Susceptibility of Jojoba (*Simmondsia chinensis*) to *Verticillium dahliae* and to *Phymatotrichum omnivorum*

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ABSTRACT

Orum, T. V., Alcorn, S. M., Herrera-Perez, T., and Miller, G. 1981. Susceptibility of jojoba (*Simmondsia chinensis*) to *Verticillium dahliae* and to *Phymatotrichum omnivorum*. Plant Disease 65:243-245.

Jojoba seedlings through 23 mo of age were susceptible to *Verticillium dahliae* under greenhouse conditions. Major symptoms associated with infection by *V. dahliae* included unilateral, marginal chlorosis of leaves and gray-green, wrinkled leaf surfaces. Vascular discoloration was frequently associated and was most obvious at nodes bearing leaves with symptoms. Defoliation and death occurred in some tests. Minimum effective inoculum concentrations for soil drenches contained 5×10^4 conidia per milliliter, but concentrations containing at least 5×10^6 were more effective, with up to 95% of the plants becoming infected. Jojoba plants (at least 3 mo old) also were susceptible to *Phymatotrichum omnivorum*. Numbers of affected plants varied with soil type and inoculum concentration. Under the most favorable conditions, 16 of 24 plants had rotted roots in 7 wk; five of these died. Strands of *P. omnivorum* occurred on nonrotted roots of some inoculated plants.

Additional key words: arid land plants, control, Texas root rot, *Verticillium* wilt

Seeds of the broad-leafed evergreen jojoba (*Simmondsia chinensis* (Link) Schneider) contain a liquid wax (jojoba oil) that is unique among plant products. This wax consists of a long-chain alcohol esterified to a fatty acid to form a structure with properties very similar to those of sperm whale oil (6). Jojoba oil, therefore, is a possible substitute for sperm whale oil. Other commercial uses for jojoba oil have been reported (7).

Because of its potential economic value and low water requirement, the jojoba, a taprooted, woody, perennial native to the Sonoran Desert of the southwestern United States and northwestern Mexico, is being promoted as a substitute for high water-use plants (3). Accordingly, plantings could involve acreage currently devoted to conventional agricultural crops.

Diseases of jojoba have been investigated only recently. Young studied foliar pathogens of jojoba in the wild (11). In a nursery, Stanghellini isolated *Phytophthora parasitica* and, occasionally, *Pythium aphanidermatum* from 6-mo-old potted jojoba seedlings with root rot (9). Because jojoba may be planted in previously cropped areas in the Southwest, it is important to determine its susceptibility to two important soilborne pathogens in this region, *Verticillium dahliae* Kleb. and *Phymatotrichum omnivorum* (Shear) Dug. Both pathogens have a wide host range among dicotyledonous plants, including many woody perennial plants (4). We present here evidence from greenhouse studies that jojoba is susceptible to both fungi. Preliminary reports have been presented elsewhere (1,2).

University of Arizona Agricultural Experiment Station No. 3337.

MATERIALS AND METHODS

***Verticillium* studies.** Jojoba seedlings were grown in 10- or 15-cm pots or in Speedling trays (Todd plant flat model 150-5, Speedling Inc., Sun City, FL 33586), which contained 128 planting spaces (4 × 4 cm, 13 cm deep) in Styrofoam, arranged in 16 rows of eight. Three rows of empty planting spaces were left between inoculated and uninoculated plants in trays. Seeds were planted in a heat-treated, standard greenhouse potting-soil mix (top soil, peat moss, and sand, 1:1:1). Seedlings began to emerge approximately 2 wk after planting and at 2 mo were 10- to 15-cm tall, with three to five pairs of true leaves. After emergence, plants were thinned to one or two per space in the trays and two to four per pot.

The source of inoculum for most experiments was DX-2, a defoliating strain of *V. dahliae* isolated in Arizona from cotton (*Gossypium hirsutum* L.). Strain 70-21, isolated in Arizona from pepper (*Capsicum frutescens* L.) and nondefoliating on cotton was used in three experiments. The fungus was grown on Czapek's medium (Difco) at room temperature (21–24 C). For inoculum, the contents of six overgrown plates (9 cm in diameter) were blended in 1 L of distilled water, giving a concentration of 10^6 to 10^7 conidia and 10^4 to 10^5 microsclerotia per milliliter. Concentrations of conidia and microsclerotia were determined by counts using a Levy Counting Chamber with double Neubauer ruling (Arthur H. Thomas Company, Philadelphia, PA 19105).

Plants were inoculated either by soil drench or stem puncture. In soil drenches, inoculum was poured into approximately 1-cm diameter holes (near the seedlings), which were then filled with soil. Three holes were used per 10- or 15-cm pot; one hole was used per space in the trays. Inoculum was applied, 9 ml for each planting in the trays and 25 ml and 50–70 ml for 10-cm and for 15-cm pots, respectively. Plants older than 3 mo at the time of inoculation were placed in 15-cm pots.

Stem injections involved depositing several drops (approximately 0.05 ml each) of suspension on either the hypocotyl or the epicotyl just below the first true leaves and piercing the stem through the drops with a hypodermic needle.

Every experiment included as many uninoculated control plants as plants for each type of inoculation.

The studies included the influence of plant age, method of inoculation, inoculum concentration, transplanting, and plant-containers on susceptibility of jojoba to *V. dahliae*. Most seedlings were grown from bulk seed. In one instance, however, seeds were collected in Arizona from one plant each near Tucson, in the Quijotoa Mountains of Pima County, and in Yuma County and were used to evaluate the influence of seed source on seedling susceptibility to *V. dahliae*.

In one test, rooted cuttings (courtesy of LeMoyne Hogan, University of Arizona [5]) were transplanted into 15-cm pots (two cuttings each) to determine if cuttings are susceptible to *V. dahliae*. Thirty cuttings were inoculated by soil drench and 18 cuttings by stem injection; 20 cuttings were left as uninoculated control plants.

Reisolation involved surface-sterilizing 1- to 3-cm stem pieces by immersion for 3–5 min in 0.5% sodium hypochlorite containing 2 or 3 drops of Tween 80 per liter. Thin cross sections from the middle of treated pieces were aseptically cultured on Czapek's agar plates at room temperature in the dark. Generally, mycelial growth was visible within 3 days and microsclerotia within 2 wk when DX-2 was reisolated.

***Phymatotrichum* tests.** The susceptibility of jojoba seedlings to *P. omnivorum* was determined in two tests. In the first test, one or two seedlings per 15-cm pot were grown for approximately 3 mo in the greenhouse planting mix. Plants in three groups of eight pots were inoculated by placing in contact with the roots 16, 8, or 4 sorghum seeds, respectively, overgrown with *P. omnivorum*. Eight pots with 24 plants were used as uninoculated controls. After inoculation, all pots were placed randomly in a "soil-temperature" chamber adjusted to 29–32 C. Approximately 11 wk after inoculation, plants were observed for aerial and root symptoms and with a hand-lens for typical strands of *P. omnivorum* on roots. Slides of representative strands and of all strandlike entities were made for microscopic confirmation of characteristic cruciform hyphae (10).

The second test varied from the first in that all plants were grown in nonsterile field soil, that there were three jojoba plants per pot, and that readings were made approximately 7 wk after inoculation.

In each test, seven or eight inoculated (eight infested sorghum seeds per pot) and seven or eight uninoculated 75-day-old Deltapine 16 cotton plants (one plant per pot), growing in nonsterile field soil, were used as controls.

Table 1. Influence of method of inoculation, fungal strain, and plant age on the susceptibility of jojoba (*Simmondsia chinensis*) to *Verticillium dahliae*

		Inoculated plants			
Fungal strain[a]	Inoculation method[b]	Total inoculated (mo.)	Age at inoculation (no.)	With vascular discoloration[c] (%)	From which *V. dahliae* was reisolated[c] (%)
DX-2	Stem	21	1	71	95
	Soil	20	1	55	85
	Stem	42	3	90	93
	Soil	32	3	22	50
70-21	Stem	6	1	67	100
	Soil	25	1	28	48
	Stem	38	3	58	90
	Soil	27	3	18	59

[a] DX-2 isolated from cotton and 70-21 from pepper plants, causing defoliation and no defoliation of cotton plants, respectively.
[b] Stem: 0.05 ml of $1–8 \times 10^6$ conidia per milliliter deposited on stems; stems pricked with hypodermic needle through the drop. Soil: 25 ml of the suspension divided among three holes in soil per 10-cm pot.
[c] Averages of results of two tests conducted in February and April 1977.

Table 2. Effect of inoculum density on the susceptibility of jojoba to *Verticillium dahliae* as evidenced by vascular discoloration

		Percent of plants infected[a] at inoculum density (conidia/ml) 5 ×			
Inoculation method[b]	Plants inoculated with each concentration (no.)	10^3	10^4	10^5	10^6
Stem	8	13	75	63	38
Soil	40	0	12.5	0	22.5

[a] 4 mo after inoculation.
[b] Stem: 0.05 ml drops of inoculum were placed on the hypocotyl. A hypodermic needle was used to prick through the drops into the stems. Soil: 1-mo-old plants were transplanted from Speedling trays to 15-cm pots on the day of inoculation; 60 ml of inoculum were then divided among three holes per pot.

RESULTS

***Verticillium* studies.** Jojoba was susceptible to the defoliating (DX-2) and nondefoliating (70-21) strains of *V. dahliae* under greenhouse conditions (Table 1). Early leaf symptoms were of two types: chlorosis, which was usually marginal and unilateral, and graying of the whole leaf combined with slight wrinkling of the leaf surface. Affected leaves usually became necrotic. Defoliation occurred in some plants both before and after leaf necrosis developed. Usually infected plants were harvested before they died, but plant death was observed occasionally.

Vascular discoloration was not consistently associated with infection (Table 1). Vascular discoloration appeared earlier and more reliably at nodes than at internodes of infected plants; generally leaves attached to such nodes had symptoms. In a survey of 270 plants showing leaf symptoms, 80% had vascular discoloration at least at one node. In a survey of inoculated plants not showing leaf symptoms, one of 84 had vascular discoloration. Uninoculated control plants never exhibited symptoms typical of *Verticillium* infection.

Reisolation was attempted from all plants in the first two tests. *V. dahliae* was recovered from 50 to 100% of the inoculated plants (Table 1). Subsequently, reisolations were attempted only from representative plants. In the July 1977 test, *V. dahliae* was reisolated in 27 of 35 attempts from plants with vascular discoloration and from three plants with no vascular discoloration. In the September 1978 inoculation series, *V. dahliae* was recovered from all 17 plants with leaf symptoms and vascular discoloration, 18 of 22 plants with leaf symptoms but no vascular discoloration,

Table 3. Influence of inoculum concentration and planting medium on the susceptibility of jojoba (*Simmondsia chinensis*) plants to *Phymatotrichum omnivorum*[a]

		Plants in soil-sand-peat (1:1:1)[c]			Plants in nonsterile field soil[d]		
Host	Inoculum concentration[b] (no. of seeds)	Total inoculated (no.)	With strands on roots[e] (%)	With root rot (%)	Total inoculated (no.)	With strands on roots (%)	With root rot (%)
Jojoba	16	16	43.8	6.3	24	100.0	37.5
	8	13	15.4	0	24	100.0	66.7
	4	11	9.1	0	24	83.3	25.0
	0	12	0	0	24	12.5	0
Cotton	8	7	85.7	42.9	8	75.0	50.0
	0	7	0	0	8	0	0

[a] One to three 3-mo-old jojoba or one cotton plant per 15-cm pot. After inoculation, pots were placed in soil temperature tanks at 29–32 C.
[b] Inoculum consisted of sorghum seeds overgrown with *P. omnivorum*. The seeds were placed in each pot adjacent to tap roots or main side roots.
[c] Cotton plants were in nonsterile field soil; all plants read approximately 11 wks after inoculation.
[d] Plants read approximately 7 wks after inoculation.
[e] Strands with hyphae forming cruciform branches are characteristic of this fungus (10). All rotted roots had strands.

and from one plant with no leaf symptoms and no vascular discoloration. In most cases (two exceptions), reisolation was successful from both the node and the internode even when vascular discoloration appeared only at the node. Accordingly, vascular discoloration was used as a conservative indicator of infection.

The use of Speedling trays permitted the inoculation of large numbers of 1-mo-old jojoba seedlings. Vascular discoloration was observed in 62% of 535 plants within 4 mo of inoculation. The Speedling tray experiments and results in Table 1 show that 1- to 3-mo-old plants are susceptible to *V. dahliae*. Older plants are susceptible as well: 6-mo-old plants inoculated by soil drench and stem injection showed vascular discoloration in more than 50% of the plants observed 12 mo after inoculation. Four of six 23-mo-old plants had vascular discoloration 5 mo after inoculation by soil drench.

Plants were susceptible at inoculum levels as low as 5×10^3 and 5×10^4 conidia per milliliter when inoculated by stem-injection and soil drench, respectively (Table 2). The nature of the planting container did not affect plant susceptibility as long as sufficient inoculum was used.

No great differences were observed in the susceptibility of jojoba seedlings from the three different seed sources to *V. dahliae*. Vascular discoloration was detected in 59, 71, and 68% of the inoculated plants grown from seed from Tucson, the Quijotoa Mountains, and Yuma County, respectively. Rooted cuttings also were susceptible to *V. dahliae*. Of 30 cuttings inoculated by soil drench, 16 developed leaf symptoms and vascular discoloration within 6 mo. However, vascular discoloration was detected in only one of 18 needle-inoculated cuttings.

***Phymatotrichum* tests.** Jojoba plants were susceptible to *P. omnivorum* (Table 3). However, the incidence of infection (ie, occurrence of root rot) was much less when plants were grown in greenhouse mix than in field soil, even though plants in field soil had an approximately 4-wk shorter incubation. The incidence of strand occurrence varied directly with the amount of inoculum. In field soil, however, the incidence of rotting roots was greatest when eight *P. omnivorum*-infested sorghum seeds were used per pot (Table 3). At the time of evaluation, eight of the inoculated plants in the field soil were dead and seven additional plants had varying degrees of dieback. Early foliar symptoms included a dull rather than shiny green color of newly expanded leaves, general leaf chlorosis, and leaf necrosis beginning usually at the leaf tip, then the margins, and extending inward. However, similar symptoms have been noted on uninoculated plants (used for other purposes) that have been pot-bound and watered excessively or insufficiently.

At the time of evaluation a number of cotton plants were infected with *P. omnivorum* (Table 3); five of 15 inoculated plants (total from both tests) were dead. Eight of the 15 had vascular discoloration in stem tissues as high as 17- to 20-cm above the groundline; one additional dead plant had brown tissues throughout. All dead plants and plants with vascular discoloration had strands on their roots. Additionally, two inoculated plants with neither root rot nor vascular discoloration had strands. Neither *Fusarium* spp. nor *V. dahliae* could be recovered from the discolored tissues. One of the 15 uninoculated cotton plants had very slight vascular discoloration at the groundline.

DISCUSSION

Our studies show that jojoba in the greenhouse is susceptible to strains of *V. dahliae* that do or do not defoliate cotton and also to *P. omnivorum*. The life expectancy of jojoba plants in the wild has been estimated to exceed 100 years (8). Since *V. dahliae* and *P. omnivorum* are capable of infecting numerous, mature dicotyledonous plants (4), the potential susceptible period of jojoba to these pathogens could be lengthy. Because of the difficulties of chemical control of these pathogens, prospective growers should avoid planting jojoba in sites with a history of diseases caused by *V. dahliae* and *P. omnivorum*.

Variations in the rate of development and the severity of symptoms caused by *V. dahliae* in jojoba suggest that screening for resistance in seedlings or cuttings might prove fruitful. The use of Speedling trays would enable testing large numbers of plants.

Added in galley: We now have isolated *V. dahliae* from naturally infected cultivated plants exhibiting vascular discoloration and foliar symptoms as described. During the summer of 1979, these were planted as rooted cuttings in a field near Bakersfield, CA, that was in barley the previous year and in cotton for two prior seasons.

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