BIOLOGY AND MANAGEMENT OF PHYTOPHTHORA CROWN AND ROOT ROT OF WALNUT

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ABSTRACT

Our objectives were to: 1) Evaluate elite Paradox hybrid clones for resistance to *Phytophthora citricola,* 2) Determine the cause for high incidence of crown and root rot in some Butte and Glenn County orchards on Paradox walnut rootstock, and 3) Determine efficacy of phosphonate injections for management of *P. cinnamomi* and *P. citricola* on walnut. The greenhouse screen proposed in Objective 1 will not be completed until spring 2006. Trees for a field evaluation of resistance to *P. citricola* were planted in May 2005 at Armstrong Tract, Department of Plant Pathology, UC Davis. The trial includes nine clonal hybrid selections: AX1, GZ1, PX1, and WIP3 (susceptible to *P. citricola* in greenhouse trials) and RX1, AZ2, VX211, NZ1, and JX2 (partially resistant to *P. citricola* in greenhouse trials). Northern California black walnut and Chinese wingnut were included in the field trial as highly susceptible and highly resistant standards, respectively. Each rootstock was planted in 12 replicate plots, six to be infested with *P. citricola* and six to remain non-infested as a control. Results from this trial are anticipated in 2006. As part of Objective 2, we tested pathogenicity of *P. parasitica* and an unidentified *Phytophthora* sp. on Paradox hybrid and Northern California (NCa) black walnut seedlings in a greenhouse. The test isolates were from orchards affected by crown and root rot on Paradox rootstock in Glenn and Butte Counties. Isolates of *P. cinnamomi* and *P. citricola* (both highly aggressive on walnut) were included as standards. Neither *P. parasitica* nor the unidentified *Phytophthora* sp. caused much disease on Paradox hybrid or NCa black walnut seedlings in the greenhouse, whereas both *P. cinnamomi* and *P. citricola* caused severe crown and root rot. The greenhouse results, which indicated little aggressiveness in *P. parasitica* and the unidentified *Phytophthora* sp. on walnut, and our field sampling results, which have included infrequent isolation of *Phytophthora* spp. from the orchards affected by crown and root rot, do not provide conclusive evidence that *Phytophthora* species are the primary cause of tree losses in the surveyed under Objective 2. Factors complicating the investigation include repeated grower applications of phosphonate in the affected orchards, significant populations of ring and lesion nematodes in at least one of the orchards, and apparent subsidence of tree losses in the last year. Our phosphonate injection trial (Objective 3) became a phosphonate chemigation and spray trial, because the 6th-leaf walnut trees designated for the research did not take on injected material at acceptable rates. Trunk injection attempts in spring and summer 2005 were unsuccessful. The modified trial includes four treatments: (1) a non-treated control; (2) three chemigations with Fosphite® (J.H. Biotech, Ventura, CA), each at 3 quarts per acre; (3) one foliar spray with Fosphite at 3 quarts per acre; and (4) three chemigations plus one foliar spray (combination of treatments 1 and 2). Treatments 2, 3, and 4 with phosphonate suppressed canker development caused by *P. citricola* by 41, 59, and 70%, respectively, compared to the non-treated control. Additional inoculations with *P. citricola* in 2006 will evaluate persistence of the fall 2005 treatments. Phosphonate treatments appear promising for walnut.

INTRODUCTION

Crown and root rots caused by species of *Phytophthora* are among the most serious diseases of walnut worldwide. In California, more than 10 species of *Phytophthora* have been implicated in the diseases, but *P. cinnamomi* and *P. citricola* were determined to be the most virulent (Mircetich et al., 1998).

There has been continued interest in comprehensive evaluation of Paradox hybrids for resistance to *Phytophthora* spp. and other desirable traits. Paradox is more resistant than Northern California (NCa) black or English seedling rootstocks to most *Phytophthora* spp. Although Chinese wingnut is the only walnut family member known as highly resistant to *P. cinnamomi*, it is not graft compatible with all English walnut cultivars and has other potential limitations (i.e., suckering, unknown yield efficiency). Paradox hybrids available from commercial nurseries are diverse, involving crosses between one or more species of black walnut and *J. regia* (Potter et al., 2002), and results of greenhouse experiments suggested that the diversity among Paradox hybrids may include important variation in resistance to *P. citricola* (G.T. Browne, *unpublished*). Development and application of propagation and acclimatization technology by Wes Hackett and the Walnut Improvement Program (WIP) provided rooted hybrid clones from previous selections made by Browne, the WIP, and McKenry.

Here we report on: (1) ongoing evaluations of resistance to *P. citricola* in Paradox hybrid clones, (2) investigations concerning losses of trees due to crown and root rot in Glenn and Butte County orchards, and (3) new research on efficacy of phosphonate treatments for control of Phytophthora crown rot.

OBJECTIVES

1) Evaluate elite Paradox hybrid clones for resistance to *Phytophthora citricola.*
2) Determine the cause for high incidence of crown and root rot in some Butte and Glenn County orchards on Paradox walnut rootstock.
3) Determine efficacy of phosphonate treatments for management of *P. cinnamomi* and *P. citricola* on walnut.

PROCEDURES

Objective 1. Evaluations of resistance to *P. citricola* in hybrid clones.

Greenhouse trials. Clonal walnut hybrids were selected from seed families. During 1997 to 1999, a total of more than 75 Paradox hybrid seed families, obtained from commercial nurserymen in California and the WIP, were planted and grown in a greenhouse. After germination and growth for 2 to 4 months, the majority of the seedlings were screened for resistance to *P. citricola* in pots of artificially infested soil. In each screen, the plants were subjected to biweekly 48-h periods of flooding for 3 months and then assessed for severity of crown and root rot. Seed families of genetic interest that had insufficient numbers of plants for screening were preserved in pots. Many individual clones of Paradox hybrids were preserved and multiplied as micro shoots, either due to their putative resistance to *P. citricola* in the screens described above or due to their unique genetic background (G.T. Browne, *unpublished*).

Selected genotypes of the micro shoots described above, as well as additional clones from the WIP (WIP3 and PX-1), were multiplied further, rooted in micro culture, transplanted to pots and acclimatized, and grown for at least 3 months in a greenhouse in 4 x 20 cm cones (Hackett et al., *unpublished*). After growth in the greenhouse, the plants were run through a cycle dormancy induced by cold storage (6 °C, 3 to 5 months). When timed properly, the chilling improves subsequent plant vigor and suitability for resistance evaluations with *Phytophthora*. Plants to be screened in 2005 were removed from chilling in fall 2004, which was considered suboptimal for subsequent plant growth (lighting in the greenhouse was poor in winter) but necessary to avoid plant degeneration during prolonged storage. The plants were not considered to be vigorous enough for screening in spring 2005 and are currently receiving natural chilling outside. These plants, as well as an additional set of rootstock clones propagated in 2005, will be screened for resistance to *P. citricola* in the greenhouse in spring 2006 using procedures described previously (Browne et al., 2004 Report to the Walnut Marketing Board).

Field trial. Trees for a field evaluation of resistance to *P. citricola* were planted in May 2005 at Armstrong Tract, Department of Plant Pathology, UC Davis. The trial includes nine clonal hybrid selections: AX1, GZ1, PX1, and WIP3 (susceptible to *P. citricola* in greenhouse trials) and RX1, AZ2, VX211, NZ1, and JX2 (partially resistant to *P. citricola* in greenhouse trials) (Table 1). NCa black walnut and Chinese wingnut were included in the field trial as highly susceptible and highly resistant standards, respectively. Each rootstock was planted in 12 replicate plots, six to be infested with *P. citricola* and six to remain non-infested as a control.

Objective 2. Determining etiology of the crown and root rot on Paradox hybrid rootstock.

Isolates of *Phytophthora* associated with the death of English walnut trees on Paradox walnut rootstock in five orchards sampled repeatedly in Butte and Glenn Counties (Browne et al., Walnut Marketing Board Report, 2004) were confirmed as *P. parasitica* and an unidentified *Phytophthora* sp., based on morphological traits. Pathogenicity of two of the isolates of *P. parasitica* (each from a different orchard in Glenn County) and one isolate of the unidentified *Phytophthora* sp. were evaluated in a greenhouse trial. For use in the trial, seeds of paradox hybrid and NCa black walnut rootstock were stratified, grown in 10-cm-diameter pots of UC potting soil mix (UCM). Two months after planting, the seedlings were transplanted into 2-liter pots (one plant per pot) filled either with UCM artificially infested with one the test isolates of *Phytophthora* (40 ml of infested V8 juice-oat medium per liter UCM) or with non-infested UCM (40 ml of sterile V8 juice-oat medium per liter UCM). There were seven replicate plants per rootstock for each isolate of *Phytophthora* and the control. The experiment was arranged in a split plot design with the inoculum treatments randomized in complete blocks of main plots and the rootstocks randomized in subplots. All plants were subjected to 48 h of soil flooding once every 2 weeks. Weekly visual disease ratings were assigned using a 0 to 5 scale (0=healthy to 5=plant dead; 1, 2, 3, and 4=gradations in between), starting 1 month after transplanting. The disease ratings were summed for each plant and used as an area under the disease progress curve (AUDPC). At the end of the experiment, the roots were washed free from the soil, and the extent of root and crown rot was determined. Root disease ratings included the percentage of root crown length affected by crown rot (measured) and the percentage of roots rotted (visual estimates). Roots were considered to be rotted when they were necrotic in the stele (center portion of root) as well as in the cortex (outer portion of root).

Excised shoot segments from rootstock plants were used for a secondary assessment of pathogenicity of *P. parasitica* and the unidentified *Phytophthora* sp. on walnut. Current season's shoots were collected from each rootstock in the Armstrong Tract rootstock trial (Objective 1). Shoot samples from each rootstock were cut into 20-cm segments and wound inoculated with the isolates of *Phytophthora* used in the greenhouse-based pathogenicity test described above. Each shoot segment was prepared for inoculation by removing a 3-mm-diameter disk of bark with a cork borer and then inoculated on the wound with a V8 juice agar disk colonized by one of the isolates of *Phytophthora* or with a sterile V8 juice agar disk (the control). The inoculated wounds were wrapped with electrical tape and incubated in humid chambers. There were seven shoots per inoculant, randomized in complete blocks. One week after inoculation, the length of necrosis in the bark was measured for each shoot.

In Nov 2005, soil and root samples were collected again from one of the Glenn County orchards visited in 2004. On the day dead and dying trees were removed with a backhoe, soil and root samples were collected from them. Each of the samples was split and subjected to: (1) procedures to detect *Phytophthora* spp. (pear baiting and bark and root culturing in PARP semi-selective medium), and (2) extractions to detect nematodes (sugar floatation for soil, mist chamber extraction for roots; Westerdahl Lab).

Objective 3. Determining efficacy of phosphonate treatments.

A walnut orchard planted at Campbell Tract by Terry Prichard in 2000 was used to evaluate efficacy of phosphonate treatments for management of Phytophthora crown rot. The western half of the orchard was used for the 2005 trial reported here, and the eastern half will be used for a proposed repeat of the experiment in 2006. Trunk injection treatments were attempted in the orchard using a Sidewinder tree injector (http://www.treeinjectors.com) in spring and summer 2005. The injections were unsuccessful; regardless of experimental variations in injection technique, unacceptably high pressure was required to drive liquid into the xylem and resulted in wood splitting and bark separation at the cambium. Interestingly, almond tree trunks accepted injections easily.

The phosphonate trial was redesigned to evaluate phosphonate chemigation and spray treatments instead of phosphonate trunk injection treatments. Using a split-split plot design, phosphonate chemigation treatments were applied through microsprinklers to soil around trees in randomly selected main plots. The mainplots were 16-tree rows served by dedicated irrigation line with one microsprinkler per tree (Bowsmith full circle, 10-foot diameter pattern, 5.7 gallons per hour, head placed 3 ft. from the tree trunk). Phosphonate sprays were applied to the foliage of trees in randomly selected subplots (multiple pairs of trees within each 16-tree row). The design was factorial, resulting in four treatments:

1. Non-treated/water control
2. Phosphonate chemigation alone
3. Phosphonate spray alone
4. Phosphonate chemigation + spray combination

The phosphonate chemigation treatment consisted of applications Fosphite on 29 Aug, 6 Sep, and 12 Sep 2005. Each application injected Fosphite® (J.H. Biotech, Ventura, CA) at 3 quarts per acre during the first 45-minutes of a 24-hr irrigation using the resident microsprinkler system. Control plots for the phosphonate chemigation treatment (16-tree rows) received the

same amount of water, without Fosphite, through microsprinklers. The foliar spray treatment consisted of one application of Fosphite at 3 quarts per acre in 100 gallons of water per acre on 12 Sep 2005. The spray was applied with a backpack sprayer to wet all aboveground parts of the trees, and care was used to avoid spray drift to adjacent control trees, which received no treatment.

One month after the completion of the phosphonate treatments (7 Oct 2005), eight trees per treatment (four for each rootstock) were wound inoculated on one side of the trunk with a 1-cm x 1-cm V8 juice agar square colonized *P. citricola* and on the other side of the trunk with a sterile square of V8 juice agar (the inoculation control). The inoculations occurred about 1 ft. above the soil surface, roughly 6 inches above the graft union. A 1-cm-wide chisel was used to remove a 1-cm x 1-cm square of bark (the wound) before the inoculants were placed in the wound. The sides of the tree trunks were assigned randomly to the inoculants. The inoculated wounds were covered with the patch of bark previously removed with a chisel and wrapped with silver duct tape to prevent drying of the wound.

Two months after inoculation (12 Dec), the resulting canker areas were measured. After the surface bark was shaved off with a hatchet to reveal the entire margin of each canker, a clear sheet of acetate plastic was used to trace each canker's margin. The area of each canker was determined by digitally scanning its trace and applying APS Assess software.

RESULTS AND DISCUSSION

Objective 1. Evaluations of resistance to *P. citricola* in hybrid clones.

Greenhouse trials. Plants to be screened in 2005 were removed from chilling in fall 2004, which was considered suboptimal for subsequent plant growth but necessary to avoid degeneration of plant quality in storage. The plants were not considered to be vigorous enough for screening in spring 2005 and are currently receiving natural chilling outside. These plants, as well as an additional set of rootstock clones propagated in 2005, will be screened for resistance to *P. citricola* in the greenhouse in spring 2006, using procedures described previously (Browne et al., 2004 Report to the Walnut Marketing Board).

Field trial. All rootstocks planted for the field screening trial (Table 1) grew well. The soil infestation treatment with *P. citricola* and the non-inoculated control will be applied in December 2005. Disease development in rootstocks with susceptibility to the pathogen is anticipated in 2006.

Objective 2. Determining etiology of the crown and root rot on Paradox hybrid rootstock.

Neither *P. parasitica* nor the unidentified *Phytophthora* sp. isolated from dead and dying trees in surveyed Glenn and Butte County orchards caused much disease in Paradox hybrid or NCa black walnut test seedlings in the greenhouse experiment. In contrast, the isolates of *P. cinnamomi* and *P. citricola* included as known aggressive standards caused severe crown and root rot on both seedling types (Fig. 1). The non-inoculated controls developed no significant disease (Fig. 1). On excised shoots of some of the rootstocks, wound inoculation with *P. cinnamomi*, *P. citricola*, or *P. parasitica* resulted in more shoot necrosis than inoculation with the agar control, but the unidentified *Phytophthora* sp. caused no measurable disease (Fig. 2).

The soil and roots sampled from diseased trees in the Glenn County orchard in November 2005 did not yield a *Phytophthora* sp. The portions of these samples submitted to the Westerdahl lab yielded significant populations of lesion and ring nematodes (700 to 6300 *Pratylenchus vulnus* and 450 to 3600 *Criconemella* sp. per liter soil; 0 to 126 *P. vulnus* per gram of root).

Cumulatively, our results have not provided clear evidence that a *Phytophthora* sp. is responsible for the anomalous tree decline on Paradox rootstock in Glenn and Butte County orchards. *Phytophthora parasitica* and the unidentified *Phytophthora* sp. have been isolated from soil and roots around the affected trees, but not consistently (see 2004 WMB report, Browne et al.). Furthermore, results of the greenhouse trial indicated relatively little aggressiveness in *P. parasitica* and the unidentified *Phytophthora* sp. on Paradox hybrid or NCa black walnut. The excised shoot inoculations provided some evidence for pathogenicity of *P. parasitica* on some walnut rootstocks, but these results should not be considered as reliable as those from the greenhouse test, which involved a more natural exposure to the pathogens.

Several complicating factors should be weighed concerning the evidence gathered for Objective 2. For example, it is possible that our ability to detect species of *Phytophthora* has been compromised by applications of phosphonate that have occurred repeatedly in the affected orchards. In controlled trials, applications of phosphonate interfered with isolation of *P. citricola* and *P. cactorum* from almond (Browne and Viveros, 2005). The detection of relatively high counts of both lesion and ring nematodes in one of the affected orchards suggests that they may be contributing to tree decline, if not death, in the orchard. We will continue to monitor incidence of tree loss in the affected Butte County orchards. Samples will be collected from trees that decline in 2006 and subjected to isolations for *Phytophthora* spp. and other pathogens.

Objective 3. Determining efficacy of phosphonate treatments.

Each of the phosphonate treatment programs resulted in significant suppression of trunk cankers caused by *P. citricola*. The triple chemigation treatment with Fosphite reduced the area of necrosis caused by *P. citricola* by 41%, compared to the cankers on inoculated trees that received no Fosphite (Fig. 3). Similarly, the single foliar spray with Fosphite reduced canker size by 59%. The strongest canker suppression (70%) resulted from the combined Fosphite treatment program involving three chemigations and one spray with Fosphite. On the trees inoculated with *P. citricola*, the statistical interaction between chemigation and foliar spray treatment was highly significant (P=0.0009). There were no significant or main or interactive effects of rootstock on canker development (P=0.11 to 0.52); this indicated that efficacy of the phosphonate treatments was not affected by rootstock.

The results suggest strongly that phosphonate treatment programs involving foliar sprays and/or chemigation treatments in fall can contribute valuably to management of crown rot of walnut caused by *P. citricola*. Additional trees that received each of the treatments in 2005 will be inoculated in spring 2006 to determine whether the systemic activity of the phosphonate applications is maintained. In almond, effects of foliar sprays with phosphonate in the fall persisted for up to 5 months (Browne and Viveros, 2005).

LITERATURE CITED

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Potter, D., Gao, F.Y., Baggett, S., McKenna, J.R., and McGranahan, G.H. 2002. Defining the sources of Paradox: DNA sequence markers for North American walnut (*Juglans* L.) species and hybrids. Scientia-Horticulturae. 94: 157-170.

Table 1. Rootstock selections established in field screening trial at Armstrong Tract, Department of Plant Pathology, UC Davis, in May 2005

Rootstock	**Genetic background**	**Testing background**
NCa black walnut	*Juglans hindsii*	Susceptible standard
Chinese wingnut	*Pterocarya stenoptera*	Resistant standard
AX1	*J. californica x regia*	Susceptible in greenhouse
GZ1	*J. hindsii x regia*	Susceptible in greenhouse
JX2	*J. hindsii x regia*	Partially resistant in greenhouse
PX1	*J. hindsii x regia*	Susceptible in greenhouse
VX211	*J. hindsii x regia*	Partially resistant in greenhouse
RX1	*J. microcarpa x regia*	Partially resistant in greenhouse
AZ2	*J. (major x regia) x nigra x regia*	Partially resistant in greenhouse
NZ1	*J. ((major x regia) x nigra) x regia*	Partially resistant in greenhouse
WIP3	*J. (hindsii x regia) x regia*	Susceptible in greenhouse

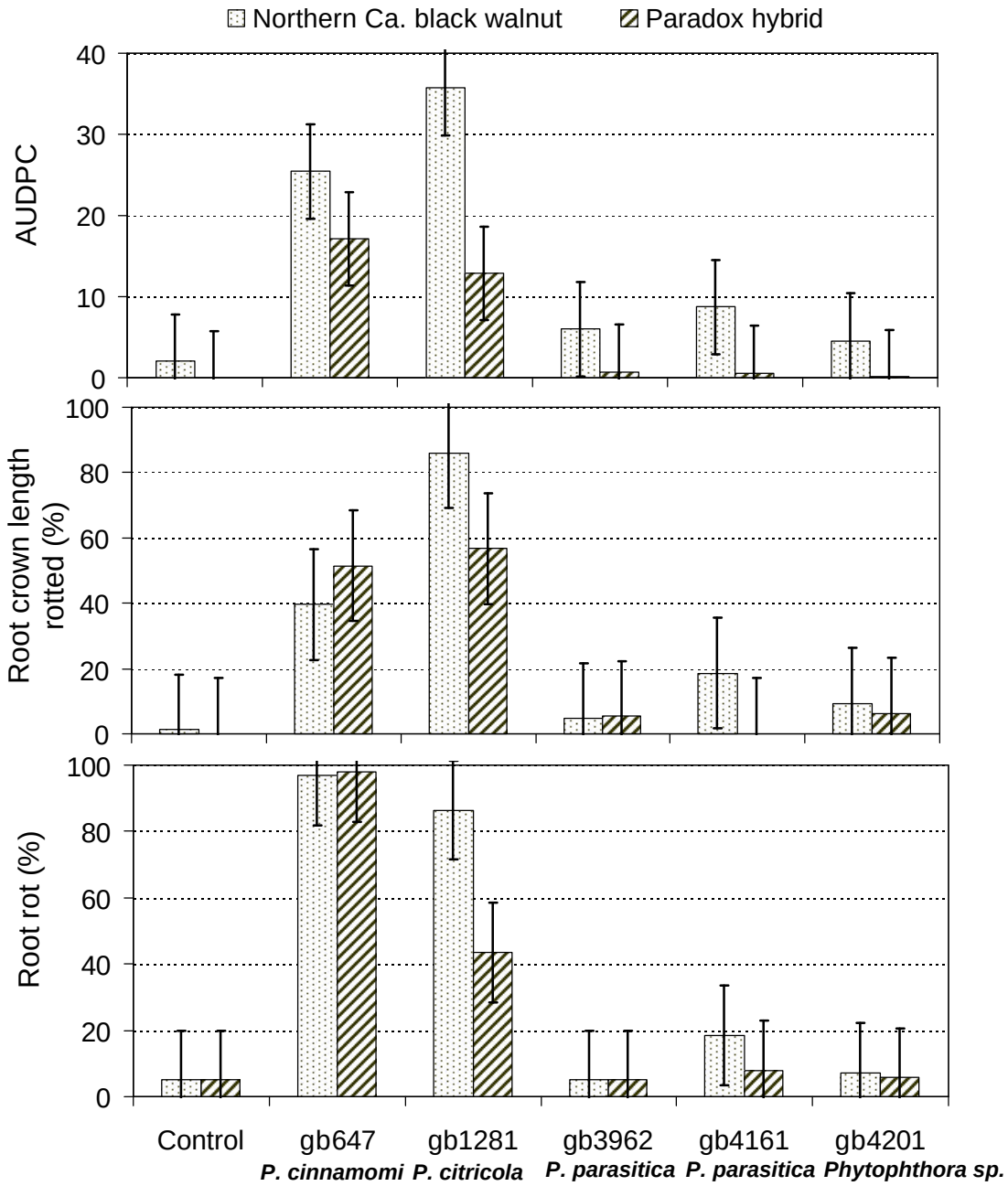

Fig. 1. Lack of aggressiveness of isolates of *Phytophthora parasitica* and *Phytophthora* sp. from Glenn and Butte County orchards. Note high aggressiveness of walnut isolates of *P. cinnamomi* and *P. citricola*, which were used as standards. The AUDPC values indicate average areas under disease progress curves, based on summations of visual disease ratings on a 0 to 5 scale (0=no disease, 5=dead plant, 1,2,3, and 4=gradations in between). The percentages of root crown length rotted were measured and the percentages of root rot were estimated visually. Vertical bars are 95% confidence intervals.

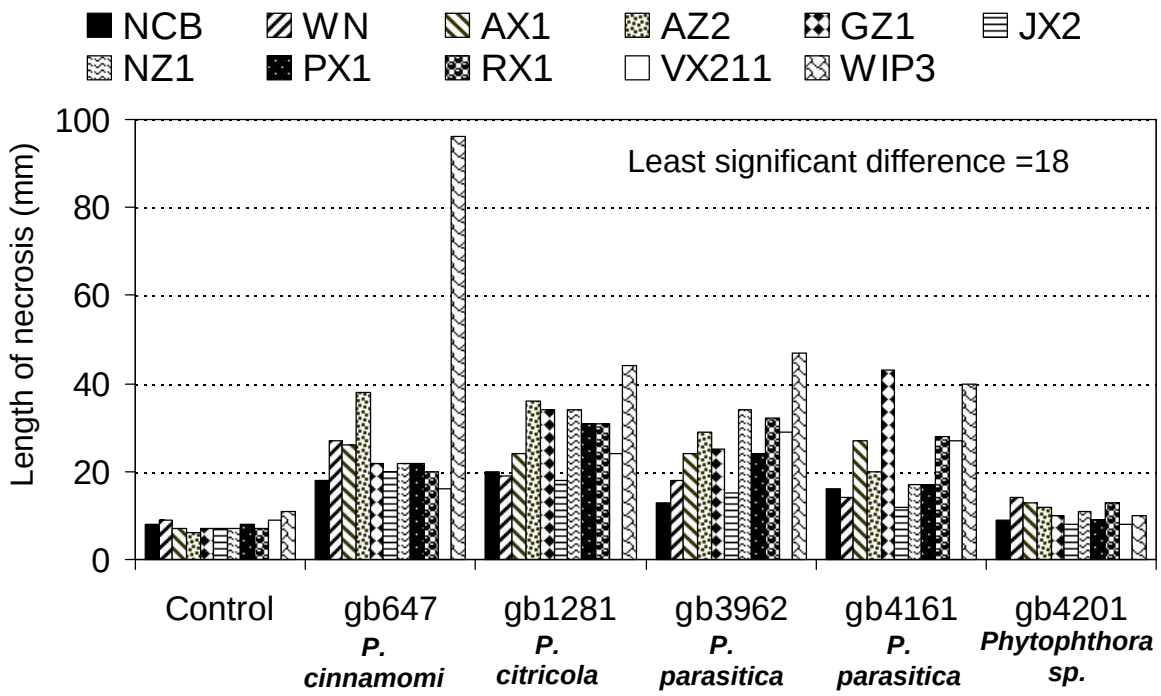

Fig. 2. Results of wounded excised shoot inoculations with isolates of *Phytophthora parasitica* and *Phytophthora* sp. from Glenn and Butte County orchards. Isolates of *P. cinnamomi* and *P. citricola* were used as standards for comparison. The least significant difference is based on 95% confidence intervals.

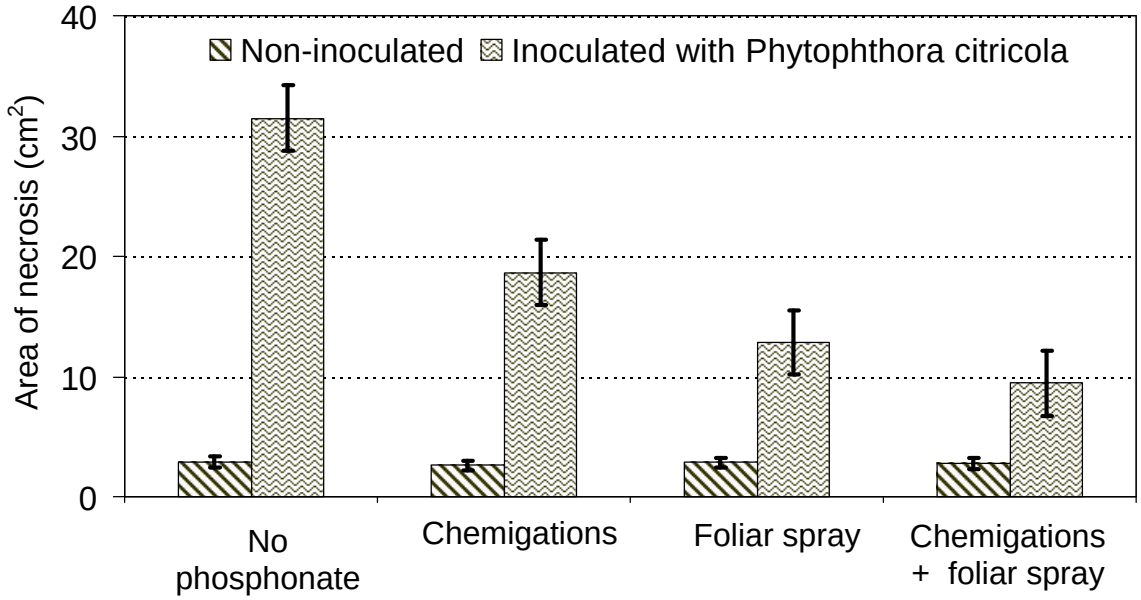

Fig. 3. Effect of pre-inoculation chemigation and foliar treatments with Fosphite®, a phosphonate fungicide, on canker development caused by *Phytophthora citricola*. The "chemigations" treatment included three applications of Fosphite, each at 3 quarts per acre through microsprinklers. The "foliar spray" treatment included one application of Fosphite at 3 quarts per acre in 100 gal water per acre. Vertical bars are 95% confidence intervals.