Rate and Extent of Colonization of Naturally and Artificially Inoculated American Elms by Ceratocystis ulmi

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ABSTRACT

When current season shoots of *Ulmus americana* in close proximity to main branches were inoculated with *Ceratocystis ulmi*, systemic infection resulted more frequently than when similar inoculations were made in the apical portion of the tree or in the terminal portion of lateral branches. The fungus could be readily recovered from leaves which had been taken from trees previously inoculated at the base with a large number of spores. However, the frequency of recovery of the fungus was much lower from leaves taken from naturally infected trees. Symptomless leaves harvested from infected shoots from naturally infected trees rarely contained the pathogen, and hence it is doubtful if leaf insects are important agents of fungal transmission in nature. Phytopathology 61: 1456-1458.

Additional key words: Dutch elm disease.

Colonization of the living host by the Dutch elm disease pathogen, *Ceratocystis ulmi* (Buis.) C. Moreau, has not been satisfactorily described. Most previous experiments have been conducted by injecting large numbers of spores suspended in a large volume of water into the xylem at the base of the tree. Such experiments have demonstrated that the spores are rapidly distributed to the terminal branches, but the technique is entirely artificial and does not measure the rate or extent of natural colonization.

The principal agents of transmission of *C. ulmi* are scolytid beetles which feed primarily in twig crotches in the periphery of the crown. Hence, except for transmission through root grafts, natural colonization follows inoculation of a few conidia in small twigs, usually without the aid of free moisture. The rate and extent of colonization of the host by the pathogen following natural infection or by inoculations which simulate the activity of elm bark beetles differed markedly from the rate and extent of colonization when inoculations were made at the base of the tree. Instead of very rapid distribution of the fungus and rapid disease development, inoculation in young twigs resulted in limited, inconspicuous symptoms in the first growing season (2,4,5).

The rate of disease development after inoculation in small twigs has been reported to fluctuate with the season of inoculation (1,5) or the age of the twig inoculated (4). Since the downward movement of the pathogen from small twig inoculations was very slow, workers suggested (2,5) that the rapid crown decline frequently observed in June resulted from inoculations during the previous year. However, differences in disease development could also be due to the location of the inoculated twig in the tree crown and its proximity to the main upward vascular flow of sap. *Ceratocystis ulmi* was frequently recovered from the leaves of small elms following inoculation in the trunk (6,8). Workers suggested (6,8) that the presence of the fungus in the leaves could hasten tree death, and could constitute a source of inoculum for the spread of the pathogen by phyllophagous insects.

The objectives of this investigation were to determine (i) the relationship between inoculation site and disease development; and (ii) the frequency of occurrence of the pathogen in leaves after natural infection, and to compare this frequency with the presence of the pathogen after different types of artificial inoculation.

MATERIALS AND METHODS.—To determine the effect of the location of the inoculation site on subsequent disease development, inoculations were made (on 26 or 27 May in *Ulmus americana* L., 7-13 m in height) by placing a drop of spore suspension (4,000 spores/cm^3) on the bark, then thrusting a sharply pointed pin through the drop into the underlying tissue several times. Each tree was inoculated at one of four different points: (i) directly into the bole ca. 1 m above the ground; (ii) the current season's terminal growth on the highest shoot on the tree; (iii) in epicormic branches (consisting entirely of current season growth) 5-10 cm from the trunk and 1 m aboveground; or (iv) the current season growth at the terminal portion of a lateral branch located ca. 1 m aboveground. The inoculation point on the lateral branch was at least 1 m from the bole of the tree. Forty trees were inoculated in each location. Symptom development was recorded at 2-week intervals during the first summer, and at monthly intervals the second summer.

The presence of the fungus in leaves from naturally infected trees was compared with the presence of the fungus in leaves from artificially inoculated trees. Samples from naturally infected trees were collected from the top of several large (25-cm diam breast height) diseased *U. americana* at intervals throughout the spring and summer of 1967 and 1968. Samples were also collected from artifically inoculated *U. americana* trees 2.5-4.0 m in height. These were inoculated by placing a single drop of a spore suspension either in a twig axil (ca. 400 spores/tree) or on the bole (ca. 50,000 spores/tree). A needle was then pierced through the drop into the bark. Trees

May; 7, 17, and 27 June,1967; and 15 May, 5 June, and 5 July 1968. Symptomless leaves were collected with 2-30 days after inoculation from trees inoculated with 400 spores/tree. Samples from trees inoculated with 50,000 spores/tree were harvested after some of the leaves began to show symptoms (ca. 2 weeks).

The leafy shoots were divided into three symptom categories based on the conditions of the leaves: symptomless, chlorotic, or partially necrotic. Symptomless leaves were collected from branches thought to be colonized by the pathogen. Samples taken from partially necrotic leaves were collected from the living portion of the leaf. Leaves and sections of the shoot between the leaves were placed without surface sterilization on the surface of elm extract agar (3) in petri plates. Tissues were considered to contain *C. ulmi* when chracteristic synnemata were present after 14 days at 22 C.

RESULTS.—Thirty-nine of the 40 trees inoculated on the trunk developed wilt; most subsequently died. In most cases, wilt was apparent 2 weeks after inoculation. Symptoms were first noted in the topmost portion of the tree, and gradually preceeded downward. The pattern of symptom development was similar for trees inoculated through epicormic branches, except that the percentage of successful infections was lower (26 of 40 trees). In most cases (17 of 26), the epicormic branch itself did not show wilt symptoms. These results indicate that rapid colonization of the tree can occur following inoculation in young growth if the downward distance the fungus must travel to reach the main transpiration stream is limited to a few centimeters.

Inoculations into 1-year-old growth at the apex of the tree crown resulted in symptoms restricted to the tissue distal to the point of inoculation in 15 cases, in 6 cases where external symptoms developed proximally 30-60 cm but symptoms the following year were absent, and in 19 cases where the trees gradually wilted from the top down and eventually died. In this latter group of trees, external symptoms developed downward as much as 1.5 m in 6 weeks and 5 m in 8 weeks. This rate (ca. 10 cm/day) of movement is faster than the fungus itself can grow; hence, the pathogen must have been carried passively by the xylary fluids in the tree.

When inoculations were made in the terminal portion of lateral branches at least 1 m from the main trunk, symptoms were restricted to 15 cm above or below the inoculation point in 26 trees. The lateral branches of four trees died, but the infection did not move into the remainder of the tree. In 10 cases, the pathogen moved out of the lateral branch and into the main trunk, resulting in rapid decline of the entire tree.

The number of attempted isolations from leaves and shoots with each type of symptom and the percentage which contained *C. ulmi* are shown in Table 1. Colonization of trees inoculated with 50,000 spores/tree was consistently more extensive than was colonization of naturally infected trees or trees inoculated with 400 spores/tree. When the trees were naturally infected or when trees were inoculated with 400 spores/tree, symptomless leaf blades rarely contained *C. ulmi* (0.6 and 0.0%, respectively) even though the fungus was commonly present in the shoot to which the leaves were attached. Not until the leaves on naturally infected trees began to show necrosis was the fungus isolated with any degree of consistency. In all cases, the frequency of *C. ulmi* was lowest in the leaf blades and highest in the shoots. As symptoms developed, the presence of the fungus increased in all tissues. No significant differences in the frequency of positive isolations were observed for different seasons of the year.

DISCUSSION.—These results indicate that differences in the rate of symptom development in naturally infected trees can be partially explained by the location of the infection court. The shorter the distance the spores must descend to reach the main sap stream, the more rapid and severe the symptom development. Inoculations in epicormic branches, due

TABLE I. Frequency of isolation of *Ceratocystis ulmi* from leaves and shoots of *Ulmus americana* showing different degrees of symptom development

	Naturally infected			Inoculated with 50,000 spores/tree			Inoculated with 400 spores/tree		
Leaf symptoms	Blade	Petiole	Associated shoot	Blade	Petiole	Associated shoot	Blade	Petiole	Associated shoot
Symptomless									
No. positive[a]	2	16	54	61	87	170	0	4	12
No. tested	356	356	167	759	759	330	242	242	121
Chlorotic									
No. positive[a]	1	9	27	16	24	53			
No. tested	111	111	76	140	140	81			
Partially necrotic									
No. positive[a]	9	29	96	69	107	182			
No. tested	184	184	186	390	390	251			

[a] Number of sections from which *C. ulmi* was isolated.

to their proximity to the trunk or major branches, may therefore be responsible for the rapid decline observed in some trees. Inoculations in the periphery of the crown resulted in infection of fewer trees and in a slower rate of symptom development in those that did become diseased because the spores must descend many feet before they reach the main sap stream. Relatively slow downward movement of xylary fluids probably accounts for the movement of the pathogen from inoculation points in the periphery of the crown to the remainder of the tree.

These data also show, as do previously published data (2,5), that the pathogen is often restricted to the small branch originally inoculated. These data do not necessarily support the hypothesis (2,5) that main branch or stem infections apparent in June result from inoculations of the previous year, as symptoms developed rapidly in trees inoculated through epicormic branches. Systemic infections may instead result from recent beetle inoculations in succulent growth adjacent to the trunk or other large branches. Even though inoculations in epicormic branches would comprise only a small percentage of the total inoculations, their proximity to the main upward vascular flow greatly increases the chance of a systemic infection.

The recovery of *C. ulmi* from leaves on trees inoculated in the trunk with a large number of spores was similar to previous reports (6,7), but this observation could not be substantiated when leaves were cultured from naturally infected trees or from trees artificially inoculated with small numbers of spores. Only when the leaves began to show areas of necrosis was the fungus isolated consistently and even then it was present in only 5% of the leaf blades. In view of these results, it is doubtful that leaf insects play a significant role in the transmission of the pathogen under natural conditions. As most leaves became necrotic before they were invaded by the pathogen, it is also unlikely that leaf death is associated with the presence of the pathogen in the leaf itself.

LITERATURE CITED

1. BROWN, M. F., R. J. CAMPANA, & M. A. ROSINSKI. 1963. Downward movement of conidia of Ceratocystis ulmi from terminal twigs of elm. Phytopathology 53:871 (Abstr.).
2. CAMPANA, R. J., & H. G. BROTZMAN. 1967. Restricted development of Dutch elm disease in small elm stems. Phytopathology 57:338-339 (Abstr.).
3. HART, J. H. 1960. A modified method for culturing elm samples. Plant Dis. Reptr. 44:806-807.
4. NEELY, D. 1968. Twig inoculations on American elm with Ceratocystis ulmi. Phytopathology 58:1566-1570.
5. NEELY, D. 1970. Dutch elm disease symptom progression. Plant Dis. Reptr. 54:127-129.
6. POMERLEAU, R. 1968. Progression et localisation de l'infection par le Ceratocystis ulmi dans l'orme d'Amerique. Phytopathol. Z. 63:302-327.
7. POMERLEAU, R. 1970. Pathological anatomy of the Dutch elm disease. Distribution and development of Ceratocystis ulmi in elm tissues. Can. J. Bot. 48:2043-2057.
8. POMERLEAU, R., & A. R. MEHRAN. 1966. Distribution of spores of Ceratocystis ulmi labelled with phosphorus-32 in green shoots and leaves of Ulmus americana. Nature Can. 93:577-582.