New strawberry genotypes tested for organic production on a *Verticillium*-infested site

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Abstract

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Nineteen new strawberry breeding clones, bred at the Institute for Breeding Research on Fruit Crops in Dresden-Pillnitz, Germany, were evaluated for their suitability for organic production at a *Verticillium*-infested site in Vienna, Austria. Soil cover, plant vitality, resistance to leaf spot diseases as well as flower damages by the strawberry blossom weevil (*Anthonomus rubi*) and by spring frost were recorded. In two consecutive years, marketable yield and fruits infested by *Botrytis cinerea* were assessed. Three breeding clones, P-7189, P-8043 and P-8071, were considered as suitable for organic strawberry production. P-7189 and P-8043 showed high tolerance to Verticillium wilt. The breeding clones P-8155 and P-8166 performed well under organic management but had very soft fruits that were not acceptable for commercial fruit production. The rest of the breeding clones were low-yielding, however, some of them could be of interest for home gardens due to their good flavour and high plant vitality.

Keywords: tolerance; resistance; root diseases; breeding; fruit quality

In organic strawberry production, there is a need for vigorous cultivars with high tolerance to pests and diseases, particularly to *Botrytis cinerea*. Root diseases (first of all *Verticillium dahliae* and *Phytophthora cactorum*) can lead to yield and plant losses on farms which lack land for a wide crop rotation, especially when using susceptible cultivars. Differences in the susceptibility of commercially used cultivars to these root diseases are well documented, however, completely resistant cultivars are not known. The described resistances (i.e. reduced susceptibility) are of quantitative nature and inherited by additive factors (Eikemo et al. 2000; Zebrowska et al. 2006; Shaw et al. 2008, 2010). Since there is no strawberry breeder in Austria, all currently used cultivars have been bred under conventional growing conditions in other European countries, particularly in Italy, France and the Netherlands. Breeding research in Austria has mainly been focusing on the evaluation of new cultivars and breeding clones from foreign breeding programs in order to identify those that perform best under existing conditions. A number of breeding clones, bred at the Julius Kuehn-Institute (JKI), Institute for Breeding Research on Fruit Crops in Dresden-Pillnitz, Germany, were tested in Vienna. The JKI is active in strawberry breeding since the early 1920's, when new cultivars were developed for home gardening. Today, the aims of the existing breeding program have been adapted to the current demands. The program focuses on the development of commercial cultivars with improved fruit flavour combined with resistance to root and

fruit diseases (Olbricht, Hanke 2008). Because disease resistance is one of the major aims of the German strawberry breeding program, it is expected that it will provide new cultivars that are suitable for organic and low input production and are tolerant to root diseases.

MATERIAL AND METHODS

Nineteen advanced breeding clones (Table 1) were selected within five years (2004–2008) of seedling evaluation in a previous screening at the experimental field at JKI. The selection was based on their good flavour characteristics and their satisfying plant performance at a field site highly infested by *V. dahliae* and *P. cactorum*. Fruit size and appearance were also included in the evaluations. Since selections were carried out in view of different production systems, both genotypes with firm and soft fruits were selected. Whereas firm fruits are required for wholesale, medium soft fruits are suitable for producers which sell their fruits directly or do processing on a small scale.

Table 1. Pedigree of the tested breeding clones

Genotype	Female parent	Male parent
MS 139	Mieze Schindler	Elsanta
P-4020	Fraroma	*
P-4043	Fraroma	*
P-4325	Fraroma	Honeoye
P-4331	Fraroma	Honeoye
P-4337	Fraroma	Honeoye
P-5357	Elsanta × *F. chiloensis* ssp. Lucida	SEL VR 96582
P-5515	Cirofine	*
P-5588	(E 2/1 × Honeoye)	Florence
P-5627	Florence	Toro
P-6737	**	**
P-7189	Alba	*
P-8043	P-5245	*
P-8071	P-5245	*
P-8083	P-4197A	*
P-8086	P-4198	*
P-8096	Toro	Florence
P-8155	Spadeka	*
P-8166	Spadeka	*

*mix of pollen obtained from different genotypes was used for hybridization, the real parent is unknown; **obtained from a former breeding program – no information available

For comparison, cvs Elsanta (reference for a fresh market cultivar), Senga Sengana (reference for a processing cultivar), Fraroma (bred at JKI Dresden-Pillnitz and recommended for home gardens) and Weiroma (bred at the Technical University Munich, recommended for home gardens and for small-scale processing) were chosen.

The experimental field was located in the research garden of the University of Natural Resources and Life Sciences (BOKU) on the north-eastern periphery of Vienna, Austria. The climate during the vegetation period in this region is warm and dry, with usually less than 600 mm of annual precipitation and a mean annual temperature of 9.8°C (ZAMG 2010).

In August 2009, the genotypes were planted in the open field with five replications and five plants per replication. The plants were placed in a shifted double row (0.25 × 0.5 m). The soil was highly infested with *V. dahliae* (15.8 microsclerotia/g soil) which was determined by the wet-sieving method (Harris et al. 1993).

The percentage of soil cover was assessed on 10. 5. 2010, by taking photographs of each plot within a frame of 1 m^2 that had been laid over the plot. The photo sections within this square meter were then analysed with the SigmaScan Pro 5 "Turf Analysis" program (Systat Software Inc., San Jose, USA) (Karcher, Richardson 2005). A colour threshold was set and the percentage of green pixels was calculated. In late June 2011, the plant vitality (dead, very weak, weak and healthy plants) was assessed using a scale from 1 to 4 for each plant. A few days later, the incidence of leaf spot diseases (*Mycosphaerella fragariae, Diplocarpon earliana*) and chlorosis was evaluated using a scale from 1 to 9. The incidence of the blossom weevil (*Anthonomus rubi*) was evaluated on two plants per plot on 10. 5. 2010. Flower damage by spring frost was assessed on 10. 5. 2011. During harvest in 2010 and 2011, the marketable yield (weight and number of fruits) and the number of fruits infected by *B. cinerea* and *P. cactorum* were determined three times a week.

Eight fruits per plot were collected for the measurements of soluble solids and fruit firmness in the second week after the beginning of the harvest, depending on the cultivar. The fruits were stored at 4°C until the samples were analysed the following day. The content of soluble solids was measured using a digital hand refractometer (model PT-32; Atago, Tokyo, Japan). Fruit firmness was assessed

Table 2. Vegetative parameters

Genotype	Soil cover (10. 5. 2010; 29. 6. 2011; 1. 7. 2011)	Plant vitality (29. 6. 2011)	Chlorosis (1. 7. 2011)	Leaf spots (1. 7. 2011)
	(%)	scale (1–4)	scale (1–9)	
Elsanta	39^{bcde*}	2.52^{abc}	7.4	4.0
Fraroma	53^{efg}	4^{d}	5.4	3.8
Senga Sengana	48^{bcdef}	3.68^{bcd}	4.8	3.2
Weiroma	35^{abc}	3.36^{bcd}	4.6	1.8
MS 139	33^{ab}	3^{bcd}	6.2	3.4
P-4020	40^{bcde}	4^{d}	4.8	2.0
P-4043	51^{defg}	3.48^{bcd}	5.8	3.2
P-4325	37^{abcd}	3.88^{cd}	6.0	3.0
P-4331	36^{abcd}	3.8^{cd}	4.2	2.8
P-4337	26^{a}	3.16^{bcd}	3.2	2.2
P-5357	40^{bcde}	3.8^{cd}	4.4	2.4
P-5515	35^{abc}	2.76^{abcd}	3.5	2.0
P-5588	56^{fg}	3.32^{bcd}	1.8	2.6
P-5627	49^{cdefg}	2.44^{ab}	5.0	3.3
P-6737	49^{cdefg}	3.48^{bcd}	7.6	4.4
P-7189	47^{bcdef}	3.75^{cd}	4.8	5.3
P-8043	62^{gh}	3.76^{cd}	3.8	6.6
P-8071	62^{gh}	3.08^{bcd}	5.8	6.4
P-8083	42^{bcdef}	3.88^{cd}	4.2	4.6
P-8086	46^{bcdef}	3.88^{cd}	4.8	4.8
P-8096	55^{fg}	1.92^{a}	6.7	3.7
P-8155	44^{bcdef}	3.95^{d}	4.0	3.3
P-8166	71^{h}	4^{d}	4.6	4.0

*ANOVA, S-N-K test, $P < 0.05$; scale (1–4): 1 – dead plant, 2 – very weak plant, 3 – weak plant, 4 – healthy plant; scale (1–9): 1 – no symptoms, 9 – very high incidence

using a penetrometer (penetration area – 1 cm^2, penetration depth – 7 mm) (model M1000E; Mecmesin, Slinfold, UK). The evaluation of intensity of taste was done by a group of three persons who agreed on a common rating.

All numeric data were analysed statistically with SPSS 15.0 (IBM, New York, USA) (ANOVA, S-N-K test, $P < 0.05$).

RESULTS AND DISCUSSION

Vegetative parameters

In the first spring after planting, the soil cover of the genotypes Fraroma, P-5588, P-8043, P-8071, P-8096, P-8166 and P-4043 (all having values above 50%) was significantly higher than the soil cover of the genotypes Weiroma, P-4337, MS 139 and P-5515 (Table 2). A dense foliage to cover the soil is a trait sparsely considered in conventional breeding, however, it is certainly worth a consideration in organic breeding programs to reduce the emergence of weeds. A correlation between dense foliage and high susceptibility to fruit rots was not seen in our experiment.

Since a very high amount of microsclerotia of *V. dahliae* were found on the site, and typical symptoms of Verticillium wilt were observed, we assumed that stunting and wilting were caused mainly by infestation of *V. dahliae*. Plant vitality was the lowest in genotypes P-8096, P-5627 and Elsanta, differing

Table 3. Damages by the blossom weevil *(A. rubi)*, by spring frost and by *B. cinerea*

Genotype	Flowers damaged (%)		Fruits infected (%) by *Botrytis cinerea*	
	by *Anthonomus rubi*	due to frost		
	10. 5. 2010	10. 5. 2010	2010	2011
Elsanta	16.7^{bcde*}	17.2^{abc}	8.5^{ab}	0.3^{a}
Fraroma	3.5^{ab}	8.0^{abc}	26.2^{c}	2.2^{ab}
Senga Sengana	4.0^{abc}	12.7^{abc}	22.9^{c}	4.6^{c}
Weiroma	1.1^{a}	3.6^{a}	6.2^{a}	0.5^{a}
MS 139	0.3^{a}	18.8^{abc}	2.5^{a}	0.1^{a}
P-4020	11.9^{abcd}	11.0^{abc}	7.5^{ab}	1.1^{ab}
P-4043	6.6^{abc}	28.4^{bc}	11.2^{ab}	0.7^{a}
P-4325	21.6^{de}	17.9^{abc}	2.8^{a}	0.6^{a}
P-4331	14.9^{abcde}	19.5^{abc}	1.2^{a}	0.7^{a}
P-4337	18.0^{cde}	21.2^{abc}	4.0^{a}	0.0^{a}
P-5357	2.9^{ab}	6.3^{ab}	10.6^{ab}	0.7^{a}
P-5515	5.4^{abc}	17.1^{abc}	15.7^{b}	0.4^{a}
P-5588	7.8^{abc}	17.5^{abc}	4.7^{a}	0.4^{a}
P-5627	2.7^{ab}	9.7^{abc}	4.0^{a}	1.1^{ab}
P-6737	15.2^{abcde}	6.9^{ab}	3.8^{a}	0.2^{a}
P-7189	8.5^{abc}	19.8^{abc}	15.7^{b}	3.1^{b}
P-8043	26.3^{e}	32.2^{c}	7.0^{a}	0.3^{a}
P-8071	10.3^{abcd}	18.0^{abc}	4.6^{a}	0.4^{a}
P-8083	8.1^{abc}	22.3^{abc}	5.1^{a}	0.9^{a}
P-8086	2.8^{ab}	18.6^{abc}	2.7^{a}	1.1^{ab}
P-8096	0.2^{a}	12.6^{abc}	1.8^{a}	1.2^{ab}
P-8155	3.8^{abc}	14.0^{abc}	2.3^{a}	1.6^{ab}
P-8166	3.4^{ab}	21.7^{abc}	3.0^{a}	0.4^{a}

*ANOVA, S-N-K test, $P < 0.05$; ■ early-flowering: BBCH > 62 on 3. 5. 2010; □ medium-flowering: BBCH > 60 < 62 on 3. 5. 2010; ▢ late-flowering: BBCH < 60 on 3. 5. 2010

significantly from the genotypes Fraroma, P-4020, P-8155 and P-8166. The genotpyes Elsanta, MS 139, P-4337, P-5515, P-5627 and P-8096 already showed wilting symptoms in 2010. The level of the so-called field resistance to *V. dahliae* depends on the conidial inoculum concentration (Shaw et al. 1997) and relies on both resistance and tolerance mechanisms (Shaw et al. 2010). It is controlled quantitatively by many genes and has never been found to be complete (Zebrowska et al. 2006; Shaw et al. 2010). Olbricht and Hanke (2008) were able to re-isolate the fungus even from symptomless plants of cv. Fraroma after artificial inoculation.

The genotypes Fraroma, P-4020 and P-8166 which had no symptoms at this site may not be regarded as tolerant in any environment, as *V. dahliae* could also be attested in a diseased plant of cv. Fraroma on another site. A high cultivar × site interaction was also shown in previous screenings on several *Verticillium*-infested sites (Weissinger et al. 2011).

Since the site has a pH of 7.5, most genotypes showed chlorosis. An exception was genotype P-5588 which had rich green leaves. The breeding clones P-7189, P-8043 and P-8071 had a high incidence of leaf spots. Usually, leaf spot diseases

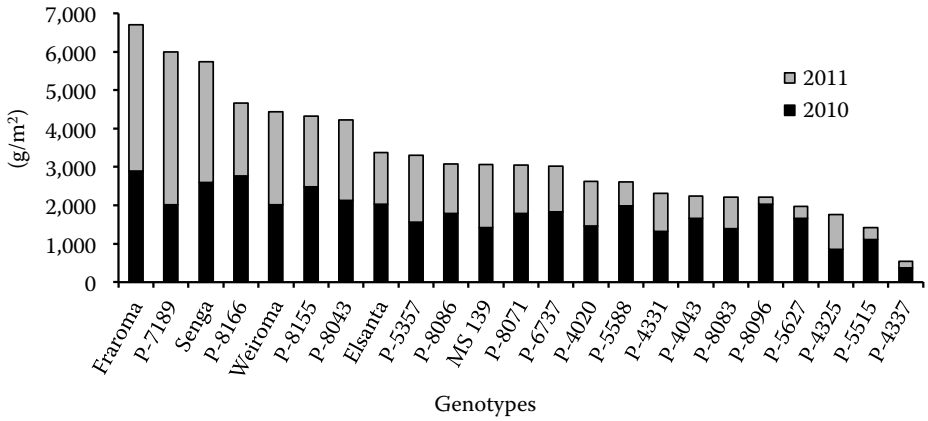

Fig. 1. Marketable yield of 2010 and 2011

are not addressed in breeding programs since only those pests and diseases that cause the largest economic damage are actively screened (Chandler et al. 2012) and leaf spots can be easily controlled in conventional production. However, breeding for resistance is important for organic production and also feasible since resistant genotypes (e.g. resistant to leaf scorch caused by *Diplocarpon earlianum* [Ell. and Ev.] Wolf) were described (Xue et al. 1997; Hancock et al. 2010). The resistance is assumed to

Table 4. Fruit quality characteristics

Genotype	Fruit weight (g)		Fruit firmness (kg/cm²)	Soluble solids (°Brix)	Taste intensity (1–5)**
	2010	2011			
Elsanta	14.41^{cd*}	8.7^{bcde}	0.64^{h}	9^{fg}	3
Fraroma	9.53^{ab}	6.77^{abcd}	0.46^{def}	8^{bcd}	3
Senga Sengana	9.25^{ab}	6.46^{abc}	0.51^{ef}	7.6^{ab}	4
Weiroma	10.2^{ab}	7.82^{bcde}	0.41^{cd}	7.8^{bc}	5
MS 139	11.19^{ab}	7.24^{abcde}	0.38^{bc}	7.6^{ab}	3
P-4020	12.3^{bc}	9.52^{de}	0.55^{fg}	8.3^{cde}	4
P-4043	12.31^{bc}	7.21^{abcde}	0.45^{def}	9.8^{h}	4
P-4325	14.33^{cd}	9.13^{cde}	0.33^{b}	10.5^{i}	5
P-4331	9.66^{ab}	8.66^{bcde}	0.51^{ef}	9.7^{h}	3
P-4337	10.85^{ab}	6.83^{abcd}	0.67^{h}	11^{j}	5***
P-5357	9.92^{ab}	6.65^{abcd}	0.46^{def}	8.5^{def}	5
P-5515	11.58^{ab}	8.49^{bcde}	0.66**	7.6**	5
P-5588	8.57^{a}	5.94^{ab}	0.48^{def}	8.5^{def}	2
P-5627	10.93^{ab}	6.79^{abcd}	0.43**	8.4**	4
P-6737	12.3^{bc}	7.81^{bcde}	0.77^{i}	8.9^{efg}	5***
P-7189	22.06^{f}	14.08^{g}	0.62^{h}	7.1^{a}	3
P-8043	18.16^{e}	12.41^{f}	0.78^{i}	7.5^{ab}	2
P-8071	17.61^{e}	9.75^{e}	0.92^{j}	9.1^{fg}	5
P-8083	15.04^{d}	8.68^{bcde}	0.6^{gh}	10^{hi}	4
P-8086	10.4^{ab}	7.1^{abcde}	0.49^{ef}	9.2^{g}	4
P-8096	10.75^{ab}	4.73^{a}	0.83**	9.7**	5
P-8155	10.13^{ab}	5.92^{ab}	0.19^{a}	8.5^{def}	3
P-8166	12.2^{bc}	7^{abcde}	0.34^{b}	10.2^{hi}	4***

*ANOVA, S-N-K test,**1 – low, 5 – high; **values only from 2010; ***similar flavour to *Fragaria vesca*

be of polygenic nature, but it is inherited at a high level and easy to score on both young runner plants and adult plants (Xue et al. 1997).

Yield parameters

Most of the late-flowering genotypes (Weiroma, MS 139, P-5357, P-5627, P-8086 and P-8166) and the medium-flowering genotype P-8096 had significantly fewer damaged buds by the blossom weevil *A. rubi* than the medium-flowering genotypes P-4325, P-4337 and P-8043 (P-8043 had 26.3% damaged buds). All early-flowering genotypes had significantly fewer damaged flowers than genotype P-8043. The fact that late-flowering genotypes had less blossom weevil damage agrees with other cultivar evaluations conducted in Eastern Austria (not published) and with experiences in Estonia (Kikas et al. 2009), but not with the results from Western Germany (Steen et al. 2011). These findings indicate the need for region-specific recommendations. Simpson et al. (1997) suggested that the damage by *A. rubi* is "not merely a function of flowering time but that susceptibility is under independent genetic control". This was confirmed by Kikas et al. (2009) and by the present study, with some significant differences within the medium-flowering genotypes. Regarding spring frost tolerance, both a late bloom and frost hardiness of styles and receptacles are important traits. The stages "Full petal" and "Petal fall" are most susceptible to freezing temperatures (Ki, Warmund 1992). In our trial, the medium-flowering cv. P-8043 was most affected by both *A. rubi* and spring frost and had significantly more flowers damaged by frost than Weiroma, P-5357 and P-6737, three late flowering genotypes. Climate change may result in earlier flowering and therefore higher exposure to frost risks (Carlen, Krüger 2008). Although screening for frost tolerance has not been part of current breeding programs, it should be part of future evaluations. Khanizadeh et al. (2004) showed that chlorophyll fluorescence could be a suitable screening method. Hummel and Moore (1997) doubted that it is enough to select for frost tolerance of flowers and suggested selecting for protective canopy architecture.

In 2010, a high percentage of fruits were infected by *B. cinerea,* whereas in 2011, the infection level was very low. In 2010, genotypes Fraroma, Senga Sengana, P-7189 and P-5515 showed a high susceptibility to *Botrytis* fruit rot. These genotypes had significantly more infected fruits than most of the others (Table 3). Leather rot caused by *P. cactorum* only occurred in genotype P-7189 to a greater extent (9.4% of the fruits were affected in 2010, 0.5% in 2011, data not shown). Generally, all breeding clones were significantly more tolerant to *B. cinerea* than the reference cvs Fraroma and Senga Sengana. However, despite high losses due to fruit rots, cvs Fraroma, Senga Sengana and P-7189 had the highest yields of marketable fruits (Fig. 1). Other high yielding genotypes were P-8043 and Weiroma. Genotypes P-8166 and P-8155 appeared promising in 2010, but dropped behind concerning yield in 2011. The genotypes P-8096 and P-5588 had similar yields to cv. Elsanta in 2010, but much lower yields than cv. Elsanta in 2011 due to wilting in genotype P-8096 and to both wilting and a decline in fruit weight in P-5588.

Fruit quality characteristics

Genotype P-7189 had the highest average fruit weight in both years (22.06 g in 2010 and 14.08 g in 2011), followed by P-8043 and P-8071 (Table 4). Genotypes with fruit weights below 10 g in the first harvest year were Fraroma, Senga Sengana, P-4331, P-5357 and P-5588. From 2010 to 2011, the greatest decline in fruit weight was measured on P-8096, probably due to Verticillium wilt.

There were significant differences in fruit firmness between the genotypes. P-8071 had the firmest fruits of all genotypes (0.92 kg/cm^2). Fruits of P-6737 and P-8043 were firmer (> 0.7 kg/cm^2) than fruits of cv. Elsanta (0.64 kg/cm^2). P-8155 produced the softest fruits (0.19 kg/cm^2), differing significantly from all other genotypes. Fruits of P-4325 and P-8166 also had rather low fruit firmness.

The genotypes P-4337, P-4325, P-8083 and P-8166 had the highest soluble solids content of average for 2010 and 2011, whereas P-7189, P-8043, MS 139, cvs Senga Sengana and Weiroma had the lowest values (Table 3). Correlations between high sugar content and low yield were reported by Faedi and Mezzetti (1998) and confirmed in our tests, with the exception of P-8166, which had a rather high yield and high sugar content.

The taste of fruits of genotypes Weiroma, P-4325, P-4337, P-5357, P-5515, P-6737, P-8071 and P-8096 was characterized as highly intense. While the taste of P-5588 and P-8043 was rather shallow, P-4337, P-6737 and P-8166 had a flavour reminiscent of *Fragaria vesca*. Fruit flavour is a complex trait in

strawberries. Sugars, acids and approximately 15 to 20 volatile compounds make up the key components of the sensory perception (Flachowsky et al. 2011). The typical "woodland strawberry-like flavour", which is preferred by consumers, is regrettably missing in most commercially used cultivars. This flavour is typical for the diploid woodland strawberry *F. vesca*, but seems to be present only in a few octoploid cultivars. The semi-volatile compound methylanthranilate (MA) is the key constituent of this taste (Ulrich et al. 1997). Breeding of genotypes with MA high levels is difficult, because this trait is only inherited by one-third of the offspring (Olbricht et al. 2008).

Recommendable breeding clones

Fraroma, Senga Sengana and P-7189 had the highest yield and a high plant vitality, but were susceptible to *B. cinerea*. P-7189 was also susceptible to *P. cactorum*. However, P-7189 could be a new interesting fresh market cultivar because of its very high, stable yield and its large and firm fruits. P-8043 and P-8071 also show fresh marketing qualities, but are less productive than P-7189. However, it showed tolerance to fruit rots and high vegetative vigour, thus suitable for production on organic farms. In 2011, the yield of P-8043 was reduced by spring frost damage and the blossom weevil, but in other years or sites the actual high yield potential could be exploited. Its soil cover and plant vitality were very high, but its taste was evaluated below average. P-8071 was rated high for taste, but showed slight symptoms of Verticillium wilt after one season of harvest.

The genotypes Weiroma, P-8155 and P-8166 combined plant vitality, high yield and tolerance to *B. cinerea* which are important characteristics for organic production. However, serious drawbacks of these genotypes are the low fruit firmness and the small fruit size which make them suitable only for home gardens or processing purposes. P-5357, P-6737 and P-8086 are interesting for home gardens only. They had low yields, but high plant vitality and a good flavour.

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