Insect Transmission of *Xylella fastidiosa* to Pecan

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ABSTRACT

Sanderlin, R. S., and Melanson, R. A. 2010. Insect transmission of *Xylella fastidiosa* to pecan. Plant Dis. 94:465-470.

Pecan bacterial leaf scorch (PBLs), caused by the bacterium *Xylella fastidiosa*, can cause economically significant crop loss to some pecan (*Carya illinoensis*) cultivars in the southeastern United States. *X. fastidiosa* is typically vectored by spittlebugs (Cercopidae) and leafhoppers (Cicadellidae). Because no vector species had been reported for pecan, an attempt was made to identify potential vectors that are capable of acquiring the bacterium from infected pecan trees and transmitting to pecan. Several spittlebug and leafhopper species collected from various sources, including sorghum and pecan, were tested as potential vectors of the pathogen from pecan to pecan. When tested in groups, the pecan spittlebug, *Clastoptera achatina*; the Johnson-grass sharpshooter, *Homalodisca insolita*; and the glassy-winged sharpshooter (GWSS), *Homalodisca vitripennis*, had transmission rates to pecan of 11.4, 19.3, and 4%, respectively, following a pathogen acquisition period on infected pecan terminals. The pecan spittlebug is common in pecan orchards in the southeastern United States, and the GWSS was observed on young vigorous pecan shoots. Limited testing with the diamond-backed spittlebug, *Lepyronia quadrangularis*, and the lateral-lined sharpshooter, *Cuerna costalis*, suggested that these could be occasional vectors of *X. fastidiosa* to pecan. There is a need for studies on the identification and population dynamics of Cicadellidae that inhabit pecan orchards to determine if management of vectors is needed in commercial pecan production to reduce the spread of PBLs.

Pecan (*Carya illinoensis* (Wangenheim) K. Koch) is one of the few economically important native horticultural crops in North America. In the United States, it is grown commercially throughout the southeastern states, in parts of the mid-south, and in some areas of the southwest (42). Pecan bacterial leaf scorch (PBLs) (35) is caused by the bacterial pathogen *Xylella fastidiosa* Wells et al., which causes disease in many horticultural crops and landscape trees including grape, peach, plum, almond, sycamore, oak, and red maple (19,20). Four subspecies of *X. fastidiosa* have been described (38-40); the pecan strain appears to belong to the *multiplex* subspecies (R. Melanson, unpublished). *X. fastidiosa* resides in the xylem tissue of the host plants it infects, producing diseases that typically cause defoliation, reduction in plant growth, and sometimes plant death (16). In pecan, *X. fastidiosa* can cause significant yield reduction and defoliation in susceptible cultivars (36). Disease development tends to be chronic, occurring at varying levels of severity annually. Disease symptoms can

occur in one or several limbs, eventually spreading throughout the tree. Visual symptoms include leaflets turning tan to brown at the margins and necrosis progressing basipetally toward the midrib and petiole, followed by abscission of affected leaflets and rachises (35).

The primary means of *X. fastidiosa* transmission is insect vectoring. Insect vectors are limited to members of the Cicadellidae (leafhoppers) and Cercopidae (spittlebugs) (19,34). Most of the insect vectors identified in the southeastern United States have been in studies of *Vitis* and *Prunus* species (19,26). PBLs has been observed to spread within pecan orchards (R. Sanderlin, unpublished), but no insect vectors have been reported for *X. fastidiosa* on pecan. Determination of insect vectors of the PBLs pathogen in pecan could lead to management measures to help reduce pathogen spread in commercial orchards. The objective of this research was to identify potential insect vectors of *X. fastidiosa* in pecan.

MATERIALS AND METHODS

Tree source and general transmission test procedures. Nongrafted pecan trees grown from seed of the PBLs-susceptible cultivar Cape Fear were used as hosts for transmission tests. Seed collected from noninfected Cape Fear trees the previous year and stored at 4 to 7°C were germinated in a growth chamber near 32°C before being transplanted individually into

pots. In 2004, germinated seeds were transplanted into 18.9-liter (5 gal) pots using Metro Mix 250 growth medium (SunGro Horticulture Distribution, Inc., Bellevue, WA). In succeeding years, the germinated seed were transplanted to 7.6-liter (2 gal) pots in Metro-Mix 702. Trees used in the transmission tests were generally 25 to 38 cm in height. Excess trees not used in transmission tests and maintained in the greenhouse (14.6 × 9.1 m) were used as checks for detection of errant transmission events (greenhouse controls).

Because of a shortage of Cape Fear seed, 42 of 261 trees used in transmission tests in 2004 and 2005 were grown from seed of cultivar Desirable, another PBLs-susceptible cultivar. Trees were produced as described for Cape Fear. Because there was no difference in percent infection between the two seed sources (chi-square test, $P = 0.41$), the results were combined.

Insects were maintained on test trees with cylindrical wire cages covered with white organza cloth. Following insect transfer, cage tops were sealed with aluminum foil. The inoculation access period (IAP) for insect nymphs was in a greenhouse. All adult (winged) insects were given IAPs in the lab to prevent escapes in the greenhouse. Following the IAP, trees were maintained in a greenhouse in a randomized design. Prior to the IAP, insects were usually given an acquisition access period (AAP) on infected pecan tissue to increase chances of exposure to the pathogen. Symptomatic terminals of infected orchard trees, symptomatic trees in pots, or symptomatic terminals cut from trees and maintained in water in the lab were used as pathogen-source plant tissue.

Spittlebug transmission tests. Pecan spittlebug, *Clastoptera achatina* Germar, nymphs and adults were collected from an orchard near Cloutierville, LA (31° 32' N, 92° 57' W). There was a high intensity of PBLs on Cape Fear trees in this orchard. Terminals with spittle masses containing nymphs were collected from 1 June through 19 August 2004 and on 6 and 20 May and 14 July 2005. Terminals from infected Cape Fear trees were cut, placed in plastic bags, and transported to the Louisiana State University AgCenter Pecan Research-Extension Station in Shreveport, LA. Terminals collected in 2004 were symptomatic. In 2005, collected terminals with nymphs were both symptomatic and asymptomatic (from known infected trees prior to symptom development). Nymphs

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Accepted for publication 30 December 2009.

used in transmission tests were transferred directly to test trees by removing them from their spittle masses with a small, soft-bristled paint brush or by placing the cut terminal with spittle masses onto the test trees. The number of nymphs placed on a tree ranged from 5 to 30. Nymphs remained on these trees until they died or molted to adults.

Adult pecan spittlebugs were collected by aspirators from terminals. Adults were collected in August and September 2004, from May through September 2005, in May 2006, and in September 2007. Adults collected from symptomatic trees were not given an AAP. Adults collected from asymptomatic tissue were given an AAP before transfer to test trees. AAPs for spittlebug adults were 1 or 2 days. The number of adults on each test tree ranged from 1 to 20. Adults remained on these trees until they died.

One other spittlebug species, the diamond-backed spittlebug, *Lepyronia quadrangularis* (Say), was also collected and tested for transmission. In September 2005, 10 *L. quadrangularis* adults were captured in sweep nets from a sorghum plot. These spittlebugs were given 1- or 2-day AAPs on detached symptomatic pecan terminals; five specimens were then transferred to each of two test pecan trees for an IAP of 17 to 18 days. After the initial IAP, the six spittlebugs still alive were given another 1-day AAP and a second IAP of 10 days followed by transfer of the four remaining insects to two test trees for 7 days.

***Homalodisca insolita* transmission tests.** From October to early November 2004, insect sweep nets were used to collect 65 *H. insolita* (Walker), the Johnson-grass sharpshooter, from a sorghum plot at the edge of an orchard at the Pecan Station (32° 21.7' N, 93° 38.6' W). The leafhoppers were given 1- to 3-day AAPs, and 1 to 10 insects were placed on each of 23 transmission test trees. *H. insolita* was again collected from a sorghum plot at the Pecan Station from 13 September to 20

September 2005 (plot destroyed by storm on 24 September). Eighty-nine *H. insolita* were given a 1-day AAP, and 24 of these were given a second 1-day AAP before transfer to fresh test plants. IAPs were 7 to 19 days with 2 to 15 insects on each of 34 trees.

***Homalodisca vitripennis* transmission tests.** In 2005, 41 *H. vitripennis* (Germar), the glassy-winged sharpshooter (GWSS), were collected from healthy pecan, plumeria, and sorghum and tested for transmission using 16 healthy pecan test trees in the initial exposure following AAPs of 1 to 5 days. In 2006, GWSS were collected from healthy pecan and citrus trees at a pecan orchard (31° 3.2' N, 92° 8.6' W) near Hessmer, LA, in June and July. They were given initial AAPs of 1, 2, or 3 days. The IAPs were from 6 to 12 days with 18 test trees used for initial exposure after the AAPs. In May and June 2007, GWSS were collected from the same location and given AAPs on PBLs symptomatic tissue of 1 (76 GWSS), 2 (51 GWSS), or 3 (14 GWSS) days followed by IAPs of generally 7 days. The initial IAPs were comprised of 49 trees with 1 to 5 GWSS on each tree. Following the initial IAPs each year, *H. vitripennis* still living were transferred to additional test trees.

Miscellaneous Cicadellidae transmission tests. Over the course of the study, a few specimens of three other leafhoppers, one *Cuerna costalis* (Fabricius), three *Oncometopia nigricans* (Walker), and two *Paraulacizes irrorata* (Fabricius), were collected from sorghum and pecan and tested for *X. fastidiosa* transmission to pecan. These leafhoppers were given AAPs of 1 to 2 days, then transferred to test trees as described in general transmission test procedures.

Data analyses. Percent transmission by insect species was compared using the least significant difference (LSD, $\alpha = 0.05$) test. Likewise, comparison of percent transmission between insects given a 1-day or more than 1-day AAP was made with the LSD test.

Serological assay of test trees and insects. In addition to visual determination of PBLs symptoms, all test trees were screened for infection by enzyme-linked immunosorbent assay (ELISA; double-antibody sandwich [DAS]-ELISA for *X. fastidiosa*, Agdia Inc., Elkhart, IN). Generally, assays included sections of three rachises of each tree, with rachises from symptomatic leaves, if present. Tissue sections were placed in sample bags (Agdia) and pulverized in 4 to 5 ml of cold extraction buffer with a pestle. Positive reactions were recorded for trees with an absorbance (640 nm) at least three times greater than tissue from noninfected trees in the greenhouse.

Test trees that developed symptoms during the same year that they were exposed to insects were assayed for infection upon

symptom development. All test trees were assayed in July or August of the year following insect exposure. All test trees from 2004 and 2005 were monitored for symptoms and assayed again 2 years following vector exposure. Because no additional infections were found during the second year after IAP for the 2004 and 2005 trees, the second-year assay was discontinued for the 2006 and 2007 trees. Trees that did not exhibit PBLs symptoms were assayed by combining tissue from three trees that were exposed to the same insect species to make a single ELISA sample. A negative reaction indicated that none of the trees in the sample were infected.

Samples of pecan spittlebug nymphs and adults and adult leafhoppers were also tested for the presence of *X. fastidiosa* by ELISA. Whole spittlebugs or heads of leafhoppers were homogenized in 1 ml of cold extraction buffer with a mortar and pestle. Spittlebug nymphs were assayed on the same day they were collected from infected trees or within 24 h of collection. Assay samples consisted of 10, 20, or 40 nymphs, and one sample of 125. Assay samples of spittlebug adults had 6, 10, or 20 spittlebugs collected primarily from the nuts of asymptomatic terminals of infected trees. *H. insolita* ELISA samples were from the specimens used in transmission tests and then stored at 4°C. Two samples were assayed for *X. fastidiosa*, one with 12 and the other with 24 insect heads. *H. vitripennis* ELISA samples were collected from transmission test trees and from AAP sources and then stored at 4°C. GWSS samples had 1 to 16 insect heads.

Insect identification. Samples of insects used in transmission tests were sent to the Louisiana State University AgCenter Department of Entomology for identification.

RESULTS

Spittlebug transmission tests. Pecan spittlebug nymphs did not transmit *X. fastidiosa* in the tests reported here (Table 1). Adult pecan spittlebugs transferred directly from symptomatic tissue to test pecan trees transmitted *X. fastidiosa* to 2% of the trees. Adult pecan spittlebugs given an AAP on infected pecan tissue transmitted the pathogen to 11.4% of the test trees (Table 1). The five test trees that were infected were exposed to 7 to 20 spittlebugs.

One of six IAP trees tested with two specimens of *L. quadrangularis* became infected, indicating pathogen transmission to pecan by this spittlebug. This is apparently the first report of this spittlebug as a vector of *X. fastidiosa*.

***H. insolita* transmission tests.** Nearly 16% of the first pecan trees exposed to *H. insolita* following AAPs were infected (Table 2). Fourteen percent of the trees exposed to *H. insolita* following transfer from the first group of test trees were infected (Table 2). *H. insolita* given a second

Table 1. Summary of transmission of *Xylella fastidiosa* to pecan by *Clastoptera achatina*, 2004-2007

Source of spittlebugs ^x	Number of insects ^y	Trees infected/trees tested ^z
Direct orchard transfer	2,351 nymphs	0/93
Direct orchard transfer	589 adults	1/49
1-2 day AAP	283 adults	4/35

^x Spittlebugs were either transferred directly from terminals they were collected on in a pecan orchard or first given an acquisition access period (AAP) on infected pecan tissue.

^y Total number of insects in a test category.

^z Numerator is number of transmission test trees that developed pecan bacterial leaf scorch symptoms and gave a positive serological assay for *X. fastidiosa*. Denominator is total number of trees in the test.

AAP following the initial IAP transmitted to 60% of the next test trees (Table 2). Transmission by *H. insolita* during the 2-year test period was with four to eight insects on a tree.

***H. vitripennis* transmission tests.** Of the first group of test trees exposed to *H. vitripennis* following AAPs, 9.6% were infected (Table 3). Successive transfers to additional test trees without another AAP resulted in transmission to 1.9% of the trees. No transmission was recorded for the eight *H. vitripennis* given a second AAP (Table 3). Transmission by *H. vitripennis* during the 3-year test period was with one to five insects on a tree.

Miscellaneous Cicadellidae transmission tests. Of the three leafhopper species for which only a few specimens were collected and tested for transmission of the pathogen to pecan, the single specimen of *Cuerna costalis*, the lateral-lined sharpshooter, transmitted *X. fastidiosa* to two pecan trees; whereas the three *Oncometopia nigricans* and the two *Paraulacizes irrorata* did not transmit.

Data analyses. Because few positive transmission events by *H. vitripennis* occurred with less than four insects on a tree and few trees had less than four pecan spittlebugs or *H. insolita*, comparisons across insects were made using only trees with four or more insects. Results from all years were combined for the analysis. There were no significant differences in the percent transmission between *C. achatina* adults, *H. insolita*, and *H. vitripennis* when each tree was exposed to four or more insects following an AAP (Table 4).

No significant differences were detected in percent transmission for GWSS with a 1-day AAP or AAPs of 2 days or more (Table 5). All IAP trees with the first exposure to GWSS following an AAP from 2005 through 2007 were used in this analysis. The analysis for the effect of length of AAP on transmission efficiency was done only with the GWSS because few insects of the other species had AAPs longer than 1 day.

Serological assay of insects for *X. fastidiosa*. *X. fastidiosa* was not detected by serological assay in *C. achatina* nymphs or adults when collected from symptomatic or asymptomatic terminals of infected trees (Table 6). One of two groups of *H. insolita* used in ELISA was positive for the bacterium (Table 6); however, these groups had not been sorted by the test trees they were on.

X. fastidiosa was detected by ELISA in 2 of 6 groups of *H. vitripennis* that had transmitted to test trees (Table 6). Conversely, the bacterium was detected in 5 of the 41 GWSS groups that did not transmit to any of their test trees (Table 6). GWSS in these five groups had been exposed to a total of 39 test trees with 7-day IAPs. None of the 39 test trees that had been exposed to at least one infectious insect

were infected. *X. fastidiosa* was also detected in 38% of the GWSS that were given an AAP but died before the IAPs.

Greenhouse controls. Throughout the pathogen transmission tests, none of the extra trees not exposed to insects developed PBLs symptoms. Serological assays of 10 or more of these trees each year were negative. Only trees that had been intentionally exposed to insects and that developed symptoms of the disease tested positive for the bacterium by ELISA.

DISCUSSION

During pecan spittlebug collection, we observed that adults tended to be more prevalent on asymptomatic terminals of infected trees than on terminals with visible PBLs. Similar host selectivity by other *X. fastidiosa* vectors was reported with citrus (24). This suggests that spittlebug feeding preference is for tissue without restricted xylem flow. This habit may affect transmission rates in nature. Even a low transmission rate, as recorded in this study, could result in significant disease increase when hundreds of spittlebugs feed

on trees during high population cycles. In California, a vineyard area with vector populations of low transmission efficiency and low vector numbers resulted in a rate of Pierce's disease spread comparable to that of a vineyard area with vector species of higher transmission efficiency (31). Results of a meta-analysis of transmission experiments indicated that transmission was positively correlated with the number of *H. vitripennis* but not with length of acquisition periods for the bacterium or length of inoculation access periods (13).

Because the number of pecan spittlebugs on pecan trees could easily be in the hundreds (43), our data suggest that this insect could be a significant vector of *X. fastidiosa* in pecan orchards. Although there has been uncertainty about the importance of pecan spittlebugs as an economic pest in commercial orchards (47,48), their identification as a vector of *X. fastidiosa* in pecan increases their significance in commercial pecan culture. Because spittle masses are easily seen prior to adult emergence, it should be possible to greatly reduce the transmission

Table 2. Summary of *Xylella fastidiosa* transmission by *Homalodisca insolita* to pecan, 2004-2005

Collection source	AAP ^w	IAP ^x	No. of insects tested	Infected trees/trees tested ^y
Sorghum	1-3	1-20	154	6/38
Test tree ^z	1	10-12	24	3/5
Test tree	0	7-11	35	2/14

^w AAP = acquisition access period, number of days insects were caged on *X. fastidiosa*-infected source. All source tissue was infected symptomatic pecan terminals.

^x IAP = inoculation access period, the number of days insects were caged on a transmission test tree.

^y Numerator is number of transmission test trees that developed pecan bacterial leaf scorch symptoms and gave a positive serological assay for *X. fastidiosa*. Denominator is total number of trees in the test.

^z Collected from test trees and transferred to additional test trees successively. Sometimes an additional AAP was given between IAPs.

Table 3. Summary of *Xylella fastidiosa* transmission by *Homalodisca vitripennis* to pecan, 2005-2007

Collection source	AAP ^v	IAP ^w	Number of insects tested	Infected trees/trees tested ^x
Host ^y	1-5	4-60	267	8/83
Test tree ^z	0	4-12	204	4/216
Test tree	1-2	7-10	8	0/5

^v AAP = acquisition access period, number of days insects were caged on *X. fastidiosa*-infected pecan tissue.

^w IAP = inoculation access period, number of days insects were caged on noninfected pecan trees following the AAP.

^x Numerator is number of transmission test trees that developed pecan bacterial leaf scorch symptoms and gave a positive serological assay for *X. fastidiosa*. Denominator is total number of trees in the test.

^y *H. vitripennis* was collected from several host species including pecan, peach, citrus, sorghum, and plumaria.

^z Collected from test trees and transferred to additional test trees successively. Sometimes an additional AAP was given between IAPs.

Table 4. Infection data for IAP^y trees with four or more insects each, 2004-2007

IAP trees	<i>Clastoptera achatina</i>	<i>Homalodisca insolita</i>	<i>Homalodisca vitripennis</i>
Number of IAP trees	35	49	91
Number of trees infected	4	11	8
Percent IAP trees infected ^z	11.4 a	22.4 a	13.2 a

^y IAP = inoculation access period, following the *Xylella fastidiosa* acquisition access period.

^z Values in a row followed by the same letter are not significantly different by LSD ($\alpha = 0.05$).

potential of this vector with insecticide applications.

In contrast to the adult pecan spittlebug, we did not detect any transmission of *X. fastidiosa* by the nymphs, which were transferred directly from infected trees to test trees, where they quickly formed new spittle masses and continued development into adults. Due to the large number of nymphs tested, even though they were not given AAPs, it was surprising that no transmission occurred, as nymphs of other vector species acquire and transmit *X. fastidiosa* (32,41). The pecan spittlebug overwinters as eggs beneath bark (43); thus, the nymphs would have to acquire the bacterium by feeding on infected pecan tissue where they hatch. When the nymphs develop in the spring, the populations of *X. fastidiosa* may be too low in the new terminal growth for efficient acquisition (17). Nonetheless, the ability of spittlebug nymphs to transmit the bacterium in nature would likely be epidemiologically irrelevant because nymphs develop within spittle masses on their pecan host and do not move to other trees until molting to adults, at which time they would lose the bacterium (32).

X. fastidiosa was not detected in pecan spittlebug nymphs or adults by ELISA, suggesting that it either was not acquired, or was below the population density necessary for detection by the ELISA test (27,33). Low bacterial numbers in the spittlebugs may be the reason for lack of detection since transmission by adults did occur.

In addition to the pecan spittlebug, which lives entirely on *Carya* species, the spittlebug *Lepyronia quadrangularis* transmitted *X. fastidiosa* when caged on pecan. This spittlebug is common throughout the eastern United States and feeds primarily on weedy hosts (14). While it is not known if this insect feeds on pecan in nature, it emphasizes the likelihood of a large number of potential vectors that may occasionally transmit the pathogen to pecan.

There are no reports of leafhopper species that typically occur in pecan orchards. However, because leafhoppers are considered to be the primary vectors of *X. fas-*

tidiosa, an attempt was made to test leafhopper species collected in and near pecan orchards for their ability to transmit the pathogen to pecan. Sorghum is sometimes recommended as a trap crop for stinkbug pests in pecan orchards (25). A sorghum plot planted for this purpose was a source of several known leafhopper vectors of *X. fastidiosa*. *H. insolita*, a vector of *X. fastidiosa* to peach in the southeastern United States (28,44), was collected from sorghum. Although it was not collected from pecan in this study, it transmitted to pecan in our tests, and has since been trapped in pecan orchards (R. Sanderlin, unpublished).

The GWSS is native to the southeastern United States and is an important vector of *X. fastidiosa* to several plant species (4,5,11,12,21,32,34). It is a major threat in the spread of the pathogen because it is a polyphagous voracious feeder (7,45). The GWSS in our study was initially captured from sorghum, and subsequently observed to be a common feeder on succulent pecan shoot growth in several orchards in Louisiana. GWSS tended to feed in groups on young vigorous pecan shoots, often lining up in rows on these shoots. The GWSS were not easily driven from pecan when disturbed, usually returning immediately to the same tree. They were present in the highest numbers on pecan from about 11 A.M. to 1 P.M., preferring terminals in sunlight over shade, and were difficult to find on pecan from dawn to midmorning in the summer months. GWSS activity on *Prunus* has been reported to be higher at night than during daylight; nighttime activity was not monitored on pecan (7).

In light of the preference for young vigorous pecan growth in GWSS, and perhaps in other vectors, it is interesting to note that PBLs is often first observed developing on current year's growth in the tops of trees where sunlight is most prevalent. Although not as efficient a vector as *H.*

insolita in the tests reported here, its feeding habit on pecan and other hosts makes GWSS a potentially significant vector for spreading *X. fastidiosa* within orchards and especially for moving the pathogen into orchards from other hosts. One GWSS that was transferred every 7 days lived on the test pecan trees a total of 183 days after collection from a pecan orchard. Such longevity when confined on pecan indicates that GWSS has the ability to feed for lengthy periods of time in pecan orchards.

Transmission rates by GWSS in our tests were variable from year to year, 0 to 22%. Conditions of AAP and IAP hosts, environment, bacterial populations, and vectors likely make transmission rates highly variable in the tests and in nature. Once an adult vector acquires *X. fastidiosa*, the insect continues to carry the pathogen throughout its life (5,41). Three of nine groups of GWSS that transmitted the bacterium to the initial tree it was placed on following AAP transmitted to a second test tree. Compared to studies with grape, initial and successive transmission rates in our tests with pecan were low (5,41). Ninety-two percent of the GWSS specimens in the groups that transmitted to the first test tree survived long enough for exposure to at least four test trees. Thus, vector mortality was not the reason for low successive transmissions. It may be that grape has higher bacterial populations than many other hosts, making vector acquisition more efficient (4,17). Population densities of *X. fastidiosa* in pecan have not been determined. Another possible explanation for the low transmission rates is the lack of vigor of the IAP trees. The test trees were growing in small pots and usually did not have the type of vigorous growth that GWSS is attracted to in orchards. However, many GWSS survived for several weeks and some for several months, indicating that the insects did feed in captivity on the IAP pecan trees. Other

Table 5. *Homalodisca vitripennis* transmission data for the initial IAP^x tree, 2005-2007

IAP trees	AAP ^y = 1 day	AAP ≥ 2 days
Noninfected trees	56	49
Infected trees	9	7
Percent infected ^z	13.8 a	12.5 a

^x IAP = inoculation access period, following *Xylella fastidiosa* acquisition access period.

^y AAP = acquisition access period, number of days that insects were caged on *X. fastidiosa*-infected pecan tissue.

^z Values in a row followed by the same letter are not significantly different by LSD ($\alpha = 0.05$).

Table 6. Serological assay of insects for *Xylella fastidiosa*¹

Species	Source ^a	No. of insects assayed ^b	No. of positive assays/ No. of assays ^c
<i>Clastoptera achatina</i>	Terminals with PBLs	2,035 nymphs	0/205
<i>C. achatina</i>	Asymptomatic terminals	270 nymphs	0/12
	from infected trees	132 adults	0/10
<i>Homalodisca insolita</i>	IAP trees	36 ^x	1/2
<i>Homalodisca vitripennis</i>	AAP source	66	6/16
<i>H. vitripennis</i>	IAP trees (infected)	24	2/6 ^y
<i>H. vitripennis</i>	IAP trees (noninfected)	119	5/41 ^z

¹ ELISA kit (Agdia) for *X. fastidiosa* used for serology. Unless noted, all insects assayed were adults.

^a Asymptomatic terminals had lost most of their original foliage from pecan bacterial leaf scorch (PBLs) and produced a second flush of foliage that was not yet symptomatic. IAP (inoculation access period) trees were used in transmission tests and the insects on them collected for assay after they died. Insects on AAP (acquisition access period) source were dead when retrieved and were not exposed to IAP trees; they were stored at 4°C until enzyme-linked immunosorbent assay (ELISA).

^b Total number of insects used in a group of assays.

^c Numerator is number of assays positive for *X. fastidiosa*. Denominator is total number of ELISAs for a group.

^x Not known if any of these insects transmitted during IAPs.

^y Each of the six groups in these assays transmitted *X. fastidiosa*.

^z None of the insects in these assays transmitted *X. fastidiosa* to any of their 39 IAP trees.

studies have shown that GWSS feed on secondary stem tissue and can transmit *X. fastidiosa* to 2-year-old stems (5,44). GWSS can also acquire and transmit the bacterium when feeding on dormant tissues (4,6). As reported for grapevine (5,32), a 1-day AAP was as efficient as longer AAPs for acquisition and transmission of *X. fastidiosa* to pecan. The observation of a lack of increase in the numbers of a vector that transmit the pathogen when given longer exposure periods to infected tissue is an important area that needs more investigation.

Similar to phony peach disease, clear differences in the ability of three species of vectors to transmit the pathogen to pecan were not observed (46). Other studies have reported both significant and not significant differences in species transmission efficiencies with both single insects and groups of insects (23,30,34). The overall transmission rate by vectors to pecan suggests that either pecan is difficult to infect, the acquisition from pecan tissue is lower than from other hosts that have been studied, or there is a lack of feeding on PBLs symptomatic tissue during AAP (3,17,24).

Detection of *X. fastidiosa* in *H. insolita* and *H. vitripennis* by ELISA was erratic. The bacterium was not detected in all groups that transmitted and was sometimes detected in groups that did not transmit. Failure of some insects to transmit the bacterium even though they were carriers based on ELISA results, and the low successive transmission rates in the tests, imply that exposure of pecan trees to infectious vectors does not always result in infection of the trees.

Only one specimen of *Cuerna costalis* was tested on pecan, but its transmission to two trees suggests that, as with peach, even though this species is primarily a grass feeder, it has the potential to be a vector on pecan (45,46). Even though *Oncometopia nigricans*, a vector on peach, grape, and citrus (1,9,46), did not transmit to pecan in our very limited testing, the fact that it was collected from pecan and survived for nearly 100 days on pecan test trees indicates that further testing of this leafhopper as a *X. fastidiosa* vector on pecan is warranted.

Several insecticides have been reported to quickly kill leafhoppers and reduce transmission of *X. fastidiosa* in experimental tests on other crops (8,10,15,18,29). Some of these insecticides are registered for use on pecan (22). Studies are needed to determine the vector species that occur regularly in pecan orchards and their population dynamics to establish if insecticides would be useful for reducing PBLs spread (2). Management of vectors in pecan orchards along with control of pathogen introduction into orchards through hot-water treatment of scions (37) may afford methods to significantly lessen the spread of PBLs in commercial orchards.

ACKNOWLEDGMENTS

We thank Christopher Carlton, Professor, and Stephanie Gil, Louisiana State Arthropod Museum, Department of Entomology, Louisiana State University Agricultural Center, for identification of leafhopper species used in this study.

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