis of forest productivity attribute mean differences between two forest types assuming datasets from whole time span as independent (without effects of occasions and interactions);
- to perform power analysis of forest productivity attribute mean differences between last measurement and target quantities for each forest type.

MATERIAL AND METHOD

Study area

The research has been conducted on data collected on ten experimental plots in beech-fir (with spruce) forests in the MU "Igman" over 50 years. MU "Igman" (area size 8.219,3 ha) encompasses the territory of mountain Igman near Sarajevo in central Bosnia and Herzegovina (Figure 1).

Mountain Igman (with Bjelašnica) make a geomorphologic complex unit of high Dinaric mountain with characteristic landscape related to frequent climate conditions (especially temperature) inversions. It is mostly a limestone mountain, with the main soil types alternating on a small area.

The permanent experimental plots were set up in forests which are related to species type and productivity the most dominant and significant in MU "Igman". Five locations were chosen on different altitude, different terrain expositions and inclinations in beech, fir and spruce forests and five in fir and spruce forests (without beech). The experimental plots size varied, ranging from 1.0 to 3.14 ha.

Owing to plant community nomenclature, plots are included in *Abieti Fagetum illyricum* Treg. (four plots), *Fagetum subalpinum* Horv. (one plot), *Abieti Piceetum Illyricum* Stef. (three plots), *Piceo Pinetum Illyricum* Stef. (one plot) and *Pyrolo-Piceetum* Fuk. community (one plot).

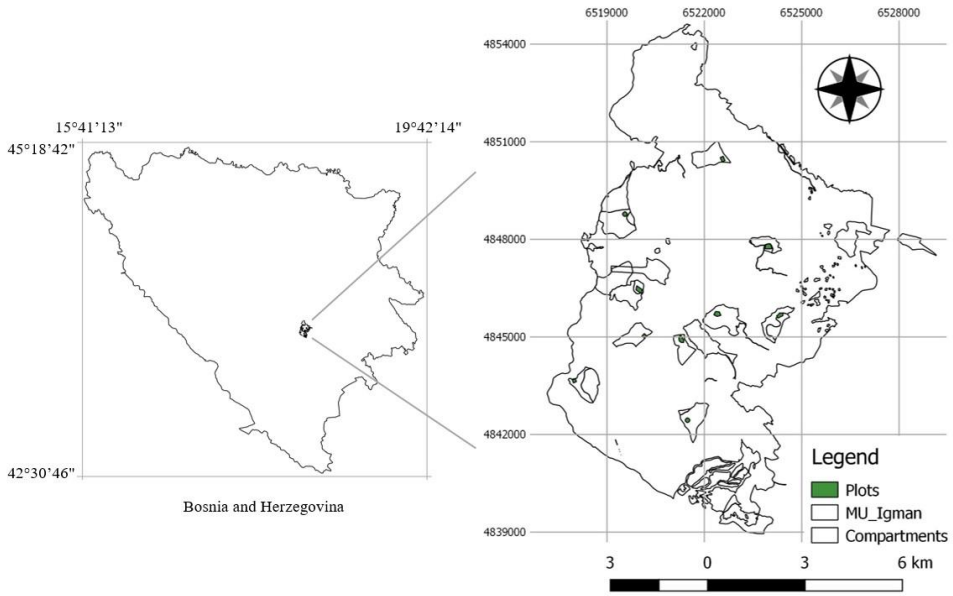

Figure 1. Study area

Experimental design

The long-term experiment with permanent plots was established in native uneven aged mixed multilayer forests in the middle of last century (between 1954 and 1958).

The experimental plots were distributed randomly in two types of forest tree species mixture: the fir-spruce mixture (FS) and the beech-fir-spruce mixture (BFS). First measurement (occasion) was conducted when plots were established, then in three occasions periodically in ten years periods. The last (fifth) measurement was conducted after twenty years period due to the war. The fifth measurement was not conducted on two experimental plots because they were in mined area so additional plots in assessable neighborhood were established and measured.

All measurements have been conducted using the unified methodology enabling connectivity of collected data and information. Detailed description about data collection and calculations of forest productivity attributes per experimental plot is given in Ibrahimspahić (2013).

Considering two different forest types ("between" effect) and successive measurements in five (four) repetitions (occasions) ("within" effect), experiment could be examined using linear mixed model (Čabaravdić and Ibrahimspahić 2017).

Statistical analysis

In this research, collected data are analyzed using descriptive statistics, analysis of variance (ANOVA) and power analysis. The basic statistics for data collected during whole period of experiment are determined: mean, standard deviations and 95% confidence interval for the most important forest productive

attributes: N (trees/ha), $N_{ingrowth}$ (trees/ha), N_{cut} (trees/ha), BA (m^2/ha), GS (m^3/ha) and CAI_v (m^3/ha/year), for two forest types.

We used a linear mixed model to assess the impact of two forest types (FS and BFS) on the forest productivity attributes across five (four) occasions (Occ.). The two forest types are assigned as the main factor (between-subject) and occasions (within-subject) as the factor where within variability is of interest. Here is combined multivariate and univariate approach related to ANOVA of within-between repeated measures (occasions). Differences within occasions and interaction between forest type and occasions were evaluated using multivariate tests of within–subjects effects. Here is evaluated Wilks lambda, F value, associated probability value, partial eta squared and estimated (observed) power. Difference between forest types was obtained using test of between–subjects effects. Here, F value, associated probability value, partial eta squared, Cohen's effect size and observed power were evaluated. The associated probability value in ANOVA points out if relationship exists, but cannot measure effect size. Here is used partial eta squared (η^2) as a measure of the degree of association between an effect (e.g., main effects, an interaction) and the value of the forest productivity attribute. A partial eta squared can be interpreted as the proportion (or percentage) of variance that is attributable to each effect. Then, partial eta-squared is used to calculate Cohen's metric f(U) to measure effect size for F-ratio in ANOVA. Then, the probability to detect the difference if difference exists was estimated and reported as power. Low power means low chance of finding significance if it exists (Faul *et al*. 2009).

In addition, the minimum detectable effects (MDE) as the minimum difference between main factor levels that yields a statistically significant result, for given sample size, power and type I error level were identified and presented on power curve. In our case, power analysis was used to determine effect size of forest attributes difference in two forest types and to identify magnitude of achieved power compared with 80% power level.

Then, using power analysis we examined what sample size is required to detect a same effect size with power of 0.80. An approach referred to look at each pairwise comparison by doing power analysis for a set of unpaired *t*-tests (Foster *et al.* 2001). Further, we compared GS and BA means and evaluated differences from last measurement with targeted values prescribed with management plans for each forest type. Finally, one-sided *t*-test was applied to identify significance between here obtained mean values and those reported in similar researches.

Here are used PASW Statistics 18 and G*Power 3.1.

RESULTS AND DISCUSSION

Descriptive statistics of forest structural and productivity attributes for two forest types over experimental period are presented in Table 1. All values are in average higher in fir-spruce forest considering whole period of measurement. The GS ranged from 198.4 m^3/ha to 612.3 m^3/ha with average of 407.1 m^3/ha in

FS forest type while range was narrower in BFS forest (from 159.2 m^3/ha to 454.9 m^3/ha) with average of 330.0 m^3/ha. In FS forest CAI_v mean of 9.51 m^3/ha/year was obtained with range between 5.74 m^3/ha/year and 12.80 m^3/ha/year. In BFS forest mean CAI_v was 6.85 m^3/ha/year varying between 3.12 m^3/ha/year and 10.71 m^3/ha/year.

Table 1. Descriptive statistics of forest structural and productivity attributes for two forest types over experimental period

Attribute	Forest type	N	Mean	Stnd. Dev.	Min	Max
N (trees/ha)	FS	5	458	140	277	819
	BFS	5	433	206	230	869
$N_{ingrowth}$ (trees/ha)	FS	4	92	89	23	343
	BFS	4	49	29	16	119
N_{cut} (trees/ha)	FS	4	68	48	6	182
	BFS	4	50	53	7	198
BA (m^2/ha)	FS	5	35.1	8.0	20.8	50.6
	BFS	5	30.3	6.0	17.5	40.5
GS (m^3/ha)	FS	5	407.1	116.9	198.4	612.3
	BFS	5	330.0	77.8	159.2	454.9
CAI_v (m^3/ha/year)	FS	4	9.51	2.09	5.74	12.80
	BFS	4	6.85	1.89	3.12	10.71

Analysis of the dynamic changes (variability within occasions)

Changes of forest productivity attributes during successive occasions (measurements) in for two different forest types are illustrated in Figure 2. Results of ANOVA with within-subjects factor (repeated occasions and interaction forest type × occasion) and between-subject factor of forest type (FT_1 and FT_2) are presented in Table 2 and Table 3. Results of multivariate test related to within-subject difference are given in Table 2. Mauchly test indicated that the assumption of sphericity had been violated (χ^2 (2) = 16.8, $p < .001$), therefore degrees of freedom were corrected using Greenhouse–Geisser estimates of sphericity ($\varepsilon = 0.98$).

Multivariate tests (within subjects) indicated:

There were significant mean differences in N, GS and CAI_v related to consecutive occasions (dynamic changes within occasions) regardless forest type:
N (Wilks Lambda = 13, F (4, 5) = 8.4, p = 0.02, partial eta squared = .975);
GS (Wilks Lambda =0.03, F (4, 5) = 48.7, $p < 0.001$, partial eta squared = .975);
CAI_v (Wilks Lambda =0.11, F (3, 6) = 15.9, $p < 0.001$, partial eta squared =0.888).

There were not significant mean differences in $N_{ingrowth}$ related to occasions (dynamic changes within occasions) nor interaction occasion × forest type ($p > 0.05$), but with low power.

There were significant mean differences in N_{cut} and BA between forest types within occasions (interaction occasions × forest type):

N_{cut} (Wilks Lambda =0.26, F (3, 6) = 5.76, p = 0.03, partial eta squared =0.742);

In this case 74.2% of variance of N_{cut} was explained by interaction between forest type and occasion;

BA (Wilks Lambda =0.18, F (4, 5) = 5.81, p = 0.04, partial eta squared =0.823);

In this case 82.3% of variance of BA was explained by interaction between forest type and occasion.

Table 2. Results of multivariate test (ANOVA within occasions and interaction occasions × forest type)

Attribute	Source	Wilks λ	F	df_1	df_2	Sig.	Part. ε^2	Est. Power
N (trees/ha)	Occ.	0.13	8.38	4	5	0.02	0.870	0.84
	Occ×FT	0.51	1.21	4	5	0.41	0.492	0.19
$N_{ingrowth}$ (trees/ha)	Occ.	0.49	2.06	3	6	0.21	0.507	0.30
	Occ×FT	0.75	0.66	3	6	0.61	0.248	0.12
N_{cut} (trees/ha)	Occ.	0.04	45.13	3	6	0.00	0.958	1.00
	Occ×FT	0.26	5.76	3	6	0.03	0.742	0.71
BA (m^2/ha)	Occ.	0.02	58.96	4	5	0.00	0.979	1.00
	Occ×FT	0.18	5.81	4	5	0.04	0.823	0.69
GS (m^3/ha)	Occ.	0.03	48.69	4	5	0.00	0.975	1.00
	Occ×FT	0.23	4.21	4	5	0.07	0.771	0.54
CAI_v (m^3/ha/year)	Occ.	0.11	15.86	3	6	0.00	0.888	0.99
	Occ×FT	0.72	0.77	3	6	0.55	0.278	0.14

Obtained statistical findings supported description of dynamic changes visible on graphical presentations (Figure 2). Analyzing dynamic changes through whole time span we noticed:

There were no significant differences of measured forest attributes (N, BA, GS) between two forest types in the first occasion. The same tendency remained in the second occasion as for the same attributes so for firstly measured attributes: $N_{inrowth}$ and CAI_v. Here appeared the significant difference of number N_{cut} in interaction occasion × forest type. It was the highest difference of cut intensity in the whole time span.

In the next (the third) occasion differences between means of all forest productivity attributes remained non-significant. In the fourth occasion the significant difference of BA was obtained for interaction occasion × forest type. Also, the significant difference of CAI_v was determined that remained in the next (the fifth) occasion too.

It seems that the higher cut intensity in the second occasion in FS forest type supported tendency of BA and CAI_v increase resulting in significant differences of CAI_v in the fourth occasion that remained next 20 years. Progressive tendency of CAI_v changes in FS forest type was consistent during the

whole time span while the tendency of CAI_v changes in BFS forest type was almost invariable with slight decrease. Bončina *et al* (2013) reported that changes were divergent in study areas in Dinaric uneven-aged forests of the NW Balkan too although stable structure over several decades were expected.

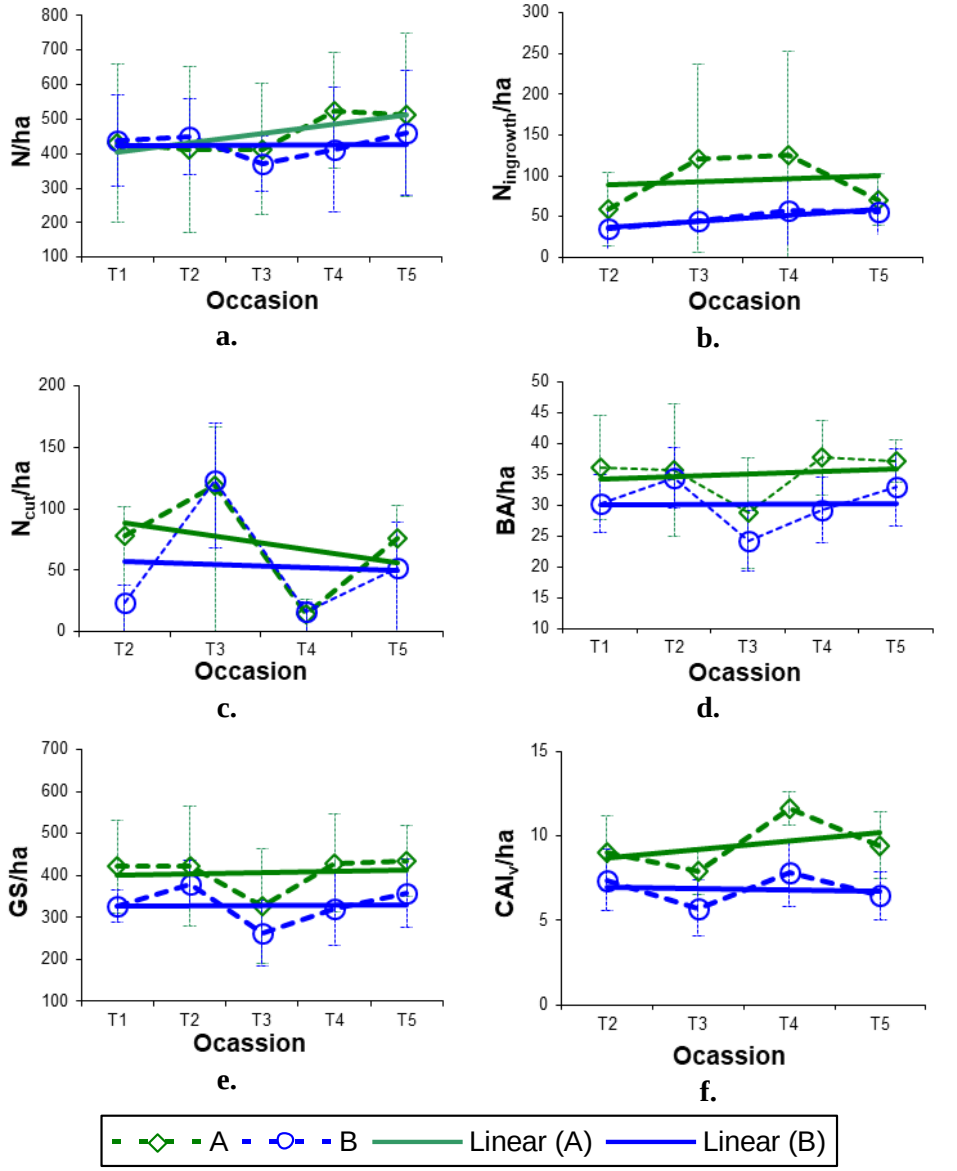

Figure 2. Changes of the observed averaged forest attributes (dotted lines) and trends (solid lines), for the number stem per ha (a), ingrowth number of trees per ha (b), number of cut trees per ha (c), basal area per ha (d), growing stock per ha (e) and current annual increment per ha (f). Error bars are standard deviations of the observed attributes.

Analyses of forest productivity attribute differences (variability between forest types)

Table 3 summarizes results of univariate tests. The main effects comparing the two forest types were not significant for all attributes ($p > 0.05$) except CAI_v ($F(1,8) = 9.32$, $p = 0.02$, partial eta squared = 0.538).

Table 3. The main factor means significances (ANOVA between forest types) and power *post-hoc* analysis of forest productivity attributes ($\alpha = 0.05$, $n_{groups} = 2$)

Attribute	N_{occ}	F	Sig.	Partial Eta Squared	Cohens f(U)	Est. Power
N (trees/ha)	5	0.11	0.75	0.013	0.12	0.06
$N_{ingrowth}$ (trees/ha)	4	1.79	0.22	0.183	0.47	0.22
N_{cut} (trees/ha)	4	4.83	0.06	0.377	0.78	0.49
BA (m^2/ha)	5	1.57	0.25	0.164	0.44	0.20
GS (m^3/ha)	5	1.70	0.23	0.175	0.46	0.21
CAI_v (m^3/ha/year)	4	9.32	0.02	0.538	1.08	0.76

The partial eta squared ranged from 0.013 (N) to 0.538 (CAI_v). Lower percentages of variance determined by forest type were obtained for BA and GS and $N_{ingrowth}$ (< 20%). The higher percentages of variance explained by forest type were found for N_{cut} (38%) and CAI_v (54%). Cohen's metric $f(U)$ of effect size points out the largest effect for CAI_v (1.08) reaching statistical significance and approaching to reliable power of 0.8 approximately. Compared to this value, effects for BA, GS, $N_{ingrowth}$ and N_{cut} could be assigned as medium (between 0.44 and 0.78) and effects for N as small (< 0.20). Then the highest association between forest types and N_{cut} (0.78), almost significant difference ($p = 0.06$) and observed power of 0.5 approximately points out probability of 50% to find out significant difference if is there. Finally, it is visible that probability to find out significant difference for other attributes, if they exist, is very low (< 25%).

Power analysis - minimal detectable effects (MDE)

Using sensitivity approach, minimal detectable effect (required effect size to reach power of 0.8) of attributes for forest type of 1.13 was determined assuming chosen experimental design.

The Figure 3 shows relationship between effect size and power for ANOVA, repeated measures, between factors (number of groups = 2, number of measurement, err prob = 0.05, total sample size = 10) produced using G*Power 3.1. It shows that power increases with larger effect size. It is visible that CAI_v approached to required effect size only. So evidence about significant mean difference between CAI_vs in two forest type reached high power was obtained and this significance could be accepted for generalization.

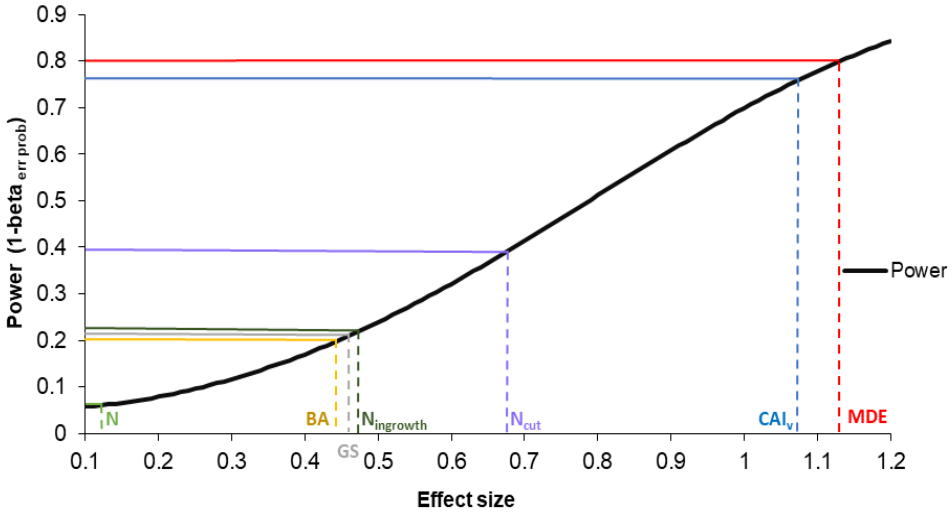

Figure 3. Power by effect size for forest productivity attributes (ANOVA repeated measures, between factors, Q*Power 3.1)

Hybrid power analysis – Sample size estimation

Many researchers emphasize that power analysis contributes the most in the planning research phase when desired effect size, significance level, test power and sample size should be chosen or estimated (Foster 2001, Di Stefano 2001). Foster (2001) demonstrated capacities of *a priori* power analysis discussion forest monitoring program in native uneven aged multispecies forests in the United States. He stated the relative mean difference of forest productivity attribute (expressed in percentage) as desired effect size and calculated required sample size and estimated power (based on data collected in the first monitoring phase). For example, desirable change over time to detect was the decrease of 20% for the canopy cover and three density, BA to remains the same and to detect the GS increase of 20%.

In this research, power analysis was performed for sample size estimation, based on data from last occasion, with an idea to compare difference between means of BA, GS and CAI_v in new simple comparative experiment using minimal sample size to reach power of 80%.

The minimum sample size CAI_v to detect this effect with exact power of 80% in new experiment is similar (n = 7 plots per forest type) (Figure 4) (G*Power 3.1 or other software).

The effect size for BA of 0.44, could be qualified as medium. With an alpha =0.05 and power = 0.80, the projected sample size needed with this effect size is approximately N =38 (19 plots per each forest type) for the pairwise comparison. The effect size for GS of 0.46, could be qualified as medium too. With an alpha = .05 and power = 0.80, the projected sample size needed with this effect size is approximately N = 48 (24 plots per each forest type).

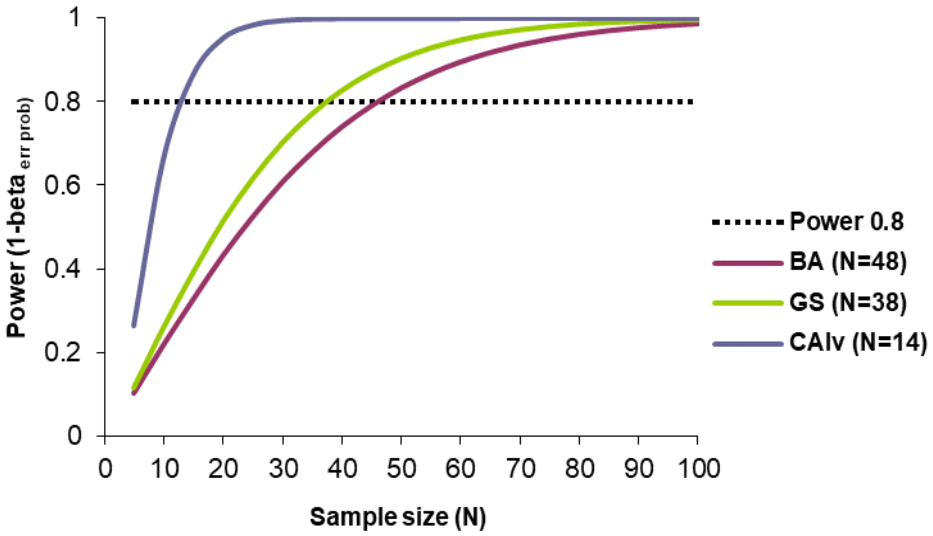

Figure 4. Power by sample size for forest attributes (*t*-test two independent groups, Q*Power 3.1)

Choosing larger sample size (24 plots per each forest type), uncertainty related to BA and GS could be clarified in the frame of common research planned as simple comparative experiment (two independent samples) (Figure 4).

If it acceptable for researchers to neglect impacts of occasions (from the practical reasons although their significances were confirmed earlier) and experimental data accept as independent observations in two forest types, then two data sets related to forest types could be used to clarify relations between effect size, sample size and power additionally. In this case we assume the independency of observation within forest type. Results of pairwise comparisons of forest productivity attributes mean differences between two forest types are given in Table 4.

This approach results in conclusions that the larges effect sizes related to CAI_v, GS, BA and $N_{ingrowth}$ (Cohens d > 0.60) become statistically significant with larger sample sizes ($n_{II\text{-}V}$ = 40 and $n_{I\text{-}V}$ = 50). Difference related to (N) remains non-significant with low power.

Significant difference between mean growing stocks in two forest types on experimental sample plots is expected and consistent with recent findings (Matić 1959, 1963, 1971, 1980; Kotar 2005). Higher conifers participations with narrow crowns and emphasized adaptation on limited light contribute to higher density enabling higher growing stock consequently (Ibrahimspahić, 2013).

The power *post-hoc* analysis

In the first phase of the experiment, plots structural and productivity characteristics were determined and then stand productivity normal quantities

proposed for each plot within forest type (Drinić 1974, Pavlič 1987). Last measurement was used in order to compare differences between observed and stated GS and CAI_v quantities within each forest type. Results are presented in Table 5.

Table 4. The main factor (forest type) means significances (unpaired *t*-test) and power analysis of forest productivity attributes ($\alpha = 0.05$, $n_{groups} = 2$) (completely randomized design)

Attribute	N	t	*p* value	Effect size	Est. Power	α level (power 0.8)	Sample Size (Power 0.8)
N (trees/ha)	50	0.66	0.512	0.18	0.099	0.753	910
$N_{ingrowth}$ (trees/ha)	40	2.22	0.033	0.70	0.584	0.174	66
N_{cut} (trees/ha)	40	1.16	0.254	0.36	0.201	0.632	240
BA (m^2/ha)	50	2.42	0.019	0.61	0.658	0.122	70
GS (m^3/ha)	50	2.77	0.008	0.79	0.779	0.059	54
CAI_v (m^3/ha/year)	40	4.29	<0.01	1.36	0.987	0.002	20

Table 5. The *post hoc* power - observed *vs.* targeted (normal) values in two forest types (n=5)

Type	Attribute	Obs.	Normal	p - value	Effect Size	ES conv.	Est. Power
FS	GS (m^3/ha)	434.6	344.8	0.06	2.63	very large	0.95
	CAI_v (m^3/ha/year)	9.44	7.21	0.07	2.47	very large	0.93
BFS	GS (m^3/ha)	357.9	357.7	1.00	0.01	small	0.05
	CAI_v (m^3/ha/year)	6.48	7.23	0.38	0.98	large	0.28

There are no statistically significant differences between GS and CAI_v means in both forest types (α=0.05, p>0.05). The *p* values in FS forest type are almost critical pointing out high risks of errors type II what is confirmed with very high estimated power (0.95 and 0.93 respectively). It means there are 95% and 93% chances of significant differences but we did not find them (probably sample size is critical). On other size, in the BFS forest types GS effect size is very small, difference is not significant and a chance of being significant is very low (5%). Also, CAI_v difference is not significant (p=0.38) although effect size is large. Obtained results support conclusions that nonsignificant differences between observed and proposed values hold very high risk in FS forest type while the same statement could be accepted for BFS forest type with very low risk.

Recently, mixed uneven aged beech, fir and spruce forests particularly have been recognized as the most challenging forest type considering competitive abilities of broadleaves and conifers exposed to dynamic environmental, economic and social conditions and changes. Their particular importance related to productivity, biodiversity and other forest functions have been identified very early. Many papers reported that old-growth BFS forests on Balkan peninsula achieve very high wood production (Keren *et al.* 2014, Motta *et al.* 2014, Chivulescu *et al.* 2016) and preserve very high biodiversity (Gazdić *et al.* 2016). Then, forest management planning strives to adapt proper silvicultural treatments in order to achieve high wood production and maintain sustainable principles. Bončina et al. (2014) reported about structural characteristics as in managed mixed forests so in old-growth virgin across region: Slovenia (SI), Croatia (CRO), Serbia (SRB), Bosna and Herzegovina (BIH) (Foča-Toholji), and Montenegro (MNE). We compared means of BA and GS obtained for experimental plots in BFS on Igman and correspondent values reported for managed stands in study areas across region (chosen as representative of selection forest management in the country) (Bončina *et al.* 2014) (Table 6). Also, we found as comparable result from similar experimental research completed in Biogradska Gora (Čurović *et al.* 2013) and reported about difference between GS mean from Igman and proposed normal value of 389 m^3/ha in this study area (Table 6). It was noticeable that reported values were higher than Igman's means mainly (GS of 330.0 m^3/ha and BA of 30.3 m^2/ha) so we examined if they were significantly higher. Comparison was based on the one-sample *t*-test (one-sided) where reported values were used for statistical research hypothesis.

Table 6. The *post hoc* power – GS and BA comparison with other study areas (managed mixed uneven aged beech and fir with spruce forest stand) in region

	Stand volume (m^3/ha)				Stand basal area (m^2/ha)			
Country	Mean	*p* value	Effect Size	Est. Pow	Mean	*p* value	Effect Size	Est. Pow
SI	428	0.06	1.96	0.97	36	0.17	1.08	0.63
CRO	436	0.05	2.18	0.99	35	0.25	0.72	0.38
SRB	493	0.01	3.78	1.00	35.6	0.20	0.93	0.35
BIH	447	0.03	2.49	0.99	41	0.02	2.87	1.00
MNE	361	0.47	0.09	0.06				
MNE[a]	389	0.22	0.87	0.49				

Note: The data in columns two and six are from "A comparative analysis of recent changes in Dinaric uneven-aged forests of the NW Balkans" (Bončina *et al.* 2014, p. 75); [a] Value from "The ratio between the real and theoretically normal number of trees in mixed fir, beech and spruce forests in the national park "Biogradska gora" (Čurović *et al.* 2013, p. 14)

Almost significant difference is obtained for GS from Slovenia (p=0.06) with high probability that actual difference exists but was not found (97%). Significant differences are found for Croatian, Serbian and BIH (Foča-Toholji) forest stands ($p \leq .05$). On other side, non-significant differences for GS related to Montenegrin forest stands confirm quantity similarities in Bosnian (Igman) and Montenegrin experimental trials. The BA differences are not significant in cases of Slovenian, Croatian and Serbian forest stands while BA obtained on Bosnian forest stand located in Foča-Toholji is significantly larger (p=0.02). Effect sizes for Slovenian and Serbian BA are very large (approx. 1) while for Croatian could be qualified as large (approx. 0.7).

The complexity of natural processes in native uneven aged mixed forests needs various analyses relevant as for ecological stability so for economic forest management on sustainability principles (Miletić 1950, Matić 1980). Power analysis applied in this research shows potentials to clarify forest productivity attribute differences between two forest types, effect sizes of their dynamic changes in time and results in similar research.

CONCLUSION

Here are demonstrated applications of linear mixed analysis of variance related to the analysis of dynamic changes and power analysis of forest productivity data collected in long-term experimental research conducted as repeated measurements (occasions) in native uneven aged multilayer forests.

Behind evaluation of their statistical significance here are identified and interpreted effect sizes of forest productivity attributes related to difference caused by forest type, occasion and their interactions. Power curves for main effect (forest type) related to effect sizes and sample sizes are determined. The minimal detectable effect size is calculated for presented experimental design pointing out that only estimated significant difference of current annual increment (m^3/ha/year) between two forest type could be generalized with adequate power (near 80%). Then, obtained data of basal area (m^2/ha) and growing stock (m^3/ha) in last occasions could be used as preliminary data to plan sample size for new experiment in order to achieve test power of 80%. Also, we performed power analysis of forest productivity attributes pairwise comparison assuming all measurements as timely independent and confirmed very high significance for current annual increment per ha. In this approach significant mean differences were obtained for growing stock (m^3/ha), basal area (m^2/ha) and ingrown trees per ha demonstrating capacities of sample sizes. Accepting this approach obtained results support expected significant differences between forest attributes in fir-spruce and beech, fir with spruce forests.

Then, obtained growing stock (m^3/ha) and basal area (m^2/ha) means are compared with targeted values from management plans for two forest types finding out no significant differences. The very large effects sizes were found in fir-spruce forest pointing out large differences between obtained and targeted values.

Finally, forest attributes comparisons with similar managed uneven mixed beech, fir with spruce stands in region were performed. The non-significant differences of growing stock (m^3/ha) were obtained between Bosnian forest stands on Igman and Montenegrin forest stands Ljubišna and Biogradska Gora only. Effect size for the first difference was small (0.06) while the second effect size was large (0.87). Effect sizes for basal area (m^2/ha) ranged from large (Croatia, Serbia) to very large (Slovenia).

In conclusion, presented research integrates possibilities to analyze long-term experimental forest structural and productivity data focusing on dynamic changes analysis and power analysis as rarely used statistical method. Here was emphasized importance of effect size and power in non-significant differences between forest attributes as within stands so between forest types on different scales.

Further research could include other forest attributes and additional available environmental data as covariates and explore their participation in effects (climate, soil, terrain and others) locally and globally.

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L-GLUTATHIONE-REDUCED ENHANCES IN VITRO ROOTING OF APPLE ROOTSTOCK MM106 (*Malus domestica* Borkh.)

SUMMARY

The experiment was carried out to study the effect of L-glutathione-reduced (GSH) on rooting, vegetative growth and callusing under *in vitro* conditions of shoot tip explants in MM106 apple rootstock. GSH was applied at 10 concentrations (0-1000 μM) in combination with 5.4 μM α-napthaleneacetic acid (NAA). After 28 weeks of culture in the rooting media, obtained results indicated that the incorporation of 10 μM GSH into the MS culture medium resulted in the maximum root number per rooted explant (11.67), greatest root length (41.11 mm) and root fresh weight (FW) (0.289 g). However, the highest rooting percentage (50%) was achieved in the 25 μM GSH + 5.4 μM NAA combination treatment. The results also cleared significant increase in shoot length (41.43 mm) and shoot FW (0.232 g) by 2.81 cm and 3.5 times, respectively compared with the control GSH-untreated explants. Callus FW (0.699 g) and callusing percentage (57.14%) were maximum by adding 10 μM GSH to the medium. Neither rooting nor callusing was recorded in the absence of GSH. In addition, the application of 5, 50 or 1000 μM GSH resulted in complete inhibition of rooting. No callus induction occurred when the explants were treated with 5, 100, 250 or 1000 μM GSH. Taking into consideration all the aforementioned it is clearly demonstrated that 10 μM GSH when applied simultaneously with 5.4 μM NAA enhanced rooting and shoot growth of microcuttings in a considerable degree. Therefore, GSH can be used as a rooting promoting agent in MM106 apple rootstock tissue culture system.

Keywords: antioxidants, apple rootstock, *in vitro* rooting, L-glutathione, *Malus domestica* Borkh, thiol compounds.

INTRODUCTION

Apple (*Malus domestica* Borkh.) is one of the most important fruits in temperate-zones. It is the third most important fruit tree in the world (FAO 2013). Apple is conventionally propagated by vegetative methods, such as budding or grafting. Although these traditional propagation methods do not ensure disease-free and healthy plants, they depend on the season; moreover, they typically result in low multiplication rates. Micropropagation provides the rapid propagation of new varieties, breeding lines or mutants in apple breeding

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because it is the most necessary stage in the regeneration of transgenic lines and determines the effectiveness of a transformation protocol (Aldwinckle and Malnoy 2009). Recently in apple, many reliable methods have been developed for both propagation of rootstocks and scions using *in vitro* techniques. Successful micropropagation of apple using microcuttings or single node cuttings is influenced by several internal and external factors, including genotype, physiological state of sampling, *in vitro* media constituents and their ratio, light, temperature and other factors (Dobránszki and Silva 2010). Micropropagation of apple rootstocks depends upon new areas of research and fruit tree propagation allowing the problems of conventional methods to be overcome and enabling rapid multiplication of disease-free fruit plants at a commercial scale (Zhu *et al.* 2005). Micropropagation of apple to produce self-rooted plants will open up new areas of research and will allow changes in traditional fruit tree propagation (Modgil *et al.* 1999). Micropropagation of apple rootstocks has opened up new areas of research and fruit tree propagation allowing the problems of conventional methods to be overcome and enabling rapid multiplication of disease-free fruit plants at a commercial scale **(**Bahmani *et al.* 2009). So far, apple micropropagation has been attempted with only varying success (Marin *et al.* 1993).

Clonal apple rootstocks have been widely used in apple growing. Among these M9 (dwarf), MM106 (semi-dwarf), and MM111 (semi-vigorous) have been well known and used in various types of soils and plantation systems. These rootstocks have been propagated by stool bed layering and rooting of hardwood cuttings. The MM106 and MM111 apple rootstocks can be rooted easily by hardwood cuttings (Hartmann and Kester 1983). MM106 is a clone of apple that has been originated as a cross between Northern spy and Malling 1 (Hartmann *et al.* 2002). Trees on MM106 are well anchored, do not sucker, are semi-dwarfing (60 - 75% the size of trees on apple seedlings), and very productive (Wiley, 1987).

Low molecular weight antioxidants, such as ascorbate, glutathione, and tocopherol, are information-rich redox buffers that interact with numerous cellular components. In addition to crucial roles in defence and as enzyme cofactors, cellular antioxidants influence plant growth and development by modulating processes from mitosis and cell elongation to senescence and death (Potters *et al.* 2004; Tokunaga *et al.* 2005). Localized activity of glutathione could also help elucidate the mechanism of stress resistance. This effect indicates that glutathione may be involved in protection against DNA damage (Lodhi, 1998). Glutathione is a small, ubiquitous molecule that is involved in a plethora of cellular processes in addition to its role as an antioxidant and in the maintenance of cellular redox homeostasis (Schafer and Buettner 2001).

Glutathione is crucial for biotic and abiotic stress management as is a pivotal component of the glutathione-ascorbate cycle, a system that reduces poisonous hydrogen peroxide (Noctor and Foyer 1998). It is the precursor of phytochelatins, side-chain and an antioxidant, preventing damage to important

cellular components caused by reactive oxygen species such as free radicals and peroxides (Pompella *et al.* 2003). Glutathione is found almost exclusively in its reduced form, since the enzyme reverting it from its oxidized form, glutathione reductase, is constitutively active and inducible upon oxidative stress. In fact, the ratio of reduced glutathione to oxidized glutathione (Anna *et al.* 2003) within cells is often used scientifically as a measure of cellular toxicity. Some scientists suggest that rooting of micropropagated plants can be improved by treatment with antioxidants (Stonier, 1971). Antioxidants can potentially protect the natural plant rooting hormones from oxidation, enhancing rooting and increasing the tolerance of plants to greenhouse conditions (Lis-Balchin, 1989).

The aim of the current research was to study the influence of the thiol compound GSH in a wide range of concentrations to induce rooting of MM106 apple rootstock microshoots in combination with the auxin NAA (α-napthaleneacetic acid), as an auxin source. The main goal of this research study was to verify if and up to what extent the combined effect of GSH+NAA exhibits better rooting results than the individual application of NAA under *in vitro* conditions.

MATERIAL AND METHODS

Plant material and culture conditions

The experimental plant material was shoot tip explants from previous *in vitro* cultures provided by the Plant Tissue Culture Laboratory "VITRO HELLAS S.A., Tree and Plant Nurseries, Alexandria, Nisseli Imathias, Greece". The initial material was certified as virus-free. The nutrient medium used was the MS (Murashige and Skoog 1962). The effects of GSH applied exogenously at ten concentrations (0, 2.5, 5, 10, 25, 50, 100, 250, 500, 1000 μM) in combination with 5.4 μM NAA were studied in order to enhance root regeneration of the apple rootstock MM106 (*Malus domestica* Borkh.) under *in vitro* conditions. The explants were grown in glass flat bottom test tubes (25×100 mm) containing 10 ml of MS medium. The culture medium was also supplemented with 30 g/l sucrose and 6 g/l agar (Bacto-Agar; Voigt Global Distribution Inc., Lawrence, USA). The pH of the culture medium was adjusted to 5.8 before adding agar and then the medium was sterilised by autoclaving at 121 °C for 20 minutes. One explant was aseptically transferred to each test tube which was capped with aluminium foil. All the cultures were incubated in a growth room under controlled environmental conditions i.e. a 150 μmol/m^2/s light intensity provided by cool white fluorescent lamps (36W, Philips), a 16 h photoperiod and a 22 ± 1 °C temperature. Mean root number per rooted explant, root length, root fresh weight (FW), rooting percentage, shoot length and shoot FW, callus FW and callus induction percentage were recorded after 28 weeks of explants' maintenance in the rooting media in order to obtain full response.

Statistical analysis

The experimental layout was completely randomised and the data were analysed with ANOVA (Analysis of Variance) using the statistical package SPSS

17.0 (SPSS Inc, Chicago, USA). The experiment was repeated twice and the reported data are the means. The experiment consisted of 10 treatments each one with a total of 10 replicates. To establish significant differences among the treatments, the Duncan's multiple range test was used at $P \leq 0.05$ for mean comparison.

RESULTS AND DISCUSSION

In the control treatment, neither rooting nor callus induction was observed. However, the incorporation of 2.5-500 μM GSH led to root formation (Figure 1a-1e) while GSH applied at 5, 50 or 1000 μM concentrations completely inhibited rooting. Better rooting results in terms of root number/rooted explant (11.67), root length (41.11 mm) and root FW (0.289 g) were achieved with 10 μM GSH. However, 25 μM GSH exhibited the highest rooting percentage (50%) (Table 1).

Table 1. Effect of L-glutathione reduced (GSH) concentration (0-1000 μM) combined with 5.4 μM NAA (a-naphthaleneacetic acid) on average root number/rooted microcutting, root length (mm), root FW (g) and rooting percentage (%) in MM106 apple rootstock.

GSH (μM)	Root number/rooted microcutting	Root length (mm)	Root FW (g)	Rooting (%)
0	0.00 ± 0.00 a	0.00 ± 0.00 a	0.000 ± 0.000 a	0 a
2.5	5.00 ± 0.45 cd	30.63 ± 0.47 e	0.278 ± 0.012 d	28.57 b
5	0.00 ± 0.00 a	0.00 ± 0.00 a	0.000 ± 0.000 a	0 a
10	**11.67 ± 0.34 e**	**41.11 ± 0.91 g**	**0.289 ± 0.031 d**	42.86 c
25	5.50 ± 0.52 d	13.20 ± 0.85 c	0.083 ± 0.012 b	**50 d**
50	0.00 ± 0.00 a	0.00 ± 0.00 a	0.000 ± 0.000 a	0 a
100	5.00 ± 0.00 cd	5.00 ± 0.00 b	0.005 ± 0.000 a	25 b
250	2.50 ± 0.07 b	19.58 ± 1.80 d	0.060 ± 0.007 b	22.22 b
500	4.50 ± 0.37 c	37.50 ± 0.37 f	0.163 ± 0.014 c	22.22 b
1000	0.00 ± 0.00 a	0.00 ± 0.00 a	0.000 ± 0.000 a	0 a
P-values	0.000***	0.000***	0.000***	0.000***

Means ± S.E. with the same letter in a column are not statistically significant different from each other according to the Duncan's multiple range test at $P \leq 0.05$, ***$P \leq 0.001$

Shoot length was augmented by 2.81 cm (from 13.33 mm in the control to 41.43 mm) and a 3-fold increase in shoot FW (from 0.064 g in the control to 0.232 g) was recorded by adding 10 μM GSH to the enriched with 5.4 μM NAA MS medium. Callusing percentage was highest (57.14%) and callus FW (0.699 g) greatest when the explants were treated with 10 μM GSH. The incorporation of 2.5-500 μM GSH led to callus formation while GSH when applied at 5, 50 or 1000 μM concentrations resulted in complete inhibition of callusing (Table 2).

Rooting is affected by numerous endogenous and exogenous factors, with the principal role of auxin as a chief regulator of adventitious root formation.

Blocking the transport of endogenous auxin to seedling rooting zone inhibits rooting (De Klerk *et al.* 1999). In the regulation of adventitious rooting process, glutathione seems to be involved in a complex interplay between auxin and other components of cellular redox systems.

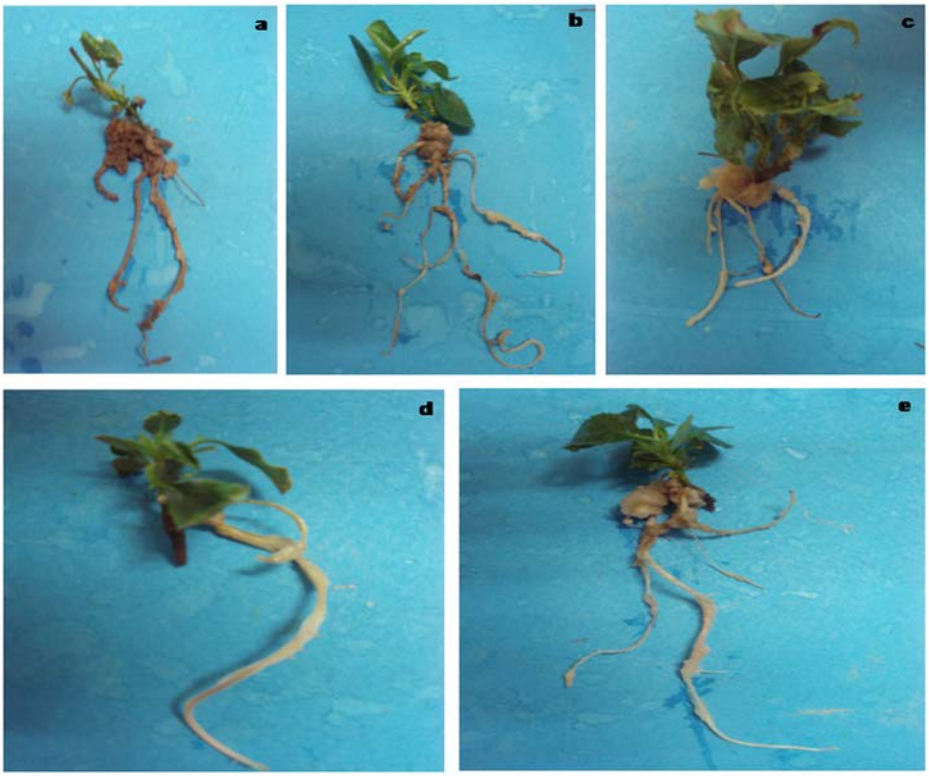

Figure 1. Effect of GSH (0-1000 μM) concentration combined with 5.4 μM NAA on *in vitro* rooting of shoot tip explants of MM106 apple rootstock: (a) 2.5 μM GSH, (b) 10 μM GSH, (c) 25 μM GSH, (d) 250 μM GSH and (e) 500 μM GSH.

In the present study employing the MM106 apple rootstock, GSH in the presence of NAA auxin significantly promoted rooting of shoot tip explants. In accordance with our findings, root formation in shoot cuttings of soybean (*Glycine max* L. 'Williams'), mungbean (*Phaseolus aureas* Mdlbg.), English ivy (*Hedera helix* L.), and apple *(Malus* x *domestica* Borkh. 'Jork 9') was stimulated by GSH in the presence and absence of auxin (IAA: indole-3-acetic acid) shock (Auderset *et al.* 1996). However, no root regeneration in MM106 apple vitroplants occurred in the absence of GSH from the MS containing NAA medium. According to Auderset *et al.* (1996), root number was positively influenced when 100 μM GSH were used simultaneously with IAA in both soybean and mungbean (*P. aureas* Mdlbg.) cuttings. The inclusion of 10 μM GSH to the medium gave the best results regarding root number and root length of MM106 apple microshoots while 25 μM GSH led to the highest rooting

percentage. Similar results were obtained by Auderset *et al.* (1996) in micropropagated apple *(Malus* x *domestica* Borkh. 'Jork 9') shoots derived from callus where 25-100 μM GSH augmented the percentage of rooted explants while root number was increased in the 50-75 μM GSH concentration range. Accordingly, *in vitro* rooting of sweet cherry *(Prunus avium* L.) cv. 'Kristiina (root number and rooting percentage) was significantly promoted by fortifying the MS containing 9.84 μM IBA medium with 25 μM GSH (Vasar, 2004).

Table 2. Effect of L-glutathione reduced (GSH) concentration (0-1000 μM) combined with 5.4 μM NAA (a-naphthaleneacetic acid) on average shoot length (mm), shoot FW (g), callus FW (g) and callus induction percentage (%) in MM106 apple rootstock.

GSH (μM)	Shoot length (mm)	Shoot FW (g)	Callus FW (g)	Callus induction (%)
0	13.33 ± 0.43 a	0.064 ± 0.004 ab	0.000 ± 0.000 a	0 a
2.5	30.00 ± 1.97 e	0.136 ± 0.013 cd	0.431 ± 0.049 c	57.14 d
5	28.00 ± 0.94 de	0.154 ± 0.006 a	0.000 ± 0.000 a	0 a
10	**41.43 ± 4.76 f**	**0.232 ± 0.047 e**	0.699 ± 0.102 e	57.14 d
25	25.00 ± 2.36 cde	0.196 ± 0.028 d	0.149 ± 0.009 b	50 c
50	13.75 ± 0.87 ab	0.073 ± 0.007 a	0.570 ± 0.000 d	25 b
100	20.00 ± 1.29 bc	0.091 ± 0.010 cd	0.000 ± 0.000 a	0 a
250	23.33 ± 2.11 cd	0.121 ± 0.018 b	0.000 ± 0.000 a	0 a
500	20.00 ± 1.29 bc	0.114 ± 0.019 c	0.239 ± 0.013 b	22.22 b
1000	14.44 ± 1.38 ab	0.037 ± 0.006 a	0.000 ± 0.000 a	0 a
P-values	0.000***	0.000***	0.000***	0.000***

Means ± S.E. with the same letter in a column are not statistically significant different from each other according to the Duncan's multiple range test at $P \leq 0.05$, ***$P \leq 0.001$.

The combined effect of GSH + NAA exhibited better rooting results than the individual effect of NAA alone, indicating that GSH and NAA act synergistically in the process of root regeneration at least in the MM106 apple rootstock. Similar findings and conclusions were reported by Imin *et al.* (2007) who found that both reduced (GSH) and oxidised (GSSG) form of glutathione markedly enhance the number of roots formed by callus derived from leaf explants of *Medicago truncatula* cultured on NAA-supplemented medium than on medium supplemented with NAA alone. In the current study with MM106 apple microshoots, GSH was applied only in combination with NAA since preliminary experiments (data not shown) showed that root formation was not stimulated due to GSH application alone in the absence of auxin. Our findings in MM106 apple rootstock are partly in line with those presented by Tyburski and Tretyn (2010) who demonstrated that supplementing the rooting medium with GSH (1-2.5 mM) increased the number of roots formed by tomato seedling cuttings grown on an auxin-free medium, however, the strongest stimulation of

root formation occurred when plants were simultaneously treated with auxin and GSH (Tyburski and Tretyn 2010). In MM106 apple rootstock, GSH when applied at 5, 50 or 1000 μM resulted in complete inhibition of rooting. Standardi and Romani (1990) reported inhibition of rooting in *Malus* due to GSH application at mM concentrations.

Shoot height of MM106 apple microcuttings was substantially enhanced due to GSH (2.5-500 μM) inclusion to the medium. The optimum GSH concentration of 10 μM resulted in an increase of shoot length by 2.81 cm in comparison to the control GSH-untreated MM106 apple explants. Our results are in agreement with those obtained by Nomura *et al.* (1998), who found that 100 μM GSH improved the development of isolated shoot tips of apple. Similarly, in soybean, 100 μM GSH increased shoot length both in the presence and absence of IAA shock alone (Auderset *et al.* 1996). Positive effects of GSH on shoot length were also reported for green onion (*Allium cepa* L., cv. Giza 6) plants (El-Awadi and Abd El Wahed 2012) and *Spilanthes calva* L. *vitro* culture (Shankar *et al.* 2012). In micropropagated MM106 apple shootlets, GSH (2.5-1000 μM) did not exert an inhibitory effect on vegetative growth. On the other hand, in gladiolus, shoot organogenesis frequency and shoot number per explant using leaf segment explants were increased with the addition of 500 μM GSH, however, higher concentrations were found to be inhibitory (Dutta Gupta and Datta 2003).

Callus growth of MM106 apple microcuttings regarding callus FW and callusing percentage was stimulated in a considerable degree by adding GSH to the medium. Similar findings were reported in apple *vitro* culture, where GSH promoted callus growth (Nomura *et al.* 1998), and also in *Pistacia vera* shoot tip culture (Tabiyeh *et al.* 2006) results indicated that GSH reduced the total phenolic compounds, promoting shoot growth. In the present study with MM106 apple rootstock, callus induction percentage was highest with 10 μM GSH. In yew (*Taxus baccata* L.), callogenesis percentage was significantly increased when 100 μM GSH were added to the culture medium (Ghafoori *et al.* 2012).

The mechanism by which thiol compounds might enhance rooting is unknown. For example, GSH reduces auxin effects by forming conjugates (Farago *et al.* 1994). However, there is no precedent for potentiation of an auxin response by thiols. Our findings may be of theoretical importance concerning a potential auxin x thiol interaction in plant growth and differentiation and of practical importance to artificial rooting of woody and herbaceous shoot microcuttings.

CONCLUSIONS

GSH participates in plant regeneration, being involved in mechanisms regulating cell divisions in newly formed meristems and participating in hormone metabolism and signalling. Diverse functions of this antioxidant open vast possibilities of using it for the improvement of tissue culture and plant regeneration methods. However, further studies are required to fully exploit the

properties of GSH for manipulating developmental processes in plant tissue culture..

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STABILITY OF EARLINESS OF AUTOCHTHONOUS POPULATION OF *PHLEUM PRATENSE L.*

SUMMARY

Phleum pratense (L.) is perennial grass. The aim of this paper was to determine the stability of maturing time of germplazm population *Phleum pratense L.* 20 autochthonous populations of *Phleum pratense L.*, originating in western Serbia. Significant differences between the examined populations were determined by analysis of variance. It is evident that the year had a statistically significant influence on the tested parameter in the population of PP20. The start time for maturing for all populations was, on average, 57.49 days for the first study year and 61.31 days for the second year studied. Stability of the examined values for the panicle forming start time, between years, was noted, Cv = 5.86%. The highest stability by years, was tested at population PP10 (CV = 1.63%).

The coefficient of variation for the length of panicle for all populations was Cv = 19.15%. The statistically significantly better productive properties were at the late populations of PP20 (17.08 cm), PP16 (17.08 cm), PP4 (12.77 cm) and PP9 (12.11 cm) compared to other tested populations.

Populations PP20, PP16, PP4 and PP9 have a good basis and can serve as good material for further breeding programs.

Keywords: autochthon populations, *Phleum pratense L.*, stability, maturing time

INTRODUCTION

Timothy - *Phleum pratense (L.)* is an important pasture species in many cool-temperate regions of the world, particularly those with cold winters and moist summers. The plant timothy originated in Europe but it was first valued as pasture grass in the United States. It is valued for hay as it retains good feed quality even when seed heads are present. When compared with the commonly used grasses, such as perennial ryegrass, timothy is late flowering, tending to head 6–10 weeks later than ryegrass. It has less production in winter, but commences spring growth early, giving it a long period with high quality leafy pasture. Commercial cultivars are usually hexaploid, with a chromosome number of 42, but diploid, tetraploid and octoploid forms also occur (Neilson and Nath

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1961, citied Charlton and Stewart, 2000). Timothy cultivars can be classified according to flowering time, with early cultivars developing seed heads around early December and late cultivars have heading around late December. Early-flowering cultivars are usually more erect and have less densely tiller than ones of later-flowering, with a greater tendency to produce flower heads in late summer. Pastures of timothy frequently provide advantages in quality and appearance of animal over those of perennial ryegrass, and this is largely explained by four following factors:

a) Compared to perennial ryegrass, later flowering of timothy's grass, postpones decline in feed quality (which is associated with emergence of seed head), allowing digestibility levels to be maintained from October to December, over a longer period than ryegrass.

b) Timothy decline in feed quality as seed heads develop is less than in perennial ryegrass and its reproductive tillers remain green longer and continue to accumulating carbohydrates, even after the seed matures. Timothy hay, cut at this mature stage, is, therefore of superior feed quality then those made from perennial ryegrass. c) Timothy, as an endophyte-free grass, is superior then endophyte-infected perennial ryegrass pastures, and this difference is more pronounced during summer and autumn, particularly when conditions become dry (Charlton and Stewart, 2000). Authors reported that Timothy prefers moist soils of moderate to high fertility, a soil pH of 5.5– 7.0 and is only slightly less tolerant of soil with aluminium than perennial ryegrass. d) Drought tolerance: Timothy has roots that are shallower and more sensitive to drought, than perennial ryegrass. Timothy responds well to irrigation in drier regions.

Timothy is an important fodder in the regions where the climate in the cultivation season is wet and not too hot. In our country, these areas are over 600-800 m above sea level where Timothy achieved a 10 t ha^{-1} of dry matter in two to three mowing. The crude protein content ranges from 14-17% (Tomić and Sokolović, 2007). High energy value of entire plant and grain is being excellently supplemented with high protein content of alfalfa, why those two feeds are regularly combined in cattle diet - TMR – total mixed ratio, (Dewhurst, 2013, Dubljević et al, 2016). Timothy has a low field emergence capacity, which can result from a large sensitivity of husked seeds to environmental factors but also from a strong response to allelopathic compounds released by other grass species during germination (Lipińska 2006). Too large seed density increases competition and makes emergences difficult, and also reduces the survival rate of seedlings (Martyniak 2005, Szczepanek and Katańska-Kaczmarek, 2012).

The aim of this study was to identify and compare the stability of the maturing time of germplazm population and panicle length of 20 populations germplasm *Phleum pratense* L. originating in western Serbia.

MATERIAL AND METHODS

The study was based on a field experiment, established at Rađevo Selo (233 m.n.v.), (Valjevo, Western Serbia), in the leached soil, in period 2008-2010.

The type of soil on which the experiment was performed was slightly acidic (pH 5.9%), medium fertility, with humus content of 3.59%. The randomized split-plot design was used in three replications. The area of plots was 10 m^2. Sowing of seeds, collected from autochthonous populations, was carried out on October 13, 2008. Tested were 20 autochthonous populations of *Phleum pratense* L., from the area of Western Serbia, Kolubara district, which belongs to the mountainous area and vary in the length of the vegetation period. Tested populations was: PP1, Bobova (453 m.n.v.); PP2, Gornje Leskovice (747 m.n.v.); PP3, Donje Leskovice (747 m.n.v.); PP4, Krčmar (650 m.n.v.); PP5, Bratačić (351 m.n.v.); PP6, Gornji Lajkovac (525 m.n.v.); PP7, Golubac (373 m.n.v.); PP8, Lopatanj (392 m.n.v.); PP9, Planinica (559 m.n.v.); PP10, Pričević (298 m.n.v.); PP11, Lelić (329 m.n.v.); PP12, Struganik (575 m.n.v.); PP13, Sušica (535 m.n.v.); PP14, Suvodanje (543 m.n.v.); PP15, Komirić (283 m.n.v.); PP16, Rožanj (973 m.n.v.); PP17, Gunjaci (395 m.n.v.); PP18, Skadar (530 m.n.v.); PP19, Popučke (161 m.n.v.); PP20, Carina (742 m.n.v.). Kolubara district extends in the north-western part of Serbia and covers an area of 2474 km^2. During the vegetation, the time of the start of panicle forming was monitored, while at the end of the vegetation, at the stage of maturity, from each repetition, 10 plants were taken for morphological analysis of the panicle length of the 20 population of timothy. The time of reaching a maturity for harvesting was different in individual years of the study. The obtained results were analysed statistically for the split-plot design, and the significance of differences was determined using StatSoft, at the significance level p = 0.05.

Meteorological data

Meteorological data were recorded throughout the entire experiment by a meteorological station (Valjevo, Serbia). The average temperature, in the first year, was 11.79°C and total precipitation was 709.80 mm and during the second year average temperature was 12.02°C while the total precipitation was 651.1 mm.

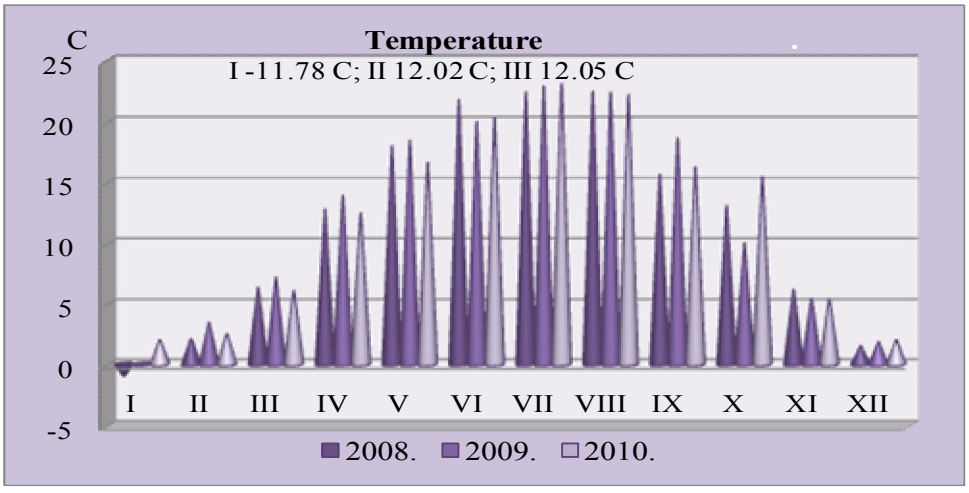


Figure 1. Monthly air temperature average (°C), Valjevo, Serbia

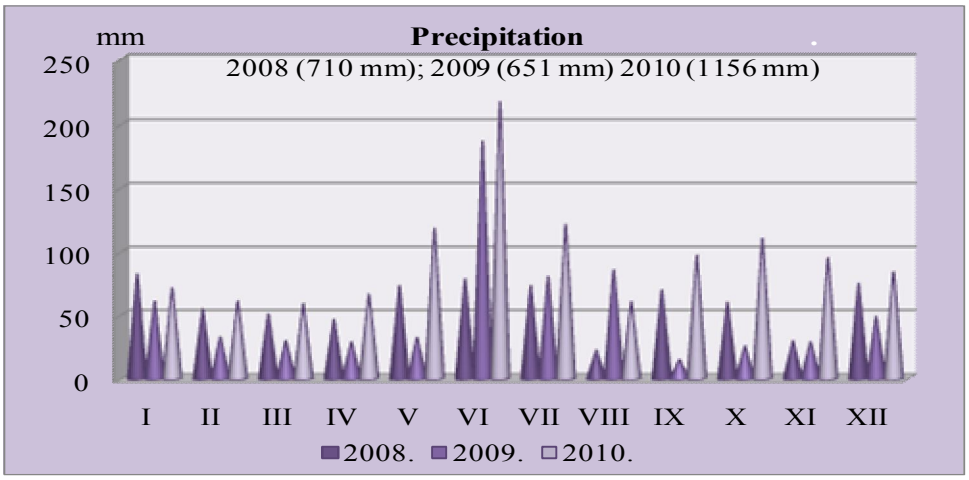


Figure 2. Total precipitation (mm), b, Valjevo, Serbia

During the third year of the average temperature amounted to 12.05°C while the total precipitation was 1156.20 mm (Figure 1 and 2). Meteorological conditions are unpredictable and variable (Popovic, 2010; 2015; Popovic et al., 2015, 2016a, 2016b). The biomass production reduces with increasing in water deficit stress; nonetheless, in major of cases, the tolerant genotypes had less reduction in biomass than ones susceptible under stress caused by drought (Salem, 2003). Water lack stress reduced not only biomass production, but also inhibited partition of photo-assimilates within the plants (Bota et al., 2004).

RESULTS AND DISCUSSION

Table 1 shows the descriptive statistics of the studied properties of the *Phleum pratense* L. populations for the examined years. The population, the year and interaction the investigated factors, had statistical significance for the tested parameters, ($p < 0.05$). The populations had been statistically significantly different. The interaction of the examined factors for the tested parameter were statistically significant ($p < 0.05$). In the second experimental year, a statistically significantly higher difference was made between the value of the tested parameter, compared to the first study year, ($p < 0.05$), tab. 1. Population PP19, PP15, PP11 and PP5 was the shortest time for beginning of panicle forming, while the populations, PP16 and PP9 (67.82 and 63.35) had later beginning of panicle forming, Table 1, Figure 3.

The stability of the examined values for the start time of the creation of panicle between years was noted, Cv = 5.86%. The variability of the examined values within the year for the start time of the creation of panicle varied from $7.35\% < Cv < 10.94\%$.

The coefficient of variation for the start time of panicle forming was ranged in the interval of $1.63\% < Cv < 10.41\%$, Table 1.

Table 1. Start time of the creation of panicle, a., and panicle length, cm, of *Phleum pratense* L.

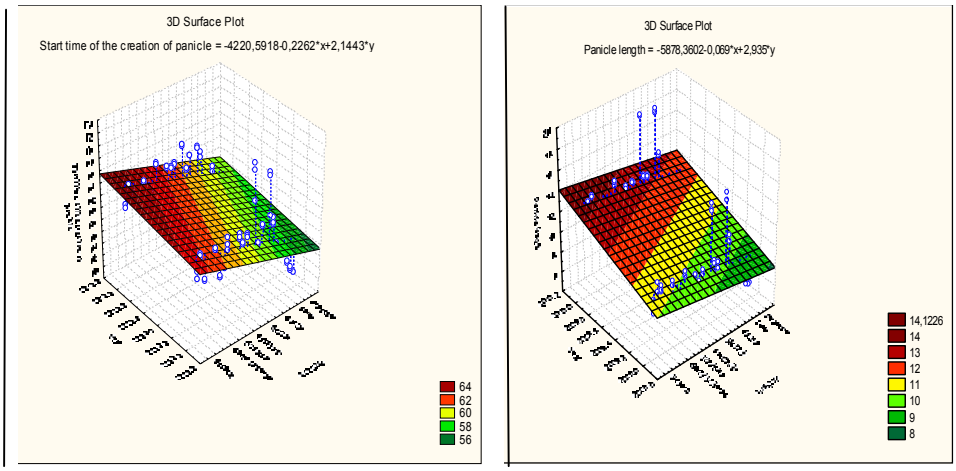
Population	2009	2010	$\overline{X}$	σ	Cv	2009	2010	$\overline{X}$	σ	Cv
	Start time of the creation of panicle, days					**Panicle length**, cm				
PP1	59.06	60.96	60.01	0.95	2.23	9.16	12.04	10.60	1.44	19.21
PP2	61.93	63.80	62.87	0.94	2.10	10.13	13.26	11.70	1.57	18.92
PP3	58.26	61.73	60.00	1.74	4.09	10.36	13.60	11.98	1.62	19.12
PP4	60.90	62.56	61.73	0.83	1.90	11.33	14.20	12.77	1.43	15.89
PP5	56.73	58.30	57.52	0.78	1.93	7.90	10.78	9.34	1.44	21.80
PP6	61.56	63.36	62.46	0.90	2.04	9.96	11.14	10.55	0.59	7.90
PP7	56.73	59.70	58.22	1.48	3.61	8.26	11.26	9.76	1.50	21.73
PP8	57.96	61.60	59.78	1.82	4.31	7.80	10.62	9.21	1.41	21.65
PP9	61.36	65.33	63.35	1.99	4.34	10.50	13.71	12.11	1.61	18.75
PP10	52.73	53.96	53.35	0.61	1.63	6.60	9.70	8.15	1.55	26.89
PP11	55.70	59.93	57.82	2.12	5.17	7.00	10.21	8.61	1.61	26.38
PP12	61.66	63.90	62.78	1.12	2.52	9.80	12.68	11.24	1.44	18.12
PP13	60.06	63.40	61.73	1.67	3.82	8.70	11.50	10.10	1.40	19.60
PP14	61.10	63.73	62.42	1.31	2.98	9.60	12.83	11.22	1.62	20.36
PP15	51.40	53,36	52.38	0.98	2.65	6.23	9.16	7.70	1.47	26.92
PP16	69.43	66.20	67.82	1.62	3.36	15.33	18.83	17.08	1.75	14.49
PP17	58.70	61.86	60.28	1.58	3.71	9.70	12.80	11.25	1.55	19.48
PP18	61.46	63.83	62.65	1.18	2.67	9.53	12.63	11.08	1.55	19.78
PP19	50.43	54.13	52.28	1.85	5.21	5.63	8.63	7.13	1.50	29.75
PP20	55.76	64.63	60.19	6.27	10.41	15.53	18.63	17.08	1.55	12.83
Average	58.64	61.31	59.40	1.59	5.86	9.45	12.41	10.93	1.48	19.15
CV	7.35	10.94	7.82	-	-	27.04	21.41	23.76	-	-

Tested parameters	**LSD**	**Genotype**	**Year**	**Genotype x Year**
Start time of the creation of panicle	0.5	1.006	0.319	1.425
	0.1	1.335	0.423	1.891
Panicle length	0.5	0.383	1.211	0.541
	0.1	0.508	1.606	0.718

The investigated populations statistically significantly differed by the length of the panicle. In the second experimental year, a statistically significantly higher value of the panicle length was achieved, compared to the first test year, ($p < 0.05$), tab. 1. The interaction of the examined factors for the tested parameter was statistically significantly different ($p < 0.05$). PP19, PP15, PP10, PP11 and PP5 had the shortest panicles while the longest had populations PP20 (17.08 cm), PP16 (17.08 cm), PP4 (12.77 cm) and PP9 (12.11 cm), Table 1, Figure 3.

The variability of panicle length, within the year, was $21.41\% < Cv < 27.04\%$. The coefficient of variation for the panicle length for all populations, between

the examined years, was 19.15%. The variability was at an interval of 7.90% <Cv <29.75%, Table 1.



a. **b.**

Figure 3. Start time of the creation of panicle, a., and panicle length, cm, of *Phleum pratense* L.

The statistically significantly better productive properties were at the late populations PP20 (17.08 cm), PP16 (17.08 cm), PP4 (12.77 cm) and PP9 (12.11 cm) compared to other tested populations, Table 1, Figure. 3.

Populations PP20, PP16, PP4 and PP9 have a good basis and can serve as good material for further breeding work.

According to Kryzeviciene [2000] higher yields of timothy seeds can be obtained in early cultivars. According to Rutkowska and Dєbska-Kalinowska [1989], early cultivars of grasses form more fertile shoots in comparison to the late ones. This results from a fast rate of development in the year of foundation and forming autumn shoots with a larger number of leaves, which has a significant effect on seed yield in the next growing season. Schöberlein [1987] indicated that at a larger number of leaves on autumn shoots the ears of timothy were longer and heavier. Optimal supply of plants with water, nutrients and in access of light.

Correlations of studied features

The results of relative dependence of examined indicators of *Phleum pratense* (L.) population were presented by Pearson's correlation coefficient and shown in Table 2. The panicle length has a positive non-significant correlation with start time of the creation of panicle ($r=0.36^{ns}$), Table 2.

Based on the obtained value and also by the appropriate choice of selection methods, we can see that excellent genotypes (PP20, PP16, PP4 and PP9) are available to us for a successful selection process in order to obtain new varieties of *Phleum pratense* (L.).

The efficiency of selection and breeding depends on the present genetic variation in the initial breeding material.

Table 2. The correlation of tested components

Parameters	Panicle length	Start time of the creation of panicle
Panicle length	1.00	0.36 ^{ns}
Start time of the creation of panicle	0.36 ^{NS}	1.00
^{ns} Stat. non significant		

Kryzeviciene [2000] indicate that the yield of an early cultivar of timothy was 3.5 times higher than that of the semi-early and late cultivars. Also, Furuya et al. [1996] report that early cultivars are more productive in growing for seeds. Varied assessment of the reproductive abilities of early cultivars of timothy in the present experiment and other studies does not give the grounds for making conclusions concerning seed productivity only on the basis of ears of grass and maturing times.

SWOT analysis of national breeding programs showed, their strengths are reach gene pool, and traditional selection methods. Institution network in range of zones, varieties adaptation especially in low, and average income segments;, intellectual property IP rights similarity to European ones, and others are also strengths. Weaknesses are old infrastructure and equipment's; limited access to modern technologies; autocratic management; poor breeders motivation etc. Opportunities are possible collaboration development; trailing in different climate zones; seed market potential growth, etc. Realty as subject of raiders attacks; agricultural policy unpredictability; “dyeing” some breeding programs due to cost; erosion of genetic resources; mutual sanctions; availability of know-how and hi-tech in breeding are threats (Goncharov, 2016). Climatic changes increase the level of investment needs required to reach food security mainly via crop breeding (Goncharov, 2016; Capone et al., 2016).

Breeding of perennial grasses, in our conditions, is primarily directed towards increasing yields or maintaining stable yields with quality improvement. Selection in a given direction is done for creating the varieties with different maturing and which are intended for different forms of exploitation (Vučković *et al*., 2007; Babić *et al.* 2015; Vasileva and Vasilev, 2012, 2017, Janković et al, 2017). Seed is a key factor of agricultural prospects, as other agricultural inputs efficacy depends on them in a big scale. Appropriate seeds quality is required to meet the demand of diverse climate conditions and cropping systems. Sustainable agricultural production depends on adapted varieties flow, crops production and efficient channels of quality seeds supply to the farmers (Alabushev, 2010; Goncharov, 2013).

New domestic varieties in Serbia have good chances to be distributed in other republics of the cis cue to similarity of geographical, cultural and economic conditions.

CONCLUSIONS

Timothy is an important pasture species in cool-temperate regions of the world, with cold winters and moist summers.

Variance analysis showed significance for all tested properties. The year, population and interaction of the examined factors had a statistically significant influence on the tested parameters .

The stability of the values for the start time of the creation of panicle, between years, Cv = 5.86% was noted.

The coefficient of variation for the panicle length for all populations was Cv = 19.15%. The statistically significantly better productive properties were at late populations of PP20 (17.08 cm), PP16 (17.08 cm), PP4 (12.77 cm) and PP9 (12.11 cm) compared to other tested populations.

The panicle length has a positive non-significant correlation with start time of its creation ($r=0.36^{ns}$).

Populations PP20, PP16, PP4 and PP9 have a good basis and can serve as good material for further breeding work.

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Gordan S. KARAMAN [1]

NIPHARGUS CYMBALUS, NEW SPECIES AND *N. JOVANOVICI* S. KAR. 1931 IN GREECE (CONTRIBUTION TO THE KNOWLEDGE OF THE AMPHIPODA 298)

SUMMARY

Two members of the family Niphargidae (Amphipoda, Gammaridea) from Greece are treated in this study: From the subterranean waters in Epirus is described and figured new species *Niphargus cymbalus*, sp. n. [type locality: wells in Glikorizo, Epirus, Greece] and its taxonomic position regarding other members of this genus are discussed. *Niphargus jovanovici* S. Karaman, 1931 [loc. typ.: Skoplje, Macedonia] is established at the first time from two localities of Greece [Viotica in Attica and Amarinthos on Euboea island] and taxonomical characteristics of these populations regarding these from Macedonia are analyzed.

Keywords: Amphipoda, taxonomy, new species, *Niphargus cymbalus*, jovanovici, Greece, subterranean waters.

INTRODUCTION

The subterranean fauna of Amphipoda in Greece is very rich but only partially investigated, presented by several families and genera with various number of species. Among the family Niphargidae, genus *Niphargus* Schiödte 1849, was presented by nearly 16+ species known from continental Greece and Greek islands (G. Karaman, 2017). During our recent studies of *Niphargus* samples collected by Italian scientists in Greece, we established one new species from the subterranean waters in Epirus region, *Niphargus cymbalus*, sp. n. and presence of *Niphargus jovanovici* S. Karaman 1931 known from Macedonia, in two localities in Greece.

MATERIAL AND METHODS

The studies material was preserved in the 70% ethanol. The specimens were dissected using a WILD M20 microscope and drawn using camera lucida attachment. All appendages were temporarily submersed in the mixture of glycerine and water for study and drawing. Later, all appendages have been transferred into Liquid of Faure on permanent slides. The body-length of examined specimens were measured by tracing individual's mid-trunk lengths (from tip of head to end of telson) using camera lucida. All illustrations were inked manually.

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Note: The authors declare that they have no conflicts of interest. Authorship Form signed online.

Some morphological terminology and setae formulae follow G. Karaman's terminology (Karaman, G. 1969; 2012) regarding the last mandibular palpus article [A= setae on outer face; B= setae on inner face; C= additional setae on outer face; D= lateral marginal setae; E= distal long setae] and propodus of gnathopods 1 and 2 [S= corner S-spine on outer face; L= lateral slender serrate L-spines; M= facial M-setae; R= subcorner R-spine on inner face]. Terms "setae" and "spines" are used based on its shape, not origin. The study was realizes based on morphological, ecological and zoogeographical research.

TAXONOMICAL PART

Family NIPHARGIDAE

NIPHARGUS CYMBALUS, sp. n.
Figures 1-4, 5A-H

MATERAL EXAMINED: GREECE:

G-17= Epirus, Glikorizo, Arta, 10 m a.s.l., freshwater well, water temperature. 11.5oC, pH 7, 24.2.1976, one exp. accompanied by *Niphargus* sp. (leg. Argano, Pesce & Bianco).

DIAGNOSIS (males only):

Body moderately stout, rostrum short, peduncular article 3 of antenna 1 short; accessory flagellum 2-articulated; urosomal segments 1-2 with spines on each dorsolateral side, urosomal segment 3 naked. Epimeral plates acute. Coxa 4 with ventroposterior lobe, coxa 5 shorter than 4; mandibular palpus article 1 naked. Maxilla 1 outer plate spines with several teeth each, palpus short. Maxilliped unknown.

Gnathopods 1-2 relatively small, article 3 with one posterior bunch of setae; propodus trapezoid, as large as coxae, with L-setae sitting laterally of S-spine and with dactylus bearing a row of setae along outer margin. Dactylus of pereopods 3-7 moderately strong, with one slender spine at inner margin. Basipodit of pereopods 5-7 large, ovoid, lobed. Pleopods 1-3 with 2 retinacula and peduncles scarcely setose. Uropod 1 peduncle with dorsointernal and dorsoexternal row of spines, rami of equal length. Uropod 2 with equal rami, spines are short. Uropod 3 unknown. Telson is relatively short, deeply incised, with 3 distal spines on each lobe.

DESCRIPTION

Male 6.1 mm (holotype): Body moderately stout. Head with short rostrum and subrounded lateral cephalic lobes, ventroanterior excavation developed (fig. 1A), eyes absent. Metasomal segments 1-3 with 2-4 dorsoposterior marginal setae each (fig. 3E). Last mesosomal segment at ventral margin with 2 median sexual tubercles (fig. 5D); urosomal segment 1 and urosomal segment 2 on each dorsolateral side are provided with 2 strong spines (fig. 5H); urosomal segment 3

naked. Urosomal segment 1 near basis of uropod 1 peduncle are with one strong spine (fig. 5H).

Epimeral plates 1-3 are sharply acute, with inclined posterior margin bearing several short setae each (fig. 3E). Epimeral plate 1 naked, provided with slightly concave ventral margin; epimeral plates 2 and 3 are with slightly convex ventral margin bearing 2-3 subventral spines each (fig. 3E).

Antenna 1 hardly shorter than half of body-length; peduncular articles 1-3 progressively shorter (ratio: 55:36:24), very scarcely setose (fig. 1B); last peduncular article is not elongated. Main flagellum is consisting of 16 articles (most of them with one short aesthetasc (fig. 1C).

Accessory flagellum 2-articulated, exceeding half of last peduncular article (fig. 1D).

Antenna 2 moderately slender, peduncular article 4 slightly longer than article 5 (ratio: 65:58), bearing several bunches of setae (the longest setae slightly exceeding the diameter of article itself); article 5 along ventral margin with 3 bunches of setae (the longest setae longer than diameter of article itself); flagellum moderately slender, scarcely setose, consisting of 9 articles (fig. 1E); antennal gland cone is short (fig. 1E).

Mouthparts well developed (maxilliped missing). Labrum is broader than long. with poorly concave distal margin (fig. 5A).

Labium is with well developed inner lobes, outer lobes entire, distally subrounded (fig. 5B).

Mandible: molar triturative. Right mandible: incisor with 4 teeth, lacinia mobilis bifurcate, accompanied by 8 rakers (fig. 1F). Left mandible: incisor with 5 teeth, lacinia mobilis with 4 teeth accompanied by 7 rakers. Palpus consisting of 3 articles: first article is short, naked; second article is provided with 7 setae (fig. 1G); third article is subfalciform, with nearly 16 D-setae and 6 E-setae, on outer face is attached group of 3 A-setae (fig. 1H), on inner face of third article are attached 4 single B-setae (fig. 1G).

Maxilla 1: inner plate is narrow, with 1-2 distal setae; outer margin with 7 spines bearing 5-6 lateral teeth each (fig. 1 I); palpus 2-articulated, not reaching distal tip of outer plate- spines, consisting of 2 articles and provided with 5 distal setae.

Maxilla 2 is with well developed lobes bearing numerous distal marginal setae each, facial setae absent (fig. 5C).

Maxilliped unknown (missing).

Coxae are of moderate size. Coxa 1 is as long as broad, with subrounded ventroanterior corner and bearing 5-6 marginal short setae (fig. 2A). Coxa 2 is poorly longer than broad (ratio: 60:59), with subrounded ventral margin bearing nearly 7 short setae (fig. 2D). Coxa 3 is remarkably longer than broad (ratio: 85:55), with convex anteroventral margin, bearing nearly 6 short marginal setae (fig. 3A). Coxa 4 is longer than broad (ratio: 87:78), with convex ventral margin bearing nearly 7 short setae and with developed ventroposterior lobe (fig. 3C).

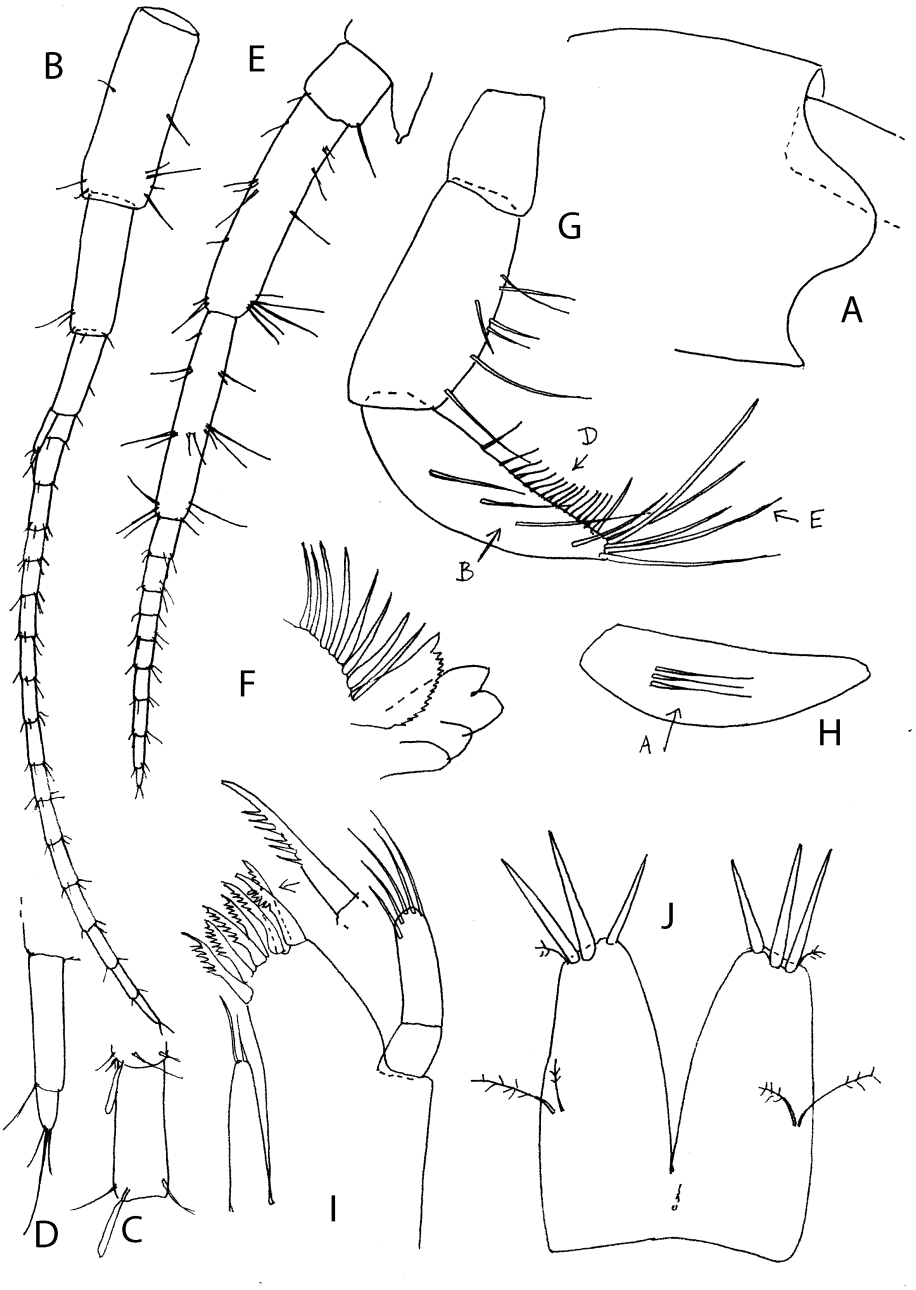

Figure 1. *Niphargus cymbalus*, sp. n., Glikorizo, Arta, Epirus, male 6.1 mm (holotype): A= head; B= antenna 1; C= aesthetasc; D= accessory flagellum; E= antenna 2; F= left mandible incisor and lacinia mobilis; G= mandibular palpus, inner face (B= B-setae, D= D-setae; E= E-setae); H= third article of mandibular palpus, outer face with A-setae; I= maxilla 1; J= telson.

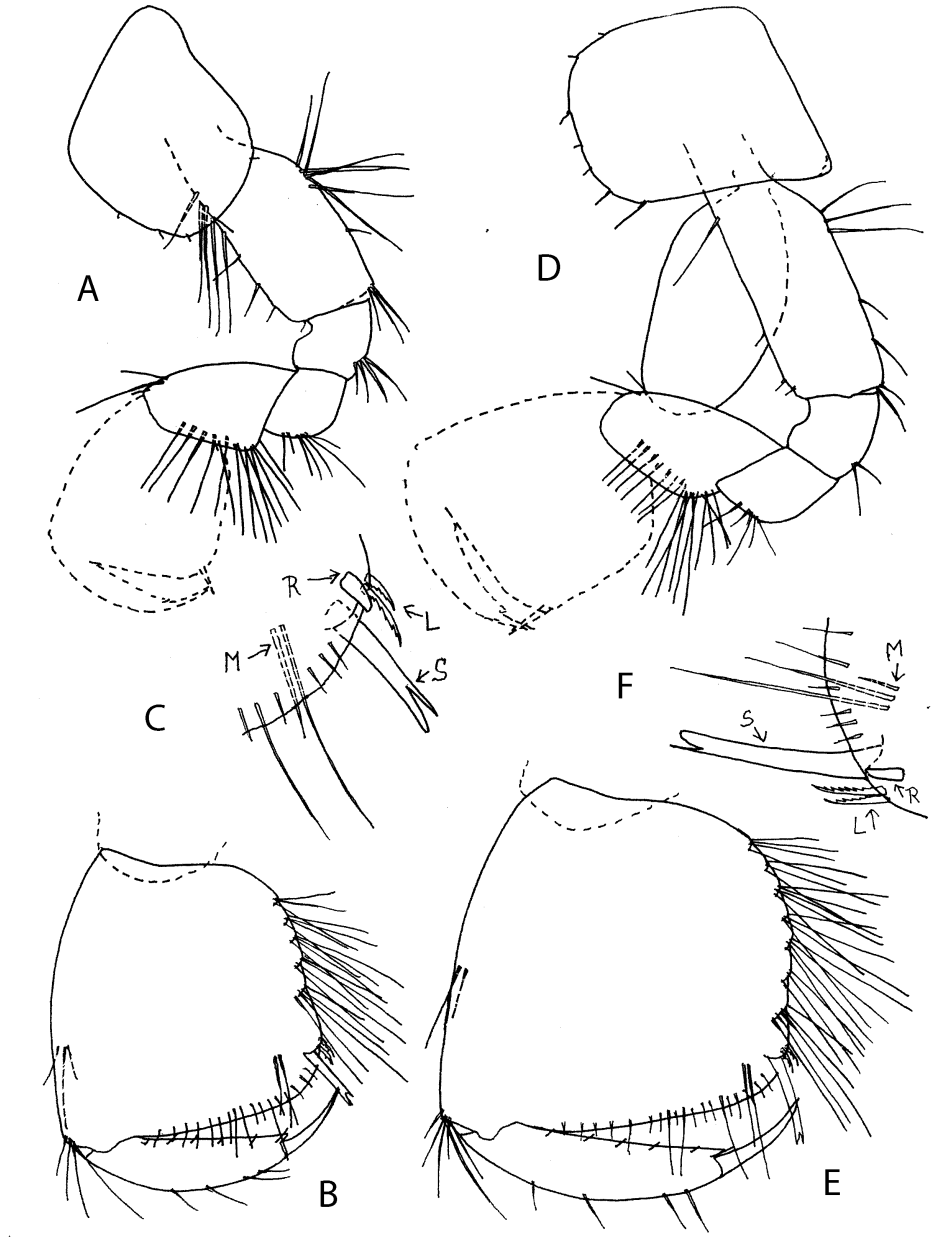

Figure 2. *Niphargus cymbalus*, sp. n., Glikorizo, Arta, Epirus, male 6.1 mm (holotype): A-B= gnathopod 1, outer face; C= distal corner of gnathopod 1 propodus (S= corner S-spine; L= lateral L-spines; M= facial M-setae); D-E= gnathopod 2, outer face; F= distal corner of gnathopod 2 propodus, inner face (S= corner S-spine; L= lateral L-spines; M= facial M-setae; R= subcorner R-spine).

Coxae 5-7 are short. Coxa 5 is broader than long (ratio: 64:43), remarkably shorter than coxa 4, anterior lobe relatively short (fig. 4A).

Coxa 6 is broader than long (ratio: 54:32), remarkably shorter than coxa 5 (fig. 4C).

Coxa 7 is entire, broader than long (ratio: 57:25) (fig. 4E).

Gnathopods 1 and 2 are relatively small, nearly as large as corresponding coxa (fig. 2A, D). Gnathopod 1: article 2 along proximal part of anterior and posterior margin, with rows of long setae, in distal part is with shorter setae (fig. 2A); article 3 at posterior margin with one bunch of setae; article 5 is slightly shorter than propodus (ratio: 42:49), along anterior margin with distal bunch of setae, along posterior margin with numerous long setae. Propodus trapezoid, slightly longer than broad (ratio: 83:75), along posterior slightly inclined margin appear 5 transverse rows of setae (fig. 2B); palm is slightly convex, inclined rather less than half of propodus-length, defined on outer face by one corner S-spine accompanied laterally by 2 serrate L-spines and with 2 facial M-setae, on inner face appear one subcorner R-spine (fig. 2C). Dactylus reaching posterior margin of propodus, along outer margin are attaches 4 single median setae (fig. 2B), at inner margin appear several short setae.

Gnathopod 2 is distinctly larger than gnathopod 1 (fig. 2D). Article 2 is in proximal part with long setae and in distal part with shorter setae; article 3 at posterior margin with one bunch of 2 median setae; article 5 is slightly shorter than propodus (ratio: 48:60), along anterior margin with one bunch of distal setae, along posterior margin with numerous setae. Propodus is trapezoid, poorly longer than broad (ratio: 100:94), along posterior margin with 7 transverse rows of setae (fig. 2E); palm slightly convex, inclined nearly 1/3 of propodus-length, defined on outer face by one corner S-spine accompanied laterally by 2 L-spines and 2 long facial M-setae, on inner face by one subcorner R-spine (fig. 2F). Dactylus reaching posterior margin of propodus, along outer margin are attached 4 single median setae, along inner margin appear several short setae.

Pereopods 3 and 4 are relatively slender. Pereopod 3: along anterior slightly concave margin of article 2 appear 2 long setae in proximal part and 2-3 short setae in distal part, along posterior margin are attached 2 proximal long setae and several short setae (fig. 3A). Articles 4-6 are of different length (ratio: 60:40:50), articles 4 and 5 along both margins with single spine-like setae; article 6 along posterior margin with 4 single short spines, at tip with one long and 2 short setae. Dactylus is much shorter than article 6 (ratio: 24:50), at inner margin with one weak spine, at outer margin with one median plumose seta; nail is shorter than pedestal (ratio: 30:45) (fig. 3B).

Pereopod 4: article 2 with long setae in proximal part and short setae in distal part; articles 4-6 of different length (ratio: 54:40:47); articles 4-5 along both margins with short setae and spine-like setae; article 6 at posterior margin with 4 groups of single short spines and setae (fig. 3C). Dactylus is much shorter than article 6 (ratio: 21:47), at inner margin with one slender spine near basis of the nail, along outer margin with one median plumose seta; nail is shorter than pedestal (ratio: 30:36) (fig. 3D).

Pereopods 5-7 are relatively short, with large article 2. Pereopod 5 is distinctly smaller than pereopods 6 and 7 (fig. 4A, C, E), article 2 ovoid, slightly

broader than long (ratio: 90:68), anterior margin remarkably convex but without lobe, provided with row of 10 short spine-like setae (fig. 4A), along posterior strongly ovoid margin appear nearly 8 short setae, ventroposterior lobe shallow. Articles 4-6 relatively slender, of different length (ratio: 40:45:52), along both margin with short and long slender spines (the longest spines exceeding the diameter of articles themselves). Article 6 with 2 long distal setae. Article 2 is remarkably longer than article 6 (ratio: 90:52). Dactylus is moderately slender, much shorter than article 6 (ratio: 23: 52), at inner margin with one slender spine near basis of the nail, at outer margin with one median plumose seta (fig. 4B); nail is shorter than pedestal (ratio: 27:46).

Pereopod 6 is ovoid, longer than broad (ratio: 100:75), along anterior convex margin appear a row of 5 median single spine-like setae and bunch of distal short spines; posterior strongly convex margin is provided with nearly 11 short setae, ventroposterior lobe well developed (fig. 4C). Articles 4-6 moderately strong, of different length (ratio: 54:60:75), along both margins with short and long spines (the longest spines exceeding the diameter of articles themselves); article 6 with 3 distal setae. Article 2 is longer than article 6 (ratio: 100:75). Dactylus is moderately strong, much shorter than article 6 (ratio: 27:75), along inner margin with one slender spine, at outer margin with one median plumose seta (fig. 4D); nail is shorter than pedestal (ratio: 32:57).

Pereopod 7: article 2 is ovoid, longer than broad (ratio: 90:73), with convex anterior margin bearing medially nearly 5 spine-like setae, anterior lobe is not developed; posterior convex margin is provided with nearly 11 short setae, ventroposterior lobe well developed (fig. 4E). Articles 4-6 are moderately strong, of different length (ratio: 46:57:82), along both margins with strong spines. Article 2 is longer than article 6 (ratio: 90:82). Dactylus is moderately strong, much shorter than article 6 (ratio: 27:82), at inner margin with one slender spine near basis of the nail, at outer margin with one median plumose seta (fig. 4F); nail is shorter than pedestal (ratio: 35:63).

Pleopods 1-3 with 2 retinacula each. Peduncle of pleopod 1 along anterior margin with 3 short setae (fig. 5E); peduncle of pleopod 2 with one median short seta at anterior margin (fig. 5F); peduncle of pleopod 3 along posterior margin with 4 single setae (fig. 5G).

Uropod 1: peduncle with dorsoexternal and dorsointernal row of strong spines (fig. 5H); inner and outer ramus are of equal length; inner ramus is with 4 lateral and 3-4 distal strong short spines; outer ramus is with nearly 6 lateral short spines and distal bunch of 4 short spines.

Uropod 2: peduncle is with 2 distal strong spines; outer and inner ramus are of equal length; outer ramus is poorly curved upwards, bearing one lateral and 4 distal short spines; inner ramus is straight, bearing 2 lateral and 4 distal short spines (fig 5H).

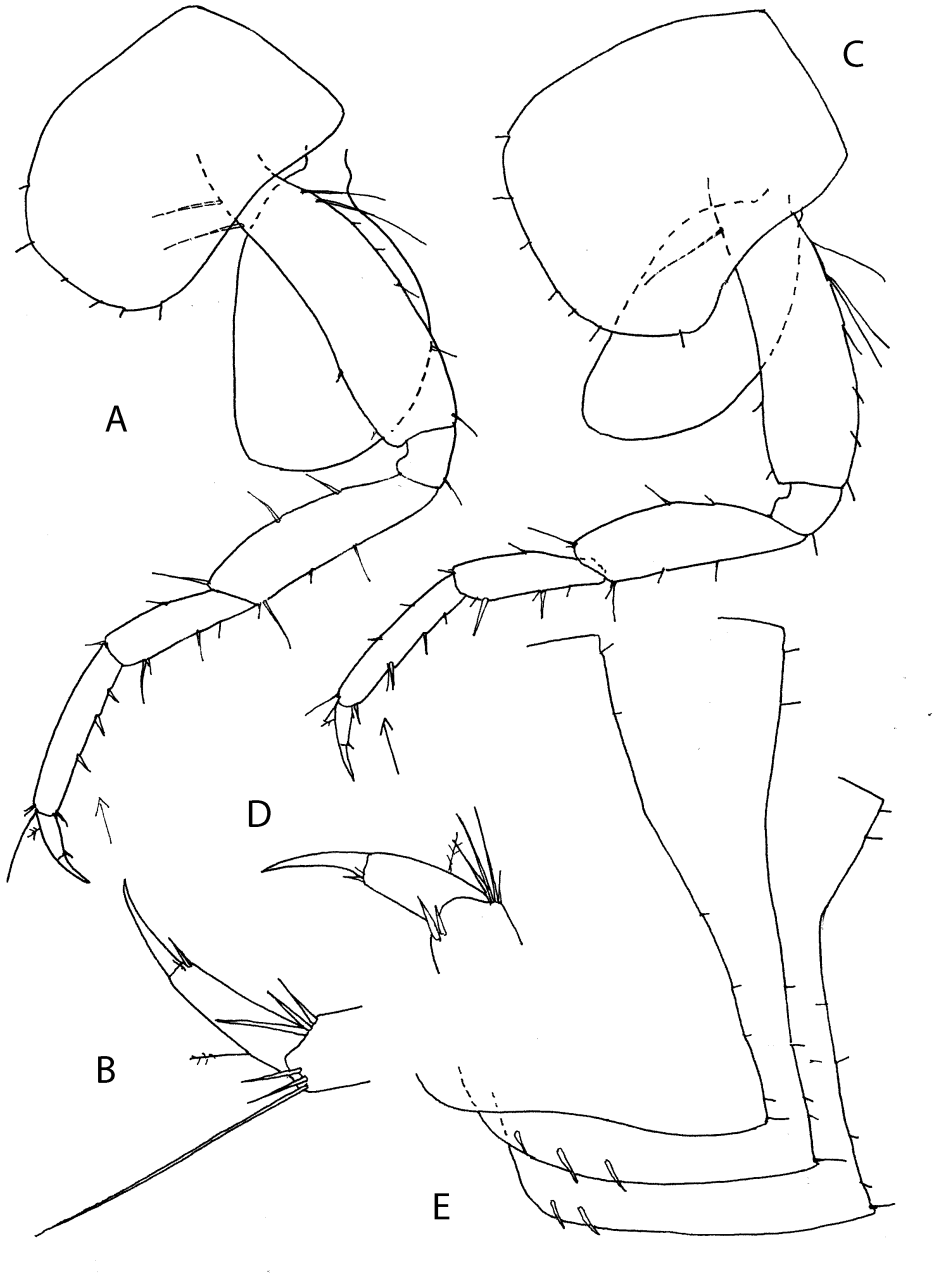

Figure 3. *Niphargus cymbalus*, sp. n., Glikorizo, Arta, Epirus, male 6.1 mm (holotype): A-B= pereopod 3; C-D= pereopod 4; E= epimeral plates 1-3.

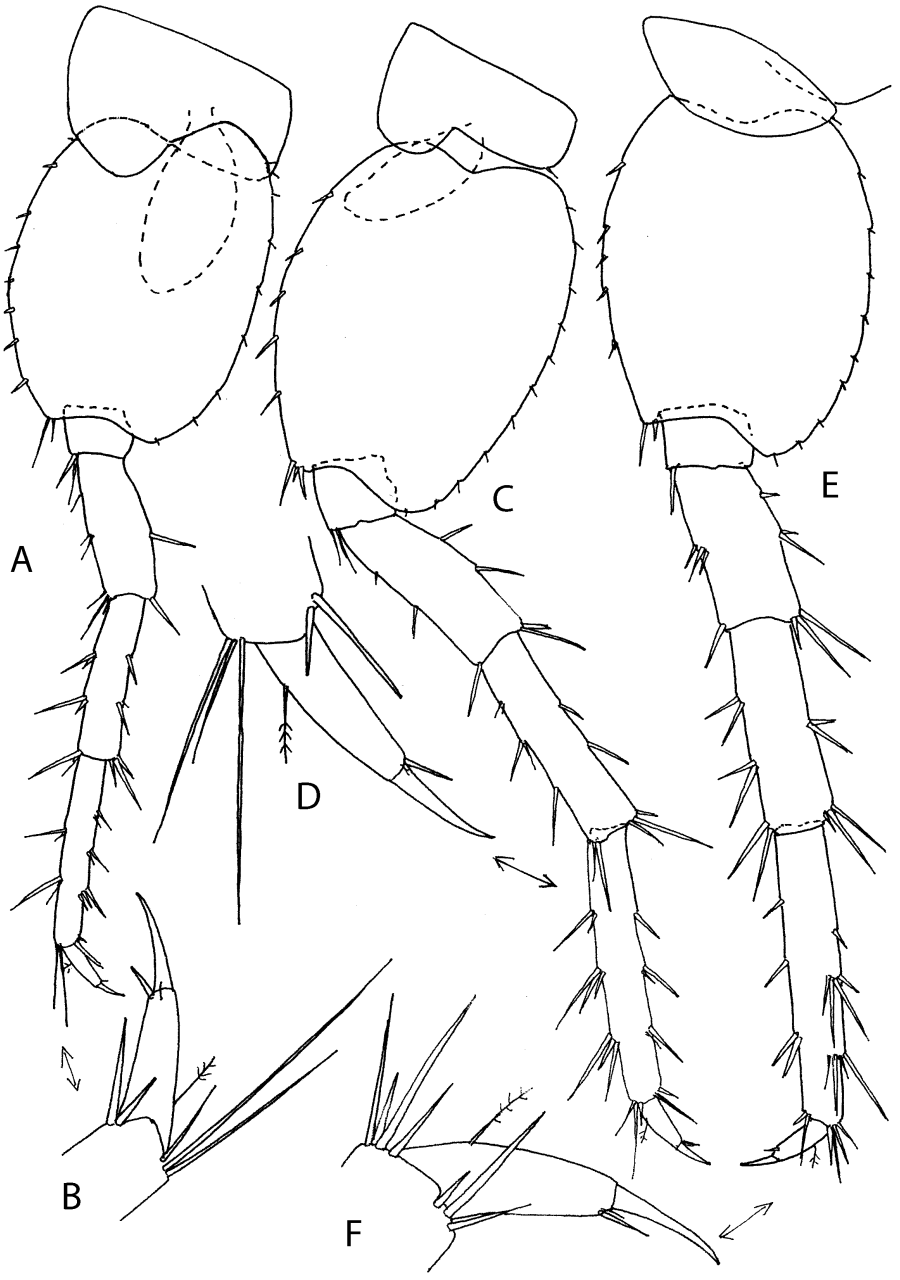

Figure 4. *Niphargus cymbalus*, sp. n., Glikorizo, Arta, Epirus, male 6.1 mm (holotype): A-B= pereopod 5; C-D= pereopod 6; E-F= pereopod 7.

Uropod 3 unknown [based on the taxonomic characteristics of the body, we suppose that uropod 3 should be short and strong, with short distal article of outer ramus].

Telson is not elongated, slightly longer than broad (ratio: 85:74), incised over ¾ of telson-length; each lobe is provided with 3 distal spines (the longest spine reaching half of telson-length), lateral and facial spines are absent; one short plumose seta is attached at external distal tip of each lobe (fig. 1J). One pair of 2 unequally long plumose setae is attached along outer margin nearly the middle of each lobe.

Coxal gills are ovoid, on gnathopod 2 and pereopods 3-4 nearly reaching ventral tip of corresponding article 2 (fig. 2D). Coxal gills on pereopods 5-6 are short, on pereopod 6 coxal gill is shorter than that of pereopod 5 (fig. 4A, C).

Females are unknown, probably similar to the males.

VARIABILITY: unknown.

HOLOTYPE: male 6.1 mm (slides G17/1, G17/2, G17/3) are temporarily deposited in KARAMAN`s Collection in Podgorica, Montenegro.

REMARKS AND AFFINITIES

Niphargus cymbalus differs from all known species of genus *Niphargus* known from Greece by very large ovoid basipodit of pereopods 5-7, but this character is present in various other species from adjacent regions.

At the first glance, *N. cymbalus* is rather similar to *Niphargus lourensis* Fišer et al. 2006, described from Greece (loc. typ.: spring of Louros River) by short uropod 3, absence of lateral and facial spines on telson, shape of gnathopods), but *N. lourensis* differs by much more narrowed basipodit of pereopods 5-7, higher number of setae on maxilla 1 inner plate, etc.

By the shape of basipodit of pereopods 5-7, lobate coxa 4, acute epimeral plates and almost pectinate outer plate spines of maxilla 1, *N. cymbalus* is rather similar to *Niphargus skopljensis* S. Karaman, 1929, known from Macedonia [loc. typ. subterranean waters in Skoplje], but *N. skopljensis* differs remarkably from *N. cymbalus* by presence of only one median seta on dactyls of gnathopods 1 and 2, by fully pectinate outer plate spines of maxilla 1, by different shape of propodus of gnathopods 1 and 2, etc.

Another species with very large ovoid lobed basipodit of gnathopods 1-2 is *Niphargus factor* Karaman & Sket, 1990 known from Bosnia and Herzegovina [loc. typ.: Vjetrenica cave near Trebinje], but this species differs from N. *cymbalus* by very long and slender dactylus of all pereopods, long and narrow telson, narrow propodus of gnathopods 1-2, etc.

Niphargus numerus Karaman & Sket, 1990 from Croatia [loc typ.: Čavlinska pećina-Cave near Obrovac, Croatia] is provided also with very large basipodit of pereopods 5-7, acute epimeral plates and telson with distal spines only, differing remarkably from *N. cymbalus* by "*kochianus*" type of gnathopods

1-2, by long slender dactylus of all pereopods, less serrate maxilla 1outer plate spines, short stout uropods 1-2, etc.

Niphargus melticensis Dancau & Andreev, 1973 from Bulgaria [loc. typ.: well in Sokolovo, Lowech district] is provided with large lobate basipodit of pereopods 5-7, but it differs from *N cymbalus* by presence of only one median seta on dactylus of gnathopods 1-2, elongated article 5 of gnathopods 1-2, etc.

Niphargus asper G. Karaman, 1972 known from Montenegro (Crna Gora) [loc. typ.: wells in Podgorica (= Titograd)] is provided also with large ovoid basipodit of pereopods 5-7, with several setae along outer margin of dactylus in gnathopods 1-2, acute epimeral plates, like these in *N. cymbalus*, but this species differs remarkably from *N. cymbalus* by different shape of gnathopods 1-2, long telson, presence of one lateral tooth on spines of maxilla 1 outer plate, etc.

In Italy appear also taxa similar to *N. cymbalus*, provided with large ovoid basipodit of pereopods 5-7, pectinate or multispinose spines of outer plate in maxilla 1, short palpus of maxilla 1, presence of several setae on outer margin of gnathopods 1 and 2 dactylus: *N. stefanellii* Ruffo & Vigna Taglianti 1968 [loc. typ.: Roma, Italy] and *N. ictus* G. Karaman 1986 [loc. typ. Grotta di Fiume-Cave, Marche, Italy], but these species differ distinctly from *N. cymbalus* by more spinose telson and combination of other characters [pectinate maxilla 1, elongated peduncular article 3 of antenna 1, etc.].

To this group belongs also *Niphargus hebereri* Schellenberg 1933 described from Croatia [loc. typ.: wells in Rovinj, Istra], species very similar to *N. cymbalus* by various characters (epimeral plates, pereopods, maxilla 1), but this species differs from later by different shape of propodus in gnathopods 1-2, by longer and narrow telson, unequal rami of uropod 2, etc.

DERIVATIO NOMINIS. The name *cymbalus* regards the association of broad pereopods of this species with the musical percussion instrument cymbal.

NIPHARGUS JOVANOVICI S. Karaman, 1931
Figures: 5 I, J, 6

Niphargus jovanovici S. Karaman 1931: 93, figs. 1-2; S. Karaman 1932: 220, 226; G. Karaman 1980: 17; Pesce & Maggi 1983: 58; G. Karaman & Ruffo 1986: 526;

Niphargus jovanovici (part.) Carausu, Dobreanu & Manolache 1955: 262, figs. 243-245;

Niphargus jovanovici jovanovici S. Karaman 1943, pl. III, figs. 43-62; Sket 1972: 10, fig. 107; Barnard J.L. & Barnard, C.M. 1983: 692;

Niphargus (*Jovaniphargus*) *jovanovici jovanovici* S. Karaman 1960: 86, fig. 5;

nec *Niphargus jovanovici jovanovici* Dobreanu, Manolache & Puscariu 1951: 579, figs. 1-2 (= *serbicus*).

MATERIAL EXAMINED:

GREECE:

G-84 Attica, Viotia, main road Thebes-Lamia, Levadia, freshwater well, water temperature 16°C, pH 6.9, NO_2 1 mg/l; one exp., 10.5.1977 (leg. Pesce, Maggi & Miranda);

G-201 (=S-7336) = Euboea island, Amarinthos, road Chalchis-Aliverion, on km 2.500, 2 wells, water temperature 15.1°C, pH 7.1; 30.6.1980, one ovigerous female (leg. G. Pesce).

MACEDONIA:

Nr.-57= Spring near Kumanovska reka-River (near Kumanovo), 2.6.1955 (leg. Kiro Bogoevski), one exp. mixed with *Niphargus skopljensis* S. Karaman, 1929;

M-16= Skopje Andja well, 1929, many exp. mixed with *Niphargus skopljensis* (leg. S. Karaman).

REMARKS.

Niphargus jovanovici has been discovered and described by S. Karaman in 1929 from the wells in Skoplje, and later was mentioned by some authors from various other localities in Macedonia only. Pesce & Maggi (1983) generally mentioned *N. jovanovici* for Ionian islands and northern Greece, but without any locality or description.

Among the studied samples of *Niphargus* collected by various samplers from Greece, we established the presence of *N. jovanovici* in samples from Amarinthos and Levadia. Taxonomic characters of these specimens agree mainly with these from Macedonia.

Ovigerous female 8.2 mm from Amarinthos: Head with short rostrum and subrounded lateral cephalic lobes. Metasomal segments along dorsoposterior margin with 4 short dorsoposterior marginal short setae each (fig. 6E). Epimeral plates 1-3 acute, with posterior margin bearing scarce number of setae (fig. 6B); epimeral plates 2 and 3 with one subventral spine each.

Urosomal segment 1 on each dorsolateral side with 1 seta, urosomal segment 2 on each dorsolateral side with one slender spine, urosomal segment 3 naked.

Antenna 1 rather shorter than body length, peduncular articles 1-3 are progressively shorter, main flagellum consisting of 20 articles. Flagellum of antenna 2 consisting of 10 articles. Mandibular palpus article 1 naked, article 2 with 9 setae; article 3 falciform, with one bunch of 3 A-setae, 4 single B-setae, nearly 18 D-setae and 4 E-setae.

Maxilla 1 inner plate with one seta, outer plate with 7 spines (6 spines with 1-2 teeth, inner spine with 3 teeth), palpus 2-articulated, short, with 3 distal spines.

Maxilliped inner plate short, with 2 distal spines and single setae, outer plate short, with 6 distolateral (mesial) pointed spines, palpus article 4 with one seta at inner margin near basis of the nail.

Propodus of gnathopods 1 and 2 is ovoid, that of gnathopod 1 is hardly longer than propodus of gnathopod 2 (fig. 5 I, J). Palm of gnathopod 1 propodus is with convex margin bearing on outer face one corner S-spine accompanied by

one lateral L-spine and 3 facial M-setae, on inner face appear one longer R-spine. Palm of gnathopod 2 propodus is on outer face with one S spine accompanied laterally by one L-spine and 2 facial M-setae, on inner face by one longer R-spine (fig. 6A). Dactylus of gnathopods 1-2 is with 2-3 short outer marginal median setae (fig. 5 I, J).

Pereopods 3-7 like specimen figured by Stanko Karaman (1943) from Skoplje.

Pereopods 5-6 like these figured by S. Karaman (1943).

Pereopod 7 is long, much exceeding posterior tip of the body; article 2 is linear, much longer than broad (ratio: 77:36), with concave posterior margin bearing proximal small lobe, along posterior margin are attached nearly 7 short setae (fig. 6C); articles 4-6 are of different length (ratio: 60:87:107), along anterior and posterior margin with numerous slender spines. Article 2 is shorter than article 6 (ratio: 77:107). Dactylus is slender, much shorter than article 6 (ratio: 40:107), at inner margin with one slender spine, at outer margin with one median plumose seta (fig. 6D); nail is much shorter than pedestal (ratio: 10:30).

Pleopods 1-3 are with 2 retinacula. Peduncle of pleopod 1 is with 3 median short setae along anterior margin; peduncle of pleopod 2 naked; peduncle of pleopod 3 is provided with 2 short median setae along posterior margin.

Uropod 1 peduncle is provided with dorsointernal and dorsoexternal row of spines. Rami of uropod 1 are of equal length, with several lateral spines and long distal spines (fig. 6E).

Uropod 2 is with equal rami bearing long distal spines (the longest spines are almost as long as rami themselves) (fig. 6F).

Uropod 3 is relatively short, with inner ramus scale-like bearing distal spine and plumose seta; outer ramus 2-articulated: first article along outer margin with 4 bunches of spines, along inner (mesial) margin with 4 bunches of longer slender spines mixed with single plumose setae (fig. 6G); second auricle is short, without plumose setae or spines.

Telson is slightly longer than broad (ratio: 54:45), incised only slightly over half of telson-length; each lobe is provided with 2 slender distal spines and one short plumose seta, accompanied laterally by 3 very long plumose setae (fig. 6H).

Specimen from Viotia (juv. 5.5 mm) agree with specimen from Amarynthos, but propodus of gnathopod 1 is hardly more narrow than that of gnathopod 2, with dactylus bearing 1-2 short median setae along outer margin. Distal spines on rami of uropods 1 and 2 are rather longer than these in specimen from Amarinthos.

VARIABILITY

Dactylus of gnathopods 1-2 in specimens of *N. jovanovici* from Macedonia is provided usually with one median seta only, but the specimen from spring near Kumanovska reka-River is provided with 1-2 median setae along outer margin. Evidently, this character is rather variable within *N. jovanovici*.

The length of distal spines on rami on uropods 1 and 2 is also rather variable within specimens from various localities in Macedonia and Greece.

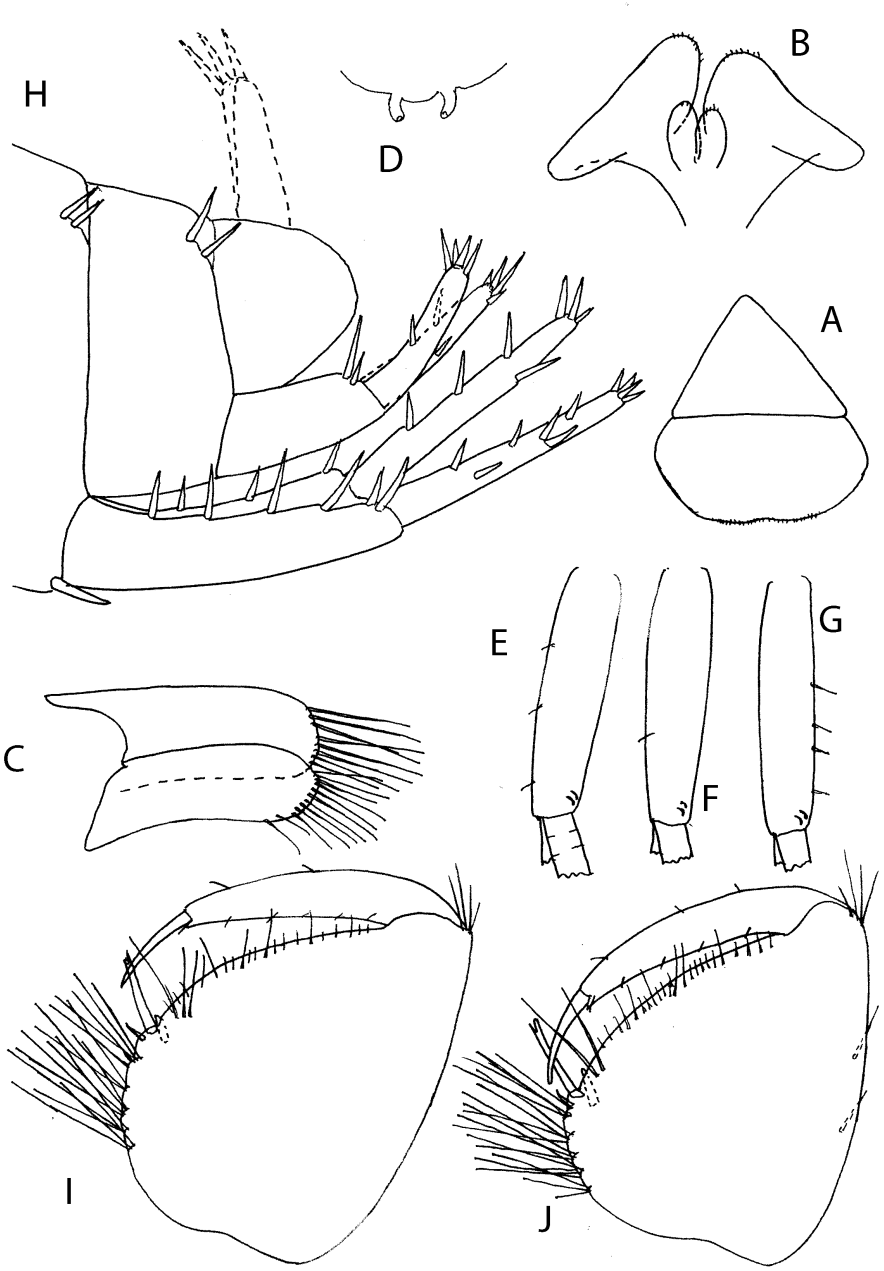

Figure 5. *Niphargus cymbalus*, sp. n., Glikorizo, Arta, Epirus, male 6.1 mm (holotype): A= labrum; B= labium; C= maxilla 2; D= sexual papillae on ventral side of last mesosomal segment; E-F-G= peduncle of pleopods 1-2-3; H= urosome with uropods 1-2. *Niphargus jovanovici* S. Karaman, 1931, Amarinthos, female 8.2 mm: I= gnathopod 1 propodus; J= gnathopod 2 propodus.

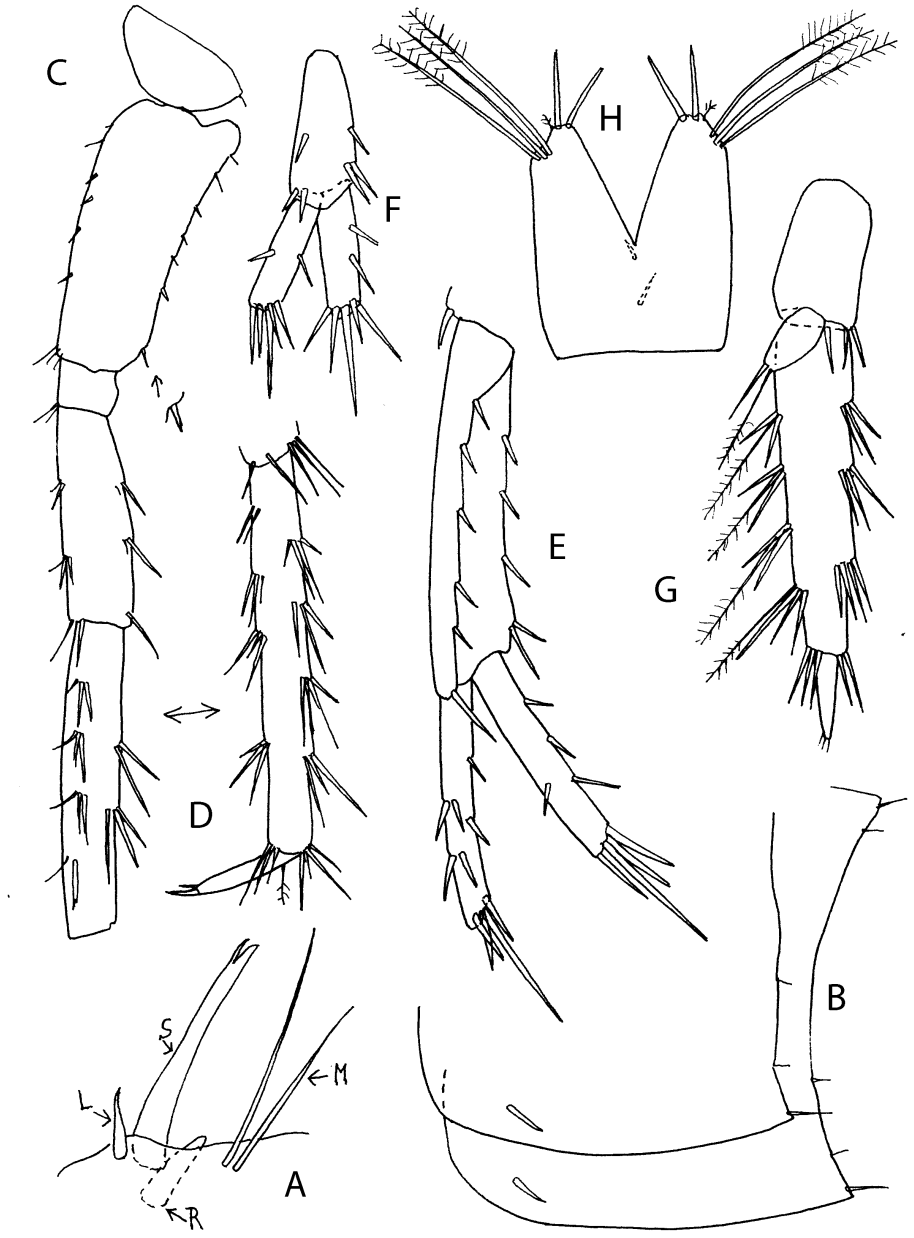

Figure 6. *Niphargus jovanovici* S. Karaman, 1931, Amarinthos, female 8.2 mm: A= distal corner of gnathopod 2 propodus, outer face (S= corner S-spine; L= lateral L-spine; M= facial M-setae; R= subcorner R-spine); B= epimeral plates 2-3; C-D= pereopod 7; E= uropod 1; F= uropod 2; G= uropod 3; H= telson.

CONCLUSIONS

Based on present research, the number of known species of genus *Niphargus* is elevated to 17+ species.

Stanko Karaman established subgenus ***Niphargus (Jovaniphargus)*** n. sbg. with typus generis *Niphargus jovanovici* S. Kar. 1931, including in it all species with long plumose setae on telson, large ovoid propodus of gnathopods 1-2, narrowed basipodit of pereopods 5-7, etc. [*N. serbicus* S. Karaman, 1960), *N. multipennatus* Sket 1956, *N. grandii* Ruffo 1937, *N. kieferi* Schellenberg 1936, *N. gallicus* Schellenberg 1935, *N. bajuvaricus* Schellenberg 1932, *N. microcerberus* Sket, 1972, *N. aberrans* Sket 1972, etc.].

It was very useful attempt to divide large amount of *Niphargus* species (now over 300 taxa) into smaller entities based on certain morphological characters. The further study of these taxa based also on other characters, will clear taxonomical position of all these species to each other and within genus *Niphargus*.

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LENGTH-WEIGHT RELATIONSHIPS AND CONDITION FACTOR OF 11 FISH SPECIES FROM THE TIMIŞ RIVER, WESTERN ROMANIA

SUMMARY

Length-weight relationship (LWR) parameters and Fulton's condition factor (K) were evaluated for eleven native fish species collected from October 2014 to May 2015 from the Timiş River, in western Romania, namely: *Alburnus alburnus* L., 1758; *Cobitis taenia* L., 1758; *Gobio gobio* L., 1758; *Leucaspius delineatus* Heckel, 1843; *Pseudorasbora parva* Temminck & Schlegel, 1846; *Rhodeus amarus* Bloch, 1782; *Romanogobio albipinnatus* Lukasch, 1933; *R. kesslerii Dybowsky*, 1862; *Squalius cephalus* L., 1758; *Sabanejewia balcanica* De Filippi, 1863 and *Vimba vimba* L., 1758.

Parameter *b* ranged from 1.339 (*Leucaspius delineatus*) to 3.277 (*Gobio gobio*), r^2 values ranged from 0.723 (*Pseudorasbora parva*) to 0.935 (*Alburnus alburnus*), whereas the average K values varied between 1.03 (*Sabanejewia balcanica*) and 2.22 (*Rhodeus amarus*).

These are the first estimated parameters of length-weight relationships for the fish species inhabiting the Timiş River basin. In addition, from all studied species data regarding LWRs for cyprinids *Romanogobio albipinnatus* Lukasch, 1933 and *R. kesslerii* Dybowsky, 1862 were unknown before to FishBase.

Keywords: Timiş River, length-weight relationship, FishBase, Romania

RESULTS AND DISCUSSION

Although the Timiş River is the main lotic system in the south-western Romania and the largest river of the Banat area, representing a site of comunity interest listed in Natura 2000 network, there is still a paucity of biometric data on fish species living in this water basin (Bănăduc et al., 2013; Năstase and Oţel, 2016).

Length-weight relationship (LWR) is usually estimated for giving information about the growth patterns of a fish species, while condition factor is used to provide the state of well-being of individuals in their habitat (Nehemia et al., 2012; Omar et al., 2015; Stavrescu-Bedivan et al., 2016).

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Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

As no study currently exists on the type of growth of fish species from the Timiş River, the aim of this report was to investigate the length-weight relationship, Fulton's condition factor and the growth pattern for the fish species collected from the above mentioned freshwater ecosystem.

This study was conducted in western Romania, along the Romanian length of the Timiş River.

Fish samples were collected from 26 different stations, covering a distance of 144 km, between Lugoj (45°41′10″N; 26°54′2″E) and Grăniceri, near Romanian-Serbian border (45°26′22″N; 20°52′59″E), respectively.

In surveys taking place monthly from October 2014 to May 2015, overall 529 fish individuals belonging to 22 species, 19 genera and five families were obtained using electrofishing from the Timiş River freshwater ecosystem, as follows: Centrarchidae (*Lepomis gibbosus* L., 1758 - pumpkinseed); Cobitidae (*Cobitis taenia* L., 1758 - spined loach, *Sabanejewia aurata* De Filippi, 1863 - golden spined loach, *S. balcanica* Karaman, 1922 - Balcan spined loach); Cyprinidae (*Abramis brama* L., 1758 - freshwater bream, *Alburnus alburnus* L., 1758 - bleak, *A. chalcoides* Güldenstädt, 1772 - Danube bleak, *Alburnoides bipunctatus* Bloch, 1782 - schneider, *Carassius auratus* L., 1758 - European crucian carp, *Gobio gobio* L., 1758 - gudgeon, *Leucaspius delineatus* Heckel, 1843 - belica, *Leuciscus aspius* L., 1758 - asp, *Pseudorasbora parva* Temminck & Schlegel, 1846 - stone moroko, *Rhodeus amarus* Bloch, 1782 - European bitterling, *Romanogobio albipinnatus* Lukasch, 1933 - white-finned gudgeon, *R. kesslerii* Dybowsky, 1862 - Kessler's gudgeon, *R. uranscopus* Agassiz, 1828 - Danubian longbarbel gudgeon, *Rutilus rutilus* L., 1758 - roach, *Squalius cephalus* L., 1758 - chub, *Vimba vimba* L., 1758 - vimba bream); Gobiidae (*Proterorhinus marmoratus* Pallas, 1814 - tubenose goby); Ictaluridae (*Ameirus nebulosus* Lesueur, 1819 - brown bullhead). Only *Ameiurus nebulosus* and *Lepomis gibbosus* are introduced, the remainder being native fish species in Romanian ichthyofauna.

The scientific names and occurence of each identified species were confirmed using the FishBase (Froese and Pauly, 2017). After sampling, fish specimens were preserved in recipients filled with 4% formalin solution and transported in laboratory for biometric analysis.

From the total of collected fish, 506 individuals from selected 11 species were considered in this study for estimating LWRs, only those with sample size ≥ 10 specimens.

All collected fish specimens were measured for length (standard length, SL ± 1 mm) and weighed (total weight, TW ± 0.001 g). It is known that, over time, the caudal fins of collected and stored fish could become brittle and break off, in which case the measurement of standard length instead of total length is preferred (Önsoy et al., 2011).

The relationship between the length and the weight of fish was calculated through linear regression (log W= log a + b *log L), where log a (intercept of the regression), and coefficient b (slope) offers information about the growth pattern (Froese, 2006).

When $b > 3$, positive allometric pattern of growth was indicated, while low values of b (< 3) exhibit negative allometric or hypoallometric growth (Karachle and Stergiou, 2012). The coefficient of determination (r^2) and confidence intervals ($CI_{95\%}$) for parameters *a* and *b*, were computed by the least-square method using PAST (Paleontological Statistics Software) version 3.04 and MS-Excel 2010.

Fulton's condition factor (*K*) of the fish species in their habitat was calculated using the equation: $K= (W/L^3)*100$ (Froese, 2006).

All 11 examined fish species are native in Romania. The biological data (parameters of LWR and their confidence intervals, range for length and weight, *K* Fulton's condition factor, r^2 coefficient of determination, allometric type of growth) for each analyzed species (sex combined) are shown in Table 1.

Length-weight equations determined from regression of log weight on log length of the selected fish indicated a negative allometric type of growth for both cobitids (*Cobitis taenia*, *Sabanejewia aurata*) and four cyprinids species (*Leucaspius delineatus*, *Pseudorasbora parva Squalius cephalus* and *Vimba vimba*), while cyprinds *Alburnus alburnus*, *Gobio gobio*, *Rhodeus amarus*, *Romanogobio albipinnatus* and *R. kesslerii* seems to increase in body thickness rather than in length, exhibiting a positive allometric pattern of growth.

The mean value of Fulton's condition factor (*K*) of the captured fish in the study area varied from 1.03 (*Sabanejewia balcanica*) to 2.2 (*Rhodeus amarus*).

No information regarding length-weight relationship was reported previously in the FishBase online database for *Romanogobio albipinnatus* Lukasch, 1933 and *R. kesslerii* Dybowsky, 1862 (Froese and Pauly, 2017). Moreover, to the best of author's knowledge, this study provides the first findings on length-weight relationship parameters for native fish species from the Timiş River.

The slope (*b*) values for selected species were within expected range of 2.5-3.5, except for *Leucaspius delineatus*, represented by a small sample size. The average coefficient of determination (r^2) was 0.874; for seven species from the total of 11, the r^2 value was > 0.874. The calculated r^2 values for all fishes could be affected by the using of combined sexes, as suggested before (Esmaeili et al., 2014).

The high values of *K* for *P. parva*, *R. amarus* and *S. cephalus* might indicate that these species are living well in the habitat and have fat deposits due to their feeding activity (Falaje et al., 2015; Banerjee et al., 2016).

More than a half of studied fishes seem to have a negative allometric growth type, but further investigations are required to detect the factors that could influence the growth patterns, e.g. sample size, fish age or water quality parameters (Stavrescu-Bedivan et al., 2016).

Considering the above mentioned statements, it can be concluded that the contributions presented in this paper will be useful references for fishery biologists in future studies on the population assessment of the species inhabiting the Timiş River freshwater ecosystem.

Table 1. Length-weight relationship parameters (*n*, sample size; range, minimum and maximum size; *a*, antilog of the intercept; *b*, slope, $CI_{95\%}$, confidence intervals of *a* and *b*, respectively; r^2, coefficient of determination), condition factor (*K*) and type of growth, tg (A+, allometric positive; A -, allometric negative) for ten fish species from the Timiş River, Romania. Bold: fish species with LWR reported here for the first time. SL- standard length (cm); W-total weight of the fish (g).

Family	Species	*n*	SL range	W range	*a*	$CI_{95\%}$ of *a*	*b*	$CI_{95\%}$ of *b*	r^2	*K*	tg
Cobitidae	*Cobitis taenia* (L., 1758)	22	2.80-9.02	0.23-6.55	0.0186	0.0066-0.0517	2.690	2.145-3.234	0.841	1.10	A-
Cobitidae	*Sabanejewia balcanica* (De Filippi, 1863)	41	3.70-7.51	0.97-3.80	0.0117	0.0055-0.0247	2.906	2.476-3.336	0.827	1.03	A-
Cyprinidae	*Alburnus alburnus* (L., 1758)	71	2.30-8.20	0.08-10.11	0.0087	0.0064-0.0117	3.215	3.012-3.418	0.935	1.22	A+
Cyprinidae	*Gobio gobio* (L., 1758)	22	3.30-8.60	0.65-10.72	0.0088	0.0041-0.0186	3.277	2.847-3.708	0.926	1.47	A+
Cyprinidae	*Leucaspius delineatus* (Heckel, 1843)	10	2.20-5.30	0.51-1.70	0.0168	0.0280-0.4038	1.339	0.720-1.958	0.756	1.88	A-
Cyprinidae	*Pseudorasbora parva* (Temminck& Schlegel, 1846)	29	3.92-5.31	1.98-3.37	0.0976	0.0414-0.2300	2.037	1.539-2.535	0.723	1.96	A-
Cyprinidae	*Rhodeus amarus* (Bloch, 1782)	154	2.30-6.60	0.25-6.84	0.0204	0.0164-0.2543	3.044	2.894-3.193	0.914	2.22	A+
Cyprinidae	***Romanogobio albipinnatus*** **(Lukasch,**	17	3.60-7.10	0.77-4.95	0.0136	0.0062-0.0297	3.008	2.533-3.483	0.924	1.41	A+
Cyprinidae	***Romanogobio kesslerii*** **(Dybowsky, 1862)**	10	3.20-6.50	0.44-4.43	0.0076	0.0018-0.0313	3.325	2.426-4.224	0.901	1.29	A+
Cyprinidae	*Squalius cephalus* (L., 1758)	96	2.95-21.07	0.40-214.33	0.0227	0.0149-0.0345	2.915	2.738-3.092	0.919	1.92	A-
Cyprinidae	*Vimba vimba* (L., 1758)	34	2.40-11.60	0.34-23.54	0.0325	0.0208-0.0506	2.588	2.372-2.805	0.948	1.44	A-

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The International System of Units (SI) should be used.

- CONCLUSIONS

The conclusion should present a clear and concise review of experiments and results obtained, with possible reference to the enclosures.

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