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Potassium Phosphite Resistance and New Modes of Action for Managing Phytophthora Diseases of Citrus in the United States

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ABSTRACT

Phytophthora diseases are economically important in citrus production worldwide. Pathogens in California include *Phytophthora parasitica, P. citrophthora, P. syringae,* and the less common *P. hibernalis.* These can cause fruit brown rot, root rot, foot rot, and trunk cankers. Resistant rootstocks, cultural practices, and fungicide treatments are used for their management. Preharvest applications of copper are effective against brown rot; whereas phosphonate and phenylamide-acylalanine fungicides are used for managing root and brown rots. Phosphonates have been used more extensively due to phenylamide resistance, lower cost, ambimobility in the tree, ease of application by chemigation or foliar treatment, and regulatory approval for phosphite salts to be included in phosphate fertilizers. Baseline sensitivity studies before registration of phosphonates are lacking. We detected a wide range of EC_{50} values for inhibiting mycelial growth with values up to 252 µg/ml for *P. citrophthora* and 142 µg/ml for *P. parasitica* and *P. syringae.* To determine if these isolates were field resistant, Navel orange fruit were inoculated with sensitive (EC_{50} = 7.6 µg/ml) and putative resistant (EC_{50} = 186 µg/ml) isolates of *P. citrophthora*. Using the sensitive isolate, brown rot was effectively managed with pre- or postharvest applications of potassium phosphite at 620 or 4000 µg/ml, respectively, but not when using a putative resistant isolate. No cross resistance was detected to mefenoxam, as well as to the new oxathiapiprolin, fluopicolide, and mandipropamid. The three new modes of action have baseline sensitivities with mean EC_{50} values of <0.001, 0.06, or 0.0035 µg/ml, respectively, for *P. citrophthora*. In field trials, they reduced root rot to near zero levels. Oxathiapiprolin and mandipropamid are also highly effective against brown rot. All three will be registered on citrus in the United States.

INTRODUCTION

Citrus crops are affected by numerous species of *Phytophthora* worldwide that may cause economically important diseases including fruit brown rot, root rot, foot rot, and trunk cankers (e.g., gummosis). In California, *P. citrophthora* and *P. syringae* are the major pathogens of brown rot, whereas *P. citrophthora* and *P. parasitica* (*P. nicotianae*) are the main causes of root rot and trunk cankers (Graham & Menge 2000; Hao et *al.* 2015). *P. hibernalis* is a less common cause of brown rot and trunk cankers during the winter months. Phytophthora diseases are especially important in areas with high rainfall, poor soil drainage, or improper irrigation practices. In California, brown rot is most serious during the winter harvest season when most of the annual rainfall occurs. The epidemiology of Phytophthora diseases on citrus is closely interrelated, and inoculum from one disease may be the source of serious losses by another disease. Therefore, they are managed in an integrated approach using resistant rootstocks, cultural practices, and fungicide treatments. Recently, *P. syringae* and *P. hibernalis* were designated quarantine pathogens in China after their detection in citrus fruit shipments from California. This has restricted the California citrus trade and subsequently initiated renewed research on control strategies (Adaskaveg & Förster 2014).

Few fungicides are currently registered in the United States for the control of *Phytophthora* diseases of citrus. Preharvest foliar and fruit applications of copper are effective against brown rot. Since the 1980s, phosphonate (e.g., fosetyl-Al; potassium phosphite, calcium phosphite) and phenylamide-acylalanine (e.g., mefenoxam) fungicides are used for managing root and brown rot. Phosphonates are applied two to three times per year. Phosphonates have been used more extensively due to phenylamide resistance, lower cost, ambimobility in the tree, and ease of application through chemigation or by foliar treatment. Potassium phosphite (K-phosphite) was also registered in 2013 as the first postharvest fungicide to manage brown rot (Adaskaveg & Förster 2014; Adaskaveg et *al.* 2015).

Baseline sensitivity studies before registration of phosphonates are lacking. With increased use of K-phosphite as a postharvest treatment, one of our objectives was to evaluate sensitivities of *P. citrophthora, P. parasitica,* and *P. syringae* isolates to this fungicide. Because isolates with reduced sensitivity were identified, field and postharvest studies using registered rates of potassium phosphite were done to determine if disease caused by these isolates can be managed or if field resistance has developed. We also initiated evaluations of possible new fungicide alternatives for the management of Phytophthora diseases of citrus.

MATERIALS AND METHODS

Isolates of *Phytophthora* were collected from brown-rotted citrus fruit and infected roots in major citrus growing areas of California. *In vitro* sensitivities to K-phosphite (ProPhyt, Helena Chemical Co., Collierville TN) were determined using the agar dilution method (with 10% clarified V8 agar), whereas for other fungicides (fluopicolide, oxathiapiprolin, mandipropamid, mefenoxam), the spiral gradient dilution method (SGD; Förster et *al.* 2004) was used. For the agar dilution method, EC_{50} values were determined by regressing log-transformed phosphite rates against logit-transformed inhibitions as compared to the non-amended controls. Regression equations were then solved for concentration at 50% inhibition. For the SGD method, EC_{50} values were calculated using a computer program.

Preharvest treatments with K-phosphite (ProPhyt; 620 µg/ml, 38 HL/Ha) were applied to navel orange fruit 10 or 0 days before harvest using an air-blast sprayer. Fruit were harvested, inoculated with zoospores of selected isolates of *P. citrophthora*, and incubated for 8 days at 20°C. Procedures used in postharvest studies were previously described in detail (Adaskaveg *et al.* 2015). Treatments were done 18 to 24 h after inoculation of navel oranges with *P. citrophthora.* In the laboratory, treatments were applied as 5- to 15-s dips, and in experimental packingline studies, 12-s drench applications were used, followed by a treatment with carnauba fruit coating. K-phosphite solutions (4,000 to 12,000 µg/ml) were at ambient temperature (25°C) or at 54°C. Fruit were then incubated at 20°C for 8 days and evaluated for the incidence of brown rot. Four replications of 12 and 24 fruit each were used in laboratory and experimental packing line studies, respectively.

RESULTS

In vitro sensitivities of Phytophthora isolates to K-phosphite

For each of the three species, there was a wide range of EC_{50} values for K-phosphite: 5.5 to 252 µg/ml for 44 isolates of *P. citrophthora*, 9.8 to 141.6 µg/ml for 44 isolates of *P. syringae*, and 12.2 to 141.5 µg/ml for 20 isolates of *P. parasitica.* Growth of putative resistant isolates was similar to sensitive isolates in the absence of phosphite.

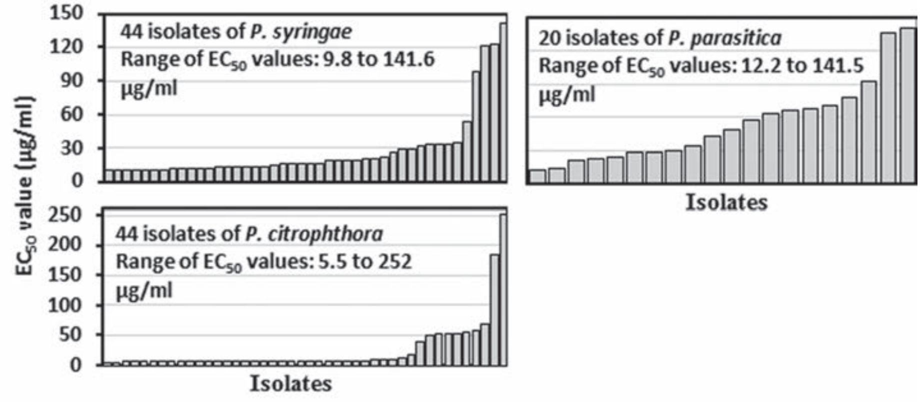

Figure 1 *In vitro* sensitivities of *Phytophtora* spp. from citrus against potassium phosphite

Efficacy of K-phosphite in pre- and postharvest studies to manage citrus brown rot

In the pre-harvest studies, brown rot developed at high incidence in the non-treated control after inoculation with *P. citrophthora* (Table 1). When K-phosphite-treated fruit were inoculated with a sensitive isolate (EC_{50} = 7.6 µg/ml), the incidence was reduced by 73.9% in

Table 1 Managing brown rot of navel orange caused by *Phytophthora citrophthora* with reduced sensitivity to potassium phosphite by preharvest treatments

	2015: Incidence of brown rot (%)[b] *P. citrophthora* with EC_{50}		2016: Incidence of brown rot (%) *P. citrophthora* with EC_{50}	
Treatment[a]	EC_{50} 7.6 µg/ml	EC_{50} 69 µg/ml	EC_{50} 7.6 µg/ml	EC_{50} 186 µg/ml
Control	95.8 a	92.7 a	100 a	100 a
K-phosphite	21.9 b	77.5 b	14.6 b	81.3 b

[a] Treatments were applied in the field at 620 µg/ml, 38 HL/Ha.

[b] In 2015, fruit were harvested 10 days after application, in 2016, fruit were harvested the same day as application. Fruit were inoculated and incubated for 8 days at 20°C.

Table 2 Managing brown rot of navel orange caused by *Phytophthora* citrophthora with reduced sensitivity to potassium phosphite by postharvest treatments[a].

			Incidence of brown rot[b]	
K-phosphite 4000 µg/ml, 25°C, 12 s	K-phosphite 4000 µg/ml, 54°C, 12 s	Fruit coating	*P. citrophthora* EC_{50} 7.6 µg/ml	*P. citrophthora* EC_{50} 186 µg/ml
---	---	x	97.6 a	100 a
x	---	x	28.0 b	100 a
---	x	x	9.7 c	100 a

[a] Fruit were treated 18 to 20 h after inoculation by in-line drench applications at 25°C or 54°C. These were followed by spray application with a carnauba fruit coating.

[b] The incidence of brown rot was assessed after 8 days of incubation at 20°C.

Table 3 Managing brown rot of navel orange caused by *Phytophthora citrophthora* with reduced sensitivity to potassium phosphite by postharvest dip treatments[a]

Treatment	Temperature	Dip time	Incidence of brown rot[b] *P. citrophthora* EC_{50} 186 µg/ml
Water	25°C	15 s	98.5 a
Water	54°C	15 s	41.7 bc
K-phosphite 8,000 µg/ml	25°C	15 s	59.2 b
K-phosphite 8,000 µg/ml	54°C	15 s	4.3 d
K-phosphite 8,000 µg/ml	54°C	5 s	25.0 c
K-phosphite 12,000 µg/ml	54°C	5 s	22.9 c

[a] Fruit were treated 22 to 24 h after inoculation by dip treatments at 25°C or 54°C.

[b] The incidence of brown rot was assessed after 8 days of incubation at 20°C.

2015 (fruit harvested 10 days after treatment) and by 85.4% in 2016 (fruit harvested 0 days after treatment). In contrast, brown rot incidence was reduced by only 15.2% in 2015 after inoculation with an isolate with an EC_{50} of 69 µg/ml, and by 18.7% in 2016 after inoculation with an isolate with an EC_{50} of 186 µg/ml.

In postharvest studies using fruit inoculated with a phosphite-sensitive isolate, brown rot incidence was significantly reduced by drench treatments with K-phosphite at 4000 µg/ml at 25°C or 54°C (Table 2). Additionally, heated treatments were significantly more effective than treatments at 25°C. Phosphite treatments, however, were not effective when fruit were inoculated with an isolate of *P. citrophthora* with reduced sensitivity (EC_{50} = 186 µg/ml).

Additional laboratory dip studies with higher rates of K-phosphite were done to improve the efficacy of phosphite on fruit inoculated with *Phytophthora* isolates with reduced sensitivity to the fungicide. Water dips at 54°C significantly reduced decay incidence as compared to dips at 25°C (Table 3). As in the previous study, heated 15-s dips at 8,000 µg/ml were significantly more effective than treatments at 25°C, and decay incidence was reduced from 98.5% in the control to 4.3%. The 15-sec dip duration was critical because 5-s dips at the same rate were less effective, and 5-s dips at 12,000 µg/ml did not improve efficacy.

Toxicity of new alternative fungicides to *Phytophthora citrophthora*

High *in vitro* sensitivities to mycelial growth of 62 isolates of *P. citrophthora* were identified for fluopicolide, oxathiapiprolin, and mandipropamid. Overall, oxathiapiprolin was the most effective (EC_{50} = $\leq$0.001 µg/ml). Mandipropamid had intermediate EC_{50} values (0.002 to 0.005 µg/ml), whereas those for fluopicolide (0.03 to 0.09 µg/ml) were similar to mefenoxam (0.013 to 0.12 µg/ml). There was no cross-resistance between K-phosphite and mefenoxam, oxathiapiprolin, fluopicolide, or mandipropamid.

DISCUSSION

A wide range of *in vitro* sensitivities to K-phosphite was detected among isolates of three *Phytophthora* species from citrus in California, and several isolates of each species showed reduced sensitivity. Field resistance in these latter isolates was confirmed because preharvest applications with K-phosphite at registered rates failed to control brown rot, and for postharvest applications, rates had to be increased, solutions had to be heated, and exposure times of 15 s had to be used.

Phosphonates have been widely used in citrus production in California since the 1990s. The only alternative fungicide for brown rot management is copper. Resistance is common to mefenoxam, the only alternative for control of the root rot disease phase. Furthermore, the regulatory approval for phosphite salts to be included in phosphate fertilizers has led to over-use. Thus, the extensive use of phosphonates likely led to the selection of less sensitive pathogen populations. Resistance to phosphonates has rarely been reported in Oomycota pathogens. For example, resistance to fosetyl-Al was found in *P. cinnamomi* from *Chamaecyparis lawsoniana* in nurseries (Vegh et *al.* 1985) and widespread insensitivity to phosphite was documented in downy mildew of lettuce (*Bremia lactucae*) where insensitive strains still grew at two-fold of the field rate (Brown et *al.* 2004). The mode of action of FRAC group 33 that contains the phosphonate fungicides is still unknown. The group has been suggested to have an unknown direct target site in the pathogen, induce host resistance, or to block the phosphate starvation response of the pathogen (Cohen & Coffey 1986; Smillie et *al.* 1989; Förster et *al.* 1998). Lack of effective control of disease caused by insensitive isolates in our studies indicates a direct target site in the pathogen.

Development of fungicides with new modes of action is ongoing as a resistance management strategy and to provide adequate control of Phytophthora diseases. The three new modes of action, the benzamide fluopicolide, the carboxylic acid amide (CAA) mandipropamid, and the piperidinyl thiazole isoxazoline oxathiapiprolin, were shown to be highly active against *P. citrophthora* in our studies. Additionally, in our field trials (data not shown), they have reduced root rot to near zero levels, and oxathiapiprolin and mandipropamid were also highly effective against brown rot. All three are scheduled for registration on citrus in the US.

REFERENCES

Adaskaveg JE; Förster H (2014). Integrated postharvest strategies for management of Phytophthora brown rot of citrus in the United States. In: *Plant Pathology in the 21st Century: Post-Harvest Pathology*, eds D Prusky & ML Gullino, pp. 123-131, Springer International Publishing: Switzerland.

Adaskaveg JE; Hao W; Förster H (2015). Postharvest strategies for managing Phytophthora brown rot of citrus using potassium phosphite in combination with heat treatments. *Plant Disease* 99, 1477-1482.

Brown S; Koike ST; Ochoa OE; Laemmlen F; Michelmore RW (2004). Insensitivity to the fungicide fosetyl-aluminum in California isolates of the lettuce downy mildew pathogen, *Bremia lactucae*. *Plant Disease* 88, 502-508.

Cohen Y; Coffey MD (1986). Systemic fungicides and the control of Oomycetes. *Annual Review of Phytopathology* 24, 311-338.

Förster H; Adaskaveg JE; Kim DH; Stanghellini ME (1998). Effect of phosphite on tomato and pepper plants and on susceptibility of peppers to Phytophthora root and crown rot in hydroponic culture. *Plant Disease* 82, 1165-1170.

Graham JH; Menge JA (1999). Root diseases. In: *Citrus Health Management*, eds LW Timmer & LW Duncan, pp. 126-135. APS Press: St. Paul, MN, USA.

Förster H; Kanetis L; Adaskaveg JE (2004). Spiral gradient dilution, a rapid method for determining growth responses and 50% effective concentration values in fungus-fungicide interactions. *Phytopathology* 94, 163-170.

Hao W; Miles TD; Martin FN; Förster H; Adaskaveg JE (2015). Distribution and frequency of *Phytophthora* species causing brown rot of citrus in the Central Valley of California. *Phytopathology* 105 (Suppl. 4), S4.57.

Smillie R; Grant BR; Guest D (1989). The mode of action of phosphite: evidence for both direct and indirect modes of action on three *Phytophthora* spp. in plants. *Phytopathology* 79, 921-926.

Vegh I; Leroux P; LeBerre A; Lanen C (1985). Detection sur *Chamaecyparis lawsoniana* 'Elwoodii' d'une souche de *Phytophthora cinnamomi*. Rands resistante au phosethyl-Al. *Revue Horticole* 262, 19-21.