Phomopsis Canker and Dieback of *Elaeagnus angustifolia*

A. L. MOREHART, R. B. CARROLL, and M. STUART, Associate Professors of Plant Pathology and Graduate Student, respectively, Plant Science Department, University of Delaware, Newark, 19711

ABSTRACT

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The pathogenicity of *Phomopsis elaeagni* was assessed on Russian-olive trees wounded at three sites and inoculated by four methods. Disease (wilt) was induced nearly equally in 2-yr-old trees inoculated by wounding the secondary phloem and cork, secondary xylem, and roots. The fungus moved into the root system and up the stem after either superficial or deep wounding in trees 60 cm high. The same pattern was evident in trees 40 cm high, except that greater upward movement occurred in 60-cm trees inoculated in the roots. Deep wounding produced the most severe disease symptoms in cuttings. Of eight *Phomopsis* spp. tested, only *P. elaeagni* produced disease in Russian-olive cuttings. Extensive culturing from soil on which diseased Russian-olive trees grew failed to yield *P. elaeagni*.

Additional key words: *Diaporthe*, oleaster

Russian-olive or oleaster (*Elaeagnus angustifolia* L.) is a small tree or shrub introduced into the United States from southern Europe and Asia (9). It has been cultivated in the ocean shore area of southern Delaware because it grows rapidly and withstands ocean salt spray, periods of drought, and deposition of windblown sand (3). In 1975, canker and dieback caused by *Phomopsis elaeagni* (Carter et Sacamano) R. H. Arnold et Carter, 1974, killed several hundred 1- to 2-yr-old Russian-olive seedlings and 600 3-yr-old saplings in a Delaware nursery planting (4). Numerous hedge plantings and ornamental specimen trees in other parts of the state also were infected with *P. elaeagni*. All the infected plants apparently were obtained from one nursery.

In 1963, Carter and Sacamano (5), found *Fusicoccum* on Russian-olive trees in Missouri. In 1968, Carter and Dodd (6) detected infected trees in several Illinois nurseries and reported that *Fusicoccum* canker was a serious threat to Russian-olives in both nursery and ornamental plantings. White and Ellett (8) later reported *Phomopsis* dieback of Russian-olive in Ohio and demonstrated its pathogenicity on landscape trees and potted greenhouse plants. The disease was reported from Canada in 1973 (1). In 1974, Arnold and Carter (2) transferred *Fusicoccum elaeagni* Carter et Sacamano to the form-genus *Phomopsis*.

The purpose of our study was to establish the infection route and susceptibility of Russian-olive trees to *P. elaeagni* and other species of *Phomopsis* suspected of occurring in Delaware soils.

MATERIALS AND METHODS

***Phomopsis* isolates.** All *Phomopsis* isolates were obtained from the American Type Culture Collection (ATCC), Rockville, MD, except *P. elaeagni* D 0081, which was isolated by the authors from

Present address of third author: E. I. du Pont de Nemours Co. Inc., Wilmington, DE 19898.

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Russian-olive (4). The isolates and hosts were as follows: 1) *P. elaeagni* (Carter et Sacamano) R. H. Arnold et Carter, D 0081—Russian-olive; 2) *P. elaeagni* (Carter et Sacamano) R. H. Arnold et Carter, ATCC 18286—Russian-olive; 3) *P. acerina* Pirone et Carter, ATCC 16408—maple; 4) *P. gardeniae* Hansen, ATCC 12110—gardenia; 5) *P. mali* Roberts, ATCC 24162—apple wood; 6) *P. obscurans* (Ell. et Ev.) Sutton, ATCC 24220—*Fragaria* sp.; 7) *P. scabra* (Sacc.) Traverso, ATCC 22584—sycamore; 8) *P. vexans* (Sacc. et Syd.) Harter, ATCC 14321—eggplant; and 9) *P. viticola* (Reddick) Goidunich, ATCC 12685—grape.

Inoculum for all tests was prepared from 6-wk-old cultures on potato-dextrose agar (PDA), with the isolates grown in the dark at 26 ± 0.5 C. The mycelial mat was transferred aseptically from 9-cm plastic petri dishes to a sterile Waring Blendor containing 300 ml of sterile distilled water. The mixture was blended at top speed for 3 min, then filtered through cheesecloth. The inoculum concentration was adjusted to 1.9×10^6 propagules per milliliter, determined by direct hemacytometer counts, and appropriate dilution was made with sterile distilled water.

Pathogenicity studies. Two-yr-old Russian-olive trees were obtained from a Virginia nursery and ascertained to be free from *Phomopsis* sp. by culturing various plant parts on PDA. The trees were divided into two groups of 60 each on the basis of root development and were placed into sterilized plastic pots (15 cm diam) containing a steam-sterilized sand, peat, and soil mixture (4:4:1, v/v). Plants with small root systems were pruned to a height of 40 cm and those with larger root systems were pruned to 60 cm. The average diameter of the 40- and 60-cm trees was 11 and 20 mm, respectively.

Three routes of infection were used to simulate wounding that occurs under nursery conditions: 1) deep wound—the bark was surface-sterilized with 70% ethanol, a 1 × 2 cm incision was made in the secondary xylem with a sterile scalpel, and 2 ml of inoculum was pipetted into the wound, which was then covered with a rubber budding band to prevent desiccation; 2) superficial wound—the secondary phloem and cork at the base of the tree was scraped with a scalpel, and 2 ml of inoculum was pipetted onto the wound; and 3) root wound—the root system was cut with a small spade, and 2 ml of inoculum was pipetted over the wounded roots. Control trees were wounded in the same manner, and sterile distilled water was used instead of inoculum. A randomized complete block design with four replicates of 10 trees each was used for all treatments. The trees were examined every day and symptoms were recorded. After 30 days, the trees were removed from the pots and the root systems were washed free from soil in a stream of running tap water. All lateral branches and roots were removed by pruning. The trees were cut into 10-cm sections with a sterile scalpel and surface-sterilized for 10 min with a 1:9 solution of commerical Clorox (5.25% sodium hypochlorite), followed by a 2-min treatment in 70% ethanol. The bark was removed, and 5-mm sections of sapwood and heartwood were placed on PDA.

Cuttings from Russian-olive trees growing on the campus were treated with rooting powder containing indoleacetic acid and were then placed on a mist bench. After the cuttings rooted, they were arranged in a randomized complete block design with four replicates of five plants in each treatment. The treatments were: 1) pin wounding through the bark at the tree base with a 2.5-cm nickel dissecting pin and dipping into the inoculum; 2) pin wounding through the bark at the tree base and soaking for 20 min in the inoculum; 3) scrape wounding through the bark with a sterile scalpel at the tree base and dipping into the inoculum; 4) scrape wounding through the bark at the tree base and soaking for 20 min in the inoculum; 5) scrape wounding at the tree base, then pouring the inoculum around the base; 6) cortical wounding by making a 1 × 2 cm incision with a sterile scalpel and pipetting inoculum into the wound; 7) no wounding and no inoculation; 8) no wounding but dipping into the inoculum; and 9) cortical wounding, then inoculation with sterile distilled water. Observation and isolation techniques were the same as those used in the previous experiment.

Isolation attempts from soil. To determine the significance of soil as a reservoir of *P. elaeagni*, soil samples were taken from the nursery site of infected trees and from the potted-plant inoculation studies and assayed for *P. elaeagni*. The experimental design was a randomized complete block with four replicates of the following: 1) nursery soil from beneath diseased Russian-olive stock; 2) nursery soil from beneath other ornamental plants; 3) soil from the pots of previous inoculation tests; 4) soil from the controls of the inoculation tests; 5) uninfested nursery soil plus soil in which diseased Russian-olive trees had been growing for 3 mo; and 6) nursery soil from beneath diseased Russian-olive stock but autoclaved for 30 min at 121 C. Samples of each soil were sieved through a 20-mesh screen and placed in sterile 20-ml vials. The screen was immersed in 10% Clorox and 70% ethanol after each sample was processed. Soil plates and soil dilution plates of PDA and rose-bengal agar were prepared as described by Johnson and Curl (7). Inoculated plates were incubated in the dark for 1 wk at 26 ± 0.5 C.

Inoculation tests with other *Phomopsis* spp. The cropping history of the nursery containing the diseased plants indicated that vegetables, apples, and grapes, in addition to many woody ornamental plants, had been grown at various times. Inoculation tests were done to determine the pathogenicity of other *Phomopsis* spp. to Russian-olive. Cuttings of Russian-olive were established as previously described. Cultures of *P. elaeagni* D 0081, *P. elaeagni* ATCC 18286, *P. acerina, P. gardeniae, P. mali, P. obscurans, P. scabra, P. vexans,* and *P. viticola* were grown on PDA, and inoculum was prepared as in other tests. The experimental design was the same as that used in previous tests except that each of the four replicates contained five trees per treatment. All trees were inoculated with 2 ml of inoculum after cortical wounding. Controls were 1) wounded and noninoculated, 2) non-wounded and inoculated, and 3) wounded and inoculated with sterile distilled water. The plants were observed for 3 wk and cultured on PDA by the same techniques used in previous tests.

RESULTS

***Phomopsis* isolates.** Each isolate grew well and fruited on PDA. Pycnidia and

Table 1. Symptoms produced in 2-yr-old Russian-olive trees wound-inoculated by three methods with *Phomopsis elaeagni*

Treatment[y]	Symptoms: Wilt, 60-cm trees (no.)	Symptoms: Wilt, 40-cm trees (no.)	Symptoms: Absence of new buds, 60-cm trees (no.)	Symptoms: Absence of new buds, 40-cm trees (no.)
Deep wound				
Inoculated	32 c[z]	27 bc	10 b	11 b
Control	8 a	17 a	4 b	6 b
Superficial wound				
Inoculated	22 bc	27 bc	14 b	9 b
Control	12 bc	12 ab	4 b	12 b
Root wound				
Inoculated	12 bc	8 a	10 b	12 b
Control	8 a	6 a	0 a	6 b

[y]Total of 40 trees in each treatment.
[z]Numbers followed by the same letter within a column are not significantly different ($P = 0.05$) according to Duncan's new multiple range test.

Table 2. Movement of *Phomopsis elaeagni* after 30 days in 2-yr-old Russian-olive trees inoculated by three routes

Treatment	Percentage recovery from given site (cm)[y]				
	−10	0	+10	+20	+30
60-cm trees					
Deep wound					
Inoculated	29 b[z]	63 d	20 bc	13 ab	20 b
Control	0 a	0 a	0 a	0 a	0 a
Superficial wound					
Inoculated	55 b	35 b	25 bc	17 b	25 b
Control	0 a	0 a	0 a	0 a	0 a
Root wound					
Inoculated	0 a	20 c	13 bc	13 ab	15 ab
Control	0 a	0 a	0 a	0 a	0 a
40-cm trees					
Deep wound					
Inoculated	27 b	33 c	13 bc	9 ab	0 a
Control	0 a	0 a	0 a	0 a	0 a
Superficial wound					
Inoculated	33 b	43 c	7 ab	22 b	0 a
Control	0 a	0 a	0 a	0 a	0 a
Root wound					
Inoculated	0 a	0 a	33 a	0 a	0 a
Control	0 a	0 a	0 a	0 a	0 a

[y]0 = inoculation site, + = upward movement, − = downward movement.
[z]Numbers followed by the same letter within the column are not significantly different ($P = 0.05$) according to Duncan's new multiple range test.

Table 3. Symptoms in rooted Russian-olive cuttings by three wounding methods and four types of inoculation with *Phomopsis elaeagni*[y]

Wound method	Inoculation method	Symptoms			
		Wilt (no.)	Leaf loss (no.)	Vascular discoloration (no.)	Apical crooking (no.)
Pin	Dip	0 a[z]	7 b	10 c	4 ab
Pin	Soak	5 ab	3 ab	3 ab	5 ab
Scrape	Dip	3 ab	4 ab	4 ab	5 ab
Scrape	Soak	14 c	4 ab	4 ab	0 a
Scrape	Pour	8 c	7 b	0 a	3 ab
Deep	Wrap	10 c	10 c	10 c	8 c
Deep	Wrap, H_2O	0 a	0 a	0 a	0 a
None	None	0 a	0 a	0 a	0 a
None	Dip	0 a	0 a	0 a	0 a

[y]Total of 20 trees in each treatment.
[z]Numbers followed by the same letter within a column are not significantly different ($P = 0.05$) according to Duncan's new multiple range test.

Table 4. Symptoms and percentage recovery of pathogen in cuttings of Russian-olive inoculated with various *Phomopsis* spp.

Phomopsis isolate	Recovery of pathogen (%)	Wilt (%)	Vascular discoloration	Apical crooking
P. acerina ATCC 16408	25	0	−	−
P. elaeagni D 0081	25	70	+	+
P. elaeagni ATCC 18286	40	80	+	+
P. gardeniae ATCC 12110	50	0	−	−
P. mali ATCC 24162	70	0	−	−
P. obscurans ATCC 24220	10	0	−	−
P. scabra ATCC 22584	45	0	−	−
P. vexans ATCC 14321	25	0	−	−
P. viticola ATCC 12685	25	0	−	−
Controls				
Wounded, noninoculated	0	0	−	−
Nonwounded, inoculated	0	0	−	−
Wounded, inoculated with H_2O	0	0	−	−

characteristic α and β conidia were observed in all isolates and were similar to published descriptions (2,8).

Pathogenicity studies. Deep wounding followed by inoculation with the pathogen was the most effective method of producing wilt in trees 60 cm high. Deep wounding and superficial wounding followed by inoculation were equally effective in producing wilt in trees 40 cm high. Root wounding and inoculation resulted in significant wilt in 60-cm trees but not in 40-cm trees (Table 1). The difference in response between 60-cm and 40-cm trees is thought to be due to vigor. Wounding alone resulted in wilting of some noninoculated trees, but the pathogen was never recovered from these trees.

The absence of new buds was not a significant index of disease in 40-cm trees, regardless of the inoculation route, but was striking in the 60-cm trees (Table 1).

Measurement of fungal growth from the point of inoculation showed that with cortical and base wounding followed by inoculation, the pathogen moved into the root system and up the trunk in 60-cm trees. A similar pattern was evident in inoculated 40-cm trees except that vertical movement was much reduced in root-wounded and inoculated trees (Table 2).

Of three wounding methods and four inoculation types tested on cuttings of Russian-olive, cortical wounding and inoculation produced the most evident symptoms of disease. The other wound-inoculation methods tested, with three exceptions, did not produce statistically significant differences between the test methods and controls (Table 3).

Isolation attempts from soil. *P. elaeagni* was not observed in direct-plate or dilution-plate cultures of nursery soil and soil from beneath inoculated plants.

Inoculation tests with other *Phomopsis* spp. When Russian-olive cuttings were inoculated with various *Phomopsis* spp., only the two *P. elaeagni* isolates produced wilt, vascular discoloration, and apical crooking after 3 wk (Table 4). Other *Phomopsis* spp. were recovered from inoculated tissues, most notably *P. mali* and *P. gardeniae*, but they did not produce disease symptoms (Table 4).

DISCUSSION

The cankers observed in this study differed from those of previous reports (5,6,8) in that gummosis was observed only occasionally at the edges of cankers. This difference may be due to environmental conditions, to vigor of the trees in respect to canker development, or to a characteristic of fungous strains. Differences in symptom expression have been noted by other researchers. For example, Carter and Sacamano (5) observed that cankers did not girdle branches 12–25 mm in diameter, whereas Arnold and Straby (1) reported that branches and

main stems of seedlings were girdled.

Disease, as indicated by wilt and vascular discoloration, was produced only in wounded, inoculated trees. The fungus was never isolated from soil from beneath diseased trees. This suggests that soil is not a reservoir for *P. elaeagni* and that mechanical injury must be avoided in the propagation of Russian-olive.

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