Response of Siberian Elm to Inoculations with *Sphaeropsis ulmicola*

J. M. KRUPINSKY, Plant Pathologist, and R. A. CUNNINGHAM, Research Geneticist, USDA, Agriculture Research Service, Northern Great Plains Research Laboratory, P.O. Box 459, Mandan, ND 58554-0459

ABSTRACT

Krupinsky, J. M., and Cunningham, R. A. 1993. Response of Siberian elm to inoculations with *Sphaeropsis ulmicola*. Plant Dis. 77:678-681.

Eighty-one clones of Siberian elm (*Ulmus pumila*) were screened for resistance to *Sphaeropsis ulmicola* (= *Botryodiplodia hypodermia*) to determine if different levels of disease reaction or canker development could be detected. In 1989, inoculations with *S. ulmicola* resulted in significant differences among clones for branch dieback and canker length. In 1990, three clones with some resistance (small cankers) and three susceptible clones (large cankers) were inoculated with six isolates of *S. ulmicola*. On the basis of branch dieback and canker length, these clones were significantly different and responded similarly to inoculations performed in 1989. No significant differences in canker development among the six isolates were detected, and the isolate × clone interactions were not significant. These studies indicate that branch inoculations of clones with *S. ulmicola* will be useful in ranking or grouping clones based on their relative susceptiblity. The putative resistant clones can be identified and utilized in an elm breeding program.

Additional keyword: windbreaks

Siberian elm (*Ulmus pumila* L.) has been widely planted in the northern Great Plains in field windbreaks to control soil erosion and in farmstead windbreaks to improve environmental conditions for man and livestock. Seed sources of Siberian elm currently used produce planting stock that is fast growing and adapted to the region. Ample numbers of mature trees are available to provide seed for planting stock. Unfortunately, canker diseases can contribute to the decline of Siberian elm in windbreak plantings (6,10–12,15,18). In 1972, Otta and Bode (11) reported that cankers on Siberian elm caused by *Sphaeropsis ulmicola* Ellis & Everh. (= *Botryodiplodia hypodermia* (Sacc.) Petr.) were severe in the eastern half of South Dakota. Girdling cankers caused the foliage on stems above the canker to wilt and die, and adventitious sprouts often developed below the cankers (14). In 1978, Riffle (13) confirmed the pathogenicity of *S. ulmicola* and found that the most rapid canker development followed inoculations in July through September. In 1979, Krupinsky (6) collected 609 cankers from Siberian elm from 56 counties in four states (Minnesota, Montana, North Dakota, and South Dakota) and evaluated them for fungi present. *S. ulmicola* was isolated from 42% of the cankers evaluated and was confirmed as the most important canker-forming pathogen of Siberian elm in the region (6).

A range of resistance to canker development has been reported for other host-pathogen combinations. Differences in canker resistance of honey locust (*Gleditsia triacanthos* L.) cultivars have been reported (1,5). Significant clone and isolate effects have been reported for canker length, branch death, and callus formation with Hypoxylon canker on aspen (*Populus tremuloides* Michx.) (4). Similarly, with Leucostoma canker on peach (*Prunus persica* (L.) Batsch), half-sib families differed for canker length (2) and heritability for canker length was relatively high, suggesting that it may be possible to select individuals resistant to Leucostoma canker (3).

Because Sphaeropsis canker can be a disease problem on Siberian elm, incorporating resistance to this disease in a tree improvement program is important. The use of resistant clones in future windbreak plantings would be the most cost-effective measure for preventing canker disease damage. This study was undertaken to screen Siberian elm germ plasm to determine if different levels of disease reaction or canker development can be detected. In order to evaluate a unique germ plasm collection without inoculating the main stem of the trees and possibly causing the loss of some clones, branch inoculations were done. A preliminary report has been published (9).

MATERIALS AND METHODS

In 1984, 486 trees (six replications of 81 clones) were planted in a randomized complete block design. Seventy-six of the 81 sources had been selected for possible canker and insect resistance on the basis of visual observations of their individual performances in field windbreaks in North Dakota and South Dakota. The remaining five trees were from common nursery stock; three (336, 338, and 339) had been selected for canker resistance after several inoculations with *S. ulmicola* and two (341 and 342) were considered susceptible to *S. ulmicola*. All trees in the planting were propagated by rooting shoots from the source tree. The planting was surrounded by a border row of Siberian elm planted at the same time as the experimental plots. After 5 yr, all 81 clones were still represented in the planting, but only 372 (77%) of the 486 trees survived. The number of trees (replicates) for each clone ranged from one to six, with a mean of 4.6. Branches on surviving trees were used for the inoculations; some inoculated branches were lost because of breakage.

Previously described procedures (6) were used to obtain isolates of *S. ulmicola* from cankers on Siberian elm. Cultures were maintained on 18% V8 juice agar (19) in a controlled-temperature room (22 C) 30 cm below continuous light (75 $\mu E \cdot m^{-2} \cdot s^{-1}$ from 40W cool-white fluorescent light) (7). For long-term storage, individual isolates were maintained by suspending spores in a 15% glycerol solution and storing them at −90 C. Inoculum was produced by transferring mycelium from an actively growing culture onto the surface of a V8 juice agar dish, sprinkling sterile wheat kernels on the surface of the dish, and allowing the fungus to grow over the kernels and sporulate (7).

Branches (7–10 mm in diameter) were wounded by excising a small twig flush with the branch. The pruner was sterilized with 95% ethanol between cuts. A fungus-infected wheat kernel was placed on the wound with forceps, wrapped in place with Parafilm M laboratory film, covered with aluminum foil, and secured with fiber tape (8). The wrappings were left in place until the cankers were measured. Branches used as controls were inoculated in the same way except that sterile wheat kernels were used. All inoculations for a particular isolate were done on the same day. Inoculated branches were evaluated for branch dieback (dead branch above the point of inoculation caused by a girdling canker) and canker length (distance from the center of the inoculation site to the canker

Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products that may also be suitable.

Accepted for publication 3 March 1993.

margin closest to the main stem of the tree), measured after cankers were excised.

Isolations were made from cankers on inoculated branches to confirm the identity of the causative fungus. Before isolations were made, canker margins were surface-sterilized by swabbing with 70% ethanol. The bark was removed with a sterile scalpel, and four wood chips were cut from the canker margin and placed on V8 agar. Dishes containing wood chips were kept below continuous cool-white fluorescent light to promote growth and sporulation. Fungal spores were examined microscopically to confirm the identity of the fungus.

Study 1: Inoculations of all clones. Two isolates of *S. ulmicola*, one obtained from Grant County in south central North Dakota (isolate 1) and one from Edmunds County in north central South Dakota (isolate 2), were used to inoculate branches. On 4 August 1989, one branch on each tree was inoculated with isolate 1, and on 7 August another branch on each tree was inoculated with isolate 2. Beginning 2 wk after inoculation, branches were evaluated weekly for symptoms of wilting and dieback above the point of inoculation until the leaves started to turn color in the fall. Branches were visually evaluated for the presence of branch dieback above the point of inoculation in the spring of 1990. Inoculated branches were excised, wrappings at the inoculation site were removed, and canker length was measured. Isolations were made from cankers obtained from one replicate for each isolate (isolate 1 from replicate 1, isolate 2 from replicate 4) and from all cankers on the branches used as controls.

Study 2: Inoculations of selected clones. In order to confirm differences among clones detected in study 1, six clones were selected for further testing. Three clones (338, 416, and 431) were selected as putative resistant types (small cankers). Clone 338 (average canker length of 106 mm) was originally selected from common nursery stock. Clones 416 and 431 (average canker lengths of 77 and 93 mm, respectively) were originally selected from field windbreaks for resistance to disease and insects, respectively. Three clones (067, 329, and 342) were selected as apparent susceptible types (large cankers). Clone 342 (average canker length of 317 mm) was from common nursery stock, clone 067 (average canker length of 317 mm) was originally selected from a field windbreak for good crown, and clone 329 (average canker length of 482 mm) was a putative hybrid of *U. pumila* and *U. rubra* originally selected in a USDA Soil Conservation Service nursery in Vermillion, South Dakota.

The six isolates of *S. ulmicola* used to inoculate the selected clones were obtained from cankers collected from a diverse area: isolate 2573-1 from Spink County and isolate 2605-1 from Deuel County in northeastern South Dakota, isolate 2636 from Swift County in western Minnesota, isolate 2974 from McCone County in northwestern Montana, and isolate 2199 from Rolette County and isolate 2479 from McLean County in north central North Dakota. In an earlier study in which various isolates were tested, these isolates were aggressive and typical (8).

Six branches on six trees of each of the six clones were inoculated on 17 July 1990 with one isolate each (36 inoculations per isolate). Beginning 2 wk after the inoculations, inoculated branches were visually rated for branch dieback. Branches were excised and canker lengths were measured during the summer of 1991. Isolations were made from the cankers on three replicates (one, two, and four) for all six isolates.

Data analyses. Data from study 1 were analyzed with a general linear models procedure (17) and data from study 2, with a standard analysis of variance procedure (16). Statistical comparisons were made with the Student-Newman-Keuls test (16,17).

RESULTS AND DISCUSSION

Study 1: Inoculations of all clones. Wilting above the point of inoculation was evident as early as 1 wk after inoculation. Two weeks after inoculation, branch dieback was evident on 21% of the inoculated branches (Table 1). This increased to 48% after 3 wk and to 52% after 4 wk, followed by only slight increases after 5 and 6 wk. The analysis of all data on branch dieback after 6 wk indicated that the clone and isolate effects were significant and that the clone × isolate interaction was not significant. Therefore, there was a significant difference among clones in their ability to resist girdling cankers 6 wk after inoculation. Isolate 1 was more aggressive than isolate 2 (Table 1).

By the end of May 1990 (43 wk after inoculation), the percentage of dead branches had increased to 69% (Table 1). Analysis of all data on branch dieback after 43 wk indicated that the clone and isolate effects were significant and that the clone × isolate interaction was not significant. These results were consistent with the 6-wk data.

Clones 405, 437, 400, 329, and 413 had the longest cankers, with mean canker lengths ranging from 350 to 500 mm. Clones 436, 440, 403, 419, 416, 214, 424, 003, 422, and 403 had the shortest cankers, with mean canker lengths ranging from 38 to 92 mm. A high level of resistance (no canker development) was not evident in the clones evaluated.

Analysis of all data on canker length indicated that the clone and isolate

Table 1. Number of dead branches resulting from inoculations of 81 Siberian elm clones with *Sphaeropsis ulmicola* (study 1)[z]

Time after inoculation (wk)	Isolate 1		Isolate 2		Total	
	No.	%	No.	%	No.	%
2	99	27	58	16	157	21
3	230	62	127	34	357	48
4	237	64	146	39	383	52
5	244	66	148	40	392	53
6	246	66	159	43	405	55
43	288	78	218	59	506	69

[z] For the ratings 2–6 wk after inoculation, isolate 1 was evaluated on 372 branches and isolate 2, on 371 branches, for a total of 743 branches. At 43 wk after inoculation, isolate 1 was evaluated on 368 branches and isolate 2, on 370 branches, for a total of 738 branches.

Table 2. Analyses of canker development (canker length) on Siberian elm clones inoculated with *Sphaeropsis ulmicola*

Study	Source of variance	df	Mean squares	*F* value	*P* > *F*
1	Replicate	5	239,487	5.2	0.0001
	Clone	80	87,227	1.9	0.0001
	Error a	286	45,825		
	Isolate	1	448,514	15.1	0.0001
	Isolate × clone	80	36,880	1.2	0.1048
	Error b	289	29,769		
	Corrected total	741			
2	Replicate	5	603,811	2.9	0.0357
	Clone	5	1,146,730	5.4	0.0016
	Error a	25	211,363		
	Isolate	5	294,174	1.7	0.1511
	Isolate × clone	25	121,969	0.7	0.8677
	Error b	150	178,660		
	Total	215			

effects were significant and that the clone × isolate interaction was not significant (Table 2). Thus, there was a significant difference among clones and isolates and a lack of specific interactions among clones and isolates. These results are similar to those obtained for branch dieback.

Sporulating cultures of *S. ulmicola* were produced by 65 (94%) of 69 cankers originally inoculated with isolate 1 in replicate one and by 30 (77%) of 39 cankers originally inoculated with isolate 2 in replicate four. This confirms the presence of *S. ulmicola* and its ability to cause cankers on Siberian elm. Six weeks after inoculation, a canker was detected on one branch used as a control that had been broken, apparently in a windstorm; the remaining 38 branches used as controls showed no symptoms. After the winter season (43 wk after inoculation), cankers were observed on six (15%) of the 39 branches used as controls, and *S. ulmicola* was isolated from these cankers. Over the winter, *S. ulmicola* naturally infected the wounded inoculation sites on branches used as controls.

Study 2: Inoculations of selected clones. Symptoms of branch dieback above the point of inoculation were present on 59% of the inoculated branches 4 wk after inoculation (Table 3). The three apparent susceptible clones had dead branches on 73% of the inoculated branches, whereas the three putative resistant clones had dead branches on only 45% of the inoculated branches (Table 3). This indicates that after 4 wk, the clones selected for long canker length in study 1 were more susceptible to girdling cankers than clones selected for short canker length. When all data were analyzed, the clone effect was significant but the isolate effect and the clone × isolate interaction was not significant. Thus, there were significant differences among the clones but differences among the six isolates were not detected. The lack of differences among isolates contrasts with an earlier study in which differences were reported (8). One can speculate that differences could not be detected because the six isolates used in this study were only the aggressive typical types identified in the earlier study (8), which included a wide range of isolates.

When canker length was measured, the clone effect was significant (Table 2) and the apparent susceptible clones had longer cankers than the putative resistant clones (Table 3), confirming results from study 1. Although the apparent susceptible and putative resistant clones cannot be clearly separated into two distinct groups, differences among clones were detected (Table 3). Apparent differences among isolates (average canker lengths varied from 147 mm for isolate 2573-1 to 359 mm for isolate 2605-1) were not significant (Table 2). The clone × isolate interaction was not significant (Table 2).

S. ulmicola was isolated from excised cankers. Sporulating cultures of *S. ulmicola* were obtained from 66 (61%) of 108 plated cankers originally inoculated with six isolates in replicates one, two, and four. Overall, *S. ulmicola* was obtained from 80% of the inoculated branches from the apparent susceptible clones, compared with 43% of the inoculated branches from the putative resistant clones (Table 4). No cankers were detected on the 25 branches used as controls.

Differences among clones of Siberian elm were detected in both studies, and the results of study 2 confirmed the clonal differences observed in study 1. Consequently, branch inoculations of Siberian elm are apparently useful in ranking or grouping clones on the basis of their relative susceptibility. Although the studies were conducted over a period of only 43 wk, we believe they reflect the relative susceptibility of the clones. Because the resistance of a perennial plant will be potentially challenged by a canker-causing organism numerous times over a period of years, these clones will be tested further with long-term field evaluations. In conclusion, it appears that germ plasm of Siberian elm can be screened for susceptibility to cankers caused by *S. ulmicola*. Clones demonstrating putative resistance will be valuable to a breeding program for Siberian elm.

Table 3. Canker development on six selected Siberian elm clones inoculated with six isolates of *Sphaeropsis ulmicola* (study 2)[v]

Clone	Type[w]	No.	Dead branches[x]		Canker length[y] (mm)
			No.	%	
431	PR	36	16	44	97 a[z]
416	PR	36	14	39	101 a
338	PR	36	19	53	160 ab
Total		108	49	45	
067	AS	36	33	92	237 ab
342	AS	36	23	64	386 bc
329	AS	36	23	64	545 c
Total		108	79	73	
Overall		216	128	59	

[v] No significant differences among the six isolates; six clones × six replicates × six isolates = 216 branch inoculations.
[w] PR = putative resistant, AS = apparent susceptible.
[x] Four weeks after inoculation.
[y] At conclusion of study.
[z] Means followed by the same letter are not significantly different at $P = 0.05$, according to the Student-Newman-Keuls multiple range test.

Table 4. Number of successful isolations of *Sphaeropsis ulmicola* from cankers on six selected clones inoculated with six isolates of *S. ulmicola* (study 2)[x]

Replicate	Number of inoculated branches per clone	Clones					
		No. 431[y]	No. 416[y]	No. 338[y]	No. 067[z]	No. 342[z]	No. 329[z]
1	6	0	2	2	5	6	4
2	6	5	2	5	6	5	5
4	6	4	3	0	5	6	1
Total	18	9	7	7	16	17	10

[x] No cankers were produced on 25 branches used as controls, i.e., inoculated with sterile wheat kernels.
[y] Putative resistant clones.
[z] Apparent susceptible clones.

ACKNOWLEDGMENTS

We thank D. Wetch, C. Flakker, and M. Peterson for technical assistance; J. F. White, Jr., for examination of fungal isolates and recommendation of the appropriate name for the fungus; and J. A. Walla, W. R. Jacobi, and anonymous reviewers for reviews and constructive comments.

LITERATURE CITED

1. Bedker, P. J., and Blanchette, R. A. 1984. Control of *Nectria cinnabarina* cankers on honey locust. Plant Dis. 68:227-230.
2. Chang, L. S., Iezzoni, A., and Adams, G. 1991. Heritability of *Leucostoma persoonii* canker resistance among diverse peach genotypes. HortScience 26:60-62.
3. Chang, L. S., Iezzoni, A., Adams, G., and Howell, G. S. 1989. *Leucostoma persoonii* tolerance and cold hardiness among diverse peach genotypes. J. Am. Soc. Hortic. Sci. 114:482-485.
4. Griffin, D. H., Manion, P. D., Valentine, F. A., and Gustavson, L. 1984. Canker elongation, branch death, and callus formation as resistance or susceptibility responses in *Populus tremuloides* and virulence or avirulence characteristics of *Hypoxylon mammatum*. Phytopathology 74:683-687.
5. Jacobi, W. R. 1989. Resistance of honeylocust cultivars to *Thyronectria austro-americana*. Plant Dis. 73:805-807.
6. Krupinsky, J. M. 1981. *Botryodiplodia hypodermia* and *Tubercularia ulmea* in cankers on Siberian elm in northern Great Plains wind-

breaks. Plant Dis. 65:677-678.
7. Krupinsky, J. M. 1982. Growth and sporulation of *Botryodiplodia hypodermia* in response to different agar media and temperatures. Plant Dis. 66:481-483.
8. Krupinsky, J. M. 1983. Variation in virulence of *Botryodiplodia hypodermia* to *Ulmus pumila*. Phytopathology 73:108-110.
9. Krupinsky, J. M., and Cunningham, R. A. 1992. Siberian elm resistance to Botryodiplodia canker. (Abstr.) Phytopathology 82:1116.
10. Krupinsky, J. M., and Walla, J. A. 1986. Tubercularia canker of Siberian elm and Russian olive. Pages 40-41 in: Diseases of Trees in the Great Plains. J. W. Riffle and G. W. Peterson, tech. coordinators. U.S. Dep. Agric. For. Serv. Rocky Mt. For. Range Exp. Stn. Gen. Tech. Rep. RM-129.
11. Otta, J. D., and Bode, F. L. 1972. Siberian elm canker in South Dakota. Plant Dis. Rep. 56:572-574.
12. Peterson, G. W., and Riffle, J. W. 1976. Protection of windbreaks from diseases. Pages 172-180 in: Proc. Symp. Shelterbelts Great Plains. R. W. Tinus, ed. Great Plains Agric. Counc. Publ. 78.
13. Riffle, J. W. 1978. Development of cankers on *Ulmus pumila* related to month of inoculation with *Botryodiplodia hypodermia*. Phytopathology 68:1115-1119.
14. Riffle, J. W. 1981. Cankers. Pages 34-42 in: Compendium of Elm Diseases. R. J. Stipes and R. J. Campana, eds. American Phytopathological Society, St. Paul, MN.
15. Riffle, J. W., and Krupinsky, J. M. 1986. Botryodiplodia canker of elms. Pages 38-39 in: Diseases of Trees in the Great Plains. J. W. Riffle and G. W. Peterson, tech. coordinators. U.S. Dep. Agric. For. Serv. Rocky Mt. For. Range Exp. Stn. Gen. Tech. Rep. RM-129.
16. SAS Institute. 1989. SAS/STAT User's Guide. Version 6, 4th ed. Vol. 1. SAS Institute, Cary, NC.
17. SAS Institute. 1989. SAS/STAT User's Guide. Version 6, 4th ed. Vol. 2. SAS Institute, Cary, NC.
18. Stack, R. W., Krupinsky, J. M., and Walla, J. A. 1990. Decline of Siberian elm on the Great Plains. (Abstr.) Phytopathology 80:1064.
19. Stevens, R. B., ed. 1974. Mycology Guidebook. University of Washington Press, Seattle.