Fruit Volatiles Inhibitory to *Monilinia fructicola* and *Botrytis cinerea*

CHARLES L. WILSON, Lead Scientist, and JERRY D. FRANKLIN and BRIAN E. OTTO, Technicians, USDA, Agricultural Research Service, Appalachian Fruit Research Station, Box 45, Kearneysville, WV 25430, and West Virginia University, Morgantown 26506-6108

ABSTRACT

Wilson, C. L., Franklin, J. D., and Otto, B. E. 1987. Fruit volatiles inhibitory to *Monilinia fructicola* and *Botrytis cinerea*. Plant Disease 71:316-319.

Sixteen volatile compounds occurring naturally in fruits were tested for their effects on spore germination and growth of *Monilinia fructicola* and *Botrytis cinerea*. Nine of these compounds (benzaldehyde, benzyl acetate, benzyl alcohol, δ-decalactone, γ-caprolactone, γ-decalactone, γ-octalactone, methyl salicylate, and γ-valerolactone) greatly inhibited spore germination of both fungi at 1,250 μl/L. Benzaldehyde totally inhibited spore germination of *B. cinerea* at 25 μl/L and germination of *M. fructicola* at 125 μl/L. Three of the compounds (benzaldehyde, methyl salicylate, and ethyl benzoate) completely inhibited growth of *M. fructicola* and *B. cinerea* at 370 μl/L. Ethyl benzoate was fungicidal against *M. fructicola* and fungistatic against *B. cinerea*, whereas methyl salicylate and benzaldehyde were fungicidal against both fungi.

Synthetic fungicides have been a major tool in controlling postharvest fruit diseases. However, under optimal conditions for disease development, control by fungicides is often ineffective. Public concern over the introduction of synthetic pesticides into our food chain is leading to restricted fungicide use, particularly for postharvest treatments made close to the time food is consumed (7). Fungicide effectiveness is further reduced by the frequent occurrence of resistance to synthetic fungicides by pathogens (4). These factors emphasize the need for new methods to control postharvest diseases.

Volatile compounds from plants can be both inhibitory and stimulatory to fungal growth and/or spore formation and germination (8–11). Natural plant volatiles also have been implicated in plant resistance mechanisms. Shaw (20) indicated that the resistance of fruit to rot in high-CO_2 storage was due to the production of high levels of acetaldehyde and ethyl acetate by the fruit in response to these conditions. Following this lead, Prasad and Stadelbacher (19) controlled *Botrytis cinerea* Pers. ex Fr. and *Rhizopus stolonifer* (Ehr. ex Fr.) Vuill. rots of strawberries and raspberries with acetaldehyde vapor. Acetaldehyde also has been used as a fumigant to control the green peach aphid on head lettuce (22); however, injury from the fumigant has been reported (23). Ethyl formate has been proposed more recently for green peach aphid control (24). Babu et al (2) examined the effects of a number of organic volatiles such as acetone on citrus rots, but they did not look at naturally occurring compounds and their tests were limited. To date, no commercial application has been made of any of these findings (5).

Our research was conducted to determine whether volatile compounds emitted from peach and other stone fruits had fungitoxic properties with the prospect that they might be used as fumigants to control fruit rots.

MATERIALS AND METHODS

Sixteen natural volatile compounds (Table 1) reported from peach and plum (6,14) were tested for their effects on

This paper reports the results of research only. Mention of a chemical, pesticide, or product does not constitute a recommendation for use by the USDA and does not imply registration under FIFRA as amended.

Accepted for publication 28 October 1986 (submitted for electronic processing).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

spore germination of *M. fructicola* and *B. cinerea*. Each was selected on the basis of its presumed safety and commercial availability. The compounds were obtained as commercial preparations of at least 95% purity from Sigma Chemical Co., St. Louis, MO, and Alpha Products, Danvers, MA.

Chambers were devised to "fumigate" germinating spores. Removawell Strips (eight wells per strip), supplied by Dynatech Laboratories, Inc., Chantilly, VA, were broken into single wells. Into these wells, 24 μl of the spore suspension (4×10^4 spores per milliliter) was added and shaken to evenly cover the well bottom. The wells were then placed into 4-ml Wheaton glass vials (one well per vial). Five microliters of the concentrated volatile compound (liquid) was added to a 6-mm disk of filter paper. This disk was placed in the indentation of a rubber serum bottle stopper, and a cheesecloth square was placed over the indentation extending over the lip of the vial when it was sealed to keep the disk from falling into the vial. The volatiles completely evaporated in the vials, making a concentration of 1,250 μl/L. Closed vials were sealed in plastic bags and placed in an incubator at 26 C for 18–20 hr. The vials were then opened and the individual wells placed in a Removawell Strip Holder. This holder was placed in the moveable tissue culture plate specimen holder of a Zeiss inverted microscope, and total germinated and ungerminated spores were counted. Ten replicates were made of each fumigant. Experiments were repeated three times.

Effects of the volatiles on growth were tested in tight-lid petri dishes 50 × 9 mm containing 3.5 ml of water agar. Four-millimeter plugs of agar and growth of *M. fructicola* and *B. cinerea* were removed from 8-day-old potato-dextrose agar (PDA) cultures and placed in the center of the water agar. One-centimeter filter paper disks were impregnated with 10 μl of concentrated volatile, moistened with one drop of sterile distilled water, and placed on the inner surface of the petri dish lid, then the lids were sealed. The diameter growth of colonies on inoculated plates was measured after 2 wk. On plates in which no growth occurred after 2 wk, a piece of the original agar inoculum was transferred to petri dishes containing PDA to determine whether the fungus was still alive.

The following concentrations of each volatile were used to study its effect on growth: 0.74, 3.7, 7.4, 37, 74, 370, and 740 μl/L. Volatiles were undiluted at the three highest levels. At the four lower levels, volatiles were diluted in mineral oil to make a total volume of 1 ml before pipetting 1 μl onto the filter paper. For effects on germination, the following concentrations were tested: 2.5, 12.5, 25, 125, and 259 μl/L. Volatiles were not diluted at the highest levels. At the four lowest levels, volatiles were diluted in 95% ethanol to make a total volume of 1:1 before pipetting 1 μl onto the filter paper. Controls were 1:1 of mineral oil for growth studies and 1:1 of 95% ethanol for germination tests. Analyses were performed using Duncan's multiple range test ($P < 0.05$). The highest available reagent-grade chemicals were used.

RESULTS

Nine of the 16 natural volatile compounds tested were consistently very inhibitory to spore germination of both *M. fructicola* and *B. cinerea* (Table 1). The same nine compounds were effective against both fungi: benzaldehyde, benzyl acetate, benzyl alcohol, δ-decalactone, γ-caprolactone, γ-decalactone, γ-octalactone, γ-valerolactone, and methyl salicylate. The relative inhibition of these compounds was similar against both *M. fructicola* and *B. cinerea* (Table 1).

Among the 16 volatiles tested against growth of *M. fructicola* and *B. cinerea* in fumigated petri dishes, three (benzaldehyde, ethyl benzoate, and methyl salicylate) consistently and completely inhibited growth of these two fungi after 2 wk (Figs. 1 and 2). Plates having water treatment or treated with the other volatiles had growth covering one-half to two-thirds of the water agar surface after 2 wk. When agar plugs were transferred from inoculum showing no growth after treatment with benzaldehyde and methyl salicylate, neither *M. fructicola* nor *B. cinerea* grew out onto PDA medium after 2 wk, indicating that these fumigants were fungicidal. Growth issued from *B. cinerea* plugs treated with ethyl benzoate but not from *M. fructicola* plugs similarly treated.

Of the four volatiles most effective in inhibiting spore germination, benzaldehyde was active at the lowest concentration (Figs. 3 and 4). It was inhibitory to *B. cinerea* at only 12.5 μl/L and *M. fructicola* at 125 μl/L. Benzyl alcohol was inhibitory at 125 μl/L for both fungi. γ-Caprolactone and γ-valerolactone were inhibitory at 125 μl/L for *B. cinerea* and 250 μl/L for *M. fructicola*. The three volatiles most effective at inhibiting growth (benzaldehyde, methyl salicylate, and ethyl benzoate) completely stopped growth at 370 μl/L for both fungi (Figs. 1 and 2).

DISCUSSION

Natural volatile compounds with fungitoxic properties may be useful for the control of postharvest rots of fruit.

Table 1. Mean spore germination of *Monilinia fructicola* and *Botrytis cinerea* in chambers with 16 volatiles at 1,250 μl/L

Compound	Percent spore germination[z]	
	B. cinerea	*M. fructicola*
d-Limonene	79.0 b	53.0 a
Water check	76.0 a	50.0 a
Hexyl acetate	66.0 ab	47.0 a
Isopentyl acetate	62.0 ab	45.0 a
Methyl isovalerate	66.0 ab	42.0 a
δ-Dodecalactone	51.0 b	42.0 a
γ-Dodecalactone	19.0 c	27.0 b
Ethyl benzoate	13.0 c	10.0 c
Methyl salicylate	7.0 c	9.0 c
δ-Decalactone	8.0 cd	6.0 c
γ-Decalactone	2.0 d	5.0 c
γ-Octalactone	1.0 d	2.0 de
Benzyl acetate	0.1 d	1.0 de
Benzaldehyde	0.0 d	0.8 e
Benzyl alcohol	0.0 d	0.6 e
γ-Caprolactone	0.0 d	0.3 e
γ-Valerolactone	0.0 d	0.1 e

[z] Each value represents the mean of 10 replicates. Percentages with the same letters do not differ significantly ($P < 0.05$) according to Duncan's multiple range test, with arc sine transformation of the data.

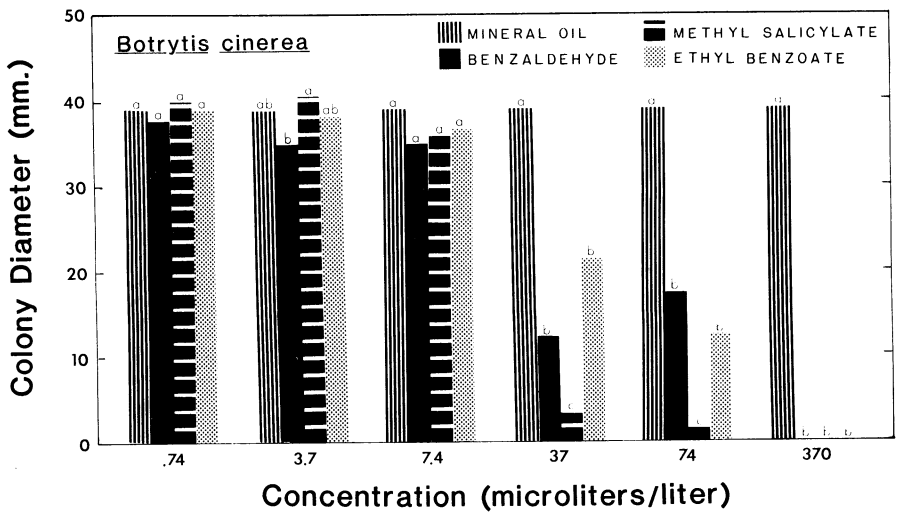

Fig. 1. Effects of different concentrations of mineral oil, benzaldehyde, methyl salicylate, and ethyl benzoate on growth of *Botrytis cinerea*. Each value represents the mean of five replicates. Treatments with the same letters do not differ significantly ($P < 0.05$) according to Duncan's multiple range test.

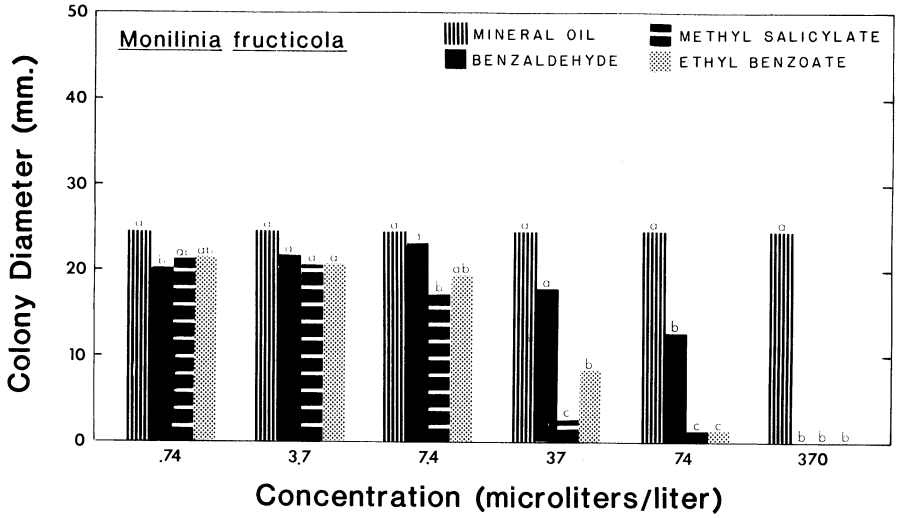

Fig. 2. Effects of different concentrations of mineral oil, benzaldehyde, methyl salicylate, and ethyl benzoate on growth of *Monilinia fructicola*. Each value represents the mean of five replicates. Treatments with the same letters do not differ significantly ($P < 0.05$) according to Duncan's multiple range test.

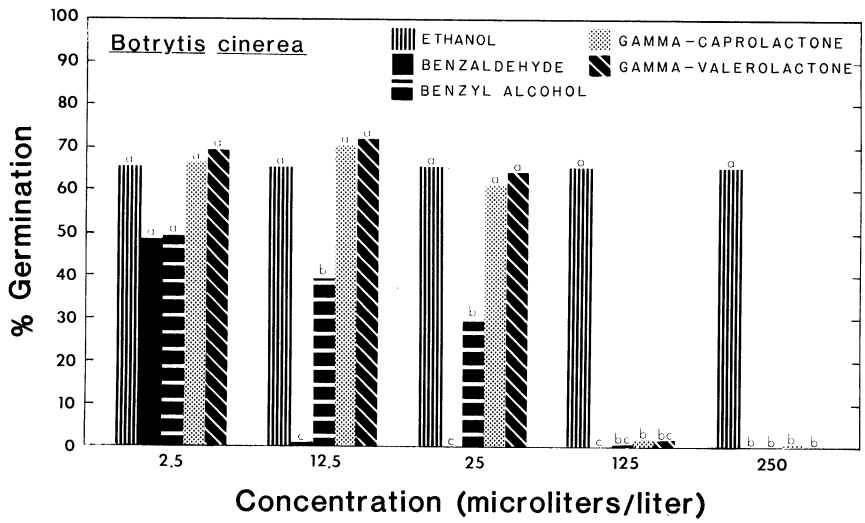

Fig. 3. Effects of different concentrations of ethanol, benzaldehyde, benzyl alcohol, γ-caprolactone, and γ-valerolactone on spore germination of *Botrytis cinerea*. Each value represents the mean of five replicates. Treatments with the same letters do not differ significantly ($P < 0.05$) according to Duncan's multiple range test.

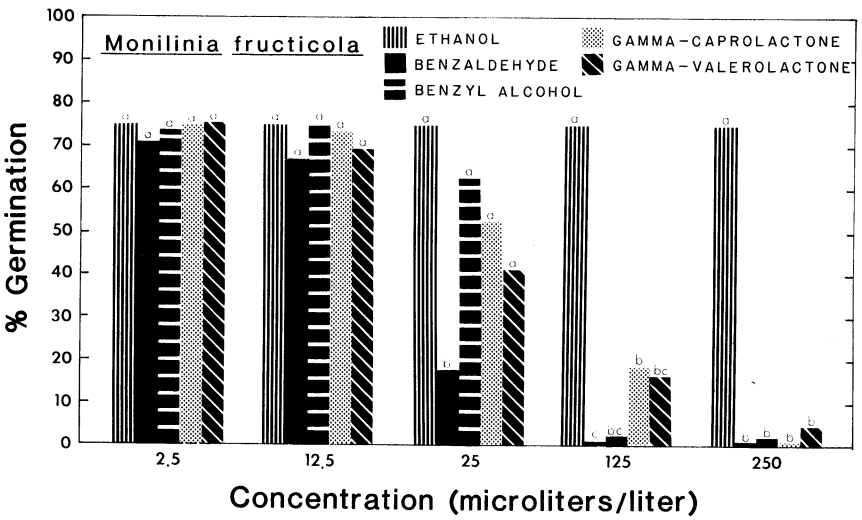

Fig. 4. Effects of different concentrations of ethanol, benzaldehyde, benzyl alcohol, γ-caprolactone, and γ-valerolactone on spore germination of *Monilinia fructicola*. Each value represents the mean of five replicates. Treatments with the same letters do not differ significantly ($P < 0.05$) according to Duncan's multiple range test.

We were surprised to find so many with antifungal properties. Fruit can be managed to promote the atmospheric accumulation of these compounds in storage (20), or the compounds can be artificially added in combination with various storage conditions. Entomologists are also exploring this approach (24). The effects of combinations of volatiles on pathogen and insect populations should be investigated.

Benzaldehyde may hold special promise as a volatile to control postharvest rots. It is thought that greater amounts of benzaldehyde account for the superior flavor of tree-ripened over artificially ripened peaches (6,16). Benzaldehyde is probably oxidized in the fruit to benzoic acid, a compound that has broad use as a food preservative (1). Benzoic acid also has been shown to be an important phytoalexin that protects apples against rot (25). In green Cavendish banana skins, 3,4-dihydroxybenzaldehyde appears to protect the fruit against rots caused by *Gloeosporium musarum* Cke. & Mass. (17). The opportunity may exist for enriching a phytoalexin in fruit (benzoic acid) by fumigation with benzaldehyde.

A large percentage of the volatiles tested were fungitoxic. Maruzzella et al (15) found that 63% of the aromatic compounds that they tested inhibited the growth of at least one of their test organisms. A rich reservoir of fungitoxic compounds may exist among fruit volatiles. Besides benzaldehyde, a number of lactones were demonstrated to have antifungal properties (12,15,18). Further investigation of other lactones as fungicides would seem indicated. Buston and Roy (3) found that 5,6-dihydro-6-methyl-2*H*-pyran-2-1 was fungistatic toward *Fusarium, Phycomyces,* and *Nematospora* spp. and was the natural resistance factor in European mountain ash (*Pyrus aucuparia* (L.) Gaertn.) berries. Also, Holden et al (13) found that 5-methylene-2(5*H*)-furanone had a broad-spectrum activity against bacteria, fungi, and protozoa.

The most effective volatiles in our tests were active at concentrations that should make them potential fumigants for postharvest disease control. Stewart et al (23) used concentrations of 38–50 μl/L of acetaldehyde to fumigate head lettuce without causing phytotoxicity. Benzaldehyde completely inhibited spore germination of *B. cinerea* at 25 μl/L.

New ways of applying fungitoxic volatiles to fruit should be explored. Perhaps volatile compounds could be added to wraps in slow-release formulations. Ethyl formate is presently being applied to vacuum-packed lettuce for insect control (24). Volatiles might be effectively applied in waxes that are used in fruit processing.

LITERATURE CITED

1. Anonymous. 1976. The Merck Index. 9th ed. Merck & Co., Rahway, NJ.
2. Babu, K. J., Laxminarayana, P., and Reddy, S.

M. 1983. Evaluation of different volatile compounds in the control of fruit rot of lemon. Pesticides (India) 17:35-38.
3. Buston, H. W., and Roy, S. K. 1949. The physiological activity of some single unsaturated lactones. I. Effect on the growth of certain microorganisms. Arch. Biochem. 22:1-7.
4. Delp, C. J. 1980. Coping with resistance to plant disease control agents. Plant Dis. 64:652-657.
5. Dennis, C. 1983. Soft fruits. Pages 23-42 in: Post-Harvest Pathology of Fruits and Vegetables. C. Dennis, ed. Academic Press, New York. 264 pp.
6. Do, J. Y., Salunkhe, D. K., and Olson, L. E. 1969. Isolation, identification and comparison of the volatiles of peach fruit as related to harvest maturity and artificial ripening. J. Food Sci. 34:618-621.
7. Eckert, J. W. 1983. Control of postharvest diseases with antimicrobial agents. Pages 265-285 in: Post-Harvest Physiology and Crop Preservation. M. Sieberman, ed. Plenum Press, New York. 572 pp.
8. French, R. C., and Wilson, C. L. 1981. The effect of nicotine and related compounds on germination and vacuolation in several species of rust uredospores. Physiol. Plant Pathol. 19:201-207.
9. French, R. C., Graham, C. L., Long, R. A., and Gale, A. W. 1979. Effect of nonanol and related fungal spore stimulators on germination of pollen of several *Pinus* species. J. Agric. Food Chem. 27:184-187.
10. French, R. C., Long, R. K., Latterell, F. M., Graham, C. L., Smoot, J. J., and Shaw, P. E. 1978. Effect of *n*-nonanal, citral, and citrus oils on germination of conidia of *Penicillium digitatum* and *Penicillium italicum*. Phytopathology 68:877-882.
11. Fries, N. F. 1973. Effects of volatile organic compounds on the growth and development of fungi. Trans. Br. Mycol. Soc. 60:1-21.
12. Harman, G. E., Mattich, L. R., Nash, G., and Nedrow, B. L. 1980. Stimulation of fungal spore germination and inhibition of sporulation in fungal vegetative thalli by fatty acids and their volatile peroxidation products. Can. J. Bot. 58:1541-1547.
13. Holden, M., Seegal, B. C., and Baer, H. 1947. Range of antibiotic activity of protoanemonium. Proc. Soc. Exp. Biol. Med. 66:54-60.
14. Ismail, H. M. M., Williams, A. A., and Tucknatt, O. G. 1981. The flavor components of plums: An examination of the aroma components present in the headspace above four cultivars of intact plums, Marjoires Seedling, Merton Gem, NAIO and Victoria. J. Sci. Food Agric. 32:498-502.
15. Maruzzella, J. C., Chiaramonte, J. S., and Garofalo, M. M. 1961. Effects of vapors of aromatic chemicals on fungi. J. Pharm. Sci. 50:665-668.
16. Mazza, G., and Hodgins, M. W. 1985. Benzaldehyde, a major aroma component of Saskatoon berries. HortScience 20:742-744.
17. Mulvena, D., Webb, E. C., and Zerner, B. 1969. 3,4-Dihydroxybenzaldehyde, a fungistatic substance from green Cavendish bananas. Phytochemistry 8:393-395.
18. Naoshima, Y., Ozawa, H., Konda, H. K., and Hayashi, S. 1983. Synthesis of racemates and (+)-enatiomers of gamma-caprolactone, gamma-dodecanolactone and delta-hexadecanolactone, lactic sex pheromones of the dermestid beetle, rove beetle and oriental hornet. Agric. Biol. Chem. (Tokyo) 47:1431-1434.
19. Prasad, K., and Stadelbacher, G. J. 1973. Control of postharvest decay of fresh raspberries by acetaldehyde vapor. Plant Dis. Rep. 57:795-797.
20. Shaw, G. W. 1969. The effect of controlled atmosphere storage on the quality and shelf-life of fresh strawberries with special reference to *Botrytis cinerea* and *Rhizopus nigricans*. Ph.D. thesis. University of Maryland. 62 pp.
21. Stadelbacher, G. J., and Prasad, K. 1974. Postharvest decay control of apple by acetaldehyde vapor. J. Am. Soc. Hortic. Sci. 99:364-368.
22. Stewart, J. K., Aharoni, Y., Hartsell, P. L., and Young, D. K. 1980. Acetaldehyde fumigation at reduced pressures to control the green peach aphid on wrapped and packed head lettuce. J. Econ. Entomol. 73:149-152.
23. Stewart, J. K., Aharoni, Y., Hartsell, P. L., and Young, D. K. 1980. Symptoms of acetaldehyde injury of head lettuce. HortScience 15:148-149.
24. Stewart, J. K., and Mon, T. R. 1984. Commercial-scale vacuum fumigation with ethyl formate for postharvest control of the green peach aphid (Homoptera: Aphididae) on film-wrapped lettuce. J. Econ. Entomol. 77:569-573.
25. Swinburne, T. R. 1973. The resistance of immature Bramley's Seedling apples to rotting of *Nectria galligena* Bres. Pages 365-382 in: Fungal Pathogenicity and the Plant's Response. R. J. W. Byrde and C. V. Cutting, eds. Academic Press, New York. 499 pp.