Influence of Flooding Duration on Development of Phytophthora Root and Crown Rot of *Juglans hindsii* and Paradox Walnut Rootstocks

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ABSTRACT

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Two-month-old seedlings of *Juglans hindsii* were grown for 3 mo in soil artificially infested with *Phytophthora cryptogea, P. citrophthora, P. citricola*, or *P. cinnamomi* and subjected to biweekly flooding periods of 0, 6, 12, 24, or 48 hr. The severity of root and crown rot caused by *P. cryptogea* and *P. citrophthora* increased as the length of the flooding periods was increased. A similar relationship was observed when Paradox seedlings were grown in soil infested with *P. citricola*. In contrast, seedlings of *J. hindsii* planted in soil infested with *P. citricola* or *P. cinnamomi*, and Paradox seedlings planted in soil infested with *P. cinnamomi*, suffered severe root and crown rot in unflooded soil as well as in soil flooded for 48 hr. The results suggest that careful soil-water management should minimize losses of English walnut trees in orchards containing rootstock of *J. hindsii* and infested with *P. citrophthora* or *P. cryptogea*, or in orchards containing Paradox rootstock and infested with *P. citricola*.

Additional key words: Persian walnut, soilborne diseases.

In California orchards, the highest incidence of root and crown rot caused in English walnut (*Juglans regia* L.) by *Phytophthora* spp. occurs on poorly drained sites subject to prolonged soil saturation and flooding (16). Field observations and experimental evidence with walnut (16) and cherry (17,23) trees suggest that severity of root and crown rot caused by several *Phytophthora* spp. increases with the length of time soils are saturated or flooded.

Reports in the literature have suggested that flooding may increase the severity of Phytophthora root and crown rot by promoting the discharge and dispersal of zoospores (6,7,10) and, when soil oxygen levels are depleted, by adversely affecting the host's physiology (3,9,23) and restricting root regeneration after decay by *Phytophthora* spp. (21). Both sporangium production and indirect germination (zoospore formation) are influenced by soil moisture. Maximum production of sporangia by *P. megasperma* Drechsler was reported to occur in saturated soil (0 millibars [mb]) matric potential (ψ_m) (18). In contrast, isolates of *P. cactorum* (Leb. & Cohn) Schroet. (19) and *P. cryptogea* Pethyb. & Laff. (4,5) produced maximum numbers of sporangia in nonsaturated soils ($\psi_m \leq 0$). Zoospore release by sporangia of *P. cryptogea* (2,10,12,23), *P. megasperma* (10,12), *P. cambivora* Petri (Buisman) (23), and *P. drechsleri* Tucker (23) was stimulated in soil at saturation ($\psi_m = 0$). When zoospore release by sporangia of *P. megasperma* and *P. cryptogea* was interrupted by drying soil to −150 mb ψ_m for up to 24 hr, subsequent rewetting to saturation resulted in completion of zoospore release (11).

The purpose of the research reported in this paper was to investigate the effects of flooding duration on disease severity in *J. hindsii* (Jeps.) Jeps. and Paradox (*J. hindsii* × *J. regia*), standard walnut rootstocks in California orchards, in soil artificially infested with *P. cinnamomi* Rands, *P. citricola* Sawada, *P. citrophthora* (R. E. Smith & E. H. Smith) Leonian, or *P. cryptogea*. Of the *Phytophthora* spp. that have been isolated from walnut, these four appear to have the most potential to devastate walnut orchards propagated on rootstock *J. hindsii* (14,15). We also investigated the influence of the duration of flooded periods on sporangium production and zoospore release by these pathogens in soil. A partial account of this work was reported earlier (13).

MATERIALS AND METHODS

Influence of flooding duration on sporangium production and germination. Six-millimeter-diameter leaf disks of *J. hindsii* (Northern California black walnut) were colonized by *P. cinnamomi, P. citricola, P. citrophthora*, or *P. cryptogea* by using previously described procedures (22). These four *Phytophthora* spp. were grown on V-8 juice agar (V8A) for 5 days at 24 C. Leaf disks were surface sterilized for 20 min in a 1% solution of sodium hypochlorite, rinsed three times in sterile distilled water, then placed adjacent to the margin of an actively growing culture of *P. cinnamomi, P. citricola, P. citrophthora*, or *P. cryptogea*. After 48 hr of incubation at 24 C, the leaf disks were colonized by the pathogens, but no sporangia were detected.

Five colonized leaf disks were placed on a 1-cm layer of U.C. potting mix (1:1 mixture of sand and peat moss) (1) in 14-cm-diameter × 12.7-cm-deep plastic pots and then covered with an additional 10-cm layer of potting mix. The potting soil in each pot was then watered with tap water until saturated, allowed to drain and covered with a plastic saucer to retard evaporation from the potting soil surface. After 2 days at 24 C, leaf disks from an unflooded pot were removed, rinsed with water, and fixed and stained with acid fuchsin in 85% lactic acid to record sporangium production prior to flooding. The remaining pots were flooded for 6, 12, 24, or 48 hr at 24 C by placement into 16.5-cm-diameter x 13-cm-deep water-filled containers, after which leaf disks were removed, rinsed, and fixed. Numbers of full and empty sporangia along the margins of disks were counted. It was presumed that empty sporangia had germinated indirectly by releasing zoospores. Each value reported represents an average of five leaf disks from each of two separate experiments for a total of 10 replicate leaf disks.

The water content of U.C. mix surrounding the leaf disks was determined 2 and 48 hr after the potting soil was thoroughly wetted

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and allowed to drain by weighing a sample, drying it for 24 hr at 105 C, and reweighing.

Influence of flooding duration on disease severity. Two-month-old seedlings of the standard rootstocks used in California walnut orchards, *J. hindsii* and Paradox (*J. hindsii* × *J. regia*), grown in Jiffy pots (14), were transplanted into 14-cm-diameter × 12.7-cm-deep plastic pots containing potting mix artificially infested with *P. cinnamomi, P. citricola, P. citrophthora*, or *P. cryptogea* and maintained in the greenhouse for 3 mo. Colonized vermiculite inoculum was prepared as described earlier (14) and mixed with steam pasteurized U.C. mix potting medium at the rate of 10 cm^3 inoculum per 1,000 cm^3 of U.C. mix. The controls received rinsed vermiculite not colonized by *Phytophthora* spp. During the experimental period, potting soil temperature ranged from 18 to 24 C. Plants were fertilized weekly with an aqueous solution of calcium nitrate, iron chelate, and Nutri-min minor element concentrate (14).

Plants were either subjected to biweekly flooding periods of 6, 12, 24, or 48 hr. or maintained without periodic flooding. Flooding was accomplished by placing plastic pots containing walnut seedlings into 16.5-cm-diameter × 13-cm-deep water-filled containers, which allowed 1 cm of water to stand on the potting soil surface and around the seedling trunk. Between flooding treatments, all seedlings were watered only as needed. Throughout

TABLE 1. Plant growth and severity of root and crown rot caused by four *Phytophthora* spp. on *Juglans hindsii* walnut rootstock in artificially infested soil with different durations of flooding

Treatment		Plant growth and disease severity[w]			
Soil artificially infested with	Duration of biweekly flooding (hr)	Fresh wt. of tops (g)	Root rot[x] (%)	Girdling index[y]	Plants with crown rot[z]
P. cryptogea	0	33 b	13 def	0 g	0 d
	6	28 bc	12 ef	0.8 ef	7 bc
	12	19 cd	32 cd	0.6 fg	6 c
	24	14 de	29 cde	1.4 de	9 ab
	48	9 e	98 a	3.8 a	10 a
P. citrophthora	0	21 cd	30 cde	1.9 cd	9 ab
	6	22 cd	39 c	2.3 c	10 a
	12	21 cd	46 bc	2.2 c	10 a
	24	19 cd	58 b	3.0 b	9 ab
	48	5 e	100 a	3.7 ab	10 a
P. citricola	0	6 e	98 a	4.0 a	10 a
	48	7 e	100 a	4.0 a	10 a
P. cinnamomi	0	6 e	96 a	3.8 a	10 a
	48	7 e	96 a	3.7 ab	10 a
Control	0	48 a	9 f	0 g	0 d
	48	46 a	8 f	0 g	0 d

[w] Average of ten replicate plants per treatment. Numbers in each column with the same letter do not differ according to Duncan's multiple range test, $P = 0.05$.
[x] Percent of root system rotted as estimated by visual observation 3 mo after inoculation.
[y] Index ranges from 0 to 4; with rating of 0 = no crown rot, 1 = 25%, 2 = 50%, 3 = 75%, and 4 = 100% canker development around seedling trunk.
[z] Plants that developed crown rot within 3 mo. Plants were 2 mo old when transplanted into artificially infested soil.

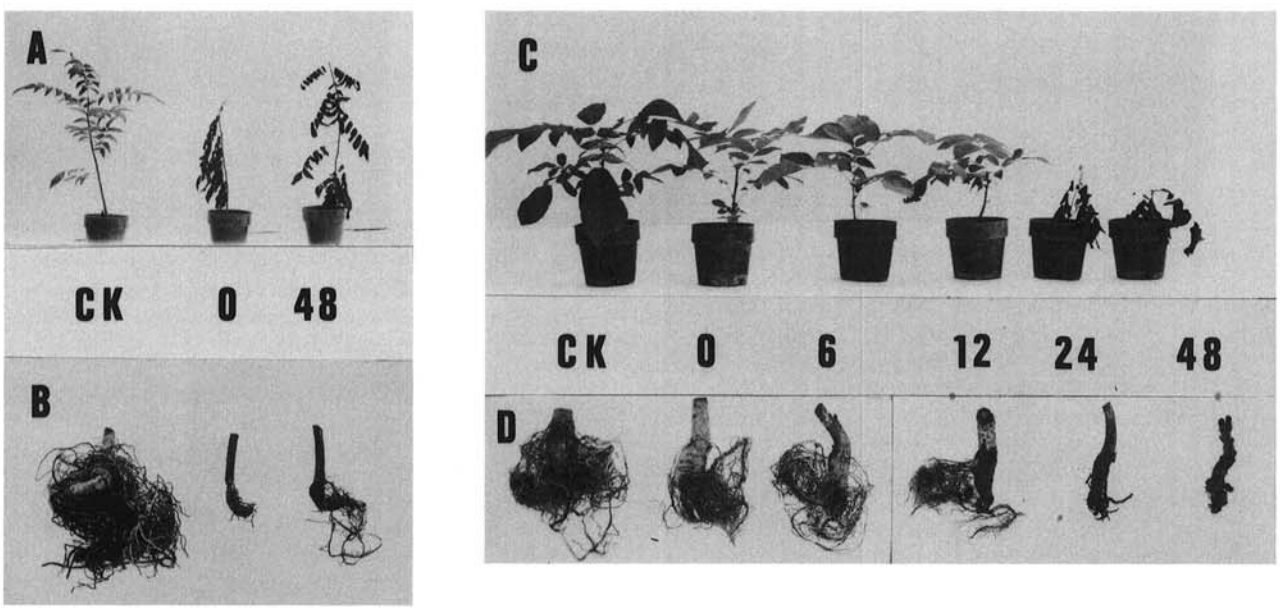

Fig. 1. Tops and roots of **A and B,** 5-mo-old Northern California black walnut (*Juglans hindsii*) and *C and D,* Paradox (*J. hindsii* × English walnut [*J. regia*]) seedlings grown for 3 mo in uninfested soil flooded biweekly for 48 hr (ck) and in soil artificially infested with *Phytophthora citricola* and flooded biweekly for 0, 6, 12, 24, or 48 hr.

the experiments, unflooded seedlings also were watered only as needed (e.g., when wilting was observed) by applying sufficient water to thoroughly wet the potting soil without prolonged saturation. Whenever flooding was not in progress, the potting soil was allowed to drain freely. Experiments were repeated at least twice and terminated 3 mo after transplanting, when disease severity was assessed. Root and crown rot were confirmed as resulting from infection by the appropriate *Phytophthora* spp. by reisolation of the pathogens from walnut seedlings.

RESULTS

Influence of flooding duration on sporangium production and germination. Known relationships of water content to matric potential (ψ_m) for U.C. mix (22) indicate that ψ_m values of soil around unflooded leaf disks were approximately −1 and −2 mb, respectively, within 2 and 48 hr after initial watering of pots. After 48 hr at these soil matric potentials, *P. cinnamomi, P. citricola, P. citrophthora*, and *P. cryptogea* produced average numbers of 41, 67, 68, and 177 sporangia per leaf disk, respectively. Virtually no indirect germination occurred until the onset of flooding.

After 6 hr of flooding ($\psi_m = 0$) the percentage of sporangia of *P. cinnamomi, P. citricola, P. citrophthora*, and *P. cryptogea* germinating indirectly was 34, 46, 14, and 84%, respectively. As the duration of flooding increased from 6 to 48 hr, the percentage of sporangia of *P. cinnamomi, P. citrophthora*, and *P. cryptogea* that germinated indirectly increased to 67, 15, and 96%, respectively, while the detectable level of indirectly germinated sporangia of *P. citricola* decreased to 22%. Detection of empty sporangia, especially of *P. citricola*, became progressively more difficult as the duration of flooding increased; therefore, the actual percentage of sporangia germinating indirectly was probably higher than we observed. We were unable to detect a secondary cycle of sporangium production by these four fungi when duration of flooding was increased from 0 to 48 hr.

Influence of flooding duration on disease severity. When seedlings of *J. hindsii* were grown in potting soil artificially infested with *P. cryptogea* or *P. citrophthora*, the severity of root and crown rot increased as biweekly flooding periods increased from zero to 48 hr; e.g., disease severity was lowest with unflooded plants and highest with plants flooded biweekly for 48 hr (Table 1). In contrast, seedlings of *J. hindsii* planted in potting soil infested with *P. citricola* or *P. cinnamomi* suffered severe root and crown rot in both unflooded and flooded treatments (Table 1, Fig. 1 B). There was no apparent effect of flooding for 48 hr on growth or presence of root rot symptoms in uninoculated control plants (Table 1).

In another experiment, Paradox seedlings were planted in potting soil infested with *P. citricola* or *P. cinnamomi* and subjected to biweekly flooding periods of 0, 6, 12, 24, or 48 hr. Results of this experiment are summarized in Table 2 and Fig. 1 C-D. *P. citricola* caused root and crown rot of increasing severity in Paradox seedlings as biweekly flooding periods increased from zero to 48 hr. However, with or without biweekly flooding treatments, Paradox seedlings planted in potting soil infested with *P. cinnamomi* suffered severe root and crown rot. There was no measureable difference in growth or root rot between unflooded and flooded plants grown in uninfested potting soil (Table 2).

DISCUSSION

Results of our investigations show that *P. cinnamomi, P. citricola, P. citrophthora*, and *P. cryptogea* produce sporangia in unsaturated soils at matric potentials of −1 to −2 mb. Sporangium formation has been reported to occur in unsaturated soil for *P. megasperma* (5,11), *P. cactorum* (10), and *P. cryptogea* (4,5). Our data also revealed that sporangia of *P. cinnamomi, P. citricola, P. citrophthora*, and *P. cryptogea* were stimulated to germinate indirectly when previously drained soils were flooded; this finding also agrees with previous reports on several *Phytophthora* spp. (2,10,12,23). Disease severity increased when seedlings of *J. hindsii* grown in potting soil infested with *P. cryptogea* and *P. citrophthora*, or Paradox seedlings grown in potting soil infested with *P. citricola*, were subjected to flooding periods that ranged from zero to 48 hr in length. However, the majority of sporangia of *P. cryptogea* and *P. citrophthora* that were stimulated to release zoospores when flooded did so within 6 hr of initiation of flooding. Disease severity continued to increase as flooded periods were extended. Apparently, increasingly long flooding periods promote increasing disease severity by enhancing release and dispersal of zoospores and perhaps by creating conditions favorable for host infection by zoospores of *P. cryptogea* or *P. citrophthora* (3,6,7,9,10,21,23).

Severe root and crown rot, with ensuing seedling death, occurred under unflooded soil conditions when *J. hindsii* was planted in soil infested with *P. citricola* or *P. cinnamomi* and when Paradox was grown in soil infested with *P. cinnamomi*. Under these conditions, only brief periods of saturation occurred when seedlings were watered. Perhaps the low resistance of *J. hindsii* and Paradox permits these pathogens to become established and to spread rapidly from relatively few infection sites, with resultant plant death. Furthermore, inoculum of *P. citricola* used in these studies contained numerous oospores, while inoculum of *P. cinnamomi* contained many chlamydospores. These propagules, in addition to

TABLE 2. Plant growth and severity of root and crown rot caused by *Phytophthora citricola* and *P. cinnamomi* on Paradox (*Juglans hindsii* × *J. regia*) walnut rootstock in artificially infested soil with different durations of flooding

Treatment		Plant growth and disease severity[w]			
Soil artificially infested with	Duration of biweekly flooding (hr)	Fresh wt. of roots (g)	Root rot[x] (%)	Girdling index[y]	Plants with crown rot[z]
P. citricola	0	38 b	43 b	1.4 b	6 b
	6	30 bc	38 b	1.7 b	9 a
	12	24 c	54 b	3.5 a	10 a
	24	12 d	81 a	3.6 a	10 a
	48	8 d	92 a	3.9 a	10 a
P. cinnamomi	0	8 d	82 a	2.9 a	10 a
	48	10 d	86 a	3.3 a	10 a
Control	0	59 a	10 c	0 c	0 c
	48	52 a	8 c	0 c	0 c

[w] Average of ten replicate plants per treatment. Numbers in each column with the same letter do not differ from each other ($P = 0.05$) according to Duncan's multiple range test.

[x] Percent of root system rotted as estimated by visual observation 3 mo after inoculation.

[y] Index ranges from 0 to 4; with rating of 0 = no crown rot, 1 = 25%, 2 = 50%, 3 = 75%, and 4 = 100% canker development around seedling trunk.

[z] Plants that developed crown rot within 3 mo. Plants were 2 mo old when transplanted into artificially infested soil.

fragments of mycelium and directly-germinating sporangia, also could initiate severe disease under the unsaturated soil conditions that would not favor numerous infections by zoospores. A report in the literature (8) does show that sporangia of *P. palmivora* in unsaturated soil can germinate directly in the vicinity of papaya roots; this suggests that sporangia of *P. citricola* and *P. cinnamomi* might also behave similarly in the presence of walnut roots. Also, Sterne et al (20) reported that chlamydospores of *P. cinnamomi* germinated and developed germ tubes when maintained in soil at −0.1 bar matric potential, which indicates that these propagules could cause disease in walnut seedlings in unsaturated soil.

Zoospore liberation and dispersal, which are enhanced by saturated soil conditions, may not account entirely for the severe root and crown rot observed in *J. hindsii* and Paradox seedlings in these experiments. While soil moisture is indeed an important parameter regulating zoospore production, liberation, and dispersal in soilborne *Phytophthora* spp., final root and crown rot severity is apparently determined by interactions between the type of walnut rootstock involved, the *Phytophthora* spp. present, the behavior of pathogen propagules other than zoospores, as well as the effects of soil moisture on zoospore production and dispersal.

Results of our investigations support the previous suggestion (16) that proper soil-water management can be an effective strategy for control of Phytophthora root and crown rot in walnut orchards. The effectiveness of this control measure, however, depends upon the walnut rootstocks involved and the *Phytophthora* spp. present. For established walnut orchards on rootstock of *J. hindsii*, avoidance of prolonged and repeated periods of saturation or standing water around tree trunks should minimize tree losses due to infection by *P. cryptogea* and *P. citrophthora*. However, the extreme susceptibility of *J. hindsii* to infection by *P. citricola* and *P. cinnamomi* under unsaturated soil conditions suggests that even the best soil-water management will not control these pathogens of walnut.

The use of Paradox rootstock along with careful soil-water management could reduce tree losses in orchard sites infested with *P. citricola*. In addition, earlier reports (14,15) and some results presented here suggest that the use of Paradox rootstock and soil-water management can help minimize root and crown rot in California walnut orchard sites infested with *P. cactorum, P. citrophthora, P. cryptogea*, and *P. megasperma*. Present studies, in addition to field observations, indicate that these control measures are likely to be ineffective in orchard sites infested with *P. cinnamomi*.

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