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ARTICLE

Variation in the Amounts of Selected Volatiles in a Model Population of *Fragaria* × *ananassa* Duch. As Influenced by Harvest Year

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Volatile metabolites are a basis for sensory and resistance traits of *Fragaria* × *ananassa*. Stability of expression is important for the selection of cultivars. For the first time, the stability of volatiles in a strawberry population after cross-combination of two distinct cultivars ('Mieze Schindler' × 'Elsanta') has been investigated. In this work, environmentally caused variations in the synthesis of 18 volatiles were studied over two years using a model population of 158 clones. The stability varied throughout the F1 seedling population between the two years, defining stable and unstable genotypes with respect to volatile synthesis. Most of the stable genotypes exhibited low values in relative volatile concentration. Merely 6 stable volatiles were detected in the parental cultivars, whereas about 40% of the F1 progeny had up to 11 stable volatiles. Consequently, a higher stability in volatile synthesis can be achieved by breeding.

KEYWORDS: Nontargeted analysis; HS-SPME; volatile organic compounds; inheritance; strawberry

INTRODUCTION

Due to its outstanding flavor, the cultivated strawberry *Fragaria* × *ananassa* Duch. is one of the most important soft fruits in the world. In the past 250 years several hundreds of different cultivars have been bred. Although the volatile organic compounds (VOCs) of strawberry were intensively investigated in the past (*1*−*5*), the sensory quality was widely disregarded in recent breeding programs. Changes in sensory quality between old and new cultivars are one aspect of the so-called "funnel effect" or "genetic erosion", which always goes along with plant domestication and breeding of high-yielding cultivars (*3*, *6*−*8*). Replacing existing cultivars by better tasting ones is the goal of present breeding programs aiming at more convenient kinds of fruits and fruits with increased nutritional and healthy values (*9*, *10*). Aditionally, secondary metabolites, including VOCs, are key substances involved in resistance interactions and physiological adaptation and acclimation. Therefore, they have become also a breeding topic with regard to other horticultural traits (*11*).

Among approximately 360 volatiles from different compound classes found in strawberry fruit, about 20 volatiles have been identified as character impact compounds, which are important for sensory impression (*3*, *5*, *12*−*14*). The furanones 2,5-dimethyl-4-methoxy-2,3-dihydro-3-furanone (DMF, mesifuran) and 2,5-dimethyl-4-hydroxy-3[2*H*]-furanone (DHF, Furaneol) (*14*), exerting a fruity and caramel-like odor, are the dominating and characteristic sensory impressions. Although the sensory impressions of the furanones are dominant in all investigated cultivars, the flavor diversity of cultivars can be discriminated especially by the content of short-chain esters and methyl anthranilate (MA). MA, with its flowery, aromatic-fruity, and typical wood-strawberry-like odor, was recognized as a kind of marker substance for well-tasting strawberry types (*3*, *15*).

Fruit flavor biosynthesis and in particular strawberry aroma compounds are still under intensive investigation (*16*−*19*). So far, the inheritance of aroma compounds is a disrespected area in breeding research (*20*). On the other hand, analytical tools for flavor quality selection have become more and more important in fruit breeding (*10*). One of the first studies of the inheritance of volatile compounds in strawberries was performed by a crossing between *Fragaria* × *ananassa* cultivars 'Mieze Schindler' and 'Elsanta' (*21*). The female crossing partner, 'Mieze Schindler' is an old pistillate cultivar that was bred in the 1920s in Dresden, Germany. There, it is considered as the cultivar with the most intensive and most typical strawberry flavor. Its soft-textured fruits are characterized by a very specific sweet and aromatic flavor, reminiscent of wood strawberries. In ripe fruits the volatile pattern contains besides low-boiling esters also moderate amounts of MA. In contrast to 'Mieze Schindler', 'Elsanta' is a modern high-yielding cultivar with medium or poor aroma. Therefore, 'Elsanta' is a member of the poor flavor category (chemotype) as defined by Ulrich et al. (*3*, *12*), with low contents of butanoic and hexanoic esters and without MA. The fruits of both cultivars differ not only in aroma characteristics but also in various attributes such as fruit size, color, and texture. Therefore, the selection of these cultivars as parents ensures a widespread variability in the

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F1 progeny. The investigation of the population during a single year brought three important results (*21*): First, for most of the 18 volatiles, the concentration range of the F1 population was much wider than the interval between the levels of the parents. Second, volatiles from biosynthetic pathways that were not detected in the parent cultivars (such as those for undecanone) were detected in some genotypes of the offspring. A simple crossing by pollination obviously enables the activation of inactive biosynthetic pathways. Third, the formerly found funnel effect for MA is a result of an inheritance pattern of this compound. In this regard, in only 25% of the offspring were detectable amounts of MA found. Therefore, MA will disappear in the progeny within a few generations in the breeding process without control of the volatile patterns by human sensory and instrumental analysis.

Besides the rules of inheritance, the stability of inherited traits in following years is an important aspect in plant genetics and practical breeding, in particular. It is a well-known fact that successful cultivars of fruits and vegetables have to possess a certain stability of characteristics regarding environment and year. Breeding lines with unstable traits are not usable for a successful development of cultivars. The environmental influence on quality traits including aroma compounds is widely known; however, to our knowledge there are no reports in the literature on a breeding population in soft fruit. Therefore, the purpose of this paper is the investigation of aroma patterns in genotypes of a model population over a period of two harvest seasons (summers of 2005 and 2006). Analytical data and their interpretation are fundamental for practical breeding work and for genomic mapping in strawberry.

MATERIALS AND METHODS

Plant Material. Two genetically distinct cultivars of *Fragaria* × *ananassa*, 'Mieze Schindler' and 'Elsanta', were chosen as parents for cross-combination. To create a model population, 200 F1 seedlings were randomly selected from a population of 438 seedlings and propagated with three plants each by cloning.

Plant Cultivation. All plants of the population were cultivated in the field at Dresden-Pillnitz (Julius Kühn-Institute, Germany) on sandy loam to loamy sand on gravel ground. The cultivation followed the principles described by Olbricht et al. (*21*). The conditions for propagation, growing, harvesting, sample preparation, and analysis were identical in both years. In the years 2004 and 2005, fresh plants of 200 clones propagated by runners were used for the planting in August of the respective years; 158 clones delivered fruits in the years 2005 and 2006.

Fruit Sampling. Only fully ripe and typical fruits from the first main harvest of F1 clones were used for analysis in June and July of 2005 and 2006. Data from the fruits of 158 clones were processed. A minimum of 10 fruits were collected per clone. To avoid differences due to fruits of different rank, fruits of ranks A (primary), B (secondary), and C (tertiary) were taken. Fruits of the cultivar 'Elsanta' were harvested three times in 2005 and nine times in 2006. Fruits of the cultivar 'Mieze Schindler' were collected at the intermediate harvest in 2005, taking all ripe fruit, and five harvests in 2006, respectively. Immediately after harvest, the fruits were brought to the laboratory, where they were washed and dried. The sepals were removed, and the prepared fruits (approximately 250 g) were homogenized in 250 mL of a solution of 18.6% (m/v) NaCl using a standard mixer for 2 min. The homogenate was centrifuged at 4 °C and 4000 rpm for 30 min. One hundred milliliters of the supernatant was mixed with 10 μL of internal standard (0.1% (v/v) 2,6-dimethyl-5-hepten-2-ol dissolved in ethanol). For each sample, four headspace vials containing 3 g of NaCl for saturation were filled with 10 mL of the liquid, sealed with magnetic crimp caps including septum, and stored at 4 °C for up to 3 weeks.

Gas Chromatographic Analysis of Volatiles. The volatiles were sampled by headspace solid phase microextraction with a 100 μm polydimethylsiloxane fiber (Supelco, Bellefonte, PA) using an MPS2 autosampler from Gerstel (Mühlheim an der Ruhr, Germany). The equilibration time before absorption was 10 min at 35 °C in the shaking operation mode (300 rpm). The fiber was exposed to the headspace for 15 min at 35 °C under further shaking. Thermal desorption was then performed for 2 min in the injector (splitless mode) at 250 °C. Afterward, additional thermal cleaning (3 min at 250 °C, split ratio 1:10) was done. The analysis was conducted with an Agilent Technologies 6890N gas chromatograph (Agilent Technologies, Germany) equipped with a flame ionization detector (FID). The compounds were separated on a polar column HP INNOWax (0.25 mm i.d.; 30 m length; 0.5 μm film thickness). The FID temperature was 250 °C. Hydrogen was used as a carrier gas with a column flow rate of 1.1 mL/min. The temperature program was the following: 1, isothermal processing at 40 °C (3 min); 2, heating from 40 to 200 °C at a rate of 3 K/min; 3, isothermal processing at 200 °C for 15 min. The volatiles were identified by parallel running of selected samples by mass spectrometric analysis (GC-MS) and coelution of authentic references. For identification, the same GC with an Agilent 5973 MSD in the electron impact ionization mode (70 eV) was used. GC run parameters were used as described above. For the identification of compounds, the Wiley 138, NIST 02, and HPCH 1607 (Allured Corp., Carol Stream, IL) libraries were used. Eighteen volatiles were considered with regard to their inheritance (**Table 1**).

Semiquantitation of GC Data. The values for the comparison of the two harvest years were calculated from raw data (exported from ChromStat) using absolute peak areas in counts given in relative concentration.

Definition of Stability. In the context of this research, a volatile was defined as stable if the concentration of the specific compound showed similar concentrations in both years. This definition of stability was made by the authors. A deviation of 10% in both directions from the diagonal line, which represents equal concentration values in the two years of investigation ($x = y$, cf. **Figure 1b**), was chosen by the breeder and applied to the sum of volatiles and the 18 individual volatiles. Using this approach, the evaluation of the stability of the parental cultivars and the F1 genotypes is possible.

RESULTS

First results about the inheritance of important volatile compounds were demonstrated by Olbricht et al. (*21*) using fruits from the F1 population 'Mieze Schindler' × 'Elsanta' (*Fragaria* × *ananassa*) in 2005. Investigations in a second year (2006) using the identical population provided information about the environmental influence on the synthesis of the volatiles over a two-year period. Of the 200 clones harvested in the first year, 158 delivered also a sufficient number of fruits in the second harvest year. The numbers of analyses performed accumulate to 460 chromatographic runs in 2005 and 360 in 2006 including blank runs and fiber test runs using a reference sample mix. The results presented in this paper are based on altogether 820 single analyses, which were performed using the same gas chromatographic instrument. Because of unavoidable changes in the chromatographic system such as fiber replacement and/or column aging, the data were processed by the nontargeted data processing method (*21*) separately for both years. As a result of this approach, the pattern recognition software detected different numbers of peaks: 199 in 2005 and 186 in 2006. Differences in time slices detected automatically by ChromStat are found only for very small and unidentified peaks (impurities, artifacts). All 18 volatile compounds were present in both years.

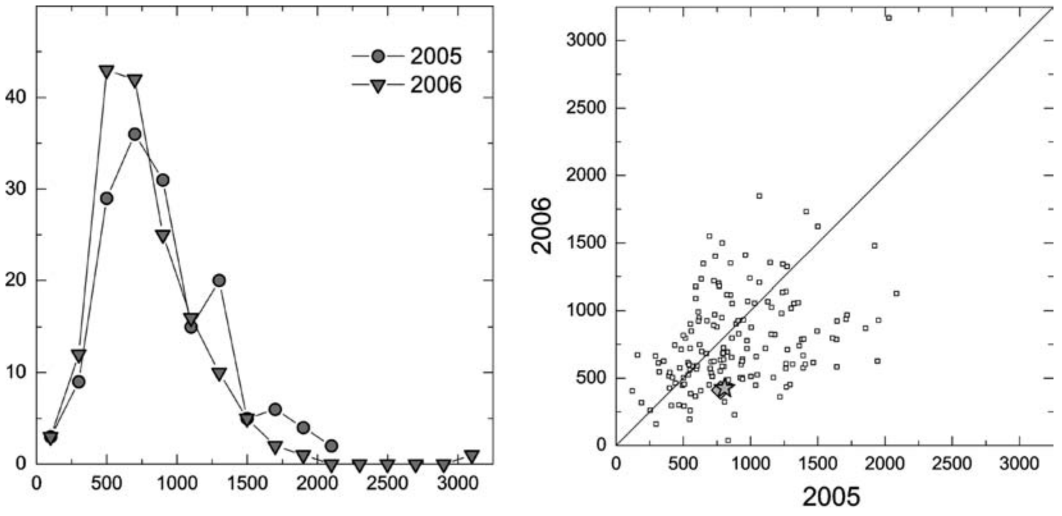
Sum of 18 Volatiles. The frequencies (158 F1 genotypes in each year) of the sum of 18 volatiles exhibited in each year are akin to a right-skewed distribution (log-normal) with a little higher mean value in 2005 of 897 compared to 772 units of relative concentration in 2006 (**Figure 1a**; **Table 1**). In contrast, the parental cultivars showed around half of the sum in 2006 compared to 2005 (**Table 1**). Their means in 2005 ranged close to the mean of the F1 progeny, but they showed only half of the F1 mean value in 2006.

Most of the F1 genotypes behaved differently in the two years, which is expressed by their distance from the diagonal ($x = y$) in **Figure 1b**. However, distinct F1 genotypes showed stability with regard to the sum of their volatiles. They are located near the $x = y$ curve. Other F1 genotypes were located extremely far from the

Table 1. Descriptive Statistics for 18 Compounds and Sum of 18 Compounds: 'Mieze Schindler', 'Elsanta', and the F1 Model Population ($n = 158$)[a]

	F1 population 2005			'Mieze Schindler' 2005	'Elsanta' 2005	F1 population 2006			'Mieze Schindler' 2006	'Elsanta' 2006
volatile (**no.**)	mean	min	max	mean	mean	mean	min	max	mean	mean
esters										
methyl butanoate (**1**)[b]	125.1	6.8	538.1	94.1	86.9	100.8	ND	832.0	46.5	20.4
ethyl butanoate (**2**)[b]	96.2	ND	657.9	33.4	81.1	136.5	ND	827.0	95.5	15.8
methyl hexanoate (**3**)[b]	150.1	9.6	581.9	211.8	83.8	16.0	ND	124.6	31.7	2.5
butyl butanoate (**4**)[b]	71.1	8.8	450.5	27.6	70.8	58.8	ND	335.2	28.9	59.5
ethyl hexanoate (**5**)[b]	103.9	ND	764.5	48.4	47.0	65.5	ND	336.0	2.4	39.8
3-methylbutyl butanoate (**6**)[c]	2.8	ND	44.6	ND	1.2	5.4	ND	84.7	ND	ND
hexyl acetate (**7**)[b]	56.4	4.3	222.6	82.6	40.6	76.2	ND	461.0	51.1	27.9
2-hexenyl acetate (**8**)[b]	102.1	8.2	329.4	233.0	64.9	78.2	ND	277.7	56.2	32.3
methyl anthranilate (**17**)[b]	1.6	ND	26.5	3.7	ND	6.7	ND	80.3	5.8	ND
terpenoids										
linalool (**10**)[b]	54.8	ND	253.4	3.4	62.5	44.2	ND	274.0	3.4	20.2
myrtenyl acetate (**12**)[b]	1.0	ND	15.0	ND	1.4	4.1	ND	39.4	ND	1.6
nerolidol (**15**)[b]	31.8	ND	148.5	ND	101.6	13.9	ND	110.4	1.8	21.9
lactones										
γ-decalactone (**16**)[b]	2.1	ND	30.8	ND	7.3	71.1	ND	379.8	7.2	85.5
γ-undecalactone (**18**)[c]	9.1	ND	38.3	ND	12.0	6.0	ND	20.6	0.5	1.3
carotenoid derived										
β-damascenone (**13**)[b]	16.0	ND	61.3	3.7	25.0	16.4	ND	86.0	22.6	21.1
miscellaneous										
benzaldehyde (**9**)[b]	5.4	ND	19.2	9.4	1.2	17.3	ND	50.2	11.0	13.8
undecanone (**11**)[b]	0.1	ND	3.3	ND	ND	2.4	ND	43.3	1.2	9.6
hexanoic acid (**14**)[b]	66.1	6.1	368.0	57.0	88.2	52.9	ND	329.8	59.1	37.7
sum	897	121	2085	808	775	772	37	3169	425	411

[a] Data processing was performed using raw data as absolute peak areas in counts (so-called relative concentration). ND, not detected = below the detection threshold of 0.1 count. Commercial sources of reference compounds: (A) Sigma-Aldrich (compounds **4**, **5**, **7**, **8**, **10**−**12**, **14**, **15**); (B) Roth, Germany (compounds **1**−**3**); (C) Fluka (compound **9**); (D) Dragoco Holzminden, Germany (compounds **13**, **16**, **17**). [b] Identified by MS library search and coelution of authentic references = "identified". [c] Identified by MS library search = tentatively identified.



(a) (b)

Figure 1. (**a**) Frequency (*y*-axis) of the sum of 18 volatiles (*x*-axis: given as relative concentration) for 158 F1 genotypes in 2005 and 2006 and (**b**) comparison of the sum of each of the 158 F1 genotypes and the parental cultivars in 2005 and 2006 (diagonal line resembles equal values in both years: $x = y$, 'Mieze Schindler', star; 'Elsanta', diamond, given as relative concentration).

position of the parental cultivars. This demonstrates a wide variety in the offspring after cross-combination. Although 51% of the F1 genotypes are found to be stable, none of the parental cultivars is stable under the above definition.

Stability of Volatiles. The values for single volatiles are summarized for the F1 progeny and the parental cultivars in **Table 1**. The stability of each volatile is shown by the distribution of the genotypes in the diagrams (**Figure 2**). A high variation between 2005 and 2006 can be observed in the diagrams for all volatiles. According to the stability criterion defined above, a certain number of genotypes can be classified as stable. The number of stable genotypes allows qualification of the degree of stability of the volatiles (**Table 2**).

Esters. Considering nine esters, 1−53% of the F1 progeny were classified as stable. For most of the esters, > 25% of the progeny are stable. Methyl hexanoate with only one stable F1 genotype and methyl anthranilate with 83 stable genotypes in the segment are the volatiles with minimum and maximum degrees of stability,

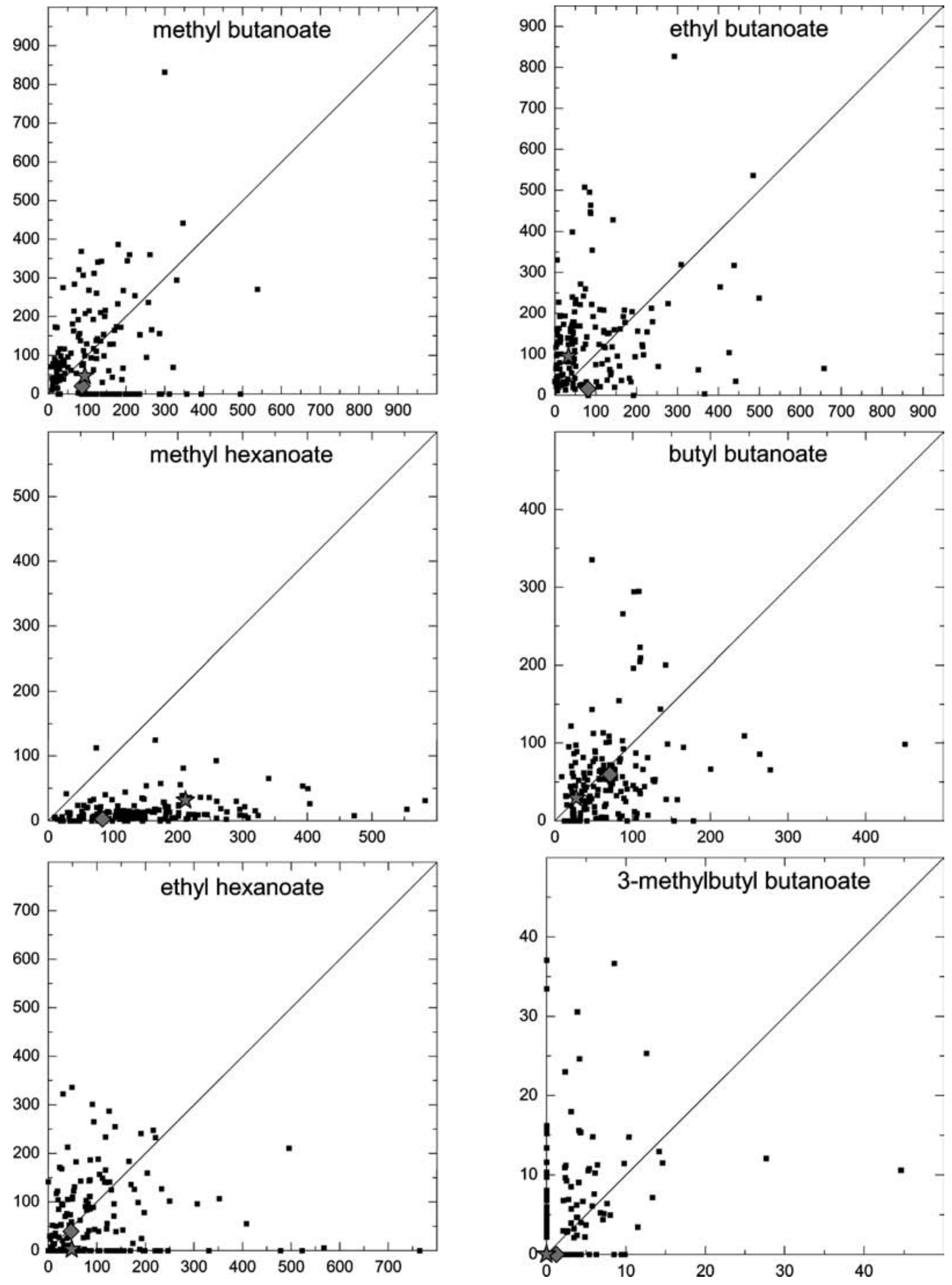


Figure 2. Continued

respectively. Only two stable esters were found in ‘Mieze Schindler’ and four in ‘Elsanta’ (**Table 2**).

Terpenoids. All three terpenoid compounds showed with a range from 26 to 49% higher stability in the F1 progeny compared to the other compound groups. Two stable terpenes were found in ‘Mieze Schindler’ and only one in ‘Elsanta’ (**Table 2**).

Lactones. Two lactones were semiquantified; decalactone resembled one of the least stable compounds, with only two stable genotypes (1% of the F1 progeny). Undecalactone showed a degree of stability of 42% of the F1 progeny. The parental cultivars were not classified as stable with regard to the lactones investigated here (**Table 2**).

Carotenoid-Derived Compounds. Damascenone was classified as stable in ‘Elsanta’ and in 34% of the F1 progeny (**Table 2**).

Miscellaneous. Undecanone showed the highest degree of stability of all 18 volatiles, being classified as stable in 138 genotypes (87% of the F1 progeny). The cultivar ‘Mieze Schindler’ was found in the stability segment of benzaldehyde and of hexanoic acid; 18 and 45% of the F1 progeny were found within the stability segment of benzaldehyde and hexanoic acid, respectively (**Table 2**).

DISCUSSION

Stability regarding environmental influences of all important plant characteristics is a general goal in breeding. Whereas traits such as yield can be easily determined, flavor is a much more

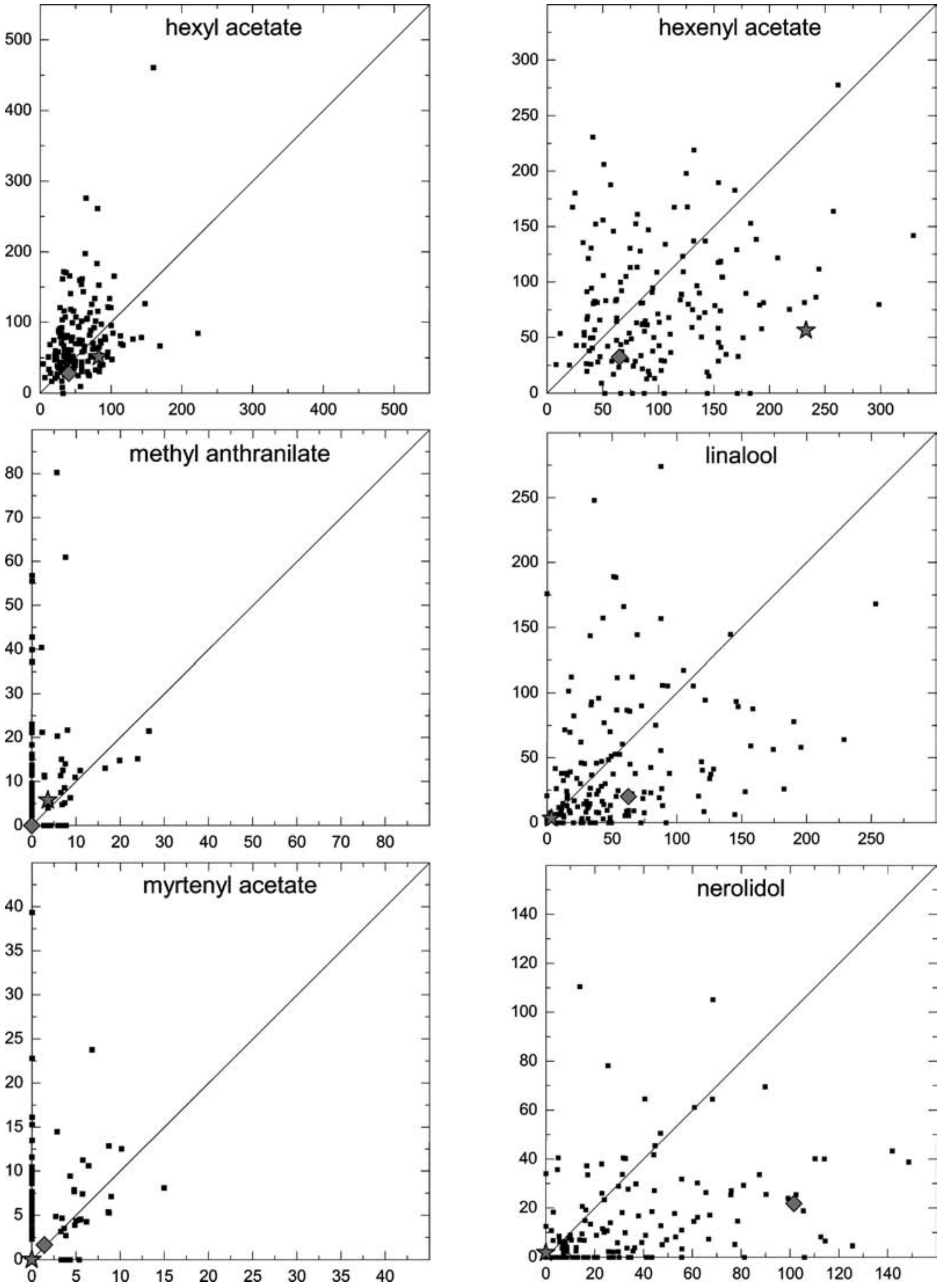


Figure 2. Continued

complex trait. However, basic research on the genetic background, including the inheritance of aroma compounds, is scarce, in particular for the very complex aroma matrices in strawberry (*16*). Carrasco et al. (*20*) reported that the heritability for aroma traits is generally low. They used progenies of crosses between *Fragaria* × *ananassa* and *Fragaria virginiana*. Zhang et al. (*22*) investigated volatiles in only two selected genotypes and their parents. They concluded that particular volatiles found in the parental plants are inherited to the F1 genotypes. On the basis of the statistical width of our two-year study including 158 F1 genotypes, it becomes evident that inheritance patterns of aroma volatiles are much more complex.

On the basis of analytical and proper sensory investigation of numerous cultivars, wild species, and breeding clones over several years, 18 volatiles could be defined as important volatiles for strawberry flavor (*3*−*5*, *12*, *23*, *24*). In a first study of a one-year analysis performed on a model population, comprehensive results could be deduced for each volatile and its degree of inheritance (*21*). Using the same F1 progeny, the segregation in the following year does provide details on the degree of stability of the volatiles in strawberry for the first time. Subsequent investigations over three or more years are certainly needed to prove the stochastic nature of a two-year analysis.

Stable Synthesis of Volatiles. Our two-year study demonstrates a high variation in the synthesis of volatiles in the F1 population

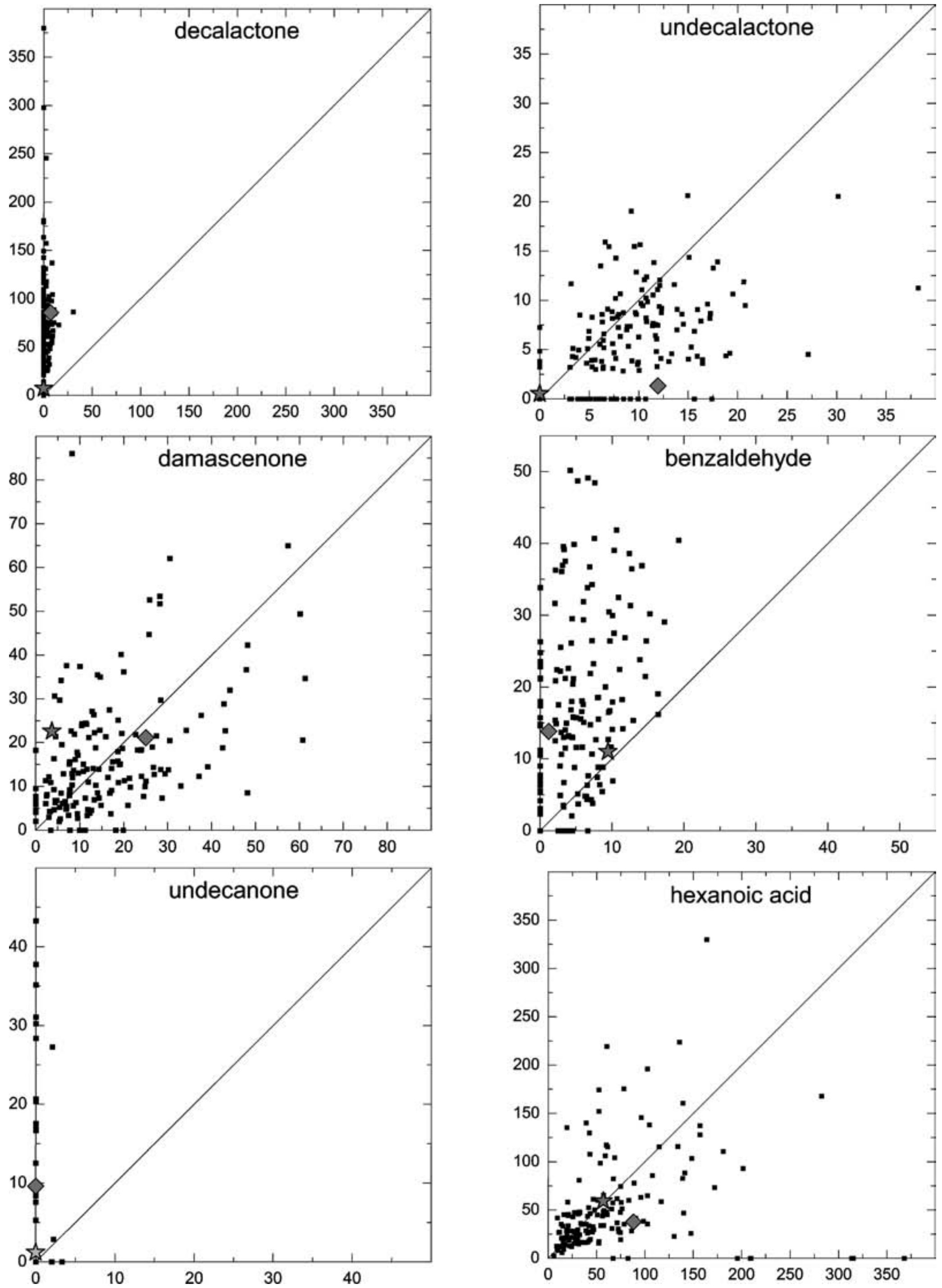


Figure 2. Volatiles of 158 F1 genotypes and the parental cultivars 'Mieze Schindler' (star) and 'Elsanta' (squares) in 2005 and 2006 given in relative concentration. The diagonal lines show equal values in both years: *x* = *y* ('Mieze Schindler', star; 'Elsanta', diamond).

of *Fragaria* × *ananassa*. There are some extremely unstable volatiles that indicate a strong environmental influence. For example, methyl hexanoate with only one and decalactone with only two stable F1 genotypes are the least stable synthesized volatiles. The highest stability is shown for undecanone and methyl anthranilate. However, from a sensory perspective it is important to consider that in these two cases the stability is mainly determined by zero values for a large number of genotypes in both years. Although in this situation the genotypes have to be classified as stable, the sensory contribution of the respective volatiles to the strawberry flavor is not given. On the other hand, these two volatiles are important compounds of the aroma pattern of a genotype.

Therefore, stable zero values of these volatiles determine the overall flavor impression of the genotypes. Additionally, with regard to zero values we have to note that there are systematic thresholds of volatile detection (detection limit) which depend on both the SPME sample preparation (recovery rate) and gas chromatographic design composed of a polar column and FID. However, there are also volatiles with high degrees of stability in the progeny, such as butyl butanoate, hexyl acetate, hexenyl acetate, and hexanoic acid, for which the stability over the two-year period is based on nonzero values. For undecalactone only a few F1 genotypes are stable due to zero values in both years, whereas most of the stable genotypes show nonzero values. The

Table 2. Frequency of 158 Genotypes in a ±10% Stability Segment Deviating from the Axis $x = y$ (Values of 2005 = Values of 2006) and Stable Volatiles for the Parental Cultivars

	frequency of genotypes in a ±10% segment from $x = y$ (values of 2005 = 2006)		values in a ±10% segment from $x = y$	
volatile	absolute	%	values of 2005 = 2006 for 'Mieze Schindler'	values of 2005 = 2006 for 'Elsanta'
esters				
methyl butanoate	38	24		
ethyl butanoate	33	21		
methyl hexanoate	1	1		
butyl butanoate	49	31	yes	yes
ethyl hexanoate	33	21		yes
3-methylbutyl butanoate	78	49	yes	
hexyl acetate	63	40		yes
2-hexenyl acetate	52	33		
methyl anthranilate	83	53		yes
terpenoids				
linalool	41	26	yes	
myrtenyl acetate	78	49	yes	yes
nerolidol	41	26		
lactones				
γ-decalactone	2	1		
γ-undecalactone	67	42		
carotenoid-derived				
β-damascenone	53	34		yes
miscellaneous				
benzaldehyde	29	18	yes	
undecanone	138	87		
hexanoic acid	71	45	yes	
sum	80	51		

stability of myrtenyl acetate and 3-methylbutyl butanoate is caused by zero values as well as by nonzero values in both years.

The character impact compounds of strawberry aroma derive from different metabolic pathways that are highly interconnected. In particular, volatiles of the lipoxygenase, terpene, and shikimate pathways were semiquantified in this research. The clones of the F1 progeny and the parental cultivars were tested regarding correlations between different compound groups (LOX-derived, carotenoid-derived, esters, terpenes, benzenoids). The retrieval of a wide range of correlation coefficients between distinct compound groups in two years confirms the differentiated influence of the environment on the biosyntheses of metabolites. The correlation coefficients amount to −0.48 for amino acid derivatives, −0.07 for terpenoid derivatives, 0.19 for carotenoid derivatives, and 0.92 for fatty acid derivatives.

The extremely high phytochemical variability within the population as well as between the two harvest years may originate from the existence of the so-called "silent metabolisms", which are defined as "occult biosynthetic capacities" that are conserved in plants as an evolutionary benefit (*25*). These inactive pathways can be activated by crossing, plant development, or environment. For example, the compound undecanone was absent (more correctly, below the detection level) in the parents in 2005; however, it was detected in 3% of the F1 progeny (**Table 1**). After the second year of analysis, it was obvious that an environmental effect was responsible for this result. Both parental cultivars showed detectable amounts of undecanone in 2006. Similarly, a higher number of F1 genotypes with nonzero values of undecanone were found in the second year (12% of the F1 progeny). A second example, which can be discussed in this context, is the branched ester 3-methylbutyl butanoate. It was not detected in 2006 in the parents; however, it was found in 83 F1 genotypes in the same year of investigation (52% of the F1 progeny). One year before, in 2005, a low level of 3-methylbutyl butanoate could be detected in 'Elsanta' and in a lower number of the F1 genotypes (44% of the F1 progeny), whereas it was also not found in 'Mieze Schindler'.

Fragaria is not only one of the most important fruit crops cultivated worldwide but also a Rosaceae model plant for genetic and molecular studies because of its short reproductive cycle and the facile vegetative and generative propagation (*26*). The results presented here are valuable findings regarding aroma biosynthesis and may have implications for molecular marker development. Our investigation shows that the search for proper molecular markers for flavor will be misleading if it is based on data from only one harvest season. Data of at least two and possibly even more years are necessary for reliable results.

The transgenic approach is an interesting and powerful tool to discover the metabolic pathways in strawberry or to develop transgenic plants with enhanced characteristics (*25*, *26*). Despite discussions about low consumer acceptance of genetically modified organisms, the high variability of metabolite patterns caused by environment effects as shown in this paper and the unpredictable expression of foreign genes are major problems in genetic engineering.

As an example, the metabolic engineering of the terpenoid pathways including linalool and geraniol in different genetic surroundings was discussed by Lewinsohn (*25*). The aspects discussed reveal a basic drawback for using genetic transfer in cultivar breeding programs due to the complex and highly regulated metabolic network.

Stable Genotypes in the F1 Progeny and Breeding Aspects. In the F1 model population, genotypes were found that exhibit stable sums of volatiles as well as stable single volatiles over the two-year period. With regard to single volatiles, only genotypes that are located in a ±10% segment deviating from the diagonal curve ($x = y$, cf. **Figure 1b**) are considered to be stable genotypes. Surprisingly, the parental cultivars are often widely deviating from the diagonal curve ($x = y$). Only 6 of 18 volatiles in 'Mieze Schindler' and 6 volatiles in

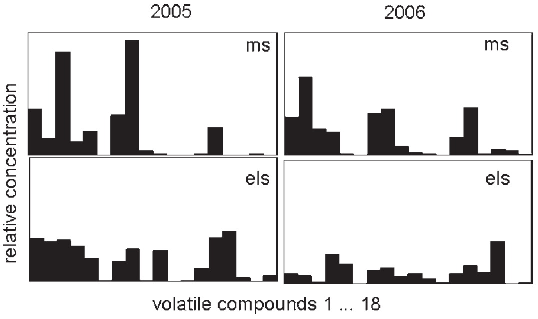


Figure 3. Aroma pattern showing 18 volatiles (relative concentration; for denomination of the no., see **Table 1**) for the parental cultivars 'Mieze Schindler' (ms) and 'Elsanta' (els) in 2005 and 2006.

Table 3. Variation of the Sum of 18 Volatiles (Relative Concentration) during Harvest of 'Elsanta' and 'Mieze Schindler' in 2005 and 2006

	'Elsanta'	'Elsanta'	'Mieze Schindler'	'Mieze Schindler'
year of harvest	2005	2006	2005	2006
first main harvest	683	643		363
intermediate harvest	954	356	808	500
final harvest	690	235		400

'Elsanta' were stable using the present definition. In contrast, the segregation after cross-combination of these two cultivars showed a distribution of 2−11 stable volatiles in the F1 genotypes. Furthermore, the aroma patterns of the parental cultivars, which are depicted in **Figure 3**, differ between the two years of investigation. The stability criterion as defined in this work might be too strong with respect to the sensory quality of the two cultivars, keeping in mind that 'Elsanta' and 'Mieze Schindler' are successful cultivars in strawberry production and in home gardens. On the other hand, 40% (63 genotypes) of the F1 progeny showed a higher number of stable volatiles (>6) compared to the parental cultivars. This result is very important for breeding; that is, genotypes with a higher stability in volatile synthesis can be obtained. Nonetheless, most of the stable genotypes show low relative volatile concentrations.

The variability of the taste and aroma of a cultivar due to seasonal changes and weather conditions is well-known and has to be considered. Neither of the two cultivars are stable in their sum of the 18 volatiles, whereas 51% of the progeny are classified as stable. In this regard it has to be noted that the sums of the 18 volatiles vary between different harvest dates in the same year, as is shown for the parental cultivars in **Table 3**. This variation is being investigated at present and will be published elsewhere. This effect, however, was minimized in the presented investigation because all ripe fruits of the F1 genotypes were taken from the first main harvest with at least 10 fruits per clone of all ranks as stated above.

Altogether, the results of this study suggest that there is a potential for selecting genotypes with a high stability in view of volatile synthesis. For the breeding process, it can be stated that several years of investigation are necessary for the selection of cultivars with stable characteristics. However, a second year of volatile analysis already provides a sufficient basis for the selection: Only those genotypes that are stable in view of volatile concentration are kept in the selection process. Modern approaches of volatile analysis including reliable sample preparation methods, powerful detectors such as mass spectrometers, and appropriate data processing are suitable tools to support breeding. However, the selection of a new cultivar is always based on a compromise because a balance between all demands on the performance of a new selection besides flavor is required. Therefore, it is not surprising that most cultivars show only average or below average values with regard to single traits. It remains to be shown whether the stringent selection regarding volatile stability over whole pedigrees will result in genotypes with higher aroma stability.

ABBREVIATIONS USED

VOCs, volatile organic compounds; HS-SPME, headspace solid phase microextraction; MA, methyl anthranilate; DMF, 2,5-dimethyl-4-methoxy-2,3-dihydro-3-furanone (mesifuran); DHF, 2,5-dimethyl-4-hydroxy-3[2*H*]-furanone (Furaneol); GC, gas chromatography; MS, mass spectrometry; FID, flame ionization detection; LOX, lipoxygenase.

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