

Resistance to *Phytophthora* and Graft Compatibility with Persian Walnut among Selections of Chinese Wingnut

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Abstract. Seedlings from seven open-pollinated selections of Chinese wingnut (*Pterocarya stenoptera*) (WN) representing collections of the USDA-ARS National Clonal Germplasm Repository at Davis, CA, and the University of California at Davis were evaluated as rootstocks for resistance to *Phytophthora cinnamomi* and *P. citricola* and graft compatibility with scions of five cultivars of Persian walnut (*Juglans regia*). Seedlings of Northern California black walnut (NCB) (*J. hindsii*) and Paradox hybrid (PH) (typically *J. hindsii* × *J. regia*) were used as standards. In greenhouse experiments, potted plants of the rootstocks were subjected to intermittent flooding in soil artificially infested with the pathogens. All WN seedlings were relatively resistant to the pathogens (means of 0% to 36% of root and crown length rotted) compared with NCB (44% to 100%) and PH seedlings (11% to 100%). Negligible disease occurred in flooded control soil without the pathogens. In 9-year graft compatibility trials in an orchard, NCB and PH rootstocks supported relatively good survival and growth of all tested scion cultivars ('Chandler', 'Hartley', 'Serr', 'Tulare', and 'Vina'; final scion survival 80% to 100%, mean scion circumference increase 292 to 541 mm), whereas results with WN were mixed. Wingnut rootstocks from all sources were incompatible with 'Chandler' (final scion survival 20% to 60%, scion circumference increase 17 to 168 mm). Conversely, all WN rootstocks from all sources were compatible with 'Tulare' and 'Vina' (final scion survival 80% to 100%, scion circumference increase 274 to 556 mm). Use of the WN rootstocks produced variable results in 'Hartley' and 'Serr' (final scion survival 10% to 100%, mean scion circumference increase 69 to 542 mm). There was a tendency for more rootstock sprouts on WN selections than on NCB or PH. In a commercial walnut orchard infested with *P. cinnamomi*, 'Hartley' survived and grew markedly better on WN selections than on PH. High resistance to *P. cinnamomi* and *P. citricola* was common to all of the WN selections. The results indicate that WN selections may be useful rootstocks for cultivars Tulare and Vina in soils infested with *P. cinnamomi* or *P. citricola* and that WN selections may contribute valuable resistance to these pathogens in walnut rootstock breeding efforts.

Phytophthora crown and root rot (PCRR) is among the most serious diseases of Persian walnut worldwide. In California, more than 10 species of *Phytophthora* have been implicated in the disease, but *Phytophthora cinnamomi* and *P. citricola* are especially aggressive and difficult to manage (Matheron and Mircetich, 1985a; Mircetich et al., 1998; Mircetich and Matheron, 1983). Incidence and severity of

PCRR generally can be minimized by careful soil water management and use of PH rootstock (typically *Juglans hindsii* × *J. regia*) (Mircetich et al., 1998). Compared with NCB (*J. hindsii*) and Persian walnut (*J. regia*) rootstocks, PH is more resistant to several species of *Phytophthora* (Matheron and Mircetich, 1985b; Mircetich and Matheron, 1983). In practice, however, the resistance of PH has been insufficient to prevent losses caused by *P. cinnamomi* or *P. citricola* (Mircetich et al., 1998). Improved management strategies are needed for these two pathogens on walnut.

Chinese wingnut (*Pterocarya stenoptera*) (WN) has been explored to a limited extent as

a rootstock for Persian walnut (Whitehouse and Joley, 1948; Zielinski, 1957). Seedlings of WN were reported to be highly resistant to *P. cinnamomi* and *P. citricola* (Browne et al., 2002; Matheron and Mircetich, 1985b), susceptible to *Armillaria* sp. (Serr and Rizzi, 1964), resistant to waterlogging injury (Catlin et al., 1977), and tolerant to *Pratylenchus vulnus* (Lownsberry et al., 1974). Although WN supported satisfactory scion growth in the Persian walnut cv. Eureka, it stunted the growth of other walnut scions (Serr and Rizzi, 1964). Chinese wingnut was hybridized with *J. regia*, and plants of the hybrids were produced using embryo rescue (McGranahan et al., 1986; Tulecke and McGranahan, 1985). The hybrids were highly resistant to *P. citricola* in vitro, but they did not survive in the field (McGranahan, unpublished data). Seedlings from different sources of *P. stenoptera* were reported to vary with respect to several vegetative growth characteristics (Li et al., 2001), but we are aware of no examinations of different wingnut seed families for resistance to *Phytophthora* or graft compatibility with current Persian walnut cultivars.

The objective of this study was to examine diverse open-pollinated seed families of *P. stenoptera* for: 1) relative resistance to *P. cinnamomi* and *P. citricola*; and 2) graft compatibility with key current cultivars of Persian walnut. It was hypothesized that superior seed sources of Chinese wingnut could be identified for use in breeding or propagating walnut rootstocks.

Materials and Methods

Plant materials. Seven sources of WN were used for evaluation of resistance to *Phytophthora* and graft compatibility with Persian walnut (Table 1). The seeds were collected from trees in September after they matured and became dry. Seed lots of NCB and PH were obtained from commercial nurseries in the fall. All of the seeds were air-dried, soaked overnight in clean tap water, rinsed thoroughly, and stratified for 3 to 4 months at 4 °C in moist sphagnum peat. The stratified seeds were planted in the spring in Jiffy Pots (8-cm diameter, 8-cm height) (Jiffy Products of America Inc., Lorain, OH) each filled with 300 mL UC Mix (Matkin and Chandler, 1957) and grown in a greenhouse (air temperature 18 to 33 °C, photoperiod 16 h). The plants were watered daily with deionized water and fertilized weekly with Grow More fertilizer (11-7-26, with micronutrients; Gardena, CA) until they were transplanted as described subsequently.

Inocula. Inocula of *P. cinnamomi* and *P. citricola* were grown on a V8 juice–oat–vermiculite substrate for 4 to 6 weeks as described previously (Matheron and Mircetich, 1985a). Three isolates collected from diseased walnut trees in different regions of California were used for each species of *Phytophthora* to represent pathogenic diversity. The isolates were grown individually in separate jars of substrate and were mixed in equal proportions

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Table 1. Half-sib seed family selections of Chinese wingnut (*Pterocarya stenoptera*) used in the study.

Selection	Location of maternal tree	Seed source of maternal tree ^y
DPTE 1.01	NCGR, ^z Davis, CA	Jiangsu province, China
DPTE 2.08	NCGR, Davis, CA	Beijing, China
DPTE 10.01	NCGR, Davis, CA	Dushanbe, Tajikistan
DPTE 11.02	NCGR, Davis, CA	Vacratot, Hungary
DPTE 17.01	NCGR, Davis, CA	Greifswald, Germany
Dairy Road	UC Davis grounds	Unknown
Minicenter	UC Davis grounds	Unknown

^zNational Clonal Germplasm Repository, USDA-ARS, Davis, CA.

^yFrom botanical gardens at listed locations.

just before introduction to soil. Sterile V8 juice–oat–vermiculite substrate was used as a control. Before mixing with soil, the inocula containing a *Phytophthora* sp. as well as the control were rinsed in sterile deionized water to remove unassimilated nutrients.

Evaluations of resistance to Phytophthora. Three experiments were conducted. Expt. 1 used seed lots collected in 1996, and Expts. 2 and 3 used seed lots collected in 1999. At 2 to 3 months of age, each seedling was transplanted into a 1-L pot of UC mix soil amended with 45 mL of sterile V8 juice–oat–vermiculite substrate (the control) or 45 mL of the substrate colonized by *P. cinnamomi* or *P. citricola*. In Expt. 1, five replicate plants were used per combination of soil inoculum treatment and seedling type (i.e., PH, NCB, and the multiple selections of WN). In Expts. 2 and 3, six replicate plants were used per treatment combination, except that there were 12 replicate plants for PH seedling.

A split plot design was used for all three experiments. The inoculum treatments were allocated to main plots, which were randomized in complete blocks. The subplots, consisting of the individual potted plants, were randomized within the main plots. Each seedling type was represented once in each main plot, except that two seedlings of PH (each in a separate pot) were included in main plots of Expts. 2 and 3.

To facilitate infection by the pathogens, soil flooding was administered 7 to 10 d after transplanting. Then and every 2 weeks thereafter, all pots in the control and inoculated treatments were placed in bowls, and cool tap water was added to the soil to maintain the water surface at ≈ 1 cm above the soil surface for 48 h. After each flooding, the pots were removed from the bowls and allowed to drain freely. Between floods, the soil was watered daily using deionized water.

Three months after transplanting, resistance to the pathogens was assessed according to the severity of root and crown rot and root fresh weights. The root systems were washed free from soil, weighed, and rated for the percentage of total root length and total crown length exhibiting necrosis. The percentage of root length rotted was estimated visually. Root length was considered necrotic when the cortex and stele tissues were black to dark brown and healthy when the same tissues were white. The percentage of crown length rotted was measured with a ruler. For rating purposes, the crown was considered to be the stem and central root system axis starting from the point

of main root convergence and extending to 3 cm above the soil surface. On the crown, a knife was used to reveal boundaries of healthy bark (white under the outer corky layer) and necrotic bark (black to brown).

Isolations were conducted from the root systems of all plants to confirm that those grown in non-infested soil remained free from *Phytophthora* spp. and that those grown in soil infested with *P. cinnamomi* or *P. citricola* had been exposed to the appropriate pathogen. Ten 1-cm-long segments of roots ≈ 1 mm in diameter were removed from each root system, cultured in PARP medium (Kannwischer and Mitchell, 1978), incubated in the dark at ≈ 18 °C, and observed after 5 d for the presence of mycelium of a *Phytophthora* sp. Species of *Phytophthora* were identified according to their morphological characteristics (Erwin and Ribero, 1996).

Evaluations of graft compatibility. Two field trials were conducted near Davis, CA, to examine graft compatibility of Persian walnut cultivars Chandler, Hartley, Serr, Tulare, and Vina on rootstocks of the seven WN selections and NCB and PH standards. In preparation for these trials, each scion–rootstock combination was propagated at two commercial walnut nurseries, one located near Oakdale, CA, (Nursery 1) and the other located near Yuba City, CA (Nursery 2). At each nursery, a split plot design was used with the rootstock selections allocated to main plots and scion cultivars allocated to subplots. At Nursery 1, there were four replicate main plots per rootstock (except for PH, which was propagated under identical conditions in adjacent rows) with a total of 16 to 20 trees (four to five per subplot) per rootstock–scion combination. At Nursery 2, there were five replicate main plots per rootstock with a total of 12 to 53 trees (two to 12 per subplot) per rootstock–scion combination. At each nursery, the main plots were randomized in complete blocks, and the subplots were randomized completely within main plots. At Nursery 1, trees were spaced 25 cm apart in the rows and 1.4 m apart between rows, whereas at Nursery 2, trees were 23 cm apart in the rows and 1.8 m apart between rows.

Briefly, propagation and grafting procedures at the nurseries were as follows: at Nursery 1, seeds collected in 1996 were stratified, grown into seedlings in 8-cm pots as described previously, and then transplanted into nursery plots in May 1997. At Nursery 2, seeds collected in 1997 were used. The PH and NCB seeds were planted directly in the nursery plots in Nov. 1997, whereas the WN selections,

stratified and grown in pots as described previously, were transplanted into the nursery plots in May 1998. Grafting of the scion–rootstock combinations at Nursery 1 was first attempted in mid-Mar. 1998 using the whip graft method (Hartmann et al., 1997) at ≈ 35 cm above the soil line. Nearly all of the grafts on WN selections failed. The grafts were removed by cutting back to the rootstock. The ungrafted WN and NCB seedlings of similar size were transplanted to new plots in four randomized complete blocks at the same nursery in early Feb. 1999. There were five trees per rootstock–scion combination arranged in a split plot design as described for the first planting. Paradox hybrid seedlings were grown and grafted in adjacent rows under the same conditions. In mid-Apr. 1999, all of the seedlings were whip-grafted at ≈ 35 cm above the soil line with the same walnut cultivars as used previously ('Chandler', 'Hartley', 'Serr', 'Tulare', and 'Vina'). Grafting of the scion–rootstock combinations at Nursery 2 also was completed in mid-Apr. 1999. The rootstocks were whip-grafted at ≈ 20 cm above the soil line. At both nurseries, the trees were dug during winter dormancy in early 2000 using conventional nursery equipment. Healthy grafted trees of each scion–rootstock combination were trimmed conventionally and stored with their roots covered in bins of moist sawdust. The trees were stored at 4 °C until transplanting to the orchard plots as described subsequently.

In Mar. 2000, the grafted rootstocks described previously were used to establish two graft compatibility trials in a field near Davis (long. 38°31.196' N, lat. 121°45.579' W). Expt. 1 included trees from Nursery 1, and Expt. 2 included trees from Nursery 2. The experiments were located adjacent to one another on Yolo loam and Yolo silty clay loam soil (fine-silty, mixed, superactive, nonacid, thermic Mollic Xerofluvents). The soil was not known to be infested with *Phytophthora* or phytopathogenic nematodes and did not have a history of walnut production. Each trial included five trees of each scion–rootstock combination in a randomized complete block design. The trees were spaced 4.6 m apart between and within rows. Scion and rootstock survival was tabulated 2 months after planting and at the end of the trial, ≈ 9 years after planting. Trunk circumference was measured at 30 cm above the graft union at the time of transplanting, every 1 to 2 years thereafter, and at the end of the trial. Scions that had died were assigned circumference values of zero. The intensity of shoot sprouting from the rootstocks was rated near the end of the experiments in May 2009. Until that time any sprouts had been trimmed back to the rootstock annually in February and in late summer. Rootstocks with no sprouts received a rating of 0, whereas those with one to five sprouts per tree, six to 10 sprouts per tree, and greater than 10 shoot sprouts per tree were assigned sprout ratings of 1, 2, and 3, respectively. Only rootstocks with living scions were rated.

Commercial orchard trial. A third field trial was established in Apr. 2000 in a commercial

walnut orchard near Linden, CA (long. 38°2.817' N, lat. 120°59.818' W) with a history of walnut tree death caused by *P. cinnamomi*. The soil was a Cognia loam (fine-silty, mixed, superactive, thermic Calcic Pachic Haploxerolls), and the trial was established in a rectangular area from which all walnut trees had been removed. The trial included only 'Hartley' grafted on the WN selections and PH, and the trees were produced at Nursery 2 along with those used in the graft compatibility trial at Davis. There were 15 to 20 trees per rootstock selection randomized in four complete blocks. Each block included a plot of three to five trees for each rootstock. Tree survival was monitored at 2 months after planting (June 2000) and at the end of the trial (July 2004). Tree circumference was measured at 1 and 3 years after planting, and dead scions were assigned trunk circumferences of zero. Trees that died and had symptoms of root and crown rot were subjected to diagnostic isolations as described previously (Matheron and Mircetich, 1985a).

Data analysis. The MIXED procedure of SAS software Version 9.2 (SAS Institute Inc., Cary, NC) was used for analysis of variance (ANOVA) of the continuous variables, i.e., percentage of root length rotted, percentage of crown length rotted, increase in trunk circumference, and root fresh weight. The percentages were transformed to arcsine values before analysis. When there were multiple observations per plot, the plot means were the values used for ANOVA. Treatment factors of inoculum, rootstock, and scion were specified as fixed effects in the model statements of the MIXED procedure, whereas block was specified as a random effect in the random statements. In repeated experiments, the random statement specified block within the experiment. Additionally, in the greenhouse experiments, the random statement specified block × inoculum interaction. In cases in which treatment effects were significant ($P \leq 0.05$) for continuous variables, 95% confidence intervals were either presented with the means (figure) or used to separate the means (tables). For clarity, non-transformed percentage means were used for presentation in tables. In the graft compatibility trials and the commercial orchard trial, Fisher's exact test (Agregi, 2007) was used to compare tree survival rates on the Paradox standard rootstock with survival rates on the other rootstocks; 2×2 contingency tables specifying counts of living and dead individuals for Paradox rootstock and each of the other rootstocks (in turn) were evaluated. Means and SE of the means were calculated for the rootstock sprout severity scores in the graft compatibility trials.

Results

Evaluations of resistance to Phytophthora. In the greenhouse evaluations of resistance to *P. cinnamomi* and *P. citricola*, there was a significant experiment × inoculum × rootstock interaction ($P < 0.0001$, $P = 0.009$, and $P < 0.0001$ for percentage of root length rotted, percentage of crown length rotted, and root fresh weight, respectively). In Expt. 1, the NCB

and PH rootstock standards were highly susceptible to *P. cinnamomi* and *P. citricola* and developed high levels of root and crown rot (68% to 100%; Table 2) and significant reductions in root fresh weight in soil with the pathogens ($P = 0.05$). In contrast, the WN selections were relatively resistant, sustaining negligible root and crown rot in soil infested with *P. citricola* and relatively low levels of root and crown rot in soil infested with *P. cinnamomi*. Neither pathogen significantly reduced root fresh weights of any WN selection.

In Expts. 2 and 3, results were similar to those of Expt. 1, except PH exhibited only low to moderate levels of root and crown rot in soil infested with *P. citricola* (11% to 53%; Table 2) and no root fresh weight reduction with either species of *Phytophthora*. The paradox hybrid still was significantly more susceptible than WN selections to both *Phytophthora* spp., except for percentage of crown length rotted in Expt. 3. All the selections of WN developed low levels of root or crown rot that were not significantly different from those of the controls (Table 2). In Expts. 2 and 3, *P. cinnamomi* significantly reduced root fresh weights of 'WN 1.01' and 'WN Minicenter' seedlings, respectively. In Expt. 3, *P. citricola* significantly reduced the root weights of

'WN Minicenter'. Root weights of the other WN selections were unaffected significantly by the pathogen.

In each trial, root isolations from the experimental plants confirmed that *Phytophthora* was absent on root systems grown in non-infested control plots and that the expected species of *Phytophthora* was present in plots infested with one of the pathogens. The pathogens were isolated from all selections of WN as well as from NCB and PH in treatments infested with *Phytophthora*.

Evaluations of graft compatibility. Tree survival and rootstock sprout results were similar in Expts. 1 and 2 and therefore were combined for presentation (Table 3). Most of the scion-rootstock combinations initially survived in high proportion after transplanting into the Davis graft compatibility trials (Table 3). Exceptions were that only six of the 10 trees of 'Chandler' on WN '10.01' and six of the 10 trees of cultivar Hartley on WN 'Minicenter' rootstock survived initially. Final scion and rootstock survival rates varied greatly among the scion-rootstock combinations (Table 3). All scion cultivars survived and grew adequately on the NCB and PH standards. In contrast, high proportions of 'Chandler' scions ultimately failed on all of the WN selections.

Table 2. Comparative susceptibility of open-pollinated seedlings from seven sources of Chinese wingnut (*Pterocarya stenoptera*) and standard walnut rootstock seedlings to *Phytophthora cinnamomi* and *P. citricola* in three greenhouse experiments.²

Inoculant ¹	Selection	Expt. 1		Expt. 2		Expt. 3	
		Root rot (%)	Crown length rotted (%)	Root rot (%)	Crown length rotted (%)	Root rot (%)	Crown length rotted (%)
Control	NCB ^x	5 a-d	0 a	7 a	3 a	6 ab	0 a
	PH ^w	2 abc	0 a	18 a	4 a	10 ab	0 a
	Wn. ^v DPTE 1.01	0 ab	0 a	4 a	0 a	3 a	0 a
	Wn. DPTE 2.08	0 a	0 a	4 a	0 a	4 a	0 a
	Wn. DPTE 10.01	0 ab	0 a	5 a	0 a	4 a	0 a
	Wn. DPTE 11.02	0 a	0 a	6 a	0 a	6 ab	0 a
	Wn. DPTE 17.01	0 a	0 a	5 a	0 a	5 ab	0 a
	Wn. Dairy Rd.	0 a	0 a	5 a	0 a	4 a	0 a
	Wn. Minicenter	0 a	0 a	4 a	0 a	4 a	0 a
<i>P. cinnamomi</i>	NCB	98 g	68 b	90 c	53 c	91 d	44 b
	PH	100 g	78 c	90 c	49 c	97 d	38 b
	Wn. DPTE 1.01	36 e	16 a	17 a	2 a	18 ab	3 a
	Wn. DPTE 2.08	22 cde	24 a	11 a	0 a	15 ab	3 a
	Wn. DPTE 10.01	21 b-e	18 a	13 a	6 a	24 bc	3 a
	Wn. DPTE 11.02	36 e	11 a	11 a	3 a	16 ab	0 a
	Wn. DPTE 17.01	25 de	7 a	11 a	5 a	15 ab	3 a
	Wn. Dairy Rd.	30 e	15 a	15 a	5 a	14 ab	12 a
	Wn. Minicenter	24 de	13 a	18 a	7 ab	6 ab	7 a
<i>P. citricola</i>	NCB	100 g	100 d	85 c	86 d	89 d	65 c
	PH	85 f	79 bc	53 b	30 bc	32 c	11 a
	Wn. DPTE 1.01	0 a	0 a	6 a	0 a	4 a	1 a
	Wn. DPTE 2.08	0 a	0 a	7 a	0 a	4 a	1 a
	Wn. DPTE 10.01	1 a	0 a	8 a	0 a	5 ab	1 a
	Wn. DPTE 11.02	0 a	0 a	13 a	0 a	5 ab	1 a
	Wn. DPTE 17.01	0 a	0 a	7 a	0 a	4 a	1 a
	Wn. Dairy Rd.	1 a	0 a	7 a	0 a	4 a	1 a
	Wn. Minicenter	0 a	0 a	6 a	0 a	5 ab	0 a

²Effect of experiment × inoculant × rootstock significant at $P < 0.0001$ for root rot and $P = 0.009$ for crown rot. Analysis of variance results and mean separations based on arcsine-transformed data. Means presented are non-transformed. Mean separations are based on 95% confidence intervals generated from the arcsine-transformed data; values in the same column without letters in common had non-overlapping 95% confidence intervals.

¹V8 juice-vermiculite-oat substrate was either sterile (the control) or colonized by the indicated pathogen.

^xNCB = Northern California black walnut (*Juglans hindsii*), a standard rootstock for walnut in California.

^wPH = Paradox hybrid (typically *J. hindsii* × *J. regia*), a standard rootstock for walnut in California.

^vWn = Chinese wingnut (*Pterocarya stenoptera*).

Table 3. Comparative performance of Persian walnut scion cultivars on rootstocks of Northern California black walnut (*Juglans hindsii*), Paradox hybrid (typically *J. hindsii* × *J. regia*), and seedlings from seven selections of Chinese wingnut (*Pterocarya stenoptera*).²

Rootstock	Scion	No. of surviving trees/total number of trees ³				Rootstock sprout severity score (and SE) ⁴
		Initial		Final		
		Rootstock	Scion	Rootstock	Scion	
NCB	Chandler	10/10	9/10	10/10	10/10	0.4 (0.2)
	Hartley	9/9	9/9	9/9	9/9	0.1 (0.1)
	Serr	9/10	9/10	9/10	8/10	0.0 (0.0)
	Tulare	10/10	10/10	10/10	10/10	0.4 (0.2)
	Vina	9/10	9/10	9/10	9/10	0.4 (0.2)
PH	Chandler	10/10	10/10	10/10	10/10	0.5 (0.2)
	Hartley	10/10	10/10	10/10	10/10	0.2 (0.1)
	Serr	10/10	10/10	10/10	10/10	0.0 (0.0)
	Tulare	10/10	10/10	10/10	10/10	0.1 (0.1)
	Vina	10/10	10/10	10/10	10/10	0.1 (0.1)
Wn. DPTE 1.01	Chandler	10/10	8/10	*4/10*	*2/10*	3.0 (0.0)
	Hartley	10/10	10/10	10/10	10/10	1.4 (0.3)
	Serr	10/10	10/10	7/10	7/10	1.6 (0.4)
	Tulare	10/10	10/10	10/10	10/10	0.6 (0.2)
	Vina	9/10	9/10	8/10	8/10	0.3 (0.2)
Wn. DPTE 2.08	Chandler	10/10	8/10	*6/10*	*4/10*	3.0 (0.0)
	Hartley	10/10	10/10	9/10	9/10	2.3 (0.2)
	Serr	9/10	9/10	*6/10*	*6/10*	1.2 (0.5)
	Tulare	10/10	10/10	10/10	10/10	0.5 (0.3)
	Vina	10/10	10/10	9/10	9/10	0.2 (0.1)
Wn. DPTE 10.01	Chandler	10/10	*6/10*	*6/10*	*2/10*	2.5 (0.5)
	Hartley	8/10	8/10	7/10	7/10	1.9 (0.4)
	Serr	9/9	8/9	7/9	7/9	0.3 (0.2)
	Tulare	10/10	10/10	9/10	9/10	0.7 (0.2)
	Vina	9/10	9/10	8/10	8/10	0.9 (0.4)
Wn. DPTE 11.02	Chandler	10/10	8/10	9/10	*6/10*	2.5 (0.5)
	Hartley	10/10	10/10	9/10	9/10	2.3 (0.2)
	Serr	10/10	10/10	8/10	*6/10*	1.7 (0.5)
	Tulare	10/10	10/10	10/10	10/10	0.6 (0.2)
	Vina	10/10	10/10	10/10	10/10	1.3 (0.3)
Wn. DPTE 17.01	Chandler	9/10	9/10	7/10	*4/10*	2.0 (0.7)
	Hartley	10/10	9/10	9/10	7/10	1.4 (0.5)
	Serr	10/10	10/10	*4/10*	*1/10*	—
	Tulare	10/10	10/10	8/10	8/10	0.4 (0.3)
	Vina	10/10	10/10	10/10	9/10	1.3 (0.3)
Wn. Dairy Rd.	Chandler	10/10	9/10	*5/10*	*3/10*	1.5 (0.5)
	Hartley	9/10	9/10	7/10	7/10	1.9 (0.3)
	Serr	10/10	10/10	*5/10*	*4/10*	1.0 (0.7)
	Tulare	10/10	10/10	8/10	8/10	0.6 (0.4)
	Vina	10/10	10/10	9/10	9/10	1.6 (0.4)
Wn. Minicenter	Chandler	9/10	8/10	7/10	*5/10*	1.0 (0.3)
	Hartley	*6/10*	*6/10*	7/10	*6/10*	0.5 (0.3)
	Serr	10/10	9/10	10/10	9/10	1.6 (0.4)
	Tulare	10/10	8/10	9/10	8/10	0.0 (0.0)
	Vina	9/10	9/10	9/10	9/10	0.2 (0.1)

²Trees grafted in replicate plots at two commercial nurseries were transplanted in Mar. 2000 for evaluations of graft compatibility in two field experiments near Davis, CA. Results of the two experiments were similar and were combined here.

³Initial and final survival ratings were made in June 2000 and May 2009, respectively. Numerators of the fractions indicate number of rootstocks or scions surviving, and denominators indicate the total number of trees planted. Within a column, proportions bracketed by asterisks differ significantly from those for the corresponding cultivar on PH ($P \leq 0.05$, Fisher's exact test).

⁴Scored in May 2009 according to the following scale: rootstocks with no sprouts received a rating of 0, whereas those with one to five sprouts per tree, six to 10 sprouts per tree, and greater than 10 shoot sprouts per tree were assigned sprout ratings of 1, 2, and 3, respectively. Only rootstocks with living scions were included.

A relatively low proportion of 'Serr' trees ultimately survived on WN '2.08', '11.02', '17.01', and 'Dairy Road' selections, but most 'Serr' scions survived on WN '1.01', '10.01', and 'Minicenter' selections. 'Tulare' and 'Vina' survived in high proportion across all WN selections (Table 3).

The scion-rootstock combinations that had high proportions of final survival tended to

produce satisfactory scion growth (Fig. 1A–B). Trunk circumference increases were affected by a significant scion × rootstock × experiment interaction ($P = 0.01$). All of the scion cultivars grew relatively well on the standard NCB and PH rootstocks, but 'Chandler' grew poorly on all WN selections. Including dead scions, which were assigned trunk circumference increases of zero, mean trunk circumference

increases for 'Chandler' on WN selections ranged from 4% to 47% of those on NCB and PH rootstocks (Fig. 1A–B). Including only the surviving 'Chandler' scions, mean trunk circumference increases during the experiment for 'Chandler' on WN selections ranged from 14% to 85% of the increases on NCB and Paradox rootstocks. A few trees of 'Chandler' on WN (i.e., one on WN '11.02', two on WN '17.01', and one on WN 'Minicenter') generated increases in trunk circumference that fell within the 95% confidence intervals for mean growth of 'Chandler' on NCB and PH. Surviving scions of 'Serr' and 'Hartley' on WN selections generally fared better than those of 'Chandler'. Mean increases in trunk circumference of surviving trees of 'Serr' and 'Hartley' on WN selections ranged from 51% to 137% of the corresponding increases on NCB and PH.

Among the trees that survived the initial transplanting on WN but failed later, it was possible to distinguish three arbitrary classes. In one category, the scions died back either partly or completely, starting with distal portions of scaffolds or shoots. In many cases, living basal portions of the affected scions initiated new growth for 1 or more years in the spring and summer, but the dieback recurred annually until the scions ultimately died. This category predominated in failed 'Chandler' scions, occurring on all WN selections (incidence 38% to 67% of trees that survived transplanting), but it also occurred in 'Hartley' (11% on WN 'Dairy Rd. '), 'Serr' (12% and 22% on WN '10.01' and '2.08', respectively), 'Tulare' (10% on '10.01', '17.01', 'Dairy Rd. '), and 'Vina' (11% on '10.01', 10% on '17.01'). In a second category, tree failure involved sudden trunk breakage at the graft union. This situation did not occur with 'Chandler' but did so with 'Hartley' (incidence 22% on WN '17.01'), 'Serr' (10% to 40% among WN '1.01', '11.2', '17.01', and 'Dairy Rd. '), and 'Vina' (11% on '1.01'). A third category of tree failure involved death of both the scion and rootstock. Its incidence was greatest with 'Chandler' on WN (12% to 22% among WN '1.01', '2.08', 'Dairy Rd. '), but it also occurred on 'Hartley' (10% on '2.08' and '11.02'), 'Serr' (10% to 20% among '1.01', '2.08', '17.01', and 'Dairy Rd. '), 'Tulare' (10% on '17.01' and 'Dairy Rd. '), and 'Vina' (10% on '2.08' and 'Dairy Rd. ').

Among the trees that survived for the duration of the Davis trials, mean severity of rootstock shoot sprouts at the end of the trial period was affected by significant rootstock × scion interaction ($P = 0.0001$), but there was no significant experiment × rootstock × scion interaction ($P = 0.16$). Mean sprout severity scores were relatively low (i.e., 0.0 to 0.5) for NCB and PH rootstocks. Sprout severity scores were relatively high (i.e., 1.0 to 3.0) for most WN rootstocks grafted with 'Chandler', 'Hartley', and, in some cases, with 'Serr' (Table 3). The limited tree survival of 'Serr' on '17.01' prevented estimation of its mean rootstock sprout score.

Commercial orchard trial. Most trees of 'Hartley' grafted on the standard seedling PH rootstock survived the initial transplant

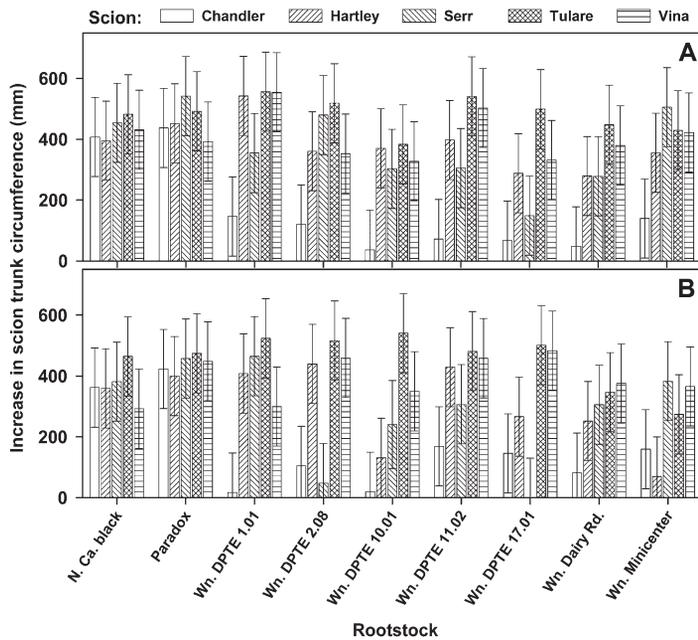


Fig. 1. Comparative growth of cultivar Hartley scions on standard seedling rootstocks of Northern California black walnut (*Juglans hindsii*), Paradox hybrid (typically *J. hindsii* × *J. regia*), and seven seed families of Chinese wingnut (*Pterocarya stenoptera*). (A) Results from Expt. 1; (B) results from Expt. 2. In each experiment, trees grafted in replicate plots at a commercial nursery were transplanted in Mar. 2000 for evaluations of graft compatibility near Davis, CA. Increases in trunk circumference were measured from the time of transplanting to May 2009. Effect of experiment × rootstock × scion interaction was significant ($P = 0.01$). Error bars indicate 95% confidence intervals for the means.

Table 4. Comparative performance of ‘Hartley’ walnut on seedling rootstocks of Paradox hybrid (typically *Juglans hindsii* × *J. regia*) and open-pollinated seed families of Chinese wingnut (*Pterocarya stenoptera*) in a commercial orchard infested with *Phytophthora cinnamomi*.^z

Rootstock	No. of surviving trees/total number of trees ^y				Increase in scion circumference (mm) ^x
	Initial		Final		
	Rootstock	Scion	Rootstock	Scion	
Paradox	17/18	16/18	2/18	2/18	10 a
Wn. DPTE 1.01	15/17	14/17	*14/17*	*14/17*	96 b
Wn. DPTE 2.08	19/20	19/20	*19/20*	*19/20*	103 b
Wn. DPTE 10.01	15/15	15/15	*15/15*	*15/15*	118 b
Wn. DPTE 11.02	16/16	16/16	*16/16*	*16/16*	107 b
Wn. DPTE 17.01	18/19	18/19	*18/19*	*18/19*	91 b
Wn. Dairy Rd.	17/18	16/18	*16/18*	*16/18*	46 a
Wn. Minicenter	16/17	16/17	*15/17*	*15/17*	87 b

^zThe selections were transplanted in Apr. 2000 into a cleared area of a commercial walnut orchard where previous trees had died in association with infection by *Phytophthora cinnamomi*.

^yInitial and final survival ratings were made in June 2000 and July 2004, respectively. Numerators of the fractions indicate number of rootstocks or scions surviving, and denominators indicate the number of trees planted. The proportions bracketed by asterisks differ significantly from those of PH in the same column ($P \leq 0.05$, Fisher’s exact test).

^xIncrease in circumference during the period from 1 to 3 years after planting. Means without letters in common have non-overlapping 95% confidence intervals.

and began growth in the commercial orchard known to be infested with *P. cinnamomi*, but few of the trees (two of 18) survived through the end of the trial (Table 4). In contrast, most trees of ‘Hartley’ grafted on WN rootstocks survived the initial transplanting and grew through the end of the trial. The mean increase in scion trunk circumference during the trial was significantly greater on all WN rootstocks except for WN ‘Dairy Road’ compared with the increase on PH (Table 4). The trees that died on PH exhibited symptoms of *Phytophthora* root and crown rot, and *P. cinnamomi* was isolated from them.

Discussion

This is apparently the first report to examine multiple seed families of *P. stenoptera* for variation in resistance to *Phytophthora* spp. and graft compatibility with current Persian walnut cultivars. Open-pollinated seed families from seven mother trees of Chinese wingnut, selected to represent genetic variation among collections of *P. stenoptera* at the USDA-ARS National Clonal Germplasm Repository and UC Davis, were highly resistant to *P. cinnamomi* and *P. citricola*, suggesting that this resistance is highly conserved in WN. None

of the seven wingnut sources yielded seedlings that were graft-compatible with all tested Persian walnut cultivars. All WN seed families exhibited incompatibility with ‘Chandler’ scions, and some of them supported relatively poor survival and growth of ‘Hartley’ and ‘Serr’ scions. ‘Chandler’ is the dominant walnut cultivar in California [used on 37% of land area devoted walnut in California (NASS, USDA, 2010)] and its lack of compatibility with WN reduces the potential usefulness of WN rootstocks in California. However, ‘Tulare’ and ‘Vina’ survived and grew as well on all of the WN seed families as on the standard rootstocks, NCB and PH, and therefore WN may prove to be very useful for growers of these cultivars on soils infested with *P. cinnamomi* or *P. citricola*.

Our findings suggest future research topics involving *P. stenoptera*. For example, it would be useful to examine other Persian walnut cultivars of world importance for their compatibility with WN rootstock selections. Also, it may be possible to use the resistance of *P. stenoptera* in rootstock breeding efforts. It is unknown whether WN-compatible walnut interstems can be used to facilitate joining of incompatible cultivars such as ‘Chandler’ on WN, and this approach is being tested commercially. We have not attempted to determine whether the few individual WN seedlings that supported adequate growth of ‘Chandler’ are in fact compatible with the cultivar. This determination would require clonal propagation of the putatively compatible WN rootstock seedlings followed by replicated testing.

We did not attempt to assess yield potential of Persian walnut on the WN selections reported here, but such work would be valuable in further assessments. A previous report compared yield of the cultivar Hartley on seedling rootstock of *P. stenoptera* from the ‘Minicenter’ tree with yields of the same cultivar on Manregian seedling rootstock (*J. regia*), PH, and NCB rootstocks in Years 5 to 9 after planting (Civerolo et al., 1996). The 5-year cumulative yield on WN rootstock was equivalent to that on NCB rootstock, but it was exceeded by the yield on Manregian rootstock. Yield on PH was intermediate between that on Manregian and the other rootstocks.

In our trials, PH expressed greater susceptibility to *Phytophthora* in Expt. 1 than in Expts. 2 and 3. This may have resulted from environmental differences among the trials and/or from genetic variation in resistance among the sources of Paradox used. Commercial seedlings of PH are known to be genetically diverse (Potter et al., 2002) and vary in resistance to *P. citricola* and *P. cinnamomi* (Mircetich et al., 1998) (Browne, unpublished data).

The results of the trial in the commercial orchard infested with *P. cinnamomi* provided field validation of the resistance WN seed families expressed in greenhouse trials, thereby confirming the practical value of these rootstocks under such circumstances. Conversely, the death of nearly all trees on PH rootstock in this orchard resulting from root and crown rot associated with *P. cinnamomi* confirmed that

in soils infested with the pathogen, conventional walnut rootstocks offer insufficient resistance to the pathogen. Results of the graft compatibility trials at Davis suggest that cvs. Tulare and Vina grafted on WN seedling rootstocks would perform as well or better than 'Hartley' on WN in orchard soils infested with *P. cinnamomi*.

Our results involving seven different sources of *P. stenoptera* confirm and expand those of previous investigators (Matheron and Mircetich, 1985b; Serr and Rizzi, 1964) who apparently used single sources of WN seedlings in their evaluations of this species. Interestingly, *Pterocarya fraxinifolia*, the Caucasian walnut, was reported to be highly resistant to species of *Phytophthora* (Banihashemi and Ghaderi, 2006; Ghaderi and Banihashemi, 2007). *Pterocarya fraxinifolia* exhibited no disease when it was grown in soil infested with *P. citricola*, *P. citrophthora*, or *P. cactorum*, whereas all tested cultivars of *J. regia* became diseased (Ghaderi and Banihashemi, 2007).

Worldwide, *P. cinnamomi* is considered to be the most important species of *Phytophthora* affecting walnut (Belisario et al., 2009). Until recently at least, a high level of resistance to both root and crown rot caused by this pathogen such as that reported here and previously in *P. stenoptera* (Matheron and Mircetich, 1985b) has not been reported among species or hybrids of *Juglans*. However, in a recent report based on inoculations of excised shoots, *J. hindsii*, *J. nigra*, and *J. mandshurica* were more resistant than *J. regia* and *J. sieboldiana* to *P. cinnamomi* (Belisario et al., 2009). In previous tests, when *J. hindsii* and *J. nigra* were planted in soil infested with *P. cinnamomi* and subjected to biweekly flooding, they developed severe root and crown rot, and the flooding caused no disease without the pathogen (Matheron and Mircetich, 1985b). Recent trials have indicated that a clonal hybrid of *J. microcarpa* × *J. regia*, 'RX1', is moderately resistant to *P. cinnamomi* (McGranahan et al., 2010). It is clear that further examination and use of resistance to *P. cinnamomi* in *Pterocarya* and *Juglans* species have potential to contribute to integrated strategies for

management of *Phytophthora* crown and root rot worldwide.

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