ASH YELLOWS OCCURRENCE AND ASSOCIATION WITH SLOW GROWTH OF GREEN ASH IN IOWA AND WISCONSIN CITIES

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Abstract. Green ash in nine cities in Iowa and Wisconsin were surveyed in August and September 1994 for occurrence and impact of ash yellows (AshY) phytoplasma infection. In each city, the survey included 12 arbitrarily selected trees in each of three crown condition categories: less than 10% crown dieback, 11 to 30% crown dieback, and more than 30% crown dieback. Up to four trees per community with witches'-brooms were also sampled. Occurrence of phytoplasmal infection in sampled trees lacking witches'-brooms ranged from 3% to 19% among the cities in the survey. The number of trees that tested positive for phytoplasmas, or that exhibited epicormic sprouts, deliquescent branching, basal bark cracks, or basal shoots, did not differ significantly among the crown dieback classes sampled. Trees that displayed witches'-brooms or basal shoots were significantly ($P<0.005$) more likely to be infected with phytoplasmas (54%) than trees lacking these symptoms (13%). The presence of epicormic sprouts, deliquescent branching, or basal bark cracks was not significantly associated with presence of phytoplasmas. Trees infected with phytoplasmas had significantly ($P<0.0001$) less radial growth than noninfected trees from 1973 to 1994.

Introduction

Ash yellows (AshY), caused by the AshY phytoplasma, affects several ash species in the northeastern, midwestern, and western United States, including green ash (*Fraxinus pennsylvanica* Marsh.). Symptoms on green ash include witches'-brooms, deliquescent branching, reduction in radial growth, and dieback; but this species seems to be less susceptible to severe damage than another common, indigenous species, white ash (*Fraxinus americana* L.) (2, 10).

Green ash is among the most commonly planted trees in midwestern U.S. cities and towns, often constituting 20 to 40% of the urban forest. If AshY poses a significant threat to this resource, substantial economic and environmental losses to homeowners and local, state, and federal governments could ensue. AshY was first identified in the Upper Midwest region of the United States less than a decade ago (15). In a survey of four states in the region, Luley et al. (6) found that AshY infection of green ash was common in woodlands; however, no information on AshY incidence or its impact on the health of urban green ash has been published. We surveyed green ash in nine cities in Iowa and Wisconsin to gain a clearer picture of the occurrence of AshY and its relationship to tree health.

Materials and Methods

Survey. Municipal foresters in six cities in Iowa (Burlington, Davenport, Des Moines, Ft. Dodge, Iowa City, and Waterloo) and three cities in Wisconsin (Madison, Milwaukee, and Waukesha) conducted the field survey in August and September 1994. In each city, 12 arbitrarily selected green ash in each of three categories based on the extent of crown dieback (7,9) were examined and sampled. These categories were: 1) less than 10% crown dieback, 2) 11-30% dieback, and 3) more than 30% crown dieback. In addition, a maximum of four trees per city with witches'-brooms on trunks or main branches were examined and sampled (10). Occurrence of epicormic sprouts, deliquescent branching, basal bark cracks, and basal shoots (shoots arising from the trunk at or below the soil line) on sampled trees also was noted (6, 10). Trees included in the survey had trunk diameters greater than 15 cm at a height of 1.4 m above the ground (dbh).

Growth measurements and diagnostic assay. A 3-mm-diameter increment core was extracted from the trunk of each tree at 1.4 m above the ground. Widths of growth rings were measured ($\pm$ 0.2 mm) by using a dissecting microscope and a ruler.

Samples of fibrous roots 3 to 30 cm below the soil surface were obtained from two locations near the trunk of each tree, sealed in plastic bags with moist paper towels, and mailed immediately to Iowa

Table 1. Number of trees with phytoplasma (Ash Y) infection or associated decline symptoms in urban green ash populations classified by percent crown dieback[a].

	Crown dieback category		
Symptom	<10%	10 to 30%	>30%
Phytoplasma (AshY) infection	13	16	13
Epicormic sprouts	54	62	66
Deliquescent branching	10	12	11
Basal cracks	14	19	24
Basal shoots	6	5	11

[a] Based on Chi-square tests (df=2) for contingency tables, frequencies of above symptoms are not associated with crown dieback.

Table 2. Number of phytoplasma-infected green ash identified in sample populations[a] exhibiting or lacking specific decline symptoms.

	Symptom present			Symptom absent			
Symptom	Infected	Total	% Infected	Infected	Total	% Infected	P[b]
Epicormic sprouts	29	193	15.0	18	137	13.1	0.750
Deliquescent branching	9	37	24.3	36	284	12.7	0.074
Basal cracks	8	56	14.2	41	277	14.8	1.0
Basal shoots	10	29	34.5	38	301	12.6	0.004
Witches'-brooms	7	13	53.9	40	313	12.8	0.001

[a] N=334. Discrepancies in column totals are due to missing (nonreported) data.
[b] Based on Fisher's exact test for 2 x 2 contingency tables. P=the probability of observing a table with equal or greater evidence for association, given the null hypothesis of equal proportions for symptomatic and asymptomatic trees (8).

State University, where they were refrigerated. Within 2 wk after receipt, root segments 1.5 cm long and 1.5 to 3.0 mm in diameter were excised and preserved in 2.5% glutaraldehyde in 0.1 M phosphate buffer (13). Segments of one root per tree were tested for phytoplasmas with the 4',6-diamidino-2-phenylindole (DAPI) DNA-staining technique (13). A second root from the same tree was tested if DAPI results from the first root were inconclusive.

Confirmation of identity of AshY phytoplasma. Phytoplasmas previously detected in *Fraxinus* spp. have all been identified to be members of the AshY group (1). To confirm the findings obtained using the DAPI technique on roots of trees from Ft. Dodge, IA, Iowa City, IA, and Milwaukee, WI, nucleic acid was extracted from these samples. The extraction procedure described by Lee et al. (4) was used in conjunction with the Prep-A-Gene DNA purification kit (Bio-Rad, Hercules, CA). These DNA preparations along with nucleic acid extracts from healthy green ash (negative control), green ash graft-inoculated with an AshY phytoplasma (positive control), and sterile distilled water were used as templates in polymerase chain reactions (PCRs). The reactions were performed using the universal primer pair R16F2R2 and published PCR conditions (5). This universal primer set amplifies DNA from all known phytoplasmas giving a product approximately 1.2 kb in size (5). Unique profiles for the various phytoplasmal groups can be obtained by digesting the PCR products with specific restriction enzymes (5). The restriction enzyme Alu1 has been identified as an enzyme which will digest the 16SF2R2 product to give a profile unique to members of the AshY group (5). Each PCR product obtained was digested with Alu1 (GIBCO BRL, Gaithersburg, MD) according to the instructions of the manufacturer. Amplified products and restriction enzyme digests were examined as described by Lee et. al. (5).

Statistical analyses. The strength of association of the three crown dieback categories with frequency of various symptoms and of AshY infection was tested by Chi-square analysis. Two-by-two contingency tables were used to determine whether the proportion of AshY-infected trees associated with the presence or absence of certain symptoms differed significantly. Analysis of variance (8) was performed on mean radial growth data for the preceding 3, 10, and 20 yr to test for differences associated with phytoplasmal infection, location (city), and tree diameter.

Results

Frequency of occurrence of phytoplasmal infection. Of a total of 315 trees for which viable roots were assayed, including 13 trees with witches'-brooms, 50 (15.9%) were DAPI-positive. Excluding the trees with witches'-brooms (because these trees were selected deliberately as likely to be infected with ash yellows), occurrence of DAPI-positive trees in the survey was 12.3% overall and in the surveyed cities was as follows: Waterloo, 19.4%; Davenport, 18.8%; Milwaukee, 16.7%; Burlington and Iowa City, 13.9%; Des Moines, Waukesha, and Fort Dodge, 11.1%; and Madison, 2.8%. Because the sample in this survey was nonrandom, occurrence of DAPI-positive trees may not represent true incidence in the population of green ash in each city.

Most of the sample sets included fewer than 40 trees. This occurred because co-operators in most communities were unable to locate four trees with witches'-brooms, and several sampled fewer than 12 trees in one or more of the crown dieback category. In addition, roots from a few sampled trees were too decayed or desiccated for DAPI assay. The number of sampled trees with viable roots that were assayed by DAPI ranged from 33 to 40 per city.

Association of crown dieback with phytoplasmal infection and other symptoms. The frequency of occurrence of infection by the AshY phytoplasma was not significantly related to crown dieback (Table 1). Similarly, frequency of occurrence of epicormic sprouts, deliquescent branching, basal cracks, or basal shoots was not significantly associated with crown dieback.

The presence of witches'-brooms or basal shoots was associated with a higher proportion of phytoplasma-infected trees than that seen in trees lacking these symptoms (Table 2). The presence or absence of epicormic sprouts, deliquescent branching, or basal cracks, however, had no detectable association with phytoplasmal infections.

Table 3. Analysis of variance in mean radial growth during 1992 to 1994 for green ash trees sampled in nine Iowa and Wisconsin cities. Analysis of mean radial growth for the past 10 years (1985 to 1994) and the past 20 years (1975-1994) indicated similar trends (unpublished data). Similar conclusions were obtained when the analysis was conducted using tree diameter as a covariate (continuous) variable.

Source	df	SS	MS	*F*	P>*F*
Phytoplasmas detected[a]	1	293.50	293.50	22.77	0.0001
City	8	90.58	11.32	0.88	0.5409
Phytoplasmas detected X city	8	20.88	2.61	0.20	0.9892
Diameter class[b]	3	43.21	14.40	1.12	0.3507
Phytoplasmas detected X diameter class	3	4.39	1.46	0.11	0.9521
City x diameter class	20	222.58	11.13	0.86	0.6299
Phytoplasmas X city X diameter class (Error I)[c]	52	670.30	12.89	2.78[d]	0.0001
Error II (sample variation)	213	988.20	4.64		
Corrected total	308	2333.64			

[a] Results of DAPI test for the presence of phytoplasmas in root sections.
[b] Tree diameter (at 1.4 m above the ground) classes of 0 to 30 cm, 31 to 50 cm, 51 to 70 cm, and 71 to 100 cm.
[c] In this survey, the number of DAPI-positive and DAPI-negative trees in each diameter class and city was not controlled (n=1 to 22). An analysis of the unweighted means for factors specified in the effects was conducted using the interaction mean square as the error term and locations (cities) as replications.
[d] The variance for individual observations (Error II) was used to test the significance of the interaction effect.

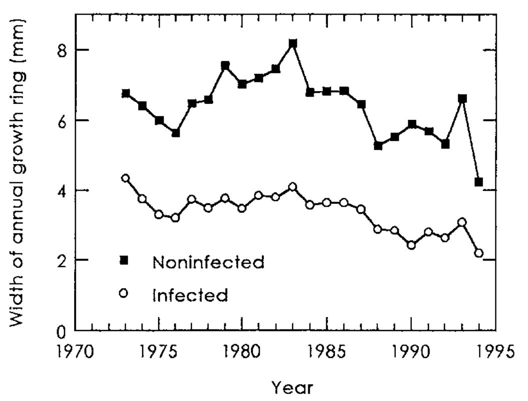

Figure 1. Mean width of annual growth rings in the years 1973 to 1994 for DAPI-positive green ash (n=49) and DAPI-negative green ash (n=264) in nine Iowa and Wisconsin cities. Mean radial growth for the last 3 yr (1992-1994), 10 yr (1985-1994), and 20 yr (1975-1994) was significantly (Prob>F = 0.0001) less in DAPI-positive than DAPI-negative trees.

Radial growth of trees infected with AshY phytoplasmas. Mean radial growth was significantly less in DAPI-positive trees than in other trees (Fig. 1 and Table 3). This pattern was significant ($P<0.0001$) for growth during the last 3 yr (1992-1994), 10 yr (1985-1994), and 20 yr (1975-1994). Mean radial growth was not affected significantly by either trunk diameter or city (Table 3).

Confirmation of identity of AshY phytoplasmas. DNA products of the predicted size (1.2 kb) were obtained for root samples from trees in Fort Dodge, Iowa City, and Milwaukee locations. Digestion of these PCR products with *Alu1 gave profiles identical to the AshY control* and to those previously reported for AshY (5).

Discussion

The results provide the first evidence that AshY infects green ash in many Iowa and Wisconsin cities. Luley et al. (6) documented widespread occurrence of the disease in forest stands of green and white ash in Illinois, Wisconsin, Missouri, and Iowa but in only 10% of surveyed sites in Wisconsin. Because our sample was nonrandom, however, data on occurrence of AshY do not represent true incidence of the disease in the population of green ash in the sampled cities.

Characteristic symptoms of AshY infection - witches'-brooms and basal shoots (11) - showed a statistically significant association with presence of phytoplasmas in our survey. Witches'-brooms, which are diagnostic for AshY, were seldom seen in our survey. Furthermore, when epicormic sprouts on trunks of urban green ash are pruned repeatedly, regrowth can resemble witches'-brooms. It is therefore possible that some epicormic sprouts were misidentified as witches'-brooms. Sinclair et al. (11) observed that at a given time, only a small proportion of infected ash exhibited witches'-brooms, the most recognizable field symptom of AshY. Luley et al. (6) found no significant relationship of the presence of basal shoots to AshY infection of green ash in woodlands, but their data pertained to plots rather than individual trees.

The absence of a significant association between phytoplasmal infection and extent of crown dieback in our survey is in accord with the findings of Luley et al. (6). Sinclair et al. (11) noted that dieback of infected green ash in woodlands in the northeastern U.S. seemed to be restricted to saplings and understory trees. The trees we surveyed, in contrast, were located in relatively uncrowded street and park sites which were likely to have been far less shaded than the understory of woodland environments.

Infection was clearly associated with reduced radial growth. On average, DAPI-positive trees exhibited about one-half the annual radial growth of DAPI-negative trees between 1973 and 1994. *Growth reduction associated with phytoplasmal* infection of green ash is consistent with the results of Sinclair et al. (12) from New York woodlands and Ferris et al. (2) in greenhouse studies with green ash seedlings. Although our findings suggest that phytoplasmal infection resulted in reduced growth rate, such a causal relationship cannot be confirmed from our data.

Our findings add to earlier evidence (6, 11, 12) that AshY infection is associated with reduction of the growth rate of green ash but seldom acts as the sole cause of decline. AshY may promote tree decline when combined with other stresses, such as drought (3), competition from neighboring trees, insect attack (10), Verticillium wilt, root disturbance, and oxygen deprivation of roots. Our survey showed that the extent of decline in the sampled green ash could not be explained by presence or absence of the AshY phytoplasma; instead, the observed decline must have been caused by other stresses. Many urban green ash in our survey have apparently tolerated phytoplasmal infection for at least several decades without suffering any symptoms other than slow growth. Nevertheless, as a precaution, management practices designed to reduce stress and promote tree vigor, such as timely watering and mulching with wood chips (10, 16), may be advisable for AshY-infected trees (11). Even without receiving special care, however, many infected green ash in urban environments in the Upper Midwest seem able to respond to favorable growing conditions. In 1993, for example, above-normal rainfall increased radial growth of both AshY-infected and uninfected green ash (Fig. 1).

This survey provides the first verification of the identity of phytoplasmas in ash shade trees in Iowa and Wisconsin. Phytoplasmas from DAPI-positive trees in three cities were confirmed to be members of the AshY group by using PCR and restriction enzymes. As the survey region is within the previously reported range of AshY phytoplasmas (1,6,14,15) it is likely that all the samples which were DAPI-positive were due to infections caused by phytoplasmas in the AshY group.

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Résumé. Les frênes de Pennsylvanie (*Fraxinus pennsylvanica*) de neuf villes de l'Iowa et du Wisconsin ont été inventoriées en août et septembre 1994 relativement au commencement et aux impacts causés par l'infection du phytoplasme jaune du frêne. Dans chaque ville l'inventaire comptait 12 arbres sélectionnées sur une base arbitraire pour chacune des trois catégories de dépérissement de cime: 10%, 11-30% et 30%. Jusqu'à quatre arbres par ville qui comportaient des balais de sorcière ont aussi été inventoriés. Le début d'infection phytoplasmique sur les arbres échantillonnés sans balais de sorcière se situait entre 3 et 19% dans les villes sélectionnées. Le nombre d'arbres qui donnaient un test positif quant aux phytoplasmes, ou encore qui exhibaient des rejets épiphytes, des branches « délinquantes », des fissures de l'écorce ou des pousses adventives, n'était significativement pas différent entre les classes de dépérissement de cime de l'échantillonnage. Les arbres qui comportaient des balais de sorcière ou des pousses anarchiques étaient, de façon significative (P 0.005), plus sujets à être infectés par les phytoplasmes (54%) que les arbres dont ces symptômes étaient absents (13%). La présence de pousses épiphytes, de branches « délinquantes » ou de fissures de l'écorce n'a pu être associée, de façon significative, à la présence de phytoplasmes. Les arbres infectés par les phytoplasmes avaient une croissance significativement (P 0.0001) moindre en diamètre que ceux non infectés entre 1973 et 1994.

Zussammenfassung. Im August und September 1994 wurden in neuh Städten in Iowa und Wisconsin an Eschen das Auftreten von Eschenvergilbung, einer Infektion des Phytoplasmas, untersucht. In jeder Stadt wurden für die Studie 12 Bäume ausgewählt, die sich in drei verschiedene Kategorien des Kronenabsterbens (<10%, 11-30%, u.>30%) unterteilen lassen. In jeder Gemeinde wurden zusätzlich noch 3 bis 4 Bäume mit Hexenbesen aufgenommen. Das Auftreten der Phytoplasma-Infektion an den aufgenommenen Bäumen rangierte von 3 bis 19%. Die Anzahl der Bäume, die im Test positiv erkrankt waren oder adventives Triebwachstum, basale Rindenrisse und dergleichen zeigten, differierten nicht zwischen den drei genannten Kategorien. Bäume mit Hexenbesen oder basalen Schoßern waren deutlich ($P<0.005$) anfälliger für die Infektion (54%) als Bäume ohne diese Symptome (13%). Das Aufteten von Adventivknospen, Rindenrissen etc. war nicht signifikant mit dem Auftreten der Phytoplasma-Infektion assoziiert. Von 1973 und 1994 zeigten Bäume, deren Phytoplasma infiziert war, deutlich ($P<0.0001$) weniger radialen Zuwachs als nicht-infizierte Bäume.