The Influence of Controlled Stresses on Susceptibility of European White Birch Stems to Attack by Botryosphaeria dothidea

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ABSTRACT

Seedlings of *Betula alba* inoculated with *Botryosphaeria dothidea* were exposed to controlled water stress, freezing stress, and defoliation stress. Susceptibility to canker formation, and to colonization of wood and bark by the pathogen, increased with decreasing water potentials, beginning at approximately the -12 bar level as measured by the pressure bomb method. Disease susceptibility induced by water stress was reversible; the rate of canker expansion declined and callus tissue formed at canker margins after seedling turgidity was restored by watering. Canker formation and bark colonization occurred following exposure of partially cold-hardened seedlings to a rapid drop in ambient air temperature from 5 C to −30 C. The extent of colonization of wood increased proportionally with exposure to minimum temperatures of −10 C, −20 C, and −30 C, respectively. Susceptibility of seedlings in a more advanced stage of cold hardiness was not affected by freezing in these tests. Canker formation, and colonization of bark and wood, occurred following 4 weeks of exposure of inoculated seedlings to defoliation stress, and increased with length of exposure. Wounding was found to be a prerequisite for invasion of stems by the pathogen in seedlings exposed to defoliation stress.

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Additional key words: predisposition, water stress, freezing stress, defoliation stress, canker.

European white birch, *Betula alba* L., is a popular tree species for landscape plantings in Illinois. In recent years, however, extensive damage due to stem cankers has appeared on this species in the central and southern portions of the state. Several fungi formed fruiting bodies in the cankers, but the species most commonly associated with margins of enlarging cankers was the *Dothiorella* stage of *Botryosphaeria dothidea* (Morig. ex Fr.) Ces & de Not., the preferred synonym of *B. ribis* (Tode ex Fr.) Gross. & Dug. according to von Arx and Müller (1).

Repeated inoculations on stems of vigorous *B. alba* seedlings with an isolate of *B. dothidea*, obtained from a canker on European white birch, did not result in the formation of stem cankers.

Although many isolates of *B. dothidea* are virulent pathogens on a wide range of host species (16), other isolates incite cankers only on hosts that are weakened or under stress. Hutton (5) reported formation of *B. ribis* cankers on apple trees suffering from drought or severe malnutrition. Toole (17) considered *B. ribis* a weak pathogen on shaded or weakened branches of sweetgum. Neely (9) associated bleeding necrosis cankers caused by *B. ribis* on sweetgum with drought stress due to several years of abnormally low rainfall. Pirone (10) observed that infection of street trees in New York by *B. dothidea* was associated with injury of lower branches by nearby leaf fires. Schoeneweiss (12) found that no cankers developed on stems of European mountain ash inoculated with *Fusicoccum* sp., later reclassified as *B. dothidea* (Schoeneweiss, *unpublished*), until inoculated host plants were weakened by transplanting.

Although a search of the literature revealed numerous allegations as to the probable influence of stresses on disease susceptibility in woody plants, most of these were based on field observations. Reports of research with controlled stresses were sparse. Increased susceptibility caused by induced water stress was reported for several host-pathogen combinations (2, 7, 18). Schoeneweiss (13) reported that cottonwood seedlings exposed to artificially-induced defoliation stress were colonized by several genera of fungi. Helton (4) reported that stems of Stanley prune trees were invaded by *Cytospora* sp. following wounding of artificially frozen tissues.

Extended drought periods in Illinois in 1970 and 1971 were followed by an apparent increase in canker damage on several woody plant species, including *B. alba*. Such damage also appeared to be more prevalent in years in which freezing damage was extensive on woody species. These observations indicated that drought and freezing stresses may influence the susceptibility of *B. alba* to attack by stem canker organisms. The studies reported in this paper were conducted to investigate the possible influence of controlled drought or water stress, freezing stress, and defoliation stress on susceptibility of *B. alba* stems to colonization by *B. dothidea*.

MATERIALS AND METHODS.—*Host plants.*—Bare-rooted, 2-year-old *B. alba* seedlings, 45-60 cm in height, were obtained from a commercial nursery in 1971 and 1972. The seedlings were planted in February in 3.8 liter containers using a potting mix composed of equal parts of loam soil and sand. In April, they were transferred to an outdoor lath house. All plants were irrigated as needed and fertilized biweekly with 100 mg/kg 45-0-45 soluble fertilizer in irrigation water for at least 4 months prior to exposure to stress conditions.

Inoculum.—An isolate of *B. dothidea* was obtained from the margin of an enlarging canker on *B. alba*. Inoculum was prepared by macerating a 10-day-old potato-dextrose agar (PDA) culture, incubated at 24 C, in 100 ml of sterile water for 2 minutes in a Waring Blendor. Cultures of the pathogen were exposed to continuous fluorescent light during the incubation period to enhance production of sporulating pycnidia, as reported by Schreiber (15).

Inoculation technique.—A horizontal hole was bored through the center of the stem at each inoculation point with a sterile 2-mm diameter drill bit, at a hieght of 10 cm above the ground line. Inoculum was injected into the hole with a medicine dropper until it flowed out the hole on the opposite side. In this manner, inoculum was placed in contact with the different tissues present in test stems. Inoculated wounds were then wrapped with grafting tape to prevent drying and contamination.

Water stress.—The pressure bomb method (14) was used to measure internal water deficits of stem tissues. This technique left stems basically intact, and permitted multiple measurements of nonosmotic xylem water potentials of excised leaves or shoots in a short period of time. Although pressure bomb determinations may differ from leaf water potentials measured with a thermocouple psychrometer (6), they provide a useful relative measure of the water status of xylem tissues (3). To avoid method error, a standard rate of pressure increase of 0.68 bars per second was used for all determinations, and the length of the excised petiole or shoot protruding from the pressure bomb was adjusted to 2 cm, as suggested by Waring and Cleary (19).

To reduce or eliminate water potential gradients present in xylem tissues of tree seedlings, test plants were placed in a specially designed humidity cabinet assembled inside a standard walk-in growth chamber. High humidity was provided by partially immersing cheesecloth wicks in pans of water placed along the inside edges of the cabinet side panels. A circulating fan inside the cabinet helped prevent the formation of humidity gradients.

To determine if there was a correlation between xylem water potential and canker incidence, or colonization of stem tissues by *B. dothidea*, three replicate tests were conducted: one in June 1971, one in July 1971, and one in June 1972. In each test, 14 seedlings were exposed to water stress by placing the containers on a covered, open-air porch and withholding water for 5 days. Two check plants were treated similarly, but received daily irrigation. All test seedlings were then placed in the humidity cabinet, and held under conditions of high relative humidity (RH)(98% ± 1%) at a constant temperature of 24 C, and exposed to a photoperiod of 6 hours per day at a light intensity of 4,304 lx (400 ft-c). After 48 hours in the cabinet, stems of all seedlings were inoculated and water potentials of three shoots on each plant were measured immediately and then at 4-day intervals for 16 days. All seedlings were then removed from the cabinet, watered, and placed on a greenhouse bench, where they were watered daily and observed for canker formation for 14 days. Measurements of the diameters of cankers formed were recorded at 4-day intervals following inoculation. At the termination of each test, sections 1-mm square by 1-mm thick were removed at 5-mm intervals from ethanol surface-sterilized bark and wood above and below inoculation points, to determine the extent of colonization by *B. dothidea*. The sections were incubated on PDA at 24 C, and recovery of the pathogen was determined on the basis of colony characteristics.

The possibility that a change in disease susceptibility induced by water stress is reversible, was also investigated. In June 1972, twenty seedlings were inoculated and exposed to water stress as described

above, until mean xylem water potentials were more negative than −12 bars. The plants were then placed in the humidity cabinet for 24 days, after which they were watered and held in the cabinet for an additional 16 days. Diameters of cankers formed were recorded at 4-day intervals following inoculation.

Freezing stress.—Freeze damage or freezing stress in woody plants is usually caused by a rapid drop in temperature, rather than by extreme low temperatures, according to Weiser (20). To create a rapid freeze environment for freezing intact seedlings in containers, a temperature cabinet similar in principle to that described by Schneider et al. (11) was constructed. The cabinet was basically a wooden frame covered with two layers of polyethylene sheeting, which provided a 5-cm layer of trapped insulating air to enhance stabilization of internal cabinet temperature. The cabinet was placed inside a walk-in freezer maintained at a constant temperature of −43 C. A fan-driven space heater was mounted in the top of the cabinet and connected to a Fenwal thermostat placed at the same height as test plant stems. The fan ran continuously, independent of the heater, to prevent the formation of temperature gradients within the cabinet. Manual adjustment of the thermostat allowed temperature control from 10 C to −30 C ± 1.5 C.

In early October 1971, 42 dormant *B. alba* seedlings were allowed to partially cold-harden in an outdoor lath house. Following exposure to several light frosts, the seedlings were transferred to a dark, cold storage room and held for 14 days at 5 C to achieve a uniform semihardened condition prior to exposure to freezing stress. Thirty-six plants were inoculated and 30 of these, plus six noninoculated plants, were placed in the cabinet. The containers were surrounded with vermiculite to a depth of 2.5 cm on the stems to prevent freezing of the root systems. The plants were then exposed to freezing stress by lowering the ambient air temperature in the cabinet from 5 C to −30 C in increments of 5 C every 30 minutes. Ten inoculated and two noninoculated plants were removed from the cabinet after 30 minutes of exposure to minimum temperatures of −10 C, −20 C, and −30 C, and thawed overnight at 5 C. The remaining six inoculated plants were held at 5 C as unfrozen checks.

An identical test was conducted in November 1971 with 42 seedlings that were in a more advanced stage of cold hardiness due to longer exposure to outdoor conditions. Both tests were repeated in the fall of 1972. In early April 1972, 42 seedlings were removed from the outdoor lath house, held at 5 C for 14 days to promote partial loss of cold hardiness, then exposed to freezing stress in the same manner. Plants from all tests were placed on a greenhouse bench after thawing, and canker formation was recorded at 4-day intervals for 30 days. At the conclusion of each test, bark and wood tissues above and below inoculation points were cultured on PDA at 24 C to determine the extent of colonization by the pathogen.

Defoliation stress.—Stems of 40 seedlings were inoculated in May 1972 and exposed to defoliation stress for 8 weeks by manually removing all leaves from test seedlings at weekly intervals. An additional five seedlings were inoculated but not defoliated, and 10 seedlings were defoliated but not inoculated. All plants were irrigated daily for the duration of the test. Two inoculated and two noninoculated plants were allowed to leaf out after 4, 5, 6, and 7 weeks of exposure. Percent of canker formation, and visual symptoms of defoliation stress, were recorded at weekly intervals.

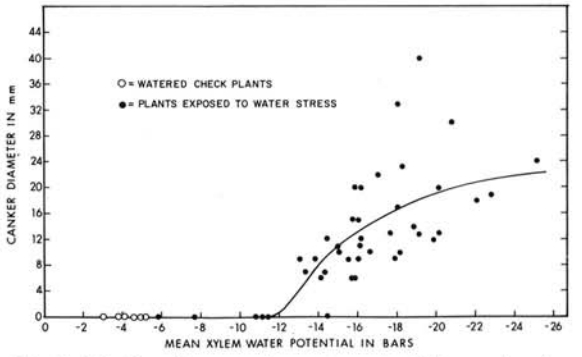

Fig. 1. Relation of mean xylem water potential to canker diameter in seedling stems of *Betula alba* inoculated with *Botryosphaeria dothidea*. Mean xylem water potentials are based on pressure bomb measurements of three shoots on each plant at 4-day intervals during a 16-day period of exposure to water stress. Each point represents the maximum canker diameter on an individual plant, measured 14 days after turgidity was restored by watering.

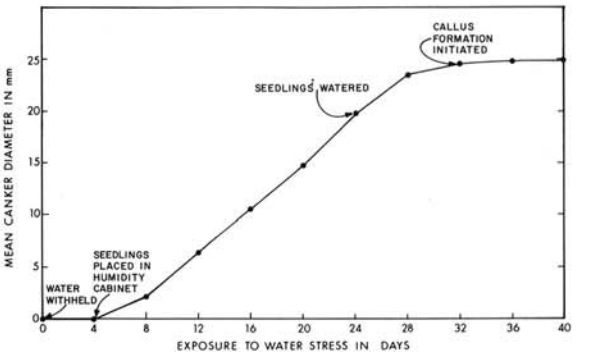

Fig. 2. Relation of length of exposure to water stress to canker diameter in stems of *Betula alba* inoculated with *Botryosphaeria dothidea*. Mean canker diameter is based on measurements at 4-day intervals of maximum diameters of cankers formed on 20 seedlings exposed to water stress levels more negative than −12 bars.

To determine whether wounding was a prerequisite for invasion of stems by *B. dothidea*, 30 plants were inoculated without a wound and exposed to defoliation stress for 8 weeks. Two methods of inoculation were used: in the first method, 1-cm squares of a PDA culture of the pathogen were placed in contact with ethanol surface-sterilized bark surfaces and held in place with grafting tape. In the second method, macerated inoculum was placed in wells made with grafting tape and petroleum jelly on sterilized bark surfaces. Stems were observed weekly for canker formation and bark and wood tissues were cultured after 8 weeks' exposure to determine the extent of colonization by the pathogen.

RESULTS.—*Water stress.*—Water potentials of plants exposed to water stress ranged from −6 to −26

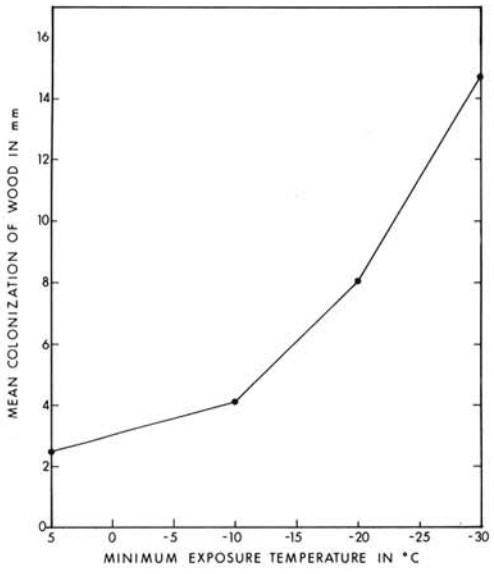

Fig. 3. Relation of minimum exposure temp to extent of wood colonization in stems of *Betula alba* inoculated with *Botryosphaeria dothidea*. Mean colonization of wood is based on data from 50 inoculated seedlings exposed to a drop in ambient air temperature from 5 C to the minimum temperature indicated at a rate of 10 C per hour. Extent of wood colonization was determined 1 month following exposure to freezing stress.

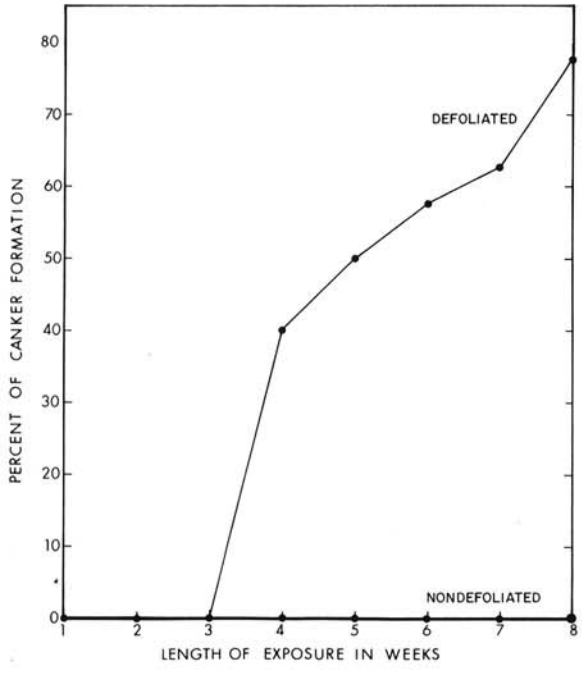

Fig. 4. Relation of length of exposure to defoliation stress to canker incidence (percent of canker formation) in stems of *Betula alba* seedlings inoculated with *Botryosphaeria dothidea*. Percent of canker formation is based on 40 inoculated seedlings which were manually defoliated at weekly intervals, and five inoculated, but nondefoliated checks.

bars. Older leaves began to turn yellow and drop at water potentials more negative than −16 bars and younger leaves began to visibly wilt at approximately −18 bars. Although pressure bomb readings from separate shoots on individual plants after 48 hours in the humidity cabinet varied less than 5%, xylem water potentials had decreased an average of 10% in nearly all test plants after 16 days in the cabinet. Therefore, the mean of the water potential measurements made during the incubation period was recorded as representing the relative water potential of each test stem.

The relationship of xylem water potential to canker formation is presented in Fig. 1. Canker formation was recorded on 36 of 37 seedlings exposed to water stress levels more negative than −12 bars. No cankers were observed at levels less negative than −12 bars. In general, canker diameters increased with decreasing water potential.

The relationship between length of exposure to water stress and expansion of canker diameters on inoculated seedlings exposed to water stress levels more negative than −12 bars, is presented in Fig. 2. Cankers began forming on all seedlings after 4 days of exposure, and continued to expand at a fairly constant rate until seedling turgidity was restored by watering. The rate of canker expansion declined after the seedlings were watered and callus tissue formation began at canker margins approximately 8 days after turgidity was restored.

Cultures made from wood and bark from all inoculated stems exposed to water stress revealed that the extent of colonization of both tissues by *B. dothidea* coincide fairly closely with canker margins.

Freezing stress.—In preliminary studies, typical freezing injury symptoms were produced on several species of container-grown woody ornamentals known to be sensitive to freezing injury, when plants were frozen as described in Materials and Methods. With *B. alba*, no visible signs of freezing injury were observed, although bud break was delayed for 7 to 10 days on most plants frozen to −30 C in October of both 1971 and 1972.

In the present studies, no canker formation was recorded on stems of inoculated plants exposed to minimum temperatures of 5 C, −10 C, or −20 C. Cankers of varying size appeared on six of 10 plants frozen to −30 C in October 1971, and on nine of 10 plants frozen to −30 C in October 1972. No cankers formed on plants frozen in November of either year, or in April 1972.

B. dothidea was recovered from cankered bark, but not from bark beyond canker margins. Bark was not colonized at exposure temperatures of 5 C, −10 C, or −20 C. The extent of wood colonization, however, increased with decreasing exposure temperature in all freezing tests, regardless of canker formation. Data on the extent of wood colonization from all five freezing tests was combined, and the relationship between exposure temperature and extent of colonization is presented in Fig. 3.

Defoliation stress.—The relationship between canker incidence (percent of canker formation) and length of exposure to defoliation stress is presented in Fig. 4. Cankers began forming on some inoculated stems after 4 weeks of exposure. Two inoculated seedlings that were defoliated for 4 weeks, then allowed to leaf out, recovered

completely without canker formation. Cankers continued to expand after initial appearance until stems were girdled on plants defoliated beyond 4 weeks, even on two plants allowed to leaf out after 5 weeks and two after 6 weeks. Noninoculated check plants recovered with no apparent damage after 6 weeks, but recovery beyond the 7th week was slow, with initial formation of chlorotic leaves. The extent of colonization of wood and bark by the pathogen coincided with canker margins; but after stems were girdled, *B. dothidea* was recovered from woody tissue above the girdle.

In tests conducted to determine whether wounding was a prerequisite for invasion by *B. dothidea*, no cankers appeared on any plants inoculated without a wound, even on plants exposed to defoliation stress beyond 8 weeks, until they eventually died. After death occurred, the pathogen was usually recovered from bark beneath inoculation points.

DISCUSSION.—Exposure of *B. alba* seedlings to controlled water stress, freezing stress, and defoliation stress revealed that all three stresses increased susceptibility of stems to attack by *B. dothidea*. The level of stress that resulted in a significant increase in susceptibility in each case was less than the level which produced visible damage in the absence of the pathogen. If diseased plants were restored to a vigorous, nonstressed condition before stems were girdled by cankers, disease development ceased.

Recovery of the pathogen in culture from inoculation points on all stressed and nonstressed plants at the termination of each test, indicated that *B. dothidea* may remain viable for many weeks in wounds on vigorous stems without extensive colonization, until plants are exposed to stress. This supports previous reports of the pathogen on elm (8), and on European mountain ash (12).

Although stress from repeated defoliation resulted in formation of cankers on inoculated stems, such extensive defoliation is rare on European white birch in Illinois, and is probably not involved in the appearance of stem cankers. A rapid drop in temperature from above freezing to −30 C is also rare; however, similar drops in temperature to −10 C and −20 C occasionally occur in the fall, and cause damage on plants that are not sufficiently cold-hardened. Since *B. dothidea* colonized wood exposed to similar freezing conditions, the pathogen may persist in frozen stems without producing disease symptoms, yet form cankers later when the plants are exposed to further stress. Extended drought conditions are fairly common in the Midwest in the summer, and are probably directly involved in the etiology of *B. dothidea* canker on *B. alba*.

The pressure bomb technique provided reliable, reproducible measurements of the nonosmotic xylem water potential of intact stems, provided test plants were held under conditions of light, temperature, and humidity conducive to the establishment of water potential equilibrium before making measurements. With this technique, correlations were made between levels of water stress and changes in disease susceptibility in birch stems. It should be possible to use this technique to make similar correlations with other host-pathogen combinations in which disease susceptibility may be influenced by water stress.

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