Phloem Necrosis of Elms: Symptoms and Histopathological Observations in Tolerant Hosts

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ABSTRACT

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The agent of phloem necrosis (PN), a lethal disease of elms indigenous to North America, was transmitted among five elm species by grafting. Infected *Ulmus americana* and *U. rubra* died, *U. laevis* showed chlorosis and epinasty, and *U. carpinifolia* and *U. parvifolia* produced witches brooms. The PN agent was transmitted by dodder (*Cuscuta epithymum*) from *U. americana* and *U. parvifolia* to periwinkle (*Catharanthus roseus*), which became broomed, stunted, and chlorotic. Mycoplasmalike organisms (MLO), not found in control plants, occurred in phloem sieve tubes of symptomatic elms and periwinkle. MLO were more easily found in tolerant species than in those killed by PN and were most conspicuous in witches brooms. In a test of the hypothesis that PN-resistant roots could protect susceptible scions, rootstocks of *U. pumila* did not prevent death of graft-inoculated *U. rubra*. In histological comparisons of healthy and infected *U. parvifolia*, secondary phloem of infected main stems appeared normal except for MLO. In witches brooms, however, the midribs of leaves contained a thicker band of secondary phloem than was seen in leaves from healthy trees. Collapsed sieve elements, scattered throughout this phloem, were more numerous in infected than in healthy samples.

Phloem necrosis (PN) of elms is known to occur only in the United States. Swingle (21,22) described the disease and established a transmissible agent as the cause. In 1972, Wilson and co-workers (23) reported mycoplasmalike organisms (MLO) in diseased phloem. The white-banded elm leafhopper, *Scaphoideus luteolus*, is the only confirmed vector (1), but there may be others (5).

Of the six elm species native to the United States, PN is lethal in five: *Ulmus americana, U. rubra, U. alata, U. serotina,* and *U. crassifolia* (19). The susceptibility of the sixth species, *U. thomasii*, is unknown.

Symptoms in native species include epinasty, yellowing, and premature casting of leaves; hyperplasia, yellow to brown discoloration, and necrosis of secondary phloem in stems and roots; and death, usually within a year after foliar symptoms appear (12,22). *U. rubra* trees (red or slippery elms) often show symptoms in two growing seasons before dying; many develop witches broom in the second season (19). Graft transmission of the PN agent to two exotic elms, *U. carpinifolia* and *U.* × *hollandica*, was reported in 1976 (18).

Objectives of our study were to identify additional plant hosts of the PN agent and to describe symptoms in the new hosts.

MATERIALS AND METHODS

Host range. Host range studies were done during 1972–1977 with the plants listed in Table 1. Bark patches (21) and approach grafts (6) were used for transmission attempts among elms. Control plants were grafted with material from healthy elms. Seven species of dodder were used in attempts to transmit the PN agent from elms to and among herbaceous plants. Healthy dodder was started from seed and maintained on healthy periwinkle. (*Catharanthus roseus*).

Most *U. americana* and all *U. carpinifolia* and *U. laevis* trees were in a plantation at Freeville, NY; PN did not occur naturally in the vicinity. The trees had been started from cuttings and were 6–10 yr old. All other plants except *U. pumila* × *U. rubra* hybrids were grown from seed in a greenhouse for up to 4 yrs. Composite trees of *U. rubra* on *U. pumila* rootstocks were kindly supplied by D. Hindal and H. S. McNabb, Jr., of Iowa State University.

The hybrid elms, *U. pumila* × *U. rubra*, found at Ithaca, NY, were identified on the basis of leaf, bud, and bark characters intermediate between those of the parent species. *U. pumila* and *U. rubra* are known to hybridize readily (10). Seedlings of *U. glabra* × *U. rubra*, from seed collected from open-pollinated *U. rubra*, were identified on the basis of leaf shape. Seedlings with three-pointed leaves, as often found on *U. glabra*, were considered to be hybrids.

Microscopy. Most samples were fixed in 3% glutaraldehyde in 0.08 M phosphate buffer (pH 7.0) for 4–5 hr at 4 C. Samples were postfixed in 2% osmium tetroxide in the same buffer for 12 hr at 4 C, dehydrated in an acetone series, and embedded in Epon-Araldite (14). Thin sections for electron microscopy were stained with uranyl acetate and lead citrate. Thick sections (1–5 μm) for light microscopy were stained with 0.05% toluidine blue in distilled water and observed with bright field or interference contrast optics. Additional samples were processed as previously described (4).

RESULTS

Host range. In both field and greenhouse trials, yellows-type symptoms developed only in inoculated plants, never in controls.

To determine the latent period after graft inoculation and to establish a standard with which the rate of transmission of the PN agent to other species might be compared, 25 plantation-grown *U. americana* were grafted on 29 June 1973 with bark patches from diseased *U. americana*. On 20 August 1973, before foliar symptoms developed, 13 inoculated trees were diagnosed as having PN on the basis of phloem discoloration accompanied, in most cases, by wintergreen odor. By 4 July 1974, 11 of the remaining trees had developed PN. During 1974, none of the infected trees showed discoloration of current-season phloem until late June or early July.

Twelve *U. carpinifolia* and two *U. laevis* were graft-inoculated three times during 1973. The inoculum was obtained twice from *U. americana* and once from *U. rubra*. Most bark patches from *U. americana* were rejected, but most of those from *U. rubra* were retained alive during the following year. When transmission occurred, therefore, *U. rubra* was considered to be the source species (Table 1).

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In July 1974, six of the inoculated *U. carpinifolia* (including the cultivar 'Christine Buisman' and two unnamed clones) began developing witches brooms, consisting of branches with abnormally short internodes, stunted foliage, and proliferated axillary shoots. Foliage on these brooming elms remained normal in color, and phloem discoloration did not develop. Electron microscopic examination revealed MLO in phloem of the brooms (Fig. 3). Many symptomatic branches failed to become dormant and died during the winter. In 1975, the previously broomed trees developed additional brooms, but progressively fewer brooms formed in 1976 and 1977. The two *U. laevis* trees had stunted foliage with epinasty and yellowing in 1974 and 1975 but showed no foliar symptoms in 1976 or 1977. Phloem discoloration did not occur. Growth suppression, dramatic in comparison with uninoculated controls, persisted through 1977 in all trees that broomed or showed foliar symptoms in 1974.

No symptoms developed in four *U. carpinifolia* 'Belgica' that were graft-inoculated in 1973 or in any of five uninoculated European elms.

Bark patches from broomed *U. carpinifolia* 'Christine Buisman' were used in 1974 to inoculate two *U. americana* and two *U. parvifolia* seedlings in the greenhouse. Typical PN developed in one *U. americana*. Four months after inoculation, both *U. parvifolia* were developing witches brooms (Fig. 1). After a further 3 mo in cold storage and return to greenhouse conditions, both plants were soon brooming systemically. Leaves on the broomed shoots were stunted but green. Phloem discoloration did not develop in *U. parvifolia*.

Attempts to transmit PN to seedlings of *U. glabra*, *U. pumila*, and *U. glabra* × *U. rubra* failed.

Two scions of *U. rubra* on *U. pumila* rootstocks were grafted with bark patches from infected *U. rubra*, and two were grafted with patches from infected *U. carpinifolia*. With each source of inoculum, both *U. rubra* scions developed typical PN and died. Extensive root necrosis was apparent at the time of scion death and *U. pumila* foliage on the rootstocks was chlorotic.

Naturally infected hybrids (*U. rubra* × *U. pumila*) were observed during two seasons. Symptomatic hybrids that most resembled *U. pumila* showed only localized brooming and chlorosis only on broomed branches. The most severely affected plants were those with the strongest expression of *U. rubra* characters. Such trees showed foliar and phloem symptoms similar to those of typical PN in *U. rubra*. MLO also were found in phloem of leaves from brooms on the hybrids.

The PN agent was transmitted via dodder (*Cuscuta epithymum*) from seedlings of two elm species, *U. americana* and *U. parvifolia*, to periwinkle. This was achieved by training healthy *C. epithymum* on healthy periwinkle, then moving an infected elm seedling into the dodder culture. The agent was then readily transmitted among periwinkle plants by *C. epithymum*, *C. ceanothi*, and splice-grafting. Infected periwinkle showed yellows-type symptoms consisting of stunted chlorotic foliage, stunted flowers, and proliferated axillary shoots (Fig. 2). When a broomed periwinkle was cut back, several healthy looking shoots usually were produced. Within 1–2 mo, such shoots developed vein clearing followed by chlorosis from base to tip. Proliferation of axillary shoots was concurrent with or began just after foliar yellowing. Yellow leaves eventually developed black, necrotic areas and then abscised. In areas that became necrotic, the upper epidermis and palisade parenchyma collapsed first; the mesophyll and lower epidermis remained intact until abscission. Among several thousand periwinkle plants grown in the greenhouse for this work during a 5-yr period, no cases of fortuitous, yellows-type disease occurred.

Attempts to transmit MLO from periwinkle back to elms or to other plants failed. Of seven species of dodder tested, only two (*C.*

TABLE 1. Transmission trials and symptoms caused by the agent of elm phloem necrosis (PN)

Species[a] (no.)	Botanical and common names; materials used	Transmission achieved: to species	Transmission achieved: by method[b]	Successful graft transmission/total attempts from species: 1	2	3	Symptoms
1	*Ulmus americana* L., American elm; seedlings, rooted cuttings	1 10	A,B D	24/25	11/26	1/2	Chlorosis, defoliation, death
2	*U. rubra* Muhl., red or slippery elm; seedlings, grafts on *U. pumila*	1,2,3,4	B		4/4	2/2	Chlorosis, brooming, defoliation, death
3	*U. carpinifolia* Gleditsch (including *U.* ×*hollandica*), European field elm, smoothleaf elm; rooted cuttings	1,2,6	B		6/12		Brooming, stunting
4	*U. laevis* Pall. European white elm; rooted cuttings				2/6		Chlorosis, epinasty, stunting
5	*U. glabra* Huds., Scots or wych elm; seedlings			0/5			
6	*U. parvifolia* Jacq., Chinese elm; seedlings	10	D	0/1		2/2	Brooming, stunting
7	*U. pumila* L., Siberian elm; seedlings			0/5		0/7	
				also 0/12 from species 6			
8	*U. glabra* × *U. rubra*; seedlings				0/5		
9	*U. pumila* × *U. rubra*; seedlings				Natural infection		Chlorosis, brooming
10	*Catharanthus roseus* D. Don (= *Vinca rosea*), periwinkle; seedlings, rooted cuttings	10	D,S				Brooming, stunting, chlorosis, suppression of bloom

[a] Additional species graft-inoculated with negative results were *Celtis laevigata* Willd. and *Zelkova serrata* (Thunb.) Mak. Species that did not develop yellows-type symptoms after exposure to dodder in cultures where transmission to periwinkle occurred were: *Chrysanthemum* × *morifolium* Ramat., *Daucus carota* L., *Lycopersicon lycopersicum* (L.) Karst ex Farw., and *Vitis labrusca* L. 'Concord.'

[b] A = approach graft, B = bark patch graft, D = dodder, S = splice graft. Dodders that transmitted the PN agent were *Cuscuta epithymum* Murray and *C. ceanothi* Behr.

epithymum and *C. ceanothi*) appeared to parasitize seedlings of American elm, and these did so only briefly. The dodder was apparently unable to maintain contact with the succulent portions of growing elm stems. Dodder cultures suitable for transmission trials could not be maintained on elms alone; *C. epithymum*, when trained on infected periwinkle, grew feebly and did not parasitize healthy elm seedlings.

Populations of MLO as related to symptoms. In all hosts examined, MLO were found only within mature sieve tube elements (Fig. 3). MLO were never observed in the control plants in transmission experiments. In sections of phloem from naturally infected and apparently healthy field-grown trees, MLO were seen only in the former. The ultrastructure of the PN agent has been described (3,4,23).

MLO were conspicuous in hosts that produced brooms and remained alive: *U. carpinifolia, U. parvifolia*, and *C. roseus*. In the latter two species, MLO could be found in almost every section of phloem from any organ, and some sieve elements seemed almost totally occluded by them. Also, some sieve elements in these hosts contained MLO considered to be senescent according to Maniloff's criteria (11).

In red elm, which usually dies in the second year of symptom expression, MLO were found sporadically throughout the plant and were most numerous in witches brooms.

In American elm, which usually dies within one year after inoculation, relatively few MLO were found. These were erratically distributed in the secondary phloem of trunks and branches (4) and also of roots. Feeder roots from two large infected trees and primary roots from several infected seedlings were sampled; a few MLO were found in each root type. Midribs of leaves from 10 infected trees were examined, and MLO were not seen.

Histopathology of *U. parvifolia*. Since *U. parvifolia* differed radically from *U. americana* in symptom development and MLO populations, a detailed cytological examination was made of one healthy and two diseased *U. parvifolia* seedlings. The secondary phloem in the stem was not obviously altered in the infected plants. Although MLO were randomly distributed within the sieve tubes in this tissue, callose deposition was no greater than that in the healthy plant. Some of the first-formed sieve tubes in both the healthy and diseased plants were collapsed. Cambial activity in the diseased plants seemed normal.

Observations were made on five healthy midribs and five midribs from stunted foliage. Excessive phloem was found in midribs from stunted foliage. Viewed in cross section, the band of abaxial phloem in the midribs of stunted leaves averaged about 45 μm wide, compared with 25 μm in the healthy leaves (Figs. 4 and 5). Collapsed sieve elements were scattered throughout the phloem in diseased midribs, but in healthy midribs only a few of the first-formed sieve elements were collapsed. Sieve element size and frequency in the healthy and symptomatic tissues were similar. Sieve tube diameter was 3.75 μm (mean of 15 measurements) in the healthy midribs, compared with 3.85 μm in the diseased tissue. Of phloem cells in the healthy midribs, 25.9% were sieve elements, compared with 26.5% in midribs from the diseased trees.

Heavy callose deposition over sieve plates, lateral sieve areas, and plasmodesmata connecting sieve elements with companion cells was usually found in *U. parvifolia* sieve tubes that contained large numbers of MLO. MLO with swollen appearance and very electron-lucent cytoplasm often completely filled the lumina of such sieve elements. Pathogen populations of this type were considered to be senescent (3,11).

DISCUSSION

Graft compatibility was a major obstacle in the host range study. Swingle (21) found that PN was transmitted only when the inoculum patch formed a union with the recipient tree. In our study, intraspecific grafts posed no problem, but interspecific grafts, particularly those involving bark patches from *U. americana*, were often unsuccessful. Because the PN agent in the secondary phloem is confined to mature sieve tube elements (4), a functional union of the phloem probably is necessary for successful inoculation. Such a union need not be maintained long, however. McLean (13) reported that the PN agent moved out of donor patches within eight days. Hildebrand (7) found that the agent of X-disease moved out of patches in about seven days. Perhaps this process takes longer when interspecific grafts are involved.

Fig. 1. Seedlings of *Ulmus parvifolia*, infected with the agent of elm phloem necrosis (right), and healthy. **2)** Periwinkle (*Catharanthus roseus*) infected with the phloem necrosis agent. The pathogen was transmitted from an infected American elm seedling via dodder (*Cuscuta epithymum*). The healthy-looking branch on the left has not yet been colonized by the pathogen.

Reaction to the PN agent varied greatly among species of elm. As previously noted (4,19,22), *U. americana* showed extensive feeder root necrosis, discoloration and collapse of the secondary phloem of large roots and the lower stem, and epinasty and yellowing of foliage throughout the crown. Infected *U. americana* generally died within 1 yr after symptoms appeared. Symptoms in *U. rubra*, also described previously (19), were similar to those in *U. americana*, except that infected slippery elms usually remained alive for two seasons after the initial appearance of symptoms, and witches broom often developed during the final season. *U. laevis*, a European species closely related to *U. americana* (15), also displayed chlorosis and epinasty during the first 2 yr after graft inoculation but only suppressed growth thereafter. Another European species, *U. carpinifolia*, developed witches brooms and also displayed continuing growth suppression. The Asiatic species, *U. parvifolia*, broomed systemically through 4 yr of observation. Phloem discoloration was not found in any of the European or Asiatic species.

Elms apparently vary in ability to support populations of the PN agent. In *U. americana*, relatively few MLO are seen (4), but in species that remain alive and form witches broom, conspicuous MLO populations develop. Such differences suggest that conditions associated with degeneration of phloem mitigate against buildup of MLO populations, as Schneider (17) noted in *Pyrus*.

None of the four *U. carpinifolia* 'Belgica' that were graft inoculated in 1973 developed symptoms. Although this cultivar may possibly be resistant to PN, it is equally likely that these results were due to graft incompatibility.

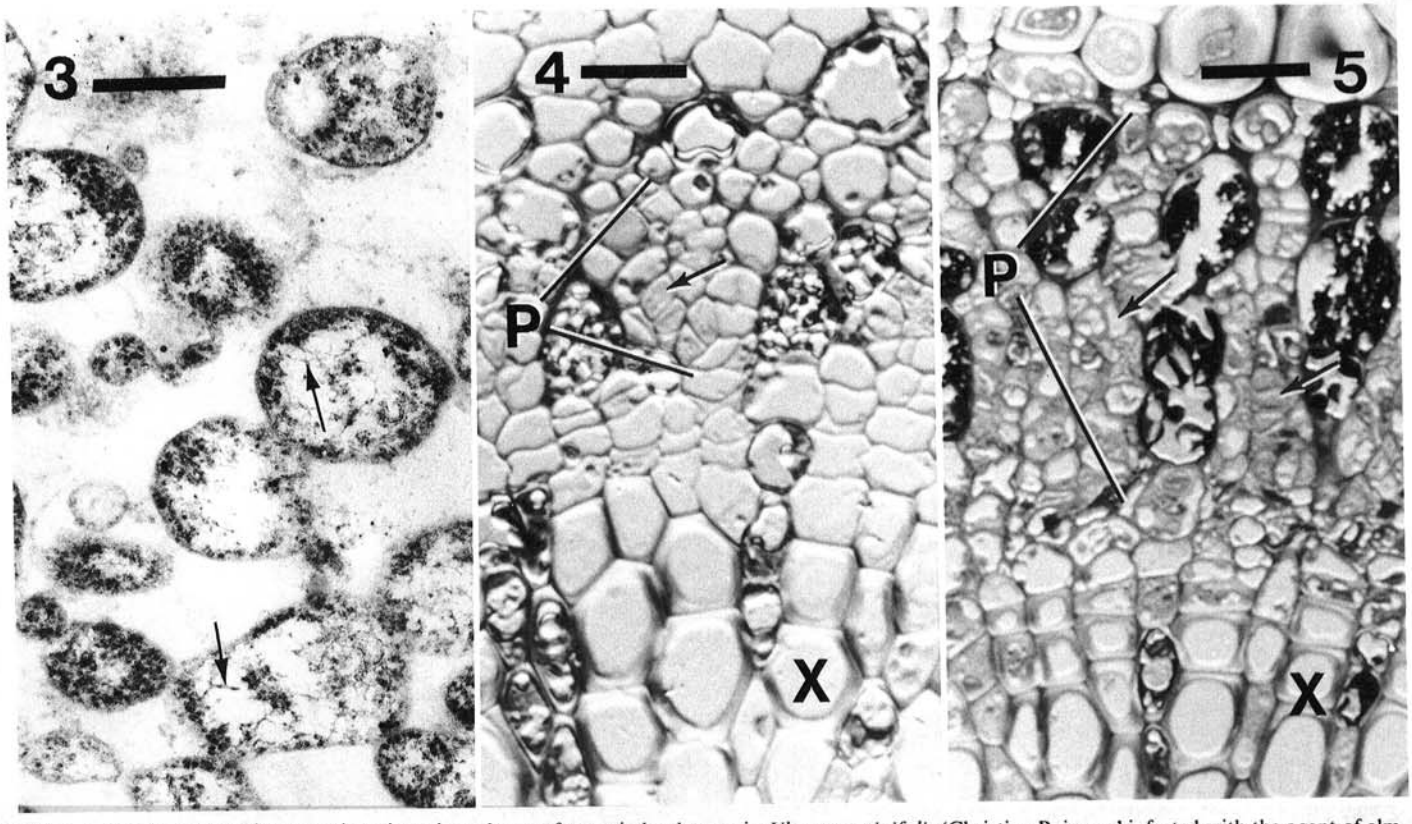
Because symptoms of PN in *U. americana* often intensify first in the roots (12,13), McLean (13) suggested that grafting on resistant rootstocks might protect PN-susceptible scions. Our results indicate that this is not a viable method of controlling PN. Whether the symptoms that developed on the *U. pumila* rootstocks were directly attributable to MLO infection was not determined, but the Siberian elm rootstocks did not protect the *U. rubra* scions from lethal PN.

Whether natural transmission of PN to any of the European or Asiatic elms can occur is unknown, but naturally infected hybrids of *U. pumila* × *U. rubra* were found. Because these hybrids tolerated infection for at least 3 yr, they may provide a perennial reservoir of the PN agent.

Despite much effort, we were not able to transmit the PN agent from periwinkle back to elm. Hildebrand (7) encountered the same difficulty with the agent of X-disease of peach. Likewise, Kunkel (9) was able to transmit the X-disease agent from peach to tomato, carrot, parsley, and periwinkle but failed to get reciprocal transmission.

MLO have been reported in phloem parenchyma cells and companion cells as well as within sieve elements (16). Beakbane et al (2) claimed to have found mycoplasmas tranversing plasmodesmatalike pores (40-nm diameter) in sieve tube walls, but their observations were not extensively documented. Hirumi and Maramorosch (8) suggested that parenchyma cells may be directly inoculated by insect vectors but that MLO do not move through plasmodesmata into adjacent cells. This hypothesis has not been critically tested, but it would explain why the PN agent was found only within mature sieve tubes of graft-inoculated elms and graft- or dodder-inoculated periwinkle.

Histopathological changes characteristic of PN in *U. americana* and *U. rubra* were not observed in the stems of brooming *U. parvifolia* seedlings. Secondary phloem in stems of diseased *U. americana* (4,12) and *U. rubra* shows excessive callose deposition followed by sieve tube collapse and hyperactivity of the vascular cambium. The hyperactive cambium produces abundant replacement phloem (16), which contains a greater percentage of sieve elements than does healthy phloem. Sieve elements produced by the hyperactive cambium are smaller than normal and soon become necrotic. In stems of PN-diseased *U. parvifolia*, secondary phloem appeared normal. Replacement phloem was found only in the midribs of stunted leaves. In this tissue, however, frequency and



Figs. 3–5. 3) Mycoplasmalike organisms in a sieve element from witches broom in *Ulmus carpinifolia* 'Christine Buisman' infected with the agent of elm phloem necrosis. Ribosomes and DNA fibrils (arrows) can be seen within the limiting cell membrane. Scale bar represents 0.5 μm (×31,000). Cross sections of typical midribs from **4)** healthy and **5)** PN-infected seedlings of *Ulmus parvifolia*. The band of phloem in the infected midrib is nearly twice as wide as in the healthy midrib although individual cells in infected and healthy tissues are the same dimensions. X = xylem, P = phloem, arrows = sieve elements. Scale bar represents 10 μm (×1,200). (Photomicrographs, interference contrast).

size of sieve elements were similar to those in midribs of healthy *U. parvifolia*. Only the amount of phloem was abnormal. Similar tissue is formed in major lateral leaf veins of some *Pyrus* cultivars affected by pear decline (17,20).

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