Growth of *Verticillium dahliae* on Sap from Five Maple Species

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ABSTRACT

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To determine the ability of the wilt disease fungus, *Verticillium dahliae*, to grow on maple tree sap, sap samples were taken at the dormant, bud-swell, bud-break, and full-leaf physiological stages of the tree and were used as growth media for the fungus. Naturally seeded *Acer negundo, A. saccharum, A. rubrum, A. platanoides,* and *A. saccharinum* were used. *Verticillium* growth on Czapek-Dox broth at various temperatures indicated that soil and tree temperatures occurring at the dormant and bud-swell stages were unfavorable and temperatures occurring at the bud-break and full-leaf stages were favorable for fungal growth and sporulation. Red and silver maple sap were the most favorable media when taken at stages when temperatures favored rapid fungal growth and sporulation. Fungal growth and sporulation on Norway and sugar maple sap reached a peak at the bud-break stage, but as temperature became more favorable, at the full-leaf stage, the sap supported less growth and sporulation. Boxelder sap was the best medium at the bud-swell stage, when the temperature was unfavorable for fungal growth, but as the temperature became more favorable, boxelder sap became a poor medium for fungal growth and conidial and microsclerotial production. This may explain the resistance of boxelder to the wilt disease. *Verticillium* growth in maple may be influenced by the nutrient level or the possible presence of inhibitors in the sap at various tree growth stages.

Additional key words: maple wilt.

Rankin (12) first reported *Verticillium* as the causal agent of maple wilt. Since then, *Verticillium* wilt of maple has been widely reported in the eastern United States and Canada (16). Generally all maple species are considered to be susceptible to wilt except *Acer negundo, A. pennsylvanicum,* and *A. spicatum* (3,7,16). The ranking of susceptibility among maple species varies among authors (17). Pirone (10) ranked *A. saccharinum* as most susceptible, followed by *A. platanoides, A. rubrum,* and *A. saccharum*. The period of maximum susceptibility of maple to *Verticillium* wilt has not been established. During the year, a peak period exists when *Verticillium* can be readily isolated from a tree and a tree can be successfully inoculated. Ease of inoculation is greatest with sugar maple in May and decreases sharply throughout the summer (2,12,20).

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This work was undertaken to determine whether the saps of different maple species differ in the ability to support fungal growth and development and to determine whether seasonal variations exist in this ability.

MATERIALS AND METHODS

Growth of *Verticillium* on sap. *Acer negundo* L. (boxelder), *A. rubrum* L. (red maple), *A. saccharum* Marsh. (sugar maple), *A. saccharinum* L. (silver maple), and *A. platanoides* L. (Norway maple) were used in the study. Five trees per species of uniform size (41–51 cm in diameter at breast height), maturity (presence of seed production), and health (high vigor) were selected. The experimental stand was a naturally seeded hardwood forest on the floodplain of the Raritan River in Piscataway, NJ.

Sap was collected in Erlenmeyer flasks connected to a hole bored into the trunk of the tree. A hand-held vacuum pump was used to increase sap flow (13). The times of collection were chosen to coincide with the dormant, bud-swell, bud-break, and full-leaf physiological stages of the trees. The collection dates and soil temperatures at a depth of 20 cm were 28 February, 4 C; 29 March, 8 C; 20 April, 12 C; and 20 May, 16 C. The sap used as a fungus growth medium was filter-sterilized through 0.45-μm Millipore membrane filters (Millipore Corp.). Aliquots (10 ml) of this medium were then aseptically dispensed into sterile, 50-ml Erlenmeyer flasks. The sap pH ranged from 5.5 to 6.4, with the lower values occurring at the bud-swell stage.

Two *V. dahliae* Kleb. isolates from Norway and red maple were used in the experiment. Both were pathogenic to maple, eggplant, and tomato in preliminary experiments.

Data were collected for each isolate on two flasks of sap from each of five trees per species and each harvest date at 4, 6, 8, 10, 12, and 14 days after inoculation. Sap from each tree was assayed separately. Microsclerotial production was rated visually as the percentage coverage of the flask bottom before the fungal mat was harvested. After microsclerotial production was rated, 0.5 ml of surfactant (Igepal CO-630, GAF Corp.) was added to each flask. The flasks were agitated on a vibrating shaker to free the conidia from the fungal mat and suspend them in the supernatant. The fungal mats were removed from the flasks, washed with distilled water to remove adhering soluble solids, placed in preweighed aluminum weighing dishes, oven-dried, and weighed. The conidial suspensions were visually rated by comparing their turbidity with counted standard suspensions, using a constant light source and distance. Each vial was marked with lines, and the suspensions were rated on the sharpness and visibility of the lines through the unknown suspension as compared to that of the lines through the known suspension. Dry weight and microsclerotial and conidial data were statistically analyzed as maximum production on or before day 8, because data taken on days 10, 12, and 14 did not change the results significantly. All data were analyzed using the H.S.D. (Honestly Significant Difference) multiple comparison test (18) at the 0.05 level of significance.

Effect of temperature on *Verticillium* growth. Isolates of *V. dahliae* were grown on 50 ml of Czapek-Dox broth (CDB) in 250-ml Erlenmeyer flasks for 10 days at 24 C in the dark. The cultures were then homogenized, washed three times by centrifugation, and resuspended in sterile distilled water. Twelve flasks of sterilized sap each received 0.1 ml of this inoculum preparation.

Verticillium was grown on CDB at 4, 8, 12, 16, 20, and 24 C for 22 days. Fungus growth, sporulation, and production of microsclerotia at the various temperatures were measured as described previously at 2-day intervals.

RESULTS

Growth of *Verticillium* on sap. Because the growth and development of both *V. dahliae* isolates on sap were very close, only the responses of the Norway maple isolate are reported. Fungus growth was significantly greater on boxelder sap taken at the bud-swell stage than on sap taken at the other growth stages (Fig.

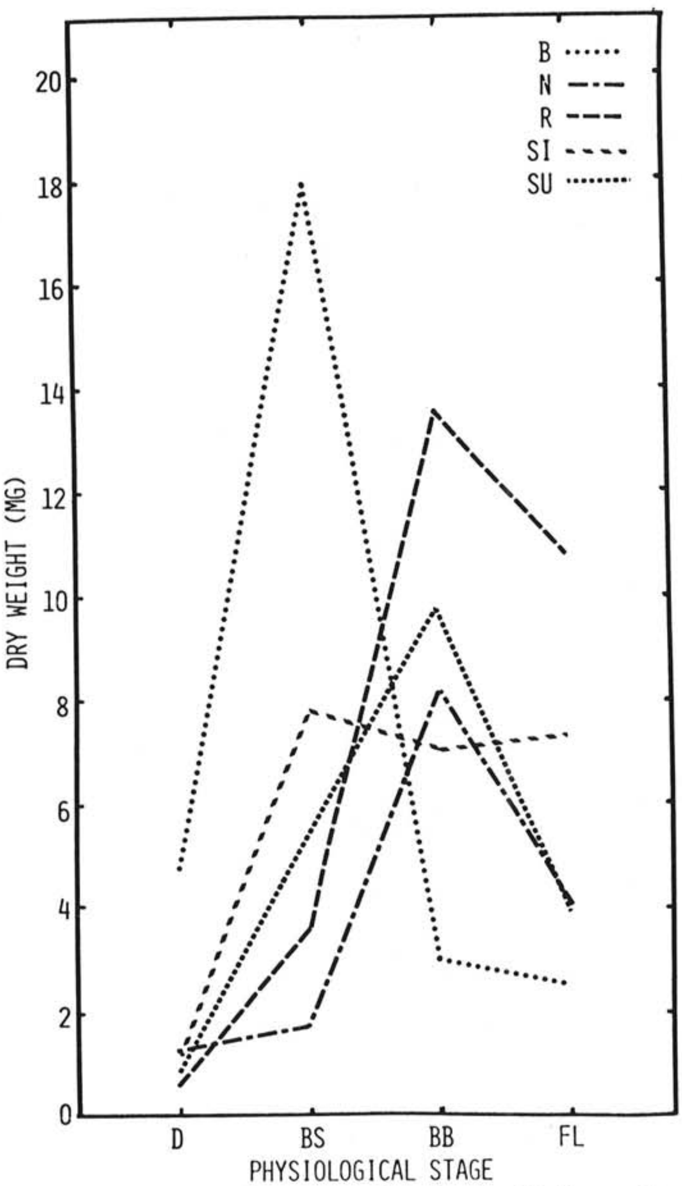

Fig. 1. Dry weight of *Verticillium* on sap from boxelder (B), Norway (N), red (R), silver (SI), and sugar (SU) maples. Sap was extracted from the trees at the dormant (D), bud-swell (BS), bud-break (BB), and full-leaf (FL) physiological stages. According to the H.S.D. multiple comparison test, $P = 0.05 = 6.3$.

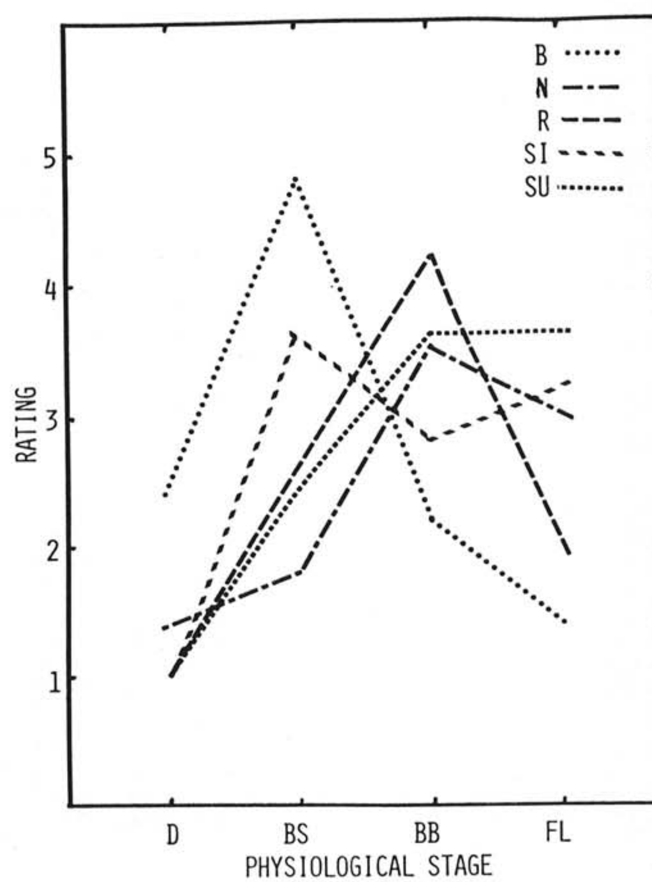

Fig. 2. Conidial production of *Verticillium* on sap from boxelder (B), Norway (N), red (R), silver (SI), and sugar (SU) maples. Sap was extracted from the trees at the dormant (D), bud-swell (BS), bud-break (BB), and full-leaf (FL) physiological stages. Conidial ratings in spores per milliliter: 1 = 0–150, 2 = 11,500, 3 = 26,000, 4 = 52,000, and 5 = 110,000. According to the H.S.D. multiple comparison test, $P = 0.05 = 1.34$.

1). Fungus growth on boxelder sap taken at the bud-break and full-leaf stages was less than on sap from other maples at the same stages.

Fungus growth on silver maple sap was greatest at the bud-swell stage and generally maintained this level on bud-break and full-leaf stage saps. Growth was better on sap of silver maple from the bud-swell stage than on similar sap from sugar, Norway, and red maple.

Red maple sap supported significantly higher fungus growth at the bud-break stage than at the two earlier stages, and growth at the full-leaf stage was only slightly lower than at the budbreak. Growth was greater than that on sap from the other trees at these two stages.

Sugar and Norway maple sap supported the best fungus growth on samples taken at the bud-break stage. Growth on the full-leaf stage sap, although less than that on red and silver maple sap, was greater than that on boxelder sap.

Conidial production of *Verticillium* on sap. Conidial production on saps from the five tree species at various growth stages followed the same general trends reported for mycelial growth (Fig. 2).

Microsclerotial production by *Verticillium* on sap. Boxelder sap taken at the dormant and bud-swell stages supported significantly greater microsclerotial production than did sap from later stages or sap from other species at all stages studied (Fig. 3). Microsclerotial production was significantly greater on silver maple sap from the bud-swell, bud-break, and full-leaf stages than on sap from the dormant stage. Although microsclerotial production on sugar maple sap was higher than on sap from other species when taken at the bud-break stage, it was lower than on red and silver maple sap at the full-leaf stage. Microsclerotial production on red maple sap from the full-leaf stage was higher than production on this sap from earlier stages.

Effect of temperature on *Verticillium*. The growth of *Verticillium* on CDB was negligible at 4 and 8 C but substantial at 12 and 16 C (Table 1). The optimum temperature for growth was 20 C. Conidial production was greatest at 20 and 24 C, and few if any conidia were produced at 4 and 8 C. Microsclerotial production was greatest at 20 C and nil at 4 and 8 C.

DISCUSSION

The optimum temperature for *Verticillium* growth and development in these tests was approximately 20 C and is in agreement with other studies on *Verticillium* (2,6,9). Temperatures of 4 and 8 C, corresponding to soil temperature at the dormant and bud-swell tree stages, allowed little growth and no conidial or microsclerotial development, even on the most favorable media. Temperatures of 12 and 16 C, corresponding to soil temperature at the bud-break and full-leaf stages, allowed significant growth and development of the fungus. This suggests that the activity of the fungus in the tree might increase during the physiological stage of the tree in which the more favorable temperatures occur. Caroselli (2) grew 10 *Verticillium* isolates on synthetic media and showed that fungal growth at temperatures corresponding to those at bud-break and full-leaf stages of the tree was 41 and 66%, respectively, of the fungal growth at the optimum temperature of 23 C.

The tree's physiological stages of dormant, bud-swell, bud-break, and full-leaf can be divided into two periods depending on tree and soil temperatures at each stage. These two periods correspond to temperatures that are unfavorable and those that are favorable to fungal growth in the laboratory. Based upon our results with *Verticillium* growing on CDB, the dormant and bud-swell stages occurred during the period in which the temperatures were unfavorable to fungal growth. The stages of bud break and full leaf occurred during the period in which temperatures were favorable to fungal growth.

These results also showed that sap collected from different maple species varies in ability to support fungal growth and development. The rate and timing of fungal growth in relation to the tree and soil temperatures and the quality of the sap as a nutrient base could influence the relative susceptibility of the species. Boxelder is considered resistant to *Verticillium* wilt (3,7). Results showed that during the dormant and bud-swell stages boxelder sap was a superior medium for growth and development of the fungus. These stages occurred during the unfavorable temperature period, which would severely limit fungal growth and development in the field. Boxelder sap lost the ability to support fungal growth, conidial production, and microsclerotial development at the bud-break and full-leaf stages. This diminished support for growth and development could reduce the inoculum and overwintering potential of the fungus.

Silver maple sap supported fungal growth and development best on samples taken at the bud-swell stage. This stage occurred during the unfavorable temperature period. However, silver maple sap maintained a high level of fungal growth on samples taken at the bud-break and full-leaf stages. These stages occurred during the favorable temperature period. This could increase the inoculum,

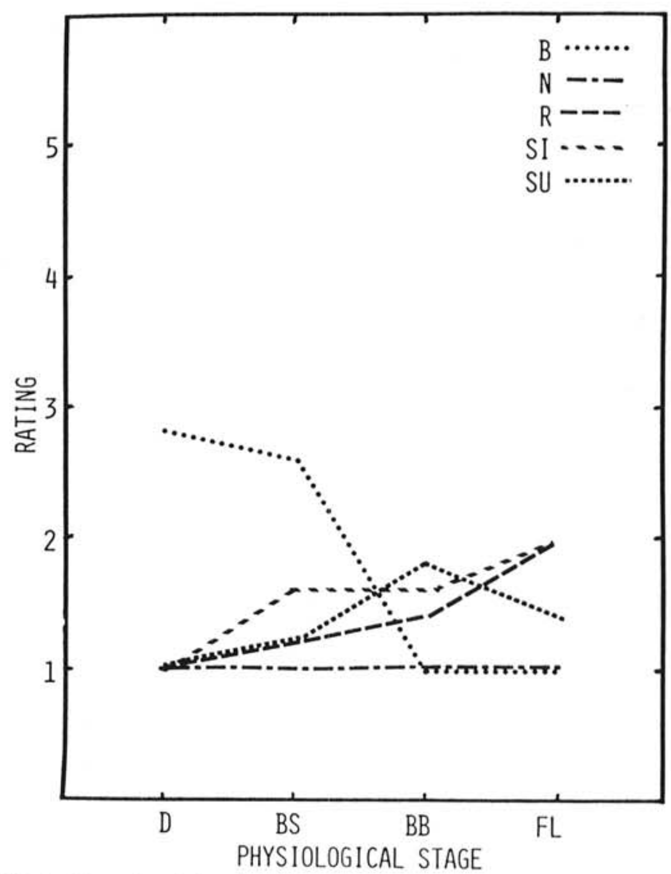

Fig. 3. Microsclerotial production of *Verticillium* on sap from boxelder (B), Norway (N), red (R), silver (SI), and sugar (SU) maples. Sap was extracted from the trees at the dormant (D), bud-swell (BS), bud-break (BB), and full-leaf (FL) physiological stages. Microsclerotial ratings in percentage of coverage of the flask bottom: 1 = no sclerotia, 2 = fewer than 10, 3 = 10–40, 4 = 41–70, and 5 = 71–100. According to the H.S.D. multiple comparison test, $P = 0.05 = 0.89$.

TABLE 1. Effect of temperature on the mycelial dry weight and conidial and microsclerotial production of *Verticillium* on Czapek-Dox broth at 22 days

Temperature (C)	Dry weight (mg)[a]	Conidia per ml	Microsclerotia per flask
20	17.50 a	52,000	20–40
24	8.25 b	52,000	1–10
16	5.30 c	26,000	0
12	4.50 c	26,000	0
8	0.30 d	0	0
4	0.25 d	0	0

[a]Values in the column followed by the same letter are not significantly different according to the H.S.D. multiple comparison test; $P = 0.05$.

overwintering, and disease potential of the fungus within the tree. Silver maple has been reported to be the most susceptible species of maple (10).

Norway, sugar, and red maples, in contrast to boxelder, may produce sap that is a poor nutrient base during the dormant and bud-swell stages. The sap of these maples showed a significant increase in the ability to support fungal growth and development when extracted at the bud-break stage. The sap of red maple tended to maintain this increased level of fungal growth at the full-leaf stage. However, sugar and Norway maple sap showed a decrease in the ability to support fungal growth and development at the full-leaf stage. Kessler (8) also found that sap from red maple taken during the summer served as a poor growth medium for *Verticillium*. Field inoculation work has shown that sugar maple trees were most susceptible to *Verticillium* wilt in May and became less susceptible during early summer (2). These results coincide with the ability of sugar maple sap taken at the bud-break stage to support fungal growth and development and the subsequent reduction of fungal growth on the sap taken at the full-leaf stage.

This work demonstrates that certain host factors determine the extent of fungal growth and development in the xylem sap. Other workers state that the severity of the *Verticillium* wilt disease is related to the quantity of conidia produced in the xylem of a host (1,4,11). Their reasoning is strongly supported by the fungal growth response on boxelder sap. Boxelder may be resistant to *Verticillium* wilt in the field because its sap does not support conidial production during the favorable temperature period for fungus growth. Suppression of conidial production could retard disease progression in the host. Apparently the active growth of *Verticillium* in the host occurs only during a short period of the year, and the fungus is dormant during the rest of the year (19,22). If the organism did not spread rapidly during this favorable period, due to nutrient-deficient sap or the presence of an inhibitor in the sap, the severity of the disease could be lessened.

If these factors were combined with a lack of microsclerotial production at the favorable temperature periods for fungal growth, an important mechanism of resistance could be established. Conidia and mycelia are viable only for relatively short periods of time, but microsclerotia are very durable (5,15,21). If an organism were unable to produce microsclerotia in a host, as in boxelder at the bud-break and full-leaf stages, the survival of the organism in the host plant from season to season might be poor.

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