Identifying Natural Volatile Compounds That Control Gray Mold (*Botrytis cinerea*) during Postharvest Storage of Strawberry, Blackberry, and Grape

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Botrytis-inoculated fruit were treated with three levels of naturally occurring volatile compounds in capped bottles and rated for *Botrytis* development and evidence of phytotoxicity during 7 days of storage at 2 °C followed by 3−6 more days at 22 °C for strawberry and 7 days at 15 °C for blackberry and grape. Hexanal, 1-hexanol, (*E*)-2-hexen-1-ol, (*Z*)-6-nonenal, (*E*)-3-nonen-2-one, methyl salicylate, and methyl benzoate exhibited potential as postharvest fumigants for control of *Botrytis* on strawberry at the lowest level tested. Ten compounds were evaluated on blackberry and grape. None caused phytotoxic responses as with strawberry, while nearly all of the compounds inhibited *Botrytis* at all three test levels. Strawberry, blackberry, and grape metabolized (*E*)-2-hexenal with reduction of the aldehyde to an alcohol and saturation of the carbon−carbon double bond adjacent to the carbonyl, but strawberry yielded more esters as major products.

Keywords: *Fragaria × ananassa; Rubus; Vitis vinifera; metabolism; antifungal; (E)-2-hexenal*

INTRODUCTION

Volatile compounds are ubiquitous in fruits and vegetables (Nijssen *et al.*, 1996), comprising the aroma component of flavor. They may also play a functional role in plant−pathogen interactions. Several volatile compounds are produced in plant tissue in response to mechanical or biological injury (Hatanaka, 1993). These "wound volatiles", usually six- and nine-carbon aldehydes or alcohols, are formed via the lipoxygenase (LOX) hydroperoxide lyase enzymatic pathway (Hildebrand, 1989), which is activated immediately following wounding. French (1985) described the stimulatory properties of many plant-derived volatile compounds on some plant pathogenic fungi. Fungistatic and fungicidal effects of volatiles also occur naturally, such as those produced by *Trichoderma* against other fungal species (Bruce *et al.*, 1984). Aldehydes were reported to have greater antifungal activity in *in vitro* bioassays than alcohols or esters (Gueldner *et al.*, 1985; Nandi, 1977; Urbasch, 1984; Zeringue and McCormick, 1989). The C_6 aldehyde LOX pathway products were more effective than the C_9 aldehyde products at inhibiting *Botrytis cinerea* growth in *in vitro* bioassays (Hamilton-Kemp *et al.*, 1992). An α,β-unsaturated bond adjacent to the carbonyl moiety increased antifungal activity (Andersen *et al.*, 1994). Thus, there are specific structural features that enhance antifungal activity.

With the exception of sulfur dioxide treatment of table grapes after harvest (Simalnick *et al.*, 1990), there are no commercially acceptable or safe postharvest treatments to control disease during storage of strawberry, blackberry, or grape. Natural volatile compounds possessing *in vitro* antifungal activity may have potential as postharvest fumigants. Incorporation of selected bioactive compounds into the storage environment at appropriate levels might inhibit or even kill postharvest pathogens, thus reducing postharvest product loss. This potential has been explored to a limited degree previously. Treatment of fruit with acetaldehyde vapor yielded contradictory evidence of its ability to inhibit mold (Aharoni and Stadelbacher, 1973; Morris *et al.*, 1979; Prasad and Stadelbacher, 1974), but it altered fruit sensory traits (Pesis and Avissar, 1990). Vaughn *et al.* (1993) evaluated selected compounds from several chemical classes, some of which inhibited *Botrytis* and *Colletotricum* molds on strawberry and raspberry fruit. The LOX pathway products 1-hexanol, (*E*)-2-hexenal, and (*Z*)-3-hexen-1-ol exhibited good antifungal activity, although the latter two compounds were phytotoxic at the levels tested. The LOX product hexanal inhibited *Botrytis* and *Penicillium expansum* development on inoculated apple slices and was metabolized so that no significant residue was apparent (Song *et al.*, 1996). The compounds benzaldehyde and methyl salicylate, naturally produced during peach ripening, exhibited antifungal activity *in vitro* (Wilson *et al.*, 1987) and on fungus-inoculated peach, nectarine, and plum fruit (Caccioni *et al.*, 1995). There are no published reports comparing a broad array of compounds for antifungal activity *in vivo* on blackberry or grape.

Many of the LOX products produced after wounding, as well as many other natural volatile compounds, have been identified in ripe strawberry, blackberry, grape, and other fruits (Nijssen *et al.*, 1996). A number of these compounds are metabolized by strawberry fruit, which may result in little or no residue (Hamilton-Kemp *et al.*, 1996). The natural origin of these compounds, their metabolism by fruit, their integral role in the human diet due to their presence in fresh fruits and vegetables, and their volatile nature may enhance consumer acceptance. Preliminary work with *Botrytis*-inoculated strawberries held at 22 °C indicated that many volatile compounds had antifungal activity *in vivo* (Archbold *et al.*, 1997). The objective of this work was

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to evaluate a wide array of volatile compounds which occur naturally, and that are produced during fruit ripening, in a simulated cold storage−shelf-life sequence to identify those with potential utility as postharvest fumigants for control of *Botrytis*, the main postharvest decay-causing fungus on strawberries, blackberries, and grapes.

MATERIALS AND METHODS

Strawberry fruit (*Fragaria* × *ananassa* Duch.) were harvested from non-fungicide-treated container-grown Tribute plants in a greenhouse and from field plots of Chandler. Chester Thornless blackberry (*Rubus* spp.) fruit were harvested from a non-fungicide-treated field plot. Emperatriz Seedless grapes (*Vitis vinifera*) were shipped from California after commercial harvest and a short period of cold storage. All fruit were fully ripe, uniformly colored, and free of evident disease, insect, or other damage. Aqueous suspensions of *Botrytis*, obtained from strawberry fruit and cultured *in vitro* (Hamilton-Kemp *et al.*, 1992), were prepared, standardized to 10^6 conidia/mL, and sprayed on the fruit.

The following volatile compounds were purchased from Aldrich (Milwaukee, WI) or were gifts from Bedoukian Chemical Co. (Danbury, CT) and were used directly from the bottles: (1) lipoxygenase-lyase pathway products and analogs hexanal, (*E*)-2-hexenal, (*E*)-2-hexenal diethyl acetal, (*Z*)-3-hexenal (50% in triacetin), (*E*,*E*)-2,4-hexadienal, nonanal, (*E*)-2-nonenal, (*Z*)-6-nonenal, (*E*,*E*)-2,4-nonadienal, (*E*,*Z*)-2,6-nonadienal, 1-hexanol, (*E*)-2-hexen-1-ol, (*Z*)-3-hexen-1-ol, and hexyl acetate; (2) aromatic compounds benzaldehyde, methyl benzoate, and methyl salicylate; (3) other volatiles ethyl butyrate, 2-carene, D-limonene, 3-hexanone, (*E*)-4-hexen-3-one, 2-nonanone, and (*E*)-3-nonen-2-one.

A single strawberry or blackberry fruit, or two or three grape berries, were placed on wire mesh over 1 mL of deionized water in a 250-mL glass bottle. Liquid samples of test compounds were bioassayed at 3 volumes, determined in preliminary work to yield a range of vapor phase concentrations. The lowest levels of the compounds were selected because they yielded measurable gas chromatograph signals. Larger quantities of less volatile compounds were often required (Hamilton-Kemp *et al.*, 1996). The volatile compounds were placed in a 10-mL glass vial within the 250-mL bottle, which was then capped tightly with a Teflon-lined plastic screw cap. The cap was solid when strawberry fruit was used, and it had a 1.9-cm-diameter hole over which a piece of low-density polyethylene (LDPE) film (Respire, Cincinnati, OH) was fixed with electrical tape when blackberry and grape were used. The film allowed some gas exchange to reduce the possibility of anaerobiosis or excessive CO_2 or ethylene accumulation. Some caps also had a gas-tight septum for vapor phase gas sampling. Controls consisted of fruit placed in the containers with no volatile compound. To simulate a commercial handling sequence, the bottles were placed in a refrigerator at 2 °C for 7 days. After 7 days, the bottles with strawberries were transferred to 22 °C storage and those with blackberries and grapes were transferred to 15 °C storage.

At intervals, fruit were inspected and rated for *Botrytis* development on a 1 to 5 scale for lesion appearance and size, and for symptoms of phytotoxicity on a 1 to 5 scale for color, texture, and other signs of deterioration. No visual evidence of *Botrytis* was 1, less than 10% of the surface infected was 2, 10−30% infected was 3, 30−70% was 4, and greater than 70% was 5. For phytotoxicity, no deterioration was 1, slight deterioration was 2, moderate deterioration was 3, severe deterioration was 4, and complete tissue collapse was 5. Symptoms of phytotoxicity included discoloration, loss of tissue turgidity and shape, and exudate appearance on the fruit surface. Fruit were rated by two or three trained observers, and the mean rating of each fruit at each date was used for statistical analysis. The experimental design was completely random, and there were 10 single-fruit replicates of each compound test level for strawberry. Due to the large number of volatile compounds tested on strawberry, weekly experiments were set up using a randomly chosen subgroup of the compounds from the complete list as well as a control group. Blackberry and grape bioassays were set up three and two consecutive weeks, respectively, with 10 replicate fruit per compound level. All compounds were tested weekly on blackberry and grape. Initial analysis of weekly results with strawberry control fruit indicated no significant variation in fungal development or phytotoxicity, so the weekly results were pooled for analysis of variance. Because the data did not exhibit homogeneity of variance, the data were analyzed by Kruskal-Wallis ANOVA for Ranks, and means were compared to the control by Dunnett's test at $P = 0.05$ (SigmaStat, Jandel Scientific, San Rafael, CA.).

The vapor phase concentrations of (*E*)-2-hexenal and its metabolites in strawberry, blackberry, and grape bioassays, using bottles with solid caps, were measured after 1, 2, 6, and 9 days by capillary gas chromatography (GC). A 0.5-mL headspace vapor sample was withdrawn with a gas-tight syringe and injected directly into a Hewlett Packard 5890 Series II GC equipped with a 30 m × 0.53 mm fused-silica DB-wax [poly(ethylene glycol)] column with a film thickness of 1 μm (J & W Scientific, Folsom, CA) and a flame ionization detector (FID). The injector and FID temperatures were 220 and 240 °C, respectively, and the column temperature was maintained at 50 °C for 5 min and then programmed at 3 °C/min to 100 °C. Helium was used as the carrier gas at a flow rate of 9 mL/min. Metabolites were identified by comparing retention times to compounds identified in strawberry (Hamilton-Kemp *et al.*, 1996). There were three replicate injections from separate bottles.

The O_2, CO_2, and ethylene concentrations were sampled from two bottles of each volatile compound level at 3, 6, 9, and 13 days. The O_2 and CO_2 levels were measured by taking a 5-mL sample from each bottle and injecting it into an oxygen/carbon dioxide headspace analyzer ZR 892 HS (Illinois Instruments, McHenry, IL). The ethylene concentration was measured by taking a 1-mL sample from each bottle and injecting it into a Varian 2100 GC fitted with a 1-m alumina column and run at 100/70/100 °C for the injector/column/FID detector temperatures, respectively.

RESULTS AND DISCUSSION

The effects of the compounds using Tribute and Chandler strawberry were similar (Table 1). Most compounds at the highest test levels inhibited *Botrytis* for the duration of the storage period. Many compounds also inhibited the fungus at the middle level, but only hexanal, 1-hexanol, (*E*)-2-hexen-1-ol, (*E*)-3-nonen-2-one, (*Z*)-6-nonenal, methyl salicylate, and methyl benzoate consistently displayed antifungal activity for the duration of the study at the lowest test concentration in both cultivars. The different responses between the cultivars suggest cultivar specificity in the fruit−fungus−volatile interaction. There was no obvious chemical structure−antifungal activity relationship evident. The C_9 LOX products were as effective as the C_6 LOX products, the alcohols were often as effective as the aldehydes, and the unsaturated bond did not appear to enhance the activity in contrast to *in vitro* results (Andersen *et al*, 1994; Gueldner *et al.*, 1985; Hamilton-Kemp *et al.*, 1992; Nandi, 1977; Urbasch, 1984; Zeringue and McCormick, 1989). Some of the compounds have been previously reported to have antifungal activity in *in vivo* bioassays with *Botrytis*-inoculated strawberry and raspberry fruit, including 2-nonanone, 1-hexanol, and (*E*)-2-hexenal (Vaughn *et al.*, 1993) but at higher levels than in this study.

The inhibitory effects of the volatile compounds were not total with some treatments. The fungus developed slowly in the presence of some compounds. In other treatments, *Botrytis* did not develop at all. Thus, both fungistatic (inhibitory) and fungicidal (lethal) effects

Table 1. Effect of Natural Volatile Compounds on *Botrytis* Development and Phytotoxic Responses of the Fruit of Tribute and Chandler Strawberry during Postharvest Storage[a]

		Tribute		Chandler	
compound	liquid vol (μL)	*Boyrytis* rating[b] at 10 days	phytotoxicity symptoms[c]	*Boyrytis* rating at 13 days	phytotoxicity symptoms
control		4.2		2.7	
hexanal	2	**1.8**	no	**1.1**	no
	10	**1.0**	at 6 days	**1.2**	at 8 days
	100	**1.0**	at 6 days	**1.1**	at 6 days
(*E*)-2-hexenal	2	**1.5**	no	**1.3**	at 10 days
	10	**1.0**	at 6 days	**1.3**	no
	100	**1.0**	at 6 days	**1.1**	at 8 days
(*Z*)-3-hexenal	4	4.6	no	3.1	no
	20	2.1	no	**2.1**	no
	200	**1.0**	at 8 days	**1.0**	at 8 days
(*E*,*E*)-2,4-hexadienal	2	**1.8**	no	3.1	no
	10	**1.6**	no	**1.6**	no
	100	**1.0**	no	**1.2**	at 8 days
1-hexanol	12	**1.1**	no	**1.1**	at 8 days
	60	**1.0**	no	**1.0**	at 8 days
	600	**1.0**	no	**1.0**	no
(*E*)-2-hexen-1-ol	12	**1.0**	no	**1.8**	at 8 days
	60	**1.0**	no	**1.1**	at 8 days
	600	**1.0**	no	**1.1**	at 8 days
(*Z*)-3-hexen-1-ol	12	**1.0**	no	2.3	at 8 days
	60	**1.0**	no	**1.0**	at 8 days
	600	**1.0**	no	**1.1**	at 8 days
hexyl acetate	2	4.0	at 6 days	4.2	at 8 days
	10	**1.1**	at 6 days	2.2	at 8 days
	100	**1.0**	at 6 days	**1.0**	at 8 days
(*E*)-2-hexenyl diethyl acetal	6	2.7	no	**1.0**	at 8 days
	30	**1.0**	no	**1.0**	at 8 days
	300	**1.0**	at 8 days	**1.1**	at 8 days
3-hexanone	2	4.0	no	**1.1**	no
	10	3.0	no	**1.3**	no
	100	**1.0**	at 6 days	**1.1**	at 10 days
(*E*)-4-hexen-3-one	2	3.8	no	**1.2**	no
	10	**1.6**	no	**1.1**	no
	100	**1.0**	at 6 days	**1.1**	at 8 days
nonanal	6	**1.0**	at 6 days	3.8	at 8 days
	30	**1.0**	at 8 days	1.8	at 8 days
	300	**1.0**	at 6 days	**1.5**	at 8 days
(*E*)-2-nonenal	6	**1.7**	at 8 days	4.4	no
	30	**1.4**	at 8 days	4.3	no
	300	**1.0**	at 6 days	2.8	at 8 days
(*Z*)-6-nonenal	6	**1.0**	at 6 days	**1.4**	at 8 days
	30	**1.0**	at 6 days	**1.0**	at 8 days
	300	**1.0**	at 6 days	2.4	no
(*E*,*E*)-2,4-nonadienal	6	3.4	no	**1.5**	no
	30	**1.6**	no	**1.2**	no
	300	**1.2**	no	**1.0**	no
(*E*,*Z*)-2,6-nonadienal	6	**1.4**	at 8 days	2.2	at 8 days
	30	**1.2**	at 8 days	**1.3**	at 8 days
	300	**1.0**	no	**1.0**	at 8 days
2-nonanone	6	**1.0**	at 6 days	4.1	at 8 days
	30	**1.0**	at 6 days	**1.1**	at 8 days
	300	**1.0**	at 6 days	**1.5**	at 8 days
(*E*)-3-nonen-2-one	6	**1.0**	at 8 days	**1.6**	at 10 days
	30	**1.0**	at 8 days	**1.2**	at 10 days
	300	**1.0**	at 8 days	**1.1**	at 8 days
methyl salicylate	12	**1.0**	at 10 days	**1.1**	no
	60	**1.0**	no	**1.4**	no
	600	**1.0**	at 10 days	**1.5**	no
methyl benzoate	12	**1.0**	no	**1.0**	no
	60	**1.0**	no	**1.0**	no
	600	**1.0**	no	**1.0**	no

Table 1 (Continued)

	Tribute			Chandler	
compound	liquid vol (μL)	*Boyrytis* rating[b] at 10 days	phytotoxicity symptoms[c]	*Boyrytis* rating at 13 days	phytotoxicity symptoms
benzaldehyde	6	3.6	no	2.0	no
	30	**1.0**	no	**1.3**	no
	300	**1.0**	no	**1.0**	no
ethyl butyrate	2	3.7	no	2.8	no
	10	2.3	at 10 days	**1.5**	no
	100	**1.0**	at 6 days	**1.0**	at 6 days
2-carene	2	4.8	at 8 days	2.3	no
	10	**2.0**	at 6 days	2.4	at 8 days
	100	**1.5**	at 8 days	2.6	at 8 days
D-limonene	2	4.0	at 8 days	2.7	no
	10	**1.4**	at 8 days	2.7	at 10 days
	100	**1.2**	at 8 days	2.3	at 8 days

[a] A liquid volume of each volatile compound was placed in a 250-mL bottle along with a single fruit, and the capped bottles were stored for 7 days at 2 °C and 3 or 6 days at 22 °C. [b] *Botrytis* development on the fruit surface was visually rated on a 1 to 5 scale for the percent of surface area infected: 1, 0%; 2, <10%; 3, 10−30%; 4, 30−70%, and 5, 70%. Mean values in bold are significantly different from the control by Dunnett's test at $P = 0.05$. [c] The first evaluation date when phytotoxicity symptoms were significantly greater than the control by Dunnett's test at $P = 0.05$. No indicates that they were not different from the controls through the final evaluation date.

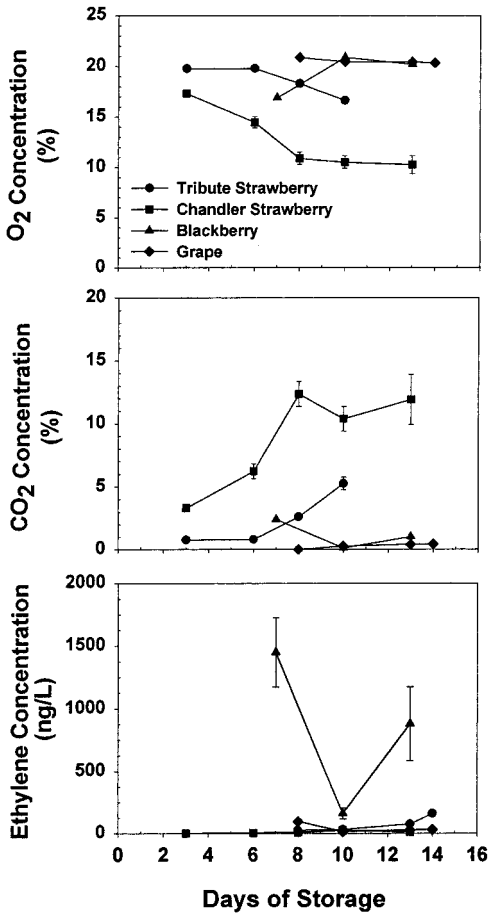

Figure 1. Concentrations of O_2, CO_2, and ethylene in bioassay bottles. Values are the means across all treatments. The vertical bar indicates the standard error of the mean and is not visible if it is smaller than the symbol.

apparently occurred. Both responses sometimes occurred with the same compound at different concentrations; for example, (*Z*)-3-hexenal exhibited complete inhibition of *Botrytis* on Tribute (Table 1) at the high rate but allowed some development at the middle level. Song *et al.* (1996) reported that hexanal inhibited but did not kill *Botrytis* at lower vapor phase levels in *in vitro* bioassays. After a 48-h treatment at 10.6 μmol/L, the fungus resumed growth in hexanal-free air. At higher exposure levels, no subsequent fungal growth was observed. The results of the present study indicate that the fungus can grow *in vivo*, albeit slowly, in the presence of low levels of many of the compounds tested.

If any of the volatile compounds have potential as postharvest fumigants, they must not have detrimental effects on strawberry fruit quality. At the conclusion of the bioassays, 10 days with Tribute and 13 days with Chandler, all fruit were significantly below market quality. Phytotoxic responses to the compounds after 6 days of cold storage and/or at 8 days, 1 day after moving the bottles to 22 °C, would occur prior to significant decline in market quality. The first date of significant phytotoxicity symptoms, if evident, is noted in Table 1. Numerous compounds at the middle and high test levels caused phytotoxic responses on one or both cultivars by 6 and/or 8 days. Of those compounds with antifungal activity at the lowest test level in both cultivars, 1-hexanol, (*E*)-2-hexen-1-ol, (*Z*)-6-nonenal, and (*E*)-3-nonen-2-one also were phytotoxic. The other compounds in that group, hexanal, methyl salicylate, and methyl benzoate, had no phytotoxic effect. Overall there was no correlation between the *Botrytis* and phytotoxicity ratings (data not shown).

Ten compounds that exhibited both antifungal activity and minimal phytotoxicity were bioassayed with blackberry and grapes. The post-cold-storage shelf life of these fruit was longer than strawberry so the bioassays were continued until 14 days. With Chester Thornless blackberry, all 10 compounds inhibited *Botrytis* at all levels and none had phytotoxic effects (Table 2). With Emperatriz Seedless grape, all compounds except methyl salicylate inhibited *Botrytis* at one or more levels and none had phytotoxic effects (Table 2).

Since the bottles were capped to hold the vapor phase of the compounds, there was concern that the atmosphere might become anaerobic and/or accumulate high CO_2 or ethylene levels, especially during the latter, warmer shelf life phase. Low O_2 below 5% and high CO_2 at 10% or more can suppress *Botrytis* (El-Goorani and Sommer, 1981). While vapor phase concentrations of O_2, CO_2, and ethylene changed during the study, the bottles did not become anaerobic or reach excessively high ethylene levels (Figure 1). However, CO_2 levels did exceed 10% in the Chandler strawberry bioassays at 8 days and after. While this may have suppressed

Table 2. Effect of Natural Volatile Compounds on *Botrytis* Development on Chester Thornless Blackberry and Emperatriz Seedless Grape during Postharvest Storage[a]

		Botrytis rating[z]			
		blackberry		grape	
compound	liquid vol (μL)	10 days	14 days	10 days	14 days
control		2.9	4.6	4.6	4.9
(*E*)-2-hexenal	2	**1.6**	**4.1**	**1.2**	**1.7**
	10	**1.2**	**1.4**	**1.0**	**1.0**
	100	**1.0**	**1.1**	**1.0**	**1.0**
1-hexanol	12	**1.1**	**2.3**	**1.3**	**1.5**
	60	**1.0**	**1.3**	**1.0**	**1.0**
	600	**1.1**	**1.1**	**1.0**	**1.0**
(*E*)-2-hexen-1-ol	12	**1.0**	**1.5**	**1.1**	**1.4**
	60	**1.0**	**1.0**	**1.1**	**1.1**
	600	**1.0**	**1.0**	**1.1**	**1.1**
(*E*)-2-hexenyl diethyl acetal	6	**1.6**	**3.8**	**1.9**	**2.3**
	30	**1.1**	**1.2**	**1.0**	**1.3**
	300	**1.0**	**1.0**	**1.1**	**1.2**
nonanal	6	**1.6**	**4.3**	3.3	4.5
	30	**1.3**	**2.7**	**1.7**	3.1
	300	**1.1**	**1.4**	**1.5**	**1.9**
2-nonanone	6	**1.7**	**3.5**	**3.4**	**4.2**
	30	**1.1**	**1.3**	**1.0**	**1.0**
	300	**1.0**	**1.0**	**1.0**	**1.0**
(*E*)-3-nonen-2-one	6	**1.0**	**2.9**	**1.6**	**2.1**
	30	**1.2**	**1.9**	**1.1**	**1.3**
	300	**1.2**	**1.9**	**1.8**	**2.1**
methyl salicylate	12	**2.0**	**3.7**	3.8	4.1
	60	**2.4**	**4.6**	3.9	4.2
	600	**2.2**	**3.9**	3.8	4.4
methyl benzoate	12	**1.3**	**2.7**	**1.9**	**3.2**
	60	**1.4**	**1.9**	**2.0**	**2.4**
	600	**1.2**	**1.4**	**1.8**	**2.1**
benzaldehyde	6	**1.6**	**4.0**	3.9	4.4
	30	**1.1**	**1.3**	**1.3**	**2.1**
	300	**1.0**	**1.0**	**1.0**	**1.0**

[a] A liquid volume of each volatile compound was placed in a 250-mL bottle along with a single blackberry or two or three grapes, and the capped bottles were stored for 7 days at 2 °C and 7 days at 15 °C. [b] *Botrytis* development on the fruit surface was visually rated on a 1 to 5 scale for the percent of surface area infected: 1, 0%; 2, <10%; 3, 10−30%; 4, 30−70%; 5, >70%. Mean values in bold are significantly different from the control by Dunnett's test at $P = 0.05$.

Botrytis development, accounting for its slower development on Chandler than on Tribute, the fungus developed nonetheless. Most importantly, there was no correlation between the levels of any of these gases and the *Botrytis* or phytotoxicity ratings (data not shown).

Strawberry, blackberry, and grape metabolized (*E*)-2-hexenal, selected as a model test compound, to several products (Table 3). The primary reactions were the reduction of the aldehyde to an alcohol and the saturation of the carbon−carbon double bond, like that previously observed with strawberry (Hamilton-Kemp *et al.*, 1996). However, neither blackberry nor grape produced an appreciable amount of the esters hexyl acetate and (*E*)-2-hexenyl acetate in contrast to strawberry. Strawberry also metabolized a greater proportion of the source compound (*E*)-2-hexenal within the same time interval.

The greater level of metabolism of (*E*)-2-hexenal by strawberry than blackberry or grape may be indirectly related to the phytotoxic effect of the compounds. More of the test volatile compound may have been absorbed or accumulated by a strawberry than by a blackberry or a grape, leading to higher levels of metabolite production. The greater accumulation of the compounds by strawberry might have also led to a greater physiological impact, i.e., the increased phytotoxicity not noted with either the blackberry or grape.

Table 3. Metabolism of (*E*)-2-Hexenal Vapor by Strawberry, Blackberry, and Grape[a]

	vapor phase concn (μg/L)			
compound	1 day	2 days	6 days	9 days
	No Fruit			
(*E*)-2-hexenal	870 ± 24	930 ± 87	500 ± 58	990 ± 121
	Strawberry			
hexanal	0	0	0	0
(*E*)-2-hexenal	48 ± 16	7 ± 1	1 ± 1	15 ± 2
hexyl acetate	9 ± 2	13 ± 3	25 ± 4	47 ± 8
(*E*)-2-hexenyl acetate	86 ± 16	85 ± 22	87 ± 13	120 ± 26
1-hexanol	3 ± 1	4 ± 1	4 ± 1	35 ± 8
(*E*)-2-hexen-1-ol	24 ± 2	38 ± 12	16 ± 2	64 ± 18
	Blackberry			
hexanal	1 ± 1	2 ± 1	3 ± 1	10 ± 2
(*E*)-2-hexenal	690 ± 54	280 ± 30	100 ± 6	230 ± 28
hexyl acetate	1 ± 1	0	0	0
(*E*)-2-hexenyl acetate	10 ± 4	3 ± 2	0	0
1-hexanol	2 ± 1	3 ± 1	5 ± 1	50 ± 2
(*E*)-2-hexen-1-ol	7 ± 2	8 ± 3	12 ± 3	36 ± 12
	Grape			
hexanal	2 ± 1	3 ± 2	2 ± 1	5 ± 1
(*E*)-2-hexenal	510 ± 89	260 ± 75	78 ± 8	100 ± 11
hexyl acetate	0	0	0	0
(*E*)-2-hexenyl acetate	0	0	0	0
1-hexanol	2 ± 1	10 ± 5	4 ± 2	46 ± 5
(*E*)-2-hexen-1-ol	4 ± 1	26 ± 10	3 ± 1	13 ± 2

[a] A liquid volume of 10 μL was placed in a bottle with a solid cap along with a single fruit. The bottles were stored at 2 °C for 7 days and at 15°C for 2 days. Values are mean ± SE of three replications.

Due to the significant level of metabolism of many of these compounds (Table 3; Hamilton-Kemp *et al.* (1996)), the headspace levels were not constant and many metabolites also accumulated in the bottles. Andersen *et al.* (1994) indicated that the vapor phase levels rose to a maximum within a few hours. After the maximum levels were reached, the vapor phase concentration declined but rose again when the temperature increased after the bottles were moved to 22 °C (Table 3). Since the vapor phase levels of the test compounds were not constant, the liquid volume of volatile compound introduced into the bottle at the start is reported. In the bioassays with blackberry and grape, the bottle caps had a LDPE film cover which allowed for some vapor phase exchange to reduce the possibility of anaerobiosis and high CO_2 accumulation. The permeability of the film to the volatile compounds likely changed with storage temperature (Song *et al.*, 1996). In addition, the metabolites may have antifungal activity, a possibility that should be considered in studies of this type.

Several of the natural volatile compounds tested in this work have potential as postharvest fumigants to control *Botrytis*. Other mold-causing fungi may also be controlled by these compounds *in vivo* (Caccioni *et al.*, 1995; Vaughn *et al.*, 1993). There appeared to be variation in species sensitivity to the compounds, as blackberry and grape exhibited no phytotoxic effects in contrast to those on strawberry. This sensitivity will need to be considered when application techniques are developed. Sustained, long-term treatment may be undesirable with some fruits. Nonetheless, if appropriate application strategies can be developed, the phytotoxic effects of the volatile compounds may be avoided.

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