BIOLOGY AND MANAGEMENT OF PHYTOPHTHORA CROWN AND ROOT ROT OF WALNUT

Greg Browne, Leigh Schmidt, Wes Hackett, Bob Beede, Bill Krueger, Terry Prichard, Gale McGranahan, Chuck Leslie, and B. Westerdahl

ABSTRACT

Our objectives are to: 1) evaluate elite Paradox hybrid clones for resistance to *Phytophthora cinnamomi and P. citricola*; 2) determine unknown causes of crown-rot induced decline of English walnut orchards on Paradox rootstock; and 3) complete evaluations of phosphonate treatments for prevention of crown rot caused by *P. citricola*. Two greenhouse trials were conducted in 2007 under objective 1; one was a repeat of a 2006 evaluation of 18 hybrid clones with *P. citricola*, and the other, which included 19 hybrid clones, was the first evaluation of the clones AZ025 [(*J. major* × *hindsii)* × *nigra*) × *J. regia*]; Burbank (BUR), GZ3, RR1, and RR4 (*J. hindsii* × *J. regia*); MW1 and CW1 (*J. nigra* × *J. regia*) and CR (at this writing background unknown, from WIP) with *P. cinnamomi* as well as *P. citricola.* Results of the 2007 screens with *P. citricola* differed from those of previous years in that all clones developed moderate to severe levels of crown rot when challenged with *P. citricola*, including RX1 and VX211, which had expressed moderate to high tolerance to the pathogen in previous trials. Nevertheless, RX1 and VX211 ranked among the most resistant clones in the 2007 trials with *P. citricola*. Only RX1 expressed very high resistance to root and crown rot caused by *P. cinnamomi*, but GZ2, GZ3, and MW1 also developed relatively low levels of root and crown rot compared to the other clones tested (AX1, AX2, AZ205, Bur, PX1, RR1, RR4, Vlach, VX211, CR, CW1, RW2, UX1, UX2, and WIP3) with *P. cinnamomi*. RX1 has performed well in a field trial led by J. Grant at an orchard site that we verified to be infested with *P. cinnamomi*. In each of the 2007 greenhouse trials, standards of Northern California black walnut (NCB; *J. hindsii*) and Chinese wingnut (*Pterocarya stenoptera*) were included as standards and were highly susceptible and highly resistant, respectively, to both species of *Phytophthora*. From the clonal trials to date we conclude that although RX1 possesses valuable resistance to *Phytophthora*, it is still subject to infection and slight to moderate levels of disease caused by *P. cinnamomi* and *P. citricola*. Concerning objective 2, our work suggests that although *P. citricola*, *P. cinnamomi*, and *Armillaria mellea* continue to be important causes of crown rot in California walnuts, there may be additional, undescribed cause(s) of crown rot on the rootstock; in some orchards on Paradox rootstock the declining trees exhibit a "watery" crown rot from which we have not been able to isolate a *Phytophthora* or *Armillaria* sp. We have isolated and characterized bacteria from this decay and will test their pathogenicity on Paradox plants. In the completion of phosphonate trials (objective 3), cankers developing from inoculations in 2007 were much larger than those resulting in 2006, but a single phosphonate spray in September 2006 still reduced the size of the cankers in 2007. In contrast, three chemigation treatments applied in September 2006 had no significant effect on canker development from the inoculations in 2007. In orchards affected by Phytophthora crown rot phosphonate treatments are advisable but should be integrated with careful soil water management and use of tolerant rootstocks for best results.

INTRODUCTION

Crown and root rots caused by species of *Phytophthora* are among the most serious diseases of English walnut trees 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 aggressive. Northern California black walnut and some selections of Paradox hybrid seedling rootstock are highly and moderately susceptible, respectively, to each of these pathogens.

It was determined that there is significant genetic diversity among Paradox hybrids and hypothesized that selection from the diversity will provide valuable resistance to pests and pathogens and superior horticultural performance. We have worked with the Walnut Improvement Program (WIP), commercial nurseries, and Wes Hackett to evaluate diverse hybrid walnut rootstocks for resistance to *Phytophthora*. We selected and micropropagated seedlings for their putative resistance to *P. citricola* and for unique genetic backgrounds of interest. The selections are being tested systematically as clones. Clones AX1 and PX1, which initially tested as moderately susceptible to *P. citricola*, and RX1, which tested as moderately resistant to *P. citricola*, have been retained as standards in all of the clonal evaluations of resistance to *Phytophthora*. In 2006 and 2007 Northern California black walnut was included in the tests as a standard highly susceptible to *P. citricola* and *P. cinnamomi*, and in 2007 Chinese wingnut was included as a standard highly resistant to each of these pathogens. This year's tests were the first to include evaluations of resistance to *P. cinnamomi* as well as to *P. citricola*.

Paradox has become widely used in the California walnut industry due to its vigor and superior resistance to most species of *Phytophthora*, but its widespread use has also revealed some of its limitations. Susceptiblity to *Agrobacterium tumefaciens* is probably its most serious weakness, but field observations suggest that, under some conditions, Paradox also is more prone than NCB to waterlogging. In addition, we have observed crown rot on Paradox roostock that appears to be distinct from waterlogging damage and has not been associated with either *Phytophthora* or *Armillaria*. Below, under objective 2, we report on our examinations of trees affected by the latter type of crown rot symptom.

We also report below on the conclusion of our Davis orchard tests of phosphonate treatments. Phophonates, which have phosphorous acid (H_2PO_3) as their active ingredient, are registered for management of Phytophthora diseases of walnut, and our tests were designed to evaluate the systemic persistence and efficacy of phosphonate treatments applied by foliar spraying and by chemigation.

OBJECTIVES

1) To evaluate elite Paradox hybrid clones for resistance to *Phytophthora cinnamomi and P. citricola.*
2) To examine contributions of pathogens to decline of English walnut orchards on Paradox rootstock.
3) Complete evaluations of phosphonate treatments for prevention of crown rot caused by *P. citricola.*

PROCEDURES

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

Greenhouse trials. Clones selected from hybrid seed families evaluated for resistance to *P. citricola* in 1997-99 were preserved and multiplied as microshoots (Browne et al., *unpublished*). Representatives of this microshoot collection as well as additional clonal selections from the WIP were multiplied further, rooted in micro culture, transplanted, and acclimatized to a greenhouse environment (Hackett et al., *unpublished*). After rooting and greenhouse-acclimatization, plants to be used for evaluations of resistance were subjected to several months of chilling at 6 °C (fall/winter 2005/06), transplanted and grown in 1-liter pots in a greenhouse (summer 2006), and held in a lathhouse during winter 2007. In late May 2007, individual plants from the 1-liter pots were transplanted into 2-liter pots filled with UC potting mix soil that was either artificially infested with *P. citricola* or *P. cinnamomi* (45 ml of V8 juice-oat-vermiculite substrate infested with one of the pathogens per liter of the potting mix) or treated as a control (45 ml sterile substrate per liter of potting mix). Two experiments were established in May 2007, one including evaluations with *P. citricola*, the other including evaluations with *P. cinnamomi* as well as *P. citricola*. In each experiment, there were 5 replicate plants in pots of non-infested soil and 10 to 20 replicate plants in infested soil in a split-plot design (main plots were inoculum treatments, subplots were rootstocks) among 5 blocks. Every 2 weeks after transplanting the soil in each pot was flooded for 48 h. Three months after transplanting, the root systems were washed free from soil and evaluated visually for incidence and severity of crown and root rot.

Objective 2. Examining contributions of pathogens to decline of English walnut orchards on Paradox rootstock.

Samples were collected from three orchards in Kings County on Paradox hybrid rootstocks where the trees exhibited distinct crown rot (as opposed to diffuse root death attributed to waterlogging in some other orchards on Paradox rootstock). Additional samples were collected from trees with similar symptoms in San Joaquin County and from trees apparently damaged by waterlogging in Glenn County. Affected root systems in the Glenn County orchard exhibited "non-focused", "spotty" necrosis, with few or no true cankers and lots of secondary decay. Samples from each of these orchards were cultured on PARP medium (for *Phytophthora* spp.) as well as general culture media to isolate fungi and bacteria.

Objective 3. Completing evaluations of phosphonate treatments.

In 2007 we completed the second of two trials evaluating efficacy of foliar and chemigation treatments with phosphonate in a walnut orchard planted at Campbell Tract by Terry Prichard in 2000. The first trial, initiated in the western half of the orchard with spray and chemigation treatments in September 2005, was completed with final canker evaluations in August 2006. The second trial, initiated with spray and chemigation treatments in the eastern half of the orchard in 2006, was completed with final canker evaluations in August 2007. In each trial, treatments were applied in a split-split plot design; a phosphonate chemigation treatment program and a water control were applied through microsprinklers to soil around trees in randomly selected main

plots. The main plots were 16-tree rows irrigated with lines of Bowsmith microjet sprinklers (full circle, 10-foot diameter pattern, 5.7 gallons per hour, one sprinkler per tree placed 3 ft. from the tree trunk). A phosphonate spray treatment was applied to the foliage of trees in randomly selected subplots (pairs of trees within each 16-tree row). The design was factorial, resulting in four treatments:

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

For the trial completed in 2007 the chemigation program consisted of three applications of Fosphite approximately 1 week apart in late August and early September 2006. 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 received the same amount of water, without Fosphite, through microjets. The foliar spray treatment consisted of one application of Fosphite at 3 quarts per acre in 100 gallons of water per sprayed acre on the date of the last chemigation treatment. The spray was applied with a backpack air-blast 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.

At approximately 1 and at 7 months after the completion of the phosphonate treatments, 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); a separate set of trees was inoculated on each of the two dates. 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 to three months after each inoculation date, 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. In the evaluation of 17 hybrid clones for resistance to *P. citricola*, all hybrid selections developed crown rot extending approximately 40% or more of root crown length (Fig. 1). In previous screens of resistance to *P. citricola*, we considered development of necrosis on 40 to 50% of crown length and circumference to be an indication of moderate susceptibility to the pathogen. This level of disease was not expected for RX1 (*J. microcarpa* x *J. regia*), which in previous experiments developed crown rot on an average of <20% of root

crown length and was considered to be moderately resistant to *P. citricola* (Browne et al., 2004-2006 reports to the Walnut Marketing Board). Still, even in the 2007 screen, RX1 was among the clones with the lowest crown and root rot scores (Fig. 1). Also, clones AX1 (*J. californica* × *J. regia*), UX1 and UX2 [(*J. californica* × *nigra*) × *J. regia*]. The standard seedlings of NCB (*J. hindsii*) and Chinese wingnut (*Pterocarya stenoptera*) were highly susceptible and highly resistant, respectively, to *P. citricola*.

It is unknown why RX1 expressed a higher level of susceptibility to *P. citricola* in 2007 than in previous years. The only potentially significant change in our screening protocol was that we initiated and completed the experiment earlier in the year, which may have influenced "physiological susceptibility" of the rootstocks. Seasonal fluctuations in susceptibility to *P. citricola* have been reported (Matheron and Mircetich 1985).

In the second 2007 greenhouse trial, which evaluated 19 hybrid clones for response to *P. cinnamomi* as well as to *P. citricola*, all clones developed necrosis on an average of approximately 40% or more of root crown length and circumference in soil infested with *P. citricola* (Fig. 2A,B). BUR (*J. hindsii* × *J. regia*), RX1, and UX1 had the lowest crown rot scores with approximately 40% of crown length and width rotted. *Phytophthrora cinnamomi* caused more root rot than *P. citricola* on most selections (Fig. 2C). Among the 19 clones, only RX1 expressed very high levels of resistance to *P. cinnamomi* (i.e., values less than 10% of root and crown rot, Fig. 2), but GZ2 (*J. hindsii* × *J. regia*) and MW1 (*J. nigra* × *J. regia*) also had relatively low crown and root disease scores. NCB and Chinese wingnut seedlings were highly susceptible and highly resistant, respectively, to both pathogens.

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

No known fungal pathogens were detected from the surveyed English walnut orchards declining on Paradox rootstock in Kings, San Joaquin, or Glenn County, although *P. cinnamomi* was detected in a block adjacent to one of the surveyed blocks in Kings County. It was judged that the orchard in Glenn County with root systems exhibiting "non-focused" "spotty" necrosis and lots of secondary decay was affected primarily by waterlogging. We did not attempt to isolate and identify bacteria from necrotic roots of the Glenn County orchard but did so from the Kings and San Joaquin County orchards sampled in 2007 and from a Butte County orchard sampled in 2006. Representative bacteria detected in necrotic root and trunk bark from the latter three orchards were identified to varying degrees by PCR amplification and sequencing of 16S rDNA (Table 1).

The fact that bacteria were isolated from the necrotic tissues is not surprising and in itself is not an indication of bacterial involvement in the problem; soil and necrotic tissues are typically permeated by saprophytic as well as potentially pathogenic bacteria. Pathogenicity tests with selected bacterial isolates will be needed and are proposed to determine whether any of the isolated bacteria are contributing to the disease losses.

Objective 3. Determining efficacy of phosphonate treatments.

In the 2006/07 trial with phosphonate, the September 2006 phosphonate spray treatment significantly suppressed canker development during the 3-month period of incubation following inoculation with *P. citricola* in late April 2007 (Table 2, Expt. 2, trees inoculated 4/30/07,

P=0.01), but the chemigation treatments had no main or interactive effects on canker development (Table 2, Expt. 2, trees inoculated 4/30/07, P=0.09).

Our results to date indicate that a single phosphonate spray treatment can provide several months of partial suppression of canker development caused by *P. citricola*. Although the triple chemigation treatment program suppressed canker development in the first experiment (Table 2, Experiment 1), the effect was not duplicated in our second experiment, suggesting that the chemigation treatments are less dependable than those of the spraying treatment and that careful soil water management before and after chemigation may be required to for sufficient uptake of phosphonate into the tree roots. If phosphonate applied by chemigation is not taken up immediately by the roots, soil microbes oxidize it to phosphate and thereby render it inactive for Phytophthora suppression. Overall, our data suggest that multiple-year programs involving foliar sprays and chemigation treatments can contribute to economical management of Phytophthora crown rot in commercial walnut production, but appears important to integrate phosphonate programs with other proven approaches for management of Phytophthora diseases, i.e., careful soil water management and judicious selection of rootstocks.

Literature cited

Matheron, M. E. and S. M. Mircetich 1985. Seasonal variation in susceptibility of *Juglans hindsii* and Paradox rootstocks of English walnut trees to *Phytophthora citricola*. Phytopathology 75: 970-972.

Table 1. Representative bacteria isolated from cankers on Paradox rootstock[a]

Bacterial taxon	No. of isolates by county location of orchard		
	Kings	San Joaquin	Butte
Bacillus	10	6	0
Unknown members of *Comamonadaceae*	4	0	0
Curtobacterium	3	0	0
Enterobacter and unknown members of *Enterobacteriaceae*	13	41	9
Microbacterium	1	0	0
Paenbacillus	0	1	0
Pseudomonas	2	2	0
Rahnella	0	0	1
Raoultella	0	0	1
Sphingomonas	0	3	0
Streptomyces	1	2	0
Unknown members of *Bacillales*	0	5	0
Unknown members of *Betaproteobacteria*	0	1	0
Unknown members of *Cellulomonadaceae*	0	1	0
Unknown members of *Gordoniaceae*	0	1	0
Unknown members of *Intrasporangiaceae*	2	0	0
Unknown members of *Microbacteriaceae*	0	5	0
Unknown members of *Promicronosporaceae*	1	0	0
Unknown members of *Rhizobiaceae*	0	3	0
Variovorax	3	0	0

[a]Isolates were cultured in 5% tryptic soy broth agar and identified by PCR and sequencing of 16S rDNA.

Table 2. Effect of pre-inoculation treatments with phosphonate on development of trunk cankers caused by *Phytophthora citricola* on English walnut [a]

Exp.	Dates defining assessment period		Trt. no.	Pre-inoculation treatment		Mean area of cankers (cm^2)[b]	
	Inoculation	Canker measurement		Dates of chemigation with phosphonate (3 qts. Fosphite/ac.)	Dates of foliar spray with phosphonate (3qts. Fosphite /ac.)	Control	Inoculated with *P. citricola*
1	10/7/05	12/13/05	1	none	none	2.8 a	31.6 a
			2	8/29/05, 9/6/05, 9/12/05	none	2.6 a	18.7 b
			3	none	9/12/05	2.8 a	12.8 b
			4	8/29/05, 9/6/05, 9/12/05	9/12/05	2.8 a	9.5 c
	4/28/06	8/8/06	1	none	none	0.1 a	47.6 a
			2	8/29/05, 9/6/05, 9/12/05	none	0.0 a	51.0 a
			3	none	9/12/05	0.0 a	27.4 a
			4	8/29/05, 9/6/05, 9/12/05	9/12/05	0.0 a	19.8 a
2	10/3/06	12/12/06	1	none	none	2.4 a	18.0 a
			2	8/28/06, 9/5/06, 9/13/06	none	2.6 a	17.5 a
			3	none	9/13/06	3.0 a	11.0 b
			4	8/28/06, 9/5/06, 9/13/06	9/13/06	2.4 a	13.2 b
	4/30/07	8/15/07	1	none	none	0.1	667.4 a
			2	8/28/06, 9/5/06, 9/13/06	none	0	615.2 a
			3	none	9/13/06	0.1	366.2 b
			4	8/28/06, 9/5/06, 9/13/06	9/13/06	0	370.3 b

[a]Formulation was Fosphite, J.H. Biotech, Ventura, CA.
[b]Values within a column and defined assessment period and without letters in common differ significantly (Waller k ratio).

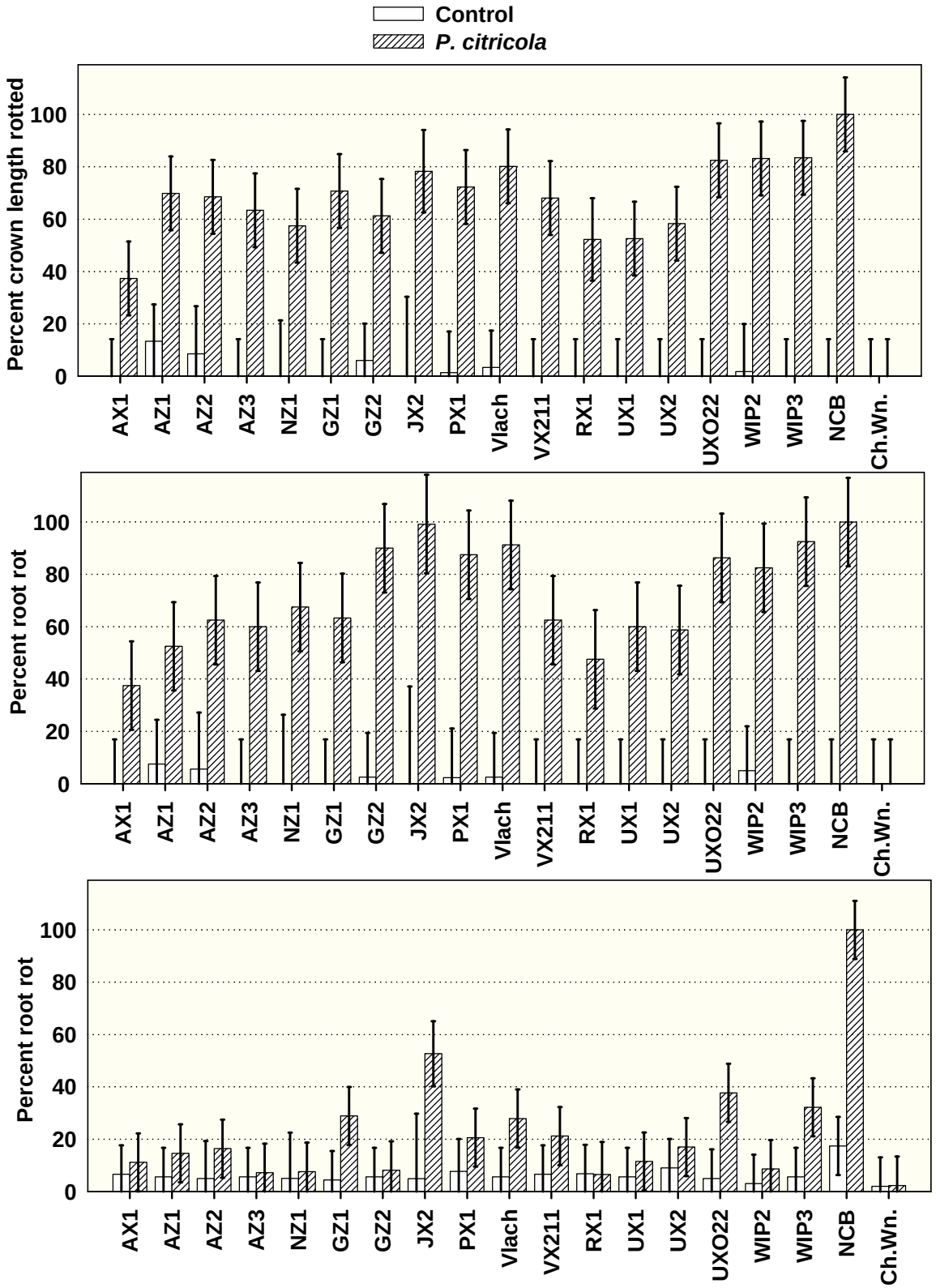

Fig. 1. Relative resistance to *Phytophthora citricola* among 17 clones of walnut hybrid rootstock, Northern California black walnut (NCB), and Chinese wingnut (Ch.Wn.) in greenhouse Experiment 1, 2007. Vertical bars are 95% confidence intervals.

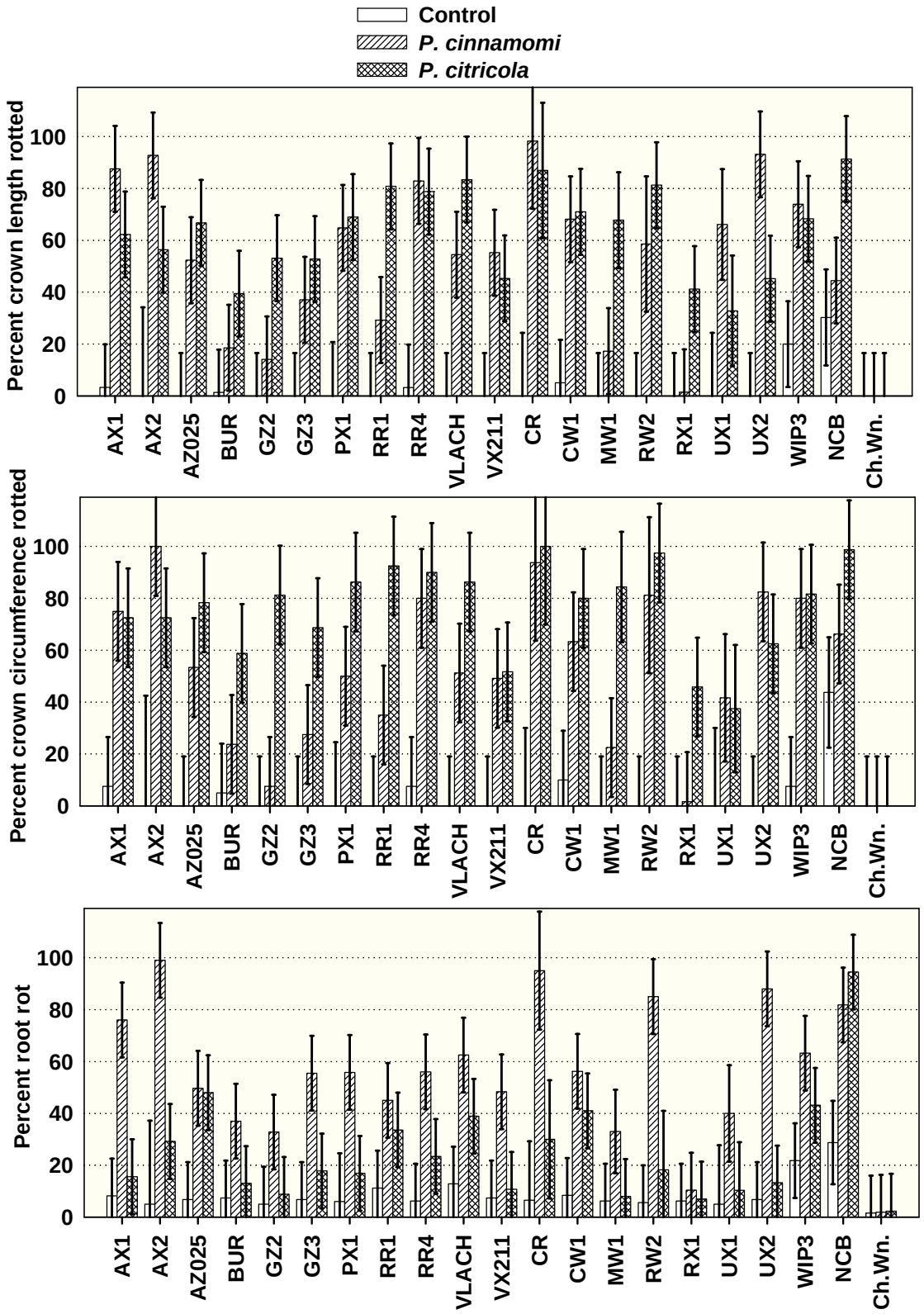

Fig. 2. Relative resistance to *Phytophthora cinnamomi* and *P. citricola* among 19 clones of walnut hybrid rootstock, Northern California black walnut (NCB), and Chinese wingnut (Ch.Wn.) in Greenhouse Experiment 2, 2007. Vertical bars are 95% confidence intervals.