

Fumigation of Stored Pome Fruit with Hexanal Reduces Blue and Gray Mold Decay

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Abstract. Stored apples and pears are subject to blue and gray mold decay incited by *Penicillium expansum* and *Botrytis cinerea* respectively. Hexanal, a C6 carbon aldehyde, used as a vapor provided effective control of both blue and gray molds in laboratory experiments on apple slices. A preliminary trial with ‘Anjou’ pears in bins showed that hexanal was not corrosive and could reduce gray mold in pears stored for 7 months. However details on the correct procedure for fumigating pome fruit were lacking, and further studies were needed to develop a reliable fumigation strategy. In trials with inoculated fruit, hexanal inactivated conidia of *B. cinerea* contaminating the pear surface when used at a rate of 2 mg·L⁻¹ for 24 hours or 4 mg·L⁻¹ for 18 hours. It was less effective on ‘Gala’ apples inoculated with conidia of *P. expansum*, but reduced blue mold decay to low levels at 15 °C. On the other hand, hexanal increased gray and blue molds when used after wounds were made in inoculated fruit. The use of a preharvest treatment with cyprodinil (0.62 g·L⁻¹) reduced both blue and gray molds in wounds with or without hexanal fumigation. Thus a strategy for controlling postharvest decay was developed by which fruit were treated 2 weeks before harvest with cyprodinil, followed by fumigation with hexanal immediately after harvest. The use of this strategy on ‘Anjou’ pears produced the highest number of mold-free fruit in 2003 and the least amount of gray and blue mold decay in 2003 and 2004 on pears stored for 4 months. Wounded apples only developed 1% rot compared with 10% in the control, indicating that hexanal fumigation of stored apples reduced contamination. Monitoring hexanal during fumigation showed that hexanal concentration declined slowly over a 24-hour period and could accurately be described by a third-order polynomial equation. Hexanal fumigation at low rates (2–3 mg·L⁻¹) was not phytotoxic and improved aroma in ‘Anjou’ pears and ‘Gala’ apples with no harmful effects on apple or pear firmness, pH, titratable acidity, or soluble solids.

Hexanal is a naturally occurring, volatile C-6 aldehyde formed via the lipoxygenase pathway in plants from linoleic acid (Hildebrand, 1989). Volatiles formed by this pathway in wounded plants have antifungal properties, as shown by early research with wounded tomato leaves in which hyphae of both *Alternaria alternata* and *Botrytis cinerea* were inhibited (Hamilton-Kemp et al., 1992). In trials conducted on *B. cinerea*, the related compound (*E*)-2-hexenal, which differs by having a double bond, was more effective against spores than mycelium of the fungus and stimulated its growth at low concentrations (Fallik et al., 1998). Thus, it was concluded that maintenance of a high vapor-phase level of (*E*)-2-hexenal was necessary to inhibit mycelial growth and to avoid enhancing postharvest mold problems.

Botrytis cinerea causes the postharvest disease known as gray mold and is considered one of the most important diseases of stored pears. Wounds or injuries are the primary infection courts for the initiation of gray mold in apples and pears (Rosenberger, 1990). The fungus forms a nest rot by growing from infected to healthy fruit at storage temperatures as low as -0.6 °C (Ogawa and English, 1991). Lennox et al. (2004) reported that, on ‘Anjou’ pears, incidence of stem-end gray mold was higher than calyx-end and puncture gray mold. Archbold et al. (1997) showed that hexanal, among other lipoxygenase pathway products, exhibited potential as postharvest fumigants, showed that when ‘Crimson Seedless’ table grapes were fumigated with (*E*)-2-hexenal, gray mold was suppressed but not completely controlled (Archbold et al., 1999).

Penicillium expansum Link causes the postharvest disease known as blue mold and is considered the most important disease of stored apples (Turechek, 2004). Hexanal vapor was successfully used to control *P. expansum* on apple slices after 48 h of continuous exposure to 100 ppm of the product (Song et al., 1996). When the fungus was allowed to grow for 48 h in air, a

subsequent treatment with 450 ppm stopped growth completely (Song et al., 1998). Fan et al. (2006) found that under similar conditions of constant exposure to hexanal, *P. expansum* lesion development on whole apples was reduced. These antimicrobial results are supported by Lanciotti et al. (1999), who found that hexanal strongly inhibited molds, yeasts, and mesophilic and psychrophilic bacteria, and extended the shelf life of apple slices. Hexanal has also been used to control postharvest decay in stone fruit incited by *Monilinia laxa* and *Rhizopus stolonifer* (Caccioni et al., 1995).

Hexanal has other properties in addition to its antimicrobial activity. Aroma volatiles are important sensory attributes of ripe fruit, but may also play functional roles in plant–pathogen interactions (Archbold et al., 2000). Hexanal treatment stimulated aroma production in ‘Jonagold’ and ‘Golden Delicious’ apple slices (Song et al., 1996, 1998). When added to modified atmosphere (MA) (70% N₂ and 30% CO₂), hexanal also prevented a browning reaction for 16 d at 15 °C (Lanciotti et al., 1999).

The goal of this study was to determine whether hexanal vapor could be used as a fumigant to prevent postharvest decay incited by either *B. cinerea* or *P. expansum* on stored apples and pears. To accomplish this objective, small quantities of inoculated fruit were fumigated to establish optimum rate, temperature, and duration for fumigation before and after fruit were wounded. Preharvest treatment of fruit in the orchard with cyprodinil was also evaluated because it was thought unlikely that hexanal would control pathogen conidia in wounds, and cyprodinil had previously been shown to be effective in preventing *B. cinerea* decay in wounds of fruit stored for 3 months (Sholberg et al., 2003a). Larger volumes of fruit were used in subsequent experiments to determine whether hexanal alone or a combination of cyprodinil and hexanal could be used for control of postharvest decay in stored fruit. Characteristics of hexanal such as concentration during fumigation, effect on fruit quality and aroma, and possibility of causing fruit damage were also studied. A preliminary report on the use of hexanal fumigation for control of postharvest decay of pome fruit has been published (Sholberg and Randall, 2005).

Materials and Methods

Decay control

Control of gray mold in inoculated ‘Anjou’ pears. ‘Anjou’ pears from the Pacific Agri-Food Research Center, Summerland, B.C., research orchard (PARC) were treated with 0, 0.31, or 0.62 g·L⁻¹ cyprodinil (Vanguard 75 WP, Syngenta Crop Protection Canada, Guelph, Ont., Canada) 2 weeks before harvest according to a completely randomized block design on 13 Sept. 2002. (The current label in the United States is for a preharvest interval of 72 d on apples.) Five single-tree replicates were sprayed until runoff with a backpack sprayer. Subsamples of 10 pears/treatment were used in the inoculated fumigation

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experiments. *Botrytis cinerea* Pers. was grown on potato dextrose agar (PDA) in plastic (10-cm diameter) Petri plates at 18 °C for 2 to 3 weeks and was used to make a spore suspension containing 1×10^4 colony-forming units (CFU)·mL⁻¹. The following procedure was followed: all fruit were inoculated, then either fumigated and wounded or alternatively wounded and fumigated. Specifically, the spore suspension was misted on each fruit with an airbrush (Paache Airbrush Company, Harwood Heights, Ill.). After the spores had dried, the fruit was placed in specially constructed 23-L chambers previously used for evaporating vinegar (Sholberg et al., 2000), and were fumigated for 18 h with 4 mg·L⁻¹ or 24 h with 2 mg·L⁻¹ with fully vaporized laboratory-grade hexanal (Sigma-Aldrich Canada Ltd., Oakville, Ont.) before or after wounding. The hexanal was vaporized by heating the liquid hexanal in an aluminum receptacle fitted with a 150-W cartridge heater (Omega Engineering, Stamford, Conn.). The volume that the fruit occupied was ≈ 2.4 L in the 23-L chamber. Each fruit was wounded at four locations equidistant over the fruit with a sterile nail (3-mm diameter \times 3-mm depth) at four locations. The fumigations were done in temperature-controlled rooms at 5, 10, 15, and 20 °C. Fruit was incubated at 20 °C for 7 d, after which the number of infected wounds was recorded.

Control of postharvest decay in stored 'Anjou' pear. A preliminary trial was conducted in 2001 on 'Anjou' pears picked at commercial maturity from orchards in Wenatchee, Wash., and Summerland, B.C. The fruit was placed in two half-bin replicates (200-kg capacity) and fumigated in an airtight room (3.0 \times 2.5 \times 3.6 m) originally designed for methyl bromide fumigation. The hexanal was poured into an aluminum frying pan, the room sealed, and the frying pan was set for 120 °C, quickly vaporizing the hexanal in ≈ 15 min, similar to previously reported studies on acetic acid fumigation (Sholberg et al., 2003b). Four large fans circulated the air in the room during the fumigation. The volume of each half bin in the room was ≈ 0.4 m³, occupying less than 2% of the total room volume. The pears were fumigated at 4 mg·L⁻¹ hexanal for 48 h at 2 °C. Control fruit was placed in 2 °C cold storage at the same time the rest of the fruit was fumigated. Immediately after fumigation, the chamber was vented and the fruit was removed and placed in 1 °C cold storage for 7 months. Upon removal from storage, each fruit was examined for decay. Decay categories recorded were tissue and pedicel infection by *B. cinerea*, and *P. expansum*. Fungi that were not obviously one of these two genera were classed as miscellaneous or unknown. Any fruit damage or obvious signs of phytotoxicity such as blackened lenticels were recorded at the same time decay was evaluated.

The PARC 'Anjou' pears harvested from the same trees in 2002 as described earlier for the inoculated trial were also used in the bin trials. The fruit was harvested into boxes and immediately placed at 15 °C and fumigated

at 2 mg·L⁻¹ for 24 h or 4 mg·L⁻¹ for 18 h. After fumigation, pears were packed in boxes and stored at 1 °C for 4 months, after which they were rated for decay and phytotoxicity as described previously. In 2003, 'Anjou' pears from PARC were treated with 0, 0.31, or 0.62 g·L⁻¹ cyprodinil 2 weeks before harvest. Each of the three single-tree replicates/treatment (Table 1) were fumigated separately in a 1-m³ chamber at 15 °C for 24 h as previously described for fumigation of table grapes with acetic acid (Sholberg et al., 1996). Immediately after fumigation, fruit was placed in a 1 °C cold storage room for 4 months, after which decay and phytotoxicity were recorded as noted earlier.

Control of blue mold in inoculated 'Gala' apples. 'Gala' apples from PARC were treated with 0, 0.31, and 0.62 g·L⁻¹ cyprodinil 2 weeks before harvest on 30 Aug. 2002 according to a randomized complete block statistical design with three single-tree replicates for each treatment. Subsamples of 10 apples/treatment were used in the inoculated apple fumigation experiments. *Penicillium expansum* grown on PDA in plastic (10-cm-diameter) Petri plates at 18 °C for 2 to 3 weeks was used to make a spore suspension containing 1×10^6 CFU·mL⁻¹. 'Gala' apples were inoculated, wounded, and fumigated as previously described for 'Anjou' pears at 5, 10, 15, and 20 °C. In this case the apples were incubated at 25 °C for 7 d, after which the number of infected wounds was recorded.

Control of postharvest decay in apples. In 2003, 'Gala' apples from PARC were treated with cyprodinil (0, 0.31, or 0.62 g·L⁻¹) 2 weeks before harvest following the same procedure as used for pears (Table 2). The apples were harvested at commercial maturity and immediately cooled to 15 °C and fumigated in bins at 3.0 mg·L⁻¹ for 24 h in the same airtight room used for pears. After fumigation, the room was vented for at least 30 min, after which the apples were placed in standard apple boxes and stored at 1 °C for 6 months. In addition to the 'Gala' apples, one bin of 'McIntosh' and two bins of 'Jonagold' apples were also fumigated as described and were stored for 5 months at 1 °C. After storage, the 'Gala' apples were ripened for 1 week at 20 °C and rated for decay. Decay categories recorded were tissue and pedicel infection by *B. cinerea* and *P. expansum*.

Fumigation of apples after storage. Three 10-apple samples were taken from each treated or untreated bin of 'Gala', 'McIntosh', or 'Jonagold' apples that had been stored for 5 months at 1 °C. Five apples from each sample were fumigated with 3.0 mg·L⁻¹ hexanal for 24 h at 15 °C. After treatment, apples were wounded as noted earlier, incubated at 20 °C for 1 week, and the number of decayed wounds was recorded.

Hexanal properties

Monitoring hexanal concentration during fumigation of fruit. Monitoring was done to ensure that the correct concentration of hexanal was applied to the fruit. Hexanal concentration was monitored during the fumigation of apples and pears by withdrawing 250-mL samples from the chambers with a vacuum pump. One-milliliter subsamples were injected into a gas chromatograph (GC model 910; Questron Technologies Corp., Mississauga, Ont.). The gas chromatograph was outfitted with a flame ionization detector and fused silica capillary column (Zebron ZB-FFAP; Phenomenex, Torrance, Calif.) for detection of hexanal. In addition to general monitoring of hexanal during fumigation, a more in-depth study was conducted during fumigation of 'Jonagold' apples in 2003. Four samples were taken each time from 5 min to 24 h after evaporation of hexanal during fumigations on 2 Oct. and 6 Oct. 2003. During fumigation, relative humidities of 74% to 80% and temperatures of 15 to 18 °C were maintained.

Effect of hexanal on pear quality. Fruit firmness (model EPT-1; Lake City Technical Products, Kelowna, B.C.), pH, titratable acidity (Metrohm AG, Herisau, Switzerland), and soluble solids (ABBE Mark II Digital Refractometer; AO Scientific Instruments, Buffalo, N.Y.) were determined for each treatment after storage of fruit in 2002 and 2003.

Effect of hexanal on 'Anjou' pear and 'Gala' apple aroma. Sensory panels were used to determine whether 'Anjou' pears or 'Gala' apples fumigated with hexanal had improved aroma over nonfumigated fruit when stored at 1 °C for 2 months. Samples of fumigated and control fruit were removed from 1 °C storage and allowed to warm to 20 °C. The next day a panel of 21 or 27 judges

Table 1. Percent decay or mold-free 'Anjou' pears fumigated with hexanal and stored for 4 months at 1 °C in 2003 to 2004.

Treatment	Stem gray mold	Gray mold	Blue mold	Mold free
Cooled fruit ^z	45.0 a ^v	7.0 a	5.0 a	33.0 a
Cyprodinil cooled fruit ^v	37.5 a	6.0 a	0.0 b	45.5 a
Control fruit ^x	30.5 a	6.5 a	4.5 a	22.0 a
Cyprodinil + hexanal ^w	33.5 a	1.5 b	0.0 b	53.0 a
Cyprodinil	29.0 a	1.0 b	0.5 b	47.5 a
Hexanal	14.5 a	1.0 b	0.0 b	68.5 a

^zCooled pears (three replicates) were immediately placed in a 1 °C cold room after picking.

^vCyprodinil at a concentration of 0.62 g·L⁻¹ was applied by spraying with a backpack sprayer 2 weeks before harvest.

^xControl pears were placed at 15 °C for 24 h and then placed in a 1 °C cold room after picking.

^wFruit were fumigated with 3 mg·L⁻¹ hexanal in bins in a 3.0 \times 2.5 \times 3.6-m room for 24 h at 15 °C.

^vMeans followed by the same letter in each column are not significantly different according to the Waller-Duncan *k*-ratio *t* test when *k* = 100.

Table 2. Percent infected or mold-free 'Anjou' pears or 'Gala' apples treated before harvest with cyprodinil and fumigated with hexanal.

Treatment ^z	Stem gray mold		Gray mold		Blue mold		Mold free	
	Anjou	Gala	Anjou	Gala	Anjou	Gala	Anjou	Gala
Control	31.7 ab ^x	24.0 a	9.0 ab	0.5 ab	13.3 ab	3.0 a	37.3 g	65.0 abc
Hexanal low	6.3 c	16.5 a	9.7 a	1.0 a	6.7 bc	2.5 a	73.7 bcd	66.0 abc
Hexanal high	13.7 c	29.0 a	6.3 abc	0.0 b	17.0 a	1.5 a	61.7 cde	61.5 abc
Cyprodinil ^y	44.3 a	13.0 a	2.3 cd	1.0 a	2.7 c	0.5 a	56.0 ef	79.5 ab
Cyprodinil + hexanal low	6.3 c	10.5 a	0.3 d	0.0 b	1.7 c	1.0 a	90.0 a	83.0 a
Cyprodinil + hexanal high	13.0 c	14.0 a	5.0 bc	0.5 ab	5.0 c	2.0 a	75.0 bc	81.5 ab

^zFruit was either left untreated or fumigated in bins (400-kg capacity) in a 3.0 × 2.5 × 3.6-m room for 24 h at 2 mg·L⁻¹ (low rate) and 18 h at 4 mg·L⁻¹ (high rate) hexanal at 15 °C. Each bin replicate (three replicates/treatment) was either fumigated or immediately placed in 1 °C cold storage. 'Anjou' pears were stored for 4 months in 2002 to 2003 and 'Gala' apples were stored for 6 months in 2003 to 2004.

^yCyprodinil was applied to pear fruit and leaves at a concentration of 0.62 g·L⁻¹ by spraying to drip with a backpack sprayer 2 weeks before harvest.

^xMeans followed by the same letter are not significantly different according to the Waller-Duncan *k*-ratio *t* test when *k* = 100.

randomly selected from PARC employees were asked to rate 'Anjou' pears or 'Gala' apples respectively for intensity of fruit aroma on a 10-cm horizontal line scale. Containers held four fruit/treatment and fruit were tested in random order twice over a 2-d interval.

Statistical analysis

Analysis of decay control and properties of hexanal. Individual fruit were considered a replicate for the inoculation experiments whereas box or bin lots were considered as replicates for the postharvest storage experiments. In the inoculation experiments, SE of the mean was calculated for each treatment and graphed; the same software was used for graphing hexanal concentration during fumigation of 'Jonagold' apples and analyzed using nonlinear regression (Prism 4, v. 4.03, GraphPad Software, San Diego). Inoculation study data were further analyzed using a three-way analysis of variance (ANOVA) with interactions between main effects identified with the general linear models (GLM) procedure (SAS Institute, Cary, N.C.), and means were separated using the least significant difference (LSD) test (*P* = 0.05). When a significant interaction occurred between two main effects, one-way ANOVA was performed and means were separated using the LSD test (*P* = 0.05). Results of postharvest decay storage trials, quality, and sensory tests were analyzed with a one-way ANOVA with the GLM procedure. In this case, means were separated using the Waller-Duncan *k*-ratio *t* test (*k* = 100). Values recorded as percentages were arcsin-transformed before analysis, although only the actual percent values are reported.

Results and Discussion

Decay control

Control of gray mold in inoculated 'Anjou' pears. Pears inoculated with *B. cinerea*, followed by fumigation with 2 or 4 mg·L⁻¹ hexanal, then wounding had almost no decay compared with high levels in the control at temperatures ranging from 5 to 20 °C (Fig. 1A). On the other hand, if fruit were inoculated, wounded, and then fumigated, control was less effective and decay increased at 5 and 20 °C when compared with nonfumigated fruit (Fig. 1B). Pretreatment of

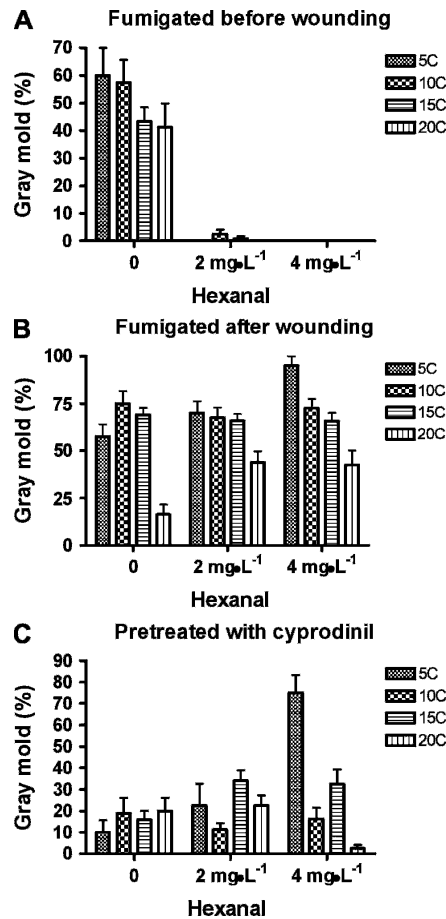


Fig. 1. (A–C) Effect of hexanal fumigation at 2 or 4 mg·L⁻¹ on 'Anjou' pears inoculated with *Botrytis cinerea* conidia (1 × 10⁴ CFU·mL⁻¹) and fumigated at 5, 10, 15, and 20 °C before wounding (A), after wounding (B), and after wounding and preharvest treatment with 0.62 g·L⁻¹ cyprodinil (C). Error bars represent SE of three replications.

pears before harvest with 0.62 g·L⁻¹ cyprodinil reduced decay by *B. cinerea* when compared with untreated fruit (Fig. 1A, C). The 0.31 g·L⁻¹ rate of cyprodinil had no effect on apple or pear decay in these inoculation or postharvest trials (data not shown). Fumigation with hexanal significantly increased gray mold in wounded fruit whereas cyprodinil reduced it (Table 3). There was no significant interaction between hexanal treatment and pretreatment with cyprodinil, although tem-

Table 3. Percent infected wounds in 'Anjou' pears or 'Gala' apples treated before harvest with cyprodinil and fumigated after wounding with hexanal at 5, 10, 15 and 20 °C.

Factor main effects	Percent infected wounds	
	Pear gray mold	Apple blue mold
Hexanal ^z (mg·L ⁻¹)		
0	35.3 c ^w	56.4 b
2	42.2 b	60.9 b
4	50.2 a	81.6 a
LSD (<i>P</i> = 0.05)	5.7	5.0
Cyprodinil ^y (g·L ⁻¹)		
0.00	61.7 a	97.6 a
0.62	23.4 b	35.0 b
LSD (<i>P</i> = 0.05)	4.7	4.0
Temperature ^x (°C)		
5	55.0 a	79.6 a
10	43.5 b	61.0 c
15	47.2 b	67.2 b
20	24.6 c	57.5 c
LSD (<i>P</i> = 0.05)	6.6	5.7
Significance		
Hexanal	<0.0001	<0.0001
Cyprodinil	<0.0001	<0.0001
H × C	0.9553	<0.0001
Temperature	<0.0001	<0.0001
H × T	<0.0001	<0.0001
C × T	<0.0001	<0.0001
H × C × T	0.0010	0.0006

^zEighty fruit per treatment were fumigated with hexanal with 2 mg·L⁻¹ for 24 h and 4 mg·L⁻¹ for 18 h.

^yOne hundred twenty fruit per treatment were treated with 0.62 g·L⁻¹ cyprodinil 2 weeks before harvest.

^xSixty fruit per treatment were fumigated in temperature-controlled rooms for 18 to 24 h at 5, 10, 15, and 20 °C.

^wMean separation within columns was according to Fisher's least significant difference (LSD) test at *P* = 0.05.

C, cyprodinil; H, hexanal; T, temperature.

perature strongly interacted with both hexanal and cyprodinil. Overall, wounded fruit treated with cyprodinil had the least decay when fumigated at 20 °C. Cyprodinil has previously been shown to reduce decay by *B. cinerea* in apples if applied about 3 weeks before harvest (Sholberg et al., 2003a). Hexanal fumigation prevented infection by spores of *B. cinerea* on the fruit surface, but was ineffective when spores were in wounds. Hexanal likely stimulated germination of spores in wounds at 5 °C, with correspondingly higher rates of decay at 4 mg·L⁻¹ than

2 mg·L⁻¹ (Table 3). Fallik et al. (1998) showed that the related compound (*E*)-2-hexenal would stimulate mycelial growth of *B. cinerea* when used at low concentrations. Apparently hexanal is similar and will stimulate growth of *B. cinerea* conidia in wounds. The effect of temperature was also apparent in these trials, with more than twice as much decay at 5 °C than at 20 °C for gray mold (Table 3). The lower amount of decay at 10, 15, and 20 °C was likely the result of a higher vapor pressure generated by hexanal at these temperatures. Gardini et al. (1997) showed that this was the case for *Aspergillus niger* between 15 and 35 °C, and our results indicate that this is likely the case for *B. cinerea* and *P. expansum*.

Control of postharvest decay in stored pears. Large quantities of ‘Anjou’ pears from Wenatchee, Wash., that were fumigated in 2001 with 4 mg·L⁻¹ hexanal for 48 h at 2 °C had less gray mold than the control fruit (Table 4). Blue mold was controlled in fruit from the second location, but increased in the first location from Washington. Possibly the low fumigation temperature and resulting low vapor pressure were responsible for poor efficacy against blue mold. Fruit storage temperature (-1 °C) after treatment did not affect antifungal activity of *trans*-2-hexenal against *P. expansum* in ‘Conference’ pears (Neri et al., 2006b). Fan et al. (2006) found with continuous application of hexanal that at 4 °C, hexanal vapor was low compared with ambient temperature, but fruit lesions incited by *P. expansum* were still substantially reduced, indicating that other factors must be involved. In 2002, preharvest treatment with cyprodinil followed by fumigation with 2 mg·L⁻¹ hexanal provided the highest number of mold-free fruit (Table 2). In 2003, hexanal fumigation reduced both gray and blue molds (Table 1). It appears that hexanal fumigation reduces natural infection of stored ‘Anjou’ pears, although it is difficult to detect control when disease levels are low.

Table 4. Percent decay or mold-free ‘Anjou’ pears fumigated with hexanal and stored for 7 months at 1 °C in 2001 to 2002.

Treatment and origin of fruit ^z	Gray mold	Blue mold	Mold free
Control			
Washington 1	62.8 a ^y	14.5 b	6.0 d
Hexanal			
Washington 1	7.2 bc	48.2 a	6.4 d
Control			
Washington 2	55.4 a	14.9 b	14.2 c
Hexanal			
Washington 2	15.6 b	4.0 c	46.6 a
Control British			
Columbia	5.8 bc	1.1 d	32.5 b
Hexanal British			
Columbia	5.2 c	2.4 cd	29.4 b

^zPears were obtained from two locations near Wenatchee, Wash., or one location in Summerland, B.C. The fruit was either left untreated or fumigated with 4 mg·L⁻¹ hexanal in bins (200-kg capacity/bin) in a 3.0 × 2.5 × 3.6-m room for 48 h at 2 °C.

^yMeans followed by the same letter in each column are not significantly different according to the Waller-Duncan *k*-ratio *t* test when *k* = 100.

Based on these results and previous research, fumigation should be conducted at 15 to 20 °C for 24 h as early as possible after the fruit are harvested and again before the pears are handled for packing. Additionally, an effective systemic preharvest treatment such as cyprodinil or pyrimethanil (Sholberg et al., 2005) should be used to protect wounds from decay because low rates of hexanal are ineffective on spores in wounds and could actually stimulate their growth (Fallik et al., 1998) (Table 3). Postharvest treatment with fludioxonil or pyrimethanil could also be used to prevent decay in wounds, although pyrimethanil should not be used if it is used as a preharvest treatment. Resistance management is very important with these new postharvest fungicides, and hexanal could provide another mode of action for use in alteration with fludioxonil and pyrimethanil.

Control of blue mold in inoculated ‘Gala’ apples. Fruit inoculated with *P. expansum*, followed by fumigation with 2 or 4 mg·L⁻¹ hexanal, then wounding had the least decay at 15 °C with 2 or 4 mg·L⁻¹ hexanal (Fig. 2A). The treatment was less effective on fruit

fumigated at 5 °C (Table 3), probably because of its low vapor pressure at this temperature (Gardini et al., 1997). As in the case of gray mold, there was significant interaction of blue mold between temperature and hexanal, and temperature and cyprodinil (0.62 g·L⁻¹). In addition there was a significant interaction between hexanal and cyprodinil with blue mold. Analysis of this interaction showed that pretreatment with cyprodinil significantly improved control of blue mold, but application of hexanal decreased the effect of cyprodinil in proportion to the concentration of hexanal. Thus the high rate of hexanal allowed 68% decay; the low rate, 21%; and the zero rate, 10%; in apples that had been pretreated with cyprodinil. In laboratory studies Neri et al. (2006c) showed that blue mold of apples and pears was controlled when fruit was wound-inoculated and fumigated continuously over a 24-h period with *trans*-2-hexenal at a concentration of 12.5 μL·L⁻¹. Continuous exposure to the fumigant at a constant rate appears necessary for control of *P. expansum* conidia in wounds. In this study, if fruit were inoculated, wounded, and then fumigated, there was no decay control (Fig. 2B). On the other hand, ‘Gala’ apples inoculated with *P. expansum* that had been treated before harvest with cyprodinil had 60% less decay than fruit that was not treated with cyprodinil (Fig. 2C; Table 3). Zhou et al. (2002) showed that cyprodinil controlled blue and gray molds by more than 90% at concentrations of 25 and 5 μg·mL⁻¹ respectively on apples. As previously found for *B. cinerea*, hexanal can also increase blue mold decay (Table 3). Analysis of the interaction of temperature with hexanal showed that the high rate of hexanal increased decay at 5, 10, and 15 °C when compared with the lower rate and the control. Neri et al. (2006a) also reported that low concentrations of the hexanal-related compound *trans*-2-hexenal enhanced conidial germination of *P. expansum*.

Control of postharvest decay in stored apples. Fruit pretreated with cyprodinil 0.62 g·L⁻¹ and fumigated with hexanal generally had less mold than fruit not treated with hexanal or cyprodinil, but did not differ significantly from the control (Table 2). ‘Gala’ apples treated with hexanal after storage for 5 months, then wounded to promote decay, developed ≈10% less decay than the control fruit whether previously fumigated or not fumigated at harvest (Table 5). Results for both ‘McIntosh’ and ‘Jonagold’ apples were not significantly different from nonfumigated fruit, probably because levels of contamination were very low. It appears that fumigation with hexanal after storage sterilizes the apple surface, reducing the chance that decay will occur by wounding. Similar results were obtained by fumigating apples with acetic acid (Sholberg et al., 2001). Fumigation with 10 μL·L⁻¹ acetic acid reduced the percentage of decayed puncture wounds in six apple cultivars to levels less than 10% after the fruit had been stored at 1 °C for several months.

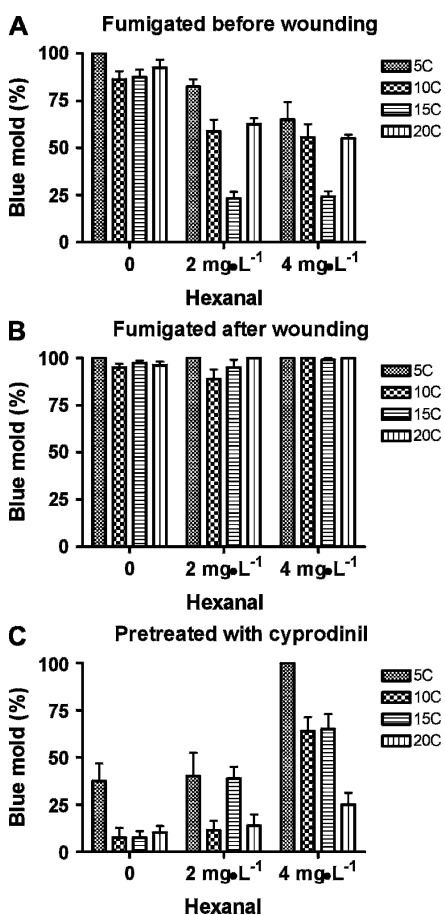


Fig. 2. (A–C) Effect of hexanal fumigation at 2 or 4 mg·L⁻¹ on ‘Gala’ apples inoculated with *Penicillium expansum* conidia (1 × 10⁶ CFU·mL⁻¹) and fumigated at 5, 10, 15, and 20 °C before wounding (A), after wounding (B), and after wounding and preharvest treatment with 0.62 g·L⁻¹ cyprodinil (C). Error bars represent SE of three replications.

Table 5. Percent infection and decay of wounded 'Gala' apples stored in bins fumigated with hexanal before and after storage at 1 °C for 5 months in 2003 to 2004.

Treatment ^z	When fumigated	Infected wounds (%)	Decay (%)
Control	Not fumigated	29.8 a ^y	10.5 a
Hexanal	At harvest	26.8 a	10.8 a
Hexanal	At harvest and after storage	3.2 b	1.2 b
Hexanal	After storage	2.5 b	0.8 b

^zFruit in bins were immediately fumigated after harvest with 3 mg·L⁻¹ hexanal for 24 h in a 3.0 × 2.5 × 3.6-m room at 15 °C and stored in a 1 °C cold room for 5 months. After storage they were allowed to warm to 15 °C for 24 h and fumigated with 3 mg·L⁻¹ hexanal for 24 h. Subsamples of 10 fruit/replicate were aseptically wounded and placed at 20 °C for 1 week, when percent infected wounds was recorded.

^yMeans followed by the same letter in each column are not significantly different according to the Waller-Duncan *k*-ratio *t* test when *k* = 100.

Hexanal properties

Monitoring hexanal during fumigation.

Hexanal vaporized completely, as recorded by the gas chromatograph during fumigation, whether in small 23-L chambers, larger 1-m³ chambers, or in the 27-m³ room. During fumigation of 'Jonagold' apples on two different dates during the first 4 h, the decline in hexanal concentration was not significantly different at any of the time points except at the 0.5-h time point (Fig. 3). Analysis of the data for fumigations on 2 and 6 Oct. 2003 using nonlinear regression showed that third-order polynomial equations would describe the data, with *R*² values of 0.9762 and 0.9803 respectively. The equations for 2 and 6 Oct. respectively were $y = 2.934 - 0.2996x + 0.01343x^2 - 0.0002092x^3$ and $y = 3.064 - 0.3317x + 0.01655x^2 - 0.0003005x^3$, where *y* is milligrams per liter of hexanal and *x* is hours.

Effect of hexanal on fruit quality. Fruit quality in 'Anjou' pears as indicated by firmness, pH, soluble solids, or titratable acidity was not significantly different among any of the treatments shortly after harvest on 1 Oct. 2003 (Table 6). After the fruit had been stored for 83 d, the fruit firmness in the control fruit and noncooled cyprodinil-

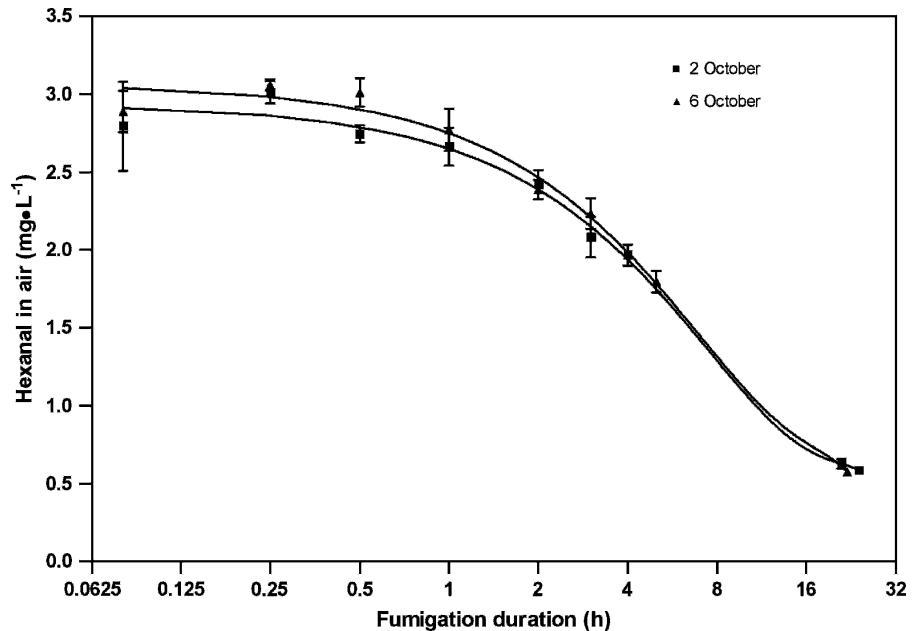


Fig. 3. Effect of time (in hours) on concentration of hexanal (in milligrams per liter) in air when 'Jonagold' apples occupying a volume of 0.38 m³ were fumigated in a 27-m³ room with 3 mg·L⁻¹ hexanal for 24 h on 2 and 6 Oct. 2003. Error bars represent sd of four replications.

treated fruit was lower than any of the other treatments, indicating that fumigation with hexanal or immediately cooling the fruit could improve fruit firmness. However, this trend did not last, and after 112 d, none of the treatments were significantly different from one another.

Hexanal-fumigated 'Anjou' pears and 'Gala' apples had fruitier aromas than non-fumigated pears (*Pr* > *F* = 0.0002) or apples. The apple aroma did not develop until the second day, when apples treated with 2 mg·L⁻¹ hexanal for 24 h had a significantly fruitier aroma (*Pr* > *F* = 0.0010) than either non-fumigated 'Gala' apples or those fumigated for 18 h with 4 mg·L⁻¹ hexanal. This result also continued into the third day of rating by the judges (*Pr* > *F* = 0.0002). Song et al. (1998) found that hexanal treatment stimulated aroma volatile production in 'Jonagold' and 'Golden Delicious' apple slices, with hexanal actively converted into the aroma volatiles hexanol and hexylacetate after 20 to

30 h of treatment. Fruit of a number of important plant species use C₆-aldehydes as precursors to aromas (Song et al., 1996). Our results with sensory evaluation of both 'Gala' apples and 'Anjou' pears support this finding for hexanal applied immediately after harvest.

Damage of fruit by hexanal fumigation. Hexanal concentrations more than 8 mg·L⁻¹ were phytotoxic to 'Anjou' pears, causing black discoloration over the entire fruit surface (Table 7). In 'Gala' and 'Golden Delicious' apples, hexanal used at 12 mg·L⁻¹ for 48 h at 1 °C or 2 mg·L⁻¹ for 48 h at 20 °C was also phytotoxic and caused a scaldlike discoloration of the fruit. 'Red Delicious' apples were resistant to damage by hexanal and were only damaged by 15 mg·L⁻¹ for 48 h at 1 °C or 4 mg·L⁻¹ for 48 h at 20 °C. Three milligrams/liter of hexanal for 24 h at 20 °C did not damage apples or pears, although 4 mg·L⁻¹ for 18 h damaged 'Golden Delicious' apples. Caccioni et al. (1995) found that hexanal at

Table 6. Effect of cooling, preharvest treatment with cyprodinil, and fumigation with hexanal on 'Anjou' pear quality after 0, 83, and 112 d storage at 1 °C.

Treatment	0 d				83 d				112 d
	Firm. ^z (kg)	pH	°Brix ^y	TA ^x (mg·L ⁻¹)	Firm. (kg)	pH	°Brix	TA (Mg·L ⁻¹)	Firm. (kg)
Cooled fruit ^w	5.2 a ^v	3.8 a	12.3 a	8.3 a	4.8 a	3.4 ab	12.6 a	7.0 a	4.7 a
Cyprodinil + cooled fruit ^v	5.1 a	3.6 a	11.9 a	7.7 a	4.8 a	3.5 a	11.6 c	5.8 a	4.0 a
Control ^u	5.2 a	3.7 a	13.2 a	8.5 a	2.3 b	3.3 b	12.5 ab	6.7 a	3.6 a
Cyprodinil + Hexanal ^l	5.1 a	3.7 a	13.1 a	8.7 a	4.1 a	3.5 a	11.8 bc	6.4 a	3.7 a
Cyprodinil	5.1 a	3.6 a	12.4 a	7.9 a	2.8 b	3.4 ab	12.4 ab	7.1 a	3.9 a
Hexanal	5.5 a	3.8 a	13.1 a	8.9 a	4.8 a	3.6 a	12.4 ab	6.3 a	4.7 a

^zFruit firmness (Firm.) measured with a Magness-Taylor penetrometer (11.1-mm tip) in kilograms.

^ySoluble solids measured with a refractometer.

^xTitratable acidity (TA) as determined by titration (pH, 8.1).

^wCooled pears were immediately placed in a 1 °C cold room after picking.

^vCyprodinil at a concentration of 0.62 g·L⁻¹ was applied to pear fruit and leaves by spraying to drip with a backpack sprayer 2 weeks before harvest.

^uControl pears were placed at 15 °C for 24 h and then placed in a 1 °C cold room after picking.

^lFruit was fumigated in bins (400-kg capacity) in a 3.0 × 2.5 × 3.6-m room for 24 h at 3 mg·L⁻¹ hexanal at 15 °C.

^vMeans followed by the same letter are not significantly different according to the Waller-Duncan *k*-ratio *t* test when *k* = 100.

Table 7. Phytotoxicity present in pome fruit treated with hexanal at different rates and temperatures.

Cultivar ^z	Fumigation at 1 °C				Fumigation at 20 °C				
	48 h, 4 mg·L ⁻¹	48 h, 8 mg·L ⁻¹	48 h, 12 mg·L ⁻¹	48 h, 15 mg·L ⁻¹	48 h, 2 mg·L ⁻¹	48 h, 4 mg·L ⁻¹	24 h, 2 mg·L ⁻¹	24 h, 3 mg·L ⁻¹	18 h, 4 mg·L ⁻¹
Anjou	No	No	Yes	Yes	Yes	Yes	No	No	No
Gala	No	No	Yes	Yes	Yes	Yes	No	No	No
Golden	No	No	Yes	Yes	Yes	Yes	No	No	Yes
Red	No	No	No	Yes	No	Yes	No	No	No

^zObservations are based on fumigation trials conducted in 23-L chambers or in the 27-m³ room from 2001 to 2005.

rates more than 2500 ppm was phytotoxic on stone fruit, but effectively controlled *M. laxa* and *R. stolonifer* at rates that did not damage the fruit. In this study, large volumes of fruit were fumigated with 2 to 4 mg·L⁻¹ hexanal consistently during the 3 years of the study without causing fruit damage. Phytotoxicity was only experienced when fruit was fumigated at 1 to 2 °C for 48 h or when it was fumigated at warmer temperatures for more than 24 h. Only in 'Abate Fetel' pears did phytotoxic symptoms develop within 3 d of treatment with *trans*-2-hexenal of several apple and pear cultivars tested by Neri et al. (2006c). In contrast to hexanal, acetic acid vapor was much more phytotoxic to 'Anjou' pears, leaving very little room for error when applied at 2 °C (Sholberg et al., 2004).

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