Development of Canker on Ulmus pumila Related to Month of Inoculation with Botryodiplodia hypodermia

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ABSTRACT

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Botryodiplodia hypodermia was confirmed, by wound-inoculation of 1-yr-old seedlings, as the cause of a canker disease of *Ulmus pumila*. Canker development was observed on 6-yr-old *U. pumila* trees in Nebraska that were inoculated with mycelial disks of a *B. hypodermia* isolate at approximately monthly intervals from March 1974 through February 1975. The fungus infected all trees that were wound-inoculated in March-September. Cankers 7.6 to 12.8 cm long developed during the first growing season on trees inoculated in March-May; by the end of the second growing season, 75% of these cankers were callused over. On trees wound-inoculated in July-September, the fungus produced numerous pycnidia on 89% of the cankers, which were 48.8 to 72.5 cm long by the end of the first growing season. These cankers enlarged by the end of the second growing season and caused dieback or mortality of 94% of the trees. Extensive adventitious sprouting occurred near the junction of dead and live tissues on more than 80% of the trees that were wound-inoculated in July-September. Only 52% of trees that were wound-inoculated in November-February were cankered by April 1975, but most of these cankers were callused over by October 1975.

Additional key words: windbreaks, Siberian elm.

Since 1935, Siberian elm (*Ulmus pumila* L.) has been planted extensively in field and farmstead windbreaks and in urban areas throughout the Great Plains of the USA. It has survived well on all sites except the shallow upland soils of the southern Plains where drought killed many elms (17). Since the late 1960's, however, planting of *U. pumila* in the northern Great Plains has declined significantly because of the tree's susceptibility to canker diseases and damage from herbicides (1, 10, 13, 14).

Many canker diseases occur on *U. pumila* (8, 9, 15). In 1969 *Botryodiplodia hypodermia* (Sacc.) Petr. and Syd., a fungus previously reported to cause stem cankers on *U. americana* (7), was identified by H. Randall (*unpublished*) South Dakota State University, as the cause of a canker disease of *U. pumila* in South Dakota. This disease has severely limited the usefulness of *U. pumila* in windbreaks (1). A 1971 survey of 21,700 *U. pumila* in 154 windbreaks in 11 South Dakota counties showed that the disease was widespread (14). Canker incidence on 4-yr-old trees ranged from 2-40%; incidence on 8-yr-old trees ranged from 13-78%. A 1976 windbreak-condition survey of five eastern forestry districts (52 counties) in South Dakota revealed windbreaks have deteriorated on 7,284 ha in the last 22 yr (19), and that one major factor involved is the high mortality rate of *U. pumila*.

A 1972 survey of 44 windbreaks in four major land resource areas in North Dakota (2) revealed that 72% of 769 *U. pumila* trees were cankered (10). Over 65% of these trees had dieback of tops or branches due to girdling by canker fungi, and nearly 6% were dead. Species of *Cytospora*, *Dothichiza*, and *Tubercularia* were isolated from 17 *U. pumila* cankers collected during the survey, but none of these fungi was considered the primary cause of its decline. Typical phenoxy herbicide damage was observed in 45% of the windbreaks examined, and the frequency of this damage led Dooling (10) to hypothesize that the primary cause of windbreak decline in North Dakota was herbicide injury. However, a 1973 survey of 473 *U. pumila* trees in 18 windbreaks in four southeastern North Dakota counties revealed that 70% of the trees were cankered, and *B. hypodermia* was isolated from 15 of 60 cankers collected (Riffle, *unpublished*).

Otta (13) sprayed 3-yr-old *U. pumia* with 2, 4-dichlorophenoxyacetic acid (2,4-D) and found that stem growth was reduced at concentrations as low as 10 μg/ml. He found leaf injury symptoms in windbreaks which were suggestive of the 10 and 25 μg/ml 2,4-D treatments, and speculated that the effects of herbicide injury, drought stress, winter injury, and various fungal leaf and stem pathogens might be working together to produce the observed deterioration of *U. pumila* in South Dakota.

Although distribution and impact of canker diseases on *U. pumila* are generally known for the northern Great Plains, no information is available on the development of *B. hypodermia* canker on *U. pumila* under field conditions. The objectives of the present study were to (i) confirm pathogenicity of *B. hypodermia* to *U. pumila*, and (ii) determine the effect of time of inoculation on canker development under Nebraska environmental conditions.

MATERIALS AND METHODS

Pathogenicity test.—One-yr-old *U. pumila* seedlings were planted in a 4:1 soil-sand mixture (4:1, v/v) in 15-cm

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diameter clay pots in February 1974. In May 1974, the bark 8 to 15 cm above the root collar on 20 seedlings was surface disinfested with 70% ethyl alcohol, and each of four seedlings was inoculated with one of four *B. hypodermia* isolates originally isolated from cankers (Fig. 1) on *U. pumila* trees growing in windbreaks. These isolates, two each from Nebraska and North Dakota, were identifield using the original description of *B. hypodermia* (16). An inverted "V" incision was made through surface disinfested bark to the sapwood with a sterile scalpel, and a 1-cm mycelial disk from the margin of a 7-day-old culture on Difco potato-dextrose agar (PDA) was inserted between the bark flap and sapwood. The bark flap was pressed back into its original position and a sterile absorbent cotton pad, moistened with sterile distilled water, was placed over the flap, covered with aluminum foil, and secured with a rubber grafting band. Four control seedlings were wounded similarly but were inoculated only with a 1-cm diameter disk of PDA. Seedlings were placed randomly on a greenhouse bench (temperature 20 to 35 C), and were examined periodically for 10 mo. After 4 mo, number of seedlings infected, lengths of cankers, number of seedlings with top dieback, and number of adventitious sprouts below inoculation sites were recorded. Canker lengths and number of adventitious sprouts per seedling also were determined after 10 mo. All seedlings then were severed at the ground line, and the bark below inoculation sites was surface disinfested with 70% ethyl alcohol. Four pieces of bark tissue (ca. 5 mm^2) were removed aseptically from each canker at the junction of live and discolored bark tissues, plated on water agar or PDA, incubated at 25 C for 25 days, and then examined for presence of *B. hypodermia*.

Monthly inoculations.—Six-yr-old *U. pumila* saplings

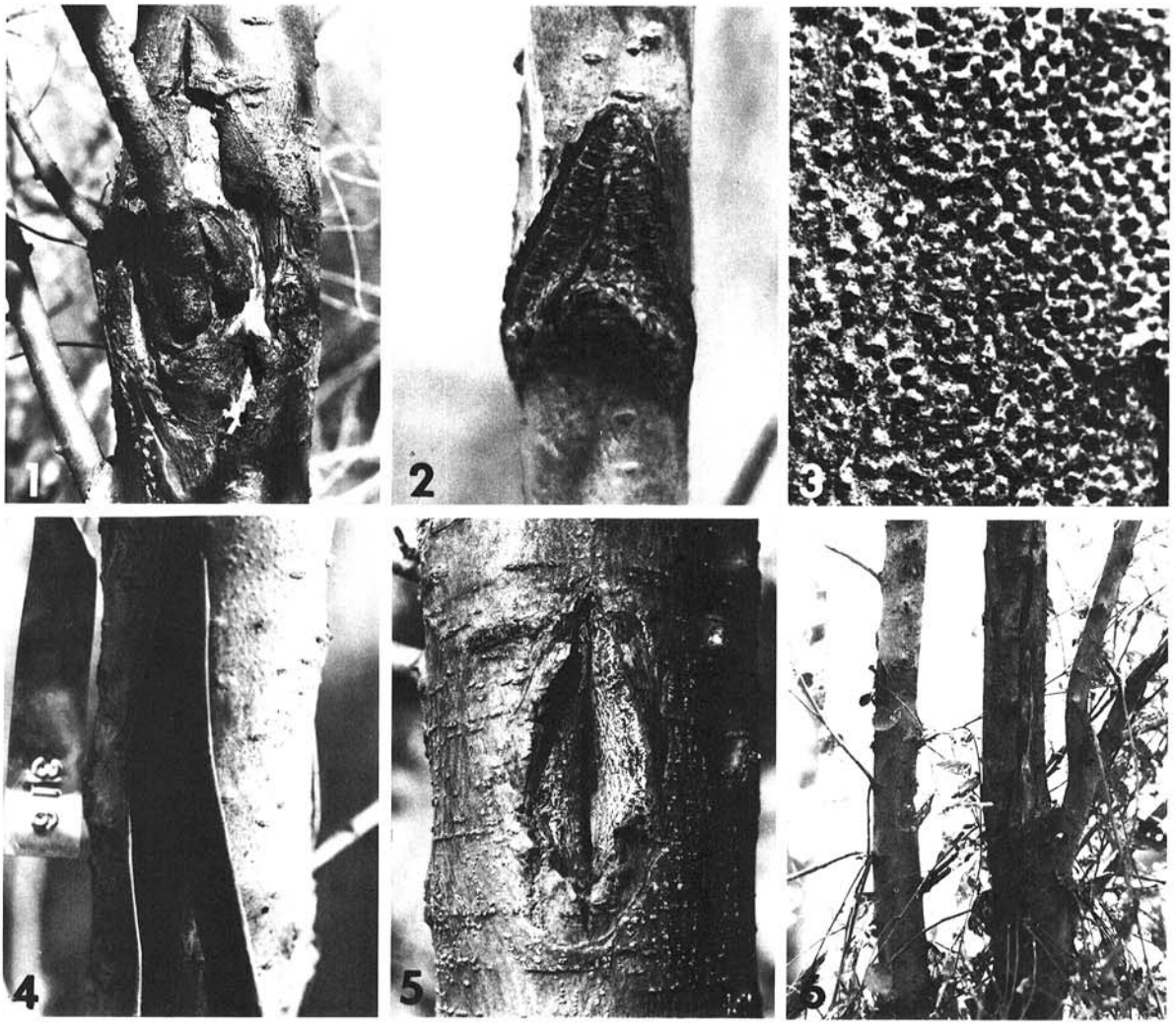

Fig. 1-6. *Botryodiplodia hypodermia* canker of *Ulmus pumila*: **1)** natural canker originating at dead lateral branch on windbreak tree; **2)** inverted "V" incision of sapling made in April 1974, callused over 190 days after wounding; **3)** erumpent pycnidia on dead bark of sapling 99 days after wound inoculation in July 1974; **4)** phellem recoiled from split in bark 43 days after wound inoculation in September 1974; **5)** canker on sapling wound-inoculated in November 1974, callused over 1 yr later; **6)** adventitious sprout development below canker on sapling 400 days after August 1974 wound inoculation.

that grew from seed on a grassland site near Hastings, Nebraska, were used for inoculation. They were inoculated with *B. hypodermia* isolate 290 at approximately 1-mo intervals from March 1974 through February 1975. This isolate was obtained from hyphal tips originating from a spore mass from a *U. pumila* canker collected from Kidder County, North Dakota, in September 1973. Only one isolate was used for field inoculations because of limited availability of experimental trees at the study location.

Eighteen *U. pumila* saplings, six replicates in each of three treatments [(i) nonwounded bark, (ii) wound into bark tissue, and (iii) wound to sapwood] were inoculated on each of 11 monthly dates. Stem diameters at inoculation sites ranged from 2.6 to 3.9 cm; heights of inoculation sites above ground were 123 to 145 cm. Bark surfaces at inoculation sites were surface disinfested with 70% ethyl alcohol. For the wounded bark treatment, approximately 1 cm^2 of periderm was removed with a sterile scalpel to expose cortical tissues. For the wound to sapwood treatment, and inverted "V"-shaped radial incision approximately 3 cm in length was made with a sterile scalpel, and the bark flap was depressed to expose the outer sapwood. Three 1-cm mycelial disks, cut from the margins of 3- to 7-day-old *B. hypodermia* cultures, were placed directly against nonwounded bark, wounded bark, or sapwood. The bark flap was pushed back into its original position after inoculation. A sterile absorbent cotton pad, moistened with sterile distilled water, was placed over the mycelial disks or bark flap, covered with aluminum foil, and secured with masking tape. These cotton pads retained moisture at the inoculation sites and were removed after 1 mo. Three trees per inoculation method were treated on each inoculation date with three 1-cm disks of Difco PDA and served as controls.

Inoculations were evaluated at the end of the first growing season (31 October-1 November 1974), and at the beginning (22-25 April 1975) and end (24-25 September and 16-17 October 1975) of the second growing season. Data recorded were: (i) percent of trees cankered, (ii) lengths of cankers, and (iii) percent of trees with top dieback. Production of callus around inoculation sites, development of adventitious sprouts on the main stem, and occurrence of *B. hypodermia* pycnidia also were recorded. In addition, samples of dying or dead bark tissues were collected 1- to 5-cm behind the margin of cankers on 155 trees for reisolation of *B. hypodermia*. Dying bark tissues were surface disinfested in 70% ethyl alcohol, and two pieces of tissue per sample were plated on Difco water agar or PDA. These cultures were incubated at 24 C and examined after 30 days. Dead bark tissues were incubated at 24 C in moist chambers for 24 hr, and examined microscopically at ×64 to ×96 for *B. hypodermia* pycnidia and spores. Two spore masses per sample were plated on water agar and incubated at 24 C for 7 days to determine if spores were viable.

RESULTS

Pathogenicity test.—Bark surfaces of inoculated seedlings were discolored dark brown, and split longitudinally 1 to 4 cm below inoculation sites 9 days after inoculation. Inner bark tissues were discolored reddish brown to black, watersoaked, and very soft. Four to 12 adventitious buds and sprouts developed on stems between the inoculation sites and root collars. Foliage on branches above cankers initially became chlorotic, and 25 days after inoculation was completely wilted and necrotic.

Cankers 4 to 5 cm long and *B. hypodermia* pycnidia on dead bark in the cankers developed on 14 of 16 inoculated seedlings within 4 mo of inoculation. Tops of the cankered seedlings were dead at this time. Cankers were 5 to 8 cm long 10 mo after inoculation, and *B. hypodermia* was isolated from distal margins of 13 of the 14 cankers. The control seedlings were not cankered and their bark incisions were callused over after 10 mo.

Monthly inoculations.—Data are presented only for inoculated trees; only 2 of 99 control trees developed cankers. No wounds were associated with cankers on

TABLE 1. Number of *Ulmus pumila* saplings cankered and length of cankers caused by monthly inoculations with mycelial disks of *Botryodiplodia hypodermia* in south central Nebraska

Inoculation dates	Type of inoculation and date of observation[a]											
	Nonwounded bark[b]				Wounded bark				Wounds to sapwood			
	10/74		9-10/75		10/74		9-10/75		10/74		9-10/75	
	(no.)	(cm)	(no.)	(cm)	(no.)	(cm)	(no.)	(cm)	(no.)	(cm)	(no.)	(cm)
22 March 1974	0	0	0	0	6	8.6	3[c]	9.6[c]	6	9.3	4[c]	13.1[c]
24 April 1974	0	0	0	0	6	7.6	...[c]	...[c]	6	9.7	...[c]	...[c]
10 May 1974	0	0	0	0	6	9.2	...[c]	...[c]	6	12.8	2[c]	12.6[c]
25 June 1974	2	8.0	...[c]	...[c]	6	12.8	2[c]	33.0[c]	6	14.7	1[c]	30.0[c]
24 July 1974	2	43.2	3	78.6	6	52.6	5[c]	114.0[c]	6	71.8	6	99.7
22 August 1974	3	40.8	3	68.3	6	72.5	6	93.8	6	59.4	6	72.1
18 September 1974	3	40.2	4	81.8	6	48.8	5[c]	117.7[c]	6	52.0	6	124.7
1 November 1974	0[d]	0[d]	0	0	0[d]	0[d]	0	0	0[d]	0[d]	0	0
9 December 1974	...	...	0	0	...	...	0	0	...	...	1	206
27 January 1975	...	...	0	0	...	...	3	10.5	...	...	1	42.0
27 February 1975	...	...	0	0	...	...	0	0	...	...	0	0

[a]Observations made after first and second growing seasons.

[b]Values in number columns represent number of six trees cankered per treatment. Values in centimeter columns are mean lengths of cankers that formed.

[c]Data for cankers that callused over after October 1974 are not presented.

[d]First reading for 1 November 1974 inoculation was 9 December 1974.

these trees, and source of the inoculum remains unknown.

Host phenology at times of inoculation was as follows: March, flowering stage with leaf buds swollen and starting to expand; April, new shoot growth 5 to 6 cm and leaves 21 mm long; May through September, trees in full leaf with leaves 44 to 50 mm long; October through November, natural leaf fall; and December through February, trees with winter buds.

Nonwounded trees inoculated prior to June 1974 and from November 1974 through February 1975 did not develop cankers (Table 1). However, two of six nonwounded trees inoculated in June 1974, and nearly half of those inoculated in July through September 1974 had developed cankers by October 1974 (Table 1).

The wounds made in March through May on control trees were callused over by October 1974 (Fig. 2). However, all wounded trees inoculated from March through September 1974 developed cankers (Table 1). Some trees wound-inoculated during November 1974 through February 1975 also developed cankers (Table 1).

Cankers about 8 to 13 cm long developed by October 1974 on trees wound-inoculated during March through May 1974 (Table 1). Reexamination of these trees in September-October 1975 showed that 75% of the cankers (21 of 36) were callused over (Table 1). Callus production was not extensive on trees wound-inoculated in June 1974; several cankers on these trees enlarged after the October 1974 evaluation (Table 1).

Botryodiplodia hypodermia was isolated from cankers on all trees that were wound-inoculated in June 1974, but not from 18 cankers sampled during the first and second growing seasons from trees inoculated prior to June 1974. Fungi isolated from these cankers included *Alternaria* and *Fusarium*. Pycnidia of *B. hypodermia* developed by October 1974 on 19% (7 of 36) of trees wound-inoculated in March through May, and on all trees similarly inoculated in June. These gray-to-black erumpent pycnidia occurred principally on dying or dead bark tissues near canker margins (Fig. 3). White spore masses containing hyaline one-celled conidia exuded from the pycnidia following rainy periods. It is not known if spores were exuded more than once from individual pycnidia, or if exudation occurred during rainy periods.

The fungus grew rapidly in bark of trees inoculated in July-September 1974. Canker lengths averaged over 40 cm on nonwounded inoculated trees, over 57 cm on wounded bark-inoculated trees, and over 60 cm on wounded-to-sapwood-inoculated trees by October 1974 (Table 1). Bark and cambium tissues of inoculated trees were rapidly killed, and the outer phellem layer, after splitting, became loose and coiled back upon itself (Fig. 4). Inner bark and sapwood were discolored dark reddish brown. Pycnidia of *B. hypodermia* developed on dead bark near the canker margins on 89% of the wound-inoculated trees. In October 1974 the fungus was isolated from three of five cankers on nonwounded trees inoculated in July through September 1974, and from 14 of 16 cankers on trees wound-inoculated in July through September 1974.

Many cankers had increased in length between October 1974 and October 1975 (Table 1), and were associated with dieback or tree mortality. Dieback and mortality were most apparent during the 1975 growing season. Of seven trees that died and 41 that showed dieback, 44 had been inoculated in July-September 1974 (Table 2). Inoculation in wounds resulted in dieback or mortality more often than inoculation of nonwounded bark (Table 2). Dead bark readily sloughed off these trees during the 1975 growing season. The two naturally infected control trees exhibited dieback by October 1975, but none of the noninfected control trees had dieback. *Botryodiplodia hypodermia* was isolated after October 1975 from nine of 10 cankers on nonwounded trees inoculated July through September 1974, and from 18 of 30 cankers on trees wound-inoculated July through September 1974.

The fungus advanced slowly in bark of trees wound-inoculated during November through February. Cankers 6 to 10 cm long developed on 52% of these trees by April 1975, but most of these cankers were callused over by October 1975 (Table 1; Fig. 5). Pycnidia of *B. hypodermia* developed on 12% (3 of 25) of wound-inoculated trees with cankers.

A host response to infection by *B. hypodermia* during months of rapid fungal movement in bark tissues (July through September) was production of adventitious buds and sprouts near and below the canker margin (Fig. 6); callus production was minimal during this period. These sprouts developed during the 1975 growing season. By October 1975, eight of 10 nonwounded inoculated trees had an average of nine adventitious sprouts, and 29 of 34 wound-inoculated trees had an average of 10 adventitious sprouts associated with stem cankers. The development

TABLE 2. *Ulmus Pumila* saplings in south-central Nebraska with mortality or dieback at end of 1975 growing season following monthly inoculations with cultured mycelium disks of *Botryodiplodia hypodermia*

Inoculation dates	Trees, of six, with mortality or dieback by treatment at end of 1975 growing season		
	Nonwounded bark	Wounded bark	Wounds to sapwood
22 March 1974	0	0	0
24 April 1974	0	0	0
10 May 1974	0	0	1
25 June 1974	0	1	0
24 July 1974	3	5	6
22 August 1974	3	6	6
18 September 1974	4	5	6
1 November 1974	0	0	0
9 December 1974	0	0	1
27 January 1975	0	0	1
27 February 1975	0	0	0

of these sprouts, similar to those formed on inoculated seedlings in the greenhouse and those observed on cankered trees in field windbreaks, gave the trees a bushy appearance.

DISCUSSION

Symptoms that developed on inoculated seedlings in the greenhouse and saplings in the field were identical to those previously observed on *U. pumila* trees with *B. hypodermia* cankers in field windbreaks in the northern Great Plains (10, 14). These symptoms and isolation of *B. hypodermia* from cankers from inoculated seedlings and saplings, and from cankers of naturally infected field windbreak trees, support the finding of H. Randall (*unpublished*) that *B. hypodermia* causes a canker disease of *U. pumila*. This fungus also has been reported to cause a wilt of elm in Norway similar to that caused by *Ceratocystis ulmi* (11).

Infection occurred on all trees that were wound-inoculated during March through September 1974. However, the most rapid disease development followed the July through September 1974 inoculations. Deficiency of rainfall during that period may have increased susceptibility of *U. pumila* to *B. hypodermia*. Examination of 1974 records of a weather station 9.6 km from the experimental area showed that total precipitation was 191 mm below normal for the period June through September, and 211 mm below normal for the year. This moisture deficiency could have resulted in reduced bark moisture content and conditions more favorable for growth of *B. hypodermia* in bark. Münch (12) reported that decreased water content of elm bark enhanced canker development by *Nectria cinnabarina*. Similarly, Bagga and Smalley (3) demonstrated that *Hypoxylon pruinatum* cankers developed most rapidly on wounded aspen cuttings grown in the greenhouse under water stress. Other investigators (4, 18) also have reported that factors contributing to moisture stress in a host increase susceptibility to certain canker pathogens.

Cankers developed most rapidly in warm weather and laboratory growth studies with the isolate used in field inoculations indicated that the fungus was adapted for growth at relatively high temperatures. Mean colony diameters on Difco PDA at constant 4, 8, 12, 16, 24, 28, 32, and 36 C for 120 hr were 10, 16, 21, 30, 38, 40, 37, and 11 mm, respectively. Percent germination of spores on 2% water agar at constant 4, 8, 16, 24, 28, 32, and 36 C for 5 hr was 0, 8, 77, 96, 96, 89, and 7, respectively. Germ tube lengths on water agar at constant 8, 16, 24, 28, and 32 C for 5 hr were 46, 90, 279, 309, and 117 μm, respectively.

Natural host factors for disease resistance, such as production of callus and tannin, operate most efficiently in tissues provided with an adequate supply of water (5, 6). Callus developed commonly at margins of wounds and cankers in March through May 1974, and November 1974 through February 1975 inoculations, months when moisture deficiencies were less than in July through September 1974. During July through September 1974, the typical *U. pumila* response to *B. hypodermia* infection was production of adventitious sprouts.

Wounding of bark enhanced but was not necessary for infection of *U. pumila* saplings. The avenue for penetration and infection of nonwounded bark on trees inoculated during June through September 1974 is unknown. Possibly *B. hypodermia* entered nonwounded bark through natural openings such as lenticels or small cracks, or through small surface wounds made by insects that were active during the summer. Depth of wounding had little effect on infection and canker development on inoculated trees (Table 1).

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