Journal of Applied Botany and Food Quality 86, 37 - 46 (2013), DOI:10.5073/JABFQ.2013.086.006

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Diversity of volatile patterns in sixteen *Fragaria vesca* L. accessions in comparison to cultivars of *Fragaria ×ananassa*

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(Received April 4, 2013)

Summary

Fragaria vesca is the most distributed wild species in the genus *Fragaria*. Due to this biogeography, a high diversity is to expect. During two harvest seasons, sixteen accessions from different locations from the most eastern habitat at Lake Baikal in Siberia, from Middle and Southern Europe and Northern Europe with Scandinavia and Iceland were investigated as well as two of the three described North American subspecies and three *F. vesca* cultivars. Five very distinct European *F. ×ananassa* cultivars were chosen to serve as a comparison. Beside brix value and acid contents, the aroma patterns including 67 volatile compounds were quantified by stir bar sorptive extraction followed by gas chromatography with mass spectrometric detection. The diversity of important volatiles of *Fragaria vesca* and *F. ×ananassa* is discussed regarding biogeography, domestication, breeding and the so-called funnel effect.

Introduction

The wild strawberry *Fragaria vesca* L. (wood strawberry) is an old food, ornamental, and medicinal plant. Already the Roman poet Ovid mentioned *Fragaria* twice and very likely meant *Fragaria vesca* (Darrow, 1966). In the Middle Ages, the wood strawberry became a Christian symbol and is to find in many paintings from that time. *Fragaria vesca* is the most widely distributed species of the genus *Fragaria* and appears circumpolar throughout Europe, Northern America, and Northern Asia. Since the Middle Ages, the so-called 'semperflorens' or 'alpine' forms of *F. vesca* ssp. *vesca* have been cultivated in European gardens (Shulaev, 2010) together with *F. moschata* L. and *F. viridis* Duch., two other wild species occurring in European forests. After the 1750's the cultivated wild-types were replaced by the garden strawberry (*F. ×ananassa* Duch.) with its bigger fruits and a unique pleasant flavor.

F. vesca (2n = 2x = 14) has a very small genome of 240 Mb. As a diploid herbaceous plant with remontant behavior in case of the forma semperflorens, it became the model plant for molecular studies in the Rose family (*Rosaceae*) (Hollender et al., 2012). In 2010, genome sequencing was completed for a Hawaiian accession of the wood strawberry (Shulaev et al., 2010).

The octoploid *F. ×ananassa* Duch. (2n = 8x = 56), which derives from a spontaneous hybridization of the two octoploids *F. chiloensis* (L.) Miller and *F. virginiana* Miller, is not a direct descendant of *F. vesca*. However, genome research revealed that the two parental species of the garden strawberry derived from different diploid ancestors including *F. vesca*. (Rousseau-Gueutin et al., 2009; Shulaev et al., 2010).

Since the Middle Ages, wild-type strawberries were praised for their intense flavor (Darrow, 2005). The aroma of wild strawberry is perceived as very aromatic and more herbaceous than those of *F. ×ananassa* cultivars (Hirvi and Honkanen, 1982). Therefore, *F. vesca* serves as a comparison with regard to flavor. In direct comparison of cultivars and wild-types, the sensory impression of the latter was described as much more sweetish-aromatic than those of the *F. ×ananassa* cultivars but with some astringent and bitter impressions (Ulrich et al., 2007). *F. vesca* is characterized by outstanding flowery notes like violet and acacia. But especially in the white mutant *F. vesca* f. *alba* (Ehrh.) Staudt, these impressions sometimes were described by the testers with negative statements like over-aromatic and perfume-like. By gas chromatography-olfactometry (GCO) experiments, the flowery impressions were assigned to the content of the aromatic ester methyl anthranilate whereas the herbaceous impressions are caused by a high content of terpenoids.

Fragaria vesca always was involved in breeding experiments. Since the typical high aromatic flavor was missed in the *F. ×ananassa* cultivars, cross-breeding started already in 1918 by Elisabeth Schiemann (Kuckuck, 1980). Successful ways of species introgression with the cultivated strawberry using cross-breeding and chromosome doubling were first described by Scott (1951). Hereby, decaploid fertile clones could be obtained. The first decaploid cultivars were introduced under the denomination *Fragaria × vescana* by Bauer and Bauer (1979), combining characteristics of *F. vesca* and *F. ×ananassa*. Synthetic octoploids were obtained from the inclusion of *Fragaria vesca* in the breeding pedigree by Evans (1977) and Bors and Sullivan (2005). Nowadays, breeding programmes with *F. vesca* focus on the inheritance of methyl anthranilate and several resistances (Pinker et al., 2012).

The aroma of the wood strawberry was investigated in detail for the first time by Drawert et al. (1973). The content of 40 identified main peaks in *F. vesca* exceeds those of the *F. ×ananassa* cultivar cv. 'Revata' by the factor of 4.7, which is in agreement with later findings of Ulrich et al. (1997) (factor 3.2 in comparison to cv. 'Elsanta'). Additionally, Aharoni et al. (2004) discussed the differences in the patterns of volatile organic compounds (VOCs) between *F. vesca* and *F. ×ananassa*. In this paper the differences are interpreted as a gain and loss of metabolic diversity caused by domestication and breeding. In plant breeding this process also is known as funnel effect (Ulrich et al., 1997; Ulrich and Olbricht, 2011) or bottlenecking (Doebley et al., 2006). A general shortcome of several published investigations of VOC profiles is the neglect of the environmentally caused dynamic of the volatile patterns. It is unquestioned that beside genetic prerequisites outer factors like maturity at harvest, location, climate, irrigation, harvest time as well as the attack by pathogens have an eminent influence on biosynthetic network and hence on the development of the whole volatile patterns (quantitative and qualitative!) (Forney et al., 2000; Azodanlou et al., 2004; Olbricht et al., 2011). To quote an example, Jouquand et al. (2008) observed changes in ester contents of fruit from cultivated strawberries between two harvest dates of the same season up to 350 %. This volatile dynamic was in the same order like those caused by the cultivars. Consequently, the use of samples from only few or even one harvest date gives results which represent only a 'snapshot' of the possible dynamic of metabolite patterns.

Therefore, the topic of this investigation is to study the diversity of volatile patterns in a collection of 16 *F. vesca* accessions in comparison to selected *F. ×ananassa* cultivars in two harvest years

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by using a sampling strategy which prevents the influence of maturity and harvest date during one season.

Materials and methods

Plant material and cultivation

Fragaria vesca is the most distributed wild species of the genus and exists with four subspecies, six formae and several cultivars (Staudt, 1962; Staudt, 1989). Due to this biogeography, a high diversity is to expect. Therefore, 16 accessions from different locations from the most eastern habitat at Lake Baikal in Siberia, from Middle and Southern Europe and Northern Europe with Scandinavia and Iceland were investigated as well as two of the three described North American subspecies and three cultivars (Fig. 1). The used accessions are listed in Tab. 1. Five very distinct European *F. ×ananassa* cultivars were chosen to serve as a comparison. They were selected with regard to their breeding history and demonstrate with different aroma patterns the process of domestication over a period of nearly 80 years.

All *Fragaria vesca* accessions were cultivated in the field at Dresden-Weixdorf, Germany, longitude 13.8°, latitude 51.145°, 200 m above sea level, on sandy loam on gravel ground (German classification: 'Ackerzahl' 37). Each accession was growing in a quadrate of 1 sqm containing plants of different ages according to their natural habitats. Neither chemical treatment nor extra fertilizer were applied, except for two applications of insecticide during the time of flowering in order to prevent damage by the strawberry weevil (*Anthonomus signatus* Say) in both years. Irrigation only was applied to avoid damage by drought. The cultivars (*F. ×ananassa*) were planted on the same location in rows on a field under the same conditions and with two additional treatments of fungicide during the time of flowering in order to prevent infection by grey mold (*Botrytis cinerea* Pers.). In 2011 and 2012, fruits of one year old plants were harvested.

Harvest and storage

All available, fully ripe, typical and healthy fruits of all *Fragaria vesca* accessions and all available fruits from six clone plants of each *F. ×ananassa* cultivar were harvested (Fig. 2). The weight per *F. vesca* accession varied depending on the fruit size (for example between 115 g with 198 fruits for 'Multiplex' and 1054 g with 902 fruits for 'Red Wonder' in 2012). All fruits were immediately frozen at minus 20 °C after the sepals were removed.

Extraction of fruit volatiles by immersion stir bar sorptive extraction (imm-SBSE)

To prepare an enzyme inhibited strawberry juice, all frozen fruits of one accession or cultivar of the whole season were pooled. One mass part of fruits without sepals were homogenized in one volume part of a solution of 18.6 % (m/v) NaCl by a household mixer for 2 min. The homogenate was centrifuged 4000 rpm for 30 min. One hundred milliliter of the supernatant were mixed with 10 µl internal standard (0.1 % (v/v) 2,6-dimethyl-5-hepten-2-ol dissolved in ethanol). For each sample, three head-space vials containing 3 g NaCl each for saturation were filled with 10 ml of the supernatant, sealed with magnetic crimp caps including septum, and stored at 4 °C for up to three weeks.

An aliquot of 8 ml of the saturated homogenate but without the solid NaCl deposit was transferred in an empty glass vial for volatile isolation by SBSE. A stir bar with 0.5 mm film thickness and 10 mm length coated with polydimethysiloxan (PDMS) was placed in the liquid (Gerstel, Mülheim an der Ruhr, Germany). The stir bar was moved at 350 rpm at room temperature for 45 min. After removal from the strawberry juice, the stir bar was rinsed with purified water, gently dried with a lint-free tissue and then transferred into a glass tube for thermal desorption and subsequent GC analysis.

Gas chromatography – mass spectrometry

The parameters for the thermal desorption unit (TDU, Gerstel) and the cold injection system (CIS4, Gerstel) were the following: thermal desorption at 250 °C, cryo trapping at -150 °C. The TDU-CIS4 unit was used in Gerstel-modus 3: TDU splitless and CIS4 with 15 ml/min split flow.

The analyses were performed with an Agilent Technologies 6890N

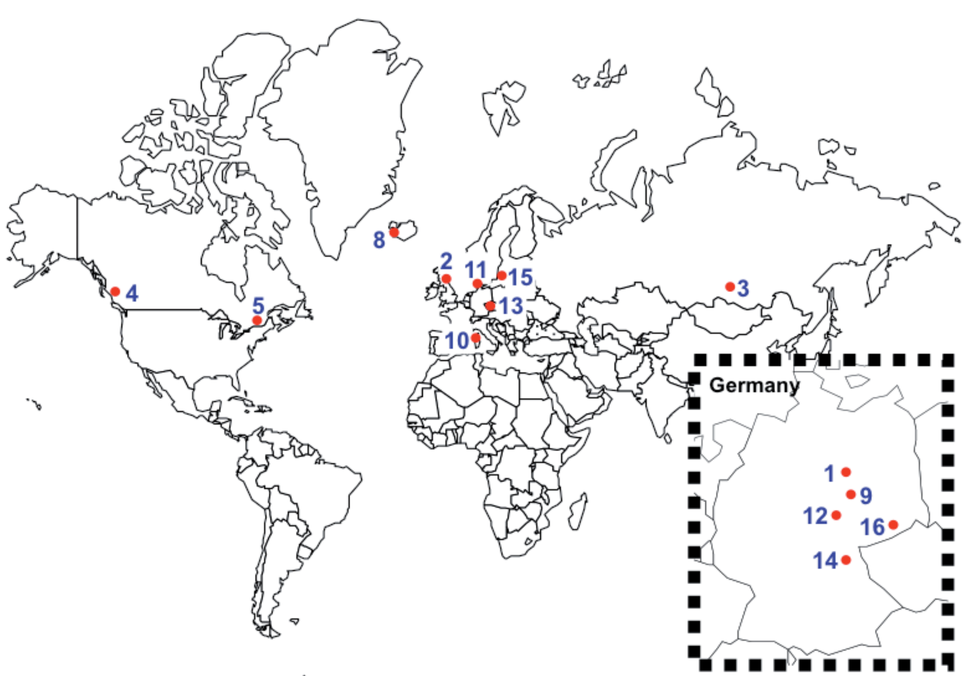


Fig. 1: Map of origins for *F. vesca* accessions. The denotation is adequate to Tab. 1.

Tab. 1: List of used accessions of *Fragaria vesca* and their origin and of five *Fragaria ×ananassa* cultivars.

number	accession / cultivar	abbreviation	origin / year of introduction
1	*F. vesca* f. *alba* St. 08/101	foralb	Gatersleben, via G. Staudt
2	*F. vesca* f. *alba* 'South Queen Ferry'	sferry	Scotland, South Queen Ferry, (near Edinburgh), United Kingdom, leg. Hohlfeld
3	*F. vesca* ssp. *vesca* St. 94, 13 'Baikal'	baikal	Lake Baikal, Siberia, Russia, leg. Popova
4	*F. vesca* ssp. *bracteata* St. 98, 04-4	bracte	Nass River, British Columbia, Canada
5	*F. vesca* ssp. *americana* St. 14324	americ	Saint-Armand, Missisquoi River, near Montreal, Canada, via Botanical Garden Montreal
6	*F. vesca* f. *semperflorens* 'Red Wonder'	redwon	cultivar
7	*F. vesca* f. *semperflorens* 'Yellow Wonder'	yelwon	cultivar
8	*F. vesca* ssp. *vesca* 'Island'	island	Botanical Garden Rejkavik, Iceland
9	*F. vesca* ssp. *vesca* 'Kaiserpfalz Tilleda'	tilled	Tilleda, Saxony-Anhalt, Germany, leg. Roentzsch
10	*F. vesca* ssp. *vesca* 'Korsika'	korsik	St. Bonifatius, Corsica, France, leg. Olbricht
11	*F. vesca* ssp. *vesca* 'Multiplex'	multip	cultivar
12	*F. vesca* ssp. *vesca* 'Weimar'	weimar	Valley of the river Ilm, Garden of J. W. v. Goethe, Weimar, Thuringia, Germany, leg. Gerischer
13	*F. vesca* ssp. *vesca* 'Böhmen'	boehme	Bohemian Sandstone Mountains, near Stimmersdorf, Bohemia, Czech Republic, leg. Olbricht
14	*F. vesca* ssp. *vesca* 'Tüchersfeld'	tuefel	Tüchersfeld, Franconia, Germany, leg . Olbricht
15	*F. vesca* ssp. *vesca* 'Süd-Öland 1'	oeland	Oeland, Sweden, leg. Drewes-Alvarez
16	*F. vesca* ssp. *vesca* 'Großolbersdorf'	olbers	Großolbersdorf, Ore Mountains, Saxony, Germany, leg. Olbricht
17	*F. ×ananassa* cv. 'Alba'	ALBA	Italy, 2003
18	*F. ×ananassa* cv. 'Mara de Bois'	MDB	France, 1991
19	*F. ×ananassa* cv. 'Mieze Schindler'	MS	Germany, 1933
20	*F. ×ananassa* cv. 'Polka'	POLKA	Netherlands, 1980
21	*F. ×ananassa* cv. 'Elegance'	ELEG	England, 2009

gas chromatograph equipped with an Agilent 5975B quadrupol MS detector. Compounds were separated on a polar column ZB-Wax plus 30 m length x 0.25 mm ID x 0.5 µm film thickness. Helium was used as a carrier gas with a column flow rate of 1.1 ml/min. Temperature programme: 45 °C (3 min), temperature gradient 3 K/min to 210 °C (30 min). The mass spectrometer was used with electron ionization at 70 keV in the full scan mode.
All samples were run with two analytical repetitions from an identical part of the supernatant.

Data processing
The resulting total ion chromatograms (TIC) were integrated using the Agilent ChemStation™ routine with an initial threshold of 15 for all samples. The value of the initial threshold was chosen in such a manner that the most intense chromatograms contain about 100 integrated peaks. For a non-targeted data processing, the integration results were imported subsequently as raw data (txt-formatted) into the chemometrical software ChromStat^{TM} 2.6 from Analyt-MTC (Müllheim, Germany). The semi-quantitative results were expressed as non-dimensional values (counts or relative concentrations).
Statistical analyses were performed using the software Statistica 7.1 from StatSoft (Tulsa, USA) and Multi Experiment Viewer from TM4 Software Development Team (www.tm4.org).

Brix and acid measurements
Brix values in °brix as a correlated value for sugar content were measured for the strawberry juice of all frozen fruits of one accession / cultivar using the portable 'Quick Brix' (Mettler Toledo, Schwerzenbach, Germany). From the same juice, an acid equivalent on the base of citric acid was determined by a semi-automatic titrator (716 DMS Titrino 06, Metrohm, Filderstadt, Germany) with given results in % acid equivalent.

Results

Volatiles
The semi-quantitative data of volatile organic compounds of 21 genotypes analyzed during two harvest seasons are displayed as so-called heat maps in Fig. 3. The concentration level is converted in a color code from deep blue ($c = 0$) to deep red ($c \leq 15$). The heat maps represent the raw data in an identical order for both harvest years. The group of *F. vesca* accessions is located above the five cultivars. The heat map patterns are similar for both years but not identical. Because of the specific entire sampling strategy of fruits described above, the concentration levels of volatiles represent an average concentration pattern of all fruits from the whole harvest season independent of sampling frequency. Since identical plant material was used in both harvest seasons, the resulting differences

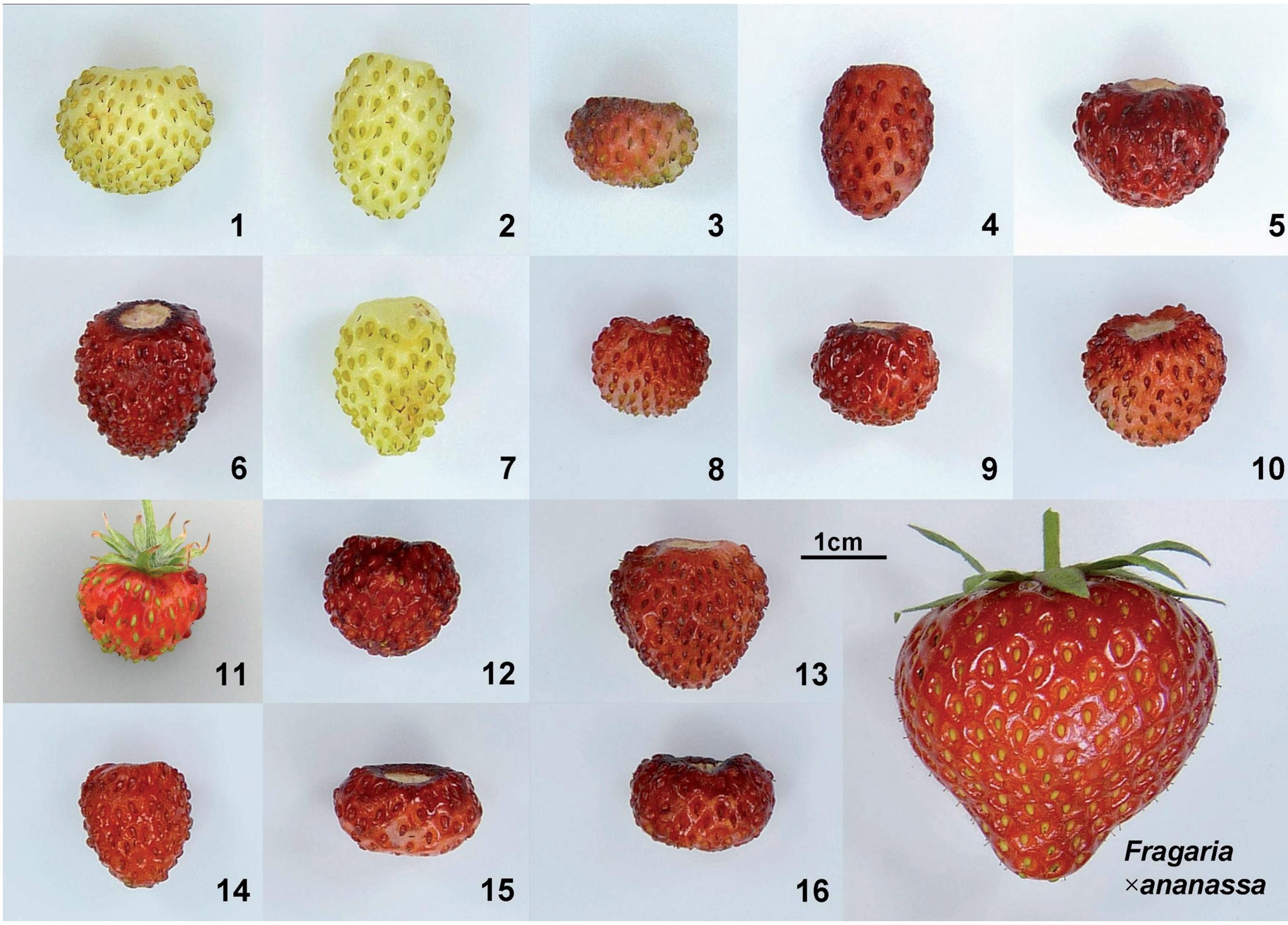


Fig. 2: Typical fruits of sixteen *F. vesca* accessions and one *F. ×ananassa* cultivar. The denotation of *F. vesca* accessions is adequate to Tab. 1.

must be caused by the climatic conditions only.

Within the *F. vesca* group, high diversity in VOCs is evident. Aroma relevant substances like ethyl hexanoate (LOX, ester biosynthesis), mesifuran (furanone), myrtenal (terpenoid) and g-decalactone (LOX pathway) vary in a wide range. Between the *F. vesca* and *F. ×ananassa,* group characteristic differences occur. Small esters like methyl butanoate, ethyl butanoate, methyl hexanoate and ethyl hexanoate as well as hexanoic acid occurs in higher amounts in the cultivars. In opposite, ketones (2-pentanone, 2-heptanone, 2-nonanone), C6-compounds like hexanol and hexenols are clearly more abundant in *F. vesca*. Another difference occurs in the group of terpenoids (e.g. terpinen-4-ol, myrtenal, myrtenyl acetate, α-terpineol and myrtenol) which were also found in higher concentrations in the *F. vesca* group. One exception is the monoterpene linalool with higher amounts in *F. ×ananassa* than in *F. vesca*. The key compound methyl anthranilate is evident in all *F. vesca* accessions. The concentrations vary in a wide range between values of 9.19 (accession no. 8, 'island') and 980 (accession no. 16, 'olbers') in 2011 and 6.75 (accession no. 8, 'island') and 359.81 (accession no. 16, 'olbers') in the season 2012. In accordance with former findings (Ulrich et al., 2009; Ulrich and Olbricht, 2011), methyl anthranilate was found in two of the five *F. ×ananassa* cultivars in medium concentration between values of 6.13 to 27.17.

In Fig 4a the diversity of some exemplary volatiles discussed above is shown as Box-Whisker-plots using the entire data from both years (2011 and 2012). The plots represent the medium values both for the *F. vesca* (left, v) and *F. ×ananassa* (right, c) group. The magnitude of metabolic diversity within these groups is characterized by the standard deviation and the minimum/maximum values. 2-Heptanone, 1-hexanol, myrtenyl acetate and methyl anthranilate represent the metabolites with generally higher amounts in wild types whereas ethyl hexanoate and linalool belong to volatiles with higher concentrations in the *F. ×ananassa* cultivars. As an exception, the ethyl butanoate content of the cultivar 'Elegance' is marked with an asterisk. The ester content of this cultivar is extremely depleted and therefore marked as outlier (see section Discussion). Several volatiles show a typical skewed distribution of the data with a mean value shifted to lower values. In general, the range between minimum and maximum values is much higher in *F. vesca* than in *F. ×ananassa* for most of the volatiles although the chosen five cultivars represent the most diverse breeding results from ca. 80 years of European breeding history. Myrtenyl acetate is not detectable in the evaluated cultivars but in the majority of investigated *F. vesca*. Generally, all median values of the demonstrated volatiles are significantly different between both groups.

For comparison of the two harvest years, a data reduction by PCA and a rank correlation analysis (Spearman's rank) was performed. The results of PCA are shown in Fig. 5 as score and parameter plots for each harvest season separately. In analogy to the heat map in Fig. 3, the results for 2011 and 2012 are similar with some differences in details. In the score plots (left), the groups of *F. vesca* and *F. ×ananassa* cultivars are clearly separated. The parameter plot of 71 parameters (67 volatiles, sum of volatile, brix value, acid content, ratio of brix and acid value) has generally a similar structure

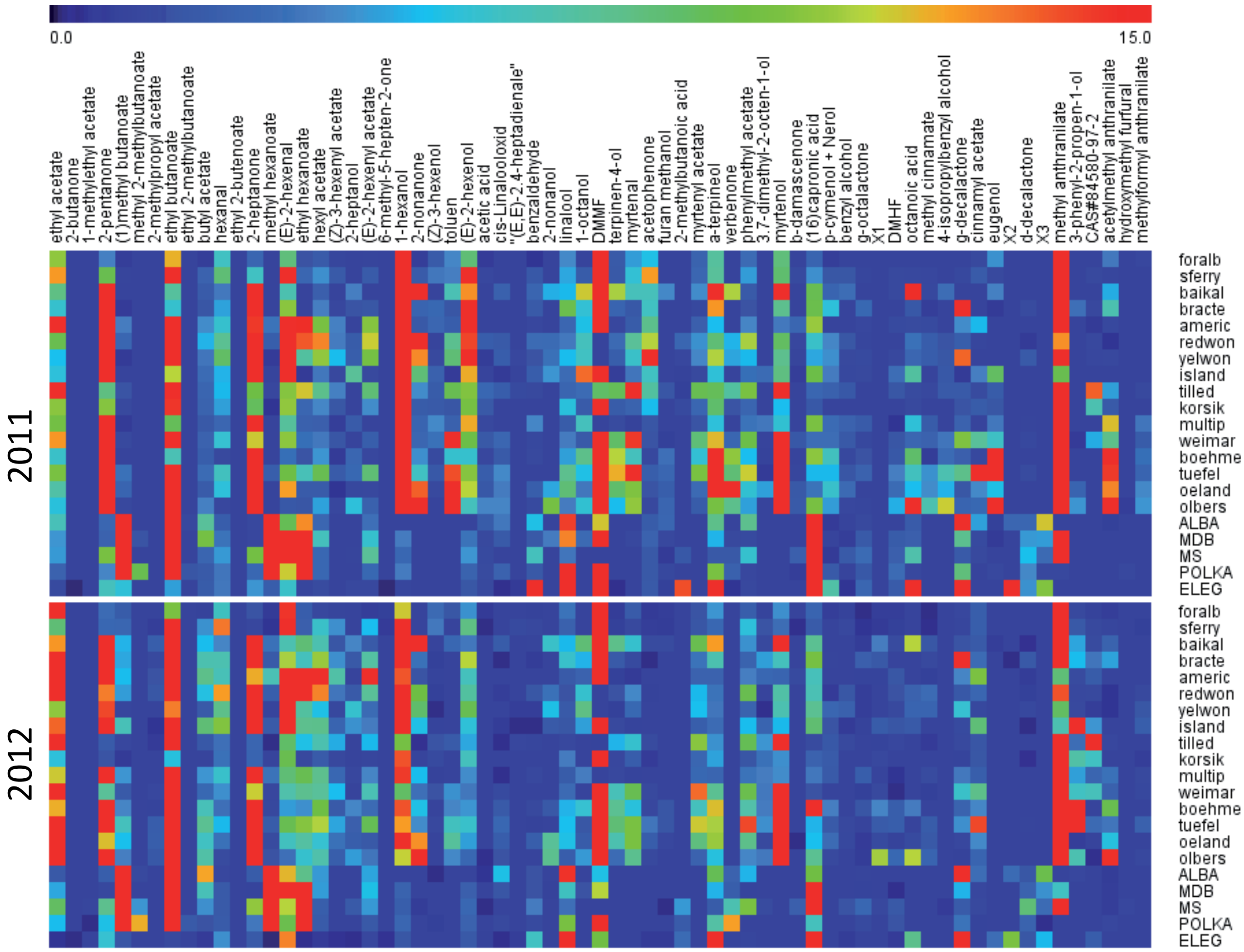


Fig. 3: Heat map of the metabolite patterns including 67 volatiles from 16 *F. vesca* accessions and four cultivars (*F. ×ananassa*). 45 volatile compounds are fully identified by co-elution of authentic substances, the remaining by MS library search. The relative concentrations of metabolites (counts) are color coded from deep blue (c = 0) to deep red (c = 15). Concentration values above c = 15 (around 10 % of all values) are also coded in deep red.

for both years. The calculation of rank coefficients (Tab. 1) gives a quantitative measure of the similarity of volatile patters between the two seasons. The coefficient values are distributed between 0.67 (*F. vesca*, accession no. 8, 'island') and 0.92 (*F. ×ananassa* cv. 'Mara de Bois') with means of 0.77 for *F. vesca* and 0.88 for the cultivars, respectively.

Non-volatiles

The distribution of brix values (brix), titratable acid (acid) as well as the ratio are shown in Fig. 4b using the entire data from both years (2011 and 2012). In contrast to the volatile data, brix and acid values are characterized by a nearly ideal Gaussian distribution. The mean values of brix are not significantly different between the wild and cultivated strawberries whereas acid values and accordingly the ratio carry significant differences. Interestingly, the range of acid values in *F. ×ananassa* cultivars is very small in comparison to *F. vesca*.

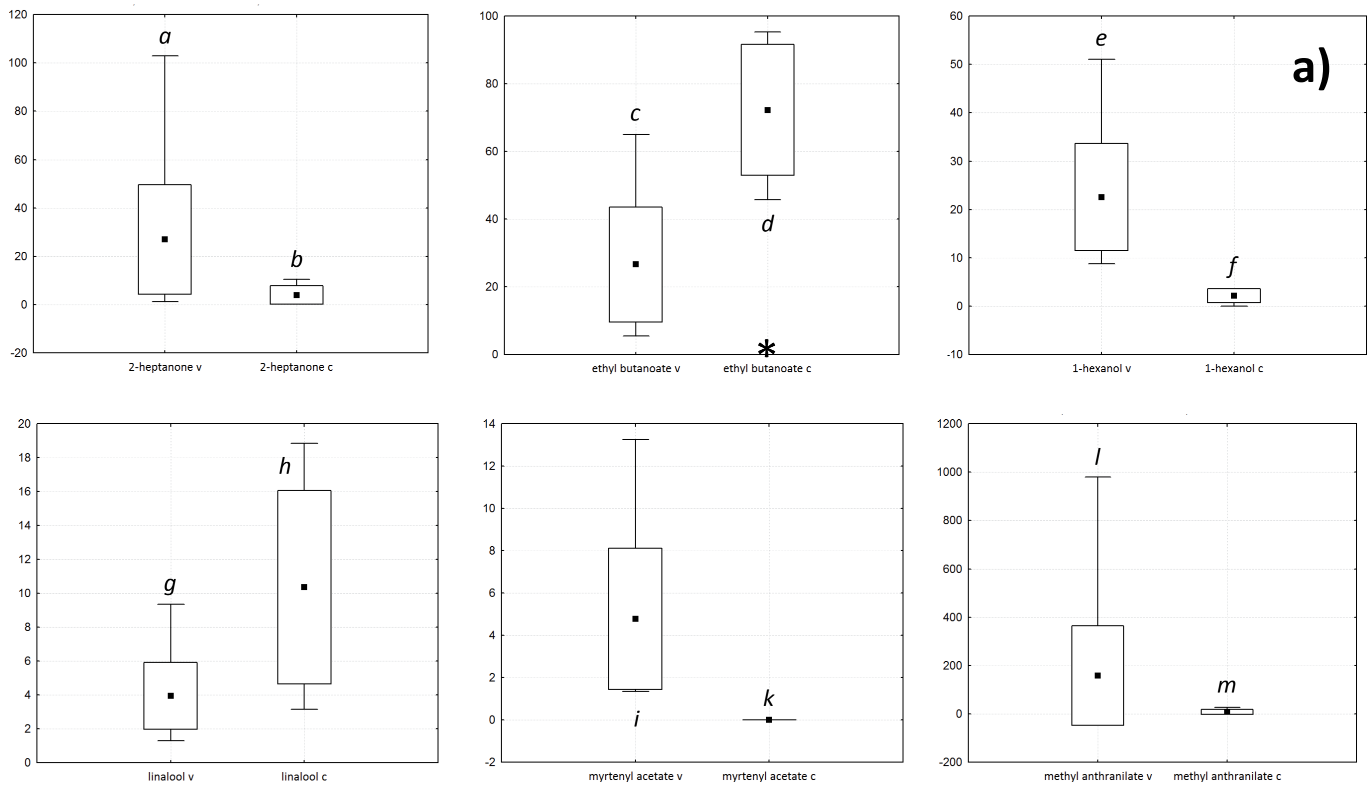
Discussion

Berry sampling method

The laborious sampling strategy for ripe and healthy berries was used to prevent temporary influences which occur between different harvest dates within the whole season. Taking into account that changes of metabolite contents between different harvest dates within one season may arise in the same order like genotype differences (Jouquand et al., 2008), the high similarity between the heat map patterns (Fig. 3) and the PCA plots (Fig. 5) is an argument for the benefits of this kind of sampling. Additionally, this fact is supported by the rank correlation results for all of 67 VOCs as well as brix, acid and the ratio brix/acid in two years (Tab. 2). The Spearman's rank show high values between 0.77 for the *F. vesca* accession and 0.88 for *F. ×ananassa* cultivars.

Strawberry aroma types

The diploid wood strawberry *F. vesca* is not a direct ancestor of our cultivated *F. ×ananassa* which is a hybrid of the two octoploid species *F. chiloensis* (L.) Miller and *F. virginiana* Miller. Nevertheless, from the view of sensory quality, the aroma of cultivars often has been judged in comparison to those of wild species, especially to those of the wood strawberry (Ulrich et al., 1997; Hoberg et al., 2000). Improving the aroma of new *F. ×ananassa* cultivars is a declared aim of all modern breeding programmes whereas the aroma of *F. vesca* often is described as the most powerful strawberry character. Therefore, the volatile patterns of sixteen *F. vesca* accession



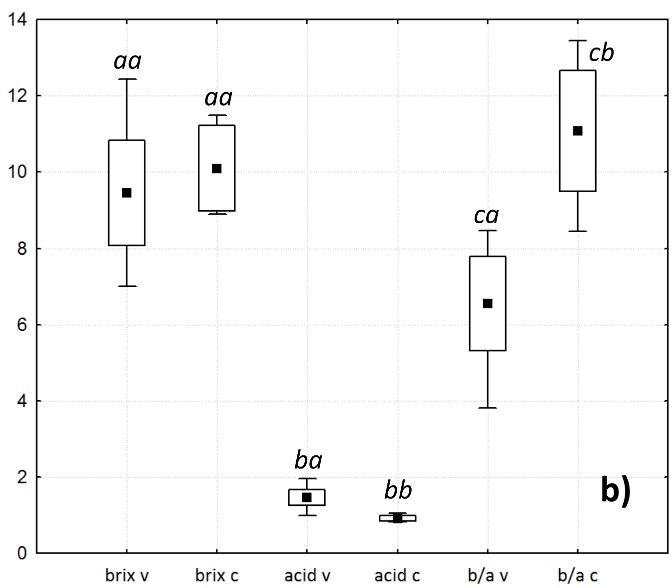


Fig. 4: Elementary statistics for the data set including the values of the harvest years 2011 and 2012. a) Box-Whisker-plots of six representative volatiles for the group of *F. vesca* accessions and *F.* ×*ananassa* cultivars. b) Box-Whisker-plots of value for brix, acid content and the relation brix/acid. Rectangle – mean; Box – standard deviation; Whisker – minimum/maximum. Nomenclature of genotypes: v – *F. vesca,* c – *F.* ×*ananassa* cultivars. Box labels with different characters indicate a significant statistical difference of the mean values. Asterisk – cultivar ‘Elegance’ is characterized by untypical low ester contents (outlier).

were compared with five *F.* ×*ananassa* cultivars. While the sixteen *F. vesca* accessions represent important habitats of their geographic distribution around the northern hemisphere (Staudt, 2009) the *F.* ×*ananassa* types represent five cultivars with very diverse aroma types regarding the classification by Ulrich et al. (1997 and 2009). This classification divides strawberries in terms of their content of methyl anthranilate (MA) and short chain esters of butanoic, hexanoic or 2-methyl butanoic acids (esters) in three main aroma types: ‘MA high’(1) and ‘MA low (2)’ with the ‘MA low’ types subdivided into an ‘ester high’ (2a) and an ‘ester low’ (2b) type . 'Mieze Schindler' (type 1) is an old German cultivar (introduction 1933) with intensive aroma which often is characterized with the notation ‘vesca-like’. 'Mara de Bois' (also type 1) is a modern highly aromatic French cultivar (introduction 1991) and an exception in the range of modern cultivars. Its rich aroma pattern is explained by the pedigree which includes the old European germplasm. This cultivar is mainly used for house gardens and as gourmet fruit. The cultivar 'Polka' (introduction 1980) represents the fresh, fruity aroma type 2a whereas the cultivar 'Alba' (type 2b) belongs to the modern high yielding cultivars with medium to low aroma strength (introduction 2003). 'Elegance' (introduction 2009) represents a high performance cultivar with firm and best looking fruit but with extremely low volatile values.

One of the most abundant volatiles in ripe *F. vesca* berries is methyl anthranilate (MA), which is a product of the shikimate pathway synthesized via chorismate (Ulrich et al., 1997; Bohlmann et al., 1996). This compound varies within the sixteen *F. vesca* accessions by a magnitude of three orders (Fig. 4a). MA is characterized by a typical sweetish-flowery smell like acacia. In combination with others, this compound causes the unique flavor of *F. vesca* and in addition the preferred sensory quality of few *F.* ×*ananassa* cultivars like 'Mieze Schindler' and 'Mara de Bois'. Because of several bioactivities, MA became an important industrial chemical, and synthetic forms are widely used as odorant, artificial flavor, bird repellent and oxidation inhibitor (Wang and De Luca, 2005). In higher concentrations MA gives an unpleasant note like soapy (Komes et al., 2006) in strawberries and foxy in grapes (Wang and De Luca, 2005).

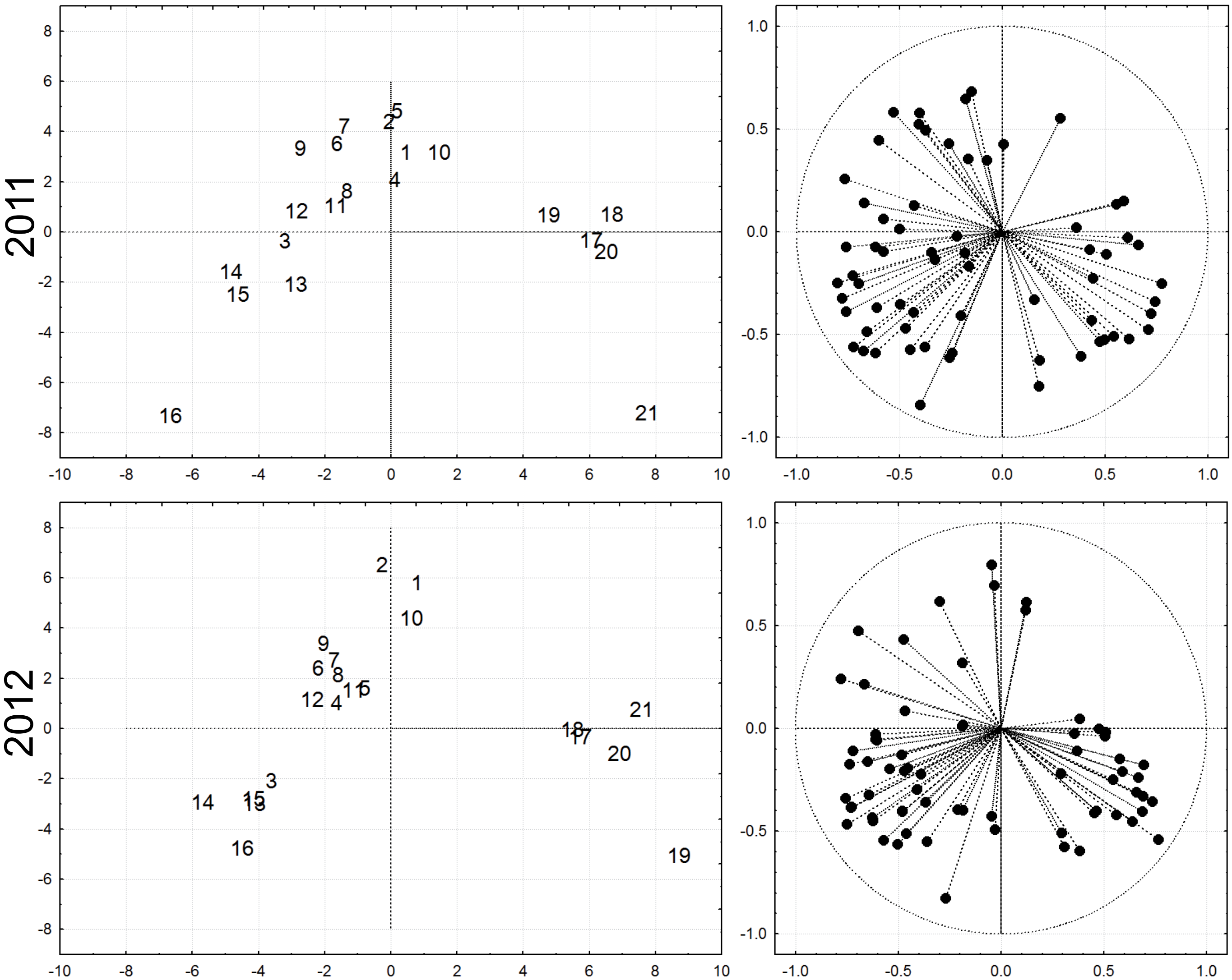


Fig. 5: Principal Component Analysis using semi-quantitative data of 67 volatiles, the sum of volatiles as well as brix value, acid content and the ratio of brix to acid value. Left – score plot of 16 *F. vesca* accession and five *F. ×ananassa* cultivars. Right – parameter plot.

Comparison of terpenoid and ester patterns

In comparison with *F. ×ananassa* cultivars, *F. vesca* accessions produce higher amounts and a higher diversity of terpenoid derived compounds (Fig. 3 and Fig. 4a). It can be interpreted as a result of the large biogeographical distribution of the wild species. The compounds mentioned above (terpinen-4-ol, myrtenal, α-terpineol, myrtenol and linalool) carry important bioactivities like attractants, pheromones, kairomones, fungizides etc. (N.N. 2013). The loss of terpenoids in cultivated strawberries may be accompanied with a loss of resistance features against different diseases and herbivores. Until now this topic is rarely investigated and also not in the focus of this research. Furthermore, this compound group has an impact on sensory characteristics due to low odor thresholds (PET'KA et al., 2012; LARSEN and POLL, 1992). The generally higher amounts of terpenoids in wild accession may have a negative influence on sensory quality induced by a turpentine-like, herbaceous, woody odor (AHARONI et al., 2004) and harsh flavor (SIMON, 1985). This is in agreement with the astringent characters found by descriptive sensory tests in comparison of wild strawberries from Europe and North America with the standard cultivar *F. ×ananassa* cv. 'Elsanta' in an earlier study (ULRICH et al., 2007). As an exception, the terpenoid linalool with a fresh-flowery, citrus-like and pleasant odor was found in higher amounts in cultivars which is, on the one hand, in agreement with the findings of CHAMBERS et al. (2012) who postulated a mutation in a nerolidol synthase (*FaNES1)* in octoploids (*F. ×ananassa*) in comparison to the diploids (*F. vesca*). On the other hand, the linalool concentrations are generally lower in *F. vesca* but not absent like postulated by CHAMBERS et al. (2012). This result supports the hypothesis that linalool synthesis may arise also from an alternative biosynthesis than *FaNES1*.

In contrast to most of the terpenoids, the group of small fruit esters (methyl butanoate, ethyl butanoate, methyl hexanoate and ethyl hexanoate) gives a positive, fruity impact to aroma and is found in higher amounts in *F. ×ananassa* cultivars.

Influence of domestication and breeding on metabolite patterns and organoleptic properties

The comparison of metabolic patterns of *F. vesca* and *F. ×ananassa* gives an interesting insight in mechanisms of domestication and breeding even if the cultivated forms do not descend directly from the diploids. On the other hand, phylogenetic research reveals that *F. vesca* shares important sequences with the genome of *F. ×ananassa* (AHARONI et al., 2004) and is considered as one of the close

Tab. 2: Rank correlation analysis between two harvest years based on semi-quantitative data of 67 volatiles, the sum of volatiles as well as brix, acid-value and the quotient of brix and acid-value.

	accession	*spearments rank 2011-2012*
1	foralb	0.81
2	sferry	0.80
3	baikal	0.74
4	bracte	0.80
5	americ	0.86
6	redwon	0.83
7	yelwon	0.80
8	island	0.67
9	tilled	0.80
10	korsik	0.75
11	multip	0.69
12	weimar	0.78
13	boehme	0.72
14	tuefel	0.76
15	oeland	0.76
16	olbers	0.71
	mean wildtypes	*0.77*
17	ALBA	0.88
18	MDB	0.92
19	MS	0.86
20	POLKA	0.85
21	ELEG	0.83
	mean cultivars	*0.88*

relatives to the octoploid species *F. virginiana* and *F. chiloensis* (ROUSSEAU-GUEUTIN and OLBRICHT, 2009). The tremendous diversity of aroma patterns within the *F. vesca* accessions is believed to be an adaptation to the different requirements of the ecosystems which were occupied by this strawberry species. Today's strawberry cultivars are characterized by rather different patterns of secondary metabolites. As described above for VOCs, early domestication steps and in particular the breeding seem to give advantages to strawberry types with pleasant flavor perceptions. In consequence, metabolites with bitter and astringent (sesquiterpenes), herbaceous, harsh and unpleasant odors (monoterpenes) were downgraded. In contrast, the content of sensorial pleasant small esters is high in cultivars. Sugar and acid contents follow the same direction. Cultivars carry high or medium sugar but low acid contents and lead to high sugar-acid ratios. The *F. vesca* accessions in this study are characterized by high acid values and very diverse brix-acid ratios. Additionally, high acid contents involve also astringency especially in combination with low sugar values.

Evidence exists that organoleptic properties of plants played an important role during domestication and even in early breeding activities. DIAMOND (2002) discussed this topic regarding the bitterness (or non-bitterness) of almond and acorns. Early selection and breeding work can be characterized as a trend toward desirable sensory sensations such as sweet, non-bitter, moderate sour and low astringent. This is because humans have an inborn preference for sweet sensations and an aversion against bitter and astringent food (SHEPHERD, 2006). In the data set presented here, this trend can be seen clearly in the comparison of the patterns of esters and the acid content (or brix-acid ratio). Excellently tasting cultivars possess high concentrations of short chain esters (butanoates, hexanoates, hexenoates and 2-methylbutanoates) in combination with high values of brix-acid ratio and a moderate firmness. In strawberries, like in other fruits, the flavors appreciated by consumers are best represented in old cultivars and so-called land races or heirloom varieties (ALSTON, 1992). Breeding activities of the past decades on *F. ×ananassa* cultivars for high-yielding and firm strawberries were accompanied with a dramatic loss of genetic diversity in modern cultivars. Between old and modern cultivars, a loss of one third of observed alleles (30 % - 35 %) was determined by molecular analysis using SSR markers (GIL-ARIZA et al., 2009, HORVATH et al., 2011). Additionally, an acceleration of this process was found because further 18 % of alleles disappeared in only one decade in the 1990s. As discussed above, this so-called funnel effect influences also qualitative and quantitative traits of volatile patterns. The most impressive example for the funnel effect in flavor caused by breeding investigated in the present research is the cultivar 'Elegance'. The ester contents of this cultivar depleted of high degree (Fig. 4a), which resulted in poor and consequently untypical *F. ×ananassa* flavor.

Methodical principals in volatile analysis

AHARONI et al. (2004) postulated a strong selective advantage of strawberry types containing the sensorial pleasant compounds linalool (sweetish, floral, citrus like note) and nerolidol (rose, apple, green note) passed on to preferred domesticated strawberries. Also in the present analyses, linalool content is significantly higher in cultivars whereas nerolidol was not detectable in all of the *F. vesca* accessions and was found in only two *F. ×ananassa* cultivars ('Polka' and 'Mara de Bois') in the first harvest year. But some important aspects are still unconsidered in this discussion.

Firstly, if correlation between metabolite profiles of the fruit and sensory perceptions are in the focus of discussion, an important prerequisite of the instrumental analysis is that the sample preparation methodology must approximately mimic the eating process. The used methodology in the present research provides for this aspect using homogenization (LOX activity of disrupted tissue) with following enzyme inhibition of the homogenate using high salt concentrations. That is that measurements using whole, undamaged fruit do not represent the metabolite profiles which are active in the human mouth. Therefore, statements about individual aroma compounds based on whole fruit (AHARONI et al., 2004; AZODANLOU et al., 2003) represent more relations to signaling processes (herbivores) than those of human sensory.

Secondly, a selective advantage based on human sensory impressions cannot directly be judged by simple instrumental analysis results (concentration values). The sensory impact of VOCs follows the so-called aroma value concept (ROTHE and THOMAS, 1963). The aroma impact in the human nose corresponds to the aroma value (AV) (or in synonym the odor activity value (OAV)) which is calculated by the ratio of the VOC concentration (c) and the odor threshold (OT) in the same matrix. Strawberry character impact compounds in cultivars differ in their concentration in a range of about two orders resulting in corresponding AVs in a range of six orders (LARSEN and POLL, 1992). The highest AVs in cultivars were found for the three esters ethyl butanoate (453,000), ethyl hexanoate (4,700) and methyl butanoate (614) as well as the furanone 2,5-dimethyl-4-hydroxy-3-(2H)-furanone (1,170). In contrast, the AVs of terpenoids are 100-fold lower (LARSEN and POLL, 1992). Based on earlier results of ULRICH et al. (1997), the AVs for the key compound methyl anthranilate can be estimated as about 100 for the *F. ×ananassa* cultivar 'Mieze Schindler' and up to 5,000 for the *F. vesca* accession no. 16, 'olbers'. These calculations confirm the findings of the present research that determining compounds for preferred strawberry aroma types are esters of butanoic and hexanoic acids in combination with methyl anthranilate and furaneol. Additionally, a high acceptance demands high sugar contents (brix value), moderate acid content (Jouquand et al., 2008) and hence a high sugar/acid ratio value. The metabolic differences and sensory relationships discussed in the present paper are in total analogy to those of other fruits and vegetables like apple and carrot (Ulrich and Olbricht, 2011).

Aroma patterns of successful cultivars are stable

An additional aspect is the VOC dynamic regarding the harvest years which is illustrated by the metabolite comparison of wild (*F. vesca*) and cultivated (*F. ×ananassa*) types in two years. The influence of the harvest year to the profiles of 20 character impact compounds in a whole F1 population of cultivated strawberries was published by Olbricht et al. (2008 and 2011). The degree of variation triggered by harvest year is very different for every single aroma compound. This effect causes a high dynamic of volatile patterns. Generally, groups of stable and unstable metabolites were found in strawberries. In the present paper, the stability of the metabolite patterns including 67 compounds as well as brix value, acid content and the ratio brix to acid value regarding the two harvest seasons can be judged using the rank correlation coefficients in Tab. 2. Obviously, the stability is significantly lower in the wild types *F. vesca* (0.77) than in *F. ×ananassa* cultivars (0.88). A conclusion for breeding is that successful cultivars like those used in this research are characterized not only by sensory suitable concentration patterns of VOCs but also by robust patterns with regard to year-dependent, environmentally caused influences. Additionally, this is supported by the two *F. vesca* cultivars (‘redwon’ and ‘yelwon’), which are a product of plant breeding too and show next to *F. vesca* ssp. *americana* comparable high values of Spearmans rank.

Acknowledgements

The authors thank Anne Doedtmann, Ursula Gerischer, Angela Ludwig, Henning Wagner, Veronika Waurich, Kirsten Weiß and Lisette Wutzky for technical realisation as well as Petra Scheewe for helpful discussion.

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