OCCURRENCE, ASSOCIATED SYMPTOMS, AND POTENTIAL INSECT VECTORS OF THE ASH YELLOWS PHYTOPLASMA IN IOWA, U.S.

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Abstract. The ash yellows (AshY) phytoplasma has been reported widely across the United States as an organism associated with ash decline in urban communities. Prior surveys conducted in Iowa communities indicated that AshY was found in up to 20% of the urban green ash (*Fraxinus pennsylvanica* Marsh.) when using the DAPI (4' ,6-diamidino-2-pheylindole·2HCl) staining method. A new survey of trees in nine Iowa communities used polymerase chain reaction (PCR) to detect the AshY phytoplasma. PCR analyses of tree tissue were positive for AshY in 4% of the 240 trees surveyed. Although 145 of the sampled trees had decline symptoms similar to those exhibited by trees infected with the AshY phytoplasma, the phytoplasma was detected in only 11 trees. Because Homoptera have been shown to vector phytoplasmas, insect populations associated with ash trees in two Iowa communities were also sampled to help determine whether the insects were carrying phytoplasmas. A total of 471 insects, representing 34 species, were collected from green ash trees; of these, 396 were assayed for the presence of phytoplasmas using PCR. Three leafhopper species were found that were reported to vector phytoplasma diseases, but phytoplasmas were not detected in any of the insects assayed.

Key Words. Homoptera vectors; mycoplasmalike organism; polymerase chain reaction.

Green ash (*Fraxinus pennsylvanica* Marsh.) and white ash (*Fraxinus americana* L.) are widely distributed and planted throughout the midwestern United States. Over the past two decades, there have been reports that ash trees are declining throughout the United States (Castello et al. 1985; Riffle and Peterson 1986; Woodcock et al. 1997). Ash yellows (AshY), caused by the AshY phytoplasma, has been widely detected and associated with declining ash trees in the northeastern, midwestern, and western United States (Matteoni and Sinclair 1985; Sinclair et al. 1990; Luley et al. 1992; Gleason et al. 1997; Walla et al. 2000). The reported symptoms of AshY have included witches' brooms, stem dieback, loss of apical dominance, and reduced growth (Sinclair et al. 1994a). Both white and green ash are susceptible to the AshY phytoplasma, with indications that the effects may be more severe on white ash (Ferris et al. 1989).

The overland spread of phytoplasmas relies on phloem-feeding insects such as leafhoppers (Cicadellidae), planthoppers (Cixiidae), and spittlebugs (Cercopidae) (Nault and Rodriguez 1985; Maixner et al. 1993; Murral et al. 1996; Tsai 1979, Weber and Maixner 1998). Nonhomopteran insects, in the genus *Halyomorpha* (Hemiptera: Heteroptera: Tingidae), have also been shown to vector phytoplasmas (Doi and Asuyama 1981). The phytoplasmas are ingested when insects feed on previously infected plant tissue, and probably increase in number in the insect's foregut. They are then passed to the plant through the insect's salivary secretions when feeding on uninfected plant tissue (Nault and Rodriguez 1985; Lefol et al. 1994).

The diagnosis of AshY has relied primarily on epifluorescent microscopic examination of tissues stained with DAPI (4', 6-diamidino-2-pheylindole·2HCl) (Luley et al. 1992; Sinclair and Griffiths 1994; Gleason et al. 1997). DAPI binds to DNA and is used to stain the genome of microorganisms, such as the AshY phytoplasma, in the phloem tissue. The DAPI staining test can be used to indicate the presence of microorganisms

in the phloem; however, it does not distinguish among different organisms (Coleman 1978).

Recent surveys conducted in the midwestern United States using the DAPI staining test have reported a high occurrence of the AshY phytoplasma. In 1990, a survey conducted in Iowa, Illinois, Missouri, and Wisconsin showed that 49% of the surveyed plots contained DAPI-positive trees that were associated with ash decline symptoms (Luley et al. 1992). In 1997, a midwestern survey of community trees showed that 15% to 20% of the surveyed trees were DAPI positive (Gleason et al. 1997). Other reports from the upper Great Plains and Rocky Mountain regions using monoclonal antibodies have shown a 47% to 60% occurrence of AshY (Walla et al. 2000).

The development of polymerase chain reaction (PCR) has allowed ultra-sensitive detection of the AshY phytoplasma in the host and the insect vector (Hill and Sinclair 2000). The only reported vectors of the AshY phytoplasma that have been identified using PCR are leafhoppers in the subfamily Deltocephalinae (Hill and Sinclair 2000). The detection of phytoplasmas in insects using PCR does not necessarily indicate that the insects are capable of transmitting the phytoplasma. However, it does help identify the insects that have the potential to transmit phytoplasmas. The objectives of this study were to determine the occurrence of the AshY phytoplasma in urban ash trees and identify potential insect vectors in Iowa using PCR.

MATERIALS AND METHODS

Tree Survey for Ash Yellows

Nine cities (Ames, Burlington, Council Bluffs, Des Moines, Dubuque, Fort Dodge, Iowa City, Mason City, and Sioux City) in Iowa were selected to be part of the survey. The sampling sites were selected without bias for species of ash, ash health conditions, or the presence of the AshY phytoplasma. The sites were located across the state and selected as representative of Iowa's urban and community forests. The trees in each of the surveyed sites were selected arbitrarily on the basis of health and separated into two groups, healthy or declining, based on visible symptoms. Symptoms characteristic of declining trees but absent in healthy trees included main branch mortality, an abundance of small twig mortality, sparse foliage, clustered foliage, loss of apical dominance, basal bark cracks, chlorotic foliage, an abundance of epicormic sprouts, basal sprouts, and the presence of witches' brooms. Both pale green foliage and bright yellow foliage, common with the presence of the AshY phytoplasma, were recorded as chlorotic (Sinclair and Davis 1996). Other symptoms recorded included the presence of decay, insect damage (feeding and boring), and any other obvious damage. Field data were also collected on the visually estimated percentage of dieback of the main stem and smaller twigs. Current-year shoot elongation was measured on each tree by taking the average growth (cm) of three different areas in the crown. Attempts were made to select branch material from 15 healthy and 15 declining trees in each community.

Twig samples at least 15 cm (5 in.) long and 2 cm (0.8 in.) in diameter were collected from the crown of each tree between July 1, 1999, and August 31, 1999. If a tree had decline symptoms that were not uniformly distributed throughout the crown, a sample from more than one part of the crown was taken for testing. For example, if a tree had witches' brooms under 2 cm in diameter on the main stem but the crown seemed to be healthy, a sample was taken from the crown as well as from the witches' brooms. Samples were packed in ice for transport to Iowa State University and stored at 4°C (39°F). DNA from the samples was extracted within 3 days of twig collection.

Tree Selection for Insect Collection

Twenty-four green ash trees in each of two cities (Ames and Iowa City, Iowa) were selected for insect sampling. The 24 trees in Iowa City were

selected in May 1997 based on decline symptoms and DAPI results from Gleason et al. (1997), including 12 DAPI-positive trees thought to be infected with AshY and 12 DAPI-negative trees without decline symptoms. Twenty-four trees in Ames were selected in May 1998 based only on the presence or absence of decline symptoms—12 healthy and 12 declining trees. The same symptoms used in the tree survey were used to identify declining trees in the insect study. Insects were collected from the trees in Iowa City during the 1997 and 1998 growing seasons. Insects were collected from trees in Ames during the 1998 and 1999 growing seasons.

Insects were collected once each week from the 48 trees using a telescoping BioQuip Monarch™ (Gardena, CA) insect net. Insect collections for each tree started in the last week of May when the insect emergence was noted and ended the first week of September when the insect populations were low. The collection times were between 8:00 A.M. and 5:00 P.M. for the convenience of city personnel and the landowners. Samples were taken from two sides of the tree, the shaded side and the side in full sun, midway in the crown. A sample consisted of four swipes of the net through the foliage on each side of the tree. The insects were then sealed in a plastic bag and packed in ice until they were returned to the laboratory, where they were stored in an ultra-low [–80°C, (–112°F)] freezer. The insects from each tree were sorted by their morphology on a cold table and identified to at least morphospecies before DNA extraction.

Plant Tissue DNA Extraction

DNA was extracted from the inner bark using a modified hot cetyltrimethylammonium bromide (CTAB) extraction method (Doyle and Doyle 1990). The twig samples were surface sterilized in a 10% bleach solution followed by rinsing with sterile water. The outer bark was stripped away using a sterile surgical scalpel to expose the living inner bark. Approximately 200 mg of fresh inner bark was stripped from each twig sample, frozen in liquid nitrogen, and pulverized in a prechilled mortar. The powdered material was placed in 600 µL of preheated CTAB extraction buffer and incubated for 20 minutes at 65°C (149°F). Samples were extracted once with phenol-chloroform-isoamyl alcohol (25:24:1) and once with chloroform-isoamyl alcohol (24:1). The aqueous DNA layer was precipitated overnight at –20°C (–4°F) with 0.6 volumes isopropanol. DNA pellets were washed with 4°C (39°F) 70% ethanol, vacuum dried, and suspended in 100 µL of sterile water.

Insect DNA Extraction

DNA was extracted from the insects using a modified hot cetyltrimethylammonium bromide (CTAB) extraction method (Doyle and Doyle 1990). Two insects of each species were ground in sterile 1.5 mL Eppendorf tubes containing 600 µL of preheated CTAB extraction buffer and incubated for 20 minutes at 65°C. Only one insect was ground from noninsectan arthropods, insects other than Hemiptera, or when only one species of Hemiptera was collected. Samples were extracted once with chloroform-isoamyl alcohol (24:1). The aqueous DNA layer was precipitated overnight at –20°C with 0.6 volumes isopropanol. DNA pellets were washed with 4°C 70% ethanol, vacuum dried, and suspended in 100 µL of sterile water.

Primer Selection

Phytoplasma-specific primers, R16mF2 and R16mR1 (Gundersen and Lee 1996), were used to amplify a portion of the 16s rDNA from DNA extractions from plant tissue. The samples were sequenced with the internal primer R16F2n (Gundersen and Lee 1996) to determine if the amplified portion of the 16s rDNA was from the AshY phytoplasma. Phytoplasma-specific primers (P1 and Tint) and AshY specific primers (fB1/rASHYS) were used to amplify a portion of the 16s rDNA from insect DNA extractions (Smart et al. 1996). The products amplified by the phytoplasma-specific primers were sequenced with the for-

ward primer P1 to determine if the amplified portion of the 16s rDNA was from the AshY phytoplasma.

DNA Amplification

A 25 μL PCR reaction containing 2.5 units of taq polymerase (Gibco BRL, Inc., Rockville, MD), buffer enzyme, 2 mM $MgCl_2$, 200 μM dNTPs, 0.5 μM of each primer, and 1 μL extracted DNA was used to amplify a fragment of the 16s rDNA. Thermal cycler (MJ Research, Inc., Waltham, MA.) conditions consisted of 35 cycles of denaturing [95°C (203°F) for 1 min], annealing [50°C (122°F) for 1.35 min], and extension [72°C (161.6°F) for 3 min]. A final extension of 72°C for 30 min was used. The PCR products were analyzed by electrophoresis with a 2% agarose gel in a 1X Tris (89 mM Tris, 89 mM boric acid, 2 mM EDTA) buffer (pH 8.0). The gel was stained with ethidium bromide and rinsed. A 100-base pair (Gibco BRL) ladder was used to determine the PCR product size.

Association of Ash Yellows Symptoms

The declining trees were separated into two groups for further analysis of symptoms: 1) trees that tested positive for the AshY phytoplasma using PCR, and 2) trees that tested negative for the AshY phytoplasma. The two categories were then tested by Chi-square using Fishers exact test ($p < 0.05$) to determine if a particular symptom was associated more commonly with the AshY-positive trees or AshY-negative but declining trees. An additional analysis of variance (ANOVA) was used to determine if there were differences ($p < 0.05$) in measured twig elongation using the Statistical Analysis System (SAS) version 6.12 (SAS Institute, Cary, NC).

Analysis of Insect Collection

Insects collected from the green ash were separated into groups by the cities from which they were collected. The insects were then separated into groups from healthy and declining trees and a one-way ANOVA was done to determine whether more insects were found on healthy or declining trees, and whether there was a significant difference in the number of insects collected in each city by date.

RESULTS

Tree Survey

A total of 145 declining trees and 95 healthy trees were sampled at the nine locations. Only 11 (4%) of the 240 trees sampled tested positive for the AshY phytoplasma using PCR. The Chi-square analysis indicated ($p < 0.05$) that a significantly greater proportion of AshY-infected trees than uninfected trees had witches' brooms and epicormic sprouts (Table 1). The Chi-square

Table 1. Percentage of ash yellows positive and negative trees with main stem or small branch dieback exhibiting individual decline symptoms.

	Ash yellows positive (% of trees)		Ash yellows negative (% of trees)		
Symptoms	Main stem dieback (n = 4)	Small branch dieback (n = 7)	Main stem dieback (n = 32)	Small stem dieback (n = 102)	P*
Sparse foliage	100	43	97	70	0.46
Clustered foliage	100	0	72	37	0.75
Loss of apical dominance	100	57	22	10	0.42
Basal sprouts	50	0	53	22	0.73
Epicormic sprouts	100	43	16	4	0.01
Witches' brooms	50	14	3	0	0.001
Chlorotic foliage	100	86	94	83	0.35
Frost cracks	0	0	3	2	0.78

*P = probability that the difference between the incidence of the symptom in ash yellows positive and negative trees is due to chance based on Chi-square analysis.

analysis also indicated that remaining symptoms for the 11 AshY positive trees did not differ from the remaining symptoms of the declining AshY negative trees (Table 1). Mean twig growth was significantly less in declining trees than in healthy trees (Figure 1). However, the difference in twig growth between the AshY infected and non-AshY declining trees was not statistically significant. The occurrence of AshY was determined for the following locations: Council Bluffs, 3%; Dubuque, 4%; Fort Dodge, 6%; Mason City, 10%; and Sioux City, 18% (Table 2). AshY-positive trees were not identified in Ames, Burlington, Des Moines, or Iowa City.

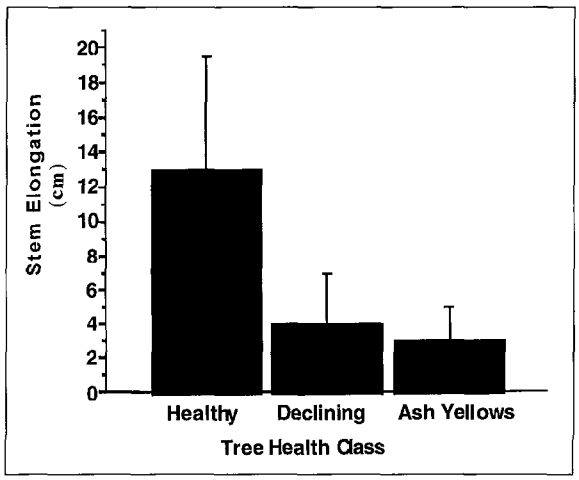

Figure 1. Mean stem elongation (cm) in current growing season for the healthy (n = 95), declining (n = 134), and ash yellows (n = 11) positive trees that were surveyed in nine communities in Iowa. Healthy trees exhibited greater enlongation than declining or ash yellows positive trees at $p < 0.05$

Insect Collection

A total of 471 insects, representing 34 different species, were collected from the surveyed green ash trees (Table 3 and Table 4). Twenty-five percent of the insects collected were Homoptera, with leafhoppers constituting 20% of the total (Table 3). The collection contained six genera of leafhoppers, two genera of spittlebugs, two genera of planthoppers, and one genus of aphids. Because of identification difficulties, many of the insects were not identified to species. The number of insects collected per city did not differ by year or community through the 2 years sampled in Iowa City (1997 and 1998) and Ames (1998 and 1999). The numbers of Hemiptera: Homoptera and Hemiptera: Heteroptera insects peaked during the month of July. The ANOVA showed that the number of insects collected from declining trees (n = 241) did not differ significantly from the number of insects collected from healthy trees (n = 230) in the two cities sampled.

Table 2. Number of surveyed declining and nondeclining ash trees that tested positive or negative for the ash yellows phytoplasma.

	Declining		Healthy	
Site	AshY-negative	AshY-positive	AshY-negative	AshY-positive
Ames	17	0	15	0
Burlington	13	0	8	0
Council Bluffs	16	1	15	0
Des Moines	14	0	11	0
Dubuque	16	1	6	0
Fort Dodge	19	2	14	0
Iowa City	7	0	6	0
Mason City	18	3	12	0
Sioux City	14	3	8	1
Total	134	10	95	1

A total of 396 insects were assayed for phytoplasmas. Only one specimen was selected for genomic DNA extraction in the Dermaptera, Orthoptera, Hymenoptera, and the noninsects because they have not been identified as likely vectors (Table 4). The 16s rDNA of phytoplasmas was not detected in any of the 396 insects screened using the P1/Tint primer set or the fB1/rASHYS primer set.

Table 3. Hemiptera: Homoptera and Hemiptera: Heteroptera collected from green ash in Iowa City and Ames during 1997, 1998, and 1999.

	Iowa City		Ames		
Insects identified	1997	1998	1998	1999	Total
Hemiptera: Homoptera					
Cicadellidae					
Draeculacephala sp.	1	1	1	1	4
Edwardsiana spp.	1	2	3	5	11
Empoasca fabae (Harris)	14	17	15	19	65
Graphocephala sp.	1	1	0	1	3
Macrosteles sp.	1	2	2	3	8
Scaphoideus sp.	3	1	0	1	5
Cercopidae					
Philaenus spumarius (L.)	1	0	2	1	4
Philaenus sp.	2	1	0	1	4
Cixiidae					
Anormenis sp.	0	1	0	4	5
Metcalfia sp.	1	2	4	1	8
Cicadidae					
Magicicada sp.	0	1	0	0	1
Aphididae					
Prociphilus	1	1	0	1	3
Hemiptera: Heteroptera					
Coreidae					
Acanthocephala sp.	0	1	0	0	1
Miridae					
Lygus spp.	4	4	6	12	26
Tropidosteptes amoenus (Reuter)	16	29	26	20	91
Tropidosteptes brooksi (Kelton)	32	35	30	26	123
Pentatomidae					
Podisus sp.	2	3	1	0	6
Reduviidae					
Pselliopus sp.	0	2	0	0	2
Tingidae					
Corythuca sp.	0	1	1	2	4
Total	**80**	**105**	**91**	**98**	**374**

DISCUSSION

Our study indicates that AshY may not be widespread in Iowa. However, it is not known if DNA extraction and PCR analysis have similar results to the DAPI and monoclonal antibody techniques used in other surveys (Sinclair et al. 1994b; Gleason et al. 1997; Walla et al. 2000). Of 240 surveyed ash trees, only 11 (4%) tested positive for the AshY phytoplasma using PCR, and we confirmed the identification by sequencing the PCR products at the Iowa State University DNA Sequencing and Synthesis Facility. This is a lower incidence than reported by Gleason et al. (1997), who found 16% of trees sampled in Iowa municipalities were AshY-positive when using the DAPI staining method. In addition, our findings indicate a large population of declining ash trees that do not have detectable levels of the AshY phytoplasma using PCR. Many declining trees that were AshY negative exhibited some of the characteristic AshY symptoms: crown dieback, reduced growth, lack of apical dominance, sparse foliage, and chlorotic foliage.

Table 4. Insects other than Hemiptera and non-insectan arthropods collected from green ash in Iowa City and Ames, Iowa during 1997, 1998, and 1999.

	Iowa City		Ames		
Insects identified	1997	1998	1998	1999	Total
Coleoptera					
Cerambycidae	1	0	0	0	1
Chrysomelidae	1	0	0	1	2
Coccinellidae	4	7	7	3	21
Curculionidae	4	3	5	0	12
Scolytidae	0	3	1	0	4
Dermaptera					
Forficulidae	0	0	1	0	1
Orthoptera					
Grylidae	1	1	2	0	4
Hymenoptera					
Formicidae	2	1	1	0	4
Diptera					
Muscidae	6	5	4	4	19
Noninsects					
Eriophyidae	4	3	1	4	12
Araneae (various spp.)	4	6	4	3	17
Total	**26**	**29**	**26**	**16**	**97**

A symptom that has commonly been used to diagnose AshY in the field is witches' brooms, although Gleason et al. (1997), Sinclair et al. (1994a), and Walla et al. (2000) have shown that many AshY-positive trees do not have witches' brooms. Based on the results of the Chi-square test, both witches' brooms and epicormic sprouts are associated with AshY positive trees.

Other researchers have analyzed growth based on incremental diameter growth of the main stem and have not found an association between AshY infected trees and loss of increment growth (Sinclair et al. 1994b; Walla et al. 2000). However, Gleason et al. (1997) did report differences in incremental growth. Based on observations of severely stunted twig growth, we considered twig elongation rather than stem diameter growth. There were significant differences in mean twig growth between healthy trees and the declining, but there was not a statistical difference in growth between AshY positive and Ashy negative but declining trees (Figure 1). These data suggest that there may be other factors responsible for the growth reduction in declining ash trees in Iowa.

None of the insects that were assayed by PCR tested positive for phytoplasmas, although several of the insects collected have been reported to transmit phytoplasmas. Using PCR, Hill and Sinclair (2000) reported various *Scaphoideus* species as potential vectors of the AshY phytoplasma in New York. Hoy et al. (1992) reported *Macrosteles* spp. to vector aster yellows on numerous plant species. Matteoni and Sinclair (1988) reported the spittlebug *Philaenus spumarius* (Linnaeus) to be a vector of both the elm yellows phytoplasma and the AshY phytoplasma.

The most common insects found in our survey were the mirid species *Tropidosteptes brooksi* (Kelton) and *Tropidosteptes amoenus* (Reuter), which are not suspected to be vectors of phytoplasmas. *Empoasca fabae* (Harris) made up the majority of the leafhopper population found feeding on the green ash in Iowa. This species had been reported to cause feeding damage on various other crops in Iowa (DeGooyer et al. 1998).

It was not surprising that we did not detect phytoplasmas in the insects screened after it was determined that the AshY phytoplasma was not detected by PCR in the surveyed trees. The sampling methods used in our survey proved to be less laborious than the root sampling typically used with DAPI and monoclonal antibodies, but it is important to reiterate that it is not yet known if DNA extractions from the inner bark of twigs will yield similar results to the DAPI staining of root tissue. Overall, we found numerous declining trees that have comparable symptoms to trees infected with the AshY phytoplasma: stunted growth, lack of apical dominance, and sparse foliage. Diverse populations of plant-feeding insects were found in the green ash trees sampled. Several species of leafhoppers and spittlebugs feeding on ash trees have been identified as likely

phytoplasma vectors in other studies and were sampled in this study, indicating that we have viable populations of these insects in the midwestern United States.

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Acknowledgements. The authors wish to acknowledge Christine Hall, Danielle Schaffert, and Barb Seifert for technical assistance in the field, and offer thanks to Joseph Steimel, Doug McNew, Daniel Hermann, and Valasia Iakovoglou for assistance in the lab. We would also like to thank city and state personnel—Terry Robinson, Raelynn Scheppers, Matt Pepper, Steve Pregler, John Murrow, John Walkowiak, Todd Redenius, and Dave Asche—for their assistance. We thank Drs. J.M. Kelly and Mark Gleason, and the anonymous reviewers for their reviews of the manuscript. Journal Paper No. J-19199 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa, Project Nos. 3413 and 3443, and supported by McIntire-Stennis, Hatch, U.S. Forest Service, and the Iowa Nursery and Landscape Association.

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Résumé. Les mycoplasmes du jaunissement du frêne ont été rapportés largement à travers tout les États-Unis comme des organismes associés au dépérissement du frêne dans les communautés urbaines. Les premiers inventaires menés dans les communautés de l'Iowa ont indiqué que le jaunissement du frêne a été observé sur jusqu'à 20% des frênes rouges (*Fraxinus pennsylvanica* Marsh.) en milieu urbain lorsque l'on utilisait la méthode du marquage par le DAPI (4' ,6-diamidino-2-pheylindole2HCl). Un nouvel inventaire des arbres dans neuf communautés de l'Iowa a été effectué au moyen de la réaction de la chaîne de polymérase pour détecter le mycoplasme du jaunissement du frêne. Les analyses de tissus d'arbres par la réaction de la chaîne de polymérase ont été positifs sur 4% des 240 arbres inventoriés. Même si 145 des arbres échantillonnés avaient des symptômes de dépérissement similaires à ceux présentés par les arbres infectés par le jaunissement du frêne, les mycoplasmes ont été détectés sur seulement 11 arbres. Étant donné que les homoptères ont été identifiés comme des vecteurs de ces mycoplasmes, les populations d'insectes associées aux frênes de deux communautés de l'Iowa ont aussi été échantillonnées afin d'aider à déterminer si les insectes transportaient des mycoplasmes. Un total de 471 insectes, représentant 34 espèces, ont été recueillis de frênes; de ces insectes, 396 ont été analysés en laboratoire pour la présence de mycoplasmes au moyen du test de la réaction de la chaîne de polymérase. Trois des espèces de punaises ont été identifiées comme des vecteurs de mycoplasmes, mais aucun mycoplasme n'a été détecté sur ces insectes.

Zusammenfassung. Der AshY wurde USA-weit mit dem Rückgang von Eschen in Städten in Verbindung gebracht. Erste Studien aus Iowa mit dem Einsatz von einer Einfärbemethode (DAPI=4,6-diamidino-2-pheylindole2HCl) zeigten, dass AshY in bis zu 20 % der *Fraxinus pennsylvanica* Marsh. Bestände vorkommt. Eine neue Studie in neun Gemeinden in Iowa verwendete PCR (polymerase chain reaction), um AshY im Phytoplasma nachzuweisen. Die PCR Analyse des Baumgewebes war bei 4 % von 240 untersuchten Bäumen positiv. Obwohl 145 der untersuchten Bäume ähnlich Absterbeerscheinungen wie die infizierten Bäume zeigten, wurde das Phytoplasma nur in 11 Bäumen bestimmt. Weil die Homoptera sich als Vektor für diese Krankheit gezeigt haben, wurde auch die Insektenpopulation der befallenen Bäume untersucht, ob sie Träger des Phytoplasmas sind. Insgesamt wurden 471 Insekten aus 34 Spezies auf den Grünen Eschen gesammelt. 396 wurden mit PCR auf Phytoplasma untersucht. Drei Grashüpfer-Arten, die unter Verdacht standen, Träger zu sein, wurden untersucht, aber es konnte kein Anzeichen von Phytoplasma gefunden werden.

Resumen. El fitoplasma del Fresno Amarillo ha sido reportado ampliamente a través de los Estados Unidos como un organismo asociado con la declinación del fresno en comunidades urbanas. Estudios anteriores en Iowa indican que el fitoplasma del fresno fue encontrado en el 20% de los árboles urbanos (*Fraxinus pennsylvanica* Marsh.), cuando se utilizó el método de coloración DAPI (4', 6-diamidino-2-fenildol2HCl). Un nuevo estudio en nueve comunidades en Iowa usó reacción en cadena de polimerasa (PCR) para detectar el fitoplasma. El análisis PCR de los tejidos del árbol fueron positivos para fitoplasma de fresno en 4% de los 240 individuos analizados. A pesar de que 145 de los árboles muestreados tuvieron síntomas de declinación similares a los exhibidos por los árboles infectados con el fitoplasma, éste fue detectado solamente en 11 árboles. Debido a que la Homóptera ha sido detectada como vector de fitoplasmas, las poblaciones de insectos asociadas con los árboles de fresno también fueron muestreadas para ayudar a determinar si los insectos estuvieron transportando fitoplasmas. Fue colectado un total de 471 insectos, representando 34 especies en fresnos; de estos, 396 fueron estudiados para detectar la presencia de fitoplasma usando PCR. Se encontraron especies defoliadoras como vectores de fitoplasma, pero éstos no fueron detectados en ningún otro insecto.