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In Vitro Fungicidal Activity of Calcium and Potassium Salts on Several Commercially Significant Plant Pathogens

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***Abstract*. In vitro dose responses of several calcium and potassium salts were determined on some commercially significant plant pathogens, including: *Helminthosporium solani*, *Fusarium oxysporum* f. sp. *pisi* race 2, *Colletotricum coccodes*, *Phytophthora cactorum*, *Phytophthora cinnamomi*, *Phytophthora erythroseptica*, *Phytophthora infestans*, *Phytophthora megasperma*, *Pythium ultimum*, and *Venturia inaequalis*. Mycelial growth inhibition was both salt-specific and dose-related. *Pythium ultimum* was completely inhibited by 75 mg·L^{-1} or greater calcium propionate, but needed 300 mg·L^{-1} or greater of calcium acetate and 40 mL·L^{-1} or greater of potassium silicate for complete inhibition. *Phytophthora infestans* was completely inhibited by 150 mg·L^{-1} or greater calcium acetate, 150 mg·L^{-1} or greater calcium propionate, or 5 mL·L^{-1} or greater potassium silicate. *Phytophthora cactorum* was completely inhibited by 300 mg·L^{-1} or greater calcium propionate, but required 600 mg·L^{-1} or greater calcium acetate and 10 mL·L^{-1} or greater potassium silicate for complete inhibition. *Phytophthora cinnamomi* was completely inhibited by calcium propionate at 600 mg·L^{-1} or greater or by 10 mL·L^{-1} or greater potassium silicate. Only potassium silicate inhibited *Phytophthora megasperma*, *Phytophthora erthroseptica*, *V. inequalis*, and *H. solani* at concentrations of 5 mL·L^{-1} or greater, 20 mL·L^{-1} or greater, 40 mL·L^{-1} or greater, or 80 mL·L^{-1} or greater, respectively. Potassium acetate did not completely inhibit any of the pathogens in this study when tested at concentrations 1200 mg·L^{-1} or less.**

Agronomic and horticultural crops in the Pacific Northwest constitute a multimillion dollar industry. Both conventional and organic cropping systems in this region are affected by several fungal and fungal-like organisms of commercial significance (Table 1). Conventional farming systems in particular have become very reliant on synthetic fungicides, and with mounting pressure from the environmental lobby, efforts must be made to identify suitable alternatives. Unfortunately, most organic remedies currently available are limited in their efficacy and given the small array of acceptable products available to organic growers, every effort should be made to quantify efficacy of potential new products with alternative modes of action to those currently in use because this will help reduce the risks associated with pesticide resistance.

Several studies have been undertaken in recent years identifying the fungicidal properties of many different inorganic salts (Biggs et al., 1997; Campanella et al., 2002; Hervieux et al., 2002; Olivier et al., 1999; Samelis et al., 2001). The following salts have shown good potential for use as fungicides: potassium silicate (Bekker et al., 2006), calcium acetate (Palou et al., 2002), and calcium propionate (Aguayo et al., 2008; Arroyo et al., 2005; Biggs, 1999, 2004; Biggs et al., 1997; Blogdett et al., 2002; Kortekamp, 2006; Mills et al., 2005; Suhr and Nielsen, 2004). However, most of these studies dealt with only some of the pathogens tested in this study and the remainder were postharvest fruit pathogens. In addition, no studies to date have evaluated the use of potassium acetate as a potential fungicide. Consequently, the present study was initiated to determine whether commercially important fungi and oomycetes in the Pacific Northwest could be suppressed before harvest using potassium acetate and other inorganic salts. This in vitro investigation is regarded as being a starting point for future field investigations and those compounds showing promise will be further tested in vivo.

Material and Methods

Fungal and oomycete isolates were obtained from culture collections maintained by Oregon State University and Washington State University (Table 1) for the purpose of testing the susceptibility of these fungal and oomycete isolates to different calcium and potassium salts. Isolates were chosen based on taxonomic diversity within the ascomycetes and oomycetes as well as economic importance as plant pathogens in the Pacific Northwest. For this study, *Helminthosporium solani*, *Fusarium oxysporum* f. sp. *pisi* race 2, *Colletotricum coccodes*, and *Venturia inaequalis* were subcultured and analyzed on Potato Dextrose Agar (PDA) (Becton, Dickinson & Co., Sparks, MD) prepared according to the manufacturer's specifications. Cultures of *Phytophthora infestans*, *Phytophthora megasperma*, *Phytophthora cactorum*, *Phytophthora erythroseptica*, *Pythium ultimum*, and *Phytophthora cinnamomi* were maintained and analyzed on Corn Meal Agar (CMA) (Becton, Dickinson & Co.).

Before inoculation, fungal and oomycetes isolates were subcultured onto these media and incubated at room temperature (≈25 °C) until the diameter of the culture was nearly that of the entire plate. Reagents were obtained from Spectrum® Chemicals and Laboratory Products (Gardena, CA) as follows: calcium acetate, anhydrous powder (FCC) [$C_4H_6CaO_4$;

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fresh weight (FW) 158.17; CAS 62-54-4]; calcium propionate anhydrous powder (FCC) ($C_6H_{10}CaO_4$; FW 186.22; CAS 4075-81-4); and potassium acetate, crystalline powder (USP) (C2H3KO2; FW 98.14; CAS 127-08-2). Potassium silicate was obtained from Monterey® Ag Resources (Fresno, CA) as Sil-MATRIX™ ($HKO_3Si.xH_2O$; 291 g·L^{-1} KSi solution in water). According to their molecular masses, different quantities of calcium acetate, calcium propionate, and potassium acetate were used to reach concentrations of 0, 75, 150, 300, 600, and 1200 mg·L^{-1} of media (Tables 2 through 4, respectively). One 4-mm plug of agar from the hyphal tip of each fungus or oomycete culture was transferred, mycelia side down, to the center of each of three replicate plates amended as described previously.

Calcium acetate, calcium propionate, and potassium acetate powder were added to the agar to achieve the targeted concentration before autoclaving. None of these salts affected the pH of the agar. Potassium silicate was sterilized by passing it through a 0.45-µm millipore filter and then added to the autoclaved agar before solidification at a temperature of 60 °C at concentrations of 5, 10, 20, 40, and 80 mL·L^{-1} of agar for each organism. Potassium silicate was added after ultrafiltration because it has a tendency to form a solidified silicon mass if added before autoclaving based on previous experiments (Bekker et al., 2006). Soluble potassium silicate raised the pH of the agar from 5.6 (unamended agar) to 10.3 and 11.7 at concentrations of 5 mL and 80 mL potassium silicate per liter of agar, respectively. Controls for those petri plates amended with potassium silicate consisted of unamended PDA and CMA agar in which the pH had been adjusted to 10.3 and 11.7 with NaOH.

Petri plates were incubated in the dark at room temperature for varying times depending on the growth rate of the particular organism and the time necessary for growth to reach nearly the edge of the petri plate. This was as briefly as 2 d for fast-growing organisms like *Pythium ultimum* and as long as 6 weeks for slower growing organisms like *Phytophthora infestans* and *V. inaequalis*. Colony diameter was recorded for each plate by measuring the distance, in millimeters, from one edge of the colony to the other at two points perpendicular to each other, and the mean was determined. Each compound was tested in triplicate in two separate experiments.

Percent organism growth was calculated according to the formula:

$$\% \text{ Organism Growth} = 100 - [(D - C)/D \times 100]$$

where D is the diameter (mm) of the petri dish of the test plate and C is the diameter (mm) of the colony on the test plate.

Statistical analysis. Data were evaluated by general analysis of variance using the statistical program GenStat® (2010) testing for differences in the percentage inhibition within and between the different fungal groups as well as effects of concentrations or Agar pH. Furthermore, the two different experiments were treated as blocks for statistical purposes.

Results and Discussion

General analyses of variance confirmed that calcium acetate, calcium propionate, and potassium silicate had significant fungicidal activity against most of the plant pathogens used in this study ($P < 0.001$). Calcium acetate (Table 2) markedly suppressed in vitro growth of *Phytophthora cinnamomi* at 1200 mg·L^{-1} (% organism growth = 1.1%) and totally suppressed in vitro growth of *Phytophthora infestans, Pythium ultimum*, and *Phytophthora cactorum* at concentrations of 150 mg·L^{-1} or greater, 300 mg·L^{-1} or greater, and 600 mg·L^{-1} or greater, respectively. The highest concentration of calcium acetate (1200 mg·L^{-1}) slightly inhibited *V. inaequalis*, *Phytophthora megasperma*, and *Phytophthora erythroseptica* but had no effect on *C. coccodes*, *H. solani*, or *F. oxysporum*. Calcium propionate (Table 3) was even more effective than calcium acetate against most plant pathogens

Table 1. Select fungal and oomycetes pathogens of commercial significance in the Pacific Northwest used in this study.

Fungal group	Class	Genus and species	Disease name	Host plant
Ascomycotes	Hypocreales	*Fusarium oxysporum* f. sp. *pisi* Race 2 (Hall) Snyd and Hans	Blight, wilt	Peas
	Phyllachorales	*Colletotrichum coccodes* (Wallr.) S. Hughes	Anthracnose wilts	Potato/various hosts
	Pleosporales	*Helminthosporium solani* Durieu & Mont.	Silver scurf	Potato
		Venturia inaequalis (Cooke) Wint.	Scab	Apples
Oomycotes	Pythiales	*Phytophthora cactorum* (Lebert & Cohn) J. Schröt.	Crown rot	Apples
		Phytophthora cinnamomi Rands	Root rot	Many tree hosts
		Phytophthora erythroseptica Pethybr.	Pink rot	Potatoes
		Phytophthora infestans (Mont.) de Bary	Late blight	Potatoes/ tomatoes
		Phytophthora megasperma Drechsler	Tuber rot root rot	Potatoes various hosts
		Pythium ultimum Trow	Leak	Potatoes

Table 2. In vitro organism growth, expressed as a percentage of average colony diameter compared with the diameter of the petri dish, of 10 commercially important fungi and oomycetes in the Pacific Northwest when grown in agar plates amended with calcium acetate at the concentrations indicated (A value of 0 = no growth of the mycelium).

	Calcium acetate concn						
Fungus/oomycete	0 mg·L^{-1}	75 mg·L-^{1}	150 mg·L^{-1}	300 mg·L^{-1}	600 mg·L^{-1}	1200 mg·L^{-1}	$LSD_{5\%}$
Helminthosporium solani[z]	80.1	82.6	82.1	82	82	81.2	2.30
Fusarium oxysporum[z]	62.3	63.1	62.2	69.6	73.2	76.6	3.58
Colletotrichum coccodes[z]	100	99.9	100	100	100	100	0.12
Venturia inaequalis[z]	64.9	59.8	54.4	57.8	54.4	52.4	6.07
Phytophthora infestans[y]	69.3	52.9	0	0	0	0	9.58
Phytophthora megasperma[y]	66.1	94.9	94.3	76.8	41.7	24.4	5.87
Phytophthora cactorum[y]	84.06	81.7	77	30	0	0	13.01
Phytophthora erythroseptica[y]	73.23	98.4	90.2	48.3	32.5	25.8	4.58
Pythium ultimum[y]	97.81	81.9	6.5	0	0	0	3.02
Phytophthora cinnamomi[y]	87.19	96.3	41.1	11.6	4.2	1.1	5.18

Agar plates consisted of either [z]potato dextrose agar (PDA) or [y]corn meal agar (CMA).
LSD = least significant difference.

Table 3. In vitro organism growth, expressed as a percentage of average colony diameter compared with the diameter of the petri dish, of 10 commercially important fungi and oomycetes in the Pacific Northwest when grown in agar plates amended with calcium propionate at the concentrations indicated (A value of 0 = no growth of the mycelium).

	Calcium propionate concn						
Fungus/oomycete	0 mg·L^{-1}	75 mg·L^{-1}	150 mg·L^{-1}	300 mg·L^{-1}	600 mg·L^{-1}	1200 mg·L^{-1}	$LSD_{5\%}$
Helminthosporium solani[z]	80.1	84.3	82.8	86.7	84.2	82.3	1.98
Fusarium oxysporum[z]	62.3	59.7	58.7	56.6	59.1	55.8	3.37
Colletotrichum coccodes[z]	100	97.9	96	90.5	80	52.3	2.14
Venturia inaequalis[z]	64.9	50.5	48.2	43.4	40.5	32.1	5.71
Phytophthora infestans[y]	69.3	1.1	0	0	0	0	4.76
Phytophthora megasperma[y]	66.1	93.3	86.8	46.6	17.3	12.7	9.22
Phytophthora cactorum[y]	84.06	75.4	18.8	0	0	0	5.51
Phytophthora erythroseptica[y]	73.23	94.2	62.3	27.8	16.2	12.6	3.21
Pythium ultimum[y]	97.81	0	0	0	0	0	0.54
Phytophthora cinnamomi[y]	87.19	66	20.2	0.9	0	0	4.44

Agar plates consisted of either [z]potato dextrose agar (PDA) or [y]corn meal agar (CMA).
LSD = least significant difference.

used in this study ($P < 0.001$). This salt completely suppressed in vitro growth of *Pythium ultimum, Phytophthora infestans*, *Phytophthora cactorum*, and *Phytophthora cinnamomi* at concentrations of 75 mg·L^{-1} or greater, 150 mg·L^{-1} or greater, 300 mg·L^{-1} or greater, and 600 mg·L^{-1} or greater, respectively. Calcium propionate partially inhibited *V. inaequalis*, *Phytophthora megasperma*, and *Phytophthora erythroseptica*, but these effects were insufficient to warrant further in vivo investigations. Interestingly, in contrast to calcium acetate, calcium propionate suppressed ≈48% of fungal growth of *C. coccodes* at the highest concentration tested (1200 mg·L^{-1}). These data suggest that calcium propionate and calcium acetate have high potential as fungicides for the control of *Phytophthora* and *Pythium* species used in this study. The fact that both of these compounds are on the U.S. Federal Insecticide, Fungicide Rodenticide Act exempt list of inert products implies that they should be recognized as organic inputs, thus enabling their use to control these devastating organisms in both organic and conventional orchards and field crops. Clearly field testing is needed to confirm efficacy, rates, and to ensure no phytotoxicity.

Potassium acetate (Table 4) was mostly ineffective in suppressing any of the fungi tested and in most cases was no better than the untreated controls. Although technically a negative result, it is nevertheless important to report this finding because future testing of this salt by others may now be avoided.

Potassium silicate (Table 5) was highly effective against several of the fungi tested and totally suppressed in vitro growth of *Phytophthora infestans*, *Phytophthora megasperma*, *Phytophthora cinnamomi*, *Phytophthora cactorum*, *Phytophthora erythroseptica*, *Pythium ultimum*, *V. inaequalis*, and *H. solani* at concentrations of greater 5 mL·L^{-1} or greater, 5 mL·L^{-1} or greater, 10 mL·L^{-1} or greater, 10 mL·L^{-1} or greater, 20 mL·L^{-1} or greater, 40 mL·L^{-1} or greater, 40 mL·L^{-1} or greater, and 80 mL·L^{-1} or greater, respectively ($P < 0.001$). The pH of the 5 mL·L^{-1} and 80 mL·L^{-1} NaOH-adjusted agar plates were pH 10.5 and pH 11.3, respectively. Consequently, additional agar plates were pH adjusted to these pH values using NaOH to determine whether these pH values were affecting fungal growth. Although the growth of the organism was suppressed at pH conditions higher than pH 5.6, the normal pH of agar, potassium silicate enhanced this suppression in a linear manner. Suppression of *Phytophthora cinnamomi*, *Phytophthora capsici*, *Sclerotinia sclerotiorum*, *Sclerotium rolfsii*, *Pythium* F-group, *Mucor pusillus*, *Drechslera* spp., *Fusarium oxysporum* pv. *cubense*, *Fusarium solani*, *Alternaria solani*, *Colletotrichum coccodes*, *Verticillium fungicola*, *Curvilaria lunata*, and *Stemphylium herbarum* by potassium silicate has been reported previously (Bekker et al., 2006). To our knowledge, however, suppression of the additional fungal and oomycetes pathogens used in the current study has not been previously reported. In a different study, Campanella et al. (2002) reported the efficacy of different calcium salts against *Phytophthora nicotiana* both in vitro and in vivo. Based on the results of that study, it is suggested that field testing of the materials that demonstrated efficacy in the current study should be evaluated using similar field concentrations (300 mL of an aqueous solution of 1200 mg·L^{-1} applied to 1.5 L of soil).

Furthermore, the recent acceptance of potassium silicate as an organic input means that organic growers may have an additional tool in the future to use to control damage caused by these fungi. Interestingly, potassium silicate did not have any effect on *Fusarium oxysporum* f.sp. *pisi* nor *C. coccodes* in the current study, whereas Bekker et al. (2006) did find it to be effective against *Fusarium oxysporum* f. sp. *cubense* and *C. Coccodes* in their study. This does raise an interesting question relating to the efficacy against different fungal species as well as localized strains and further investigations are warranted. Field testing of calcium acetate, calcium propionate, and potassium silicate as fungicides are also warranted and in vivo testing is currently underway in Lofthus, Norway.

Table 4. In vitro organism growth expressed as a percentage of average colony diameter compared with the diameter of the petri dish of 10 commercially important fungi and oomycetes in the Pacific Northwest when grown on agar plates amended with potassium acetate at the concentrations indicated.

	Potassium acetate concn						
Fungus/oomycete	0 mg·L^{-1}	75 mg·L^{-1}	150 mg·L^{-1}	300 mg·L^{-1}	600 mg·L^{-1}	1200 mg·L^{-1}	LSD$_{5\%}$
Helminthosporium solani[z]	80.1	80.4	81.2	77.7	74.6	77.1	3.19
Fusarium oxysporum[z]	62.3	63.7	63.2	64	69.2	65	2.82
Colletotrichum coccodes[z]	100	95.9	98.2	99.8	99.9	100	1.33
Venturia inaequalis[z]	64.9	55.2	57.3	54.9	49.6	48.6	5.77
Phytophthora infestans[y]	69.3	57.2	53.4	52.5	47.1	45.3	7.39
Phytophthora megasperma[y]	66.1	57	48.4	36.6	23.7	18.2	2.89
Phytophthora cactorum[y]	84.06	81.7	70.4	60	52.9	58.8	5.32
Phytophthora erythroseptica[y]	73.23	63.5	53.5	51.2	42	34.7	3.30
Pythium ultimum[y]	97.81	90.3	84.8	77.5	88.9	44.2	6.28
Phytophthora cinnamomi[y]	87.19	97.5	96.5	94.6	93.6	79.1	5.06

Agar plates consisted of either [z]potato dextrose agar (PDA) or [y]corn meal agar (CMA).
LSD = least significant difference.

Table 5. In vitro organism growth, expressed as a percentage of average colony diameter compared with the diameter of the petri dish, of 10 commercially important fungi and oomycetes in the Pacific Northwest when grown on agar plates amended with soluble potassium silicate (291 g·L^{-1})[z] (A value of 0 = no growth of the mycelium).

	Potassium silicate (291 g·L^{-1}) concn								
Fungus/oomycete	0 mL·L^{-1} (pH 5.6)	0 mL·L^{-1} (pH 10.3)	0 mL·L^{-1} (pH 11.7)	5 mL·L^{-1}	10 mL·L^{-1}	20 mL·L^{-1}	40 mL·L^{-1}	80 mL·L^{-1}	LSD$_{5\%}$
Helminthosporium solani[y]	80.1	79.8	78.3	70.9	73.2	71.1	27.6	0	2.54
Fusarium oxysporum[y]	62.3	65.1	61.5	75.1	78.5	79.2	77.6	62.5	2.75
Colletotrichum coccodes[y]	100	98.6	97.7	99.8	99.9	99.3	99.3	99	0.64
Venturia inaequalis[y]	64.9	61.4	61.8	44.4	32.7	16.6	0	0	7.18
Phytophthora infestans[x]	69.3	34.6	28	0	0	0	0	0	4.36
Phytophthora megasperma[x]	66.1	45.3	26.7	0	0	0	0	0	4.55
Phytophthora cactorum[x]	84.1	82.5	72.4	11.2	0	0	0	0	4.20
Phytophthora erythroseptica[x]	73.2	67.1	50	14.4	4.1	0	0	0	4.88
Pythium ultimum[x]	97.8	95.7	94	61.8	37.5	6.2	0	0	3.62
Phytophthora cinnamom[x]	87.2	77.3	54.5	16.6	0	0	0	0	7.22

[z]Soluble potassium silicate raised the pH of the agar from 5.6 (unamended agar) to 10.3 and 11.7 at concentrations of 5 mL and 80 mL potassium silicate per liter of agar, respectively. Increased pH in the absence of potassium silicate only partially inhibited mycelial growth.
Agar plates consisted of either [y]potato dextrose agar (PDA) or [x]corn meal agar (CMA).
LSD = least significant difference.

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