Hexanal Vapor Is a Natural, Metabolizable Fungicide: Inhibition of Fungal Activity and Enhancement of Aroma Biosynthesis in Apple Slices

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Abstract. Hexanal vapor inhibited hyphae growth of *Penicillium expansum* and *Botrytis cinerea* on potato dextrose agar (PDA) and on apple (*Malus domestica* Borkh.) slices. After 48 hours exposure to 4.1 μmol·L^{-1} (100 ppm) hexanal, the hyphae growth of both fungi was about 50% that of untreated controls. At a concentration of 10.3 μmol·L^{-1} (250 ppm), neither fungus grew during the treatment period, however, some growth of both fungi occurred 120 hours after treatment. At concentrations of hexanal vapor of 18.6 μmol·L^{-1} (450 ppm) or more, the growth of both fungi ceased and the organisms were apparently killed, neither showing regrowth when moved to air. When fungi were allowed to germinate and grow for 48 hours in hexanal-free air, a subsequent 48-hour exposure to 10.3 μmol·L^{-1} hexanal slowed colony growth relative to controls for several days and a 48-hour exposure to 18.6 μmol·L^{-1} stopped growth completely. Concentrations of hexanal that inhibited fungal growth on PDA also retarded decay lesion development on 'Golden Delicious' and on 'Jonagold' apple slices. Hexanal was actively converted to aroma volatiles in 'Jonagold' and 'Golden Delicious' apple slices, with hexanol and hexylacetate production strongly enhanced after 20 to 30 hours treatment. A small amount of butylhexanoate and hexylhexanoate production was also noted. Within 16 hours after treatment, no hexanal could be detected emanating from treated fruit. Since hexanal was metabolized to aroma-related volatiles by the fruit slices, the possibility of hexanal being an essentially residue-less antifungal agent seems likely. The possibility of developing a system for treating apple slices with hexanal in modified-atmosphere packages was also examined. The permeability of low-density polyethylene (LDPE) film to hexanal and hexylacetate was, respectively, about 500- and 1000-fold higher than LDPE permeability to O$_2$. The permeability of both compounds increased exponentially with temperature, with hexanal permeability increased 6-fold while hexylacetate increased only 2.5-fold between 0 and 30 °C.

Loss of perishable fruit and vegetables during the postharvest period is estimated to range from 20% to 25% despite the use of sophisticated postharvest storage facilities and techniques (Jeffries and Jeger, 1990; Kelman, 1989). Of these losses, decay accounts for a significant proportion. Many kinds of synthetic fungicides have been used to prevent the decay of perishable plant products, although, some may pose carcinogenic risks, which has led to public concern (Wisniewski and Wilson, 1992). As a result, fungicides will face greater restrictions and limitations in the future. Using natural products with fungistatic properties may be an attractive alternative to prevent decay. Wilson et al. (1991) noted that a number of natural volatile compounds have fumigative effects. Six-carbon (C$_6$) aldehydes have been found to inhibit the hyphae growth of *Alternaria alternata* and *Botrytis cinerea* (Hamilton-Kemp et al., 1992). Six-carbon aldehydes, with or without double bounds, are dominant compounds released by plant material through the lipoxygenase pathway after tissue damage (Vick and Zimmerman, 1987). It has not been determined if these wound volatiles are involved in the active or passive defense systems in the plant.

Six-carbon aldehydes are also important precursors for the formation of C$_6$ alcohols and C$_6$ esters, which are among the most abundant volatile compounds in apple, pears, and bananas and contribute to typical fruity odors (Paillard, 1986, 1990). Headspace treatment with C$_6$ aldehydes can enhance total aroma production in 'Golden Delicious' apple fruit in low O$_2$ storage (De Pooter et al., 1983). Even immature fruit, for which volatile biosynthesis is extremely low, respond to C$_6$ aldehyde application with enhanced aroma volatile biosynthesis (Song and Bangerth, 1994).

In this study, our objective was to explore the potential of using hexanal vapor as a natural, metabolizable fungicide for minimally processed (sliced) apple. We wished to determine the effect of hexanal vapor on hyphae growth of *Penicillium expansum* and *B. cinerea* on artificial medium and on apple fruit slices. In addition, we intended to determine the extent to which aroma volatile production of apple slices was enhanced by the interconversion of hexanal vapor to aroma volatiles and whether hexanal itself emanated from treated fruit at detectable levels. Finally, we sought to measure the permeability of low-density polyethylene (LDPE) film to hexanal and hexylacetate over a temperature range of 0 to 30 °C to determine the feasibility of using hexanal in packaged apple slices.

Materials and Methods

Biological technique. Pure strains of the fruit pathogens *P. expansum* and *B. cinerea* were obtained, respectively, from the American Type Culture Collection (Rockville, Md.) and the laboratory of Rodney Roberts (USDA–ARS, Wenatchee Tree Fruit Research Station, Wenatchee, Wash.). In this research, the general medium potato dextrose agar (PDA) was used for culturing both fungi. Stock cultures of *P. expansum* and *B. cinerea* were stored in glycerol at –70 °C and the stock culture was thawed in an ice bath before use. Inoculum was obtained by diluting the stock culture with sterilized water to a spore or conidia concentration of about 3×10^5 cells/mL.

Bioassay system. A flow-through vapor exposure system similar to that of Gardner et al. (1990), with some modification, was constructed. Under sterile conditions, 5 μL of inoculum was pipetted onto the surface of apple slices and PDA-filled petri plates. There were three inoculation sites per PDA petri dish and two inoculation sites per apple slice. Exposure chambers were 10-L glass desiccators, each containing four uncovered petri dishes and four apple slices. Desiccators and tubing were sterilized and contamination from nonsterilized tubing and inlet air was avoided using in-line microbial filters (0.45-μm pore size). Concentrated hexanal vapors were generated by passing sterilized air through or over liquid hexanal (99.9%, Sigma Chemical Co.) in a glass container. The hexanal vapor was mixed with hexanal-free air to obtain target hexanal concentrations by adjusting the air flow rates with glass microbore capillary tubes and a pressure regulator. Air containing hexanal was directed into the desiccator with 3-mm-i.d. flexible Teflon tubing impermeable to hexanal. The flow rate through the desiccator was 20 to 25 mL·min^{-1}. Treatment concentrations of hexanal were about 4.1, 10.3, 18.6, 26.9, and 55.8 μmol·L^{-1} (100, 250, 450, 650, and 1350 ppm, respectively). Gas samples for hexanal and aroma volatile analysis were obtained at a sampling port fitted with a Teflon-lined septum at the outlet of the exposure chamber. Gas concentrations measured at this point were taken to represent desiccator concentrations. The atmosphere in the desiccator was humidified by passing the diluted hexanal vapor through sterilized water in a 500-mL beaker located in the desiccator. The relative humidity in the system was >90%, as determined by a electronic hydrometer (model 15-3001; Hydrodynamics).

Plant material. Apple fruit ('Jonagold' and 'Golden Delicious') were harvested from the Michigan State Univ. Clarksville Horticultural Experiment Station and stored at 0 °C until used (within 5 to 10 d after harvest). Fruit were warmed to ambient temperature (22 ± 1 °C, 12 h equilibration), sliced into wedges (20 to 25 g), inoculated, and exposed to air with or without hexanal.

Antifungal assessments. Inoculated apple slices and petri plates were exposed to untreated air and hexanal vapor concentrations for 48 h in five successive experiments. For the 10.3- and 18.6-μmol·L^{-1} treatments, an additional study was performed to determine if previously growing colonies responded to hexanal similar to germinating spores. Three inoculated petri plates for each fungus were allowed to grow in hexanal-free air for 48 h, then transferred to the treatment chamber for a 48-h exposure period. After treatment,

the petri plates were moved again to hexanal-free air.

Hyphae growth of fungi (diameter of the colonies) was monitored daily from outside the desiccator using a scale marked on the bottom of the inverted clear glass petri dishes. Because of the difficulty of measuring the change of the colony diameter on the surface of the apple slices, only the presence (positive) and absence (negative) of a decay lesion was noted.

Hexanal analysis. A hexanal standard concentration was generated by evaporating a small amount of liquid hexanal (0.5 to 10 μL) into a specially made 4.4-L glass jar fitted with an Mininert gas-tight sampling valve (Alltech Assoc., Deerfield, Ill.). After the liquid had volatilized, a 20-μL gas sample was removed using a gas-tight syringe (Hamilton no. 1810) with a removable stainless-steel needle. Pumping the syringe plunger 30 times (which apparently saturated the adsorption sites inside the needle) gave a consistent gas chromatography (GC) analysis with a cv of 2% to 5%. The hexanal concentration in the treatment chambers was determined at the outlet of the desiccator. A GC (Carle GC series 400) equipped with a flame ionization detector (FID) was used to measure hexanal vapor concentrations. The column (3.3 m long, 3 mm i.d., 10% DEGS-PS, 80/100 mesh; Supelcoport) was maintained at 140 °C. Helium was used as carrier gas at flow rate of 20 mL·min^{-1}.

Volatile analysis. The sampling of aroma volatiles was via a solid-phase micro-extraction (SPME) device (Supelco, Bellefonte, Pa.) fitted with a fiber coated to a thickness of 100 μm with polydimethylsiloxane. The fiber was inserted through the Teflon-lined septum in the outlet of the desiccator for 20 s. Absorbed volatiles were desorbed from the fiber into the injection port of a GC (model 3400; Varian). The injector was a SPI on-column injector maintained at 200 °C. The desorption time was 40 s. Volatiles were separated using a HP-5 capillary column (10 m, 0.1 mm i.d., 0.34 μm film thickness; Hewlett Packard). Volatile detection was by a time-of-flight mass spectrometer (MS) with an electronic ionization source (FCD-650, LECO Corp, St. Joseph, Mich.). The carrier gas used was ultra purified helium (99.999%) at a flow rate 1.2 mL·min^{-1}. The temperature was isothermal for 4 min at 40 °C and then raised at the rate of 10 °C/min to 230 °C and held at 230 °C for 15 min. The transfer line temperature was 220 °C. Mass spectra were collected at a rate of 50 spectra/s over a mass range m/z 40 to 250. The electron ionization energy was 70 eV.

Identification of volatile components was confirmed by comparing collected mass spectra with those of authentic compounds

Table 1. Effect of 48 h exposure to hexanal vapor on the hyphae growth of *Penicillium expansum* on potato dextrose agar in a humidified, aerobic environment at 22 °C.

Expt.	Hexanal (μmol·L^{-1})	Hyphae growth of fungi (mm)[z]						
		Time (h)						
		24	48[y]	72	96	120	144	168
1	0	8.0 ± 0.05	13.7 ± 0.9	19.7 ± 1.2	28.7 ± 0.5	38.6 ± 2.53	41.3 ± 0.9	50.0 ± 1.69
	4.1	0	0	6.7 ± 0.9	14.6 ± 0.5	22.3 ± 2.1	29.6 ± 0.9	35.5 ± 0.5
2	0	6.3 ± 0.94	9.3 ± 0.47	17.2 ± 0.7	27.7 ± 0.9	34.3 ± 0.91	45.3 ± 1.4	54.6 ± 2.1
	10.3	0	0	0	5.3 ± 0.5	10.3 ± 0.5	22.3 ± 2.1	25 ± 2.1
3	0	8.67 ± 0.470	16.0 ± 4.22	22.7 ± 0.94	29.3 ± 0.9	41.3 ± 2.5	44.0 ± 2.820	50.7 ± 0.9
	18.6	0	0	0	0	0	0	0
4	0	5.3 ± 0.47	7.7 ± 0.5	18.6 ± 0.9	28.7 ± 0.9	31.0 ± 1.4	38.3 ± 1.2	41.0 ± 0.8
	26.9	0	0	0	0	0	0	0
5	0	5.7 ± 0.47	9.4 ± 0.94	22.7 ± 0.47	33.7 ± 0.9	40.7 ± 1.6	42.7 ± 1.6	48.0 ± 1.6
	55.8	0	0	0	0	0	0	0

[z]Mean ± sd (n = 3).
[y]End of treatment period.

and by comparison to spectra in the National Institute for Standard Technology (NIST) mass spectra library (Search version 1.0).

LDPE permeability to hexanal and hexylacetate. LDPE permeability to hexanal and hexylacetate was determined via a specially built permeation cell. The permeation cell was constructed of two hemispheric glass chambers, each fitted with inlet and outlet ports. The donor and receiver hemispheres were separated by the test film with an area of 7.55 cm^2. Purified and humidified air with (donor side) and without (receiver side) hexanal was metered through the two chambers. On the donor side, the volatile concentration was generated in the same manner as for the vapor exposure chambers and was maintained between 2.2 and 3.5 μmol·L^{-1}. A 50-μL gas sample was withdrawn using a gas-tight syringe fitted with stainless steel jacketed capillary tube as a needle. Gas analysis was the same as described previously for hexanal; however, the coating of the capillary needle accentuated the sorption of vapors, resulting in a greater amount of material being retained in the needle, thereby increasing sensitivity.

Permeability was measured at 0, 5, 10, 15, 20, 25, and 30 °C by placing the entire apparatus in a controlled-temperature chamber. Donor- and receiver-side concentrations were determined 10 or more times at each temperature for each of two pieces of 0.00254-cm (1-mil)-thick LDPE film. Permeability is expressed as mol·m·m^{-2}·s^{-1}·Pa^{-1}.

Results

Effect of hexanal on germination and growth of fungi. The 4.1-μmol·L^{-1} treatment inhibited growth of *P. expansum* and *B. cinerea* completely during the 48-h exposure period (Tables 1 and 2). After an additional 48 h in hexanal-free air (hour 96), the treated colonies had grown to 50% of the control colony diameter. A concentration of 10.3 μmol·L^{-1} prevented fungal growth during the exposure period, but also had an additional carryover effect. No growth of *B. cinerea* occurred for the next 24 h, and *P. expansum* had no growth for 48 h after treatment (Tables 1 and 2). Both organisms subsequently recovered and reached about 50% of control growth by 168 h. At concentrations of hexanal above 18.6 μmol·L^{-1}, growth of both fungi was completely arrested during the exposure period and for the subsequent 120 h of the experiment.

Effect of hexanal on growth of established colonies. During exposure, hexanal concentrations of 10.3 and 18.6 μmol·L^{-1} stopped hyphae growth of established fungal colonies that had previously grown for 48 h in hexanal-free air (Fig. 1 a and b). Following cessation of exposure to hexanal, hyphae growth resumed for the 10.3-μmol·L^{-1} treatment, but not following the 18.6-μmol·L^{-1} treatment. After the 10.3-μmol·L^{-1} treatment, regrowth of *P. expansum* was about 50% less than the control, but regrowth of *B. cinerea* was similar to that of controls. No regrowth of *P. expansum* occurred following treatment with a hexanal concentration of 18.6 μmol·L^{-1} (Fig. 1b). Five days after exposure to 18.6 μmol L^{-1} hexanal, only a slight regrowth of *B. cinerea* (10%) was measured (Fig. 1b).

Effect of hexanal on decay of inoculated apple slices. The growth of fungi in apple slices of 'Golden Delicious' and 'Jonagold' were entirely inhibited during exposure to 10.3 and 18.6 μmol·L^{-1} hexanal vapor (Table 3). Following exposure, no lesion expansion was observed. No effect of hexanal vapor on the color of either the cortex or the skin was observed for any of the treatments.

Effect of hexanal on aroma. Aroma volatiles exiting the control (hexanal-free) chamber were not detectable for either 'Jonagold' or 'Golden Delicious' slices (data not shown). However, there was significant aroma production by apple slices after only 0.5 h exposure to 10.3 μmol·L^{-1} hexanal for both cultivars (Fig. 2 a and b). Aroma data are presented as the ratio of aroma to hexanal concentration in the exit line as an estimate the relative rate of metabolic conversion of hexanal to aroma volatiles by the apple slices. For 'Jonagold', hexanal interconversion to hexanol was immediately detected; however, hexylacetate production began to increase only after 5 h (Fig. 2a). After 7.5 h, the production of butylhexanoate and hexylhexanoate was detectable; by the end of the exposure period (50 h), their ratio relative to hexanal in the chamber exit line increased to 0.06 and 0.08, respectively. Additionally, after 24 h, traces of hexylbutanoate, 2-methyl-propylhexanoate, propylhexanoate, and butylbutanoate were also identified by GC–MS (data not shown) and had the same temporal trends as hexylacetate. At 30 h, the chamber hexylacetate concentration reached the highest level, about 1.5 times that of hexanal. This maximum was followed by a decline in the hexylacetate/hexanal ratio to about 0.5 by hour 50. 'Golden Delicious' slices, like those of 'Jonagold', continuously converted hexanal to hexanol, hexylacetate and hexylhexanoate, but at somewhat different rates (Fig. 2b). The ratio of hexanol to hexanal was 1.5 after 18 h; however, the highest ratio for hexylacetate was just 0.4 and occurred after 26 h. The ratio of butylhexanoate and hexylhexanoate to hexanal only reached 0.1 at the end of the exposure period,

Table 2. Effect of 48 h exposure to hexanal vapor on the hyphae growth of *Botrytis cinerea* on potato dextrose agar in a humidified, aerobic environment at 22 °C.

		Hyphae growth of fungi (mm)[z]						
		Time (h)						
Expt.	Hexanal (μmol·L^{-1})	24	48[y]	72	96	120	144	168
1	0	4.7 ± 0.05	21.7 ± 2.3	31.3 ± 0.9	44.3 ± 0.5	50.7 ± 1.25	54.6 ± 1.3	59.4 ± 0.9
	4.1	0	0	0	11.0 ± 0.8	23.3 ± 2.35	37.6 ± 0.5	42.0 ± 1.6
2	0	6.3 ± 0.94	9.3 ± 0.47	17.6 ± 0.7	27.7 ± 0.9	34.3 ± 0.9	45.3 ± 1.4	54.6 ± 0.9
	10.3	0	0	0	0	4.3 ± 1.2	12.7 ± 0.9	22.7 ± 0.9
3	0	7.33 ± 0.5	19.3 ± 2.35	30.7 ± 0.94	40.3	46.7 ± 1.24	50.3 ± 1.24	56.7 ± 0.5
	18.6	0	0	0	0	0	0	0
4	0	5.3 ± 0.4730	10.3 ± 0.5	14.0 ± 0.9	23.3 ± 1.3	38.7 ± 0.9	46.7 ± 2.4	58.0 ± 0.5
	26.9	0	0	0	0	0	0	0
5	0	5.3 ± 0.47	19.4 ± 0.94	24.2 ± 0.47	34.3 ± 0.9	40.7 ± 0.9	47.4 ± 2.05	56.7 ± 2.05
	55.8	0	0	0	0	0	0	0

[z]Mean ± SD (n = 3).
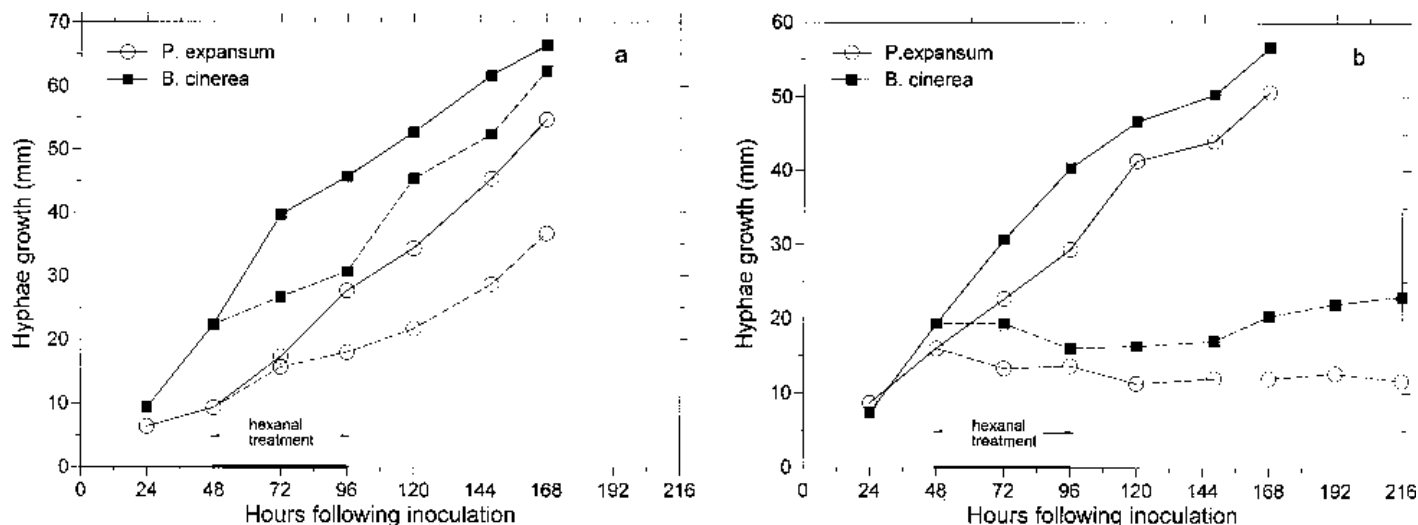[y]End of treatment period.

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Fig. 1. (**a**) Hyphae growth of *Penicillium expansum* and *Botrytis cinerea* after exposure to hexanal vapor at concentration of 10.3 µmol·L⁻¹ (100 ppm) for 48 h. Dashed line indicates treated cultures. (**b**) Hyphae growth of *P. expansum* and *B. cinerea* after exposure to hexanal vapor at concentration of 18.6 (µmol·L⁻¹) for 48 h. Dashed line indicates treated cultures.

which was slightly higher than for 'Jonagold'.

Hexanal and aroma emanations after treatment cessation. Hexanal could not be detected emanating from treated slices 16 h after treatment with hexanal at a concentration of 10.3 µmol·L⁻¹ (Fig. 3). The lower limit of detection of our system was about 4.13×10^{-5} µmol·L⁻¹ (1 ppb), which is below the odor threshold for humans (Devos et al., 1990). A rapid decrease in hexanol, hexylacetate, and hexylhexanoate occurred following treatment, with hexanol declining more rapidly than the other four primary metabolic products of hexanal and hexylacetate declining least rapidly.

LDPE permeability to hexanal and hexylacetate. The permeability of hexanal and hexylacetate increased exponentially with temperature increase (Fig. 4). The natural log of the permeability changed linearly with reciprocal of temperature (K) according to the Arrhenius equation:

$$\ln(P_i) = -E_a/RT + \ln(A)$$

where P_i is hexanal or hexylacetate permeability (mol·m·m⁻²·s⁻¹·Pa⁻¹); E_a is the energy of activation (kJ·mol⁻¹); and R is the gas constant (0.0083144 kJ·mol⁻¹·K⁻¹). The slope of the fitted line is $-E_a/R$ (Fig. 4, insert). The E_a for permeation of the gases was calculated from the fitted line and was 44.6 kJ·mol⁻¹ for hexanal and 23.6 kJ·mol⁻¹ for hexylacetate with respective coefficient of determination (r^2) values of 0.99 and 0.94. The equations of the fitted lines were rearranged to facilitate expression of $P_{hexanal}$ and $P_{hexylacetate}$ for the LDPE film at any T (K) as follows:

$$P_{hexanal} = 0.53495 \times \exp(-5364.1/T)$$

$$P_{hexylacetate} = 0.00032 \times \exp(-2832.6/T)$$

Hexanal permeability was lower than that of hexylacetate. The permeability of both compounds increased exponentially with temperature, with hexanal permeability increased 6-fold while hexylacetate increased only 2.5-fold between 0 and 30 °C.

Discussion

Lipoxygenase-related volatiles may play a role in plant response to wounding and other related stresses (Hildebrand et al., 1988).

A burst of lipoxygenase activity accompanying wounding of the cell leads to the production of fatty acid hydroperoxides, or their decomposition to compounds like C_6- and C_9-aldehydes (Galliard, 1978). Drawert et al. (1973) reported that the identified C_6-aldehydes, hexanal, (Z)-3-hexenal, and (E)-2-hexenal produced enzymatically when cellular structures are disrupted in green vegetables. These lipoxygenase reaction products can act as toxic agents to invading pathogens (Hildebrand et al., 1988). The findings of significant antifungal effects of hexanal in our experiment using the quantitative vapor system demonstrated that hexanal was effective in reducing fungal activity during the exposure period and, at higher concentrations, had a residual effect, preventing fungal regrowth probably due to a fungicidal action.

The inhibitory effect of hexanal vapors on the growth of *P. expansum* and *B. cinerea* is well supported by the literature (Wilson et al., 1991). A number of natural volatile compounds have been found to have antipathogenic effects. These compounds fall in various chemical categories but are primarily aldehydes and ketones. Natural antifungal volatiles examine for commercial applicability include acetaldehyde (Aharoni and Stadelbacher, 1973; Avissar et al., 1989), 2-nonanone (Vaughn et al., 1993), ethyl benzoate (Wilson et al., 1987), acetic acid (Sholberg and Gaunce, 1995), and C_6-aldehydes such as hexanal and trans-2-hexenal (Hamilton-Kemp et al., 1992).

A primary concern in the application of antifungal volatile compounds having aromas is the alteration of fruit flavor. Acetaldehyde, for instance, has been reported to cause off-flavor (Paillard, 1990) as well as improved flavor (Paz et al., 1982). The strong

Table. 3. Effect of hexanal vapor exposure for 48 h on lesion growth of *Penicillium expansum* and *Botrytis cinerea* on slices of 'Jonagold' apple fruit. Symbols indicate presence (+) or absence (–) of decay lesions.

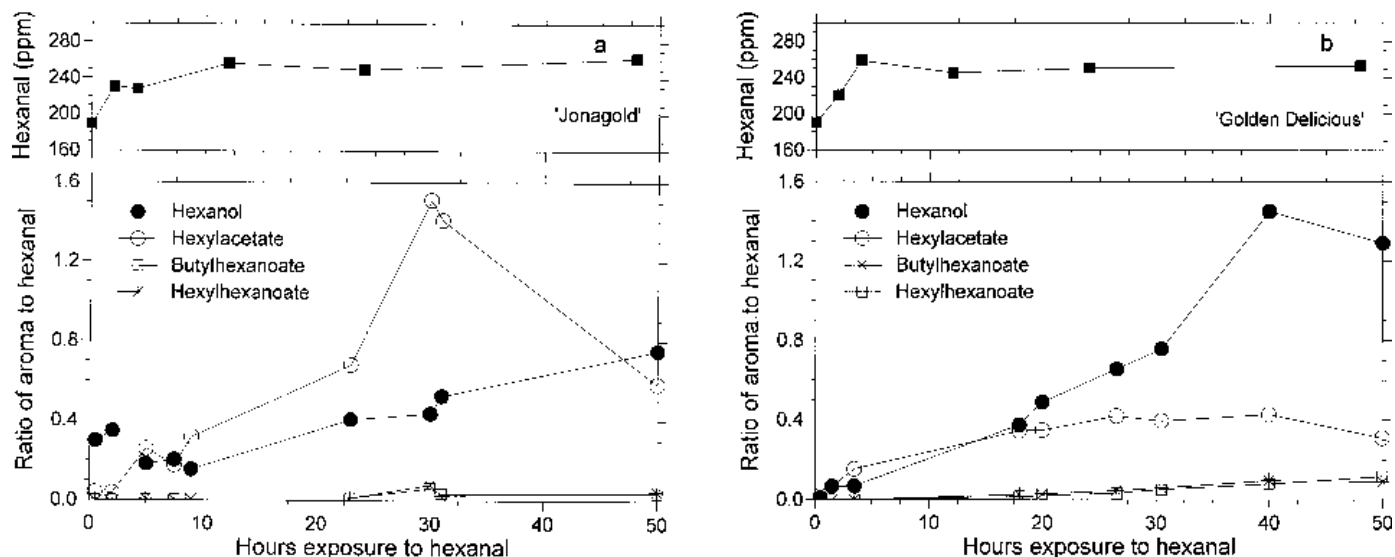| Expt.	Hexanal (µmol·L⁻¹)	*Penicillium expansum*			*Botrytis cinerea*		
		Time (h)					
		24	48	72	24	48	72
1	0	–	+	+	–	–	+
	10.3	–	–	–	–	–	–
2	0	–	+	+	–	–	+
	18.6	–	–	–	–	–	–

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Fig. 2. (**a**) Volatile compounds emanating from 'Jonagold' apple slices during hexanal vapor exposure at concentration of 10.3 µmol·L⁻¹. (**b**) Volatile compounds emanating from 'Golden Delicious' apple slices during hexanal vapor exposure at concentration of 10.3 µmol·L⁻¹.

fruity–floral aroma of 2-nonanone may be incompatible with some fruit types, such as apple. Additionally, for apple, concentrations of 2-nonanone below that needed to inhibit decay organisms induced browning of the apple skin (Leepipattanawit, unpublished data). Hexanal treatment, however, may not pose similar problems. Aldehydes such as hexanal and their oxidation products such as hexanol and hexanoic acid act as precursors for a wide range of 'fruity' aroma volatiles important to fruit flavor (Bartley et al., 1985; Drawert et al., 1973). Thus, hexanal vapor is consumed as it is converted to compounds that enhance aroma in apple (De Pooter et al., 1983; Song, 1994). The metabolism of hexanal would explain our inability to detect hexanal emanations from treated fruit by either instrument or smell 16 h after cessation of hexanal treatment. The ability of apple tissues to metabolize hexanal is also consistent with the rapid decline in hexanol and the other aroma volatiles we detected following cessation of hexanal treatment. Similarly, Song (1994) found hexanal enhancement of aromas to last only a few days following exposure.

The enzyme system responsible for this interconversion of aldehydes to alcohols to esters is the sequential reactions of alcohol dehydrogenase (ADH, E.C 1.1.1.1) and alcohol acetyltranferase (AAT, E.C 2.3.1.84). These enzymes were apparently highly active in apple slices. It is interesting to note that the small mass of apple tissue (about 80 g) being used in our experiment converted most of the supplied hexanal to hexanol and, subsequently, hexylacetate and related compounds. The dynamics of the conversion of hexanal to hexanol and hexanol to hexylacetate in 'Jonagold' and 'Golden Delicious' slices is consistent with earlier findings that the precursor availability, rather than enzyme activities, is perhaps the most important factor for regulating volatile production in apple fruit (Bartley et. al, 1985; Song and Bangerth, 1994). The two cultivars in this study differed somewhat in their response to hexanal application. While 'Jonagold' fruit exhibited a significant capacity to synthesize hexanol at the initiation of the experiment, 'Golden Delicious' did not. In fact, the 'Golden Delicious' fruit exhibited a continued increase in their capacity to convert hexanal to hexanol, which may be indicative of an increase in the capacity of ADH over time. 'Jonagold' fruit, on the other hand, appeared to undergo an increase in the competence of AAT activity with time.
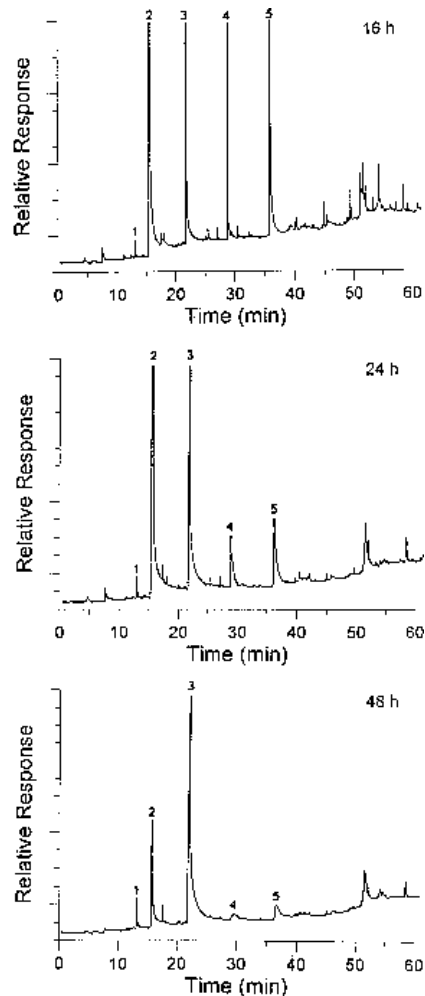

Fig. 3. Decline in volatile compounds emanating from 'Golden Delicious' apple slices with time after exposure to a hexanal vapor concentration of 10.3 µmol·L⁻¹. (1, butylacetate; 2, hexanol; 3, hexylacetate; 4, butylhexanoate; 5, hexyl-hexanoate).
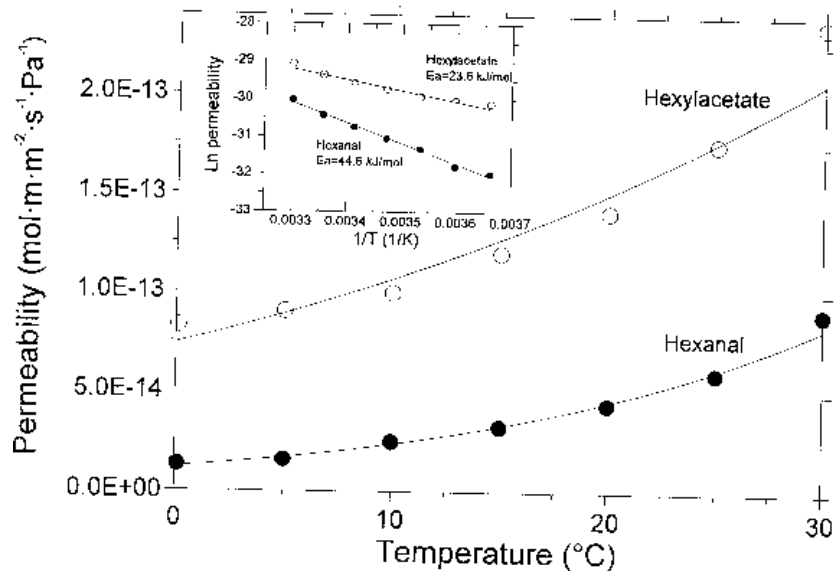
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Fig. 4. Effect of temperature on LDPE permeability to hexanal and hexylacetate ($P_{hexanal}$ and $P_{hexylacetate}$). Insert: Arrhenius plot of hexanal and hexylacetate permeability for typical film sample.

Modified-atmosphere packaging storage has some potential as an atmospheric control system to prolong the storage life of perishable products (Beaudry et al., 1992; Cameron et al., 1995). If the concept of atmospheric control is expanded to include introduced volatiles, the application of volatiles in a packaging system to prevent decay may be appropriate for apple slices (Lakakul, 1994). The permeability data here show that LDPE is a relatively poor barrier to hexanal and hexylacetate, and the data are consistent with reported values (Delassus et al., 1988). This suggests hexanal introduced into the package headspace would escape rapidly. The higher Ea for hexanal compared to hexylacetate indicates hexanal retention would be relatively better at lower temperatures. The permeability of hexanal and each of its metabolic products can be used to develop models to describe their flux similar to models developed for O_2 and CO_2 (Cameron et al., 1994). If need be, a gaseous release mechanism such as that described by Vaughn et al. (1993) might be used to release a predictable level of vapor into the package headspace to compensate for that lost from the package. It is also conceivable that more polar films might be appropriate (Debeaufort and Voilley, 1994).

Our data suggest hexanal vapor has a number of attributes that may be important to consumers demanding more natural control measures for fruit diseases and fewer toxic residues. In addition, hexanal-derived aroma enhancement may also prove desirable. Significantly, fruit of a number of horticulturally important plant species utilize C_6-aldehydes as precursors to aromas. This suggests hexanal and similar aldehydes can be used rather widely as antifungal agents. Fruit having some potential for aldehyde application include pears, strawberries, bananas, pineapple, and melons. Aldehyde use in packaging of lightly processed products of these commodities also seems to be a possible future option.

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