Postharvest Strategies for Managing *Phytophthora* Brown Rot of Citrus using Potassium Phosphite in Combination with Heat Treatments

J. E. Adaskaveg, W. Hao, and H. Förster, Department of Plant Pathology and Microbiology, University of California, Riverside

Abstract

Adaskaveg, J. E., Hao, W., and Förster, H. 2015. Postharvest strategies for managing *Phytophthora* brown rot of citrus using potassium phosphite in combination with heat treatments. *Plant Dis.* 99:1477-1482.

Phytophthora brown rot, caused by several species of *Phytophthora*, is an economically important disease of citrus in areas with rainfall during the late stages of fruit development. Recent export restrictions of California orange fruit to China due to the presence of brown rot caused by the quarantine pathogen *Phytophthora syringae* have mandated more rigorous disease management. We evaluated postharvest applications with the phosphonate fungicide potassium phosphite in combination with heat treatments. In timing studies, potassium phosphite at 1,500 µg/ml was most effective when applied within 18 h after inoculation of orange fruit with *P. citrophthora*, reducing the incidence of decay by >96% as compared with the control. Potassium phosphite was also highly effective in inoculations with *P. syringae*. Heated water treatments at 60°C were consistently and highly effective in reducing the incidence of brown rot after inoculation with *P. citrophthora*, whereas treatments at 55 or 50°C were more variable and generally less effective. Two-stage

treatments of fruit were conducted in the laboratory to simulate current packinghouse practices and to evaluate any interaction of the efficacy of potassium phosphite with treatments of two commonly used post-harvest fungicides (i.e., imazalil and thiabendazole [TBZ]) or a post-harvest carnauba-based fruit coating. In these studies, an aqueous imazalil-potassium phosphite (2,000 µg/ml) dip at ambient temperature that was followed by a spray treatment of imazalil and TBZ prepared in fruit coating significantly reduced the incidence of brown rot from the control. When the aqueous dip was applied at 54°C, brown rot developed in only 1% of the fruit as compared with 76% in the water control. The efficacy of potassium phosphite was also demonstrated in commercial packinghouse treatments. Based on our research, this fungicide was registered for postharvest use against brown rot of citrus and is exempt from tolerance in the United States and many other countries.

Phytophthora brown rot is an economically important disease of all citrus species in areas with rainfall during the late stages of fruit development (Brown and Eckert 2000; Graham and Menge 2000). In California, the disease is mainly observed in the winter Navel orange harvest season, when most of the annual rainfall occurs. Fruit with brown rot have a distinctive pungent, aromatic odor, and the rind has an olive-brown discoloration (Brown and Eckert 2000). Fruit remain firm and leathery but they may be invaded by secondary pathogens such as *Penicillium* and *Geotrichum* spp. that cause rapid decay. Infected fruit usually fall to the ground. At very high humidity, they become covered by delicate, white fungal growth. Losses from brown rot may occur in the orchard or as postharvest decays. One of the most serious aspects of the disease is when fruit are infected before harvest and do not show symptoms. If infected fruit get mixed with healthy fruit, the disease may spread quickly in storage and during transit by nesting, resulting in many fruit becoming infected. In addition, brown rot-infected fruit are readily colonized by other decay pathogens.

The main species of *Phytophthora* occurring on citrus in California are *Phytophthora parasitica* Dastur (syn. *P. nicotianae* Breda de Haan), *P. citrophthora* (R. E. Sm. & E. H. Sm.) Leonian, *P. syringae* (Kleb.) Kleb., and *P. hibernalis* Carne. These pathogens all cause brown rot of fruit and some cause root rot, foot rot, or trunk cankers (e.g., gummosis) (Erwin and Ribeiro 1996). They are found in all growing regions of the state, with *P. hibernalis* the least frequently isolated. The relative importance of these species varies with time of the year. *P. parasitica* is most prevalent from late spring to early fall, whereas the other species are more abundant in the cooler seasons (Dirac et al. 2003).

The *Phytophthora* pathogens persist in the soil, in decaying roots, or in fallen, diseased fruit. Free water is required for production and dissemination of inoculum and for infection of the host. Sporangia develop within 18 h from germinating chlamydospores or oospores or from mycelium. Species with caducous sporangia may have their sporangia carried by wind-driven rain to foliage and fruit in the canopy. Zoospores, the most important infective propagules for species with either caducous or noncaducous sporangia, are released into films of water. Zoospores may then be splashed by rain or irrigation onto low-hanging fruit. At temperatures between 14 and 23°C, a continuous wetness period of 3 h is necessary for fruit infection (Eckert and Eaks 1989).

Recently, brown rot has become a quarantine issue with one of California's main trading partners. Specifically, some species of *Phytophthora* such as *P. syringae* occur in California but have not been reported in China. To maintain trade with this market, effective brown rot management programs are required that reduce the amount of disease to below detection levels.

A cultural practice to reduce losses from brown rot is removing lower tree branches ("tree-skirting"), where most of the fruit infections occur, or waiting for infected low-hanging fruit to drop (Eckert and Brown 2000; Phillips et al. 1990). Others have suggested not harvesting low-hanging fruit or diverting these fruit to domestic or local markets (Adaskaveg et al. 2015). Additional cultural methods include proper irrigation, mowing of ground vegetation to reduce wetness periods, and maintaining adequate soil drainage (Eckert and Brown 2000). Chemical control of citrus brown rot historically has been done with field applications of Bordeaux mixture (copper sulfate and hydrated lime) or fixed or neutral coppers (e.g., copper hydroxide or copper oxide) mixed with hydrated lime prior to rainy periods during the harvest season (Adaskaveg et al. 2014; Klotz 1978; Oren and Solel 1978). Additional fungicide field treatments have been evaluated over the years (Cohen 1981; Cohen and Coffey 1986) and, currently, phosphonate (e.g., fosetyl-Al; potassium phosphite and calcium phosphite) and phenylamide (e.g., mefenoxam) fungicides are registered as soil and foliar or soil treatments only, respectively, of citrus trees in the United States (Adaskaveg et al. 2014).

Corresponding author: J. E. Adaskaveg; E-mail: jim.adaskaveg@ucr.edu

Accepted for publication 26 May 2015.

These applications can be very effective and can provide control of brown rot infections for several weeks after treatment. Soil treatments reduce inoculum, whereas foliar field applications directly protect fruit from infection. To potentially increase the level of brown rot control in an integrated approach, to prevent the spread of brown rot to healthy fruit in storage, and to inhibit incipient or quiescent infections of apparently healthy fruit from developing into decay during fruit storage, transportation, and marketing, we evaluated the efficacy of postharvest treatments. These could be especially valuable when preharvest treatments were not applied.

Postharvest treatments for citrus brown rot control have been evaluated previously by others in some countries but have never been pursued for registration in the United States until very recently. In the 1980s and 1990s, the phosphonate fungicide fosetyl-Al (Gutter 1983; Tuset and Portilla 1990) and the phenylamide metalaxyl (Cohen 1981; Cohen and Coffey 1986) were shown to reduce the incidence of decay. In assays with zoospore inoculum, pre- and post-infection activity of fosetyl-Al was demonstrated (Tuset and Portilla 1990). Additionally, postharvest treatments with heated water were investigated previously as a management strategy (Fawcett 1936; Klotz and DeWolfe 1961) but these were not utilized commercially due to their long exposure times of several minutes at the temperatures used. The objective of our study was to evaluate the phosphonate potassium phosphite in combination with heat treatments for use in an integrated citrus postharvest brown rot management strategy and to expand on our preliminary investigations (Förster et al. 2013). Laboratory studies were conducted to optimize treatment parameters, and packingline tests were done to evaluate efficacy under commercial conditions. Phosphonate fungicides are exempt from residue tolerance in the United States and many other countries, and several formulations of potassium salts recently received postharvest registrations.

Materials and Methods

Fungal isolates, culture, and zoospore production. Isolates of *P. citrophthora* (isolates 4569, 4572, 4866, and 4867) and *P. syringae* (isolates 4868, 4876, 4879, and 4884) obtained from Navel orange fruit with brown rot symptoms at different locations in California were cultured on 10% clarified V8 agar (Ribeiro 1978) at 25°C (*P. citrophthora*) or 20°C (*P. syringae*). Stock cultures were stored in liquid nitrogen. For zoospore production, approximately 50 mycelial agar plugs (5 mm in diameter) from 5- to 7-day-old cultures were placed into a 10-cm petri dish, 15 ml of 10% clarified V8 broth was added, and plates were incubated at 25°C (*P. citrophthora*) or 20°C (*P. syringae*) for 2 days in the dark to initiate new growth. Mycelial plugs were then washed three times with sterile distilled water, 15 ml of sterile water was added, and plates were incubated under fluorescent lights (2,200 lux; Daylight Full Spectrum, 5,000 K, 40W; Osram Sylvania, Danvers, MA) for 2 days for sporangial development under the same temperature conditions. To induce zoospore release, plates were incubated for 20 min at 4°C and then at 25°C. Zoospores were released within 30 min, and the concentration was adjusted to 2×10^4 to 6×10^4 zoospores/ml.

In vitro toxicity of potassium phosphite against *Phytophthora* spp. The direct toxicity of potassium phosphite against mycelial growth of *P. citrophthora* and *P. syringae* was evaluated in agar dilution tests. Potassium phosphite (Prophyt; Helena Chemical Co., Collierville TN; 34.3% phosphorous acid) was added to molten, cooled 10% clarified V8 agar to obtain final concentrations of 0 (control), 1, 2, 5, 10, 25, 50, 100, 500, or 4,000 µg/ml. Mycelial agar discs (6 mm in diameter) from 5- to 10-day-old cultures of *P. citrophthora* or *P. syringae* were placed at the center of each of three replicate petri dishes for each concentration. After incubation for 4 days at 20°C, radial growth was determined. Percent inhibition in the presence of potassium phosphite was calculated as compared with the control for each isolate.

Efficacy of potassium phosphite applied at selected times after inoculation for control of brown rot of orange fruit caused by *P. citrophthora* in laboratory studies. Navel or Valencia orange (*Citrus sinensis* L. Osbeck) fruit from the University of California, Riverside field station that were not field treated with fungicides were

washed with tap water and placed into commercial fruit trays in plastic boxes with 24 fruit per box and four replicate boxes per treatment. Fruit were inoculated by placing a 20-µl drop of zoospores (2×10^4 to 6×10^4 /ml) of *P. citrophthora* onto the surface. After inoculation, a fruit tray misted with water was placed upside down onto the fruit to provide high humidity conditions, and fruit were incubated at 20°C for 6, 18, 24, or 30 h. Potassium phosphite at 1,500 µg/ml was applied as aqueous 15-s dips. Control fruit were dipped in water. Fruit were then returned to the plastic boxes, incubated at 20°C, and evaluated for the incidence of brown rot after 7 to 8 days. This experiment was done twice.

Efficacy of potassium phosphite for control of brown rot of orange fruit caused by *P. syringae* in laboratory studies. Most procedures were similar to the experiment with *P. citrophthora* described above, except that fruit were adjusted to 12°C before inoculation with zoospores of *P. syringae* isolate 4884 (2×10^4 to 4×10^4 /ml). Inoculum was applied to wounds (2 by 2 by 2 mm) and fruit were incubated at 12°C. Fruit were dip treated after 24 or 48 h using potassium phosphite at 2,000 µg/ml or water (control), continued to be incubated for 21 days, and were evaluated for the incidence of brown rot. In the two repeated experiments, Valencia orange fruit were used.

Efficacy of heated water treatments for control of brown rot of orange fruit caused by *P. citrophthora* in laboratory studies. Navel or Valencia orange fruit were prepared and inoculated as described above and treated after 20 h by dipping in water at ambient temperature (24 to 29°C) or at 50, 55, or 60°C. Temperatures of the treatment solutions were measured before and after dipping each of the four replications and were readjusted once a decrease of more than 7°C occurred. Thus, temperatures indicated are the approximate average for the four replications. Fruit were then continued to be incubated at 20°C until evaluation for decay. For the two repeated experiments, Navel or Valencia orange fruit were used.

Efficacy of potassium phosphite in combination with other postharvest fungicide and fruit coating treatments for control of brown rot caused by *P. citrophthora* in laboratory studies. Navel or Valencia orange fruit were treated 20 h after inoculation. Two-stage treatments were conducted to simulate current packinghouse practices. In the first study, the primary treatment was an aqueous 15-s dip in potassium phosphite at 2,000 µg/ml, imazalil (Deccoil; DECCO US Post-Harvest, Inc., Monrovia, CA) at 1,000 µg/ml, a mixture of these two compounds, or water (control). All treatments were done at ambient temperature but the mixture was also applied at an average temperature of 54°C. Fruit were then air dried (simulating sponge roller drying on a packingline), and the inoculated side was sprayed or not sprayed with a mixture of imazalil at 1,000 µg/ml and thiabendazole (TBZ; Decco Salt Number 19; DECCO US Post-Harvest, Inc.) at 3,500 µg/ml that was prepared in a carnauba-based packing fruit coating (Citrus-Brite 330; DECCO US Post-Harvest, Inc.) using a hand sprayer. In the second study, fruit were treated 24, 36, or 48 h after inoculation with potassium phosphite at ambient temperature or a staged application was done with a mixture of potassium phosphite at 2,000 µg/ml and imazalil at 1,000 µg/ml at an average temperature of 57°C as the primary dip treatment and a mixture of imazalil at 1,000 µg/ml and TBZ at 3,500 µg/ml prepared in the carnauba coating as the secondary treatment. Fruit were then incubated as described above. There were four replications of 20 to 24 fruit; Valencia fruit were used for the first study and Navel fruit for the second study and each study was repeated.

Evaluation of postharvest treatments for management of citrus brown rot caused by *P. citrophthora* in commercial packingline studies. In commercial packingline studies with Navel orange, experimental fruit inoculated approximately 20 h before treatment were treated alongside commercial fruit on rotating roller beds. Experimental fruit that were marked with a felt pen for easy retrieval among commercial fruit were placed on the packingline just before an 11-s flood treatment with a heated (52 to 55 or 55 to 58°C for the first and second study, respectively) fungicide solution consisting of imazalil at 1,000 µg/ml with or without potassium phosphite (Fungi-Phite; Plant Protectants, LLC, Visalia, CA) at 500 to 4,000 µg/ml. Between treatments with different rates of

potassium phosphite, the packingline was run for 30 min to allow equilibration of the application system. Fruit then moved over a sponge roller bed to remove excess liquid, were spray treated with imazalil at 1,000 µg/ml and TBZ at 3,600 µg/ml in a carnauba-based packing fruit coating (Citrus-Brite 330), and dried by heat exposure. Control fruit were left untreated. Fruit were then incubated in a packinghouse storage room for 8 days at 20°C and 85 to 90% relative humidity, and evaluated for the incidence of brown rot. For each treatment, there were eight replications of 24 fruit (first study) or 10 replications of 100 fruit (second study).

Data analysis. All experiments were done at least twice. For calculating 50% effective concentration (EC₅₀) values, percent inhibition as compared with the control was first determined for each potassium phosphite concentration using average values from the two repeated experiments. EC₅₀ values were determined by fitting regression lines of the natural log-transformed phosphite concentrations against the logit-transformed percent inhibition as compared with the nonamended control for each isolate (SAS ver. 9.4; SAS Institute, Cary, NC). Regression equations were then solved for concentration at 50% inhibition.

For studies on the comparative efficacy of postharvest treatments in the laboratory and packinghouse, data for brown rot incidence were arcsine transformed. Bartlett's test for homogeneity of variances was performed for repeated experiments. In each study, variances were homogeneous ($P < 0.05$) and, thus, combined data sets were analyzed using a balanced one-way treatment structure. Transformed values were analyzed using general linear model or analysis of variance (ANOVA) and least significant difference mean separation procedures of SAS.

Regressions of timing of application after inoculation or treatment temperature on mean percent incidence of decay or reduction in decay as compared with the control were done using SAS. Reduction in decay incidence was first normalized for each experiment by dividing the incidence value of each treatment timing or temperature by the decay incidence of the nontreated control. For comparison of midpoint and slope of regression lines, the general linear model procedure of SAS was used.

Results

In vitro toxicity of potassium phosphite against *Phytophthora* spp. EC₅₀ values for mycelial growth for *P. citrophthora* were determined as 5.9, 7.7, 8.1, and 7.8 µg/ml for isolates 4569, 4572, 4866, and 4867, respectively, with an average of 7.4 µg/ml. For *P. syringae*, values were 53.1, 9.2, 10.6, and 9.6 µg/ml for isolates 4868, 4876, 4879, and 4884, respectively, with an average of 20.6 µg/ml.

Efficacy of potassium phosphite applied at selected times after inoculation for control of brown rot of orange fruit caused by *P. citrophthora* in laboratory studies. The effect of treatment time after inoculation on incidence of brown rot was linear, significant ($P \leq 0.05$), and had a high r^2 value of 0.902 (Fig. 1). Treatments with potassium phosphite at 1,500 µg/ml that were applied within 18 h of inoculation were highly and consistently effective, reducing the incidence of brown rot from 58.9 or 93.2% in the water control to an average of 2.1% or less in the two experiments. Average decay incidence was 7.7 and 17.2% for the 24- and 30-h treatment timings, respectively. Variability among replications and experiments was higher for the 24- and 30-h timings than for the earlier timings (Fig. 1, standard error bars).

Efficacy of potassium phosphite for control of brown rot of orange fruit caused by *P. syringae* in laboratory studies. Brown rot only developed when fruit were inoculated into wounds but not when inoculum was placed onto nonwounded fruit. The incidence of decay in the water control was 73.6% for the 24-h inoculation-treatment timing and 81.6% for the 48-h timing. No decay was observed on any of the fruit treated with potassium phosphite (2,000 µg/ml).

Efficacy of heated water treatments for control of brown rot of orange fruit caused by *P. citrophthora* in laboratory studies. The effect of temperature on reduction of decay followed a linear regression ($r^2 = 0.996$, $P \leq 0.038$; Fig. 2). Heated 15-s dip treatments with water reduced the incidence of brown rot from that of the control, where 69.8 or 93.2% of the inoculated fruit developed decay in the

two experiments, respectively. Brown rot incidence was reduced by an average of 96.7% using a water temperature of 60°C but only by an average of 58.7 and 12.0% at 55 and 50°C, respectively. The 60°C treatment was consistently effective, whereas treatments at lower temperatures were more variable in their effectiveness (Fig. 2, standard error bars).

Efficacy of potassium phosphite in combination with other postharvest fungicide and fruit coating treatments for control of brown rot caused by *P. citrophthora* in laboratory studies.

Two-stage treatments of fruit were conducted to simulate current packinghouse practices and to evaluate any effect on the efficacy of potassium phosphite of subsequent treatments of two commonly used postharvest fungicides (i.e., imazalil and TBZ) and a postharvest carnauba-based fruit coating. The model of the ANOVA was highly significant ($P < 0.0001$). In these studies, a treatment with an aqueous imazalil dip at ambient temperature (i.e., stage 1) that was followed by a spray treatment of imazalil and TBZ prepared in fruit coating

Fig. 1. Effect of treatment time after inoculation on the efficacy of potassium phosphite (1,500 µg/ml) in managing brown rot of orange fruit in laboratory studies. Fruit were inoculated with zoospores of *Phytophthora citrophthora*, incubated at 20°C for selected times, and dip treated for 15 s. The incidence of fruit decay was evaluated after 7 to 8 days at 20°C. Vertical bars represent standard errors for each timing from data of the two repeated experiments.

Fig. 2. Regression of reduction in the incidence of brown rot of orange fruit on temperature of water dip treatments in laboratory studies. Fruit were inoculated with zoospores of *Phytophthora citrophthora*, incubated for 20 h at 20°C, and dip treated at ambient temperature (24 to 29°C) or at 50, 54, or 60°C for 15 s. Fruit decay was evaluated after 7 to 8 days at 20°C and the reduction in incidence by heated treatments as compared with the ambient treatment was calculated. Vertical bars represent standard errors for each temperature from data of the two repeated experiments.

(i.e., stage 2) had no effect on brown rot development and was not significantly different from the control (Table 1). In contrast, a dip application with potassium phosphite at 2,000 µg/ml at ambient temperature significantly reduced the incidence of brown rot from 76% in the nontreated control to 9.4% (Table 1). When potassium phosphite was mixed with imazalil and an imazalil-TBZ-fruit coating application was used in the stage-2 treatment, efficacy with 19.8% decay incidence was statistically similar to potassium phosphite alone; however, there was a trend for reduced effectiveness. When the latter combination treatment was applied at 54°C, brown rot developed in only 1% of the fruit.

To further characterize treatment efficacy of potassium phosphite under simulated packinghouse conditions, another timing study was done. Using potassium phosphite at 2,000 µg/ml at ambient temperature or the staged treatment (aqueous potassium phosphite-imazalil followed by imazalil-TBZ in fruit coating) at 57°C, the decrease in efficacy over application time after inoculation (i.e., 24, 36, or 48 h) could be described as linear regressions with significant models ($P < 0.01$) and high r^2 values (Fig. 3). Linear regressions were similar and they did not differ in midpoint ($P = 0.398$) or slope ($P = 0.434$). As with the treatment timing study with potassium phosphite at 1,500 µg/ml that was described above, standard errors were lower in the 24-h treatment than for the later timings. Efficacy of both treatments was similar for each timing with a 95.2, 79.5, and 41.2% reduction in brown rot as compared with the control for the potassium phosphite treatment and a 98.7, 81.3, and 41.8% reduction for the staged treatment for timings of 24, 36, and 48 h, respectively. Control fruit and fruit treated with the heated, staged fungicide application 24 h after inoculation are shown in Figure 4.

Evaluation of postharvest treatments for management of citrus brown rot caused by *P. citrophthora* in commercial packingline studies. In two studies with staged floodler and fruit coating applications, a heated (average temperatures of 53.5 or 56.5°C) floodler treatment with imazalil significantly reduced the incidence of brown rot of inoculated fruit, and this treatment was especially effective in the second study (Table 2). Potassium phosphite at 500 µg/ml mixed with imazalil was used in the first trial and was not effective; whereas an application with 1,000 µg/ml significantly reduced the incidence of brown rot from 45.0% in the control to 8.8%. In the second study, the heated imazalil treatment was similarly effective to the potassium phosphite-imazalil mixture at 2,000 µg/ml (Table 2). The potassium phosphite-imazalil treatment at 4,000 µg/ml resulted in the lowest incidence, with 3%, as compared with 29.2% brown rot in the control.

Discussion

Phytophthora diseases are a continuous challenge during wet environmental conditions in all citrus-growing areas worldwide. Cultural and preharvest chemical management practices have been established over the years to minimize losses (Adaskaveg et al. 2014; Klotz 1978). However, recent export restrictions of California orange fruit to China, one of the major markets, due to the presence of brown rot caused by *P. syringae*, has mandated more rigorous disease management. Incidence levels below thresholds of detection are required

for a quarantine pathogen such as *P. syringae*. Thus, for the first time, postharvest chemical treatments were considered to manage brown rot in the United States. In the current study, we demonstrated the high efficacy of the phosphonate fungicide potassium phosphite and we optimized treatment strategies. Based on our research for brown rot management, this fungicide is now registered in the United States for postharvest use on citrus. In our initial screenings, additional newer oomycete-specific fungicides, including the quinone outside inhibitor azoxystrobin, the benzamide fluopicolide, and the carboxylic acid amide mandipropamid, all effectively reduced the development of brown rot when fruit were inoculated after treatment (Adaskaveg and Förster 2014; Förster et al. 2013). Only potassium phosphite, however, also showed postinfection activity.

The direct toxicity of potassium phosphite to *Phytophthora* spp. was demonstrated previously, and growth of *P. cinnamomi*, *P. palmivora*, and *P. parasitica* in a liquid medium amended with potassium phosphite at 8,000 µg/ml was inhibited by more than 90% (Smillie et al. 1989). In our studies, EC_{50} values were determined as a standard reference point for fungicide toxicity. Values for *P. citrophthora* and *P. syringae* ranged between 5.9 and 53.1 µg/ml, and higher variability in sensitivity was observed among isolates of *P. syringae*. More isolates, however, will have to be evaluated to obtain full baseline sensitivity ranges.

Although *P. syringae* currently is of more concern for the California citrus industry, *P. citrophthora* was used in most of our efficacy

Fig. 3. Regression of reduction in the incidence of brown rot of orange fruit on treatment time after inoculation in laboratory studies. Fruit were inoculated with zoospores of *Phytophthora citrophthora*; incubated for 24, 36, or 48 h at 20°C; and dip treated with potassium (K)-phosphite (2,000 µg/ml) at ambient temperature (24 to 29°C) or with imazalil-potassium phosphite at an average temperature of 57°C. The latter treatment was followed by a spray application of imazalil (1,000 µg/ml) and thiabendazole (3,500 µg/ml) in a carnauba-based fruit coating. Fruit decay was evaluated after 7 to 8 days at 20°C and the reduction in incidence was compared with the water-treated control. Vertical bars represent standard errors for each timing and treatment from data of the two repeated experiments.

Table 1. Efficacy of postharvest treatments for control of brown rot of orange fruit inoculated with *Phytophthora citrophthora* in staged, simulated packinghouse applications in the laboratory

Stage 1 treatment: 15-s aqueous dip ^x	Stage 2 treatment: spray with fungicides in fruit coating ^y	Incidence of brown rot (%) ^z
Water (control)	None	76.0 a
Imazalil 1,000 µg/ml	Imazalil 1,000 µg/ml + TBZ 3,500 µg/ml	81.3 a
K-phosphite 2,000 µg/ml	None	9.4 bc
K-phosphite 2,000 µg/ml + imazalil 1,000 µg/ml	Imazalil 1,000 µg/ml + TBZ 3,500 µg/ml	19.8 b
K-phosphite 2,000 µg/ml + imazalil 1,000 µg/ml at 54°C	Imazalil 1,000 µg/ml + TBZ 3,500 µg/ml	1.0 c

^x Treatments were applied 20 h after inoculation with *P. citrophthora* (20,000 zoospores/ml). K-phosphite = potassium phosphite.

^y Fungicides were mixed in a carnauba-based fruit coating and applied using a hand-sprayer after air-drying of the dipped fruit. TBZ = thiabendazole.

^z Incidence of brown rot was evaluated 8 days after inoculation and was based on the number of diseased fruit of the total number of fruit using four replications of 24 fruit for each treatment. Values followed by different letters indicate significant differences among treatments according to an analysis of variance ($P < 0.0001$) and least significant difference mean separation procedures ($\alpha < 0.05$).

studies with inoculated fruit. Both species are considered major causes of brown rot in the winter Navel orange harvest season (Eckert and Eaks 1989). Decay incidence after inoculation with *P. syringae*, however, was highly variable and injuries generally had to be provided. Moreover, fruit had to be incubated at 12°C to develop decay, and this fruit temperature would have made the evaluation of heated treatments difficult. Still, we demonstrated a similar high efficacy of potassium phosphite for controlling brown rot caused by *P. syringae* in repeated studies. Considering the lower cardinal temperatures of this pathogen as compared with *P. citrophthora* (Erwin and Ribeiro 1996), heated treatments should be very beneficial for both pathogens.

Fig. 4. Postharvest laboratory treatments of Valencia orange fruit for management of brown rot. **A**, Inoculated, water-treated control fruit and **B**, inoculated fruit after treatment with fungicides. Fruit were inoculated with zoospores of *Phytophthora citrophthora* and treated after 24 h at 20°C. Fruit decay was evaluated after 7 days at 20°C. Lines on fruit indicate inoculation sites.

Timing studies indicated that treatments with potassium phosphite at 1,500 µg/ml were highly effective when applied within 18 h after inoculation. Treatments after 24 or 30 h, however, were also effective and significantly reduced the incidence of brown rot from the control. Microscopic studies indicated that fruit penetration by germinating zoospores cysts of *P. citrophthora* occurs after 3 to 4 h at 20°C (H. Förster and J. E. Adaskaveg, unpublished). Thus, in the timings used for fruit treatments, infections are already at an advanced stage and the treatment effectiveness is noteworthy. In subsequent studies, treatments using 2,000 µg/ml were done 20 h after inoculation and incubation at 20°C. Under commercial conditions, fruit are picked in the morning and treated the same day or sometimes the following morning. Considering that most infections occur during a rainy period and fruit cannot be picked immediately thereafter due to potential rind damage, the time period from infection to postharvest treatment may be much longer than 20 h. Temperatures in the field during the winter, however, are generally much lower than 20°C, and this will slow the infection process and make postharvest treatments effective.

Heated (reservoir temperature 52 to 58°C) dip or drench applications of aqueous bicarbonate and fungicide solutions that are followed by a nonheated fungicide spray in a fruit coating are currently a common strategy in many California packinghouses. These treatments are followed by heated-air drying of fruit. We found heated water dip treatments for 15 s to be effective in reducing the development of brown rot, indicating that *Phytophthora* infections are very heat sensitive. This characteristic was deployed as a component of the postharvest management program. Temperatures of 60°C were most consistently and highly effective, whereas treatments at 55 or 50°C were more variable and generally less effective. Although temperatures of 60°C could potentially be accurately maintained in a commercial setting, packinghouses are reluctant to use this temperature because of potential phytotoxicity to the fruit rind. Heated-water treatments were evaluated previously by others. For example, Fawcett (1936) found that 2-min dip treatments at 49°C were effective even when done 30 h after inoculation, whereas Klotz and DeWolfe (1961) used 4-min exposures at 48°C to eradicate the pathogen in infected fruit after 30 h of incubation at 25°C or 60 h at 12°C. Incubating inoculated fruit at fluctuating grove temperatures, with averages of 12 to 15°C or 6 to 11°C, Schiffmann-Nadel (1969) found that water fruit dips at 48°C after 3 to 4 days or up to 7 days after inoculation, respectively, controlled the disease. Therefore, longer incubation times at lower temperatures can also be very effective; however, fruit rind injury such as oleocellosis may occur with these exposure times (e.g., 2 to 4 min at 48°C) if the rind is turgid (Eckert and Eaks 1989). Furthermore, timings longer than 15 s are not feasible in most packinghouses during the main harvest season when large amounts of fruit

Table 2. Efficacy of postharvest treatments for control of brown rot of orange fruit inoculated with *Phytophthora citrophthora* in staged, commercial packing-house applications

Experiment	Stage 1 treatment: aqueous flooder application ^x	Stage 2 treatment: spray with fungicides in fruit coating ^y	Incidence of brown rot (%) ^z
1	Control	None	45.0 a
	Imazalil 1,000 µg/ml	Imazalil 1,000 µg/ml + TBZ 3600 µg/ml	28.1 b
	K-phosphite 500 µg/ml + imazalil 1,000 µg/ml	Imazalil 1,000 µg/ml + TBZ 3600 µg/ml	37.5 ab
	K-phosphite 1,000 µg/ml + imazalil 1,000 µg/ml	Imazalil 1,000 µg/ml + TBZ 3600 µg/ml	8.8 c
2	Control	None	29.2 a
	Imazalil 1,000 µg/ml	Imazalil 1,000 µg/ml + TBZ 3600 µg/ml	5.9 b
	K-phosphite 2,000 µg/ml + imazalil 1,000 µg/ml	Imazalil 1,000 µg/ml + TBZ 3600 µg/ml	5.5 b
	K-phosphite 4,000 µg/ml + imazalil 1,000 µg/ml	Imazalil 1,000 µg/ml + TBZ 3600 µg/ml	3.0 b

^x Stage 1 treatments were applied as aqueous solutions in a heated flooder (average temperature 53.5°C in the first experiment and 56.5°C in the second experiment). Each treatment had 8 replications of 24 fruit each or 10 replications of 100 fruit each in the first and second experiment, respectively. K-phosphite = potassium phosphite.

^y Fungicides were mixed in a carnauba-based fruit coating and applied with a controlled droplet applicator after sponge roller drying of the treated fruit. TBZ = thiabendazole.

^z Incidence of brown rot was evaluated 7 days after inoculation and was based on the number of diseased fruit of the total number of fruit used. Values followed by different letters indicate significant differences among treatments according to an analysis of variance (experiment 1. $P < 0.004$ and experiment 2. $P < 0.0001$) and least significant difference mean separation procedures ($\alpha < 0.05$).

are being processed. Thus, our short-duration aqueous heat treatments that did not cause phytotoxicity can only be used in a multicomponent strategy that also involves the use of fungicides such as the phosphonates.

Additional laboratory studies were done to simulate current commercial practices and work toward a postharvest treatment that is effective against *Phytophthora* brown rot as well as *Penicillium* decays. For these latter decays, imazalil and TBZ have been industry standards for many years. In the staged postharvest applications, an aqueous imazalil dip that was followed by a spray treatment with imazalil and TBZ prepared in a fruit coating had no effect on reducing the development of brown rot. The addition of potassium phosphite to the imazalil dip resulted in a low incidence of decay, statistically similar to using potassium phosphite alone. In several experiments, we observed a trend to reduced efficacy when the acidic potassium phosphite was used in combination with an alkaline fruit coating application. This trend could be negated when applying the aqueous application at higher temperature.

In two commercial packinghouse studies, the incidence of brown rot of control fruit was relatively low (45.0 and 29.2%). Because fruit were inoculated in the laboratory, warm temperatures during transport to the packinghouse could have inactivated some of the infections. Heated flooders and spray treatments with imazalil and TBZ were more effective in reducing brown rot in the second study, likely because treatment temperature was higher in the second study. Potassium phosphite treatments significantly reduced the incidence of brown rot to low levels when used at 1,000 µg/ml or higher. Due to variable conditions in commercial applications such as treatment and fruit temperatures, as well as application timings after the infection period in the field, rates of 2,000 to 4,000 µg/ml are currently recommended.

Our data indicate that postharvest potassium phosphite applications can be effective for the management of brown rot of citrus fruit caused by *P. citrophthora* and *P. syringae* using existing packingline equipment. The efficacy against species such as *P. palmivora* and *P. parasitica* that cause brown rot in production areas with warmer climates (Timmer et al. 2000) will need to be evaluated. Because very low levels of disease have to be achieved for a quarantine pathogen, these treatments have to be part of an integrated management approach that includes orchard practices and preharvest fungicide applications. If heated postharvest treatments are used, reservoir and fruit temperatures as well as exposure times have to be carefully monitored. An integrated approach using pre- and postharvest practices minimizes fruit losses from decay in all steps of the postharvest handling chain and will aid in the international marketing of California fruit. As indicated above, potassium phosphite has an “exempt from tolerance” status in the United States but not in many export markets. The compound is labeled in the United States for use against green mold and brown rot. Rates required to obtain a reduction in green mold, however, are extremely high (i.e., 8,000 to 12,000 µg/ml; Cerioni et al. 2013) compared with rates needed for brown rot management. Efforts are underway for registration of potassium and calcium phosphites through the U.S. Interregional Program 4 (IR-4) to establish international maximum residue limits with our trade partners. Potassium phosphite is also currently being registered for pre- and postharvest use in China (J. E. Adaskaveg, personal communication).

Literature Cited

- Adaskaveg, J. E., and Förster, H. 2014. Integrated postharvest strategies for management of *Phytophthora* brown rot of citrus in the United States. Pages 123-131 in: *Post-harvest Pathology, Plant Pathology in the 21st Century*. D. Prusky and M. L. Gullino eds. Springer International Publishing, Cham, Switzerland.
- Adaskaveg, J. E., Förster, H., Hao, W., and Cary, D. 2015. Management of brown rot of citrus for exporting California oranges to China—From a chore to a necessity. *Citrograph* 6:36-40.
- Adaskaveg, J. E., Förster, H., and Mauk, P. A. 2014. Fungal diseases. Pages 307-326 in: *Citrus Production Manual*. L. Ferguson, and E. E. Grafton-Cardwell, eds. Agric. Nat. Resour. Publ. 3539. University of California, Richmond.
- Brown, G. E., and Eckert, J. W. 2000. Brown rot. Page 39 in: *Compendium of Citrus Diseases*, 2nd ed. L. W. Timmer, S. M. Garnsey, and J. H. Graham, eds. American Phytopathological Society, St. Paul, MN.
- Cerioni, L., Rapisarda, V. A., Doctor, J., Fikkert, S., Ruiz, T., Fassel, R., and Smilanick, J. L. 2013. Use of phosphite salts in laboratory and semicommercial tests to control citrus postharvest decay. *Plant Dis.* 97:201-212.
- Cohen, E. 1981. Postharvest control of *Phytophthora citrophthora* with metalaxyl and its relation to other fungi pathogenic to citrus fruit. *Proc. Int Soc. Citricult.* 2:793-796.
- Cohen, Y., and Coffey, M. D. 1986. Systemic fungicides and the control of Oomycetes. *Annu. Rev. Phytopathol.* 24:311-338.
- Dirac, M. F., Menge, J. A., and Madore, M. A. 2003. Comparison of seasonal infection of citrus roots by *Phytophthora citrophthora* and *P. nicotianae* var. *parasitica*. *Plant Dis.* 87:493-501.
- Eckert, J. W., and Brown, G. E. 2000. Brown rot. Page 39 in: *Compendium of Citrus Diseases*, 2nd ed. L. W. Timmer, S. M. Garnsey, and J. H. Graham, eds. American Phytopathological Society, St. Paul, MN.
- Eckert, J. W., and Eaks, I. L. 1989. Postharvest disorders and diseases of citrus fruits. Pages 179-260 in: *The Citrus Industry*, Vol. V. W. Reuther, E. C. Calavan, and G. E. Carman, eds. University of California, Division of Agriculture and Natural Resources, Oakland.
- Erwin, D. C., and Ribeiro, O. K. 1996. *Phytophthora Diseases Worldwide*. American Phytopathological Society, St. Paul, MN.
- Fawcett, H. S. 1936. *Citrus Diseases and Their Control*, 2nd ed. McGraw-Hill Book Co., New York.
- Förster, H., Vilchez, M., and Adaskaveg, J. E. 2013. Phosphonate, carboxylic acid amide, and benzamide treatments for pre- and postharvest management of citrus brown rot. (Abstr.) *Phytopathology* 103:S2.138.
- Graham, J. H., and Menge, J. A. 2000. *Phytophthora*-induced diseases. Pages 12-15 in: *Compendium of Citrus Diseases*, 2nd ed. L. W. Timmer, S. M. Garnsey, and J. H. Graham, eds. American Phytopathological Society, St. Paul, MN.
- Gutter, Y. 1983. Supplementary antimold activity of phosetyl-Al, a new brown rot fungicide for citrus fruits. *Phytopathol. Z.* 107:301-308.
- Klotz, L. J. 1978. Fungal, bacterial, and nonparasitic diseases and injuries originating in the seedbed, nursery, and orchard. Pages 1-66 in: *The Citrus Industry*, Vol. IV. W. Reuther, E. C. Calavan, and G. E. Carman, eds. University of California, Division of Agriculture and Natural Resources, Oakland.
- Klotz, L. J., and DeWolfe, T. A. 1961. Limitations of the hot water immersion treatment for the control of *Phytophthora* brown rot of lemons. *Plant Dis. Rep.* 45:264-267.
- Oren, Y., and Solel, Z. 1978. Control of brown rot of citrus fruit by application of fungicides via sprinkler irrigation system. *Phytoparasitica* 6:65-70.
- Phillips, P. A., O'Connell, N. V., and Menge, J. A. 1990. Citrus skirt pruning—A management technique for *Phytophthora* brown rot. *Citrograph* 44:6-7.
- Ribeiro, O. K. 1978. A Source Book of the Genus *Phytophthora*. J. Cramer, Vaduz.
- Schiffmann-Nadel, M. 1969. Research on *Phytophthora* diseases of citrus fruit in Israel. Pages 1201-1205 in: *Proc. 1st Int. Citrus Symp.* H. D. Chapman, ed. University of California, Riverside.
- Smillie, R., Grant, B. R., and 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.
- Timmer, L. W., Zitko, S. E., Gottwald, T. R., and Graham, J. H. 2000. *Phytophthora* brown rot of citrus: Temperature and moisture effects on infection, sporangium production, and dispersal. *Plant Dis.* 84:157-163.
- Tuset, J. J., and Portilla, M. T. 1990. Control of *Phytophthora* brown rot of citrus fruits. *Bull. OEPP/EPPO Bull.* 20:153-161.