Variation in Virulence of *Botryodiplodia hypodermia* to *Ulmus pumila*

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ABSTRACT

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Variation in spore type and virulence of 218 isolates of *Botryodiplodia hypodermia* was examined. Seven percent of these isolates were considered to be atypical. Spores from atypical isolates were slightly narrower and longer than spores from typical isolates; however, the two types could not be differentiated by spore size. Approximately 50% of the spores from mature cirrhi of atypical isolates were septate; spores of typical isolates were aseptate. Atypical isolates were less virulent than typical isolates. Branches above the point of inoculation were killed on 20% of 132 branches inoculated with atypical isolates and on 73% of 266 branches inoculated with typical isolates. Atypical isolates should not be used in evaluating germ plasm. Because there is some variation in virulence among typical isolates, several typical isolates should be used to evaluate the resistance of *Ulmus pumila* germ plasm.

Siberian elm, *Ulmus pumila* L., has been widely planted in the northern Great Plains during the past three decades mainly because it grows rapidly and is adapted to the region. *Botryodiplodia hypodermia* (Sacc.) Petr. and Syd. is an important canker-forming pathogen of Siberian elm (3,4,6,8). Botryodiplodia canker has severely limited the usefulness of Siberian elm in windbreaks in the eastern half of South Dakota (6). In 1979, 609 cankers were collected from Siberian elm windbreaks throughout the northern Great Plains. Collections were made in a total of 56 counties in four states (Minnesota, Montana, North Dakota, and South Dakota) (4). *B. hypodermia* was isolated from 256 of those cankers and was considered the most important pathogen isolated (3,4). Selection for resistance to this fungus is a major research objective of the Siberian elm improvement program at Mandan, ND. The research reported in this paper was conducted to study the effect of wounding on canker development, and variation in spore types and virulence among isolates of *B. hypodermia*.

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MATERIALS AND METHODS

B. hypodermia was isolated, as described previously, from cankers collected in a 1979 survey of windbreaks on the northern Great Plains (4). Sporulating cultures of *B. hypodermia* were maintained on V-8 juice agar at 21 ± 1 C under cool white fluorescent light (2,5) by transferring cirrhi every 3–4 wk. For long-term storage (12 mo), sporulating cultures were maintained at 4 ± 1 C on sterile wheat kernels on V-8 juice agar in screw cap test tubes. After long-term storage, cultures were easily rejuvenated by pressing the infested kernels into V-8 juice agar in petri plates. Inoculum was prepared by transferring cirrhi to V-8 juice agar and sprinkling sterile wheat kernels on the surface (2,5). After 14 days at 21 ± 1 C under cool white fluorescent light, *B. hypodermia* sporulated on the kernels. Isolates used in each study were transferred at the same time and grown under the same conditions for the same period of time.

Siberian elm trees, obtained as common 2-yr-old bare-root nursery stock (Lincoln-Oakes Nursery, Bismarck, ND 58501) were used. Before inoculation, these seedlings were grown for 3 mo in a glasshouse in a peat moss-vermiculite mixture (1:1, v/v) and fertilized twice a week with a balanced macronutrient and micronutrient fertilizer. Trees used for field inoculations were planted during the summer of 1978 and inoculated on 4 June 1980. Trees with two or

three branches of comparable size were selected for inoculation. The size of branches inoculated ranged from 5 to 12 mm in diameter. Two or three isolates were compared on branches of comparable size on the same tree, so that virulence of different isolates could be compared on host material that was genetically the same.

Different inoculation procedures were evaluated in preliminary studies in an attempt to inoculate with minimum damage to the host in order not to circumvent possible mechanical resistance. Such resistance might be expressed with minor wounding, such as the loss of a twig from a branch, but not with major wounding, such as an incision through the bark and the formation of a bark flap. In all studies in which isolates were compared, branches 5–12 mm in diameter were wounded by cutting off small twigs 2–5 mm in diameter to provide a wound opening rather than an incision, except where noted otherwise. The inoculum, consisting of a sterile wheat kernel overgrown with sporulating *B. hypodermia*, was placed over the wound, wrapped in place with parafilm, and covered with aluminum foil. In field studies, the aluminum foil was secured with fiber tape. In the glasshouse, symptom development, which consisted of wilting of leaves above the point of inoculation, was evaluated daily for the first 3 wk and weekly from the fourth through the 12th wk. In the field, symptom development was evaluated weekly. Branches were considered dead when leaves dried and fell off the branch above the point of inoculation.

Effect of wounding. The effect of wounding on infection was investigated in three preliminary studies conducted in the glasshouse. In the first study, 20 branches on 20 trees were not wounded and 10 branches on 10 trees were wounded before inoculation with 78-702-1(T), a typical isolate of *B. hypodermia*. In the second study, 20 trees with two branches of comparable size, one wounded and one not wounded, were inoculated with 79-2605-1(T), a typical isolate. In the third wounding study ~20 trees with two branches of comparable size, one wounded and one not wounded, were inoculated with isolate 79-2636(T).

A fourth study was conducted in the field. Three 5-yr-old trees (Mandan accession 13327) were used. Twenty-four branches on each tree were inoculated: Seven branches not wounded, seven wounded by cutting off a twig, seven wounded by cutting an inverted "V" incision with a sterile scalpel, and three inoculated with sterile wheat kernels as controls. Since Riffle (8) found that the most rapid disease development in the field followed inoculations made from July through September, the trees were inoculated 22 July 1980. Notes on branch cankers were taken weekly for 11 wk.

Comparison of isolates. *Spore type.* Growth of 218 isolates under standard culture conditions described above was examined. Spores from the cirrhi were mounted on slides with lactophenol (11), and cover slips were sealed with clear fingernail polish. The slides were kept at least 24 hr before spores were measured and septation noted. To determine if spores remained nonseptate upon aging, older black cirrhi were collected from 1- to 5-mo-old cultures. Because of apparent differences in size of spores between typical and atypical isolates, measurements were made of 100 spores from five atypical and nine typical isolates used in inoculation studies. Septation of spores was also noted.

Inoculations. Isolates were compared in five inoculation studies in the glasshouse (Table 1). In each of these five studies, the branches on 20 trees were inoculated with *B. hypodermia* and three branches on three trees were inoculated with sterile kernels for controls. In the fifth and sixth studies, three branches on each tree were inoculated with a different isolate. In the seventh, eighth, and ninth studies, two branches on each tree were inoculated. In the field, isolates of *B. hypodermia* were compared in three studies (Table 1). The branches on 18 trees were inoculated in the 10th and 11th studies, and the branches on 17 trees were inoculated in the 12th study, plus trees for control inoculations. Overall, five atypical and 14 typical isolates from 16 counties in four states (North Dakota, South Dakota, Montana, and Minnesota) were used in these studies.

TABLE 1. Dead branches produced when isolates of *Botryodiplodia hypodermia* were inoculated onto branches of Siberian elm seedlings

Study	Isolates			Branches inoculated (no.)[c]	Location study	Dead branches (no.)[d]				Dead branches (%)[e]
	Ident. no.	Type[a]	Canker[b]			1-wk	2-wk	3-wk	8-wk	
5	79-2085	A	Branch	20	Glasshouse	2	2	4	4	20
	79-2974	T	Branch	20		12	16	16	16	80
	79-2605-1	T	Branch	20		17	18	18	18	90
6	79-2010	T	Basal	20	Glasshouse	11	14	15	15	75
	79-2946	T	Basal	20		7	11	12	12	60
	79-2636	T	Basal	20		15	18	18	18	90
7[f]	79-2052-1	A	Basal	20	Glasshouse	0	0	0	0	0
	79-2053-1	T	Basal	20		0	8	9	10	50
8[f]	78-696-1	A	Branch	20	Glasshouse	0	2	4	5	25
	78-699-1	T	Branch	20		0	3	11	12	60
9[f]	79-2663	A	Basal	20	Glasshouse	0	3	4	4	20
	79-2672-1	T	Branch	20		0	8	9	9	45
10	79-2895-1	A	Basal	18	Field	1	1	1	1	5
	79-2479	T	Branch	18		10	12	12	12	67
	79-2521	T	Basal	18		9	11	12	12	67
11	79-2663	A	Basal	18	Field	7	8	8	8	44
	79-2199	T	Basal	18		16	18	18	18	100
	79-2573-1	T	Branch	18		14	15	15	15	83
12	79-2085	A	Branch	16	Field	6	6	6	6	38
	79-2632	T	Basal	17		13	13	13	13	76
	79-2930	T	Basal	17		15	15	15	15	88

[a] A = an atypical isolate, T = a typical isolate.
[b] Type of canker from which isolate was obtained.
[c] For each study, isolates were compared on different branches of the same set of trees; control branches are not listed.
[d] Branches above cankers were dead.
[e] Based on total number of branches inoculated.
[f] Both isolates were from the same windbreak planting.

RESULTS AND DISCUSSION

Effect of wounding. In the first three studies conducted in the glasshouse, cankers did not develop on the 60 unwounded branches that were inoculated with three isolates of *B. hypodermia*. Of the 50 branches wounded by cutting off small twigs and inoculated 62% were dead above the point of inoculation after 12 wk. The field inoculations of the fourth study also indicated that wounding was necessary for infection and canker formation. No dead branches were observed after 6 wk on unwounded inoculated branches. Severe wounding was necessary for the formation of cankers in the field. Fifteen of the 21 branches wounded prior to inoculation by cutting with a sterile scalpel were dead above the point of inoculation after 6 wk. One of the branches wounded by cutting off a twig was dead above the point of inoculation after 6 wk. Perhaps the accession of Siberian elm used in this experiment was resistant to *B. hypodermia* with moderate wounding, but not with a more severe incision. If the expression of resistance for some accessions of Siberian elm depends on the severity of wounding, this would be an important consideration in evaluating trees for resistance.

Comparison of isolates. *Spore type.* Most isolates of *B. hypodermia*, which were considered as typical isolates, had dark gray to black mycelium in culture and their cirrhi were white and contained aseptate spores that were hyaline when first extruded from pycnidia. After 4 to 6 wk, the cirrhus turned black and the spores turned tan to brown but remained aseptate even after 4–5 mo. The average spore size of isolates was within the reported size range of *B. hypodermia* (7,9). Fifteen out of 218 isolates that were considered atypical isolates had a light gray to white mycelium in culture and their cirrhi were white and contained aseptate hyaline spores when first extruded, but after their cirrhi turned black the spores turned tan to brown, and some became septate. Since Buisman stated that the brown spores of *B. hypodermia* isolated from *Ulmus foliacea suberosa* (the old name in the literature) now known as *U. carpinifolia* var. *suberosa* (Moench) Rehd. are nearly always two-celled (1), and that two-celled brown spores are described for *B. hypodermia* (9), these atypical isolates are still considered to be *B. hypodermia*. Only isolate 79-2895(A) had spores with more than one septum. Some spores from this isolate had two or three septa and were longer than previously reported for *B. hypodermia*.

In a study of taxonomic characters of *Diplodia* and *Diplodia*-like fungi, Satour et al (10) noted that "the only character that was stable enough to be of some value was the size of pycnidiospores." The mean size of spores of five atypical and nine typical isolates was 33×16 μm and 30×18 μm, respectively. Although spores of atypical isolates were slightly longer and narrower than spores of typical isolates, atypical isolates could not be distinguished from typical isolates by size of spores. Septations were present in 265 of 500 spores from atypical cultures but were present in only one of 900 spores from typical cultures. Thus, the presence of septa in spores from black cirrhi was good evidence of an atypical isolate.

Inoculations. When 20 isolates of *B. hypodermia* were compared, symptoms continued to develop during the second and third week in all studies except in the 12th study where symptoms remained stable after the first week. Most branches that ever showed symptoms did so by the third week.

Only one tree of the 20 inoculated for study five and one of the 20 inoculated for study six did not have dead branches (Table 1). Thus, these two trees were considered resistant to the three isolates. Other trees in the studies in the glasshouse were susceptible to either one, two, or three isolates. Fifty-one percent of 240 branches inoculated in the glasshouse were dead above the point of inoculation. Only 16% of 80 branches inoculated with atypical isolates were dead, but 69% of 160 branches inoculated with typical isolates were dead.

Only two trees of 18 inoculated for the 10th study did not have dead branches. In the 11th and 12th studies, all trees had at least one dead branch. Sixty-three percent of the field inoculated branches were dead above the point of inoculation. Only 29% of the 52 branches inoculated with atypical isolates were dead, but 80% of the 106 inoculated with typical isolates were dead. Atypical isolate 79-2085(A), which produced the fewest cankers (20%) in the glasshouse, also produced the fewest cankers (38%) in the field when compared with two other isolates in the 12th study even though a different set of trees was used (Table 1).

In both glasshouse and field studies the atypical isolates of *B. hypodermia* consistently caused less disease development on Siberian elm seedlings than did typical isolates. Only 20% of 132 branches inoculated with atypical isolates were dead above the point of inoculation compared with 73% of the 266 branches inoculated with typical isolates. Thus, atypical isolates should not be used when screening germ plasm for resistance. Even though the typical isolates appeared to produce slightly more cankers in the field with 80% of the saplings showing dead branches compared with the 69% showing dead branches in the glasshouse, a direct comparison was difficult because there were differences in host germ plasm and in isolates. Differences in virulence were found among typical isolates in four of the five studies in which more than one typical isolate was used (Table 1). Because inoculated trees did not respond uniformly to all typical isolates, more than one typical isolate of *B. hypodermia* should be used to screen Siberian elm germ plasm. Four of 93 trees inoculated with more than one typical isolate appeared to be resistant. Apparently several inoculations on the same tree with several virulent isolates of *B. hypodermia* may allow an evaluation of a tree's level of resistance, but this needs to be examined further.

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