Epidemiology of a Slow-Decline Phytoplasmal Disease: Ash Yellows on Old-Field Sites in New York State

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ABSTRACT

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Incidence and increase of ash yellows were studied in six white ash (*Fraxinus americana*) populations on four sites in central New York State in 1990–1994. Each of 110–307 ash per population was observed for symptoms and tested with DAPI for detection of phytoplasmas annually for 3 or 4 yr. Yellows incidence based on symptoms was correlated with incidence based on DAPI tests ($r = 0.89$). Symptom detection usually lagged 1–2 yr behind phytoplasma detection, but 23% of ash infected for 3–4 yr did not show symptoms. Reliability of sampling one root per tree per year for the DAPI test was estimated to be 94% based on the frequency of positive results for 2 consecutive years in the same trees. Initial incidence of ash yellows based on DAPI test results varied among populations from 5 to 45%. Rates of annual increase averaged over 3–4 yr varied from 0 to 8% of the initial populations and were not related to initial incidence level. The average rate of increase was 4.9% of the initial population per year based on DAPI tests and 4.0% based on symptoms. Higher incidence and higher rates of increase occurred in pure ash populations than in ash mixed with other species. New infections and new symptoms were detected more often in trees with crowns exposed to the sky than in shaded trees. Disease incidence was not significantly greater in crowded than in scattered ash trees. A proximity test revealed no significant difference between the average distance from newly diseased to previously diseased trees and the average distance from newly diseased to previously healthy trees. Pathogen transport over meters to tens of meters from diseased to healthy ash was indicated by proximity test results, similarity of disease incidence in clustered and scattered trees, and greater incidence of yellows in trees with crowns exposed to sky than in shaded trees on the same sites.

Additional keyword: mycoplasmalike organism

Ash yellows causes slow growth of various ash (*Fraxinus*) species and decline of highly susceptible species, notably white ash (*F. americana* L.) (9,17,18,21). The disease is considered to be caused by noncultivable phytoplasmas (also called mycoplasmalike organisms) in the ash yellows strain cluster (3,4,8). Ash yellows phytoplasmas are thought to be restricted to *Fraxinus* and the closely related genus *Syringa* in nature, because phytoplasmas in the ash yellows strain cluster were not identified among those detected in other plant species on sites of ash yellows occurrence (4). Vectors of ash yellows are still unknown.

Epidemiological characteristics of ash yellows, such as rates and spatial patterns of increase and final levels of disease in affected populations, have not been reported, although incidence levels >50% have been observed in particular white ash and velvet ash (*F. velutina*) populations (9,19). Also unreported is whether the majority of infections occur within a brief period early in the life of a stand, or whether ash yellows incidence rises over many years. Knowledge of rates and patterns of increase would permit predictions about the future incidence and impact of the disease in ash populations affected while young. Such knowledge could also provide a basis for inferences about abundance, efficiency, or continuity of vector populations in affected stands.

Objectives of the work described here were to characterize ash yellows increase over 3–4 yr in diverse populations of white ash,

determine whether new infections occur randomly or are spatially related to previously diseased trees, compare incidence assessments based on symptom observations to those based on microscopic diagnoses, and utilize epidemiological information to interpret patterns of disease incidence and increase.

MATERIALS AND METHODS

Ash populations. Six populations of white ash in which ash yellows had been detected were selected at four locations in central New York State. Populations 1, 2, and 3 occurred at one location. Population 1 was a dense stand of saplings and trees 13–17 yr old at one edge of an abandoned field. Population 2 was a stand of saplings and trees 10–20 yr old, scattered across the field. These stands were nearly pure white ash. Population 3 consisted of ash seedlings <1.4 m tall scattered among shrubs and herbaceous species beneath the canopy of population 2. Dominant understory species associated with the ash seedlings were *Cornus racemosa, Lonicera tatarica, Rosa multiflora, Solidago* sp., and *Viburnum dentatum.*

Population 4 was a dense stand of seedlings <1.4 m tall beneath an overstory of mixed deciduous species. Occasional specimens of *C. racemosa* and *Viburnum lentago* occurred among the ash. The overstory species were *Acer saccharum, Crataegus* sp., *F. americana,* and *Malus domestica.*

Population 5 was a dense stand of seedlings and saplings in the understory of a failing experimental plantation of *Prunus serotina* and *Pinus strobus.* White ash had begun to thrive as overstory trees died. Understory associates of the ash were *Fragaria* sp., *Prunus virginiana, Rhamnus cathartica, Rubus allegheniensis, Solidago rugosa, Viburnum lentago,* and *V. dentatum.*

Population 6 was composed of saplings and trees within an uneven-aged stand of mixed species. The ash saplings were 10–18 yr old; ash trees were 25–45 yr old. Overstory species in addition to white ash were *Acer rubrum, Carya* sp., *Pinus strobus,* and *Populus tremuloides.* Common woody understory species, in addition to white ash, were *Amelanchier* sp., *Cornus sericea, C. racemosa, Fagus grandifolia, R. multiflora, V. dentatum,* and *V. lentago.*

In 1990–1991, rectangular plots were established, one each in ash populations 1, 4, 5, and 6, and three replicate plots on the site of populations 2 and 3. Plot areas varied from 120 to 1,400 m^2 with the smallest plots in dense populations. The number of ash per plot varied from 110 to 442. All ash on three plots, and every second or third ash on the three plots with the most dense populations, were designated for examination and diagnostic testing. The number of designated ash per population varied from 110 to 307. The canopy position of every designated ash was classified as shaded or exposed to the sky.

Disease assessment. Designated ash on every plot were examined annually from 1990 or 1991 through 1994 for symptoms of ash yellows and were scored as healthy, possibly diseased, or diseased. Disease was indicated by combinations of the following symptoms (9,17): sudden and permanent reduction in annual twig growth, deliquescent branches, dwarfed basal sprouts, witches'-brooms, simple leaves on seedlings or basal sprouts, abnormally large leaflets or simple leaves, dwarfed simple leaves, diffuse interveinal chlorosis on simple or dwarfed compound leaves, light green or chlorotic foliage, branch or mainstem dieback. All symptom evaluations were made by the same observer.

Each designated plant was assayed annually in summer from 1990 or 1991 through 1993 for phytoplasma detection by means of the DAPI (4′,6-diamidino-2-phenylindole·2HCl) fluorescence test (20). Roots 1–4 mm in diameter were sampled preferentially. Petioles were substituted for roots of many seedlings in the final year of sampling, as it had become difficult to obtain suitable roots from the smallest plants without destroying them. Root segments or petioles were fixed in 2.5% glutaraldehyde in phosphate buffer, pH 7, and stored at 4 C until they were sectioned. Longitudinal sections were treated with DAPI and examined with an epifluorescence microscope. A tree was scored as infected if fluorescent specks characteristic of phytoplasmas were observed in sieve tubes of any section from it (20). Continuity of records for each tree or seedling was obtained by carrying forward the most recent symptom score and DAPI test result if the plant died or was not found or a sampled root was unsatisfactory for testing.

Analyses. Symptom incidence in each population and year was expressed as proportion symptomatic. Incidence of phytoplasmal infection in each population and year was expressed as proportion of samples scored positive in the DAPI test. Year-to-year changes in these proportions were interpreted as disease increase. Data from replicated plots were combined for incidence estimates in populations 2 and 3. The relationship between disease incidence based on symptoms and that based on DAPI test results was examined by correlation analysis (23). Consistency of ash yellows diagnosis by DAPI tests and separately by symptom evaluation was evaluated in terms of the proportion of diagnoses that changed from positive to negative in successive years.

Relationship between exposure of a tree crown (shaded versus exposed) at the beginning of the study and ash yellows onset during the study was evaluated by contingency analyses (23). The hypothesis tested was that exposed and shaded trees have equal probability of contracting ash yellows. Data from populations 1, 2, 5, and 6 were used for this evaluation, because they contained both shaded and unshaded plants in which new infections occurred during the observation period. DAPI test results and symptom observations were analyzed separately. Trees for which positive DAPI results or ash yellows symptoms were recorded in the first year of observation were excluded, because such trees may have been diseased for several years and could occupy subordinate canopy positions as a result of disease-induced growth loss. Data were pooled across populations to obtain samples with large enough numbers for analyses.

The temporal relationship between phytoplasma detection and onset of ash yellows symptoms was examined in two ways. First, symptom records for 138 trees in which phytoplasmas were initially detected by DAPI tests during the second or subsequent annual samplings were used to plot the cumulative proportion of DAPI-positive trees with ash yellows symptoms over time beginning 2 yr before phytoplasma detection. Second, the incidence of diseased trees remaining asymptomatic at least 3 yr after phytoplasma detection was determined by calculating the proportion of trees that were asymptomatic in 1994 among those in which phytoplasmas were detected in 1990 or 1991.

Grid maps showing the locations of trees in population 2 (three replicate plots) and in population 6 were prepared for the purpose of assessing randomness versus clustering of diseased trees when first sampled and the spatial relationship of individuals in which phytoplasmas were detected subsequently to trees initially found infected. These plots all had areas >600 m^2 and initial incidence of phytoplasma infection between 13 and 28%. The other populations were unsatisfactory for these tests because of smaller plots or lack of disease increase. Cells on each grid map were 2 m square and were numbered serially.

Clustering of ash trees and therefore of trees with ash yellows was evident from inspection (e.g., Fig. 1) and was confirmed by calculation of Pielou's index of nonrandomness (24). Pielou and Foster's test (12) was used to compare incidence of disease in scattered versus crowded trees. This test assigns a probability value to the difference between the proportion of trees in a population that are diseased and the proportion found diseased in a sample of N trees, biased in favor of scattered trees. If the probability of a tree being diseased is independent of its degree of isolation from others, then the two proportions will be the same. The test is performed by sampling a population randomly (or examining all trees on a plot) and determining the proportion diseased, p. Then a sample biased in favor of scattered trees is drawn by selecting the trees nearest to N random point locations in the plot. The number, x, of trees found diseased in this sample is then compared with the expected number Np. The difference between x and Np is represented by the expression $(Np-x)\sqrt{(Np(1-p))}$, which is treated as a standardized normal

variate with probability determined by reference to a table of the normal distribution. Random locations for this test were the centers of grid cells identified by drawing random numbers between 0 and i, where i = the number of cells on the grid map of the plot. Each number was utilized only once.

The spatial relationship of trees in which phytoplasmas were detected in 1992 or 1993 to those in which phytoplasmas were previously detected was assessed by a Student's t test that we call the proximity test. The mean of the distances D_d from each newly diseased tree to the nearest previously diseased tree was compared with the mean of the distances D_h from each newly diseased tree to the nearest previously healthy tree in a random sample equal in number to the previously diseased trees. The probability associated with inequality of these averages was assessed by a one-tailed Student's t test with significance declared when $P \leq 0.05$. Random previously healthy trees were obtained by generating a list of random grid cells as above and selecting previously healthy trees located within the listed cells until the required number of trees was obtained. If a cell contained more than one previously healthy tree, the one nearest to a newly diseased tree was taken. The rationale for this test is that if close proximity of a healthy tree to a diseased one enhances the probability that the healthy tree will become diseased, then the average D_d will be less than the average D_h, assuming equal numbers of previously diseased and previously healthy trees. Requirements for the test are discussed in a later section.

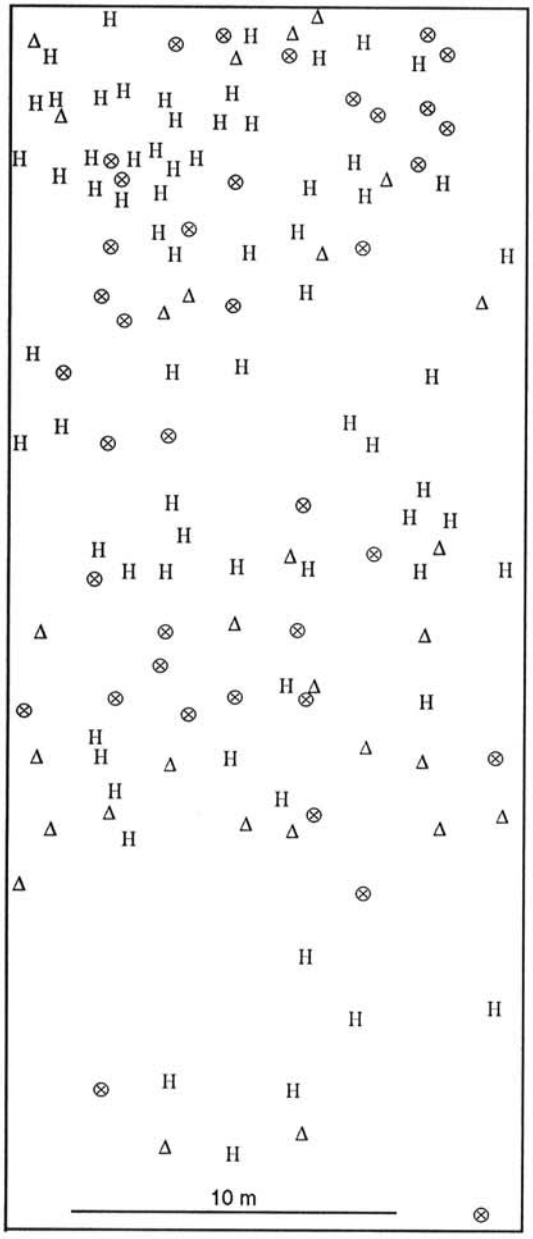

Fig. 1. Map of replicate plot A in white ash population 2 at the end of 1993, showing uneven distribution of trees but similar incidence of ash yellows, as diagnosed by means of DAPI tests, in crowded and scattered trees. Disease incidence was 48%. H = healthy; circles with Xs = disease detected in 1990-1991; triangles = disease detected in 1992 or 1993.

RESULTS

Initial incidence and rates of increase. Initial DAPI tests revealed ash yellows incidence varying from 10 to 45% among populations. Disease increase occurred each year in five of the six populations (Fig. 2A). In 1993, the final year of DAPI testing, incidence varied from 18 to 61% in these five populations. Single-year increases varied from 0 to 10% of the initial population, and average annual increase within populations varied from nil in population 3 to 8.2% in population 5. The unweighted average rate of increase across all populations, based on DAPI tests, was 4.5% of the initial population per year.

Symptom evaluations yielded estimates of ash yellows incidence and increase that were similar to those obtained by DAPI testing. Initial incidence varied from 5 to 47%, annual increases from 0 to 10%, and averages over 3–4 yr from 0.3 to 5.8% (Fig. 2B). The overall unweighted average rate of increase in symptom incidence was 4.0%.

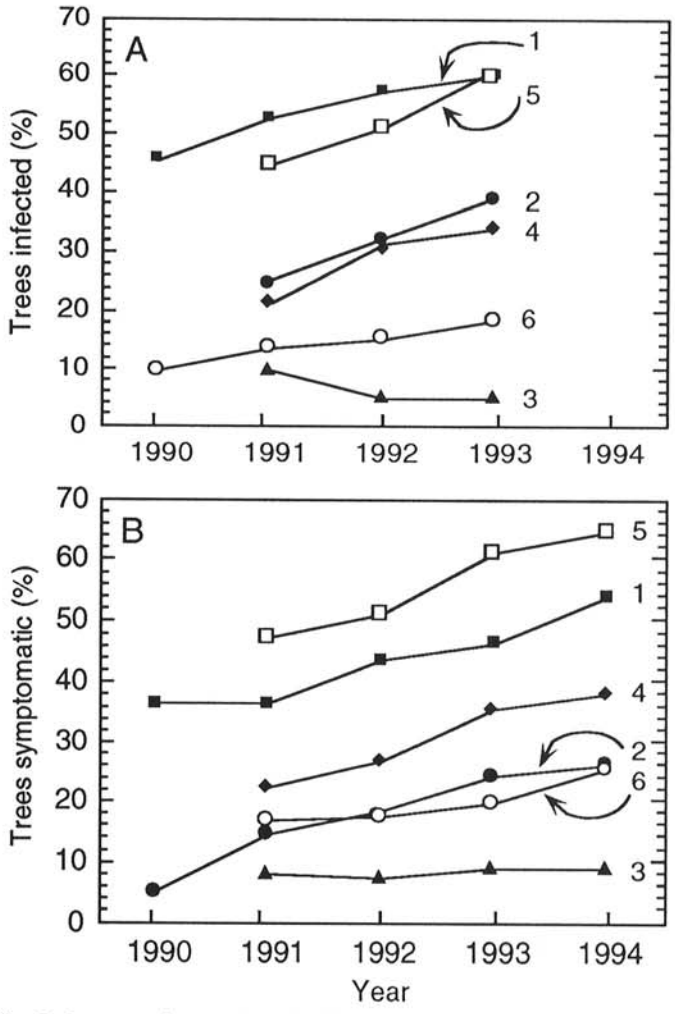

Fig. 2. Increase of ash yellows incidence over 2–4 yr in six white ash populations as revealed by **A,** DAPI tests, and **B,** symptoms. The average rate of disease increase based on DAPI tests was 4.5% and that based on symptoms was 4.0% of the initial population per year, respectively.

The lowest incidences of infection and of yellows symptoms occurred in the two populations that were interspersed with other plant species. Incidence did not increase in the population of ash seedlings <1.4 m tall interspersed with other species (population 3), although increase occurred annually in the overstory on the same site (population 2).

Agreement and consistency of symptom scores and DAPI test results. The proportions of trees with ash yellows symptoms and the proportions in which phytoplasmas were detected with DAPI were highly correlated ($r = 0.89$, $P < 0.01$, Fig. 3). In four of the six ash populations, all annual estimates of disease incidence based on symptom assessment agreed to within 5% with those from DAPI testing. In populations 1 and 2, however, the percentages of trees scored as symptomatic in each year were 7–21 points lower than the percentages of trees in which phytoplasmas were detected with DAPI. This disparity was associated with a lag period between phytoplasma detection and symptom expression, which was greater in these populations than in the others.

Trees that became diseased during the 4-yr observation period most often began to show symptoms during the first year of detected infection or 1 yr later (Fig. 4). Incidence of symptoms in trees in which phytoplasmas were first detected in the second or third year of DAPI testing was 35% at the time of phytoplasma detection but rose to 69% within 2 yr after detection (Fig. 4). Among trees found to be infected when first tested, 77% were symptomatic 3–4 yr later. Across all populations and years, ash yellows symptoms were recorded at least once for 82% of trees in which phytoplasmas were detected with DAPI. For individual trees, changes in DAPI test results from positive in one year to negative the next occurred at a frequency of 5.9% ($n = 660$ positive scores). The frequency of change of symptom score from diseased in one year to possibly diseased or healthy in the next year was 9.1% ($n = 807$). Symptom evaluations were less consistent than DAPI tests because of year-to-year fluctuations in trees' appearances and diminishing visibility of crowns of some trees growing in dense clusters. Sample sizes for these evaluations differed because fewer data were missing for symptoms than for DAPI results.

Effect of crown exposure. In populations 1, 2, 5, and 6 the proportion of trees with unshaded crowns at the beginning of the study varied from 27 to 63%. Contingency analyses led to rejection of the hypothesis of no interaction between crown exposure and onset of ash yellows. New cases of the disease, whether based on DAPI tests or symptoms, were more frequent in trees with crowns exposed to the sky than would have been predicted on the basis of the frequency of exposed plants in the samples. In the pooled sample of 485 DAPI test results, 58% of the 115 new infections occurred in exposed trees, whereas the expected proportion was 41% (χ^2, 1 df = 17.11, $P < 0.005$). In the pooled sample of 540 symptom observations, 58% of the 146 instances of new symptoms occurred on exposed plants, whereas the expected proportion was 47% (χ^2, 1 df = 10.04, $P < 0.025$). Populations 3 and 4 were unsuitable for these analyses, because no disease increase occurred in the former, and all plants in the latter were shaded.

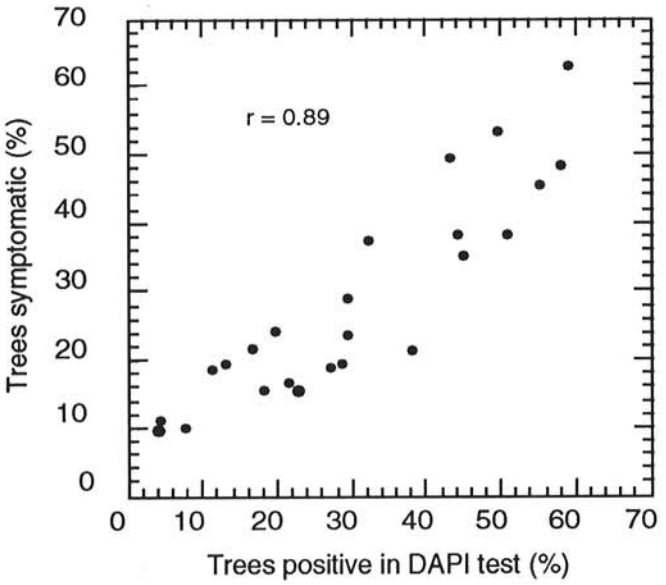

Fig. 3. Relationship between incidence of ash yellows symptoms and incidence of phytoplasmal infection based on DAPI tests. Each point ($n = 24$) represents one plot in one year.

Spatial analyses. Clustering of ash trees could be discerned on all plots used for spatial analyses (e.g., Plot 2A, Fig. 1). Trees with ash yellows were numerous in the clusters, while areas between clusters contained scattered healthy and diseased individuals. Pielou's index of nonrandomness for spatial arrangement of initially diseased trees was calculated for each plot using samples of 30 distances from random points to the nearest diseased plant. Values of this index significantly greater than 1 indicate clustering (24). All calculated values were >1.74 with associated probability >0.99 (24). Thus, diseased trees tended to be clustered, although this clustering only reflected the arrangement of each ash population. Pielou and Foster's test (12), performed separately for each plot, revealed no significant tendency for frequency of ash yellows to be higher in crowded trees than in scattered ones (Table 1).

In proximity tests conducted on four plots, trees in which disease was first detected in 1992 or 1993 were not significantly more closely associated with previously diseased trees than with members of a random sample of previously healthy trees equivalent to the number of previously diseased trees (Table 1). The average value of the ratio of distance D_d (from a newly diseased tree to the nearest previously diseased tree) to D_h (from a newly diseased tree to the nearest of a sample of previously healthy trees) varied among plots from 0.67 to 1.14.

DISCUSSION

The average rates of ash yellows increase detected on different sites over 3 or 4 yr were all between 2.9 and 8.2% of initial population per year, even though the ash populations varied widely in stage of stand development, levels of initial disease incidence, and associated vegetation. These rates of increase may be characteristic of ash yellows in central New York State. The overall rate of increase of 4.5% per year based on DAPI tests was close to reported rates of increase in incidence of ash decline in areas where ash yellows now occurs in New York State (2). Ash yellows had not been described when the decline data were gathered.

Phytoplasmal diseases of trees that have been studied exhibit two types of epidemic-development dynamics. One type is

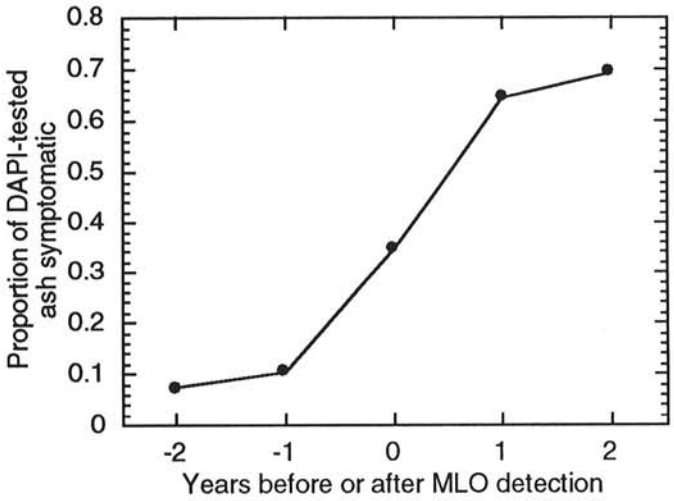

Fig. 4. Cumulative incidence of ash yellows symptoms as related to time of phytoplasma detection in white ash tested annually with DAPI. Data from 138 trees from all populations were pooled for this analysis. Trees in which phytoplasmas were detected in the initial sampling were excluded.

characterized by quick decline of individual trees and exponential increase in early stages, leading to large annual changes in incidence (e.g., elm yellows and lethal yellowing of coconut palm). Even though diseased plants are available as inoculum sources for only a few weeks to a few months before death, the population is decimated within several years (1,7,10,16). Logistic models of increase have been applied to these diseases (10,16). X-disease of *Prunus,* although not as rapidly lethal as the elm and palm diseases, also seems to fit this model (11,14). The other disease type is characterized by slowly declining tree vitality and gradual increase in incidence (e.g., ash yellows and sandal spike). Although potential inoculum accumulates in diseased individuals, the rate of disease increase does not reflect this accumulation. Sandal spike has been observed to increase only 1.6–3.6% per year (13). Average rates of ash yellows increase in this study were all below 9% of the initial population per year. Steady increase over many years, rather than sporadic large increases, apparently accounts for the high incidence of ash yellows observed on some sites. Such a disease may still have a major impact on the susceptible population, but growth losses and mortality accumulate slowly and may be partially offset by ingrowth of nonhosts (22).

Gradual increase of ash yellows over many years explains the pattern of symptoms commonly observed in affected stands of white ash. Symptoms other than dieback are more noticeable in suppressed trees and those of intermediate canopy position than in dominant and codominant trees, although many trees in the latter groups may be infected based on DAPI test results. This pattern develops because growth of most trees with ash yellows is retarded (18), resulting in diminished competitive ability. Many diseased individuals, although initially among the largest in the population (18 and this study), come to occupy subordinate canopy positions and die from suppression (22). If many trees in the stand are of pole size or larger before infection, gradual increase of ash yellows may lead to a high proportion of slowly growing trees with abnormally high incidence of dieback, as observed by various authors (2,5,9,21).

Observations of phytoplasmal disease incidence, increase, and spatial pattern permit inferences about vector behavior. The apparent independence of initial incidence and rate of increase of ash yellows could result from low populations, inefficiency, or limited movement of vectors, or from *Fraxinus* spp. being dead-end hosts for ash yellows phytoplasmas transmitted from other plants. We favor the explanations related to vector behavior, because ash yellows phytoplasmas compose a discrete group (3,4,8,15) that has been detected only in *Fraxinus* and the closely related genus *Syringa* in the field (4,6). Alternative hosts were previously sought without success (4) on three of the sites of the present work. The greater incidence of new infections in ash with unshaded crowns than in those shaded by other plants may reflect more vector movement to exposed ash than to those beneath other vegetation. This suggestion is consistent with previous observations that the first plants to show yellows-associated growth decline in ash sapling populations were those with crowns above the prevailing vegetation (18).

The contrast between lack of yellows increase in ash population 3 and steady increase in populations 4 and 5 is of interest, because all three populations consisted of small plants and inoculum was available on all three sites. The ash overstory above population 3 experienced yellows increase averaging 7% per year. Population 3 differed from populations 4 and 5 in being scattered among other plant species, whereas the latter two populations were the predominant understory vegetation on their respective sites. Lack of yellows increase in population 3 could reflect interference of other plant species with ash detection by phytoplasma vectors, or vectors may prefer the overstory if ash is an important component of it.

Spatial patterns of trees with ash yellows were of interest because a finding of discrete foci of disease at the scale of observation used in this study (meters to tens of meters) would implicate very short-range, tree-to-tree spread of the causal agent, whereas absence of foci would be consistent with an hypothesis of vector flights through or above the canopy or possible transmission of phytoplasmas to ash from alternative plant hosts. Our results and the previous failure to detect alternative hosts (4) are consistent with the hypothesis that, on a given site, insect vectors transmit ash yellows phytoplasmas to healthy ash that are randomly arranged with respect to those already diseased.

The proximity test was useful for assessing the spatial relationship of newly diseased to previously diseased trees. The test requires equal numbers of previously healthy and previously diseased trees that are nearest neighbors of newly diseased trees, because inequality would bias the calculation of mean distances D_d and D_h. For example, if previously healthy trees were more numerous than previously diseased ones, and all previously healthy trees had equal probability of becoming infected, then mean D_h would be less than mean D_d. Equal numbers would rarely be encountered on a field plot. Therefore, if initial disease incidence is <50%, the previously healthy neighbors must be members of a random sample having the same number of trees as the previously diseased group. If the initial incidence of disease on a plot is >50%, sampling procedure would be inverted; a random sample of previously diseased trees equal in number to the previously healthy trees would be needed. This test is only applicable when the frequencies of diseased individuals in clustered versus scattered trees do not differ significantly. The test of Pielou and Foster (12) can be used to address this prerequisite.

Diagnosis of ash yellows based on symptoms was strongly correlated with results of DAPI tests (Fig. 3) and thus was useful for corroborating disease increase over time, but symptom assessment gave conservative estimates of the proportion of ash infected with phytoplasmas. This disparity occurred because phytoplasmas were usually detected before symptoms appeared and because 18% of the infected trees did not show yellows symptoms during the period of observation. The delay between phytoplasma detection and symptom expression was longest and the proportion of infected trees remaining asymptomatic was highest in populations 1 and 2, in which newly diseased trees were larger than in the other populations. This information is consistent with pre-

TABLE 1. Tests of spatial relationships among diseased and healthy white ash on four plots

Ash population and plot	Number of ash	Pielou and Foster's test[a] $\frac{Np-x}{\sqrt{(Np(1-p))}}$	Pielou and Foster's test[a] P	Proximity test[b] D_d (m)	Proximity test[b] D_h (m)	Proximity test[b] t	Proximity test[b] P
2A	134	1.40	0.16	2.6	2.3	0.32	>0.5
2B	131	0.06	0.95	3.0	2.8	0.87	>0.3
2C	155	1.76	0.08	1.8	2.6	0.21	>0.5
6	252	1.48	0.14	3.2	4.8	0.11	>0.5

[a]This test compares frequency of disease in crowded vs. scattered trees within a population, as explained in text. Null hypothesis: probability of a tree being infected is independent of its degree of isolation. Difference in frequencies is represented by $(Np - x)/\sqrt{(Np(1-p))}$, which is treated as a standardized normal variate with probability determined by reference to a table of the normal distribution (12). If $P > 0.05$, the null hypothesis is accepted.

[b]Null hypothesis: mean distance D_d from a newly diseased tree to nearest previously diseased tree does not differ from mean distance D_h from a newly diseased tree to nearest previously healthy tree in a random sample equal in size to the sample of previously diseased trees. Sample sizes in this test ranged from 14 to 35 trees.

vious reports of asymptomatic phytoplasma-infected ash (20,21). Diagnosis based on symptoms other than witches'-brooms is not reliable for large trees of white ash or for trees of any size in phytoplasma-tolerant ash species such as *F. velutina* (17). Symptoms were recorded for some trees before phytoplasmas were detected in them (Fig. 4). These records are attributed to erroneous symptom interpretation and false negative DAPI test results.

The rates of ash yellows increase reported here may slightly underestimate the true rates in the populations studied for two reasons. First, many plants (approximately 15% of the total) died or their identities were lost before the end of the study. The final available record for each such plant was carried forward. Some of the lost plants tallied as healthy might have become diseased during the remainder of the study. Second, incidence was underestimated because of false negative DAPI test results, indicated by positive-to-negative changes in results for certain trees. False negative results may reflect faulty sampling, discontinuous phytoplasmal distribution in diseased trees, or failure of observers to notice evidence of phytoplasmas that were present in the sections examined. The estimated frequency of false negative results, 5.9%, is based in part on the assumption that every positive result was correct, an assumption known to be flawed because of occasional errors sampling roots in the field. This estimate is higher and probably more reliable than that obtained previously by multiple sampling of 15 diseased white ash at one time (20). The approximate true incidence of phytoplasmal infection, I_t, in a given sample can be computed by means of the formula $I_t = I_o + f(1 - I_o)$, where I_o is the proportion of ash in which phytoplasmas were detected and f is the estimated frequency of false negative results. For our data, the highest incidence observed, 61.1% diseased in population 5 in 1993, could thus be adjusted to 63.4%.

Year-to-year changes in symptom ratings from diseased to possibly diseased or healthy were more common (9.1%) than the changes in DAPI test results. Symptom assessment was anticipated to be less reliable than DAPI tests because of delayed symptom expression and because subtle symptoms of ash yellows are unreliable for diagnosis (17). We encountered particular difficulty interpreting symptoms on small plants because of slow growth and broomlike deformities induced by deer browsing, foliar deformities of unknown cause, and occasional infestation by oystershell scale, *Lepidosaphes ulmi.*

Mycoplasmal diseases other than ash yellows that cause slow decline of landscape and forest trees are little known. This is the first epidemiological study of such a disease in North America. The approaches and techniques used for studies of ash yellows may be helpful as other phytoplasma-induced decline-type diseases are investigated.

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