Seasonal Variation in Susceptibility of *Juglans hindsii* and Paradox Rootstocks of English Walnut Trees to *Phytophthora citricola*

M. E. Matheron and S. M. Mircetich

Former graduate student and professor, respectively. Agricultural Research Service, U.S. Department of Agriculture, Department of Plant Pathology, University of California, Davis 95616.

Present address of senior author: Department of Plant Pathology, University of Arizona, Yuma Mesa Agricultural Center, 6425 West Eighth Street, Yuma 85364.

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ABSTRACT

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Juglans hindsii and Paradox (*J. hindsii* × *J. regia*) walnut seedlings were evaluated for seasonal variation in susceptibility to *Phytophthora citricola*. At monthly intervals, seedlings were removed from a lathhouse, placed in a growth chamber, and flooded for 48 hr in soil artificially infested with *P. citricola*. Disease severity was assessed 14 days after commencement of flooding under controlled conditions. The incidence and severity of crown rot in *J. hindsii* was highest in June; 60% of the trees were affected by crown rot and the stem girdling index was 1.9. Lowest disease incidence and severity occurred in January; 3% of the trees were affected by crown rot and the stem girdling index was 0.1. Similarly, disease incidence and severity in Paradox rootstocks were highest in June (50% of the trees were affected by crown rot and the stem girdling index was 1.0), while the lowest disease incidence and severity occurred in October-November and January-May, when no crown rot was observed. Direct inoculation of stems and excised stem tissue of *J. hindsii* with mycelium of *P. citricola* yielded seasonal susceptibility variation results similar to those obtained in infested soil. The suitability of each assay method for investigations of relative rootstock resistance and the implications of seasonal variation in susceptibility for disease control in California walnut orchards are discussed.

Additional key words: resistance, soilborne disease.

Surveys of commercial walnut orchards in California revealed a high incidence of root and crown rot on sites subject to prolonged soil saturation or periodic flooding due to excessive rainfall or irrigation (6,7). *Phytophthora citricola* Sawada is one of eleven *Phytophthora* spp. implicated as a cause of root and crown rot. It is commonly associated with high mortality of English walnut trees on *Juglans hindsii* (Jeps.) Jeps. and Paradox (*J. hindsii* × *J. regia* L.) rootstocks in commercial orchards in California (6,7).

P. citricola is present in many California walnut-producing areas that are subject to prolonged flooding during the late fall, winter, and early spring months by the Sacramento-San Joaquin River system, and/or by heavy winter or spring rainfall. However, a high incidence and severity of crown rot caused by this species is usually observed during mid- and late-summer months and is often associated with periods of excessive and repeated irrigation in commercial orchards. This observation suggests that the susceptibility of *J. hindsii* and Paradox walnut rootstocks to *P. citricola* may change with the season.

Reports in the literature suggest that there is a seasonal fluctuation in susceptibility of apple trees to infection by *P. cactorum* (Leb. & Cohn) Schroet. (2–4,8,9). By inoculating excised apple stems at various times during the year, previous workers (2-4) found the highest susceptibility of apple inner bark tissues to colonization by *P. cactorum* to occur at or near blossom time.

This paper reports on seasonal variation in the susceptibility of *J. hindsii* and Paradox walnut rootstocks to invasion by *P. citricola*. We also investigated the reliability of different methods to assess the existence and magnitude of a seasonal fluctuation of walnut rootstock susceptibility to *P. citricola*.

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MATERIALS AND METHODS

Plant material and isolate of *P. citricola*. In early April, *J. hindsii* (Northern California black walnut) and Paradox (*J. hindsii* × *J. regia*) walnut seeds were planted individually in 10-cm-diameter × 10-cm-deep plastic pots containing sterilized U.C. potting mix (sand:peat; 1:1, v/v) (1). Pots were placed in the lathhouse where the seeds germinated and the resulting seedlings were maintained until needed the following year for monthly inoculations. In early January, and in the beginning of every month thereafter, seedlings and excised stems were removed from the lathhouse, inoculated, and immediately placed in a growth chamber for 2 wk at 24 C with a 16-hr photoperiod and 42 $W \cdot cm^{-2}$ of light. During the experiment, seedling ages ranged from 9 mo in January to 20 mo in December. Physiologically, they were completely dormant in Jan-Feb, breaking dormancy in March-April, actively growing from May to September, beginning to show leaf senescence in late October, and had returned to full dormancy by December. Seasonal differences in susceptibility of walnut seedlings were evaluated by three separate methods of inoculation with isolate 21-2-1 of *P. citricola*. Isolate 21-2-1 and all other isolates of *P. citricola* recovered from widely distributed walnut orchards were highly virulent to *J. hindsii* (6).

Soil flooding. *P. citricola* was grown on V-8 juice agar (V8A) for 7 days at 24 C. Ten 6-mm-diameter agar disks were removed from the edges of actively growing cultures of *P. citricola* and placed randomly on the soil surface of each of 10 plastic pots containing rootstock seedlings of *J. hindsii* or Paradox walnut. The agar disks contained only actively growing mycelium and no sporangia or oospores. The pots containing seedlings and mycelial disks then were placed for 48 hr in water-filled plastic pans, 23 cm wide × 31 cm long × 9.5 cm deep, so that 1 cm of water stood on the soil surface in each pot. During the 48-hr period of flooding, the agar disks were prevented from touching the stems of trees; therefore, zoospores were the infective propagules. Pots were removed from the pans of water after 48 hr, allowed to drain freely and maintained in a growth chamber for 12 additional days, and watered only as needed. The presence and severity of crown rot was recorded 14 days after commencement of flooding.

Plants were rated for stem canker severity by using a stem-girdling index in which: 0 = no crown rot; 1 = up to 25% of stem circumference cankered; 2 = up to 50% of stem circumference cankered; 3 = up to 75% of stem circumference cankered; and 4 = the stem completely girdled and the plant dead. Variation in the susceptibility of seedlings of *J. hindsii* to crown rot was studied monthly for two consecutive years, while that of Paradox walnut was studied for 1 yr. Each treatment had 10–20 replicate plants each month.

To monitor possible seasonal fluctuations in sporangium production and germination by *P. citricola*, irrigation water was drained and collected from control trees following the 48-hr flooding treatment and analyzed for its ability to induce sporangium production. Ten 6-mm-diameter agar disks from a V8A culture of *P. citricola* were placed in a 10-cm-diameter petri dish, covered with 50 ml of collected floodwater, and incubated for 48 hr at 24 C. Subsequently, empty (presumably indirectly germinated) and full sporangia formed on the edges of the agar disks were counted.

Stem inoculations. *P. citricola* was grown on V8A for 6 days at 24 C. Six-millimeter-diameter agar disks were removed from the edge of actively growing cultures and placed directly into 6-mm-diameter wounds approximately 10 cm above the soil surface on the stems of seedlings . At the wound site, bark and phloem tissue were completely removed, leaving exposed cambium. The agar disk bearing the mycelium was placed on the exposed surface and wrapped with plastic tape. The inoculated seedlings of *J. hindsii* were maintained for 5 days at 24 C in the growth chamber. Disease severity was determined by measuring the length of the canker that developed at the inoculation site. Every month, 10 seedlings of *J. hindsii* were inoculated and evaluated for disease severity.

Excised stem inoculations. Inoculum was prepared as for stem inoculations. Stems of seedlings of *J. hindsii* were cut into 10-cm-long pieces. The stem pieces were wounded by removing a 6-mm-diam plug of phloem, then inoculated by placing a 6-mm-diameter mycelial disk into the wound. Excised stem pieces were subsequently placed in a moist chamber (100% relative humidity) and incubated for 5 days at 24 C in a growth chamber. Disease severity was determined by measuring the lengths of cankers that developed at the inoculation sites. Each monthly inoculation consisted of 10 replicate stems of *J. hindsii*.

TABLE 1. Seasonal fluctuation in severity of crown rot on seedlings of *Juglans hindsii* and Paradox hybrid (*J. hindsii* × *J. regia*) walnut rootstocks caused by *Phytophthora citricola* in artificially infested soil

	Average disease severity[v]			
	J. hindsii[w]		Paradox hybrids[x]	
Month	Girdling[y] index	Plants with crown rot[z] (%)	Girdling index[y]	Plants with crown rot[z] (%)
January	0.1 e	3 e	0 b	0 c
February	0.1 e	7 e	0 b	0 c
March	0.3 de	10 de	0 b	0 c
April	0.3 de	10 de	0 b	0 c
May	1.1 bc	40 abc	0 b	0 c
June	1.9 a	60 a	1.0 a	50 a
July	1.3 ab	47 ab	0.8 a	40 ab
August	1.0 bcd	33 bcd	0.4 ab	20 bc
September	1.2 bc	33 bcd	0.4 ab	10 c
October	0.5 cde	23 cde	0 b	0 c
November	0.3 de	17 de	0 b	0 c
December	0.5 cde	13 de	0.1 b	10 c

[v] Numbers within a column with the same letter do not differ according to Duncan's multiple range test, $P = 0.05$.
[w] Average of 30 replicates per month representing data from two consecutive years.
[x] Average of 10 replicates per month.
[y] 0 = no crown rot, 4 = stem completely girdled with canker. See text for details.
[z] Determined 2 wk after inoculation.

RESULTS

Soil flooding. The greatest incidence and severity of crown rot occurred when inoculations were done as seedlings of *J. hindsii* emerged from dormancy and resumed active growth from May through July (Tables 1 and 2). Disease severity following subsequent inoculations gradually decreased, with the lowest disease incidence and severity observed during January and February, when *J. hindsii* seedlings are fully dormant (Tables 1 and 2). Paradox seedlings exhibited a similar and significant increase in susceptibility to disease that coincided with the break of dormancy and subsequent active growth (Table 1).

Quantitative determination of sporangial production showed that an average of 500, 580, 670, and 750 sporangia were produced when 10 agar disks of *P. citricola* were incubated in soil leachates collected from flooded seedlings of *J. hindsii* or Paradox in January, April, August, and November, respectively. There was no correlation between the number of sporangia and the observed incidence and severity of crown and root rot on *J. hindsii* and Paradox seedlings.

Stem inoculation. The severity of disease induced by *P. citricola*, as determined by length of stem cankers 5 days after inoculation, was greatest in May, with significantly smaller cankers formed throughout the remainder of the year. With the exception of cankers formed in June and July, disease severity as measured by stem canker length was greater when direct inoculation of stems was compared to soil flooding, especially considering that cankers resulting from direct stem inoculations were measured 5 days after inoculation, whereas those resulting from soil flooding experiments were measured 14 days after inoculation (Table 2).

Excised stem inoculation. Direct inoculation of excised stems of *J. hindsii* resulted in development of the largest cankers in May; smaller cankers formed during the remainder of the year. Comparison of stem canker development resulting from inoculations of stems and excised stems revealed that the excised-stem technique consistently yielded larger cankers than the stem inoculation technique (Table 2).

DISCUSSION

Of 11 *Phytophthora* spp. isolated from declining English walnut trees, *P. citricola* is both widely distributed and highly destructive to walnut orchards; therefore, this pathogen is potentially the most serious threat to the walnut industry in California (6,7). Our research demonstrates the existence of seasonal variation in susceptibility of *J. hindsii* and Paradox walnut rootstocks to invasion by *P. citricola*. These findings have important implications for the proper timing of measures to control this disease and for reliable evaluation of rootstocks for resistance to *P.*

TABLE 2. Canker development on *Juglans hindsii* resulting from three different methods of inoculation with *Phytophthora citricola*

	Length of canker[x] (mm) resulting from inoculation of:		
Month	flooded soil[y]	intact stem[z]	excised stem[z]
January	0 d	15 f	21 ef
February	0 d	16 ef	24 cde
March	4 cd	19 cde	26 bcd
April	4 cd	21 bcd	30 b
May	12 bcd	27 a	38 a
June	42 a	24 b	27 bc
July	22 b	21 bcd	30 b
August	17 bc	23 b	27 bc
September	14 bcd	22 bc	27 bc
October	6 bcd	19 cde	25 cde
November	4 cd	18 de	19 f
December	8 bcd	18 ef	22 def

[x] Average of 20 replicates per month representing data from two consecutive years. Numbers within a column with the same letter do not differ from each other according to Duncan's multiple range test, $P = 0.05$.
[y] Determined 2 wk after inoculation.
[z] Determined 5 days after inoculation.

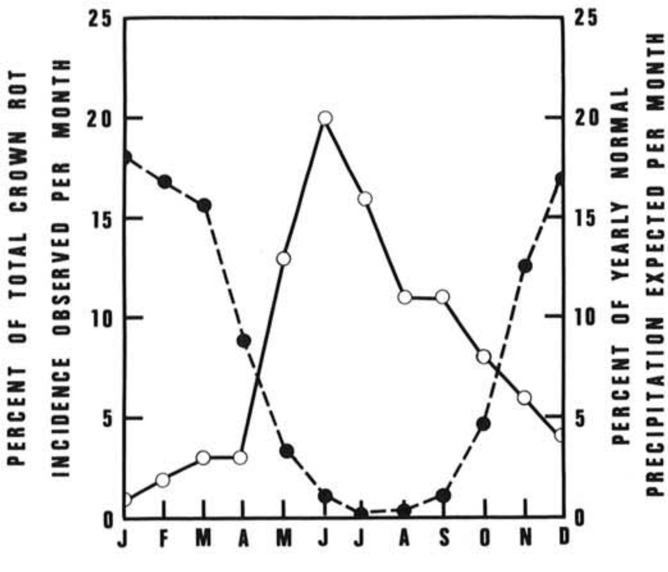

Fig. 1. Comparison of average precipitation (∵) in major California walnut-producing regions with seasonal fluctuations in crown rot incidence (o) on initially 9-mo-old seedlings of *J. hindsii* flood-irrigated for 48 hr with water containing agar disks from cultures of *Phytophthora citricola*. Each data point for crown rot represents the percentage of the total number of seedlings with crown rot observed from 30 plants of *J. hindsii* each month over a 2-yr period.

citricola. Highest precipitation and a high probability for prolonged periods with saturated soil conditions occur between November and April in California's walnut-growing areas (Fig. 1). High water levels in the Sacramento-San Joaquin River system during this period may raise the soil-water table and/or flood walnut orchards located near these rivers and their tributaries. However, our studies show that during these months *J. hindsii* and Paradox, the standard rootstocks of English walnut trees in California, have a relatively low susceptibility to *P. citricola* (Fig. 1). It is later in the season, when orchards are maintained with irrigation, that walnut rootstocks are most susceptible. Thus, proper management of irrigation water to avoid prolonged and repeated episodes of soil saturation might greatly minimize losses due to crown and root rot caused by *P. citricola*.

Disease severity, as measured by length of stem canker on *J. hindsii* seedlings, was higher with stem inoculations than with soil infestation (Table 2). Also, disease severity was always higher with inoculation of excised stems of *J. hindsii* than with inoculation of stems of this rootstock (Table 2). Apparently, inoculation of stems or excised stems indicate a higher degree of plant susceptibility to *P. citricola* than is shown by the soil infestation technique. These differences in observed susceptibility to *P. citricola* may be due to differences in inoculum relationships. In wound experiments with stems, a much greater amount of inoculum was present than in soil flooding experiments in which individual zoospores were the infective propagule.

Excised-stem assays have been used to study the susceptibility of apple trees to *P. cactorum* and to compare relative virulence of isolates within a species (2,3,5). Recently, an excised stem assay has been suggested as a method to determine relative resistance of different apple scion and rootstock cultivars to crown rot induced by *P. cactorum* (5). Caution should be exercised when relying primarily on excised stem inoculation data for determination of relative resistance. Our data indicate that resistance of excised stems may be altered by physical detachment of plant pieces from the growing plant and/or by changes in the physiology of excised stem pieces. Also, utilization of wounded excised stems or trunks only evaluates resistance mechanisms once the pathogen has gained entrance into host tissue, totally neglecting passive and active defense mechanisms operative in tree bark or root tissue. For example, direct inoculation of wounded stems of *Pterocarya stenoptera* DC (Chinese wingnut) with mycelial disks of *P. citricola* resulted in development of sizeable stem cankers, although this species is considered relatively resistant to disease (M. E. Matheron, *unpublished*). Of the three methods employed for determination of walnut rootstock susceptibility to invasion by *P. citricola*, the soil infestation technique appears most appropriate and realistic. Stem and excised-stem inoculation can also be useful techniques for determination of plant susceptibility to *P. citricola*, if relative and not absolute levels of disease are being assayed.

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