Enhanced Resistance to Side Rot in Pears Treated with Calcium Chloride During the Growing Season

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ABSTRACT

Sugar, D., Powers, K. A., and Hilton, R. J. 1991. Enhanced resistance to side rot in pears treated with calcium chloride during the growing season. Plant Dis. 75:212-214.

Incidence and severity of side rot in Bosc pears were reduced in fruit from trees treated with $CaCl_2$ sprays during the growing season. Trees were treated every 2 wk beginning in early July for a total of three applications of $CaCl_2$ at 0, 1.2, 3.6, or 6.0 g/L of calcium. Mature fruit were wound-inoculated postharvest with spore suspensions of *Phialophora malorum* at 0, 10, 10^2, 10^3, or 10^5 spores per milliliter. Lesion diameter was measured after 3 mo of storage at 0 C. A significant interaction between rate of $CaCl_2$ and spore concentration was observed. At 6.0 g/L of calcium, the mean area of decay was reduced at spore concentrations $>10^2$ per milliliter. At 3.6 g/L of calcium, lesion area reduction was significant at 10^2 and 10^5 spores per milliliter. At 1.2 g/L of calcium, lesion area reduction was significant only at 10^2 spores per milliliter. $CaCl_2$ treatment reduced incidence of side rot in naturally infected fruit in 3 yr of trial. $CaCl_2$ did not reduce incidence of decay of pear by *Penicillium expansum*. Calcium concentration in mature fruit peels was related to level of $CaCl_2$ treatment.

Increased calcium content in fruit and vegetable crops has been associated with decreased incidence of physiological disorders, improved storage life (9,13), and reduced severity of fungal and bacterial decay (2,4–6,7,11). Conway (4) reported reduced lesion size in apples pressure- or vacuum-infiltrated with calcium chloride ($CaCl_2$) solutions after wound inoculation with *Penicillium expansum* Link. Lesion size in apple was not reduced after fruit dips in $CaCl_2$ solutions at atmospheric pressure (4), nor was lesion size reduced by $CaCl_2$ preharvest sprays in peaches inoculated with *Monilinia fructicola* (G. Wint.) Honey (5). Postharvest pressure-infiltration of $CaCl_2$ solutions into peaches did, however, reduce lesion size of decay caused by *M. fructicola* (5). A proposed mechanism for fungal inhibition in $CaCl_2$-treated fruit suggests hindrance of fungal pectolytic enzyme activity by Ca^{++} ions associated with intercellular pectic substances in fruit (6,7). A similar calcium-related resistance mechanism was found by Bateman and Lumsden (1,2) to inhibit activity of polygalacturonase produced by *Rhizoctonia solani* Kühn in bean hypocotyls.

Oregon Agricultural Experiment Station Technical Paper 9288.

This project is supported in part by the Winter Pear Control Committee and the Fruit Growers' League of Jackson County, Oregon.

Accepted for publication 17 September 1990 (submitted for electronic processing).

Side rot, caused by *Phialophora malorum* (M.N. Kidd & Beaumont) McColloch (10), is an important postharvest disease of pear (*Pyrus communis* L.) in southern Oregon (16). *P. malorum* is not sensitive to fungicides currently registered for postharvest use on pear, and incidence of side rot may be exacerbated by fungicide application that reduces competition from other pathogens (18). Studies of the colonization of pear fruit in infested soil suggested that *P. malorum* is a relatively weak pathogen and may be particularly vulnerable to biological control or control via enhanced fruit resistance to decay (17).

The objective of this study was to evaluate the effect of treating pears with calcium chloride during the growing season on the incidence and severity of side rot during fruit storage. An abstract of a portion of this work has been published (19).

MATERIALS AND METHODS

Decay incidence. A commercial orchard of mature Bosc pear trees near Medford, Oregon, with a history of side rot problems during storage was used as a treatment site during 1984–1987. Each year, five different single-tree replicates were selected for each treatment in a completely randomized design. In 1984, $CaCl_2$ at 6.0 g/L of calcium was applied to each tree by handgun sprayer to runoff, with 0.16 ml/L of Ortho X-77 included as a surfactant. Check treatments included X-77 alone and untreated trees. In each subsequent year, treatments included $CaCl_2$ at 1.2, 3.6, and 6.0 g/L of calcium, plus surfactant only and untreated controls. Each treatment was applied every 2 wk for a total of three applications, beginning in the first week of July. At maturity, approximately 100 fruit were harvested from each tree. After cooling to 0 C, the fruit were floated through a commercial immersion dump tank solution containing sodium carbonate (specific gravity 1.05) and 0.3% sodium *o*-phenyl phenate (SOPP) in water. After a fresh water rinse, fruit were given a line-spray of benomyl (300 mg/L), were packed into polyethylene-lined fiberboard cartons (20 kg of fruit per carton), and were stored at −1 C. After 5 mo, the fruit were removed from storage and evaluated for incidence of side rot. Identity of the causal fungus was confirmed by isolation from lesion margins on potato-dextrose agar (PDA). The data for percentage of fruit infected by *P. malorum* were transformed to arcsine-square root percent, and means were separated using Fisher's protected least significant difference test.

The effectiveness of calcium chloride solution applied by concentrate sprayer under commercial conditions was evaluated in 1988 in large-scale plots at the Southern Oregon Experiment Station. Four 0.1-ha blocks of Bosc pear trees (four rows with four trees per row, spaced 7.6 × 7.6 m) were treated with $CaCl_2$ at 14.37 g/L, with 153.2 L applied per hectare. Four 0.1-ha blocks were left untreated as controls. Plots were treated every 2 wk beginning 11 July for a total of three applications. At maturity, approximately 200 fruit (total) were harvested from the interior four trees of each block. One-half of the fruit from each block were treated with a line-spray containing benomyl (300 mg/L) and a spore suspension of *P. malorum* and *Penicillium expansum* (10^5 spores per milliliter) prepared by washing the surfaces of 4-wk-old colonies on PDA. The remaining fruit from each block were treated with the spore suspension only. Fruit were placed in polyethylene-lined cartons, stored for 5 mo at −1 C, and evaluated as above.

Decay severity. In 1985 and 1987, an additional 100 fruit were harvested from each $CaCl_2$-treated and untreated tree. In the laboratory, fruit were surface-sterilized for 5 min in 0.5% NaOCl and stabilized on fiberboard trays. Each fruit was then wounded in four locations on the upper surface to a depth of 3 mm with a 6-mm-diameter finishing nail. A spore suspension of *P. malorum* was

prepared by washing the surface of 4-wk-old colonies on PDA with distilled water, and spore concentration was determined using a hemacytometer. A 0.05-ml drop of spore suspension containing 10, 10^2, 10^3, or 10^5 spores per milliliter was placed in each wound (100 wounds per spore concentration). Trays of inoculated fruit were covered with polyethylene bags and stored at 0 C. After 4 mo, lesion diameters were measured and areas of decay calculated.

Fruit calcium content. Calcium content was determined in fruit peels from handgun-applied plots in 1985. Ten fruit were harvested from each replicate tree in each treatment. The fruit were washed in mild detergent with hand rubbing, triple rinsed in distilled water, and allowed to air-dry before being peeled. A 1-cm-wide strip of peel from the stem end to the calyx was removed from each fruit. All peels were air-dried, then sent to the Oregon State University Plant Analysis Laboratory for ICP emission spectroscopy analysis.

RESULTS

Decay incidence. In all years of treatment except 1986, $CaCl_2$ application reduced incidence of side rot in pears during storage (Table 1). In 1986, side rot was not observed in any treatment. In 1985, all application rates of $CaCl_2$ significantly ($P = 0.05$) reduced decay as compared with the untreated control, but the three rates did not differ significantly from one another. In 1987, decay was reduced by $CaCl_2$ at 3.6 and 6.0 g/L of calcium by a significantly greater amount than at 1.2 g/L. $CaCl_2$ at 6.0 g/L of calcium did not significantly improve control over 3.6 g/L in any of the years of evaluation. Mild leaf phytotoxicity was observed in treatments with 3.6 g/L of calcium and consisted of blackening at leaf tips where droplets may have coalesced and concentrated during drying. At 6.0 g/L of calcium, leaf blackening extended around most of the leaf margin, in addition to leaf tips. No fruit phytotoxicity was observed, and fruit size was not adversely affected by treatment (*data not shown*).

$CaCl_2$ also reduced side rot incidence when applied by concentrate sprayer (Table 2). However, $CaCl_2$ treatment did not significantly reduce the incidence of decay by *Penicillium expansum*, the predominant fungus causing decay of fruit in treatments without benomyl.

Decay severity. A significant interaction was observed between inoculum concentration of *P. malorum* and level of $CaCl_2$ treatment in influencing the area of fruit decay lesions. The mean area of decay was not significantly different among rates of $CaCl_2$ at an inoculum concentration of 10 spores per milliliter (Fig. 1). At 10^2 spores per milliliter, all rates of $CaCl_2$ reduced lesion area significantly, whereas at 10^3 and 10^5 spores per milliliter, mean lesion area was significantly reduced at 6.0 g/L and at 3.6 and 6.0 g/L of calcium, respectively.

Fruit calcium content. The calcium concentration of fruit peels was increased by all $CaCl_2$ treatments, and peel calcium concentrations correlated well ($R^2 = 0.98$) with side rot incidence in 1985 (Fig. 2). Generally, the calcium content of peels increased with increasing concentration of $CaCl_2$ in the treatments.

DISCUSSION

Treatment with $CaCl_2$ during the growing season significantly enhanced the resistance of Bosc pears to side rot during fruit storage. This treatment appears to be advantageous to pear growers for the prevention of side rot, particularly since no effective fungicide alternatives are currently available. Treatment during the growing season makes use of common application equipment, in contrast to postharvest infiltration of $CaCl_2$ solutions using a pressure or vacuum apparatus. However, $CaCl_2$ spray treatment does not appear to significantly reduce incidence of decay by *Penicillium expansum*, control of which currently depends on fungicide application. Benomyl or thiabendazole has been commonly applied to pears before storage to prevent decay by *Penicillium expansum, Botrytis cinerea* Pers.:Fr., and *Pezicula malicorticis* (H. Jacks.) Nannf. (3). Resistance to benzimidazole fungicides in *Penicillium expansum* has been widely reported (3,13). The differential effect of $CaCl_2$ treatment, depending on the challenging fungus, may be due to differences in the nature or amount of pectolytic enzymes produced by each fungus. Effects of $CaCl_2$ without addition of benomyl on the development of *P. malorum* lesions were masked by the *Penicillium expansum* infection (Table 2). Benomyl application controlled *Penicillium expansum*, which allowed *P. malorum* to become the causal fungus of decay lesions (18). Although Conway (4) demonstrated that pressure- or vacuum-infiltration of apples with $CaCl_2$ reduced the area of decay lesions caused by *Penicillium expansum*, the effect on disease incidence was not reported. Conway et al (6) showed that high inoculum concentration may partially overcome tissue resistance to maceration by fungal enzymes, and inoculum concentration was a significant factor in the effect of $CaCl_2$ on lesion area reported herein. Therefore, it is possible that incidence of decay by *Penicillium expansum* in pears treated with $CaCl_2$ could have been reduced at lower inoculum concentrations. However, significant control of postharvest fungal pathogens of pear by $CaCl_2$ treatment may be limited to relatively weak pathogens such as *P. malorum, Alternaria* spp., and *Cladosporium herbarum* (Pers.:Fr.) Link (12).

Although no visible fruit injury resulted from our $CaCl_2$ treatments to Bosc pears, injury to d'Anjou pear fruit was reported after four summer applications at 1.7 g/L of $CaCl_2$ (14). Bosc is a naturally russeted pear cultivar, which may have masked fruit injury caused by high rates of $CaCl_2$ treatment. Use of $CaCl_2$ during the growing season may be limited to naturally russeted or otherwise $CaCl_2$-tolerant cultivars.

The concentrations of calcium found in fruit peel tissues are generally higher

Table 1. Effect of three summer applications of calcium chloride on incidence of side rot in Bosc pears

Season	Treatment[a]	Percent side rot[b]
1984–85	Untreated	6.2
	$CaCl_2$ 6.0 g/L Ca	0.8
	Surfactant only	6.8
	LSD ($P = 0.05$)	2.58
1985–86	Untreated	2.9
	$CaCl_2$ 1.2 g/L Ca	1.2
	$CaCl_2$ 3.6 g/L Ca	0.6
	$CaCl_2$ 6.0 g/L Ca	0.6
	Surfactant only	2.5
	LSD ($P = 0.05$)	1.31
1986–87	Untreated	0.0
	$CaCl_2$ 1.2 g/L Ca	0.0
	$CaCl_2$ 3.6 g/L Ca	0.0
	$CaCl_2$ 6.0 g/L Ca	0.0
	Surfactant only	0.0
1987–88	Untreated	10.1
	$CaCl_2$ 1.2 g/L Ca	7.2
	$CaCl_2$ 3.6 g/L Ca	4.2
	$CaCl_2$ 6.0 g/L Ca	3.2
	Surfactant only	8.8
	LSD ($P = 0.05$)	2.08

[a] All $CaCl_2$ treatments included 0.16 ml/L of Ortho X-77 as a surfactant.

[b] Values represent means of five replicate trees from each of which approximately 100 fruit were harvested. Decay was evaluated after 5 mo of storage at −1 C.

Table 2. Decay in Bosc pears treated with three summer applications of calcium chloride solutions by concentrate sprayer

Treatment[a]	Total decay (%)	Percent of total decay caused by *Phialophora malorum*	Percent of total decay caused by *Penicillium expansum*
Untreated	14.2	6.3	93.7
Untreated + benomyl	11.5	100.0	0.0
$CaCl_2$	10.7	0.0	100.0
$CaCl_2$ + benomyl	4.5	100.0	0.0
LSD ($P = 0.05$)	5.6		

[a] Trees were treated with $CaCl_2$ solutions (14.37 g/L) at 153.2 L/ha in three biweekly applications beginning 11 July 1988. After harvest, fruit were treated with a line-sprayed spore suspension of *P. malorum* and *Penicillium expansum* (10^5 spores per milliliter) with or without benomyl (300 mg/L). Values represent means of four replicate orchard blocks.

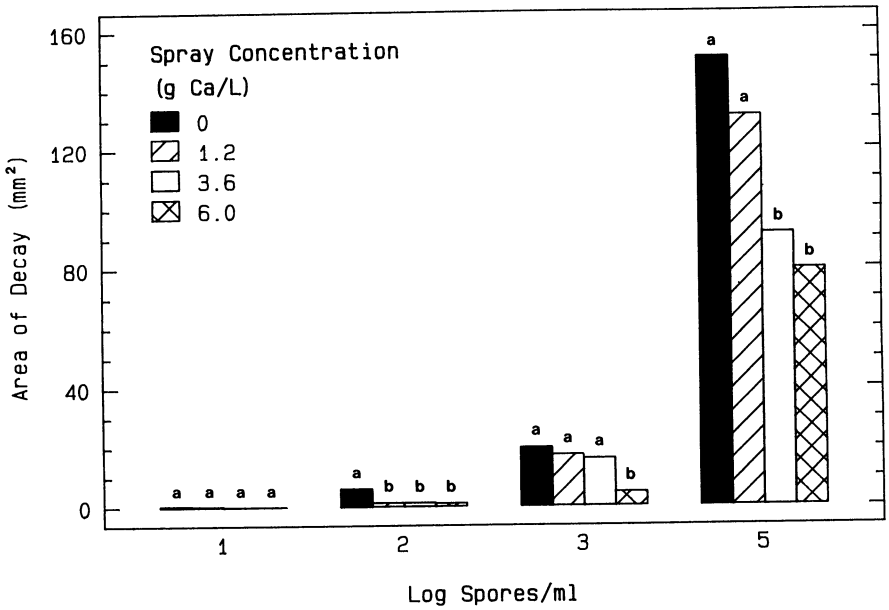

Fig. 1. Mean area of decay in Bosc pears treated with four concentrations of $CaCl_2$ during the growing season and inoculated postharvest with *Phialophora malorum* at various inoculum concentrations. Columns within each spore concentration level without the same small letter above are significantly different according to Fisher's LSD ($P = 0.05$).

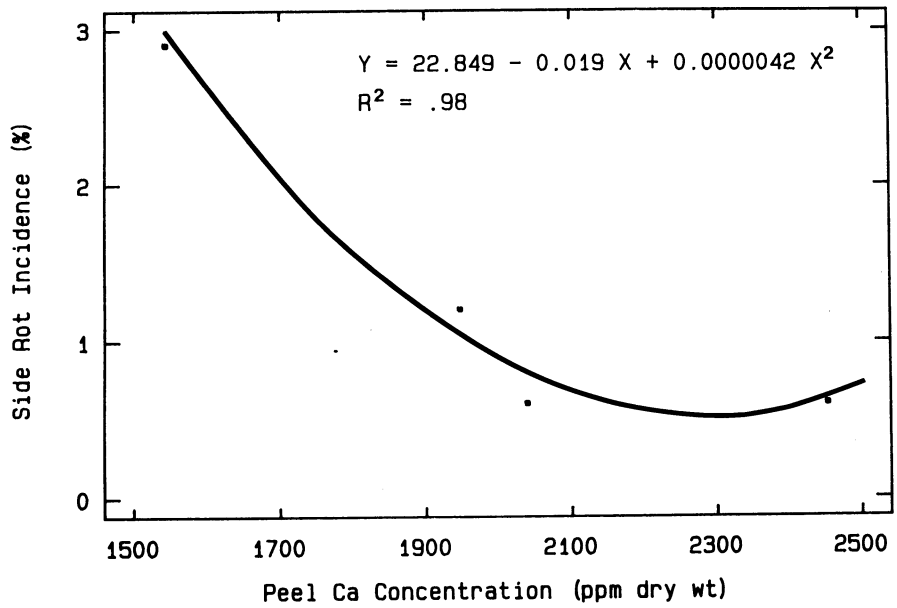

Fig. 2. Relationship between incidence of side rot in Bosc pears sprayed with $CaCl_2$ solutions during the growing season and peel calcium concentration. Data points indicate the mean values resulting from applications at (left to right) 0, 1.2, 3.6, and 6.0 g/L of calcium.

than the concentrations found in fruit cortical tissues (8,13–15). Despite differences in magnitude, calcium concentration in peel tissues has been shown to be strongly correlated with cortical values and may be a preferred tissue for analysis, since it is easier to collect and handle samples (8,15). Application of calcium salts to trees during the growing season has been shown to be an effective means of increasing fruit calcium for control of bitter pit of apple and the related disorder cork spot of pear (9). The effectiveness of calcium sprays generally increases with frequency of application and concentration of calcium salt (9).

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