# Relative Resistance of Different Rootstocks of English Walnut to Six *Phytophthora* spp. That Cause Root and Crown Rot in Orchard Trees

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## **ABSTRACT**

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Seedlings of Juglans ailantifolia, J. californica, J. cinerea, J. major, J. microcarpa, J. neotropica, J. nigra, J. regia, and Pterocarya stenoptera were compared with seedlings of J. hindsii and Paradox (J. hindsii × J. regia), standard rootstocks, for their relative resistance to Phytophthora citricola in artificially infested soil. Under conditions conducive for disease development, i.e., soil flooded for 48 hr biweekly, only Pterocarya stenoptera was highly resistant to Phytophthora citricola. J. ailantifolia, J. nigra, Paradox, and Pterocarya stenoptera were subsequently compared with J. hindsii for their resistance to Phytophthora citricola, P. cinnamomi, P. cryptogea, P. citrophthora, P. cactorum, and P. megasperma. J. hindsii was highly susceptible to P. cryptogea, P. citrophthora, P. cactorum, and P. megasperma only when flooded biweekly for 48 hr, but it was highly susceptible to P. citricola and P. cinnamomi in both nonflooded and periodically flooded treatments. J. ailantifolia showed significant resistance to P. cinnamomi, whereas Paradox, J. ailantifolia, and J. nigra were resistant to P. citricola and P. cryptogea under nonflooded conditions; however, this resistance was overcome by periodic flooding. Pterocarya stenoptera was highly resistant to Phytophthora citricola, P. cinnamomi, P. cryptogea, P. citrophthora, P. cactorum, and P. megasperma, even when flooded for 48 hr biweekly.

Surveys of commercial walnut orchards in California have revealed the association of 11 *Phytophthora* spp. with root and crown rot of English walnut (*Juglans regia* L.) trees (7,10). These *Phytophthora* spp. are *P. cactorum* (Leb. & Cohn) Schroet., *P. cinnamomi* Rands, *P. citricola* Sawada, *P. citrophthora* (R. E. Smith & E. H. Smith) Leonian, *P.* 

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cryptogea Pethyb. & Laff., P. drechsleri Tucker, P. megasperma Drechs., P. parasitica Dast., and three unidentified Phytophthora spp. (isolates 489R, 1029, and 2833). Their pathogenicity to J. hindsii (Jeps.) Jeps., a common walnut rootstock, has been demonstrated (7,10). Paradox (J. hindsii  $\times$  J. regia), another standard walnut rootstock in California commercial orchards, was highly susceptible to P. citricola, P. cinnamomi, and P. cryptogea but was more resistant than J. hindsii to infection by P. cactorum, P. citrophthora, P. megasperma, and an unidentified Phytophthora sp. (isolate 1029) (7,10).

Although it has not been used widely, another walnut rootstock, *Pterocarya stenoptera* DC., has been tried to a limited extent in California. *P. stenoptera* is used extensively in China as a walnut rootstock (12) and was reported to be highly resistant to *P. cinnamomi* when grown in soil artificially infested with a mixture of *P. cinnamomi* isolates from host plants other than walnut (11). These investigations were undertaken to evaluate and compare commonly used and potential English walnut rootstocks

for resistance to *Phytophthora* spp. known to cause root and crown rot of walnut trees in California. A brief account has appeared (6).

### MATERIALS AND METHODS

In the first series of experiments, seedlings of the following walnut rootstocks were evaluated for their relative resistance to P. citricola in artificially infested soil: J. ailantifolia Carr. (syn. J. sieboldiana Maxim, Japanese walnut); J. californica S. Wats. (Southern California black walnut): J. cinerea L. (butternut); J. hindsii (Northern California black walnut): J. major (Torr.) Heller (Arizona walnut); J. microcarpa Berland. (little walnut); J. neotropica Diels (South American black walnut); J. nigra L. (eastern black walnut); J. regia L. 'Eureka,' 'Carpathian,' and 'Manregian'; Paradox (J. hindsii $\times$  J. regia); and Pterocarya stenoptera (Chinese wingnut). Inoculum of P. citricola was prepared as described previously (7) and mixed with steampasteurized U.C. mix (1:1 sand/peat) potting soil (1) at the rate of 10 cm<sup>3</sup> of inoculum per 1,000 cm<sup>3</sup> of U.C. mix. The controls received rinsed vermiculite but no Phytophthora spp. Two-month-old rootstock seedlings were grown in pasteurized U.C. mix in pressed peat moss pots  $(7.6 \times 7.6 \text{ cm}; \text{ E. C. Geiger},$ Harleysville, PA), then transplanted into plastic pots (14 cm in diameter × 12.7 cm deep) with artificially infested potting mix and maintained for 3 mo in the greenhouse. Soil temperature ranged from 17 to 25 C. Plants were fertilized weekly with an aqueous mixture of calcium nitrate, iron chelate, and Nutrimin minor element concentrate (E. C. Geiger). Plants were flooded biweekly for 48 hr by placing them into watertight containers (16.5 cm in diameter × 13 cm deep) and flooding so that 1 cm of free water stood on the soil surface and around the seedling trunk. Between flooding treatments, the soil drained freely, and all seedlings were watered as needed with enough water to thoroughly

moisten the soil without prolonged saturation.

In the second series of experiments, rootstocks that had shown a degree of resistance to *P. citricola* were tested by the same methods for resistance to isolates of *P. citricola*, *P. cinnamomi*, *P. citrophthora*, and *P. cryptogea* from walnut.

In a third series of tests, we studied the relative resistance of *J. hindsii*, *J. nigra*, Paradox, and *Pterocarya stenoptera* to *Phytophthora cactorum* and *P. megasrma* in soil flooded biweekly for 48 hr.

All experiments ended 3 mo after seedlings were transplanted into infested

soil. Each experiment was repeated twice, and because all experiments yielded similar and comparable results, data from one representative experiment are presented. Trunk canker severity was rated with an index (9) where 0 = no crown rot, 1 = up to 25% of the trunk circumference killed, 2 = up to 50%, 3 = up to 75%, and 4 = girdled trunk, dead plant. Severity of root rot was visually estimated and confirmed to result from infection by the appropriate *Phytophthora* spp. by reisolation of the pathogens.

#### RESULTS

When periodically flooded, J.

Table 1. Growth and disease severity in 13 walnut rootstocks planted in soil infested with *Phytophthora citricola* and flooded for 48 hr biweekly

	Average growth and disease severity <sup>x</sup>					
Rootstock (seedlings)	Increase in stem diameter as percentage of control	Plants dead <sup>y</sup>	Duration of survival <sup>2</sup>			
Pterocarya stenoptera	96	0 с	92 a			
Juglans ailantifolia	45	8 b	68 b			
J. nigra	41	9 a	54 c			
Paradox hybrid	25	8 b	50 c			
J. cinerea	8	9 a	38 d			
J. regia 'Carpathian'	22	10 a	37 d			
J. neotropica	7	10 a	22 e			
J. microcarpa	0	10 a	16 ef			
J. regia 'Eureka'	0	10 a	15 ef			
J. major	6	10 a	15 ef			
J. californica	1	10 a	15 ef			
J. hindsii	4	10 a	14 ef			
J. regia 'Manregian'	0	10 a	9 f			

<sup>&</sup>lt;sup>x</sup>Average of 10 replicates per rootstock. Duration of experiment was 92 days. Numbers in each column with the same letter do not differ (P = 0.05) according to Duncan's multiple range test. Uninfested controls showed no plant mortality.

Table 2. Relative resistance of five walnut rootstocks to four *Phytophthora* spp. as influenced by biweekly flooding of soil for 48 hr

Treatment Rootstock	Average disease severity <sup>x</sup>							
	P. citricola		P. cinnamomi		P. cryptogea		P. citrophthora	
	Root rot <sup>y</sup> (%)	Girdling index <sup>z</sup>	Root rot (%)	Girdling index	Root rot (%)	Girdling index	Root rot (%)	Girdling index
Flooded biweekly								
Juglans hindsii	100 a	4.0 a	96 ab	3.7 a	98 a	3.8 a	100 a	3.7 a
Paradox hybrid	83 ab	3.8 a	86 b	3.3 a	67 b	2.4 b	24 bc	1.0 c
J. ailantifolia	77 b	3.9 a	100 a	4.0 a	100 a	4.0 a	17 bcd	1.1 c
J. nigra	82 ab	4.0 a	89 ab	3.8 a	96 a	4.0 a	15 bcd	1.9 b
Pterocarya								
stenoptera	14 c	0.1 b	31 c	0.6 b	9 c	0.0 c	9 cd	0.0 d
Nonflooded								
J. hindsii	98 a	4.0 a	96 a	3.8 a	13 a	0.0 a	30 a	1.9 a
Paradox hybrid	64 b	2.5 b	82 b	2.9 b	10 a	0.0 a	20 abc	0.5 bc
J. ailantifolia	14 c	0.4 c	31 c	1.8 c	9 a	0.0 a	17 abc	0.8 bc
J. nigra	26 c	1.9 b	78 b	3.4 ab	8 a	0.0 a	8 bc	0.3 bc
P. stenoptera	11 c	0.0 c	24 c	0.8 d	8 a	0.0 a	8 bc	0.0 c

Average of 10 replicates per treatment. For flooded or nonflooded plants, numbers in each column with the same letter do not differ (P = 0.05) according to Duncan's multiple range test. Uninfested controls sustained 5-16% root rot and no crown rot.

ailantifolia, J. nigra, J. cinerea, three clones of J. regià, J. neotropica, J. microcarpa, J. major, J. californica, J. hindsii, and Paradox were highly susceptible to P. citricola (Table 1). Seedlings showed reduced growth and high rates of mortality. In contrast, Pterocarya stenoptera was highly resistant and sustained no mortality in the same experiments.

Although seedlings of most Juglans spp. developed severe root and crown rot and died in soil infested with Phytophthora citricola, there were significant differences among species in the time that elapsed before seedlings died (Table 1). For example, J. ailantifolia, J. nigra, and Paradox survived significantly longer than most other Juglans spp. tested, including the standard J. hindsii rootstock. We also observed that prior to initiation of biweekly flood treatments, J. hindsii had a high rate of seedling mortality when grown in soil artificially infested with P. citricola. In contrast, J. ailantifolia, J. nigra, and Paradox had low rates of seedling mortality under the same conditions.

Seedlings of J. hindsii, Paradox, P. stenoptera, J. ailantifolia, and J. nigra were evaluated for resistance to P. citricola, P. cinnamomi, P. cryptogea, and P. citrophthora in the presence or absence of biweekly flooding treatments (Table 2). All J. hindsii seedlings developed crown rot and died in soil artificially infested with P. citricola whether flooded or not. In nonflooded soil, seedlings of J. ailantifolia, J. nigra, and Paradox had significantly less root and crown rot than J. hindsii. This was not the case in periodically flooded soil. Pterocarva stenoptera was highly resistant to Phytophthora citricola in the presence or absence of flooding treatments, developing no measurable root rot and very limited crown rot on one of 10 plants (Table 2).

When exposed to *P. cinnamomi*, seedlings of *J. hindsii*, *J. nigra*, and Paradox developed severe root and crown rot without appreciable differences in disease severity between flooded and nonflooded treatments (Table 2). *J. ailantifolia* was significantly less affected by *P. cinnamomi* than were *J. hindsii*, Paradox, and *J. nigra* in nonflooded soil treatments (Table 2). Of the five rootstocks tested, only *Pterocarya stenoptera* resisted *Phytophthora cinnamomi* regardless of the soil-water regime (Table 2).

J. hindsii, Paradox, J. ailantifolia, J. nigra, and Pterocarya stenoptera appeared highly resistant to Phytophthora cryptogea when grown in infested soil that was not flood-irrigated (Table 2). However, when soil was flooded for 48 hr every 2 wk, J. hindsii, J. ailantifolia, and J. nigra were highly susceptible, Paradox was moderately resistant, and Pterocarya stenoptera, which developed no measureable disease,

<sup>&</sup>lt;sup>y</sup> At 3 mo after planting in artificially infested soil.

Values represent the average elapsed times in days from planting to death of plants. Plants were considered dead when trunks were completely girdled and leaves were permanently wilted.

y Percentage root system rotted as estimated by visual observation 3 mo after inoculation. The four *Phytophthora* spp. were reisolated from necrotic roots of all plants grown in infested soil but not from those grown in uninfested soil.

 $<sup>^{2}0 = \</sup>text{No crown rot}, 4 = \text{seedling girdled}.$ 

was highly resistant to this pathogen (Table 2).

After *P. stenoptera* seedlings were planted in soil infested with *Phytophthora citrophthora*, they grew as well as seedlings in uninfested soil and showed no measurable disease regardless of flooding. Paradox, *J. ailantifolia*, and *J. nigra* developed moderate root and crown rot when grown in either flooded or nonflooded soil infested with this pathogen (Table 2). Of the five rootstocks tested, *J. hindsii* was the least resistant to infection by this pathogen when flooded biweekly (Table 2).

The relative resistance of seedlings of J. hindsii, Paradox, J. nigra, and Pterocarya stenoptera to Phytophthora cactorum and P. megasperma in soil flooded for 48 hr once every 2 wk is shown in Table 3. J. hindsii was most susceptible to root and crown rot caused by P. cactorum, whereas J. nigra appeared moderately resistant and Paradox and Pterocarya stenoptera were highly resistant to the same pathogen (Table 3). In soil artificially infested with Phytophthora megasperma, disease symptoms were severe on J. hindsii and not significant on J. nigra, Paradox, or Pterocarya stenoptera seedlings (Table 3).

In all experiments, the inoculated *Phytophthora* sp. was reisolated from necrotic roots of all plants grown in infested soil. No *Phytophthora* spp. were isolated from plants in uninfested soil. No plants tested were immune to *Phytophthora* spp.

## **DISCUSSION**

P. citricola is found in most walnutgrowing regions of California (7,10). It is associated with root and crown rot of many fruit, nut, and ornamental trees (8,9,14), and isolates from several hosts were highly virulent to J. hindsii seedlings in greenhouse tests (7). Therefore, any potential walnut rootstock should possess a high degree of resistance to this pathogen if it is to warrant further testing for horticultural characters and suitability for commercial walnut orchards. Of the rootstocks challenged with Phytophthora citricola, Pterocarya stenoptera was highly resistant, whereas J. ailantifolia, J. nigra, and Paradox were more resistant than J. hindsii. Our investigations also showed J. hindsii to be highly susceptible to Phytophthora cinnamomi, P. cryptogea, P. citrophthora, P. cactorum, and P. megasperma; thus, this rootstock should not be used in orchards infested

**Table 3.** Relative resistance of four walnut rootstocks to *Phytophthora cactorum* and *P. megasperma* infection in artificially infested soil flood-irrigated biweekly for 48 hr

	Average disease severity <sup>w</sup>					
	P. cactorum				а	
Rootstock	Root rot <sup>x</sup> (%)	Girdling index <sup>y</sup>	Plants with crown rot <sup>z</sup>	Root rot (%)	Girdling index	Plants with crown rot
Juglans hindsii	62 a	3.2 a	10 a	26 a	1.1 a	5 a
Paradox hybrid	8 b	0.3 c	2 b	8 b	0.0 b	0 b
J. nigra	8 b	1.0 b	9 a	10 b	0.6 ab	5 a
Pterocarya stenoptera	6 b	0.0 c	0 b	5 b	0.0 b	0 b

w Average of 10 replicates per rootstock. Numbers in each column with the same letter do not differ (P=0.05) according to Duncan's multiple range test. Uninfested controls sustained 5–8% root rot and no crown rot.

with these pathogens and subjected to periods of soil saturation.

Flooded soil may increase disease severity by promoting the discharge and dispersal of zoospores (3,4), by predisposing plants through adverse affects on host physiology (2,5,14), and by causing depletion of soil oxygen levels, which restricts root regeneration after decay by *Phytophthora* spp. (13). Paradox, J. ailantifolia, and J. nigra resisted P. citricola and P. cryptogea under nonflooded conditions; however, this resistance was overcome by periodic flooding. These findings suggest the potential utility of J. ailantifolia and J. nigra as rootstocks in walnut orchard sites not subjected to periodic soil saturation. Paradox, already an established rootstock, would appear superior to J. hindsii as a rootstock in orchard sites not subjected to soil saturation but infested with P. cactorum, P. citricola, P. cryptogea, P. citrophthora, or P. megasperma.

Significant resistance to *P. cinnamomi* was observed only in nonflooded *J. ailantifolia* and in nonflooded or flooded *Pterocarya stenoptera*. Additional testing of these species for their suitability as commercial rootstocks is warranted.

Chinese wingnut (P. stenoptera) showed high resistance to six Phytophthora spp. associated with severe root and crown rot of walnut orchard trees in California. It should be evaluated as a potential walnut rootstock in California orchards.

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<sup>&</sup>lt;sup>x</sup> Percentage of root system rotted as estimated by visual observation 3 mo after inoculation. *P. cactorum* or *P. megasperma* was reisolated from necrotic roots of all plants grown in infested soil but not from those grown in uninfested soil.

y 0 = No crown rot, 4 = seedling girdled.

<sup>&</sup>lt;sup>2</sup> At 3 mo after planting.