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* in greenhouse and field trials; 2) Determine the cause for high incidence of crown and root rot in selected Glenn County orchards on Paradox walnut rootstock; and 3) Determine efficacy of phosphonate chemigation and foliar spray treatments for management of Phytophthora crown rot. A greenhouse screen of resistance to *P. citricola* in 17 hybrid walnut rootstock clones was completed. Moderate resistance to *P. citricola* was expressed by the clones RX1 and VX211, which also had performed favorably in previous screens with the pathogen. Clones that expressed moderate to high susceptibility to *P. citricola* included AX1, AZ1, AZ2, AZ3, NZ1, GZ1, GZ2, JX2, PX1, Vlach, UX022, UX1, UX2, WIP2, and WIP3. Nine of these clones (AX1, AZ2, NZ1, GZ1, JX2, PX1, RX1, VX211, and WIP3) also were evaluated for resistance to *P. citricola* in a field trial at Davis. In the field trial, only clones AX1, GZ1, PX1, and WIP3 developed crown rot, and the severity was mild; NCB, the susceptible standard in the field trial, suffered 59% mortality due to Phytophthora crown rot. In surveys of orchards suffering from decline on Paradox rootstock, we did not determine a single cause for the disease; visits to affected orchards suggested that multiple factors, varying with orchard, are contributing to decline. Our ongoing field trial evaluating efficacy of phosphonate spray and chemigation treatments confirmed that a fall spray with phosphonate (Fosphite, 3 qts./a) suppresses cankers caused by *P. citricola*, but chemigation was less effective in 2006 than in 2005. Although phosphonate treatment programs appear economical, our results suggest they should be integrated with cultural and genetic strategies for optimal management of Phytophthora crown and root rots.

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 virulent.

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 black (NCB, *Juglans hindsii*) or English seedling rootstocks (*J. regia*) to most species of *Phytophthora*. Although Chinese wingnut (*Pterocarya stenoptera*) is the only walnut family member known to be 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*, and results of greenhouse experiments suggested

that the diversity among Paradox hybrids may include important variation in resistance to *P. citricola*. 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) orchard surveys to determine factors contributing to decline of on English walnut trees on Paradox rootstock, and 3) ongoing trials 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 crown rot caused *P. citricola.*

PROCEDURES

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

Greenhouse trials. As in previous years, the greenhouse evaluations of hybrids for resistance to *P. citricola* in 2006 focused on clonal selections made from seed families for putative resistance to *P. citricola* or unique genetic backgrounds. The seed families originated from commercial walnut nurserymen and the WIP. The families were evaluated for resistance to *P. citricola* in 1997-99, and the clones selected them were preserved and multiplied as microshoots (G.T. Browne, *unpublished*). In recent years, 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, the plants that were to be used for screening resistance to *P. citricola* in summer 2006 were first submitted to cycles of chilling and growth, which tended to equalize the size of plants as they grew and kept them small enough to facilitate mass screening. The cycles included dormancy induced by storage at 6 °C for 3 to 5 months (2004), growth in a greenhouse for 1 year (2005), and natural dormancy in a lath house followed by growth in a greenhouse (winter and spring 2006, respectively).

The screen for resistance was initiated in August 2006 by transplanting individual plants from 1-liter pots into 2-liter pots filled with UC potting mix soil that was either artificially infested with *P. citricola* (45 ml of *P. citricola*-infested V8 juice-oat-vermiculite substrate per liter of soil) or treated as a control (45 ml sterile substrate per liter of soil). There were 5 replicate plants planted in non-infested soil and 10 to 20 replicate plants in infested soil (exceptions were AZ1 and NZ1, with 5 replicate plants in infested soil), evenly distributed 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.

Field trial. Selections of nine clonal hybrid rootstocks, including 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) were planted in May 2005 at Armstrong Tract, Department of Plant Pathology, UC Davis. Northern California black walnut (NCB) and Chinese wingnut were planted with the clones to serve as highly susceptible and highly resistant standards, respectively. A randomized block split-plot design was used; for each rootstock, there were six four-tree plots to be infested with *P. citricola* (on Jan. 23, 2006, 100 ml of V8 juice-oat mixture infested with the pathogen was mixed into the upper 5 cm of soil around the trunk of each tree) and six single-tree plots to serve as non-infested controls (treated the same as the pathogen-infested plots, except sterile V8 juice-oat mixture was used). Each of the trees was irrigated with a single 4 liter/h emitter, placed within 5 cm of the tree trunk base. Starting the first week of June 2006, and continuing through the summer, the drip system was run at weekly intervals; each run included 3 days of 12-h-on-1-h-off cycles. On Nov. 21, 2006 the trees were assessed for growth in trunk circumference since planting and development of crown rot (as indicated by trunk cankers extending up from the soil surface).

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

Visits were made to several Sacramento Valley walnut orchards suffering from tree decline on Paradox rootstock. Becky Westerdahl and Bill Krueger collected root and soil samples to assay for nematodes from healthy and diseased areas of two Glenn County orchards sampled for *Phytophthora* spp. in previous years. Root systems in an additional six orchards suffering from decline on Paradox or Paradox and NCB rootstocks in Butte, Yuba, and Solano Counties were inspected in collaboration with UC, UCCE, and consultants. Where possible, a backhoe was used to assist in the examinations. In each orchard, attempts were made to deduce likely causes of the tree decline from the associated symptoms.

Objective 3. Determining efficacy of phosphonate treatments.

In 2006 we continued trials evaluating efficacy of foliar spray 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 in 2005, was completed in August 2006. The second trial, initiated in the eastern half of the orchard in 2006, will be completed in August 2007. In each trial, treatments were imposed in a split-split plot design; a phosphonate chemigation treatment program (and a water control) was applied through microsprinklers to soil around trees in randomly selected main plots. The main plots were 16-tree rows served by dedicated irrigation lines with one Bowsmith microjet head (full circle, 10-foot diameter pattern, 5.7 gallons per hour, placed 3 ft. from the tree trunk) per tree. 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 combination

The chemigation treatment program consisted of three applications of Fosphite approximately 1 week apart in late August and early September. 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.

One month 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). 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. In the 2005 experiment, inoculations and control treatments were applied to another set of eight trees per treatment using the same methods as described above.

Two to three months after inoculation, 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 2006 greenhouse screen for resistance to *P. citricola*, NCB expressed the expected high susceptibility to the pathogen, indicating that conditions were conducive to disease development in the test (Fig. 1). Among the 17 clonal hybrids evaluated in the screen, RX1 and VX211 (maternal parents [m.p.] of *J. microcarpa* and *J. hindsii*, respectively) were relatively resistant to *P. citricola* (Fig. 1). These clones also had exhibited resistance to *P. citricola* in previous screens (Browne et al., 2001 and 2004 Walnut Marketing Board [WMB] research reports). In contrast, the clones AZ2, AZ3, NZ1 (m.p. [*J. major* × *hindsii*] × *nigra*); UX1 (m.p. *J. californica* × *nigra*); and JX2 (m.p. *J. hindsii*) were among the relatively susceptible clones in 2006 (Fig. 1), although all of them had been relatively resistant in previous screens with *P. citricola* (Browne et al., 2001 and 2004 WMB reports). Clones AX1 (m.p. *J. californica*), GZ1 and PX1 (m.p. *J. hindsii*), UX2 (m.p. *J. californica* × *nigra*), and WIP3 (m.p. *J. hindsii* × *regia*) were among the most susceptible clones to *P. citricola* in 2006, as they had been in our previous screens. Clones AZ1 (m.p. [*J. major* × *hindsii*] × *nigra*), GZ2 and Vlach (m.p. *J. hindsii*), UX022 (m.p. *J. californica* × *nigra*), and WIP2 (m.p. *J. hindsii* × *regia*), screened for the first time in 2006, were all relatively susceptible to *P. citricola*.

RX1 and VX211 are the only hybrid clones that have consistently expressed moderate resistance to *P. citricola* in our greenhouse screens. Although greenhouse and field experience with these rootstocks is still relatively limited, they are prime candidates for continued intensive evaluations. The differing parentages between RX1 and VX211 make an interesting compliment. We are proposing to include these rootstocks in evaluations of genetic resistance to *P. cinnamomi*, and it seems prudent to evaluate their responses to *Agrobacterium tumefaciens* and waterlogging.

Field trial. The nine clonal rootstocks, including AX1, AX2, NZ1, GZ1, JX2, PX1, VX211, RX1, WIP3, and the susceptible and resistant species standards, NCB and Chinese wingnut (*Pterocarya stenoptera*), respectively, all grew well with no symptoms of disease in 2005. After applying the soil infestation and control treatments in 2006, crown rot developed in 15 of 24 (62%) of the NCB trees in plots infested with *P. citricola* and 1 of 6 NCB trees in control plots (Table 1). Among the other stocks, AX1, GZ1, PX1, and WIP3 suffered only low incidence of crown rot (1 to 2 trees of 24; 4 to 8%), while AZ2, NZ1,JX2, VX211, and wingnut developed no crown rot. Only NCB trees suffered mortality from the crown infections; 14 of 24 (58%) of NCB trees in infested plots and 1 of 6 NCB trees in control plots were dead or dying by Nov. 2006. Other than the low incidence of crown rot described above, all of the trees of other rootstocks appeared healthy. Soil infestation with *P. citricola* suppressed growth in trunk circumference in the stocks JX2, VX211, and wingnut compared to the controls, but it is noteworthy that these stocks were relatively vigorous compared to the others, with or without soil infestation. We are proposing to continue the field trial in 2007 and 2008 by adding *P. cinnamomi* to the plots infested with *P. citricola* previously.

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

Results of Westerdahl's and Krueger's 2006 sampling of roots and soil for parasitic nematodes in the two orchards near Butte City suffering from decline of trees on Paradox rootstock did not reveal a clear, consistent association between the disease and nematode parasites. In both orchards, the lesion nematode generally was detected in high counts from healthy as well as diseased trees. In only one of the orchards, ring nematode was detected in high counts from soil around some of the diseased trees but was generally not detected from healthy trees.

In six additional walnut orchards observed in 2006 with declining trees on Paradox rootstock in Yuba and Butte Counties, a range of symptoms and circumstances was observed. In four of the orchards, soil profile and root system examinations suggested a history of suboptimal soil-water conditions; declining trees in two of these four orchards had shallow root systems, few feeder roots, and were situated in a shallow layer of loam soil underlain by course sand. The observations suggested that the shallow, relatively weak root systems that probably suffered from intermittent water perching above the sand and inadequate available water storage during periods of high evapotranspiration. In the third of these four orchards, declining trees were situated on a poorly drained clay loam soil subject to a high water table. The fourth orchard suffered from many weeks of standing water in the spring due to its proximity to the Feather River and a high water table. In this orchard, the rootstock alternated in rows; roughly half of the rows were on NCB and half on Paradox, and the trees on Paradox suffered much greater mortality than those on NCB. In these four orchards, the spatial distribution of declining trees appeared to be generally associated with the deficiencies of the soil environment (i.e., severe texture stratification, intermittent waterlogging).

In another three orchards suffering from decline of English walnut on Paradox rootstock, the spatial pattern of decline incidence suggested involvement of a soilborne pathogen (i.e., the distribution of declining trees in the orchard was "spotty" with diseased trees typically among many healthy trees). Inspection of the dying trees in one of these orchards in Yuba County revealed clear association of the decline with infection by *Armillaria*. In the second of these orchards, young trees on Paradox rootstock in Butte County had, depending on the declining tree, been girdled partially or completely with crown rot, but there were no signs of *Armillaria* and no *Phytophthora* sp. was detected in isolations from the necrotic tissue. Interestingly, most symptomatic trees in this second orchard were not girdled completely, and their crown cankers were "healing over" at the margins, suggesting that the infections had occurred much earlier in 2006 (the trees were observed in September) and the original pathogen had died out or been contained by the host. In a third orchard, which was located in Solano County, declining trees appeared to have less healthy feeder roots than healthy trees, and the most severely affected trees had necrotic major roots, but there was not a consistent, clear association of the decline with severe crown and root rot.

The observations in the declining English walnut trees on Paradox rootstock suggest that, despite the rootstock's potential for high vigor and superior tolerance to most species of *Phytophthora*, it can be vulnerable to suboptimal soil environments, known soilborne pathogens including *Armillaria*, and perhaps to yet unknown or undetected soilborne pathogens. Continued field observations, replicated field trials, and supporting lab and greenhouse diagnostics will be

required to adequately characterize the performance of Paradox genotypes under different edaphic and biological environments.

Objective 3. Determining efficacy of phosphonate treatments.

In the 2006 conclusion of Expt. 1 (treatments applied Aug.-Sep. 2005), analysis of variance indicated that the 2005 phosphonate spray treatment significantly suppressed canker development during the 3-month period of incubation following inoculation with *P. citricola* in late April 2006 (P=0.04), but the 2005 chemigation treatment had no main or interactive effects on canker development resulting from April 2006 inoculations (P=0.26 to 0.87) (Expt. 1, Table 2).

In the 2006 portion of Expt. 2 (treatments applied Aug.-Sep. 2006), the spray treatment with phosphonate significantly suppressed development of trunk cankers caused by *P. citricola* during the 2.5 month incubation period after inoculation in Oct. 2006 (P<0.0001) (Expt. 2, Table 2), but there was no significant main or interactive effect of the chemigation treatment program (P=0.11 to 0.87, Expt. 2, Table 2). We will complete a second inoculation for Expt. 2 in April 2007 to assess residual effects of the phosphonate treatments applied in September 2006.

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 improved disease control in the first experiment, the effect was not duplicated in the second experiment, suggesting that its suppressive effects are less dependable than those of the spray treatment. It is likely that multiple-year programs involving multiple foliar sprays and chemigation treatments will contribute to economical management of Phytophthora crown rot in commercial walnut production, but it 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.

Table 1. Field performance of clonal Paradox hybrids, Northern California black walnut, and Chinese wingnut rootstocks in non-infested soil and soil infested with *Phytophthora citricola*, Davis[a]

Clone (or species)	Maternal background of hybrid (or species of standard)	Soil treatment (January 2006)	Incidence of crown rot (%)	Percent of trunk circ. necrotic	Incidence of tree mortality (%)	Increase in trunk circ. (mm)
AX1	*californica*	Control	0 c	0 c	0 c	163 c
		P. citricola	4 c	1 c	0 c	146 cde
AZ2	*(major x hindsii)x nigra*	Control	0 c	0 c	0 c	116 fg
		P. citricola	0 c	0 c	0 c	117 fg
NZ1	*(major x hindsii)x nigra*	Control	0 c	0 c	0 c	116 fg
		P. citricola	0 c	0 c	0 c	130 def
GZ1	*hindsii*	Control	0 c	0 c	0 c	157 cd
		P. citricola	4 c	1 c	0 c	150 cd
JX2	*hindsii*	Control	0 c	0 c	0 c	166 bc
		P. citricola	0 c	0 c	0 c	135 def
PX1	*hindsii*	Control	0 c	0 c	0 c	169 bc
		P. citricola	8 bc	1 c	0 c	157 cd
VX211	*hindsii*	Control	0 c	0 c	0 c	191 b
		P. citricola	0 c	0 c	0 c	147 cde
RX1	*microcarpa*	Control	0 c	0 c	0 c	112 fg
		P. citricola	0 c	0 c	0 c	116 fg
WIP3	*hindsii x regia*	Control	0 c	0 c	0 c	100 g
		P. citricola	8 bc	2 c	0 c	121 efg
(NCB)	*(J. hindsii)*	Control	16 b	17 b	17 b	68 h
		P. citricola	62 a	59 a	59 a	57 h
(Wingnut)	*(Pt. stenoptera)*	Control	0 c	0 b	0 c	226 a
		P. citricola	0 c	0 b	0 c	193 b

[a]All trees were planted May 2005. The assessments of crown rot and mortality were made 21 November 2006. Means within a column and without letters in common are significantly different (Waller k ratio).

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

Expt.	Dates defining assessment period		Pre-inoculation treatment			Mean area of cankers (cm^2)[b]	
	Inoculation	Canker measurement	Treatment no.	Dates of chemigation with phosphonate (3 qts. Fosphite/acre)	Dates of foliar spray with phosphonate (3qts. Fosphite /acre)	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

[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).

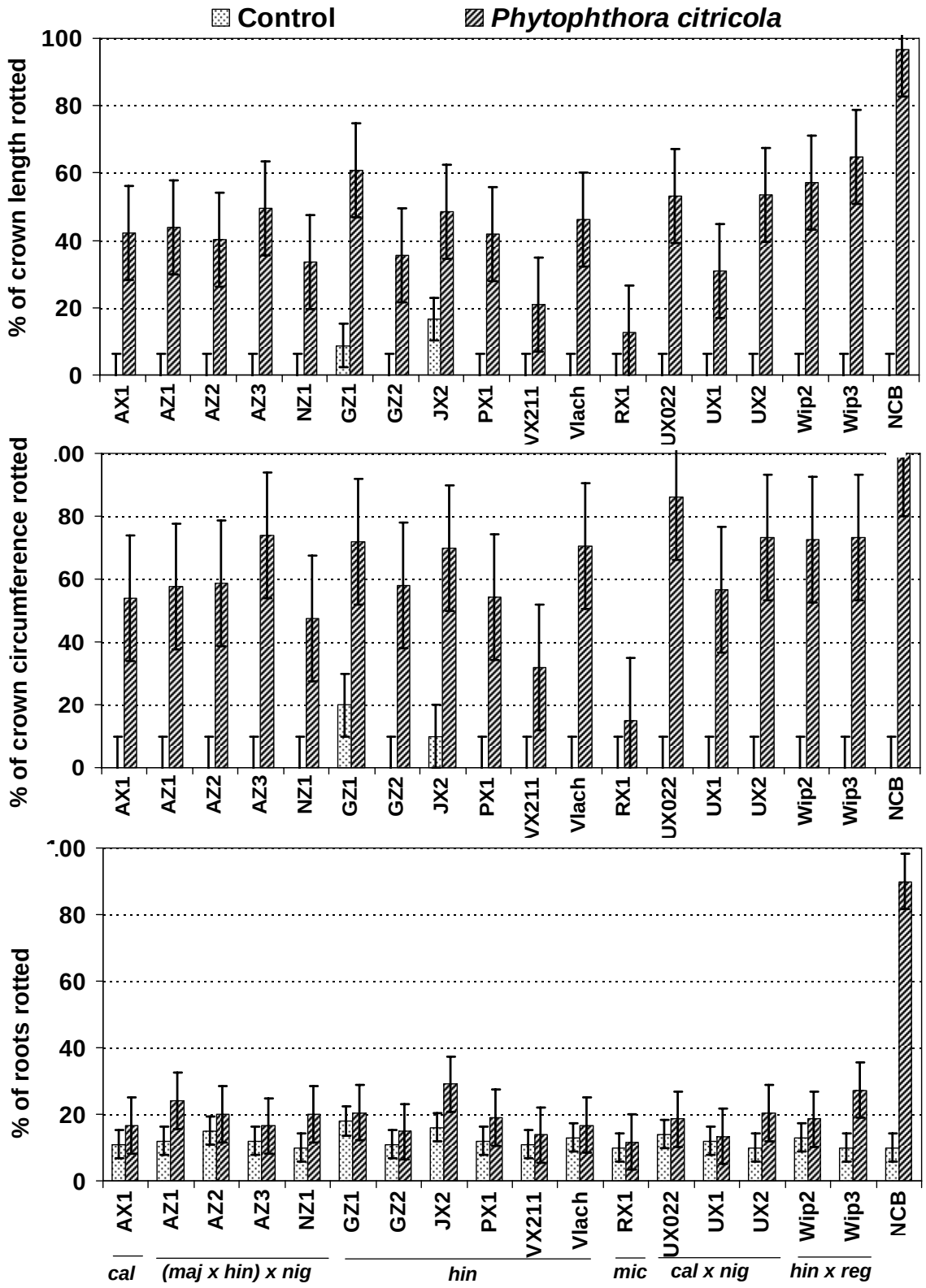

Fig. 1. Relative resistance to *Phytophthora citricola* among 17 clones of walnut rootstock hybrids and Northern California black walnut (NCB, *J. hindsii*) in a 2006 greenhouse experiment. Maternal parents of the hybrids are abbreviated on the lowest x-axis in italics.