Effect of Calcium Salts on Growth, Pectic Enzyme Activity, and Colonization of Peach Twigs by *Leucostoma persoonii*

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ABSTRACT

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The effects of several calcium salts on in vitro growth, pectic enzyme activity, and colonization of excised peach twigs by the peach canker fungus, *Leucostoma persoonii*, were investigated. Fungal growth was determined on potato-dextrose agar amended with various calcium salts. The greatest growth reduction (85%) was caused by calcium propionate, followed by calcium hydroxide (76%) and calcium silicate (73%). The fungicides captan, iprodione, and thiophanate-methyl completely inhibited fungal growth. Lesion length was reduced when excised peach twigs were wounded with a cork borer, dipped for 15 or 60 min in the various calcium solutions, and inoculated with *L. persoonii*. For 15-min dips, lesion length was reduced more than 70% by calcium silicate, iprodione, and calcium propionate. After 15 min, calcium in the bark was not greater than that in the distilled, deionized water control. When twig segments were dipped for 60 min, lesion length was reduced more than 70% by calcium acetate, calcium sulfate, calcium heptagluconate, calcium oxide, calcium succinate, calcium chloride, calcium hydroxide, and the fungicide iprodione. No lesions occurred when twigs were treated with calcium propionate, captan, and thiophanate-methyl. After 60 min, only twigs dipped in calcium sulfate showed significantly elevated levels of bark Ca^{2+}. Canker length and calcium content of bark were negatively correlated ($r = -0.26$, $P \leq 0.05$) after the 15-min treatment. No correlation was found with the 60-min dip, even though calcium content of the bark had increased significantly. When *L. persoonii* was grown on Ca^{2+}-amended media, pectin lyase activity was not reduced significantly by any treatment. Calcium oxide and calcium propionate, and calcium silicate treatments reduced polygalacturonase activity after 7 and 15 days, respectively.

Peach canker, caused by the fungi *Leucostoma persoonii* Höhn. (anamorph = *Leucocytospora leucostoma* (Sacc.) Höhn.) and *Leucostoma cincta* (Fr.:Fr.) Höhn. (anamorph = *Leucocytospora cincta* (Sacc.) Höhn.), continues to be a major limiting factor in peach (*Prunus persica* (L.) Batsch) production in the northern areas of North America. The pathogens initiate disease in wounds created by pruning, leaf abscission, freeze injury, and insect damage. The disease appears as perennial cankers on trunks, scaffold limbs, and branches, and causes crop losses due to reduction in bearing surface and premature tree death. All the currently grown peach cultivars are susceptible to these pathogens, and no known treatment prevents infection over the long term (4).

Histological studies of wounds and fungal infections of peach bark have demonstrated the importance of lignin and suberin in the cell walls of new tissues (3,6). Similar processes have been described for other stone fruit pathosystems, including the role of lignin in the resistance of almond to infections by *Phytophthora syringae* (13) and the correlation between suberin level in a peach tree's wound response and the relative field performance of several cultivars (3,6).

For many woody plant–pathogen interactions, wounding followed by a period of recovery before exposure to the pathogen precludes or reduces disease (5). Incidence and/or severity of infection depend on environmental conditions and the length of the recovery period. One approach to increasing disease resistance would be to accelerate the plant's wound-healing response, which occurs more slowly in some species or cultivars, or in individuals predisposed by their environment.

Calcium content of the host cell wall may be associated with increased resistance to pathogens (1,9–11). We previously demonstrated the effectiveness of calcium chloride applications in reducing incidence and severity of peach canker disease in the laboratory (7). The mechanism(s) by which calcium chloride reduced the disease was not clear in that earlier investigation, although increased depositions of lignin and suberin apparently were not involved. The objectives of this study were to examine the effects of several calcium salts on in vitro growth, pectic enzyme activity, and colonization of excised peach twigs by *L. persoonii*.

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MATERIALS AND METHODS

Effect of calcium salts and fungicides on fungal growth in culture. Analytical grade calcium salts used in this study included calcium acetate, calcium chloride, calcium formate, calcium gluconate, calcium heptagluconate, calcium hydroxide, calcium lactate, calcium nitrate, calcium oxide, calcium pantothenate, calcium phosphate (dibasic), calcium phosphate (tribasic), calcium pyrophosphate, calcium succinate, calcium propionate, calcium tartrate, calcium silicate, calcium silicide, and calcium sulfate. All salts were prepared in sterile deionized, distilled water to yield final concentrations of 600 mg/L Ca^{2+}. Solutions were incorporated into warm (45–55 C), autoclaved 2% potato-dextrose agar (PDA) and poured into 9-cm-diameter petri dishes. The pH of the supplemented medium was determined in preparations that were not autoclaved. The fungicides triadimefon (Bayleton 50WP, 36.9 μg a.i./ml), captan (Captan 50WP, 1.2 mg a.i./ml), flusilazole (Nustar 40DF, 14.8 μg a.i./ml), myclobutanil (Nova 40W, 59.1 μg a.i./ml), fenbuconazole (Indar 2EC, 52.8 μg a.i./ml), iprodione (Rovral 50WP, 1.2 mg a.i./ml), fenarimol (Rubigan 1E, 27.0 μg a.i./ml), and thiophanate-methyl (Topsin-M 85WDG, 0.38 mg a.i./ml) were prepared as stock suspensions and incorporated into warm PDA before pouring. Each dish received a 5-mm-diameter agar disk taken from an actively growing culture of *L. persoonii* isolated from peach and thought to be representative of the local population. Dishes were incubated in the dark at 20 ± 2 C and were evaluated after 48, 96, and 144 hr. Growth was assessed by measuring colony diameter on each dish at two locations at right angles to each other. The experiment was performed twice with four replicate dishes per treatment. In an additional experiment, the fungus was grown in stationary flasks containing 2% potato-dextrose broth amended or nonamended with calcium oxide, calcium hydroxide, calcium propionate, or calcium silicate. Growth was assessed after 7 and 14 days by determining the dry weight of the mycelium. The experiment was performed twice with three replicate flasks per treatment.

Data were analyzed with analysis of variance, and means were separated with the Waller-Duncan Bayesian k-ratio t test (21).

Colonization of calcium-treated peach twigs by *L. persoonii*. Peach twig segments were prepared by removing approximately 12 cm from the apical and basal ends of 1-yr-old dormant shoots and cutting the remaining shoot into 12-cm-long segments. Segments were surface-disinfested with 70% ethanol, and each was wounded through the bark to the xylem with a 5-mm-diameter cork borer. Twenty replicate segments were prepared for each of the calcium salts (prepared as solutions to yield Ca^{2+} at 400 mg/L) or fungicides. Of the 20 segments, half were immersed for 15 min and half for 60 min in solutions or suspensions, and then were kept overnight in autoclaved mason jars with lids. Segments were inoculated the next day by placing a 5-mm-diameter mycelial disk of *L. persoonii* into the wound, which was then sealed with cellophane tape. Controls (segments dipped for 15 or 60 min in sterile deionized, distilled water) were inoculated similarly. Inoculated segments were incubated for 10 days at room temperature in closed, autoclaved mason jars containing 10 ml of sterile distilled water. Lesion length was measured as the extent of visible bark necrosis above and below the point of inoculation. The experiment was performed twice, and data were analyzed as described above.

Calcium accumulation in bark. Twig segments from the above experiment were analyzed for calcium content of bark tissue (milligrams of Ca^{2+} per gram of dry weight). Nine of the 10 replicates per treatment were subdivided into three groups of three segments, and the three segments were pooled to serve as one replicate (for a total of three replicates per treatment). Xylem tissue was separated (and discarded) from the phloem, cortical, and periderm tissues (bark), which then were ashed at 425 C for 8 hr in a muffle furnace. The ashed samples were dissolved in 1 ml of concentrated HCl and diluted with 19 ml of double-deionized water and 0.5 ml of 4% $CsCl_2$. Samples were analyzed for total Ca^{2+} with atomic absorption spectrophotometry (Perkin-Elmer Model 4000) (15). Calcium content was determined at the site of inoculation (including diseased tissues for most treatments) and at the noninfected apical portion of the segments. Pearson's correlation coefficient was calculated to assess the relationship between calcium content and canker length (21).

Effect of selected calcium salts on fungal pectin lyase and polygalacturonase activity. Five-millimeter-diameter plugs of *L. persoonii,* grown on PDA, were transferred to stationary 250-ml flasks containing 50 ml of 2% potato-dextrose broth that was nonamended or amended with one of four different calcium salts (calcium hydroxide, calcium oxide, calcium propionate, or calcium silicate) to yield a final Ca^{2+} concentration of 600 mg/L. The pH value in each flask was adjusted to 5.8 with HCl or NaOH after adding the calcium salts. Flasks were incubated for 7 and 15 days at 20 ± 2 C under constant fluorescent light, after which the filtrates were collected and analyzed immediately for pectin lyase (PNL) and polygalacturonase (PG) activity (22). Enzyme activity was determined with a Beckman spectrophotometer at 510 nm (PG) and 550 nm (PNL), and expressed as 100× the increase in absorbance per hour per milliliter of broth per gram of dry weight of fungus mycelium. Activity of PNL was tested on citrus pectin at pH 8.5 in 0.05 M Tris-HCl buffer. PG activity was tested on sodium polypectate at pH 4.5 in 0.1 M acetate buffer. Each treatment had two replicates, and the entire experiment was conducted twice. Data were analyzed as described above.

RESULTS

Effect of calcium salts and fungicides on fungal growth in culture. Mycelial growth of *L. persoonii* on PDA amended with calcium salts was highly variable among the treatments. Growth was reduced relative to the control for most treatments after 2 and 4 days in culture (Table 1). However, after 6 days in culture, only the calcium treatments containing chloride, hydroxide, oxide, propionate, silicate, and succinate exhibited significant reductions in fungal growth. The greatest growth reduction was caused by calcium propionate (84.7%), followed by calcium hydroxide (76.2%) and calcium silicate (73.1%). The fungicides captan, iprodione, and thiophanate-methyl caused complete inhibition of fungal growth (Table 2). The sterol-inhibiting fungicides exhibited a range of activity, although none resulted in complete inhibition, and they were less effective than the aforementioned materials ($P \leq 0.05$). Fenarimol and flusilazole were similar and showed greater activity against *L. persoonii* than did myclobutanil, fenbuconazole, and triadimefon. The latter three fungicides were increasingly less effective and exhibited significant differences from each other (Table 2).

When *L. persoonii* was grown in liquid cultures amended with four selected calcium treatments, dry weight of mycelium was reduced significantly after 7 and 14 days for all treatments. Calcium hydroxide caused the least inhibition (33%) at 7 days. Greatest growth reduction occurred in broth amended with calcium propionate at 7 (83.2%) and 14 (83.1%) days. Intermediate between these two treatments were calcium oxide (70.4%) and calcium silicate (75.0%), which were similar.

Effect of calcium salts on calcium content and colonization of peach twigs by *L. persoonii*. When twig segments were dipped for 15 min in the various calcium and fungicide solutions or suspensions and then inoculated with *L. persoonii,* all treatments resulted in smaller lesions relative to the control (Table 3). Over 70% reduction in lesion length was

Table 1. Reduction in colony diameter (percent of control) of *Leucostoma persoonii* on potato-dextrose agar (PDA) amended with various calcium salts (600 ppm Ca^{2+}) after 2, 4, and 6 days

	Reduction in colony diameter (%)[x]		
Calcium salt (pH in PDA)	**2 days**	**4 days**	**6 days**
Acetate (5.8)	7.4 c–f[y,z]	18.0 hi	0.0 a
Acetate, hydrate (6.1)	19.7 g	14.9 d–f	0.0 a
Chloride (4.4)	45.7 j	36.9 j	15.9 c
Formate (4.9)	8.6 ef	10.3 c	0.0 a
Gluconate (4.8)	4.9 a–d	15.4 e–h	0.0 a
Heptagluconate (4.8)	38.3 i	14.9 e–g	0.0 a
Hydroxide (11.1)	80.2 l	75.4 m	76.2 f
Lactate (4.8)	6.2 b–e	13.8 de	0.0 a
Nitrate (4.2)	3.7 a–c	6.2 ab	0.0 a
Oxide (10.9)	58.0 k	62.6 k	70.9 d
Pantothenate (5.5)	9.9 ef	3.6 a	0.0 a
Phosphate, dibasic (5.0)	8.6 d–f	11.8 cd	0.0 a
Phosphate, tribasic (4.8)	19.8 g	6.7 b	0.0 a
Propionate (5.8)	100.0 m	80.0 n	84.7 g
Pyrophosphate (4.3)	9.9 f	16.9 f–h	0.0 a
Silicate (7.3)	80.2 l	68.2 l	73.1 e
Silicide (4.2)	1.2 a	6.2 ab	0.0 a
Succinate (6.0)	48.2 j	20.0 i	11.2 b
Sulfate (4.2)	32.1 h	10.8 c	0.0 a
Tartrate (4.3)	2.5 ab	17.4 g–i	0.0 a

[x]Percent reduction = [(diameter control − diameter treatment)/diameter control)] × 100.
[y]Each value is the mean of eight observations from the combined data of four replicates from two experiments.
[z]Different letters in columns denote significant differences according to the Waller-Duncan Bayesian k-ratio t test ($P \leq 0.01$).

observed with preexposure to calcium silicate (73.7%), iprodione (79.8%), and calcium propionate (84.1%). No lesions formed when twigs were treated with captan or thiophanate-methyl. Posttreatment calcium contents in twig bark were similar among the treatments, with the exception of calcium gluconate and calcium tartrate. Both had less Ca^{2+} than the control and nine other treatments (Table 3). After treatment and inoculation, only the bark exposed to calcium tartrate showed less Ca^{2+} than the control and 12 other treatments (Table 3). There was a low but statistically significant negative correlation between bark calcium content and lesion length ($r = -0.26$, $P \leq 0.05$), with smaller lesions associated with higher levels of bark Ca^{2+}. When data from treatments strongly inhibitory to fungal growth in culture (hydroxide, oxide, propionate, and silicate) were deleted from the correlation analysis, the r value dropped slightly and was no longer significant ($r = -0.23$, $P \leq 0.13$).

When twig segments were dipped for 60 min and then inoculated with *L. persoonii,* lesions were smaller than the control for all treatments (Table 4). Lesion length was reduced over 70% with preexposure to calcium acetate (72.9%), calcium sulfate (80.2%), calcium heptagluconate (82.4%), iprodione (84.9%), calcium oxide (87.1%), calcium succinate (90.1%), calcium chloride (94.8%), and calcium hydroxide (96.0%). No lesions formed when twigs were treated with calcium propionate, captan, or thiophanate-methyl. Posttreatment calcium content in twig bark was similar among treatments, except for calcium sulfate, in which more Ca^{2+} had accumulated than in the control and six other treatments (Table 4). After treatment and inoculation, Ca^{2+} content was not significantly different (Table 4). No correlation was observed between bark calcium content and lesion length following the 60-min dip treatments ($r = 0.0036$, $P \leq 0.98$).

Twigs dipped for 60 min had significantly greater calcium accumulation in bark than twigs dipped for 15 min, averaging an increase in Ca^{2+} of 6 mg/g in posttreatment bark and 5 mg/g in posttreatment, postinoculation bark ($P \leq 0.05$). Ca^{2+} content of posttreatment vs. posttreatment, postinoculation bark was similar (26.0 vs. 26.2 mg/g, respectively, $P \leq 0.05$). Treatment × dip time interaction was significant, due largely to increased calcium accumulations after 60 min in bark treated with calcium gluconate, calcium heptagluconate, calcium pantothenate, calcium phosphate dibasic, and calcium tartrate. For posttreatment, postinoculation bark tissue, Ca^{2+} levels were higher after 60 min of exposure to calcium gluconate, calcium lactate, calcium nitrate, calcium pantothenate, calcium phosphate dibasic, and calcium tartrate.

Effect of selected calcium salts on fungal pectin lyase and polygalacturonase activity. After 7 days, culture filtrates from flasks amended with calcium oxide and calcium propionate treatments had elevated PNL activity compared to the control, which had the lowest PNL activity. PNL activity from calcium hydroxide and calcium silicate treatments were similar to the control and were not significantly different from each other (Table 5). After 15 days, the calcium oxide treatment had the highest PNL activity, followed by and significantly different from calcium hydroxide and calcium propionate, all of which were significantly different from the control. Relative PNL activity in the calcium silicate treatment and the control were similar at 15 days.

Calcium oxide and calcium propionate reduced polygalacturonase activity at 7 days (Table 5), whereas PG activity for the calcium hydroxide and calcium silicate treatments were similar to the control. After 15 days, only calcium silicate exhibited reduced PG activity relative to the control. Calcium hydroxide had elevated PG activity relative to the control and all other treatments except calcium oxide.

Table 2. Reduction in colony diameter (percent of control) of *Leucostoma persoonii* after 5, 10, and 15 days on potato-dextrose agar (PDA) amended with various fungicides

	Reduction in colony diameter (%)[x]		
Fungicide (mg a.i./ml)	**5 days**	**10 days**	**15 days**
Captan (1.2)	100.0 a[y,z]	100.0 a	100.0 a
Fenarimol (0.027)	84.2 b	62.5 c	31.2 c
Fenbuconazole (0.053)	40.4 d	0 e	0 e
Flusilazole (0.015)	83.5 b	74.7 b	61.6 b
Iprodione (1.2)	100.0 a	100.0 a	100.0 a
Myclobutanil (0.059)	76.2 c	50.0 d	30.0 d
Thiophanate-methyl (0.38)	100.0 a	100.0 a	100.0 a
Triadimefon (0.037)	1.5 e	0 e	0 e

[x] Percent reduction = [(diameter control − diameter treatment)/diameter control)] × 100.
[y] Each value is the mean of eight observations from the combined data from two experiments.
[z] Different letters in columns denote significant differences according to the Waller-Duncan Bayesian k-ratio t test ($P \leq 0.05$).

Table 3. Effect of 15-min exposure to various calcium salts (400 ppm Ca^{2+}) on calcium content and colonization of excised peach twig segments by *Leucostoma persoonii* measured 10 days after inoculation

Treatment	**Lesion length (mm)[x]**	**Posttreatment Ca^{2+} (mg/g)[y]**	**Posttreatment postinoculation Ca^{2+} (mg/g)[y]**
Control	37.2 a[z]	25.5 a	23.8 a–d
Acetate	17.3 fg	20.2 ab	24.9 a–d
Chloride	11.6 jk	22.6 ab	25.6 a–d
Formate	23.9 de	25.4 a	24.0 a–d
Gluconate	25.7 d	16.7 b	21.3 b–e
Heptagluconate	30.5 bc	19.7 ab	27.5 ab
Hydroxide	14.6 hi	25.6 a	29.7 a
Lactate	15.5 gh	25.6 a	25.8 a–d
Nitrate	28.2 c	26.4 a	21.3 b–e
Oxide	12.4 ij	24.9 a	26.0 a–d
Pantothenate	23.3 e	22.3 ab	22.7 b–e
Phosphate, dibasic	21.9 e	23.4 ab	21.4 b–e
Phosphate, tribasic	17.6 fg	27.4 a	26.8 a–c
Pyrophosphate	22.6 e	21.9 ab	20.3 de
Succinate	22.7 e	21.0 ab	22.0 b–e
Propionate	5.9 m	24.7 a	24.9 a–d
Tartrate	31.2 b	16.1 b	17.1 e
Silicate	9.8 kl	22.2 a	20.6 c–e
Sulfate	19.3 f	22.6 ab	24.2 a–d
Captan	0 n	...	...
Thiophanate-methyl	0 n	...	...
Iprodione	7.5 lm	...	...

[x] Lesion length = length of necrotic area along the longitudinal axis of the stem minus 5.0 mm. Each value is the mean of 20 observations from the combined data from two experiments.
[y] Each value is the mean of six observations from the combined data from two experiments.
[z] Different letters in columns denote significant differences according to the Waller-Duncan Bayesian k-ratio t test ($P \leq 0.05$).

DISCUSSION

In a previous study on the application of exogenous materials to peach bark wounds, the most promising treatment for reducing disease caused by *L. persoonii* was calcium chloride (7). Although this compound did not stimulate the production of lignin or suberin,

it was the most effective chemical for reducing both infection frequency and the size of cankers. Calcium chloride reduced disease severity in this study as well. However, some other calcium compounds exhibited greater toxicity to the fungus in culture. When applied to excised twig segments, several calcium compounds were as effective as calcium chloride, but only calcium propionate provided a higher degree of protection to pretreated shoots.

The mode of action of other active calcium salts, such as calcium chloride, in this system is unknown. Possibly, the Ca^{2+} ion stimulates the synthesis of phytoalexins and/or phenols, as suggested by Kohle et al (16). Alternatively, Ca^{2+} ions may reduce the effectiveness of fungal polygalacturonase enzymes by forming cation cross bridges between pectic acids in the plant cell walls, thus making the cell walls more resistant to digestion (11). The latter possibility is supported by the observation that breakdown of the pectins present in peach bark cell walls is part of the disease process (2). The sequestration of calcium by fungal oxalic acid was shown to occur during pathogenesis by *L. persoonii* (23). However, the high concentrations of Ca^{2+} that must be present in cell walls to impede polygalacturonase activity in apples (about 800 μg/g of dry weight, W. S. Conway, *personal communication*) were not achieved in this study. The weak correlation between posttreatment Ca^{2+} and lesion length after the 15-min dips suggests some beneficial effect of relatively low Ca^{2+} levels on the ability of the host to resist colonization. However, this correlation was not present after the 60-min dip, even though the longer dip time resulted in generally higher calcium levels. It is possible also that the effects of calcium hydroxide and calcium oxide on in vitro growth may be indirect, due to the relatively high pH of the supplemented medium.

The effects of selected calcium compounds on the activity of PG in this study suggest a third possibility. Ca^{2+} or certain undissociated calcium compounds may act directly on the pathogen. Modification of membrane permeability, electron transport, and/or enzyme activity could cause reduced virulence or, in the extreme, fungistasis. Calcium silicate, calcium oxide, and calcium propionate effectively reduced production of PG by *L. persoonii.* None of the calcium salts in the present study were fungicidal at the concentrations examined.

Calcium propionate, the hemicalcium salt of propionic acid (a three-carbon organic acid), has a long history as a food additive and is well known as an inhibitor of certain molds and bacteria in stored grain (17,20,24), hay (14,18), and bread (8), and has the potential for widespread use in the preservation of other foods (8). Punja and Gaye (19) recently demonstrated the utility of calcium propionate dips in reducing black root rot, caused by *Chalara elegans,* on fresh market carrots. The pK_a of propionate is 4.87; and at a pH of 4.0, 88% of the compound is undissociated; while at a pH of 6.0, only 6.7% remains undissociated (8). The pH of our dip solutions was 5.8. The mechanism of action of calcium propionate is due to fungistasis and is thought to be due mostly to the molecule in its undissociated state (8). The lipophilic, undissociated molecule is readily soluble in cell membranes; and once there, it may interfere with the permeability of the microbial cell membrane, causing uncoupling of both substrate transport and oxidative phosphorylation from the electron transport system. In less acidic environments, where a large proportion of the molecules are dissociated, the Ca^{2+} moiety may act to inhibit fungal PG isozymes (W. S. Conway, *personal communication*).

Short-chain organic acids could have a role as disease control agents for wound pathogens. The fungistatic nature of these substances could provide the time required for host defense mechanisms to provide more effective resistance. Addi-

Table 4. Effect of 60-min exposure to various calcium salts (400 ppm Ca^{2+}) on calcium content and colonization of excised peach twig segments by *Leucostoma persoonii* measured 10 days after inoculation

Treatment	Lesion length (mm)[x]	Posttreatment Ca^{2+} (mg/g)[y]	Posttreatment postinoculation Ca^{2+} (mg/g)[y]
Control	40.3 a[z]	25.5 b	25.4 a
Acetate	10.9 h	23.5 b	25.4 a
Chloride	2.1 mn	35.1 ab	25.6 a
Formate	19.6 ef	27.3 ab	32.6 a
Gluconate	16.1 g	34.5 ab	32.2 a
Heptagluconate	7.1 ij	27.1 ab	24.5 a
Hydroxide	1.6 no	27.8 ab	31.9 a
Lactate	19.1 f	32.2 ab	32.9 a
Nitrate	26.0 c	30.2 ab	32.2 a
Oxide	5.2 kl	29.5 ab	30.7 a
Pantothenate	22.4 d	30.2 ab	32.5 a
Phosphate, dibasic	22.8 d	31.0 ab	33.0 a
Phosphate, tribasic	16.5 g	30.0 ab	30.0 a
Pyrophosphate	21.4 de	24.8 b	25.8 a
Succinate	4.0 l	22.1 b	25.3 a
Propionate	0 o	24.7 b	24.9 a
Tartrate	28.6 b	27.0 ab	28.0 a
Silicate	3.8 lm	29.7 ab	26.6 a
Sulfate	8.0 i	41.6 a	30.0 a
Captan	0 o	...	...
Thiophanate-methyl	0 o	...	...
Iprodione	6.1 jk	...	...

[x] Lesion length = length of necrotic area along the longitudinal axis of the stem minus 5.0 mm. Each value is the mean of 20 observations from the combined data from two experiments.
[y] Each value is the mean of six observations from the combined data from two experiments.
[z] Different letters in columns denote significant differences according to the Waller-Duncan Bayesian *k*-ratio *t* test ($P \leq 0.05$).

Table 5. Effect of four calcium salts on relative activity (RA) of pectin lyase and polygalacturonase activity of *Leucostoma persoonii*[w]

	7 days		15 days	
Treatment	PNL (Activity/ml/mg)[x]	PG (Activity/ml/mg)[y]	PNL (Activity/ml/mg)	PG (Activity/ml/mg)
Control	6.78 c[z]	6.40 a	6.09 c	5.60 b
Calcium hydroxide	9.74 c	4.14 ab	9.99 b	8.42 a
Calcium oxide	14.74 ab	2.05 bc	16.01 a	7.08 ab
Calcium propionate	18.81 a	1.01 c	11.17 b	5.84 b
Calcium silicate	11.26 bc	5.80 a	6.36 c	1.83 c

[w] Enzyme activity = 100× the increase in absorbance per hour per milliliter of broth per gram dry weight of mycelium.
[x] Pectin lyase. Activity was tested on citrus pectin at pH 8.5.
[y] Polygalacturonase. Activity was tested on sodium polypectate at pH 4.5.
[z] Each value is the mean of four observations from the combined data from two experiments. Different letters in columns denote significant differences according to the Waller-Duncan Bayesian *k*-ratio *t* test ($P \leq 0.05$).

tional research should address optimal concentrations of specific organic acids, the use of additives or synergists, and carriers that would maintain effective concentrations of materials for an effective time period.

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