Role of the Mycoplasmal Disease, Ash Yellows, in Decline of White Ash in New York State

J. A. Matteoni and W. A. Sinclair

Department of Plant Pathology, Cornell University, Ithaca, NY 14853. Senior author presently research scientist, Agriculture Canada, Research Station, Vineland Station, Ontario L0R 2E0.

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ABSTRACT

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A disease caused in *Fraxinus* spp. by mycoplasmalike organisms (MLO) and called ash yellows is described from observations of naturally infected and graft-inoculated white ash (*F. americana*). MLO in phloem sieve tubes were detected by electron microscopy or Dienes' stain. Witches'-brooms and deliquescent branching were diagnostic symptoms of MLO infection. Other symptoms included reduced apical and radial growth, early onset of apical growth in spring, elevated diffusive resistance of leaves, formation of abortive sprouts, dieback, and death. Trees with yellows were abnormally susceptible to cambial injury by freezing, which caused bark cracks and cankers at trunk bases. Witches'-brooms and deliquescent branching were present throughout the region where ash decline is conspicuous. In observation plots, 57% of white ash were found to be infected by MLO. Death rates of infected and apparently noninfected trees during 17 mo were 10 and 1.9%, respectively. The year of onset of decline, indicated by reduced annual growth, varied among trees on the same site, and the frequency distribution of trees according to year of onset varied among sites. *F. pennsylvanica* was also found infected and declining.

Decline of white ash (*Fraxinus americana* L.), known since the 1920s, became an important cause of ash mortality in the northeastern United States during the 1950s (2) and has persisted without adequate explanation. A decline syndrome named "ash dieback" (2,10,17) has been considered to be a discrete disease caused by multiple factors. In this paper, decline refers to gradual failure of health, and dieback refers to abnormal mortality of twigs, branches, and trunks. To minimize confusion in references to previous work, we enclose the name "ash dieback" in quotation marks.

Symptoms of "ash dieback" include reduced radial and apical growth, subnormal leaf size, chlorosis, and premature autumn coloration. Leaves appear sparse or tufted at the tips of slowly growing shoots. Smooth bark often becomes pinkish gray in contrast to the normal gray of large branches and greenish gray of small ones. Cankers are common on trunks and branches. Eventually a progressive dieback of twigs and branches begins, and epicormic sprouts or occasionally witches'-brooms may form along the trunk. Trees of all sizes and ages are affected and commonly die 2–10 yr after the onset of symptoms (10,17). Some trees persist longer, but recovery is rare (10).

Factors associated with "ash dieback" include water deficit (17,24), root injury caused by low temperature (15), viruses (7–9,12), fungi that cause cankers (16,19), and mycoplasmalike organisms (MLO) in witches'-brooms (11,18). From experience with elm yellows (3,21), we suspected that infection of ash by MLO is more frequent than formation of witches'-brooms, and that additional diagnostic symptoms of MLO infection could be identified. Moreover, informal observations of the random onset of decline in young ash trees indicated the liklihood of an infectious causal agent.

Our objectives in this study were: to identify symptoms of MLO infection in white ash, to describe the syndrome caused by mycoplasmal infection (= ash yellows), and to assess the onset and incidence of decline in relation to the incidence of yellows.

MATERIALS AND METHODS

Observation plots. In May–June 1981, plots for observation of 2,741 white ash were established at seven locations in central and

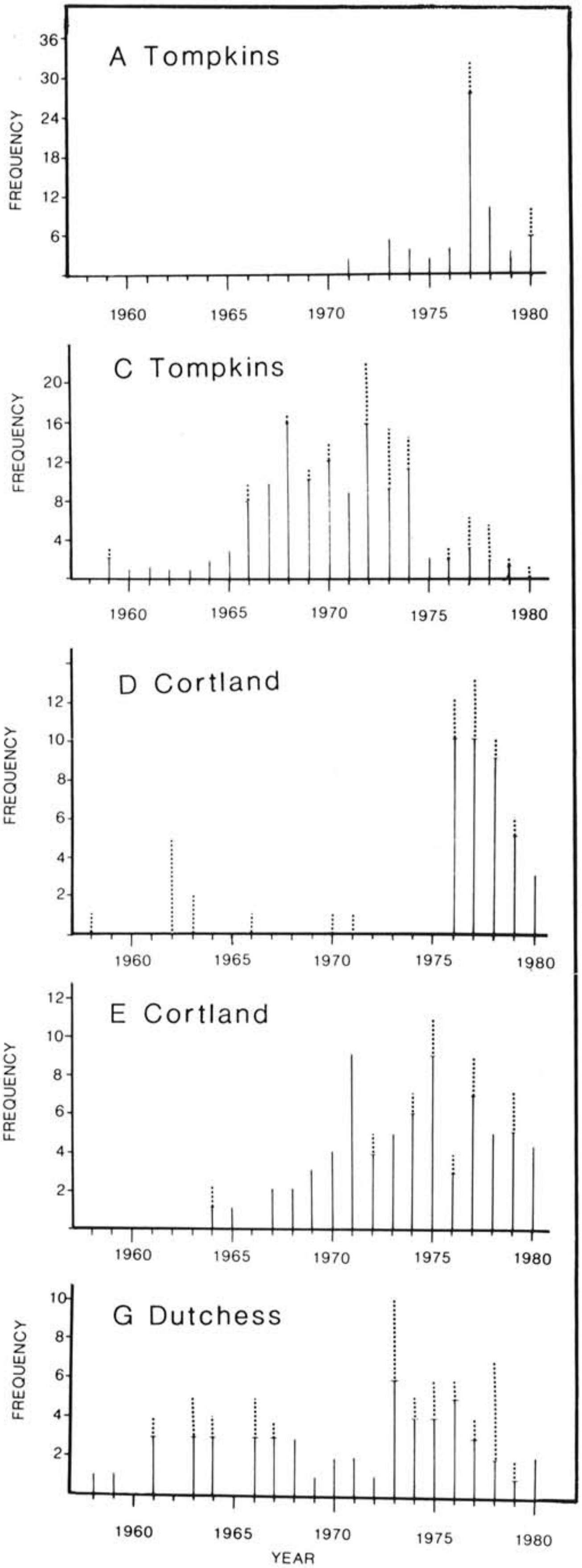

Fig. 1. Dates and frequencies of sudden, permanent reduction in radial growth of white ash trees at five locations during 22 yr. Solid bar indicates reduction to less than 50% of previous rate; dashed bar indicates smaller reduction. Locations are identified by county. Sample sizes on plots A, C, D, E, and G were 73, 157, 77, 80, and 70 trees, respectively.

southeastern New York State including three sites of previous research on "ash dieback" and ash yellows (11,17,20) and three sites of concurrent research on ash viruses (1,4,9). Four natural old-field stands, one hedgerow, one white ash plantation, and one white ash understory within a failing pine plantation were represented. Areas of plots ranged from 0.1 to 0.4 ha, and the number of white ash $\geqslant 3$ cm in diameter at 1.4 m above ground varied from 126 to 962. All such trees in five of the plots were tallied in three vigor classes on the basis of the growth of topmost twigs during approximately 1976–1980: vigorous, slowly growing, and dying back. Vigorous trees grew at least 20 cm/yr as estimated by observations from the ground. Trees classed as slowly growing (<20 cm annual twig growth) were equivalent to those classed by Ross (17) as in early stages of "ash dieback." Trees classed as dying back were growing slowly as above and bore more dead twigs or branches than could be explained by normal attrition of low branches. In two plots (B and D), observations were confined to approximately 60 trees per vigor class.

For each tree, the following symptoms were recorded if present: witches'-brooms as described by Hibben and Wolanski (11); deliquescent branching (lack of apical dominance; abnormally short twigs, all of similar length) of one or more limbs or of epicormic sprouts; early onset of apical growth in spring, determined by observations at a time in May when some trees were growing but buds of others had not opened; premature autumn coloration (yellowish to purplish leaves in late August to early September); and vertical cracks in bark and/or tangential separations of bark and wood at trunk bases indicating damage by freezing. Associations between MLO infection and various symptoms were each tested by contingency table (22).

A transverse cut was made part way through the butt of up to 60 trees per vigor class in each of six plots for a total of 774 trees. Equal numbers of trees with and without witches'-brooms were wounded where possible. The basal wound was intended to induce epicormic sprouts that would be tested for presence of MLO. Hand saws were soaked at least 3 min in 2% NaOCl between cuts. Trees were observed for two growing seasons after wounding.

Wounded trees in five plots (699 trees total) were each cut again close to the first wound and at an angle so as to produce a sector of wood with one face nearly perpendicular to the grain. Radial growth rate and, for declining trees, the year of onset of decline were determined from measurements of growth rings along one arbitrary radius in the sector from each tree. Declining trees were identified as those exhibiting dieback or chronic slow growth that began with a sudden reduction in annual ring width to less than half the width of rings formed in previous years. The year of onset of decline was defined as the year in which ring width decreased to less than 50% of the average width of the previous two rings. Lesser permanent growth reductions were also noted and recorded.

Rates of radial growth in 1979 and 1980 were converted to relative values. For declining trees, total width of the 1979 and 1980 rings was expressed as a percentage of total width of the last two rings formed before the onset of slow growth. For trees that displayed no sudden decrease in growth within the ring record of the wood sector (5–20 yr for most sectors), or that displayed a sudden decrease of less than 50%, the total width of 1979 and 1980 rings was expressed as a percentage of the total width of the widest two consecutive rings along the chosen radius.

Mortality in the five plots in which all white ash were tallied was calculated as the percentage dead in relation to the total number.

In 1984, 1,489 trees previously marked in five plots were reexamined to detect changes in symptoms since 1982 and to evaluate the validity of deliquescent branching as a diagnostic criterion for ash yellows. If deliquescent branching was a valid criterion, trees that showed this symptom in 1982 should have been more likely than others to form witches'-brooms or abortive sprouts during 1983 or 1984. Sprout abortion had been identified as a symptom of yellows during 1981–1982. Symptoms were tallied as above, and changes between 1982 and 1984 were summarized.

To supplement to plot observations, we made an informal survey of the incidence of ash yellows in relation to ash decline along highways in New York State and adjacent areas. Where dead and dying ash were seen, symptoms and identities of affected species were noted and specimens were collected for microscopic examination as described below.

Tree infection by MLO. Initially, infected trees could be identified only by presence of witches'-brooms in which MLO were detected by transmission electron microscopy (TEM). For this, segments from petioles, leaflet midribs, and small stems were cut into pieces 1 mm long and fixed in 5.0% glutaraldehyde (11) in the field and then dehydrated and embedded in Spurr's resin (23). Sections 300–500 nm thick were poststained for 20 min in uranyl acetate (6), then for 3–5 min in lead citrate (25) and were examined at 80–100 kV. Comparable tissues from nonsymptomatic white ash were sampled, treated, and examined similarly.

Diagnostic staining with Dienes' stain (5) was the principal technique used to detect MLO infection in trees without witches'-brooms. The Dienes' stain test is applied to phloem sieve tubes of the most recent growth increment. The only organisms known to occur systemically there and to be incapable of decolorizing the stain are MLO (5), which stain dark blue. Longitudinal sections 25–100 μm thick were cut from stem nodes, immersed 30 min in stain, cleared in 50% Karo® light corn syrup in distilled water on microscope slides, and incubated 0.5–24 hr before observation. False negative results were minimized by examining samples from one or two additional branches per tree if MLO were not detected in the first one.

Reliability of Dienes' stain was assessed in two ways. Stained sections from witches'-brooms on 100 trees (including all 15 witches'-brooms examined by TEM) were examined as MLO-infected standards. For a "blind" test, twigs were collected from 10 apparently healthy white ash trees, five with deliquescent branches, and five with witches'-brooms. Stained sections from each tree, identified only by serial numbers, were presented for microscopic examination to a person who had seen neither the source trees nor the twigs that were sectioned, and who did not know how many samples came from each tree category. The examiner classified each tree as healthy or infected based on the Diene's stain test, and the results were compared with records of symptoms.

Diffusive resistance of leaves (14) was also assessed as a diagnostic criterion. Measurements were made on abaxial surfaces of sunlit leaves by using a Lambda LI-65 diffusive-resistance porometer with procedures and precautions described previously (14).

Inoculations. In 1979, 24 3-yr-old white ash seedlings were graft-inoculated with bark patches (14) from white ash trees that had witches'-brooms. In September 1980, after two seasons of growth in the greenhouse, these and eight uninoculated seedlings were cut back to 60 cm height and planted in a nursery. In October 1981, the growth of every twig was measured. In October 1981 and June 1983, the presence of dieback beyond one internode was recorded. Data were checked for homogeneity of variance, and means were compared by Student's t-test (22).

RESULTS

Ash decline in observation plots. In 1981, in five plots in which all white ash trees (total 2,363) were examined, 19 to 62% (avg 40%) of living trees were classed as vigorous, and 38 to 81% (avg 60%) of living trees were growing slowly or dying back. Thirteen to 56% (avg 28%) of all white ash were dead. The dates of sudden growth reduction varied among trees within plots, and the frequency distribution of trees according to year of onset of slow growth varied among plots (Fig. 1).

Detection of MLO. Witches'-brooms from white ash at 15 locations were examined by TEM. All contained typical MLO profiles in phloem sieve tubes but in no other cell type. MLO were also found in phloem sieve tubes in sections from 13 of 16 deliquescent branches, each from a different tree that lacked witches'-brooms. Sections 300–500 nm thick were examined in order to detect helical forms if present, but only pleomorphic profiles were seen. No MLO were detected in sections from 12 apparently normal trees.

Witches'-brooms from 100 trees and deliquescent branches from 114 trees that lacked witches'-brooms were sectioned and Dienes' stain was applied. These samples included all witches'-brooms and deliquescent branches that had been examined by TEM. Phloem sieve tubes stained dark blue (Fig. 2) indicating presence of MLO (5) in sections from 90 witches'-brooms and 103 deliquescent branches on the first attempt. The remainder stained positive for MLO when sampled a second time. A positive staining result was more likely in sections from witches'-brooms or deliquescent branches than in sections from other parts of the same trees.

In the "blind" test of Dienes' stain, the examiner identified all of five witches'-broom-bearing and five deliquescently branched trees as infected, and classified eight of ten apparently healthy trees as noninfected. Phloem sieve tubes of two apparently healthy trees reacted in the same manner as phloem from trees with deliquescent branching.

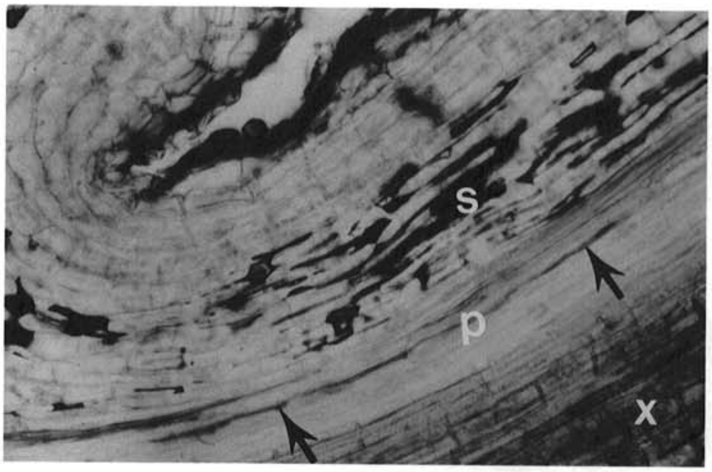

Fig. 2. Positive Dienes' stain reaction in longitudinal section from near a leaf gap in a twig of a deliquescent white ash branch. X = xylem, S = sclerenchyma bundle, and P = phloem. Sieve tubes containing mycoplasmalike organisms stain dark blue (arrows) and can be identified by bulges at sieve plates. Noninfected phloem remains light blue. ×138.

TABLE 1. Percentage of white ash trees infected[a] by mycoplasmalike organisms in relation to the condition of the trees at five locations in New York State

Condition[b]	Location					Totals and averages[c]
	A	C	E	F	G	
Vigorous	12%/51	35%/334	35%/57	21%/217	63%/49	33%/708
Growing slowly	40%/35	65%/364	66%/56	22%/27	67%/51	52%/533
Dying back	96%/54	84%/144	80%/232	62%/116	86%/58	82%/604
All classes	51%/140	57%/842	67%/345	35%/360	73%/158	57%/1,845
Chi-square	77.3	116.2	191.5	56.5	8.5	309.0
P	<0.005	<0.005	<0.005	<0.005	0.014	<0.005

[a] MLO infection detected on one or more of the following bases: witches'-brooms, production of abortive sprouts, deliquescent branching, positive reaction to Dienes' stain, abnormally high leaf diffusive resistance, or visualization of MLO with electron microscopy. Numbers following the slashes are sample sizes.
[b] Trees with topmost twigs elongating >20 cm/yr classed as vigorous unless growth rings indicated an abrupt, permanent growth reduction >50%; slow growth based either on twig elongation <20 cm/yr or radial growth rate reduction >50%. Based on observations in June 1982.
[c] Averages not weighted for varying sample sizes.

Dienes' stain was used to assess 213 additional trees with witches'-brooms or deliquescent branching, and MLO were detected in 96% of these trees. On this basis, witches'-brooms and deliquescent branches were identified as diagnostic symptoms of MLO infection, and all trees with either of these symptoms were tallied as having ash yellows. Dienes' stain also revealed MLO in 10 of 67 trees lacking diagnostic symptoms. These trees were uniformly distributed among vigor classes.

Diffusive resistance of leaves of 149 trees was measured. Those judged noninfected on the basis of symptoms or phloem staining had diffusive resistance values less than 6.0 $sec \cdot cm^{-1}$. Trees infected by MLO as judged from diagnostic symptoms or positive reaction to Dienes' stain had diffusive resistance values from 1.3 to 96.6 $sec \cdot cm^{-1}$, and 20% of these values were in the normal range of 1.3–6.0 $sec \cdot cm^{-1}$. Thus, high values reliably identified MLO-infected trees, but 20% of infected trees would have gone undetected by the use of diffusive resistance measurements alone.

Frequency of MLO infection in relation to symptoms. Each of 1,845 living white ash $\geqslant$3 cm diameter at breast height (dbh) in five plots was classed as vigorous, slow-growing, or dying back. Then, on the basis of diagnostic symptoms (witches'-brooms and deliquescent branches) or testing by TEM, Dienes' stain, or measurements of leaf diffusive resistance, each tree was identified as MLO-infected or noninfected. The average proportion of trees infected across all vigor classes in 1982 was 57%, and the range among plots was 35–73% (Table 1). At each site, the frequency of MLO infection was significantly ($P < 0.02$) related to initial vigor classification. Of MLO-infected trees, 23% were growing normally, 28% were growing slowly, and 49% were dying back. Corresponding proportions for trees apparently not infected with MLO were 57, 30, and 13%. During the 1981–1982 dormant season, 130 trees (7% of total) died. Of these, 117 were tallied as infected by MLO and constituted 10.1% of the infected trees. Among apparently noninfected trees, only 13 (1.9%) died.

Mean relative radial growth, determined by examining 699 xylem sectors, varied significantly ($P = 0.01$) among vigor and infection classes. In 1979–1980, noninfected trees classed as vigorous, slow-growing, and dying back grew at rates equivalent to 81, 59, and 45%, respectively, of the previous standards. Corresponding relative growth rates for infected trees were 59, 43, and 35%, respectively. Of 490 trees tallied as infected with MLO, 85% had sustained sudden reduction in radial growth (Fig. 3). Among the 209 apparently noninfected trees, however, 36% had also sustained a sudden reduction. The growth reduction associated with MLO infection was assessed by means of linear comparisons (22). On average across-all-vigor categories, radial growth of apparently noninfected trees in 1979–1980 was 62% of the previous standards, and radial growth of MLO-infected trees was 46% of the standards. The difference was significant at $P = 0.05$.

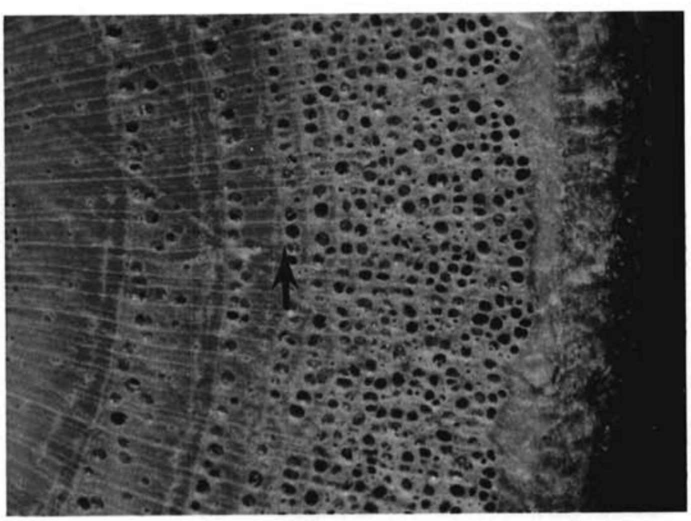

Fig. 3. Transverse section from the butt of a declining white ash, showing sudden, permanent reduction in radial growth beginning 13 yr before sampling. This tree was classified as being in decline beginning 12 yr before it was sampled (arrow). Approximately ×8.

The relationship of apical growth rate to MLO infection was similar to that of radial growth: 77% of infected trees versus 25% of apparently noninfected trees displayed slow (<20 cm/yr) growth.

Basal sprouts formed after wounding on 208 (41%) of 511 trees that initially lacked witches'-brooms (although many had deliquescent branches). The sprouts were of three types: small witches'-brooms on 138 trees, dwarfed leafless shoots that either aborted during the growing season or died during the next winter on 57 trees, and normal appearing leafy shoots on 13 trees. Dienes' stain indicated MLO in 87 of 89 trees with witches'-brooms, 32 of 33 trees with abortive shoots, and three of six trees with normal-appearing shoots that were tested. Abortive shoots were occasionally seen on nonwounded trees. Witches'-brooms or abortive shoots formed on 30% of 329 wounded trees versus 9% of 791 nonwounded trees that displayed neither witches'-brooms nor deliquescent branches at the time of wounding. Thus, wound-induced sprouts were the basis for detecting MLO infection in approximately 69 trees (30% minus 9% of 329) that if not wounded, would have been classed as noninfected. Wounded trees that sprouted were distributed unequally among vigor classes: 23% of vigorous trees, 30% of those growing slowly, and 48% of those showing dieback ($\chi^2 = 8.10$, $P = 0.02$). Among 182 wounded trees that had deliquescent branches as prior evidence of infection, on the other hand, the distribution of sprouting trees among vigor classes did not differ significantly from the average 52%.

Brooms or abortive sprouts occurred on 63% of the nonwounded, infected trees. Deliquescent branching occurred on 74% of infected trees.

Many trees with witches'-brooms or deliquescent branches began growth before the normal time in spring (Fig. 4). To assess this as a possible symptom of MLO infection, we examined 530 trees in three plots in 1981, and 785 trees in four plots in 1982, classified each as early or normal in beginning apical growth, and compared its classification with records for MLO infection. In 1981, 75% of MLO-infected trees versus 38% of noninfected trees began growth early ($\chi^2 = 72.6$, $P = 0.0001$). In 1982, 85% of MLO-infected trees versus 39% of noninfected trees were early ($\chi^2 = 171.2$, $P = 0.0001$).

Premature autumn color in relation to MLO infection was evaluated similarly for 966 trees on five plots in 1981. This symptom was displayed by an average 56% of infected trees versus 43% of those noninfected ($\chi^2 = 16.6$, $P = 0.0001$). Among infected trees, 55–58% displayed premature color regardless of vigor class. Among the 455 noninfected trees, however, the proportion displaying premature color rose from 33% of vigorous trees to 44% of slow-growing ones and 67% of those with dieback.

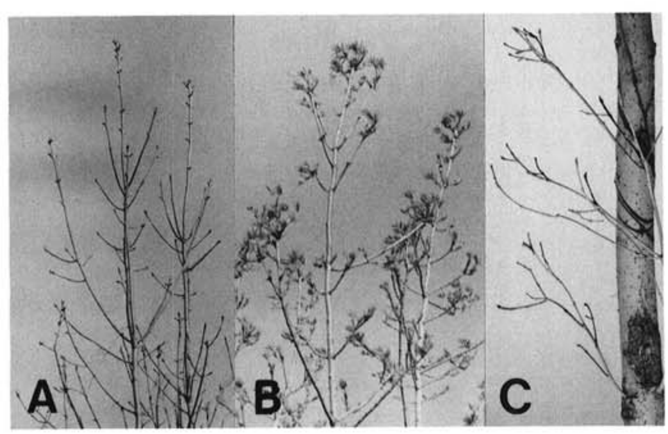

Fig. 4. Branches on adjacent white ash trees in early May: **A,** normal; **B,** growing precociously while showing the deliquescent habit associated with infection by mycoplasmalike organisms. **C,** Deliquescent branching of dormant epicormic sprouts on white ash.

Bark cracks, tangential separations of bark and wood, and cankers, all caused by freezing (Fig. 5), were found on 10.5% of trees apparently free from MLO and on 36.8% of infected trees. Regardless of MLO infection, vigorous trees were less often found damaged (9 and 28% incidence in noninfected and MLO-infected groups) than were slow-growing trees (12 and 40%) or those with dieback (13 and 39%) ($\chi^2 = 221.9$, $P = 0.0001$).

Changes between 1982 and 1984. By the end of the growing season in 1984, among 1,489 previously labeled trees that could be identified in five plots, 1.5% were tallied as vigorous, 81.9% as growing slowly or dying back, and 16.5% as dead to ground level since 1982 (witches'-brooms at trunk bases remained alive on some of these). Yellows was indicated by deliquescent branches, witches'-brooms, or abortive sprouts on 73.3% of all trees and on 81.3% of those dead or dying back. Of 676 trees that lacked diagnostic symptoms of yellows in 1982, 59% showed deliquescent branching, witches'-brooms, or abortive sprouts in 1984.

Deliquescent branching was found to be a frequent precursor of witches'-brooms or abortive sprouts. Among trees that in 1982 showed only deliquescent branches as evidence of yellows, 34% developed witches'-brooms or abortive sprouts during 1983–1984. Among trees that showed no diagnostic symptoms of ash yellows in 1982, 15% developed witches'-brooms or abortive sprouts during the next 2 yr. A chi-square value of 37.9 ($P < 0.001$) was calculated for the comparison of proportions of trees with abortive sprouts or witches'-brooms in three vigor classes of trees that showed deliquescent branching versus those that lacked this symptom in 1982.

Inoculations. Of 24 inoculated seedlings, 20 developed yellows symptoms in the greenhouse as described previously (14), and MLO infection was confirmed before these and eight uninoculated controls were moved to the nursery. The controls were negative for MLO on the basis of Dienes' stain reaction, normal diffusive resistance of leaves, and for two trees, EM examination. During the first year in the nursery, two control plants became infected through natural means. Twig growth of the 20 inoculated, infected trees averaged only 24% of that of the six remaining controls, 5.5 versus 22.0 cm/yr ($P < 0.005$). After one winter, 13 MLO-infected trees displayed twig dieback, compared to one noninfected tree, and two infected trees were dead. By June 1983, 16 infected trees had died back to within 25 cm of the ground, and four previously infected trees were dead. Noninfected trees sustained only minor twig dieback associated with browsing by deer (χ^2 for the comparison of amount of dieback in previously infected versus noninfected trees was 9.33, $P < 0.005$).

Observations of ash decline and of MLO infection in the northeastern region. Based on observation and collection of witches'-brooms and deliquescent branches along highways, and subsequent testing with Dienes' stain, we found MLO associated with declining ash trees in a region extending from west of Lake Ontario and Lake Simcoe in Ontario to the Green Mountains in Vermont, the Connecticut River Valley in Massachusetts, and the southern Hudson Valley in New York State. In western Vermont and in Canada along Lake Ontario, red ash and green ash [*F. pennsylvanica* Marsh., and *F. pennsylvanica* var. *lanceolata* (Borkh.) Sarg., respectively], were found declining and infected with MLO. We did not attempt to learn the overall distribution of infection by the MLO or of decline in ash species.

DISCUSSION

Hibben and Wolanski (11) described witches'-brooms apparently caused by MLO in white ash, and referred to these as representing a yellows-type disease. Our findings indicate that formation of witches'-brooms is but one facet of a syndrome (apparently caused by MLO) for which the name ash yellows is appropriate (13). Symptoms include slow growth and light green or chlorotic foliage, elevated diffusive resistance of leaves (14), deliquescent branching, formation of witches'-brooms or abortive sprouts, precocious growth in spring, premature autumn color, dieback, and death. Freeze damage to the vascular cambium at the base of the trunk is abnormally common in infected trees and may contribute to their debilitation. Trees of all sizes and ages are affected. Few, if any, show all symptoms in a single year. Witches'-brooms and deliquescent branching were the only macroscopic symptoms judged reliable for diagnosis.

Among trees we observed, abnormal pinkish color of bark was common but was not recorded because this was influenced by degree of exposure to sun and wind. Foliar symptoms (subnormal size, light green color, mottling, tendency to fold along the midrib, and various viruslike symptoms) were also common but were not recorded because leaf color and texture could not be judged consistently in the canopy or against varying sky conditions.

In work not described here, attempts to cultivate the ash MLO in various media suitable for growth of spiroplasmas and other mollicutes were unsuccessful.

Association of ash yellows with widespread decline of white ash. In observation plots, the dates of sudden onset of slow radial growth, which is considered to signal the onset of decline, varied among trees, and the most frequent dates of onset varied between plots in the same locality (eg, Cortland County [Fig. 1]) and among plots in different localities. Such variation is characteristic of infectious disorders although it is not exclusively associated with them. Three lines of evidence indicate a close association of MLO with decline. First, yellows as indicated by witches'-brooms and deliquescent branching is common throughout the region of severe ash decline, and 96% of the symptomatic plants that were tested gave a positive Dienes' stain reaction for the presence of MLO. Second, MLO infection was closely associated with slow growth, dieback, and death of white ash. More than half of slowly growing trees (52%) and a large majority of trees with dieback (82%) were judged infected in 1981–1982. Death during a 17-mo period was five times as common in trees naturally infected with MLO (10% mortality) as in apparently noninfected trees (1.9%). Third, MLO transmitted to small trees by grafting were associated with reduced growth, dieback, and death.

Our estimate of the frequency of MLO infection among white ash in the observation plots (57% in 1981–1982) was probably conservative. Some infected trees may have gone undetected because they lacked diagnostic symptoms, they either were not tested or gave a false negative reaction with Dienes' stain, leaf diffusive resistance either was normal or not measured, or the tree was not wounded to encourage production of sprouts. Among trees that lacked diagnostic symptoms in 1982 and that were reexamined in 1984, 59% had developed deliquescent branches, witches'-brooms, or abortive sprouts.

The standards for relative radial growth gave conservative estimates of the degree of growth retardation associated with MLO. If in declining trees the current 2-yr growth increment had been expressed as a percent of the maximum for any 2 yr period,

Fig. 5. White ash, infected with mycoplasmalike organisms, with witches'-brooms at the ground line and cracked bark overlying a basal injury caused by freezing.

current values would have been smaller than those that were reported. Also, trees that grew slowly throughout the period of record received high values for relative growth.

Our estimate of 71% of white ash dead or declining in 1982 is similar to survey estimates published in 1968 (20) and 1978 (10), but it does not indicate a static condition. During 1968 to 1982, many trees died or began to decline, and many healthy saplings joined the ash populations that we observed.

Relationship of MLO to other etiologic agents in "ash dieback." MLO causing ash yellows are closely associated with "ash dieback" in New York State. However, some decline occurs in white ash with no recognizable symptoms of ash yellows. In our plots in 1981–1982, no specific yellows symptoms or MLO were detected in 18% of trees that were dying back, or in 48% of the slowly growing trees. Although by 1984 yellows symptoms occurred in a large proportion of such trees, there remained about 25% in which yellows was not diagnosed. Also, secondary factors apparently contribute to decline. These factors include facultatively parasitic fungi that cause cankers (17,19), cambial damage by freezing (this report), and drought stress (17,24). We would expect an additive influence of MLO and water stress because both cause stomatal closure (14,24) and retard growth in white ash. Viruses are widespread in the ash population affected by decline (4,7,8,9,12), and are common in trees simultaneously infected with MLO (9, and the observations [*unpublished*] of J. D. Castello and the present authors). Thus, the concept of multiple causes of "ash dieback" (10,17) still seems valid as a basis for studying the individual and combined roles of MLO and other agents.

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