Water Relations in American Elm Infected with Ceratocystis ulmi

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ABSTRACT

Changes in foliar water loss and water transport pathways as related to the appearance of foliar wilt and chlorosis after inoculation of American elm trees with *Ceratocystis ulmi* conidia were studied under controlled environmental conditions. The wilt syndrome is associated with reduction of water available to the leaves. The appearance of foliar symptoms accompanied or followed a decrease in water loss. At no time did infected trees show an increase in water loss. In check trees, the transpiration rate either remained unchanged or increased gradually. Distribution of dye in infected branches revealed that the decrease in transpiration was associated with blockage of infected water-conducting tissue. Dye distribution within xylem elements was uniform in noninfected twigs, but diminished progressively toward the shoot tips in infected branches. One-year-old twigs and greenshoots were the most common areas for vascular dysfunctions associated with wilt symptoms. Leaves rarely wilted when vascular elements of current shoots were healthy, even if water-conducting elements of older twig sections were extensively blocked. The extent, degree, and type (chlorosis or wilting) of initial foliar symptoms expressed are believed to depend upon the speed and extent of fungal distribution and development and subsequent vascular dysfunction within the terminals of infected branches.

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In a healthy American elm (*Ulmus americana* L.), a balance between water intake and transpiration permits leaves to remain turgid and functional. In the Dutch elm disease, there is a reduction in leaf turgor with resultant wilt and chlorosis. These symptoms indicate an imbalance in water economy. Opinions differ, however, concerning the cause and mechanisms of the apparent water shortage. Foliar symptoms could occur if there were either (i) a reduction in water available to the leaves; or (ii) an increase in water loss from leaves (1, 7, 12).

The causal relationship between internal water stress and wilting has been investigated in other plants, including tomato (10, 11), banana (2, 3), oak (4), and maple (6). Foliar wilt and chlorosis appear to result from desiccation because the water-conducting capacity of invaded xylem elements has been reduced. However, the relationship between internal water stress and the appearance of foliar symptoms is not clear in the Dutch elm disease. The purpose of this study was to determine changes in (i) transpiration; and (ii) water transport as related to the appearance of foliar wilt and chlorosis after inoculation of American elm trees with conidia of *Ceratocystis ulmi* (Buism.) C. Moreau.

MATERIALS AND METHODS.—*Transpiration study.*—In the fall of 1968, 18 dormant American elms 3 to 5 years old were planted in 250-cm diam containers and placed in a cold room at 7 C for at least 5 months. The trees were transferred in the spring to a greenhouse. After full leaf expansion, three trees were placed in each of two controlled

growth chambers. A 15-hr photoperiod was maintained throughout each experiment. The temperature was held at either 24 or 30 ± 1 C. Illumination of 1,500 to 2,000 ft-c in the leaf zone was provided by six fluorescent and eight incandescent bulbs. The experiment was replicated 3 times.

The trees were held in the chamber for 1 week before inoculation. A composite spore suspension (35,000 spores/ml) was prepared from four isolates of *C. ulmi* cultured on potato-dextrose agar for 1 to 2 weeks. Approximately 1 ml of spore suspension was inoculated through an incision into the stem of each experimental tree. Control trees were treated with sterile distilled water. The incision was made through a short, soft piece of plastic tubing shaped to fit and closely appressed to the stem. The inoculum was added to the tube before the incision was made.

Continuous measurements of water loss were made on individual branches using the apparatus diagrammed in Fig. 1. Tygon tubing connected the various parts. The air pump (A) provided a steady, continuous flow of air through the apparatus. The water content of this air was regulated by four bubblers (B) containing sulfuric acid, specific gravity 1.455. Air passed from the moisture-regulating bubblers through an acid-overflow flask containing cotton (C) to a flowmeter (D). We maintained a regulated flow rate of 3 liters/min by adjusting a hose clamp on by-pass K. Air from the flowmeter passed through a polyethylene connector (E) into a clear plastic bag (G) enclosing a branch of the tree. The air circulated over the leaves and passed through another polyethylene connector (F). We made the bag essentially airtight at the base by tying the open end securely with string. Each bag had a capacity of 8 liters of air. Air leaving the plastic bag passed over a lithium chloride type hygrometer (H) attached to a Dynalog Dew Point Recorder (J) (Foxboro Co., Foxboro, Mass.). The water content of the air was continuously recorded as a dew point temperature which was converted to mg of water/g of dry air, and plotted against time. We recorded the water content of the air entering a plastic bag by opening hose clamp L and attaching tube M to the hygrometer ('Dewcel element"). Since the water content of the air entering and leaving the plastic bag was known and the rate of air flow over the leaves was known and constant, water loss from a branch could be calculated. A branch was enclosed by the plastic bag only when water loss was being recorded.

Transpiration measurements were begun before inoculation and continued daily through symptom development. Measurements on five inoculated and one noninoculated trees were made at the end of each 9-hr dark period. A recording began about 10 min before the lights went on and generally terminated less than 1 hr later, when the recorded dew point temperature reached a constant level. Transpiration measurements on the remaining trees were made after they had been exposed to light for stated periods. Under these circumstances, constant water loss usually was obtained in less than 15 min.

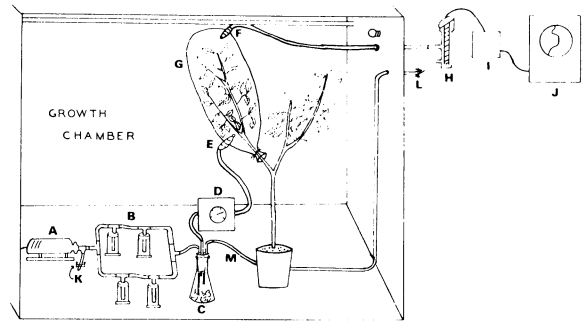Each day after inoculation, leaves were carefully

Fig. 1. Diagram of transpiration measuring apparatus: A = air pump; B = sulfuric acid bubblers; C = overflow trap; D = flowmeter; E, F = polyethylene connectors; G = plastic bag; H = humidity sensitive element; I = power unit; J = recorder; K, L = hose clamps; M = tygon tubing.

inspected for the appearance of foliar symptoms, i.e. yellowing, marginal and blotch necrosis, wilting, and abscission. Foliar symptoms were noted the day they first appeared and plotted on the transpiration graphs.

Vascular staining studies.—Ten branches from five of the trees used in the transpiration study were selected for water conduction tests. The cut ends of excised branches were inserted into the lower end of gravity-fed staining columns for identification of vascular elements capable of conducting water within infected elm twigs and foliage. A staining column consisted of a funnel attached to polyethylene tubing 100 cm in length filled with 1.0% light green SF dye solution. Immediately after twig removal from a column, free-hand transverse and longitudinal sections were cut with a sharp razor blade from leaf nodes, petioles, and midribs as well as twig nodes and internodes. The sections were then observed microscopically to obtain a pattern of dye flow through functional tracheary elements.

The distribution of dye in uninfected branches bearing artificially desiccated leaves was examined to ascertain the effect of foliar wilt per se on the transport capacity of the water-conducting elements. Several branches were excised and placed on a laboratory table for 2 days until all leaves had wilted. The branches were then attached to staining columns.

RESULTS.—*Uninfected branches.*—The rate of water loss in uninfected branches remained constant or increased gradually, both in the light and in darkness (Fig. 2-A). Following treatment with dye solution, all large springwood vessels in the current year's growth ring of these branches were uniformly and heavily stained green. When the bark was removed, exposed water-conducting elements appeared solidly stained. Mid rib and lateral veins of leaves and the three leaf traces of each petiole were deeply stained. All stem and leaf tissue appeared healthy and uninjured, i.e. there was no apparent cell degradation or necrosis. The distribution of dye in uninfected branches bearing artificially desiccated leaves was identical to dye distribution within healthy

branches. Thus, changes in dye distribution within infected branches bearing wilted leaves result from the infection process and are not merely a consequence of desiccation.

Infected branches.—Infected branches were grouped into four categories based on their patterns of water loss and foliar symptom development.

Category 1. Decrease in water loss accompanied or followed by widespread foliar symptoms.—Symptom development was most widespread in branch Q (Fig. 2-B). Water loss remained constant prior to wilting. Wilting appeared concurrently with a sudden and severe (87%) decrease in transpiration. Wilting was followed by necrosis and abscission, but little chlorosis. In branches O and P (Fig. 2-C, D), foliar symptoms appeared after 3 to 8 days of a more gradual decline in transpiration. Water loss during darkness declined only slightly (Fig. 2-D). Transpirational decreases of 60% (branch O), 74% (branch P), and 80% (in branch C, not shown) were recorded at, or prior to, the time of symptom appearance. Chlorosis was followed by necrosis and abscission, but little wilting occurred in these branches.

In branch P (Fig. 2-D), most springwood vessels in the current growth ring of 1- to 3-year-old stem sections were discolored and failed to take up dye. Dye movement was mainly confined to the current season's summerwood, where its intensity was light in shoots with symptomatic leaves. Widespread yellowing occurred in a second branch adjacent to the inoculated branch P. Dye intensity was likewise light and confined to the outer water-conducting elements of the current annual ring of this second branch. Browning was extensive at the branch node and along green shoots. In several twigs bearing symptomless leaves, however, discoloration ended abruptly at the location of the previous year's terminal bud scale scars. Vascular tissues above this point, including petioles, appeared healthy, and water-conducting elements were deeply stained green.

Category 2. Decrease in water loss followed by localized foliar symptoms.—In each branch selected for measurement in this category, only a small percentage of leaves developed disease symptoms. Wilting and yellowing appeared 4 to 11 days after the initial reduction in transpiration. Transpiration decreases of 23, 33, 47, 50, and 56% were recorded in five branches at the time of symptom appearance.

Eight leaves on one branch wilted and several others were chlorotic following a 4-day period during which the transpiration rate dropped 50%. All seven leaves on one terminal green shoot wilted. Vascular occlusion at the shoot node was apparently responsible for this wilting. Considerable dye was present in tracheary elements immediately below the node, but dye penetration ended less than 5 mm along the shoot. Apparently all vessels were plugged. There was widespread brown discoloration at the node and in vascular elements entering the symptomatic shoot. Conversely, there were no symptomatic leaves on a second terminal shoot. Although one or more leaf traces was discolored in each of several leaves, dye was always present in the

leaf midrib. At the junction of the two terminal shoots, where only a few tracheary elements contained dye, nearly all stained tracheary elements extended into the shoot with symptomless leaves.

Category 3. Decrease in water loss without foliar symptom development.—Decreases in water loss of 22, 57, and 59% were recorded for three branches that showed no symptoms. The decreases in water loss were very gradual, and began within 8 days after inoculation. In one branch, brown discoloration extended distally only 15 cm from the inoculation point. Some springwood vessels within the discolored stem section were deeply stained green. Wood exposed when the bark was removed had many green-stained water-conducting elements scattered among discolored elements. Distal to this discolored area, dye distribution was unobstructed and similar to that in noninoculated trees.

Category 4. Water loss remained unchanged, and no foliar symptoms appeared.—Although most branches on one tree developed widespread wilting, the branch previously selected for transpirational measurements showed only a slight reduction in water loss on the 9th and 10th days after inoculation. This slight reduction was followed by apparent recovery. Discoloration occurred only at the base of the selected branch, where only water-conducting elements of the current summerwood contained dye. In the terminal segment of this branch, the green dye was intense in scattered tracheary elements of the current annual ring.

Widespread wilting did develop, however, in the other branches of this tree. Two lateral branches joined the terminal branch within 3 cm of each other. A 5-cm segment was cut from just below this junction and attached to a dye column. No dye appeared at the exposed end within 15 min, indicating considerable resistance to flow, but 0.4 ml of dye passed through a 5-cm section removed from the terminal branch just above the junction during the same period. The current growth ring of the junction between the above segments was extensively discolored.

Two other branches from the same tree were placed in the staining columns after leaves on several shoots had wilted. Dye passed into the shoot nodes, but ended less than 2 mm along the shoots. All seven leaves on one shoot wilted, and dye penetration ended just below the lowermost wilted leaf.

In another tree, water loss from the branch selected for transpiration measurements again remained constant, and no foliar symptoms developed. The 1- and 2-year-old branch segments showed extensive brown streaking in the current annual ring. Only summerwood elements of the current annual ring contained dye, whereas springwood elements were discolored. The green shoots, however, were free of discoloration and were deeply stained green.

Changes in water loss curves following exposure to light.—Continuous measurements of water loss were recorded each day for five infected branches and one uninfected branch during the final 10 min of a 9-hr
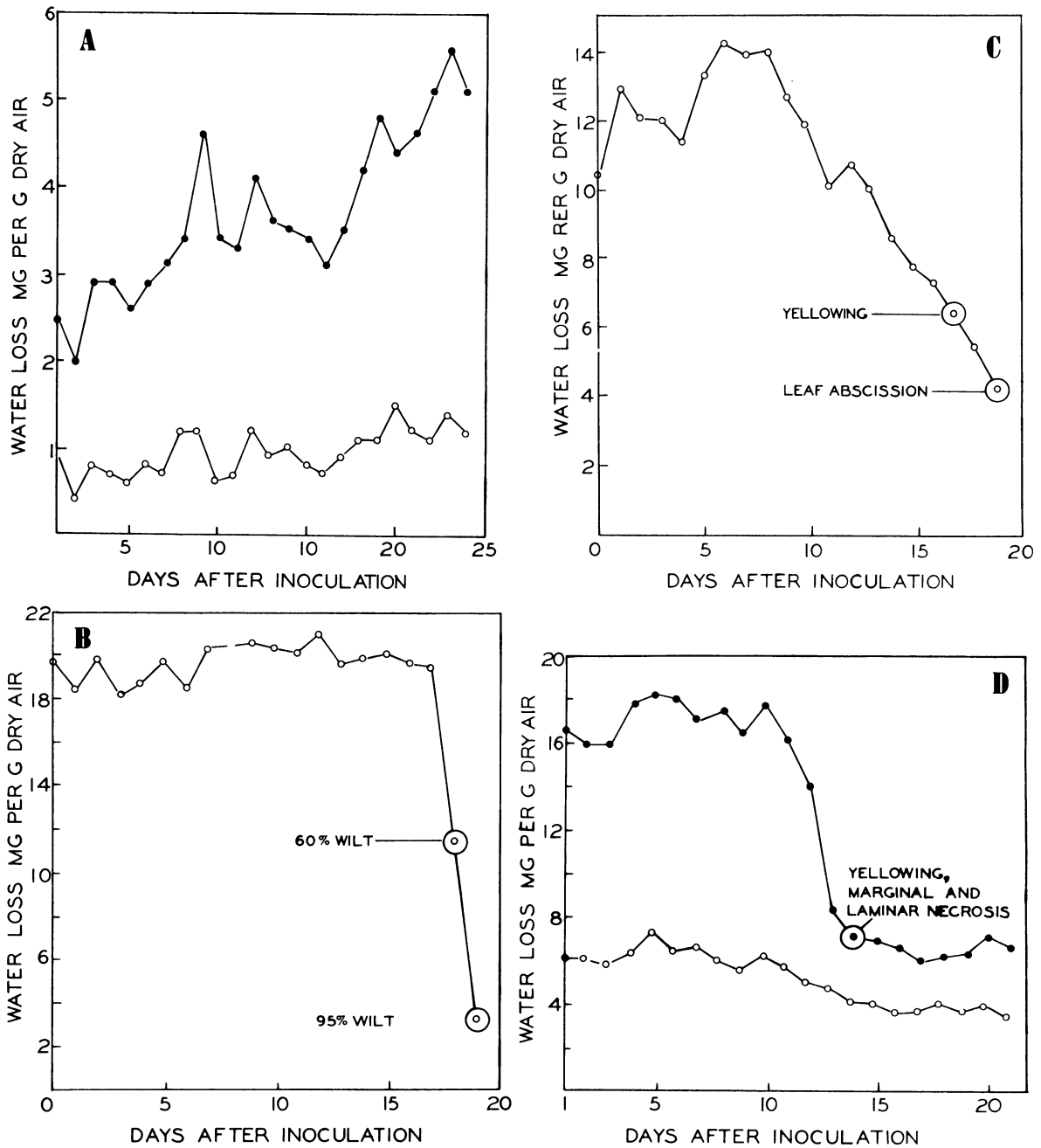
Fig. 2. Water loss from inoculated and noninoculated American elm trees. **A)** Daily water loss from noninoculated control branch during illumination (●–●) and darkness (O–O). **B,C)** Daily water loss from inoculated branches Q and O, respectively, during illumination in relation to foliar symptom appearance. **D)** Daily water loss from inoculated branch P during illumination (●–●) and darkness (O–O) in relation to foliar symptom appearance.

dark period and the first 45 min of a 15-hr light period. Time zero on the graph was used to represent water loss during darkness (Fig. 3). In the noninoculated branch, transpirational water loss increased steadily for 20-30 min after illumination, at which time it leveled off. At no time was there a fluctuation in water loss once a plateau was attained. In three infected branches which developed foliar symptoms, "normal" curves were recorded for the first 12 days after inoculation. From the 13th day through the remainder of the experiment, the curves reached a "normal" peak and then showed either an undulating pattern or a gradual decline before leveling off.

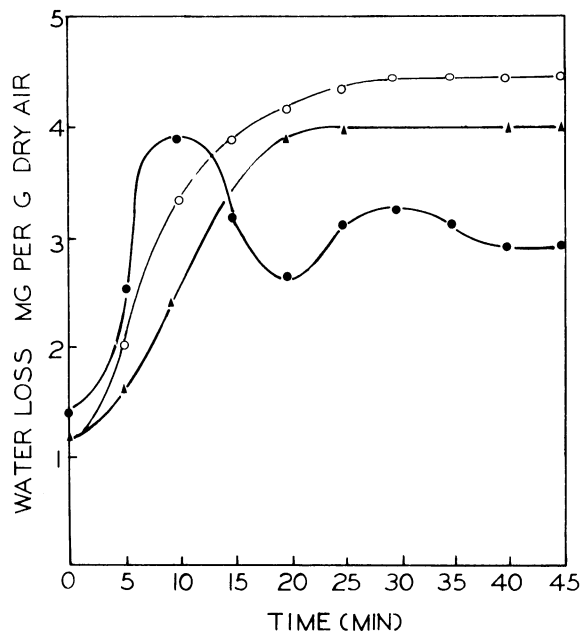*Principal sites of vascular blockage and their*

Fig. 3. The course of water loss from branches exposed to continuous light (beginning at time 0) after 9 hr of darkness. The data shown were from noninoculated control (O—O), and an inoculated branch 1 day (▲—▲) and 23 days (●—●) after inoculation.

relation to foliar symptom expression.—Dye distribution experiments were conducted to determine the critical points of vascular blockage associated with foliar symptoms. In noninoculated branches, dye always became distributed throughout stems and leaves, but in inoculated branches showing foliar symptoms, dye distribution was always interrupted. Dye was never observed within the veins of a wilted leaf. The uppermost limit of dye distribution in symptomatic branches was found once in 2-year-old internodes, 4 times in 1-year-old internodes, 14 times in current-year's shoots, and 23 times in leaf nodes or petioles. Thus, the complete vascular blockage associated with foliar symptoms occurred most often in green shoots, leaf nodes, and petioles. Foliar wilt symptoms appeared not only in the inoculated branches, but also in branches located along the main stems of infected trees, both above and below the inoculated branches. Blockage was never extensive, however, in older portions of the main stems below inoculated crotches.

DISCUSSION.—Transpirational water loss was used as a criterion to indicate the water economy within infected branches. Since transpiration has a direct effect on, and is affected by, moisture stress within a plant, it provides a good means for measuring the effect of *C. ulmi* on the water availability in infected elm twigs.

It is concluded from results presented herein that the cause of foliar wilt and chlorosis in the Dutch elm disease is a reduction in the amount of water transported to the leaves. The appearance of foliar symptoms followed or accompanied a reduction in water loss. At no time was an increase in water loss associated with symptom development in infected branches, either during dark or illuminated periods. Roberts (9) has reported an increase in transpiration of *Ulmus americana* seedlings during the 1st week after inoculation with *C. ulmi* conidia using the pot-weight method. No such increase was noted in this experiment.

In addition, the undulating pattern of transpiration in inoculated plants following a dark period was indicative of limited water availability. This pattern was similar to that of banana leaves severed (5) or infected with *Pseudomonas solanacearum* (2), and indicates the following course of events. The opening of stomate populations was initially synchronized by the exposure to light. Stomates opened normally because the water supply was plentiful after a long dark period. They soon began to close because water loss exceeded the reduced water supply. When a fair proportion of the stomates had closed, water again began to accumulate. Stomates reopened after water was gradually resupplied and guard cells regained turgor. The undulations in transpiration curves reflect the opening and closing of stomates in unison. Gradually, however, opening and closing within the stomate populations became more random until a reduced "equilibrium" curve resulted.

A comparison of dye distribution within the water-conducting tissue of uninfected and infected branches indicated that vascular dysfunction was responsible for the water deficit associated with foliar symptoms. Dye distribution was uniform in uninfected twigs, but diminished progressively towards the shoot tips in infected branches. The nodes of leaves, green shoots, and 1-year-old twigs were the sites at which the interruption in water conduction was most closely associated with foliar symptoms. These are also the sites where radioactive *C. ulmi* spores have been found to accumulate soon after inoculation (8). Hence, the findings here support Pomerleau & Mehran's hypothesis (8) that leaves and green shoots are killed following the distribution of the pathogen to young twigs and green shoots and the subsequent dysfunction of water-conducting tissue at these sites of local infection.

Expensive vascular dysfunction in the main stem and larger branches was observed, but was not a decisive factor contributing to initial foliar wilt. Leaves rarely wilted if the current shoots were free of blockage, even though water-conducting elements of older twig sections were sometimes extensively blocked. For example, in three branches, foliar symptoms did not develop and the rate of water loss remained unchanged throughout the 3 weeks of measurement after inoculation. Dye distribution patterns and discoloration indicated that transpiration rates were maintained because the summerwood vessels remained functional and the current year's shoots remained free of infection. In three additional branches, water loss gradually declined 22, 51, and 59% beginning within 8 days after inoculation. Dye

patterns and distribution data suggested that the lack of foliar symptoms was associated with host response mechanisms that limited longitudinal distribution of the pathogen. Twigs and new shoots remained free of infection, and interruption in water flow was restricted to older branch sections just above the point of inoculation.

Thus, foliar wilt and chlorosis appear to result from desiccation of leaves after the water-conducting capacity of invaded vascular elements, especially those of green shoots and petioles, has been reduced to a considerable degree. The type and degree of foliar symptom development is dependent not only upon the rate and degree, but also the precise location, of this vascular blockage. Blockage in the main stem and large branches apparently did not cause symptoms because a considerable number of interconnections provided many alternate channels of flow. Blockage of tracheary elements in twigs and green shoots was more "critical" than that in larger branches because few such alternate channels were present.

LITERATURE CITED

1. BECKMAN, C. H. 1964. Host responses to vascular infection. Annu. Rev. Phytopathol. 2:231-252.
2. BECKMAN, C. H., W. A. BRUN, & I. W. BUDDEN-HAGEN. 1962. Water relations in banana plants infected with Pseudomonas solanacearum. Phytopathology 52:1144-1148.
3. BECKMAN, C. H., S. HALMOS, & M. E. MACE. 1962. The interaction of host, pathogen, and soil temperature in relation to susceptibility to Fusarium wilt of banana. Phytopathology 52:134-140.
4. BECKMAN, C. H., J. E. KUNTZ, A. J. RIKER, & J. G. BERBEE. 1953. Host responses associated with the development of oak wilt. Phytopathology 43:448-454.
5. BRUN, W. A. 1961. Photosynthesis and transpiration of banana leaves as affected by severing the vascular system. Plant Physiol. 36:577-580.
6. CAROSELLI, N. E. 1957. Verticillium wilt of maples. Rhode Island Agr. Exp. Sta. Bull. 335:1-84.
7. DIMOND, A. E. 1967. Physiology of wilt disease, p. 100-120. In C. J. Mirocha & I. Uritani [ed.]. The dynamic role of molecular constituents in plant-parasite interaction. Nat. Sci. Foundation, Amer. Phytopathol. Soc., St. Paul, Minn.
8. POMERLEAU, R., & A. R. MEHRAN. 1966. Distribution of spores of Ceratocystis ulmi labelled with phosporus-32 in green shoots and leaves of Ulmus americana. Naturaliste Can. 93:577-582.
9. ROBERTS, B. R. 1966. Transpiration of elm seedlings as influenced by inoculation with Ceratocystis ulmi. Forest Sci. 12:44-47.
10. SAALTINK, G. J., & A. E. DIMOND. 1964. Nature of plugging material in xylem and its relation to rate of water flow in Fusarium-infected tomato plants. Phytopathology 54:1137-1140.
11. SCHEFFER, R. P., & J. C. WALKER. 1953. The physiology of Fusarium wilt of tomato. Phytopathology 43:116-125.
12. WOOD, R. K. S. 1967. Physiological plant pathology. Blackwell Sci. Publ. Ltd., London. 510 p.