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ARTICLE

Characterization of 14 Raspberry Cultivars by Solid-Phase Microextraction and Relationship with Gray Mold Susceptibility

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Fourteen raspberry varieties were evaluated over two cropping seasons by solid-phase microextraction (SPME) followed by gas chromatography−mass spectrometry. Thirty-six compounds were fully identified, and 10 more compounds were tentatively identified. Despite interannual variability, raspberry varieties can be divided in two main groups on the basis of terpenes and C-13 norisoprenoids. Susceptibility toward *Botrytis cinerea*, one of the most relevant pathogenic fungi for soft fruits during storage, was also evaluated. On the basis of volatile profiles, it was possible to highlight the relationship between different volatile compounds and resistance to *B. cinerea*. Volatile profiles and *Botrytis* susceptibility of the different raspberry varieties evaluated should assist future breeding programs.

KEYWORDS: Raspberry; SPME; GC-MS; *Rubus idaeus* spp.; *Botrytis cinerea*; fungus; PLS regression; terpenes

INTRODUCTION

Raspberry (*Rubus idaeus* L.) is a member of the Rosaceae family, and the red fruited variety is the most commercially grown berry. It is most productive in regions with mild winters and long moderate summers (*1*). However, because of their economic value, there is great interest in growing cane fruits in southern areas of Europe, such as Spain, Portugal, and Italy. Trentino is an alpine area in northern Italy with a long tradition of berry cultivation (*2*), where soft berries still guarantee high economic return. In this region, raspberries are mainly produced for the fresh market; hence, fruit flavor and quality are of primary importance. Raspberry fruits are an important dietary source of antioxidant compounds, in particular, polyphenols (*3*, *4*), which are renowned for their health benefits (*5*, *6*). Their typical flavor makes these fruits easily recognizable and appreciated not only for their health impact. Raspberry flavor is a complex combination of hundreds of volatile compounds, of which more than 200 have been identified (*7*, *8*). Major classes of compounds identified in raspberry include C13-norisoprenoids, monoterpenes, aliphatic and aromatic hydrocarbons, aldehydes, ketones, alcohols, and sesquiterpenes (*9*). Among them are some compounds that are recognized as typical of ripened raspberry fruits such as α- and β-ionone, α- and β-phellandrene, and ethyl and acetate esters (*10*). Other volatile compounds, such as benzaldehyde, 1-hexanol, 2-nonanone, *trans*-2-hexenal, and *cis*-3-hexenol, are reported to have inhibitive effects against fungi (*11*). The volatile compound profiles of different raspberry genotypes can be very different, both qualitatively and quantitatively (*12*−*14*), and they are influenced by many agronomical and technological parameters (*15*, *16*). Thus, this is an important aspect to consider for the characterization and evaluation of a particular cultivar. This soft fruit is highly susceptible to fungal diseases, particularly to gray mold caused by *Botrytis cinerea* (*16*). Gray mold may inflict significant crop losses in raspberry, particularly when no tunnels are used in cultural management. Although the use of tunnels and cultural practices can lower the incidence of gray mold, the disease still represents a problem in postharvest conditions and can contribute to the high perishability of this crop and to the decay of the fruit on market shelves. Cultivar differences involved in tolerance mechanisms have been recorded (*17*) in raspberry and related species (*18*). Some genotypic resistances were attributed mainly to cuticle and wax characteristics and anatomical features and, thus, cultivar selection plays a major role in fungus management (*19*). Plants possess a range of preformed and inducible defenses for combating the infection, and many of these defenses include secondary metabolites (*20*). For example, the volatiles emitted from crushed tomato leaves have a strongly inhibitory activity against the spore germination and hyphal growth of *B. cinerea* (*21*)

In this study, volatile organic compounds of 14 raspberry varieties, grown in a controlled environment, were monitored over two cropping seasons. The purpose of the present investigation was the rapid characterization by solid-phase microextraction (SPME) of the volatile organic compounds released by these raspberry varieties. The study aims to obtain useful information for the breeding programs to be used in Italian raspberry production. Because volatile organic compounds are also involved in plant defense, gray mold susceptibility of the considered raspberry varieties was also evaluated. A correlation between

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SPME/gas chromatography−mass spectrometry (GC-MS) results and gray mold susceptibility was attempted to investigate possible relationships between the volatile organic compounds naturally emitted by these fruits and their resistance to *B. cinerea*.

MATERIALS AND METHODS

Samples. Berries from 14 raspberry varieties (**Table 1**) produced under standardized conditions (*4*) were collected from the experimental field located at 700 m asl in Vigolo Vattaro (Trentino, Italy) during 2006 and 2007 seasons. Berries were harvested manually, from plants having mature fruits, placed immediately in ice packs, and transported to the laboratory, where samples were stored at 4 °C prior to analysis the next day. For each variety three different batches were evaluated on three different days to take into account possible variability during the production period. At each sampling the mass (g) of the single berries was recorded.

SPME/GC-MS Analysis. Samples were analyzed according to the procedure extensively described in previous work (*9*). Briefly, four or five berries (18−20 g) were introduced into a 100 mL glass flask and gently mashed with a spatula; then 10 μL of 2-octanol (12.4 μg/kg) was added as internal control. The sealed vial was immersed in a water bath and held at 35 °C to equilibrate. After 10 min, a 2 cm fused silica fiber coated with divinylbenzene/carboxen/polydimethylsiloxane 50/30 μm (DBV/CAR/PDMS) (Supelco, Bellefonte, PA) was introduced in the vial and exposed to the headspace environment for 30 min. The fiber was preconditioned before the analyses, according to the manufacturer's instructions. Volatiles adsorbed on the SPME fiber were desorbed in splitless mode for 5 min in the GC injector at 250 °C (AutoSystem XL gas chromatograph coupled with a Turbo Mass Gold Mass Spectrometer, PerkinElmer, Norwalk, CT). Separation was achieved on a HP-Innowax fused-silica capillary column (30 m, 0.32 mm i.d., 0.5 μm film thickness; Agilent Technologies, Palo Alto, CA). The GC oven temperature program consisted of 60 °C for 3 min, raised from 60 to 220 at 8 °C min^{-1}, 220 °C for 5 min, raised from 220 to 250 at 10 °C min^{-1}, and 250 °C for 5 min. Helium was used as carrier gas with a constant column flow rate of 1 mL min^{-1}. The transfer line temperature was kept at 220 °C. The mass spectrometer operated in electron ionization mode (EI, internal ionization source; 70 eV) with a scan range between m/z 30 and 300. Compound identification was based on mass spectra matching in the standard NIST-98/Wiley library and retention indices (RI) of authentic reference standards. Linear retention indices were calculated after analysis, under the same chromatographic conditions, of a C10−C24 *n*-alkane series (Supelco).

Chemicals. Hexanol, 2-heptanol, hexanal, *trans*-2-hexenal, 2-heptanone, hexanoic acid, hexyl acetate, β-pinene, limonene, linalool, theaspirane B, and β-damascenone were obtained from Aldrich (Milan, Italy). Benzaldehyde, *cis*-3-hexenol, and acetoin were obtained from Merk Chemical Ltd. (Darmstadt, Germany). Ethyl acetate, 1-octen-3-ol, 1-octanol, α-pinene, α-phellandrene, β-myrcene, γ-terpinene, *p*-cymene, 4-terpineol, geraniol, caryophyllene oxide, α-ionone, α-ionol, and β-ionone were obtained from Fluka (Milan, Italy). Benzyl alcohol, acetophenone, and acetic acid were purchased from Carlo Erba Reagents (Milan, Italy).

Gray Mold Susceptibility. Gray mold susceptibility, caused by *B. cinerea*, was detected by visual inspection, by an experienced technician, of the fruits on plants during the harvesting season and on the fruit samples stored at 4 °C for 72 h. Varieties were scored, based on a subjective index of conidia development, in a range from 0, when no development of the fungus was present, to 5, when the attack was severe. Although this procedure is highly dependent on the skills of the inspector and is possible only when the fungus is sporulating, it is the most widely used in recording gray mold (*22*).

Data Analysis and Statistics. Summary statistics were performed using software package Statistica 8.0 (StatSoft, Inc., Tulsa, OK). For semiquantitative comparison among different varieties, data were normalized versus 2-octanol added as control. Multivariate data analyses were computed by the software The Unscrambler 8.5 (Camo Process AS, Oslo, Norway). Data were normalized for each variety and within each season to total chromatogram area and further log-transformed prior to multivariate data analyses.

RESULTS AND DISCUSSION

SPME/GC-MS. The 14 cultivars of raspberry fruits were analyzed over two consecutive years (2006 and 2007) to determine the qualitative and semiquantitative composition of the volatile compounds emitted by these fruits. In the GC-MS chromatograms, 46 peaks were considered as described in previous work (*9*), extending the peak identification from 26 to 36 compounds in the present work. Ten more compounds were tentatively identified. Peak identification and summary statistics (over the two seasons) are reported in **Table 2** (detailed results for each variety separately reported over the two years can be found in the Supporting Information, Tables S1 and S2). The last column of **Table 2** reports the chromatogram area percent (CA %) that represents a measure of the contribution of each peak to the total chromatogram. The most represented class of compounds is the monoterpene family, with 12 compounds that quantitatively contribute about 29% (as total area chromatogram) of the volatiles detected, followed by C13-norisoprenoids including 10 compounds accounting for about 32% of volatiles detected; only 3 esters were identified (acetate esters), and their quantitative contribution to the recorded chromatogram is $>17\%$. The other classes of compounds contribute each no more than 5%. Among the volatile compounds detected, we tentatively identified a hydrocarbon, present in relatively high amount (CA of 3.7%), as *trans*-3-methyl-1,3,5-hexatriene. The origin of this compound is not clear, but its structure suggests a possible link with monoterpenes. We found that its occurrence is significantly ($p < 0.01$) correlated to different terpenes and, in particular, to α- and β-pinene, which showed the highest correlation coefficient ($r = 0.87$). The compound could be a degradation product or a rearrangement of terpenes.

Comparing the data over the two years, we observed that the total amount of volatiles emitted by the berries was higher in those collected in the 2007 season with the exception of Pokusa, Polka-I, and Popiel, as summarized in **Figure 1** (detailed results are available in Tables S1 and S2 of the Supported Information). In different crops, high isoprene emissions are involved in thermoprotection mechanisms from high temperatures (*23*); in raspberry and grape a stronger volatile emission is often associated with cool temperatures (*15*, *24*, *25*). In the Vigolo Vattaro (Trento, Italy) experimental fields, the mean temperatures recorded in the production period were 1−3 °C colder in 2007 compared to 2006. Thus, the higher amount of volatiles observed for the 2007 season could be associated with the lower temperature, according to the observations reported in the literature for other crops. Despite the high variability between the two seasons and within the varieties, the differences among varieties were

Table 1. Raspberry Varieties Considered in This Study with Their Type of Fruiting and Pedigree

variety	fruiting[a]	pedigree
Anne	yellow, PC	Amity × Glen Garry
Autumn Bliss	red, PC	complex cross
Caroline	red, PC	Geo-1 (Autumn Bliss × Glen Moy) × Heritage
Heritage	red, PC	(Milton × Cuthbert) × Durham
Himbo-top	red, PC	Heritage in its derivation
Josephine	red, PC	complex hybrid with Amity in the parents
Opal	red, PC	Heritage in its derivation
Pokusa	red, PC	P 86594 x P 87432 (Autumn Bliss, Heritage, *Rubus odoratus*, and *Rubus occidentalis*)
Polana	red, PC	Heritage in its derivation
Polesie	red, PC	Autumn Bliss and Heritage in its derivation
Polka-P[b]	red, PC	Opof 89141, which has Autumn Bliss in its derivation
Polka-I[b]	red, PC	Opof 89141, which has Autumn Bliss in its derivation
Popiel	red, PC	Autumn Bliss and Heritage in its derivation
Tulameen	red, FC	Nootka × Glen Prosen

[a] PC, primocane; FC, floricane. [b] P stands for Polka variety from Poland and I for Polka variety from Italy.

Table 2. Levels of Volatile Compounds in the Headspace of the 14 Raspberry Varieties As Determined by Solid-Phase Microextraction and Gas Chromatography−Mass Spectrometry: Peak Identification and Summary Statistics

				descriptive statistics[c]						
no.	compound	RI[a]	ID[b]	mean	median	min	max	SD	CV%	CA %[d]
1	2-heptanol	1336	A	22.84	12.58		144.89	30.56	134	1.29
2	hexanol	1372	A	14.84	8.77	0.92	89.52	16.30	110	0.84
3	*cis*-3-hexenol	1408	A	46.95	31.24	2.24	475.92	59.55	127	2.65
4	1-octen-3-ol	1484	A	0.20	0.15		1.46	0.22	113	0.01
5	1-octanol	1599	A	0.86	0.69	0.16	2.42	0.50	58	0.05
6	benzyl alcohol	1902	A	0.28	0.20	0.02	1.59	0.28	99	0.02
	total alcohols			**85.96**						**4.86**
7	hexanal	1055	A	40.66	22.32	0.12	185.28	41.71	103	2.30
8	*trans*-2-hexenal	1215	A	44.93	32.69	2.20	561.38	63.38	141	2.54
9	decanal	1546	A	2.01	1.44	0.08	10.78	1.93	96	0.11
10	benzaldehyde	1565	A	0.50	0.38	0.04	2.28	0.42	86	0.03
	total aldehydes			**88.10**						**4.98**
11	2-heptanone	1172	A	56.26	40.52	3.69	371.41	53.85	96	3.18
12	acetoin	1223	A	4.69	2.32		49.50	7.20	154	0.26
13	acetophenone	1695	A	2.38	1.59	0.52	19.19	2.58	108	0.13
14	δ-decalactone	2175	A	0.70	0.59	0.02	3.07	0.59	84	0.04
	total ketones			**64.04**						**3.62**
15	acetic acid	1476	A	20.26	8.50	0.08	225.04	33.24	164	1.14
16	hexanoic acid	1878	A	34.80	16.24		389.38	56.62	163	1.97
	total acids			**55.06**						**3.11**
17	ethyl acetate	832	A	118.16	101.56	8.80	570.36	92.41	78	6.68
18	hexyl acetate	1283	A	22.44	9.76		191.66	36.78	164	1.27
19	*cis*-3-hexenyl acetate	1338	A	163.80	119.54	2.64	825.61	146.85	90	9.26
	total esters			**304.40**						**17.20**
20	α-pinene	992	A	121.81	94.16	0.08	498.05	124.72	102	6.88
21	β-pinene	1079	A	7.39	3.60		35.90	8.82	119	0.42
22	α-phellandrene	1150	A	122.41	84.04		651.00	141.74	116	6.92
23	β-myrcene	1151	A	46.31	24.08		306.57	62.68	135	2.62
24	limonene	1190	A	18.07	12.94	0.88	88.88	17.64	98	1.02
25	γ-terpinene	1249	A	7.15	2.56		45.71	9.99	140	0.40
26	*p*-cymene	1281	A	25.30	13.96	0.08	175.26	32.18	127	1.43
27	linalool	1590	A	62.62	26.31	0.22	631.28	102.68	164	3.54
29	4-terpineol	1639	A	7.27	2.32		120.52	15.60	215	0.41
30	geraniol	1879	A	17.54	12.85	0.41	110.61	17.98	102	0.99
31	β-phellandrene		C	73.43	48.48		387.96	88.91	121	4.15
32	α-cyclogeranyl acetate		C	1.32	0.91	0.12	8.02	1.26	96	0.07[d]
	total monoterpenes			**510.61**						**28.85**
33	*trans*-caryophyllene	1647	A	20.26	3.01		245.30	42.90	212	1.15
34	caryophyllene oxide	2010	A	0.45	0.05		5.14	0.91	202	0.03
35	unidentified sesquiterpene		C	12.73	4.30	0.02	195.64	24.26	191	0.72
	total sesquiterpenes			**33.45**						**1.89**
28	theaspirane B	1591	A	1.90	1.28	0.32	6.96	1.44	76	0.11
36	β-damascenone	1861	A	3.11	2.64	0.02	17.36	2.60	84	0.18
37	α-ionone	1886	A	201.00	192.67	17.52	652.13	104.42	52	11.36
38	α-ionol	1930	A	10.84	7.04	0.03	56.26	11.76	109	0.61
39	β-ionone	1965	A	167.42	166.22	21.76	436.52	83.75	50	9.46
40	cycloionone I/edulan		B**	147.86	120.12	15.75	537.33	103.04	70	8.36
41	dehydro-β-ionone		C	25.48	18.40	0.48	107.72	23.47	92	1.44
42	*trans*-β-ionon-5,6-epoxide		C	0.58	0.48	0.06	1.89	0.39	67	0.03
43	dihydro-β-ionol		B***	2.11	1.59		11.32	1.79	85	0.12
44	3,4-didehydro-β-ionone		C	2.13	1.94	0.04	8.72	1.45	68	0.12
	total C13-norisoprenoids			**562.42**						**31.78**
45	5-ethyl-(3*H*)-furan-2-one		C	0.61	0.44	0.11	3.36	0.54	88	0.03
46	*trans*-3-methyl-1,3,5-hexatriene		C	64.96	50.80	1.11	246.17	56.90	88	3.67

[a] Retention indices on PEG (polyethyleneglycol) column. [b] Reliability of the identification proposal: A, mass spectrum and RI agreed with standards; B, mass spectrum and RI agreed with literature data (*, ref *34*; **, ref *35*; ***, ref *9*); C, mass spectrum agreed with mass spectral database. [c] Data normalized to the amount of 2-octanol added as internal control. [d] CA %: chromatogram area percentage.

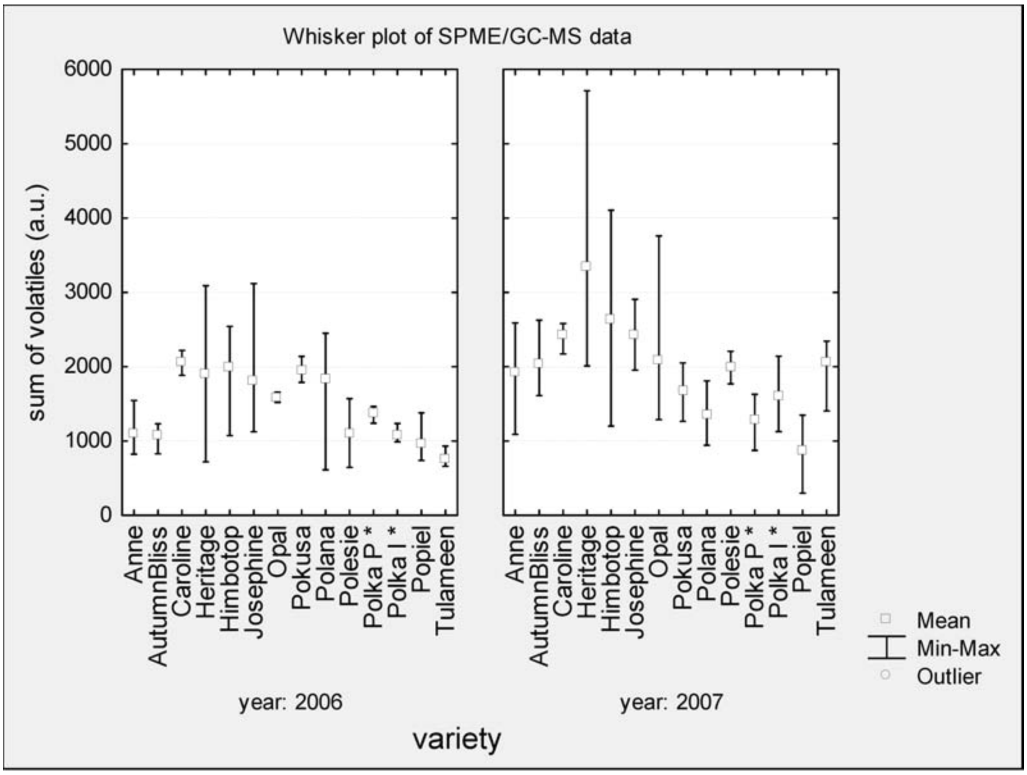

Figure 1. Total volatile organic compounds in the raspberry headspace by SPME GC-MS analysis. Data were normalized to the amount of 2-octanol added as internal control. *, P stands for Polka variety from Poland and I for Polka variety from Italy.

evident (**Figure 1**). According to our biannual data, Polka-I, Polka-P, and Popiel can be considered low volatile emitters, whereas Caroline, Heritage, Himbo-top, and Josephine can be considered higher volatile emitters.

For a rapid data exploration SPME/GC-MS results were submitted to principal component analysis (PCA). The biplot of the first two scores and loadings is given in **Figure 2**. To help the visualization/interpretation, each data point was averaged over the replicates within each variety and year. Along the second component, which accounts for 15% of the variance of the data, it is possible to observe a clear effect of the season; in fact, the 2007 samples (blue symbols) are almost always above the 2006 corresponding samples (red symbols) for each variety. As stated above, the volatile amount was higher in the samples harvested in the 2007 season (**Figure 1**). The dotted line in **Figure 2** divides the samples into two groups. The first group includes six varieties (Anne, Polana, Polesie, Polka-I, Polka-P, and Popiel) characterized by higher levels of terpene alcohols and C13-norisoprenoid compounds. The second group includes eight varieties (Autumn Bliss, Caroline, Heritage, Himbo-top, Josephine, Opal, Pokusa, and Tulameen) characterized by higher levels of monoterpenes (α-pinene, β-pinene, β-myrcene, α-phellandrene, β-phellandrene, limonene, *p*-cymene, and γ-terpinene) and sesquiterpenes (*trans*-caryophyllene and caryophyllene oxide). Furthermore, the biplot (**Figure 2**) also shows that the Tulameen variety is characterized by higher levels of C6 compounds (aldehydes and alcohols) and their esters. These compounds are responsible for herbaceous odor notes, and our finding is in accordance with previous observation on commercial samples (*9*).

Gray Mold Susceptibility. In **Table 3**, the *Botrytis* susceptibility, evaluated for the raspberry varieties studied, is reported as an index on a scale ranging from 0 (no fruits damaged) to 5 (completely damaged fruits). Fruit weights are also reported to give an indication of the variability of berries dimension. The genotypes that showed a lower degree of susceptibility were Caroline and Josephine, whereas Popiel and Polesie showed lower tolerance to *B. cinerea*. Postharvest rotting caused by *B. cinerea* Pers.: Fr. is one of the most important and widespread fungal diseases worldwide, and it has a heavy impact on fresh productions. The evaluation of gray mold was subjected to cultural and environmental pressure so that further data to support the screening are needed. However, because the different genotypes were cultivated under the same conditions, the comparison can be taken as a preliminary screening. Because fully resistant cultivars are not known, any amelioration of genotypic

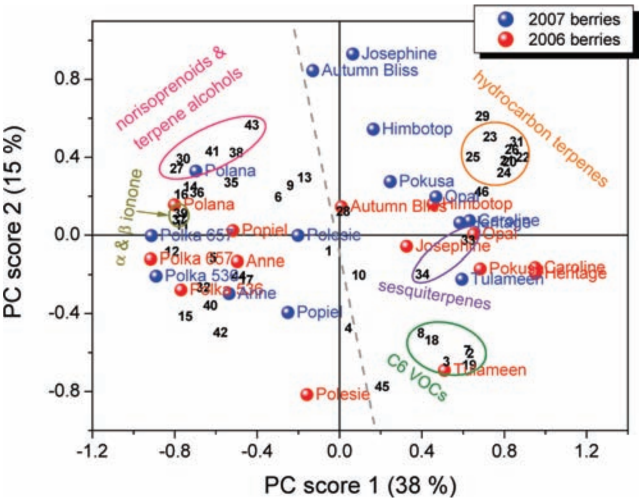

Figure 2. Biplot of the first two components of the PCA on SPME/GC-MS data. Blue symbols represent samples harvested during 2007 cropping season; red symbols represent samples harvested during 2006 cropping season. Loadings are indicated with numbers as reported in the first column in **Table 2**. Ellipses highlight loadings associated with the same class' compounds; dashed line delimits the two groups (see text).

Table 3. Raspberry Weight and *Botrytis* Susceptibility Index

variety	sbw (g) 2006[a]	sbw (g) 2007[a]	*Botrytis* susceptibility[b]
Anne	4.8 ± 0.9	5.0 ± 0.8	4
Autumn Bliss	4.2 ± 0.7	4.3 ± 0.7	3
Caroline	4.3 ± 0.6	4.2 ± 1.0	0
Heritage	4.6 ± 0.8	2.7 ± 0.6	2
Himbo-top	3.1 ± 0.6	4.3 ± 0.8	1
Josephine	3.6 ± 0.5	3.2 ± 0.6	0
Opal	4.4 ± 0.8	3.2 ± 0.6	3
Pokusa	4.3 ± 0.8	6.3 ± 1.6	4
Polana	5.3 ± 1.5	3.7 ± 0.8	4
Polesie	3.3 ± 0.9	6.0 ± 1.1	5
Polka-P[c]	5.2 ± 0.7	4.3 ± 0.9	4
Polka-I[c]	4.4 ± 1.0	4.4 ± 0.7	4
Popiel	8.2 ± 1.9	5.1 ± 1.0	5
Tulameen	4.6 ± 0.9	4.8 ± 0.9	2

[a] sbw, single berry weight (data averaged over 20 fruits). [b] 0 very low or absent; 5 highest. [c] P stands for Polka variety from Poland and I for Polka variety from Italy.

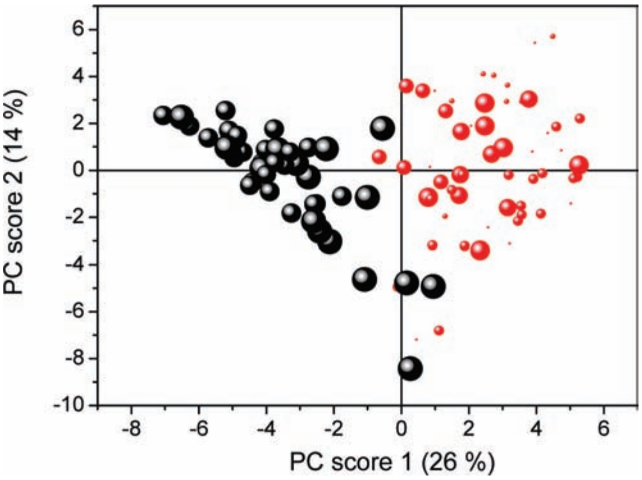

Figure 3. Symbol size is proportional to *Botrytis* susceptibility. Black and red symbols are used to distinguish between the same groups as delimited by the dashed line in **Figure 2**.

control can be useful to reduce the treatments per season that are necessary to control the pathogen (*26*).

Relationship between Volatile Compounds and Gray Mold Susceptibility. Overlaying the information of the independently measured *Botrytis* susceptibility with information condensed in the PCA of volatile compounds, the plot appears as reported in **Figure 3**, where the symbol size of the samples is proportional to *Botrytis* susceptibility. Examination of these two figures (**Figures 2** and **3**) suggests a possible role of monoterpene compounds, which characterize the second group (red symbols) designed in **Figure 3**, in contrasting gray mold disease. In several works, terpenes have been reported for their different antifungal efficacy (*27*−*29*) against *B. cinerea*. To objectively highlight the relationship between volatile compounds in raspberry and *Botrytis* susceptibility, data were analyzed by partial least-squares (PLS) regression (*30*, *31*). PLS regression is the most suitable technique for use in the presence of low-rank data matrices, characterized by highly collinear X-block descriptors, and involves the simultaneous decomposition of both the X and Y matrices to find a set of maximally covarying latent vectors. With respect to the latent vectors computed in PCA, the PLS components do not focus on explaining all of the variance of the predictor matrix, but only that portion which is maximally correlated with Y.

A reasonable model was built using six PCs with an R^2 of 0.85, which expresses the fraction of the variance in y that is explained by the model. From this model, the regression coefficients, summarizing the relationship between the predictors (chromatogram peaks) and the response (*Botrytis* susceptibility), were calculated. After Martens' uncertainty test (*32*), nine compounds resulted negatively correlated to *Botrytis* susceptibility: α-pinene, *β*-phellandrene, *p*-cymene, 2-heptanol, 4-terpineol, *trans*-caryophyllene, *β*-damascenone, dehydro-*β*-ionone, and caryophyllene oxide. These results indicate that quantification of the highlighted compounds in raspberry can be used as an indicator of resistance toward *B. cinerea*. Further investigation to assess a direct action of the single volatile compounds or combination of them is envisaged.

Quantitative and qualitative differences in fruit volatile emission among different raspberry varieties are relevant even when plants are harvested in the same controlled agronomic and environmental conditions, suggesting a wide genetic variability. These differences confer a range of properties to the berries that not only can be reflected in sensory perception (*9*) but can also contribute, beyond the role of polygalacturonase-inhibiting proteins (*33*), to pathogenic resistance as suggested in the present work. These observations confirm the importance of volatile profiling within breeding programs of fruit crops and suggest that the role of naturally emitted volatile compounds against pathogen diseases should be further investigated.

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Supporting Information Available: Tables of volatile compounds found in raspberry fruits harvested during 2006 and 2007. This material is available free of charge via the Internet at http://pubs.acs.org.

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