Fumigation of Sweet Cherry (*Prunus avium* 'Bing') Fruit with Low Molecular Weight Aldehydes for Postharvest Decay Control

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ABSTRACT

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Bing sweet cherries were inoculated with conidia of *Penicillium expansum* and then fumigated with acetaldehyde, propanal, butanal, or pentanal vapors. Conidial germination was prevented at the higher concentrations of acetaldehyde, propanal, and butanal, but extensive stem browning and fruit phytotoxicity also occurred. Stem browning was induced at lower aldehyde concentrations than fruit phytotoxicity. Treatment combinations (concentration, exposure duration) were identified that minimized decay in the absence of fruit phytotoxicity, indicating a potential use of aldehyde fumigation for processing applications.

Additional keywords: fruit storage

The postharvest storage period of sweet cherries (*Prunus avium* (L.) L.) is limited by such factors as water loss, softening, development of surface pitting, stem browning, and decay (10). Sweet cherries are susceptible to decay caused by several pathogenic fungi, including *Penicillium expansum* Link, *Botrytis cinerea* Pers.:Fr., *Cladosporium herbarum* (Pers.:Fr.) Link, *Alternaria* sp., and *Rhizopus* sp. (4). Postharvest fungicides are used commercially to minimize decay incidence, but resistant strains of *Penicillium* and *Botrytis* have developed (20,23). Fungicide resistance, along with market and regulatory pressure to minimize the use of synthetic agrichemicals, has encouraged the search for alternative means of controlling postharvest decay.

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Tompkins (26) reported a reduction in germination of fungal spores following treatment with acetaldehyde vapors. This effect has also been observed following fumigation of spore suspensions of several common postharvest pathogens of fruits and vegetables (1,3,5,17). Fumigation with acetaldehyde resulted in inhibition of spore germination on strawberries (15,19), raspberries (18), and apples (24) inoculated with postharvest pathogens. Enhanced fruit quality of blueberries, tomatoes, pears (11), citrus (14), strawberries (15), and grapes (16) has been observed following fumigation with acetaldehyde vapors. Acetaldehyde and a number of other aliphatic aldehydes are produced by sweet cherry cv. Bing fruit during development and ripening (7), and these compounds contribute to sweet cherry flavor and aroma (22). The objective of this study was to evaluate several aliphatic aldehydes for efficacy against pathogenic fungi in inoculated Bing sweet cherries.

MATERIALS AND METHODS

Aliphatic aldehydes—C_2 to C_{10}, molecular weights: acetaldehyde, 44; propanal, 58; butanal, 72; pentanal, 86;

Table 1. In vitro growth of mycelium of five fungal species[y] incubated at 18 C for 5 days after exposure to aldehyde vapor

Aldehyde	1 Hour					2 Hours					4 Hours				
	A	B	C	P	R	A	B	C	P	R	A	B	C	P	R
Acetaldehyde	−[z]	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Propanal	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−
Butanal	+	+	+	+	+	−	+	−	+	+	−	−	−	−	−
Pentanal	+	+	+	+	+	+	+	−	+	+	−	−	−	−	−
Hexanal	+	+	+	+	+	+	+	−	+	+	−	−	−	−	−
Heptanal	+	+	+	+	+	+	+	+	+	+	−	+	−	+	+
Octanal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nonanal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Decanal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

[y] A = *Alternaria* sp., B = *Botrytis cinerea*, C = *Cladosporium herbarum*, P = *Penicillium expansum*, R = *Rhizopus* sp.
[z] + = Mycelial growth observed, − = no mycelial growth observed. All treatments suppressing growth of mycelium also inhibited germination of conidia.

hexanal, 100; heptanal, 114; octanal, 128; nonanal, 142; decanal, 156—normally produced by sweet cherries (6) were evaluated. *Alternaria* sp., *B. cinerea, C. herbarum, P. expansum*, and *Rhizopus* sp. were previously isolated from sweet cherry fruit. Conidial and sporangiospore suspensions at 1.25×10^4 conidia per milliliter were prepared in sterile deionized water from an isolate of each fungus, and 20 μl was pipetted into four wells of a 96-well plate. Four replicate plates were placed into 4-L glass jars, sufficient aldehyde to saturate the vapor phase was pipetted directly into each jar (500 μl of acetaldehyde, propanal, butanal, pentanal, or hexanal and 250 μl of heptanal, octanal, nonanal, or decanal), and each jar was sealed with a metal lid. Plates in a jar with no aldehyde were used as controls. Aldehydes were used separately, and exposure times were 1, 2, or 4 hr at 20 C. Following fumigation, 100 μl of apple juice was added to each well and the plates were incubated at 18 C. After 5 days, wells were rated visually for presence or absence of mycelial growth. Wells with no visible mycelium were also examined with an inverted microscope for conidial germination. Results were used to identify the most resistant fungal isolate and the most inhibitory aldehydes for fruit fumigation treatments.

Sweet cherries were harvested at commercial maturity from orchards near Wenatchee, Washington. Fruit were surface-disinfested by immersion in a 67 mM NaOCl solution for 60 sec, then rinsed with sterile deionized water and allowed to air-dry.

Prior to fumigation treatments, cherries were wounded (2 mm depth) twice with a 26-gauge tuberculin needle. Following wounding, a thin-layer chromatography sprayer was used to spray cherries to runoff with either a conidial suspension of *P. expansum* (1×10^4 cells per milliliter) or distilled water. The fruit was then air-dried for 30 min in a laminar flow hood before being treated by aldehyde fumigation.

Acetaldehyde, propanal, butanal, and pentanal were selected for evaluation on the basis of results of in vitro studies. Fumigation treatments were applied in 4-L glass jars. One tray each of inoculated and noninoculated cherries, 25 per tray, were placed into each jar along with a flask containing the aldehyde solution or a distilled water control. Jars were sealed with metal lids. Headspace aldehyde concentrations were established by varying the amount of aldehyde (0, 5, 10, 20, or 40 ml of acetaldehyde; 0, 5, 10, 15, or 20 ml of propanal; 0, 1.25, 2.5, 5, or 10 ml of butanal; and 0, 0.25, 0.5, 1, or 2 ml of pentanal) in distilled water (100 ml total) contained in an Erlenmeyer flask. Concentrations ranged from 0 $\mu l \cdot L^{-1}$ to saturation of the vapor phase. Fumigation duration was 0, 1, 2, 3, or 4 hr. Each aldehyde concentration and fumigation duration was replicated twice, and experiments were repeated once. All treatments were performed at 20 C.

Immediately before the jars were opened at the end of the treatment period, a 1-ml gas sample was collected and used for analysis of headspace aldehyde concentration. All gas chromatographic analyses were conducted with a Hewlett-Packard 5880 GC equipped with a packed column (Porapak Q, 80–100 mesh, 30 cm long, 0.32 cm i.d.) and a flame ionization detector. Flow rates for N_2 carrier, H_2, and air were 20, 20, and 250 $ml \cdot min^{-1}$, respectively. Column temperatures were 120 C for acetaldehyde, 160 C for propanal, 190 C for butanal, and 220 C for pentanal.

After removal of fruit from the jars, 10 fruit (five noninoculated and five inoculated) were enclosed in 1-L glass jars for 1 hr, and fruit aldehyde accumulation during the fumigation treatments was estimated by measuring headspace

Table 2. Analysis of variance for aldehyde, concentration, and fumigation duration on incidence of decay (caused by *Penicillium expansum*), stem browning, and fruit phytotoxicity in Bing sweet cherries

Source	df	Decay		Stem browning		Fruit phytotoxicity	
		MS	$P > F$	MS	$P > F$	MS	$P > F$
Aldehyde (A)	3	6,278.41	0.0001	19,859.57	0.0001	54.56	0.0001
Concentration (C)	4	4,780.35	0.0001	86,515.02	0.0001	116.48	0.0001
Duration (D)	4	380.28	0.1260	22,864.71	0.0001	50.66	0.0001
A × C	12	685.40	0.0001	4,684.86	0.0001	14.74	0.0001
A × D	9	54.31	0.9809	1,753.00	0.0001	10.90	0.0001
C × D	12	156.39	0.6586	2,011.17	0.0001	9.11	0.0001
A × C × D	36	163.67	0.7458	1,847.26	0.0001	4.80	0.0001
Error	239	197.48		310.53		267.59	

Table 3. Incidence of decay (caused by *Penicillium expansum*), stem browning, and fruit phytotoxicity after aldehyde fumigation of Bing sweet cherries

Aldehyde[y]	Decay (%)	Stem browning (%)	Fruit phytotoxicity (%)
Acetaldehyde	2.7 a[z]	89.8 a	40.3 a
Propanal	14.5 b	62.9 b	32.8 a
Butanal	17.0 b	50.8 bc	16.2 b
Pentanal	28.8 c	41.2 c	3.2 c
Control	27.6 c	0	0

[y] Number of observations for each aldehyde was 64 for decay and 128 for stem browning and fruit phytotoxicity.
[z] Mean separation at the 5% level by Tukey's HSD; 0 = values not included in mean comparison.

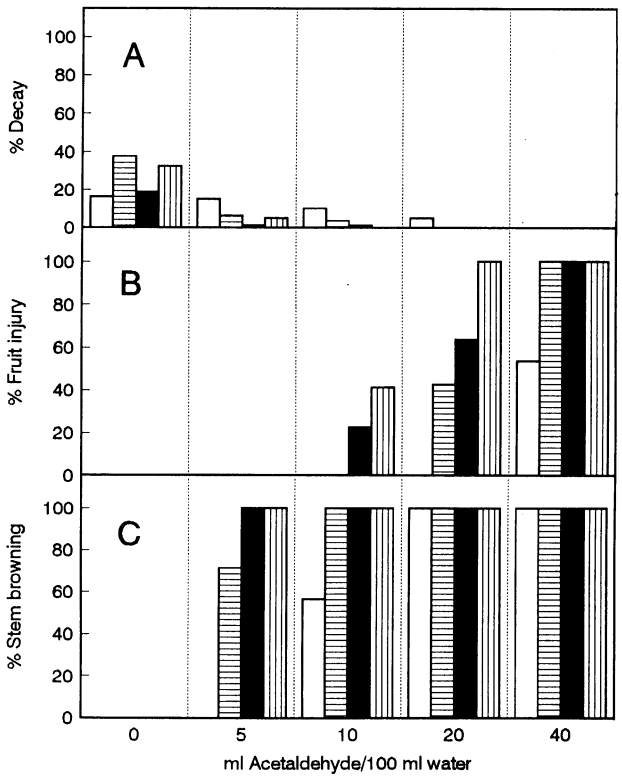

Fig. 1. Incidence of **(A)** fruit decay, **(B)** fruit injury, and **(C)** stem browning in Bing sweet cherries that were fumigated with acetaldehyde for 1 hr □, 2 hr ▤, 3 hr ■, or 4 hr ▥, then stored at 20 C for 7 days.

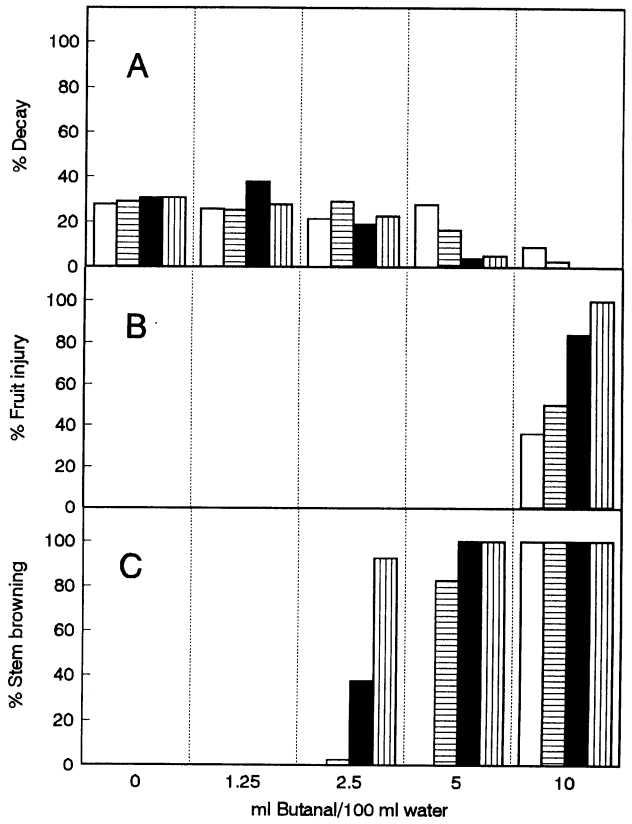

Fig. 2. Incidence of **(A)** fruit decay, **(B)** fruit injury, and **(C)** stem browning in Bing sweet cherries that were fumigated with butanal for 1 hr □, 2 hr ▤, 3 hr ■, or 4 hr □, then stored at 20 C for 7 days.

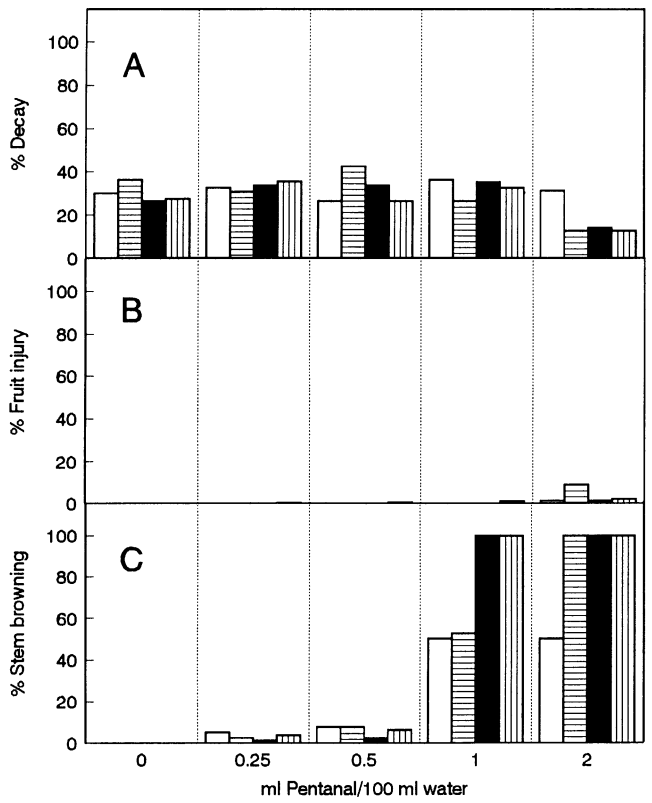

Fig. 3. Incidence of **(A)** fruit decay, **(B)** fruit injury, and **(C)** stem browning in Bing sweet cherries that were fumigated with pentanal for 1 hr □, 2 hr ▤, 3 hr ■, or 4 hr ▥, then stored at 20 C for 7 days.

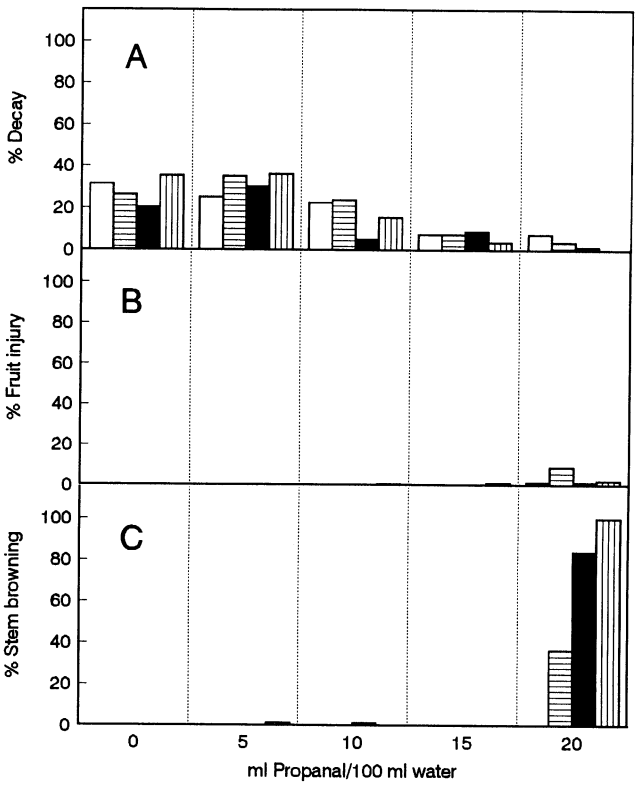

Fig. 4. Incidence of **(A)** fruit decay, **(B)** fruit injury, and **(C)** stem browning in Bing sweet cherries that were fumigated with propanal for 1 hr □, 2 hr ▤, 3 hr ■, or 4 hr ▥, then stored at 20 C for 7 days.

aldehyde concentration. The remaining fruit (20 controls, 20 inoculated with *P. expansum*) were placed on surface-sterilized Styrofoam trays and held at 20 C in moist chambers for 7 days, then evaluated for evidence of decay and the occurrence of stem browning or flesh necrosis. Hartley's *F*-max test of homogeneity of variance indicated that results from both experiments were similar. Therefore, data were pooled and analyzed as percent fruit developing decay, stem browning, or flesh necrosis. Values were arcsine transformed and subjected to analysis of variance and regression analysis (21).

RESULTS AND DISCUSSION

Inhibition of conidial germination and mycelial growth in apple juice was directly related to duration of fumigation (Table 1). The degree of inhibition decreased with increasing aldehyde molecular weight. Treatments resulting in no visible mycelial growth also prevented germination of conidia. *B. cinerea, P. expansum*, and *Rhizopus* sp. were the least affected by fumigation treatments. *P. expansum* was selected for fruit inoculations because it is a major postharvest pathogen of sweet cherry in the Pacific Northwest.

The incidence of decay caused by *P. expansum* in inoculated fruit was significantly impacted by aldehyde identity and concentration and by the interaction of those two factors (Table 2). Duration of fumigation had no significant effect on decay. Incidence of decay caused by *P. expansum* was relatively low in the inoculated control (Table 3; Figs. 1A, 2A, 3A, and 4A), possibly because iprodione (Rovral 4F) had been applied in the orchard. Decay incidence of inoculated sweet cherries was significantly ($P \leq 0.05$) reduced by fumigation with acetaldehyde, propanal, or butanal (Table 3). As observed after conidial fumigations, control of decay decreased as aldehyde molecular weight increased. Decline in aldehyde vapor pressure and water solubility with increase in molecular weight (2) resulted in lower headspace concentrations in the treatment jars (Fig. 5) and less accumulation of aldehydes in the fruit (Fig. 6). The incidence of decay decreased as the concentration of acetaldehyde, propanal, or butanal increased (Table 4; Figs. 1A, 2A, and 3A). Decay control was not significantly related to duration of fumigation in these tests (Tables 2 and 4). This may be due to the relatively slow increase in fruit aldehyde concentration over the exposure period for the lower aldehyde concentrations (Fig. 6). No significant aldehyde × fumigation duration, concentration × fumigation duration, or aldehyde × concentration × fumigation duration interactions were observed for decay control (Table 2). All factors and factor interactions did result in significant effects on stem browning and fruit phytotoxicity (Table 2).

The incidence of fruit phytotoxicity was inversely related to the molecular weight of the aldehyde (Table 3), and phytoxicity increased significantly with concentration for all aldehydes evaluated (Table 4; Figs. 1B, 2B, 3B, and 4B). Aldehyde concentrations necessary to induce fruit phytotoxicity were higher than those that resulted in stem browning, whereas significant phytotoxic effects with increased exposure time were observed only for acetaldehyde and propanal. Stem condition is a critical component of sweet cherry quality. Because stems lose water readily (10), maintaining a fresh, green stem appear-

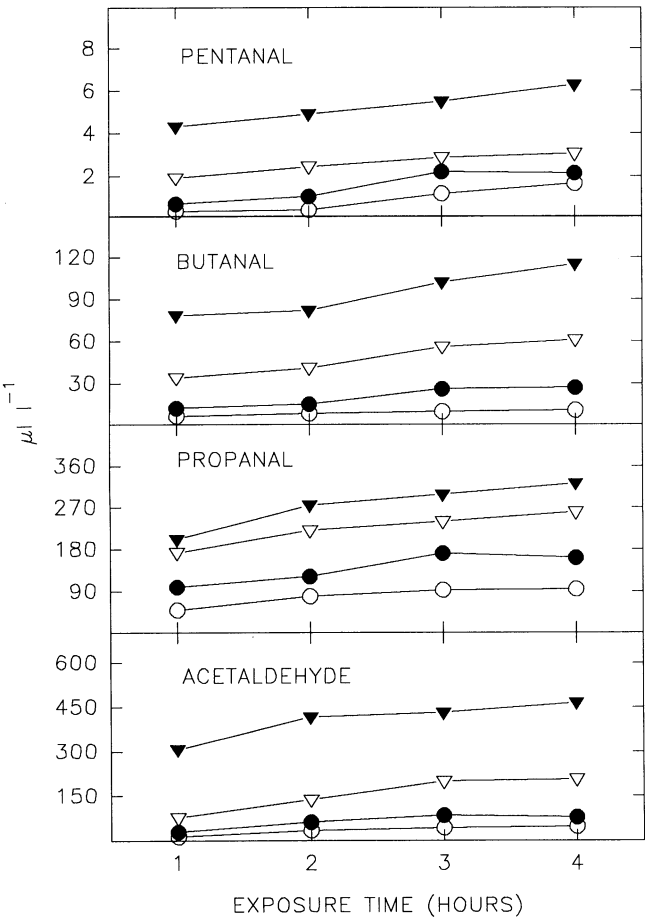

Fig. 5. Headspace aldehyde concentrations in treatment jars; samples were collected immediately before the jars were opened at the end of each fumigation treatment. Milliliters of aldehyde in 100 ml of H_2O: for pentanal, 0.25 ○, 0.5 ●, 1 ▽, 2 ▼; for butanal, 1.25 ○, 2.5 ●, 5 ▽, 10 ▼; for propanal, 5 ○, 10 ●, 15 ▽, 20 ▼; and for acetaldehyde, 5 ○, 10 ●, 20 ▽, 40 ▼.

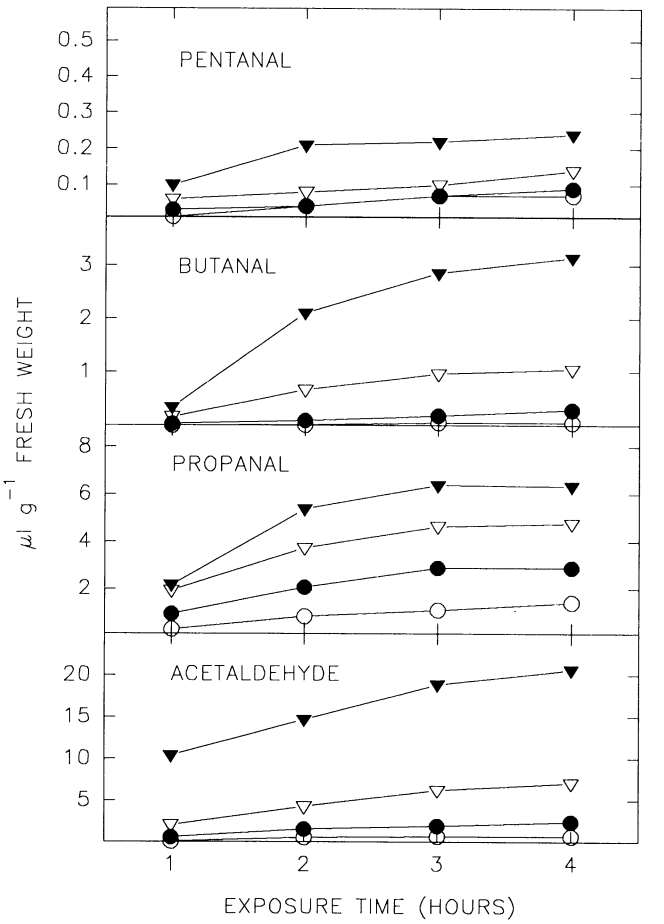

Fig. 6. Sweet cherry tissue aldehyde concentrations after fumigation treatments; concentrations were estimated from headspace samples collected from 1-L jars containing 10 fruit. Milliliters of aldehyde in 100 ml of H_2O: for pentanal, 0.25 ○, 0.5 ●, 1 ▽, 2 ▼; for butanal, 1.25 ○, 2.5 ●, 5 ▽, 10 ▼; for propanal, 5 ○, 10 ●, 15 ▽, 20 ▼; and for acetaldehyde, 5 ○, 10 ●, 20 ▽, 40 ▼.

Table 4. Coefficients of determination (R^2) and probability values for control of decay (caused by *Penicillium expansum*), stem browning, and fruit phytotoxicity in inoculated Bing sweet cherries after aldehyde fumigation[y]

Condition		Probability[z]	
Aldehyde	R^2	Concentration	Exposure time
Decay			
Acetaldehyde	0.27	0.0001	0.32
Propanal	0.51	0.0001	0.42
Butanal	0.33	0.0001	0.08
Pentanal	0.04	0.10	0.45
Stem browning			
Acetaldehyde	0.60	0.0001	0.001
Propanal	0.68	0.0001	0.0001
Butanal	072	0.0001	0.0001
Pentanal	0.54	0.0001	0.04
Fruit phytotoxicity			
Acetaldehyde	0.54	0.0001	0.0001
Propanal	0.62	0.0001	0.0001
Butanal	0.34	0.0001	0.095
Pentanal	0.12	0.0007	0.085

[y] Fruit were stored for 7 days at 20 C after fumigation treatments.
[z] Probability of a greater value due to chance alone.

ance is difficult during sweet cherry storage and handling. Treatments resulting in the most effective control of decay also resulted in the greatest incidence of stem browning (Table 3; Figs. 1C, 2C, and 3C). Stem browning increased significantly with fumigation duration for all aldehydes used (Table 4).

Phytotoxicity resulting from acetaldehyde vapors has been observed for lettuce (25), apples (24), and strawberries (19). Aldehyde vapor concentrations preventing growth of wheat fungal pathogens also resulted in reduced germination of the seeds (9). Toxic effects of acetaldehyde in solution were reported for carrot cell cultures (12). Acetaldehyde has been reported to inactivate ribonuclease (8) and to bind to other proteins (6,13), but the mechanism of aldehyde toxicity to fungal spores and plant tissue is unknown.

Although several aldehyde concentrations (particularly of acetaldehyde and propanal) effectively controlled decay caused by *P. expansum*, they also induced excessive stem browning. This would limit commercial use of this technique for sweet cherries sold on the fresh market. Some aldehyde concentrations effectively controlled decay (95% of fruit free of decay) without causing fruit phytotoxicity (Figs. 1, 2, and 3). Because stem quality of sweet cherries used for processing is less of a concern, aldehyde fumigation may present an alternative to use of fungicides for these fruit.

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