Phomopsis Canker of Russian-Olive in Southeastern Michigan

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ABSTRACT

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Phomopsis elaeagni caused 52 of 53 cankers found on a sample of 100 Russian-olives located throughout southeastern Michigan. Fifteen randomly chosen isolates all caused typical disease symptoms and were subsequently reisolated. Host colonization rates varied among the isolates in a field experiment, and isolates varied similarly when retested in the greenhouse. Seedlings were least susceptible to an aggressive isolate when inoculated at budbreak. *P. elaeagni* extended less than 15 cm below the canker in 23 of 24 infected branches.

Russian-olive (*Elaeagnus angustifolia* L.), an important ornamental tree also used in windbreaks (3,8), is susceptible to a number of canker diseases including that caused by *Phomopsis elaeagni* Arnold & Carter (1). This fungus has been detected in Missouri (6), Illinois (5), Ohio (10), Delaware (4,8), and New York (9) and in several Canadian provinces on imported stock (2).

The objectives of this research were to 1) identify and determine the incidence and severity of canker-causing fungi of Russian-olive in southeastern Michigan, 2) evaluate rates of host colonization by a number of *Phomopsis* isolates, 3) examine host susceptibility to *P. elaeagni* at several seasonal growth stages, and 4) measure the extent of colonization beyond the visible canker region.

MATERIALS AND METHODS

Survey. During autumn 1979, 100 landscape specimens of Russian-olive at least 2.5 m tall throughout southeastern Michigan were examined for cankers. Tissue from canker margins was excised, aseptically placed onto potato-dextrose agar (PDA), and incubated at 20 C in darkness for 3 wk. Identification of the fungi isolated from tissues was then attempted. Where no fungi were isolated, a second attempt was made (7).

Pathogenicity. Pathogenicity was studied in one field test and two greenhouse tests. In the field test, mycelium from each of 15 isolates of *P. elaeagni* chosen at random from among those obtained during the survey was inoculated into the main stems of 10 replicate seedlings (7–11 mm diam.) in August 1980. Mycelium on an agar plug was placed under a bark flap cut to the sapwood. Checks received sterile PDA and all wounds were wrapped with Parafilm. After 1 mo, one seedling from each group was chosen at random for attempted reisolation.

In the first greenhouse experiment, mycelium from each of the 15 isolates was inoculated into the main stem of three seedlings per isolate. In the second greenhouse experiment, inoculum consisted of spore tendrils from three isolates chosen at random from the 15 used in the preceding experiments. Inoculation and reisolation procedures for greenhouse experiments were the same as those employed in the field.

Isolate comparison. Field-grown seedlings were also used to compare host colonization rates of isolates as indicated by lesion lengths. A lesion was identified by characteristic dark brown staining of the sapwood (6) and/or dead bark containing characteristic pycnidial stromata. Total lesion length was measured 1 and 6 mo after inoculation and proximal length was measured after 6 and 12 mo.

To compare results from the greenhouse and the field and determine if host development affected susceptibility, two isolates were tested in the greenhouse, one having shown rapid and the other slow host colonization in the field. Groups of 22 dormant bare-root seedlings were planted 42, 28, 14, and 3 days before inoculation. Budbreak occurred about 2 wk after planting for most of the seedlings. Half of each group was inoculated with each isolate and lesion length was measured 2 and 4 wk later. Two seedlings were randomly selected from each treatment and reisolations were made from canker margins.

Extent of colonization. Four branches with cankers at least 76 cm from the main stem were collected from each of six mature, lightly infected trees. Hyphal extension within the host was measured by cutting sapwood chips at 2.5-cm intervals for a distance of 60 cm below the lower canker margin and incubating these on PDA for detection of *P. elaeagni*.

Statistics. Two-way analysis of variance was used to analyze colonization rates and periods of susceptibility. Dunn's procedure was used for pairwise comparisons of host colonization rates after 12 mo in the field because of highly unbalanced cell sizes caused by different mortality rates among isolate groups (alpha = 0.00055 per comparison). All other pairwise comparisons were made with Tukey's t procedure (alpha = 0.05). Cell sizes were slightly unbalanced in the experiment comparing host susceptibility at various growth stages and when isolate comparison measurements were made in the field 1 and 6 mo after inoculation. Therefore, sample sizes were equalized at random to $n = 9$ and $n = 7$, respectively, before Tukey's t procedure was applied.

Because many seedlings had died back, especially after 12 mo, the linear correlation coefficient (r) between total canker length and proximal canker length at 6 mo was calculated to determine if the two were comparable measures of host colonization. The linear correlation coefficient was also used to determine if variations in stem diameter of the test seedlings affected canker

Table 1. Canker length on Russian-olive inoculated in the field with 15 isolates of *Phomopsis elaeagni*

Isolate no.	Average canker length (cm)[z]
15	10.0 ab
12	9.4 ab
1	9.0 a
5	8.8 a
13	8.5
9	7.5
2	7.1
4	7.0
7	6.1
6	5.5
14	5.4
10	4.6
8	4.2
11	2.9 b*
3	2.4 a*

[z] Pairwise comparisons were made using Tukey's t procedure. Values followed by a letter and an asterisk are significantly different only from those higher values followed by the same letter ($P < 0.05$). n = Seven replicates (seedlings) for each cell; cankers were measured 30 days after inoculation.

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length. Such an effect would bias isolate comparisons. Residuals from all experiments were tested for serious departures from normality and equal variances.

RESULTS

Survey. Of the 100 trees sampled, 53 had cankers. *P. elaeagni* was cultured from 52 and *Tubercularia ulmea* Carter from one (7).

Pathogenicity. All mycelial and spore inoculations resulted in characteristic cankers from which the fungus was reisolated. Checks did not develop cankers and remained uninfected.

Isolate comparisons. One and 6 mo after the field inoculations, mean canker length by isolate ranged from 2.4 to 10.0 cm and from 3.2 to 24.4 cm, respectively. Mean canker length below the inoculation site ranged from 2.6 to 10.8 cm at 6 mo and from 2.7 to 27.4 cm at 12 mo for seedlings that were still alive.

After 1 mo, a minimal significant difference (5% level) of 6.1 cm showed that the four isolates producing the largest cankers were significantly different from one or both of the two isolates producing the smallest cankers. No other combinations were significantly ($P < 0.05$) different (Table 1). Measurements made after 6 and 12 mo disclosed that about the same number of significant differences in average canker length between isolates remained, whereas the identity of the isolates associated with the significant differences shifted. Over all three measurement times, one isolate was consistently among the one-third that most rapidly colonized the host, another was among the one-third that colonized the host most slowly, and another remained in the intermediate one-third. All others changed in rank.

Canker length and mortality were related. One month after test seedlings were inoculated, the groups of isolates able to quickly, moderately, and slowly colonize host trees had caused 80, 0, and 20% of the mortality, respectively. During the next 5 mo, these groups caused 63, 28, and 9% of the additional mortality, respectively. During the next 6 mo, they caused 70, 5, and 25% of the mortality during that period. Cumulative mortality for all isolates 1, 6, and 12 mo after inoculation was 4, 9, and 25%, respectively. Cumulative dieback 1, 6, and 12 mo after inoculation was 42, 46, and 44%, respectively.

On greenhouse seedlings, cankers caused by the most aggressive isolate were significantly longer ($P < 0.001$) than those caused by the least aggressive isolate (avg. 7.8 vs. 1.3 cm after 4 wk). Cankers on field-inoculated plants were longer than those on greenhouse plants. After 1 mo, average canker lengths on field-inoculated trees were 4.7 and 10.0 cm for the nonaggressive and aggressive isolate, respectively. The aggressive isolate caused more mortality in the greenhouse than in the field (70 vs. 11% after 1 mo). The nonaggressive isolate did not cause mortality in either location.

Periods of susceptibility could not be discerned with the nonaggressive isolate, but minimum susceptibility to the aggressive isolate coincided with inoculation 2 wk after planting (Table 2). *Phomopsis* was reisolated from 100% of the treatment representatives.

Extent of colonization. *P. elaeagni* was recovered 15 cm below the canker margin in one of the 24 sample cankers (Table 3). The average proximal extent of colonization was 2.5 cm from the canker margin, with a sample variance of 4 cm.

Cankers sampled for the extent of colonization were of two types: a small, discrete, ovoid canker at the base of an epicormic branch cluster or an elongate canker extending from a branch tip toward the bole. Numerous pycnidial stromata were always present within cankers of both types.

Statistics. There was a strong positive linear correlation between canker length and proximal canker length ($r = 0.91$, $P < 0.05$). Therefore, the two measurements seemed to be comparable measures of host colonization rates. There was no significant linear relationship between stem diameter of the test seedlings and canker length ($P > 0.05$). All residuals met the criteria of normality and equal variance.

Table 2. Average canker length (cm) on Russian-olive 4 wk after inoculation at different developmental stages with either aggressive or nonaggressive isolates of *Phomopsis elaeagni*[z]

Isolate	Time (wk) between transplanting and inoculation			
	0	2	4	6
Aggressive	7.1 a	3.4 b	9.8 a	10.5 a
Nonaggressive	0.6 b	1.6 b	1.6 b	1.4 b

[z]Comparisons made with Tukey's *t* procedure. Values followed by a common letter are not significantly different ($P > 0.05$). n = Nine replicates (seedlings) per cell.

Table 3. Recovery of *Phomopsis elaeagni* at various distances below the canker margin on 36 mature Russian-olives

Distance below canker margin (cm)	Sapwood chips yielding *Phomopsis*[a] (%)
0.0	100
2.5	50
5.1	20
7.6	16
15.0	4
17.5	0

[a]n = 24 Cankers, four per tree.

DISCUSSION

Results of the survey by Etz (7) and subsequent pathogenicity trials indicate that *P. elaeagni* is a major canker-causing pathogen of Russian-olive in southeastern Michigan. Both field and greenhouse studies showed that all *P. elaeagni* isolates were pathogenic but that there was considerable variation in the rate at which isolates were able to colonize the host. Canker formation, upon which host colonization rates were judged, varied within isolates when evaluated over 1 yr. Therefore, further testing should encompass a time interval sufficient for stabilization of the disease reaction. Because of the wide range in disease reaction, an array of isolates should be used to test for potential resistance. Both mycelium and spores were infective.

Although isolates selected from the field trials retained their relative host colonization rates in the greenhouse, mortality and absolute canker length caused by them in the greenhouse were different from their counterparts in the field. These differences are unexplained but suggest the superiority of field testing whenever possible.

Russian-olive appeared most resistant at budbreak, when cells of the vascular and cork cambia would be metabolically most active and rapidly growing. Such cells may resist colonization.

Isolations indicated that the hyphae of *P. elaeagni* extend only a few centimeters beyond the visible canker. Pruning cankered branches would be practical where small trees or infrequent infections are encountered.

Because all sampled cankers were consistently associated either with the base of an epicormic branch cluster or with branch tips, infection may occur in small wounds on new growth.

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