ABSTRACT

FLOYD, LEAH EVELYN. Reservoir Hosts and Vectors of *Xylella fastidiosa*, Causal Agent of Pierce's Disease of Grapevines, in North Carolina. (Under the direction of Turner Bond Sutton).

Pierce's disease (PD) of grapevines is caused by the xylem-limited bacterium *Xylella fastidiosa* (*Xf*) which is transmitted by leafhoppers and spittlebugs. Leafhopper populations were surveyed in six vineyards across North Carolina's three geographic regions, in 2006 and 2007. Populations of two phloem-feeders, *Agallia* spp. and *Paraphlepsius irroratus* were compared with populations of known PD vectors, *Graphocephala versuta* and *Oncometopia orbona* as well as other leafhoppers and planthoppers. *G. versuta* was the dominant species in the Piedmont and Mountain regions in 2007 and in one Coastal Plain vineyard in 2006. *Agallia* spp. were most common in the Coastal Plain in 2007, and in the two Mountain and Piedmont vineyards in 2006. The possibility that a phloem-feeder may transmit *Xf* to grapevines was examined. The clover leafhopper, *A. sanguinolenta*, a phloem-feeder, did not transmit *Xf* to grapevines and the bacterium was not detected in its mouthparts using RT-PCR analysis. A reservoir host list of *Xf* was developed for North Carolina. This was developed using ground vegetation surveys conducted in three vineyards in the spring and fall of 2007-08 to identify and quantify plant species growing on the vineyard floor. Plant samples were collected and tested for the presence of *Xf* with ELISA and PCR. Fourteen of 40 plant species surveyed tested positive with ELISA and two were confirmed with PCR. Plant hosts of *Xf* identified in this study that have not been previously reported are: *Chamaesyce maculata*, *Trifolium arvense*, hop clover, *Trifolium* spp., *Geranium carolinianum*, *Oxalis stricta*, *Festuca* sp., *Setaria* sp., *Hordeum pusillum*,

Poa trivialis and *Ranunculus* sp. Plant hosts of *Xf* identified that were previously reported to host PD-strains of the bacterium include: *Trifolium repens*, *Plantago lanceolata*, *Digitaria* sp., and *Cynodon dactylon*. To further examine the importance of potential groundcovers as reservoir hosts, experiments were conducted to find a groundcover that the known PD vector, *Graphocephala* spp. does not prefer for feeding. None of the plant species tested were found to be poor reservoir hosts, with regard to *G. versuta* feeding preference. Additionally, the effect of the presence of fungal endophytes on the survival of *G. versuta* was investigated. Those present in *Festuca rubra* spp. *rubra* and *Festuca rubra* ssp. *commutata* did not affect the survival of *G. versuta*. We recommend removing broadleaf weeds from the vineyard floor, and studies to find a suitable groundcover should be continued.

Reservoir Hosts and Vectors of *Xylella fastidiosa*, Causal Agent of Pierce's Disease of Grapevines, in North Carolina

by
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A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Plant Pathology

Raleigh, North Carolina

2008

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BIOGRAPHY

Leah Evelyn Floyd was born July 16th 1984. She grew up in Oxford, North Carolina and attended J.F. Webb High School. Upon graduation, she began an undergraduate degree in the Department of Botany at North Carolina State University, where she became interested in plant pathology. Leah worked for the NCSU Plant Disease and Insect Clinic and the Department of Plant Pathology's Fungal Genomics laboratory as an undergraduate. She began working for Dr. Turner Sutton during her senior year, and began her Master of Science degree in Plant Pathology at North Carolina State University in 2006 under his direction.

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INTRODUCTION

Pierce's disease of grapevines (PD) is caused by the xylem-limited, gram-negative bacterial endophyte, *Xylella fastidiosa* (*Xf*) (Wells et al., 1987). All xylophagous insects with piercing-sucking mouthparts potentially serve as vectors of *Xf*. However, some of the more well-studied, important vectors include the green sharpshooter, *Draeculacephala minerva* (Ball), the blue-green sharpshooter, *Graphocephala atropunctata* (Signoret), the red-headed sharpshooter, *Xyphon fulgida* (Nottingham) the glassy-winged sharpshooter (GWSS), *Homoladisca vitripennis* (Germer) and species of *Oncometopia* (Redak et al., 2004).

Leafhopper vectors of *Xf* found in North Carolina include *Graphocephala versuta* (Say), and *Oncometopia orbona* (Fabricius) (Myers et al., 2007). GWSSs have also been found (Villanueva, *unpublished data*) but no experiments have been conducted to determine whether or not the GWSSs were carrying *Xf*. Additionally, *Xf* was detected in the mouthparts of 33% of the bespeckled leafhopper, *Paraphlepsius irroratus* (Say), using PCR analyses (Myers et al., 2007). *P. irroratus* is a phloem-feeder and an important vector of X-disease of peach (Gilmer et al, 1966). Although there have been other reports of phloem-feeders carrying *Xf*, none of these insects have been capable of transmitting the pathogen and Almeida et al. (2005) have suggested that transmission ability is related to foregut morphology or probing behaviors specific to xylem-feeders. No prior evidence suggests that phloem-feeders may be capable of transmitting *Xf* to grapevines, but the percentage of *P. irroratus* trapped in North Carolina vineyards testing positive for *Xf* with PCR was higher than the percentage of infective *G. versuta* or *O. orbona* (Myers et al., 2007). Therefore,

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objectives of this research were to examine the possibility that a phloem-feeder may be a vector of *Xf*. The specific objectives were to (i) survey the populations of two pholem-feeders, *Agallia* spp. and *P. irroratus* in NC vineyards and compare their abundance to known vectors of *Xf* and other leafhopper species, (ii) conduct greenhouse transmission experiments to determine whether or not the clover leafhopper (CLH), *Aceratagallia sanguinolenta* transmits *Xf* to grapevine and (iii) test the CLH for the presence of *Xf* using RT-PCR and determine which strains of *Xf* it may be carrying.

MATERIALS AND METHODS

Leafhopper population surveys. Leafhopper populations were monitored in 2006-07 in six vineyards across North Carolina's three regions: the Mountains, Piedmont and Coastal Plain. Two vineyards were located in the southern Mountains in Polk Co., and were planted in Vinifera grapevines in 1996 and 1998. One vineyard, located in Alamance Co. in the Piedmont, was planted in Vinifera and French-American hybrid vines in 2001. The Wake Co. vineyard, located in the Piedmont, was planted in Vinifera and French-American hybrid vines in 2000. Two vineyards, located in Currituck Co. in the Coastal Plain, contain Vinifera, French-American hybrid and muscadine grapevines and were planted in 2002 and 1991. In each vineyard, yellow sticky traps (Great Lakes IPM Inc., Vestaburg, MI) measuring 30.5 x 14.5 cm were attached to the cordon wires in the vineyard's trellising systems, approximately 1 m from the ground, with binder clips (Office Depot Inc., Delray Beach, FL). Traps were placed along the perimeter of each vineyard. In 2006, 13 traps were placed in each of three vineyards in Wake, Alamance and Polk counties, and seven traps were placed in a second Polk Co. vineyard, due to its smaller size. Only four traps were placed in the two Currituck Co. vineyards. Traps were replaced approximately every 14 days. When removed from the cordon wires, the traps were wrapped in clear, plastic, cling wrap to prevent them from sticking to one another. In 2006, trapping periods began on 19 April and ended on 29 September. In 2007, the number of traps was reduced to four in all vineyards. The 2007 trapping period was expanded to begin on 2 March and ended on 26 September. Traps were stored at 4°C prior to counting insect catches. Numbers of known *Xf* vectors, *G. versuta* and *O. orbona*, and two phloem-feeding leafhoppers, *P. irroratus* and

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Agallia spp. were counted and recorded for each trapping period. *Agallia* spp. were not identified to the species level, due to the presence of at least two, and possibly four species present in the trapping area that are extremely similar and difficult to distinguish from one another. All other leafhoppers, including all members of the families Cicadellidae and Membracidae, were counted and categorized as "others." Upon initial trapping in 2006, a small subset of these taxonomic groups was sent to the NCSU Plant Disease and Insect Clinic for identification. These identified individuals served as references for all future identifications.

Transmission experiments. The phloem-feeding clover leafhopper (CLH*)*, *A. sanguinolenta,* was collected from vineyards in Alamance and Johnston counties with a sweep net. The insects were placed into 50 ml plastic tubes that were kept inside a cooler during transport to the laboratory. Ten individuals from the Alamance Co. vineyard were placed on each of five Chardonnay grapevines planted in 15 cm diameter clay pots and enclosed with a 15 cm diameter plastic cage with a mesh top. The pots and cages were housed in a greenhouse at approximately 21 to 24 °C. The grapevines were watered twice daily. After 10 days, the cages were removed and plants were monitored weekly for symptoms of PD. No CLH were found alive at the end of the inoculation period.

After 3 months of monitoring for symptom development the grapevines were destructively sampled. Ten petioles were removed arbitrarily from each vine and combined into one sample for ELISA testing. ELISA tests were conducted using a *Xylella fastidiosa* double-antibody sandwich kit (Agdia, Elkhart, IN) following the manufacturers protocol. ELISA plates were read in a Model 680 Microplate Reader (Biorad, Hercules, CA) and

positive cutoff values were determined by adding twice the average of the negative controls, to three times the standard deviation of the negative controls (2Avg. + 3 Std.dev), with anything above this limit considered positive.

RT-PCR analyses. Twenty CLH from the same trapping date and location as those used in the transmission experiments, and four CLH from a Johnston county vineyard, were tested for the presence of *Xf* using PCR. The heads of the CLH were severed with a No. 3 insect pin (Morpho, Czech Republic) while holding the body steady with a pair of forceps and visualizing the insect with a dissecting microscope. The insects were pinned through the head, between the eyes, being careful not to pierce the eyes as they have been shown to contain PCR-inhibiting compounds (Bextine et al., 2005). Each pinned head was placed in a 1.5 ml microcentrifuge tube and 500 µl of phosphate buffered saline (PBS) was added to the tube. A vacuum extraction procedure was performed as described (Bextine et al., 2005; Myers et al., 2007) to flush the insects' mouthparts with buffer. DNA extraction was carried out using a Qiagen DNEasy Tissue Kit (Qiagen Inc., Valencia, CA), following the manufacturers protocol for DNA extraction from animal tissues, with the exception that in the final step 100 µl of elution buffer was added instead of the recommended 150-200 µl.

PCR reactions were carried out using a 20 µl total reaction volume containing 10 µl of iQ SYBR Green Supermix (Bio-Rad Laboratories Inc., Hercules, CA), 2 µl sterile deionized water, primers BBgyrBINF2 and BBgyrBINR1 (Bextine and Child, 2007) at a concentration of 10 µM and 4 µl of DNA template. Sterile deionized water was added to the above mixture instead of DNA template, as a negative control. DNA extracted from *Xf* in pure culture using the above DNA extraction procedure was added to one reaction as a

positive control. PCR cycles were carried out in an Applied Biosystems 7000 Real-Time

PCR System (Applied Biosystems Inc., Foster City, CA) and consisted of an initial

denaturing step of 95 ºC for 3 min, followed by 40 cycles at 95 ºC for 30 s, 55 ºC for 30 s,

and 72 ºC for 30 s (Bextine et al. 2005).

RESULTS

Leafhopper population surveys. Of the two phloem-feeders, *Agallia* spp. were the most abundant. The relative proportion of *Agallia* spp. varied from 3 to 29% in 2006 and 4 to 18% in 2007. They were generally most abundant in the Alamance Co. vineyard during the 2 years of study and least abundant in Polk Co. vineyard # 1 (Figures 1-6). The proportion of *Agallia* spp. was similar in four vineyards but declined in the Alamance Co. vineyard and increased in Currituck Co. vineyard # 2 from 2006 to 2007 (Figures 3 and 6). Populations of *Agallia* spp. reached peak densities during various trapping periods, across all locations in both years. In Polk Co. vineyard # 1, populations of this genus peaked in early-August 2006 and early-September in 2007. In Polk Co. vineyard # 2 the populations peaked in early-September 2006, but in mid-July in 2007. In Alamance Co., population patterns were similar to those observed in Polk Co. vineyard # 2, with peaks occurring in late-August 2006 and in mid-July 2007. In Wake Co., populations peaked in mid-July 2006 and mid-August 2007. In 2006, in Currituck Co. vineyard # 1, populations of *Agallia* spp. experienced two peaks, one in late-June and a second in late-August. At this location in 2007, populations peaked in late-June only. Similar trends were observed in Currituck Co. vineyard # 2 (Figure 7).

The proportion of *P. irroratus* was small compared to *Agallia* spp. and ranged from 0 to 6% during the 2 years of study (Figures 1-6). In general, *P. irroratus* population densities peaked in late-May at all locations for both years (Figures 8). Exceptions occurred in Polk Co. vineyard # 2 in 2006 when three population peaks were observed in late-May, mid-June

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and again in late-September. In the Alamance Co. vineyard in 2006, *P. irroratus* populations reached peak densities in early-June (Figure 8A).

The proportion of *G. versuta* was much greater in vineyards in the Mountains and Piedmont than either of the two phloem-feeders. In 2006 it ranged from 11 to 31% and in 2007 from 27 to 60% (Figures 1-4). Populations of this vector were much less in the two Currituck Co. vineyards. The proportion of *G. versuta* in Currituck Co. vineyards # 1 and 2 was 9 and 1% and 4 and 6% in 2007 and 2008, respectively (Figures 5-6). In general, peak trap catches of *G. versuta* occurred in mid-July in both years and at most locations. Exceptions occurred in Polk Co. vineyard # 1 in 2007 when *G. versuta* populations peaked three times: in mid-April, late-June and again in late-July. In Alamance Co., populations peaked in early-June in 2006 and late-June in 2007. In Currituck Co. vineyard # 2, populations of this species peaked in late-May of 2006, and in early-July and early-August of 2007 (Figure 9).

O. orbona was present in very low numbers in all vineyards throughout both years of the study. This species comprised 1% or less of all leafhoppers trapped in the Mountain and Piedmont regions (Figures 1-4). *O. orbona* made up a slightly larger proportion of leafhoppers in the Coastal Plain vineyards (Currituck Co. vineyards # 1 and 2), at 4% for both locations in 2006, and 1 and 2 %, respectively, in 2007 (Figures 5-6). *O. orbona* populations typically peaked from late-May to mid-June in all locations and years (Figure 10).

All other members of the Cicadellidae (leafhoppers) and Membracidae (treehoppers) not specifically mentioned were grouped in the category "others." The relative proportion of

"others" was similar in the Mountain region vineyards in both years. "Others" comprised 78 and 70% in 2006, and 63 and 61% in 2007, of total leafhoppers trapped in Polk Co. vineyards #1 and 2, respectively (Figures 1-2). Similar proportions were observed in the vineyards in the Piedmont in both years. The relative proportion of "others" was 56 and 58% in 2006, and 24 and 27% in 2007, in Alamance and Wake counties, respectively (Figures 3-4). In the Coastal Plain, "others" made up very large proportions of total leafhoppers trapped. The relative proportion of "others" was 80 and 91% in 2006, and 82 and 74% in 2007, in Currituck Co. vineyards 1 and 2, respectively (Figures 5-6). In the Mountain and Piedmont vineyards, populations of "others" peaked in late-June during both years of the study. In Currituck Co. vineyard # 1, "others" peaked in mid-June and early-September in 2006 and 2007. In Currituck Co. vineyard # 2, "others" reached peak population densities in mid-June in 2006 and early-July in 2007 (Figure 11).

Transmission experiments. Symptoms did not develop on any grapevines during the 3 month observation period following feeding by the CLH for 10 days. Petiole samples collected from each vine and tested with ELISA were negative for *Xf*.

RT-PCR analyses. *Xf* was not detected in the mouthparts of CLH collected from Alamance and Johnston counties, NC with RT-PCR.

DISCUSSION

A much more diverse population of leafhoppers, in the same vineyards as those studied by Myers et al. (2007) in 2004 and 2005, was found. They found that four species, *P. irroratus, G. versuta, O. orbona* and *A. constricta* were the most abundant. They also reported that all other members of the Cicadellidae comprised between 2 and 9% of total leafhoppers trapped. However, in our study the proportion of leafhoppers categorized as "others" ranged between 24 and 91%. We also included planthoppers, members of the Membracidae in our category, "others". However, it is unlikely that Membracids alone account for the increase in relative proportion of "others." Myers et al. (2007) reported trapping a total of 9 leafhopper species. We did not differentiate the total species, however >20 species were trapped, including: the GWSS, *Paraulacizes irrorata*, *Querna* sp., *G. coccinea, Draculocephala* sp.*, Acertagallia sanguinolenta, Empoasca* sp. and *Erythroneura sp.* At least three species of Membracids were trapped. As a result of the increased proportion of "others" found in our research, two of our species of interest, the xylem-feeder *G. versuta,* and the phloem-feeder *Agallia* spp. were observed in smaller proportions than those reported by Myers et al (2007). We observed similar proportions of the xylem-feeder *O. orbona* and the phloem-feeder *P. irroratus*. The time of occurence of peak population densities of each species, was similar in both years of our study and similar to what Myers et al. (2007) reported in 2004 and 2005.

We trapped fewer total leafhoppers in the Coastal Plain vineyards than in the Mountains and Piedmont vineyards in both years. The cause of the lesser number of leafhoppers in Coastal Plain vineyards is unknown, but was also reported by Myers et al.

(2007). The proportion of leafhoppers also differed between the regions. In the Coastal

Plain, *Agallia* spp. and *O. orbona* were the most commonly observed species as opposed to

G. versuta.

In general, the number of *P. irroratus* trapped was less in 2006 than 2007. This may

be due to a hard freeze (-1 to -3 °C) that occurred across our trapping area on 8 April 2007

(Brooks, M., personal communication), just prior to when population densities of *P. irroratus*

usually peak. This decrease was not observed in Polk Co. vineyard # 2 or Currituck Co.

vineyard # 1.

The abundance and proportion of *G. versuta* increased from 2006 to 2007. This

increase was particularly dramatic in the two Piedmont vineyards. The average daily high

temperature for the month of July, when *G. versuta* populations typically peak, was similar in

both years (NC State Climate Office, CRONOS database), thus, it is not likely that the

increase in the number of *G. versuta* is related to temperature differences.

We did not detect *Xf* in any of the CLH tested with PCR, and transmission studies

were all negative. The CLH is a phloem-feeder and probably does not acquire *Xf,* as *Xf* is

limited to xylem tissue. If *Xf* is acquired during probing behaviors, the CLH likely does not

acquire *Xf* in a sufficient titer to transmit the bacteria. Myers et al. (2007) reported *Xf*

occurring in the mouthparts of *P. irroratus*, also a phloem-feeding leafhopper, however the

feeding preferences and behaviors of these two leafhoppers may differ significantly.

CLH feeds on species of clover, which is a reported host of *Xf* (Wistrom and Purcell,

2005). However, due to contamination when attempting to isolate *Xf* from white clover,

Wistrom and Purcell (2005) were unable to determine whether or not the species supports

systemic movement, or has sufficiently high titers of the bacterium to serve as a source of inoculum. It is possible that the CLH did not transmit *Xf* because, in addition to reasons discussed above, their plant hosts are not good sources of inoculum.

Studies to test for transmission ability of *Aceratagallia sanguinolenta* should be conducted in the future by caging the insects on *Trifolium* sp. inoculated with *Xf*, and transferring them to grapevines. After the inoculation period, PCR analysis of their mouthparts should be conducted to determine whether or not they acquired *Xf* from the *Trifolium* sp. and, if positive, vines should be monitored weekly for 6 months for the development of PD symptoms and tested for *Xf* if this occurs. This would provide insight as to whether or not agallian leafhoppers acquire *Xf* but fail to transmit it to grapevines, or do not acquire the bacterium at all.

Future studies involving phloem-feeders as potential vectors of *Xf* should also focus on leafhopper species that are abundant and known to prefer grapevines as a host as these factors would favor spread of PD. *Agallia* spp. were abundant in North Carolina vineyards while *P. irroratus* was not abundant in 2006 and 2007. Both leafhoppers exhibit an extremely broad host range and are likely capable of utilizing grapevines as a feeding host.

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CHAPTER 2

INTRODUCTION

Pierce's disease (PD), caused by *Xylella fastidiosa* (*Xf*) (Wells et al., 1987) is a devastating disease of grapevines in the more temperate growing regions of the US (Hewitt, 1958). The bacterium multiplies and plugs the xylem vessels of its hosts (Esau, 1948), preventing proper uptake of water and therefore causing symptoms similar to drought stress. Symptoms of PD on grapevines were originally reported by Newton Pierce in 1892 and include marginal leaf scorch (Pierce 1892), defoliation leaving the petiole attached to the shoot (Gubler et al., 2005), irregular maturation of the bark causing what are known as green islands (Hopkins, 1981), stunting, fruit shriveling and, potentially, vine death. *Xf* is transmitted by xylophagous insects including leafhoppers (Order: Hempitera, Family: Cicadellidae) (Frazier & Freitag, 1946) and spittlebugs (Order: Hemiptera, Family: Cercopidae) (Severin, 1950).

Xf has an extremely wide host range and is found both in symptomatic and asymptomatic hosts (Freitag, 1951). According to the University of California at Berkeley's College of Natural Resources *Xylella* website (Purcell, A. H., Almeida, R., personal communication), the PD strain of *Xf* has been found in over 140 plant hosts and non-PD strains have been found in at least 17 plant hosts. Recently 17 plant species have been identified in Texas as reservoir hosts of *Xf* (McGaha, et al. 2007). Through the use of RFLP and QRT-PCR, at least two *Xf* taxa were identified in Texas, a grape strain and a ragweed strain, corresponding to *Xf* subsp. *piercei* and *Xf* subsp. *multiplex* (Schaad et al., 2004), respectively (Morano et al. 2008). In their reservoir host studies, 14 *Xf* isolates were

examined and all isolates from the reservoir hosts belonged to the ragweed strain, with the exception that the grape strain was found in wild species belonging to the genus *Vitis* (Morano et al. 2008). *Xf* subsp. *multiplex* has not been reported to cause disease in grapevines, leaving questions regarding the extent to which the reported host plants are contributing to the occurrence of PD in Texas vineyards (Schaad et al., 2004). Wistrom and Purcell (2005) defined criteria that a plant host must meet in order to be considered a significant source of inoculum, indicating that the extent to which plant reservoir hosts contribute to the occurrence of PD ranges. A plant must (i) develop infections when inoculated (ii) support systemic movement of the bacterium beyond the point of inoculation, (iii) support high populations of the bacterium and (iv) be a food source for leafhopper vectors in order to contribute significantly as a source of *Xf* inoculum.

Preferred plant hosts of leafhoppers have been evaluated using a variety of methods. Brodbeck et al. (1990) determined the preference of the glassy-winged sharpshooter (GWSS) on 19 species of host plants by planting the hosts randomly and observing the number of GWSS feeding on each species 2 to 3 times weekly. Alternatively, cages may be constructed to house a leafhopper on a section of stem tissue with an attached vial for the collection and measurement of leafhopper excrement, as a function of feeding preference for a given host (Mizell III, R., personal communication). One host plant factor potentially affecting leafhopper feeding preference is the presence of fungal endophytes. Muegge et al. (1991) observed 28 total leafhopper and froghopper species on endophyte infected and non-infected tall fescue. They found that four leafhopper species and one froghopper species had significantly higher population densities on non-infected grasses, compared to endophyte-

infected grasses. The specific effects of a plant-endophyte interaction vary greatly from one insect species to another, but may become an important consideration when investigating potential reservoir hosts of *Xf*.

Preliminary studies conducted in North Carolina in 2002 determined that *Xf* was found in 18 alternative hosts including trees, shrubs, vines and grasses when the plants were tested with ELISA (Sutton and Harrison, *unpublished data*). These plants were collected from areas surrounding vineyards known to have PD.

The focus of this study is to examine potential reservoir hosts of *Xf* on the vineyard floor. It is common for North Carolina vineyard managers to allow native and weedy vegetation to proliferate on the vineyard floor. Alternatively, some growers plant and maintain groundcovers for which the recommended industry standards are red fescue, *Festuca rubra*, and tall fescue, *Festuca arundinacea* (Mitchum, 2007). Growers have expressed a desire for a groundcover that is known to be a poor host of *Xf*, and therefore will not provide a source of inoculum within vineyards. The specific objectives of this research are (i) to describe the typical North Carolina vineyard floor in terms of vegetative composition (ii) to test the plant species found on the vineyard floor for *Xf* using ELISA, PCR, and isolation into pure culture, creating a host list for North Carolina and (iii) to identify a suitable groundcover that is not a good host of *Xf*.

MATERIALS AND METHODS

Ground vegetation surveys. Surveys were conducted in the spring and fall of 2007-08 in three vineyards in Polk, Yadkin and Guilford counties to describe and quantify the vegetation present on the vineyard floor. Grapevines were planted in 1991 in the Polk Co. vineyard, located in the Mountain region of North Carolina. The surveys were conducted in an area planted exclusively to Chardonnay. The Yadkin Co. vineyard vines were planted in 1997 and the survey area was planted exclusively to Chardonnay. The Guilford Co. vineyard vines were planted in 2002, and cultivars in the survey area included Chardonnay, Nebbiolo, Sangiovese and Merlot. Yadkin and Guilford counties are located in North Carolina's Piedmont region. All locations had wooded areas bordering the vineyard on at least two sides. Beginning at an arbitrarily selected vine of Chardonnay, a 50 meter long measuring tape was stretched along the floor of the vineyards at approximately 45º in relation to the rows of vines. Ten 1 m^2 sites were randomly selected along this 50 m transect. A 1 m^2 wooden frame was placed at each site, and the percentage of each species of plant present in the frame was estimated and recorded. Samples of the most abundant species were collected and kept in a cooler during transport to the laboratory. The identity of each plants species was determined at the NCSU Herbarium or the NCSU Plant Disease and Insect Clinic Turf Diagnostics Laboratory. Samples for ELISA and PCR analyses were not necessarily taken from the same plant. Samples were kept at 4 ºC, until tested for the presence of *Xf* with ELISA.

Testing potential reservoir hosts for *Xf*. ELISA assays were conducted using a *Xylella fastidiosa* double-antibody sandwich kit (Agdia, Elkhart, IN) following the

manufacturers protocol. In 2007, assays were conducted using 0.5-0.8 g of fresh plant material, including stems, petioles and sometimes leaf tissue, depending on the anatomy of the plant species. In 2008, 0.5 g of fresh plant tissue was used for most ELISA assays; however occasionally between 0.2 and 0.5 g was used, if the samples taken were small. Petioles, stems, and leaf tissue, were used for ELISA assays in 2008 if the plant was a grass. At least one negative control of general extraction buffer only, and one positive control provided by Agdia, was used per assay. ELISA plates were read in a Model 680 Microplate Reader (Bio-Rad Laboratories Inc., Hercules, CA) and positive cutoff values were determined by calculating two times the average of the negative controls, plus three times the standard deviation of the negative controls (2Avg. + 3 Std.dev), with any value above this limit considered positive.

The plant tissue that was ground in the ELISA general extraction buffer for use in the ELISA procedure was also used for DNA extraction and PCR analyses. DNA extraction was conducted following the manufacturer' s protocol using a Qiagen DNEasy Plant Mini Kit (Qiagen Inc., Valencia, CA) with 100 µl of ground plant extract as starting material. DNA was quantified using a nanodrop and diluted to 10 ng/µl with sterile deionized water.

PCR reactions were carried out using a 25 µl total reaction volume containing 12.5 µl of GoTaq Green Mastermix (Promega, Madison, WI), 3.5 µl sterile, deionized water, primers RXYgyr907 and FXYgyr499 (Morano et al., 2008) at a concentration of 800 nM and 5 µl of DNA template. Sterile deionized water was added to the above mixture instead of DNA template, as a negative control. DNA extracted from *Xf* in pure culture using the DNA extraction procedure above was added to one reaction as a positive control. PCR cycles were

carried out in a Biorad MyCycler thermal cycler (Bio-Rad Laboratories, Hercules, CA) and

consisted of an initial denaturing step of 94 ºC for 3 min, followed by 35 cycles at 94 ºC for 1

min, 60 ºC for 1 min, and 72 ºC for 2 min, followed by a final extension step of 72 ºC for 5

min.

PCR products were visualized using horizontal electrophoresis of 1% agarose tris-

boric acid-EDTA (TBE) gels, stained with ethidium bromide, in TBE buffer. The PCR

product was 408 base pairs (bp) long and resulting bands were compared to a Quick-Load

100 bp DNA ladder (New England Biolabs, Ipswich, MA).

Culture of *Xf* was attempted from plant samples that tested positive with ELISA. In a

laminar flow hood, 0.1 g of fresh tissue (stem, petiole and possibly leaf) from the original

sample was surface disinfested in 90 % EtOH for 1 min., 2% NaOCl for 1 min., and rinsed

with sterile deionized water three times for 1 min. each rinse. The sterilized plant material

was then macerated in 2 mL sterile succinate-citrate-phosphate (SCP) buffer (Hopkins, 1982)

with an autoclaved mortar and pestle. A dilution series was made using 100 μl of the original

ground material to make a 1:10, 1:100 and 1:1000 dilution, using sterile SCP as a diluent. 50

μl of each dilution, including the undiluted original was then pipetted onto a petri plate of

periwinkle-wilt-gelrite (PWG) medium (Hill and Purcell, 1995) and spread with a sterile

glass rod. Dishes were held in a dark incubator at 28°C, and were monitored for up to 30

days for the formation of *Xf* colonies. Resulting potential *Xf* colonies were transferred and

spread onto PW medium (Davis et al., 1981) with a sterile bacterial loop.

Host plant suitability studies. Tests were conducted to assess leafhopper feeding

preferences on various reservoir host plants. Plants included in the study were tall fescue,

Festuca arundinacea; white clover, *Trifolium repens*; fine fescue, *Festuca* spp.; perennial ryegrass, *Lolium perenne*; and grape plants grown from Chardonnay seeds were included as a control. The plants were selected based either on their abundance in the vineyards surveyed, as was the case with white clover and tall fescue, or due to interest expressed by growers in determining which plants would make a suitable groundcover, as was the case with fine fescue and perennial ryegrass. Grape, tall fescue and white clover plants were grown and maintained in greenhouses kept at 21-24 ºC and watered twice daily. These plants were not exposed to any insecticides, but were occasionally treated for powdery mildew with myclobutanil (Nova; Rohm and Haas, Philadelphia, PA). Plants were not treated with myclobutanil within at least 1 month of the insect feeding experiments. The perennial ryegrass and fine fescue plants were obtained from another laboratory, and had been maintained in a different greenhouse. These plants were not exposed to any insecticide within at least 6 months of the insect feeding preference experiments.

Three replications of each plant species were used in the experiment. One pot (7 cm diam., 9 cm height) was placed inside each cage. Cages were cylindrical-shaped plastic storage containers, open at the top, measuring 15 cm in diameter and 17 cm in height. A piece of insect impermeable mesh was stretched across the open top of each cage and secured with a rubber band. This allowed the plants to be watered overhead in addition to providing ventilation for the insects. Four small holes were pierced into the bottom of each pot to allow water to drain out. A Petri dish lid (size 60 x 15 mm) was placed under the bottom of the pot to catch drainage. The bottom of each cage was lined with paper towels to prevent standing water in the cages. In 2007, 10 *Graphocephala versuta* were caged on plants in each pot and

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the number of individuals found alive was counted daily for 1 week. The plants and insects were kept in cages in the laboratory at approximately 24 °C and exposed to a 15 hr daylight cycle of fluorescent lights. All pots were watered overhead daily with 30 ml of water using a squirt bottle.

G. versuta used in the experiments were collected from vineyards in Alamance and Johnston counties with a sweep net and transported back to the laboratory in centrifuge tubes in a cooler. The G. versuta from the Alamance Co. vineyard were caught while feeding on V. vinifera while the G. versuta trapped in Johnston Co. were feeding on Sida rhombifolia. G. versuta from each location were combined. The number of live insects was counted each day. At the end of the experiment all insects, dead or alive, were recovered.

In 2008, only five leafhoppers were caged on plants in each pot due to low populations in and around the vineyards, and these insects were a mixture of G. versuta, G. coccinea, and Draeculacepha sp. All of the leafhoppers used were collected from the Johnston Co. vineyard with a sweep net and were feeding on a mixture of vegetation including Sida rhombifolia, Ipomoea sp., and Ambrosia artemisiifolia. The grape controls used in 2008 were rooted cuttings of Chardonnay.

Results were analyzed using SAS (SAS Institute, Cary, NC). Effects on leafhopper survival among the different plant treatments were analyzed using PROC MIXED with type 3 tests of fixed effects using an arcsine-square-root transformation of the proportion of leafhoppers surviving.

Fungal endophyte effects on leafhopper survival. Based on the results of the initial host suitability study, a study was conducted to determine the effects of the presence of

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fungal endophytes on the survival of leafhoppers. Seed from three grasses, each infected

with endophyte, was obtained from Scotts Seed Company (Scotts, Marysville, OH).   Grasses

included in the study were *Festuca rubra* spp. *commutata*, Chewings fescue, variety Treazure

I and *Festuca rubra* ssp. *rubra*, creeping red fescue, variety Florentine GT. The grasses had

a 96% and 74% incidence of endophyte-infected seed, respectively. The grasses were grown

from seed in flats in Fafard potting mix and kept in a greenhouse for 10 months at 21-24 °C

and watered twice daily. The grasses were not treated with insecticides. Seven cm diam.

plugs, were cut from the flats, with roots attached and the root system was washed to remove

as much potting mix as possible. Three plugs from each grass were soaked in water for 8 h.

Three plugs from each grass were also soaked in a 0.6 mL/liter solution of 250 a.i./liter

tebuconazole (Brand name: Elite, Bayer Crop Science) for 8 h, as modified from the methods

of Dongyi and Kelemu (2004) for curing plants from endophyte. The grass plugs were then

repotted into small pots containing Fafard 2P potting mix. Each pot was placed in a plastic

cage measuring 9.5 cm in diam. and 15.5 cm in height, with an open top which was covered

with mesh. Five leafhoppers were placed inside each cage. Cages remained in the laboratory

at approximately 24 °C, subjected to a 15 hour daylight cycle, and watered overhead daily

with 30 ml of water from a squirt bottle. Chardonnay grapevines were used as a control. The

number of living leafhoppers was counted each day for seven days and at the end of the

experiment the cages were opened and all leafhoppers bodies were removed. Several sprigs

of all plant types and treatments included in the leafhopper feeding studies for 2007 and 2008

were sent to the Jim White laboratory at Rutgers University, New Brunswick, NJ for testing

to determine whether or not they were infected with endophyte. Endophyte infection was

determined by staining leaf sheaths, or segments of stem, with aniline blue in 85% lactic acid

and visualization with microscopy. Results were analyzed statistically as described above.

RESULTS

Ground vegetation surveys. White clover, *Trifolium repens*, was the most abundant plant species present in the Polk and Yadkin Co. vineyards in the springs of 2007 and 2008 (Tables 1A-B and 2 A-B). Grasses that were not producing seed, and therefore could not be identified beyond family, were the most dominant group overall at these two locations, in the spring of 2008 (Tables 2A-B). The Guilford Co. vineyard vegetation was very diverse in the spring of 2007 and no one species or group dominated the groundcover. In the spring of 2008, hop clover, *Trifolium* spp. dominated, comprising 12.7% (Tables 1C and 2C).

Crabgrass, *Digitaria* sp., was the most abundant plant species present in all three vineyards in the fall of 2007 (Table 1). In the fall of 2008, bermudagrass, *Cynodon dactylon*, was the most abundant plant species in the Polk Co. vineyard at 17.3%. *T. repens* was also very abundant, comprising 17.1% of the vineyard floor (Table 2A). In the Yadkin Co. vineyard, dallisgrass, *Paspalum dilatatum*, and sterile grasses were the most abundant groups, comprising 24.4% and 21.4% of the vineyard floor, respectively (Table 2B). In the Guilford Co. vineyard sterile grasses dominated the vegetation, at 33.5% (Table 2C).

In the Polk Co. vineyard the amount of bare ground increased between spring and fall each year. However it decreased between spring and fall in the other two vineyards in both years of the study. Overall, the amount of bare vineyard floor was much greater in 2007 than in 2008, across all seasons and locations (Tables 1 and 2).

Plant species comprising ≤1% of the vineyard floor were grouped into the category, other. Therefore, the proportion of the other category is one indication of the species diversity in each vineyard. In 2007, the greatest plant species diversity was observed in the

Yadkin Co. vineyard, followed by Guilford and Polk counties. In 2008, the greatest diversity

was observed in the Guilford Co. vineyard, followed by Yadkin and Polk counties (Tables 1

and 2).

Testing potential reservoir hosts for *Xf*. Overall, 14 of 40 plant species surveyed

tested positive for *Xf* with ELISA. In 2007, seven of 27 plant species, and five of 16 plant

species tested positive for *Xf* in the spring and fall, respectively. Species testing positive in

2007 belonged to the Fabaceae and Poaceae. In 2008, eight of 21 plant species, and two of

14 plant species tested positive for *Xf* in the spring and fall, respectively. Species testing

positive in 2008 belonged to the Plantaginaceae, Ranunculaceae, Poaceae, Euphorbiaceae,

Fabaceae, Oxalidaceae, and Geraniaceae (Table 3). PCR assays confirmed the presence of *Xf*

in two species, little barley, *Hordeum pusillum* and buttercup, *Ranunculus* sp. (Figure 1).

Due to high levels of microbial contamination, attempts to isolate *Xf* from reservoir hosts

were unsuccessful.

Host plant suitability studies. In 2007, *G. versuta* survival was significantly less

when the insects were caged exclusively on fine fescue, *Festuca* spp., and perennial ryegrass,

Lolium perenne, compared to all other treatments and the grapevine control (Figure 2A). In

2008, there were no significant differences in leafhopper survival across all treatments and

the control until the fourth day of the experiment (Figure 2B).

Fungal endophyte effects on leafhopper survival. There were no significant

differences in leafhopper survival when the insects were caged exclusively on endophyte-

infected grasses compared to non-infected grasses, for both creeping red fescue and

Chewing's fescue (Figure 3).

DISCUSSION

Several hosts of *Xf* were identified in our studies that have not been previously reported: *Chamaesyce maculata*, *Trifolium arvense*, hop clover, *Trifolium* spp., *Geranium carolinianum*, *Oxalis stricta*, *Festuca* sp., *Setaria* sp., *Hordeum pusillum*, *Poa trivialis* and *Ranunculus* sp. Other members of the genera *Hordeum* and *Poa* have been previously identified as hosts (Freitag, 1951; Perring 2008; Purcell, A. H., Almeida, R., personal communication). In addition, hosts of *Xf* identified in our studies that had been previously reported to host PD-strains of the bacterium include: *Trifolium repens*, *Plantago lanceolata*, *Digitaria* sp., and *Cynodon dactylon* (Freitag, 1951; Raju, 1980; Hill and Purcell, 1997; Wistrom and Purcell, 2005; Purcell, A. H., Almeida, R., personal communication). Several species that tested negative for *Xf* in our studies have previously been identified as reservoir hosts. These include: *Conyza canadensis, Ipomoea* sp*., Trifolium pratense, Echinochloa crus-galli, Paspalum dilatatum, Lolium multiflorum, Sorghum halapense,* and *Rumex crispus* (Freitag, 1951; Raju, 1980; Black, 2008; Perring, 2008; Wistrom and Purcell, 2005; Purcell, A. H., Almeida, R., personal communication).

Multiple species in the Fabaceae and Poaceae tested positive for *Xf*, indicating that these families may be particularly important in the epidemiology of PD. Because white clover, *Trifolium repens* and crabgrass, *Digitaria* sp. tested positive for *Xf* multiple times and were abundant in the ground vegetation surveys, they may serve as important inoculum sources in North Carolina. However, the *Xf* strain infecting these plants needs to be determined. Black (2008) described *T. repens* as a high-risk species based on susceptibility to multiple strains of *Xf,* including PD-strains, and high OD readings when tested for *Xf* with

ELISA. Red clover, *T. pratense*, was also categorized as high-risk by Black (2008), although the species tested negative for *Xf* in our studies. Wistrom and Purcell (2005) were unable to determine whether *T. repens* was a good host of *Xf* because of high rates of microbial contamination when they attempted to isolate the bacteria.

Bermudagrass, *Cynodon dactylon,* which tested positive for *Xf* in our studies, provides a good breeding host for several vectors (Hill and Purcell, 1997), but it does not support multiplication or systemic movement of *Xf* (Wistrom and Purcell, 2005). Consequently, it may not be an important inoculum source within the vineyard. Horseweed, *Conyza canadensis,* although negative for *Xf* in our studies, was previously determined to be a good host of *Xf* as it accepted inoculations >50% of the time and supported sufficient titers and systemic movement of *Xf* beyond the point of inoculation (Wistrom and Purcell, 2005). Morningglory, *Ipomoea purpurea* is also considered a good host based on Wistrom and Purcell's criteria, although it tested negative for *Xf* in our studies. Yellow woodsorrel, *Oxalis stricta*, was not analyzed using Wistrom and Purcell's (2005) criteria, but may be considered an important host because in our studies it tested positive for *Xf* with ELISA on seven of 10 occasions.

Italian (annual) ryegrass, *Lolium multiflorum*, was considered a low-risk species by Black (2008), based on the criteria described above, which is consistent with our work, as it tested negative for *Xf* on 18 occasions. *Paspalum dilatatum*, though negative in our studies, is an important reservoir host of *Xf* and a preferred host of *G. atropunctata* (Raju, 1980).

Much of the bare ground observed on the vineyard floor in the vegetation surveys was the area directly beneath the grapevines. This strip is typically treated with herbicides

(Mitchum, 2007). However, considerably more bare ground was observed in 2007 than in 2008 across all locations because of an extended drought (North Carolina Drought Management Council, personal communication). These conditions likely contributed to the greater percentage of bare ground observed at all locations in 2007 compared to 2008. The conditions of moderate or extreme drought occurring in Polk Co. throughout all surveys likely accounted for the lack of plant species diversity observed at this site, compared to the other two locations.

Leafhopper host suitability studies did not result in finding a suitable groundcover that is also a poor host of *Xf*. Our findings indicate that *G. versuta* is capable of surviving on a variety of hosts, including grasses and broadleaf weeds for at least one week, which is sufficient time to acquire and transmit *Xf* (Purcell and Finlay, 1979). Therefore, none of the plant species we tested can be considered poor reservoir hosts, with regard to *G. versuta* survival.

In 2007, the effect of host plant on leafhopper survival was significant (p = 0.0008, F = 11.95, df = 4) at the the 95% confidence level. We observed mortality of all *G. versuta* within one day when they were caged exclusively on fine fescue, *Festuca* spp. Complete mortality was observed within three days when *G. versuta* were caged exclusively on perennial ryegrass, *Lolium perenne*. These results led us to hypothesize that these plants may have been naturally infected with fungal endophytes. For 2008 host suitability studies and fungal endophyte effects on leafhopper survival studies, the effect of host plant on leafhopper survival was significant (p = 0.0346, F = 2.63, df = 8) at the the 95% confidence level, however, significant differences among treatments were not observed until day 4 of the

experiment, and indicated that survival was significantly higher on tall fescue, *F. arundinacea*, compared to all other treatments except white clover, *T. repens*. Significant differences in survival were also observed between insects caged on *T. repens* and Chardonnay. *Graphocephala* spp. survived on *Festuca* spp. and *L. perenne* plants throughout the 7-day experiment. The *Festuca* spp and *L. perenne* plants used in 2007 were grown in a separate greenhouse from the other plants used in this study and we hypothesize that these plants may have unintentionally been exposed to insecticide prior to obtaining them. It was later determined that these plants were not infected with endophytes.

Our studies into the effects of fungal endophytes of Chewings fescue and creeping red fescue on leafhopper herbivory demonstrated that leafhoppers can survive exclusively on endophyte-infected plants, when necessary. The presence of *Epichloe typhina* in Chewings fescue has been shown to negatively affect the hairy chinch bug, *Blissus leucopterus hirtus* (Montandon) (Siegel et al., 1987). Additionally, Koppenhofer et al. (2003) observed reductions in survival of the oriental beetle, *Exomala orientalis* (Waterhouse), when feeding on endophyte-infected tall fescue, *Festuca arundinacea*. However, the same effects did not occur when the insects fed on endophyte-infected creeping red fescue, *Festuca rubra* spp. *rubra*. Fungal endophyte effects on leafhoppers have not been extensively studied.

In 2008, insect survival on the Chardonnay grapevine controls, a known host of *G.* spp., was considerably less than in 2007. The plants used in 2007 were grown from Chardonnay seeds. Plants used in 2008 were grown from rooted Chardonnay cuttings. The exact cause of the decreased survival in 2008 is unclear.

Due to the experimental design, we could not determine whether or not the endophyte-infected plants deterred feeding. For future work, feeding choice tests should be conducted to assess whether leafhoppers prefer to feed on endophyte-infected or uninfected grasses. Koppenhofer et al. (2003) also observed field populations of beetles in areas planted with endophyte-infected and uninfected grasses, and assessed effects on larval development by counting and weighing individual larvae. It is possible that the presence of fungal endophytes in grass hosts does negatively affect leafhoppers. However, populations need to be assessed and compared under field conditions, and additional measurements such as weight may be necessary. Future work may also focus on the effects of the presence of endophytes on breeding and oviposition.

Purcell et al. (1999) observed dramatic reductions in the abundance of the blue-green sharpshooter, *G. atropunctata*, as a result of removing plant breeding hosts of the insects from areas bordering vineyards in California. Based on our studies growers in North Carolina should eliminated broadleaf weeds from the vineyard floor. Clovers, *Trifolium* spp., and yellow woodsorrel, *Oxalis stricta*, are particularly important to remove, because these species consistently tested positive for *Xf*. Additionally, *Trifolium* spp. were abundant on the vineyard floor in spring when overwintering adults of vectors emerge and begin feeding. *Xf* acquired by the vectors at this time may result in early season infections which are more likely to become systemic than those occurring later in the year (Feil and Purcell, 2001; Purcell, 1981). We were not able to identify a suitable grass that did not support feeding of the vector *G. versuta*. Italian (annual) ryegrass, *L. multiflorum*, may be a good groundcover, as it consistently tested negative for *Xf* in our studies, and was listed as a low-risk species for

harboring PD by Black (2008). In general, warm-season grasses may be the best choice for a

groundcover, because these grasses will be dormant in the early spring when overwintering

leafhopper populations emerge and begin feeding.

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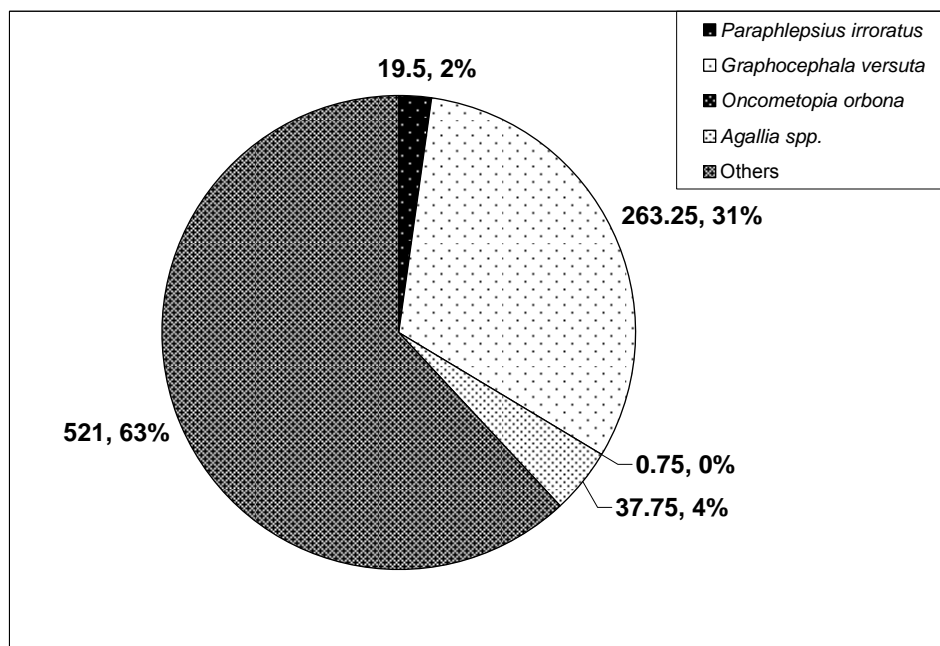Wells, J. M., Raju, B. C., Hung, H. Y., Weisburg, W. G., Mandelco-Paul, L., and Brenner, D. J. 1987. *Xylella fastidiosa* gen. Nov. sp. Nov.: Gram-negative, xylem-limited fastidious plant bacteria related to *Xanthomonas* spp. Int. J. Syst. Bacteriol. 37: 136-143.

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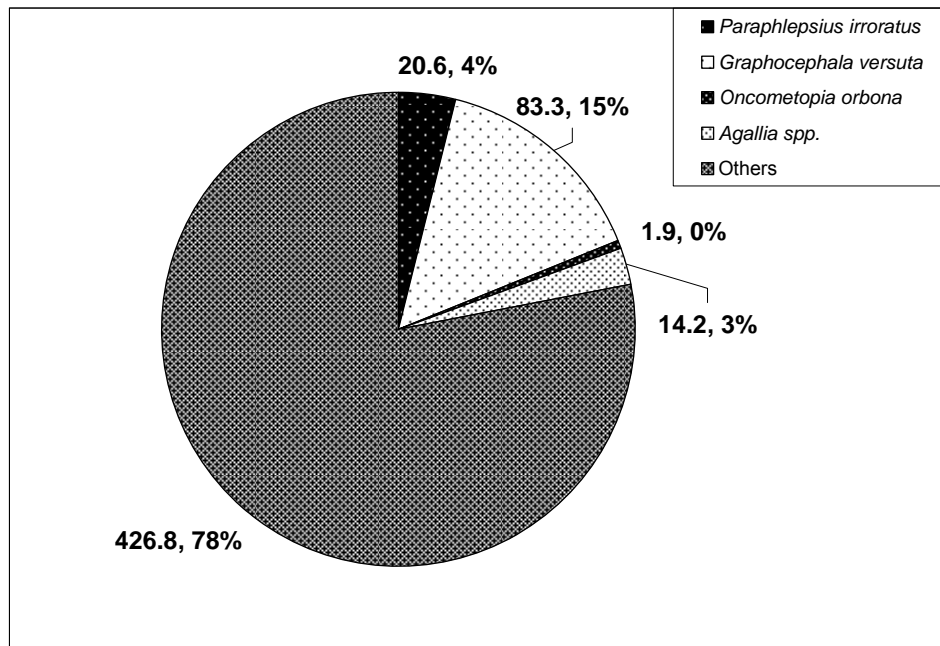
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Figure 1. Proportion of leafhoppers found in Polk Co. NC vineyard # 1 in (A) 2006 and (B) 2007, showing average number of leafhoppers per trap, followed by percentage. Category "others" refers to all other members of the Cicadellidae and the Membracidae.
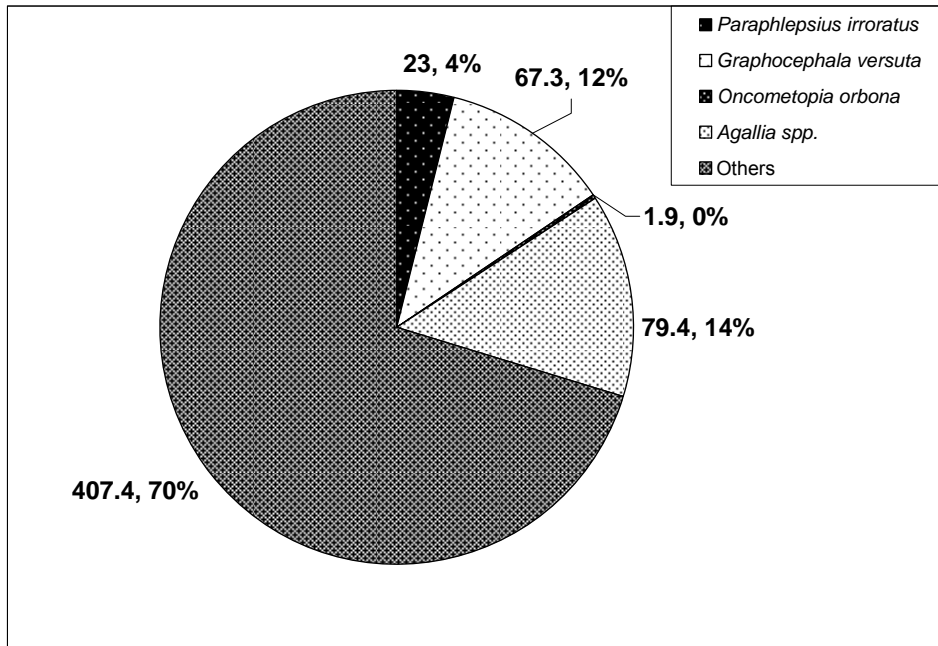
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B

Figure 2. Proportion of leafhoppers found in Polk Co. NC vineyard # 2 in (A) 2006 and (B) 2007 showing average number of leafhoppers per trap, followed by percentage. Category "others" refers to all other members of the Cicadellidae and the Membracidae.

A

B
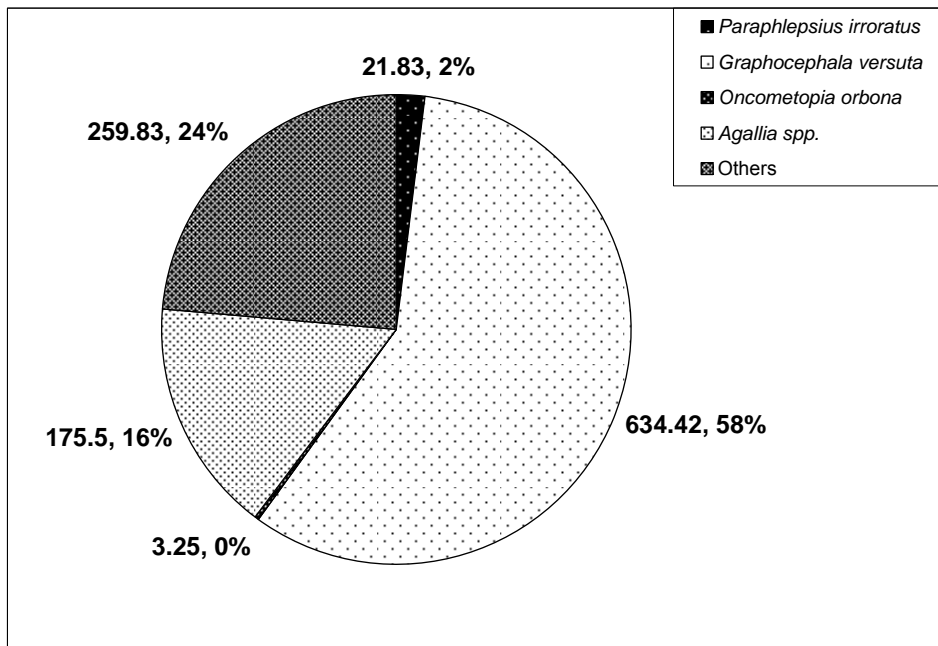
Figure 3. Proportion of leafhoppers found in Alamance Co. NC vineyard in (A) 2006 and (B) 2007 showing average number of leafhoppers per trap, followed by percentage. Category "others" refers to all other members of the Cicadellidae and the Membracidae.
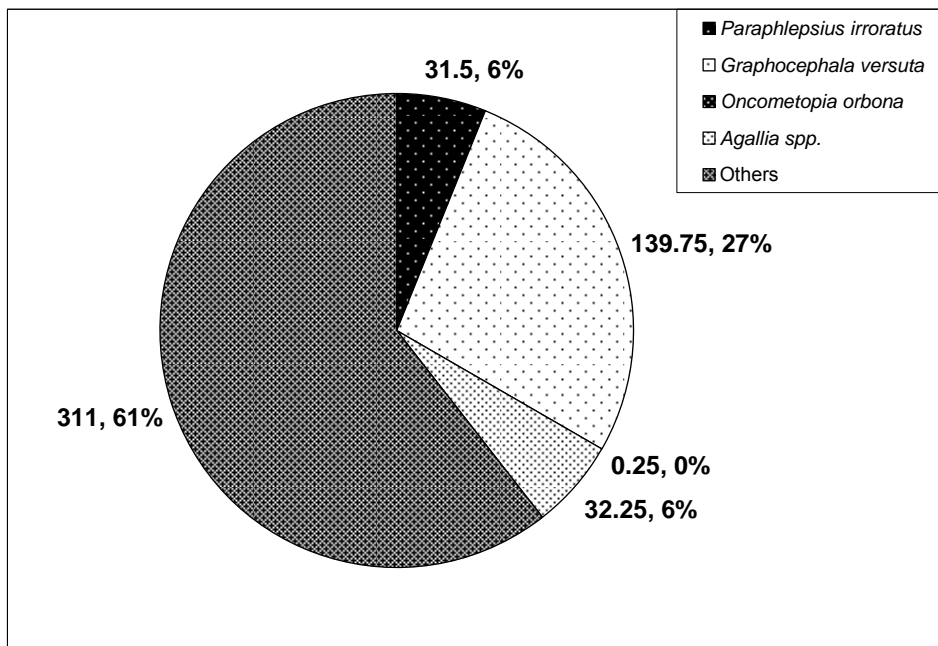
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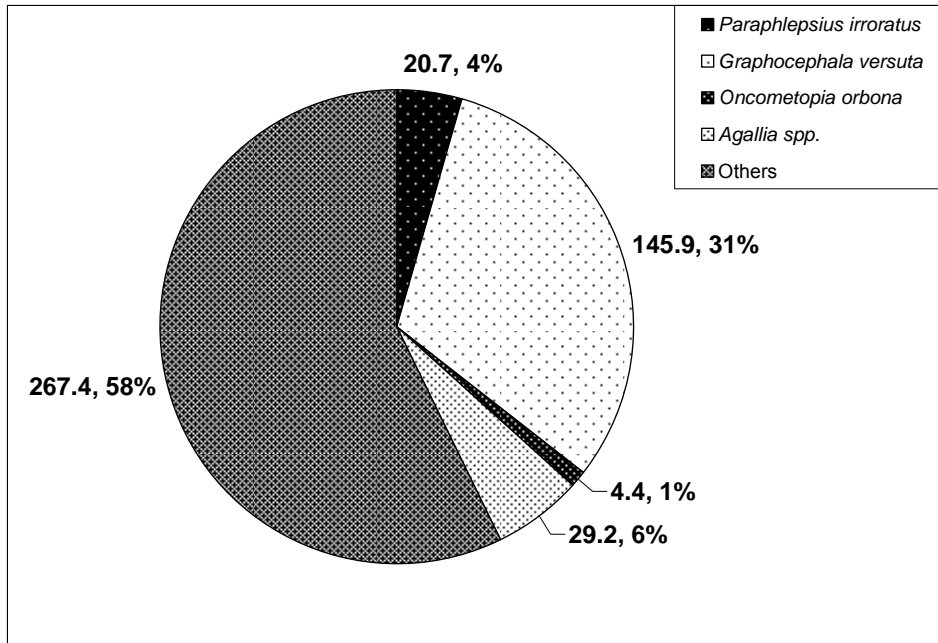
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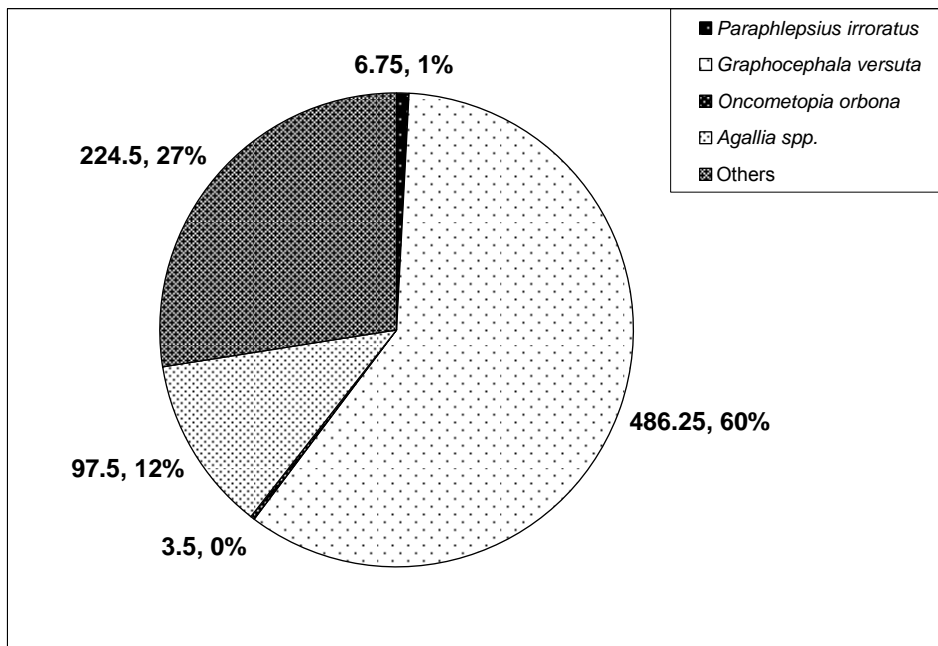
Figure 4. Proportion of leafhoppers found in Wake Co. NC vineyard in (A) 2006 and (B) 2007 showing average number of leafhoppers per trap, followed by percentage. Category "others" refers to all other members of the Cicadellidae and the Membracidae.

A
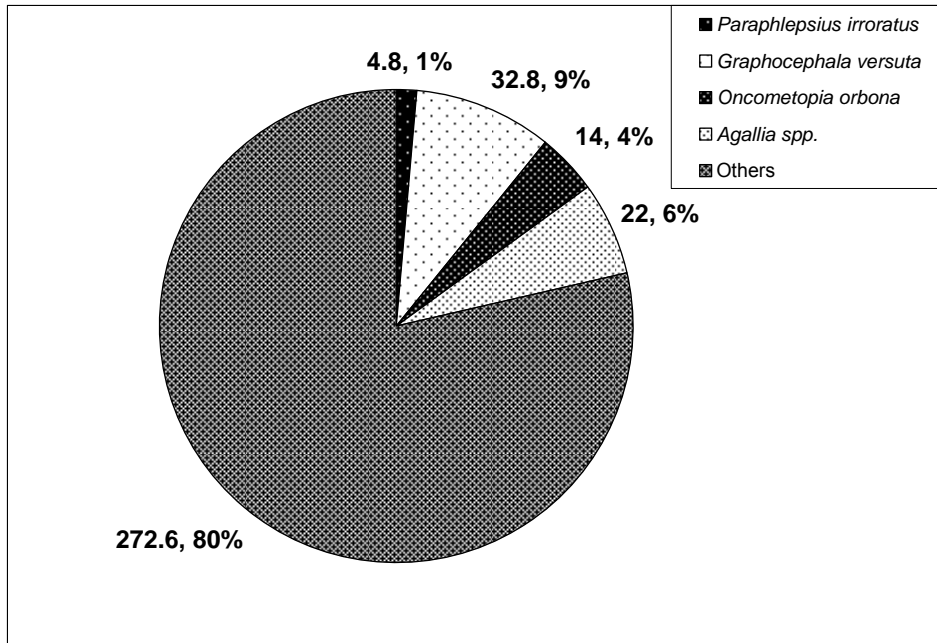
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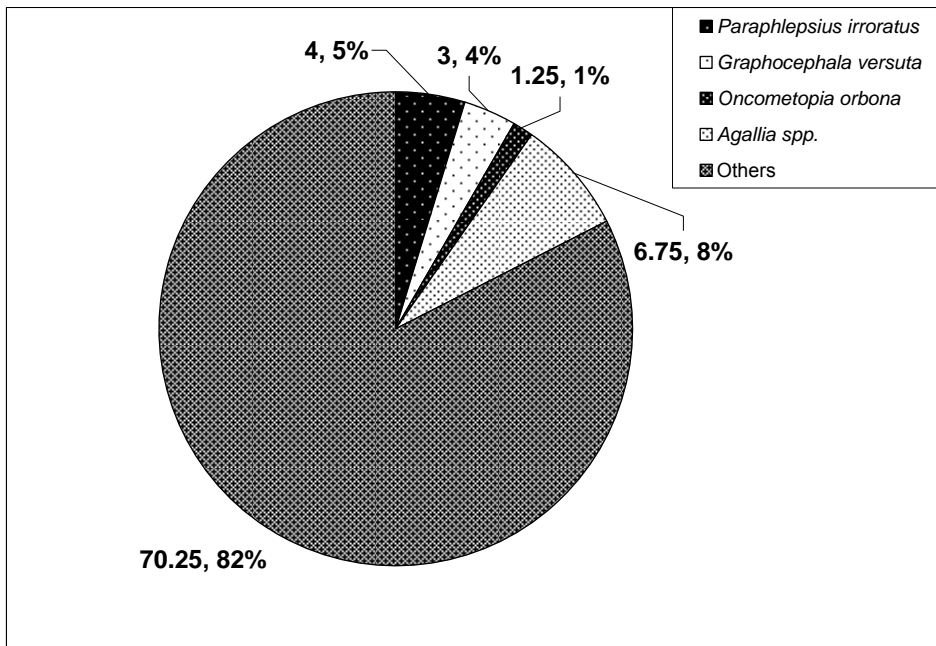
Figure 5. Proportion of leafhoppers found in Currituck Co. NC vineyard # 1 in (A) 2006 and (B) 2007 showing average number of leafhoppers per trap, followed by percentage. Category "others" refers to all other members of the Cicadellidae and the Membracidae.
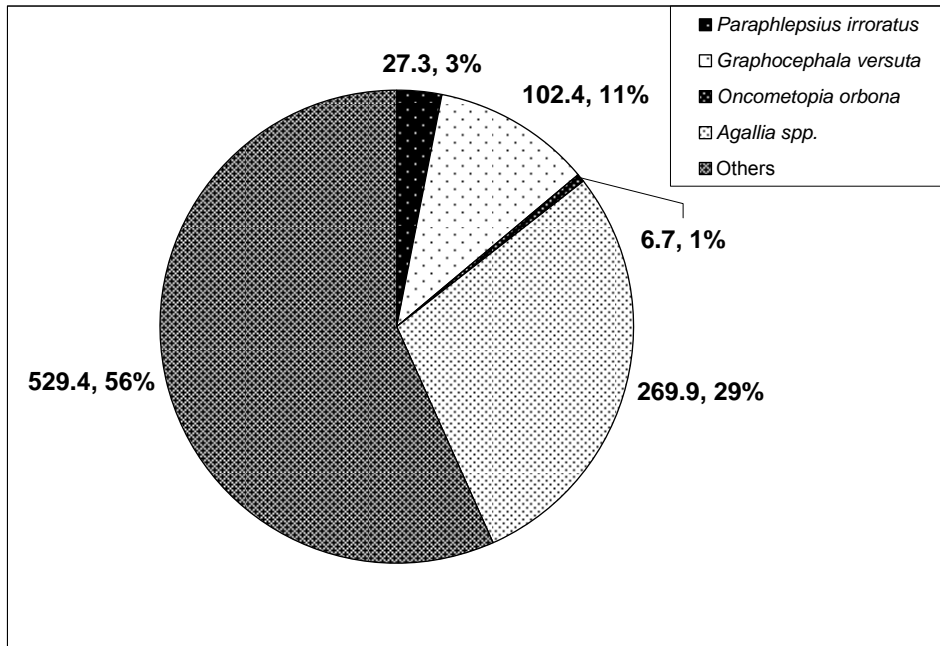
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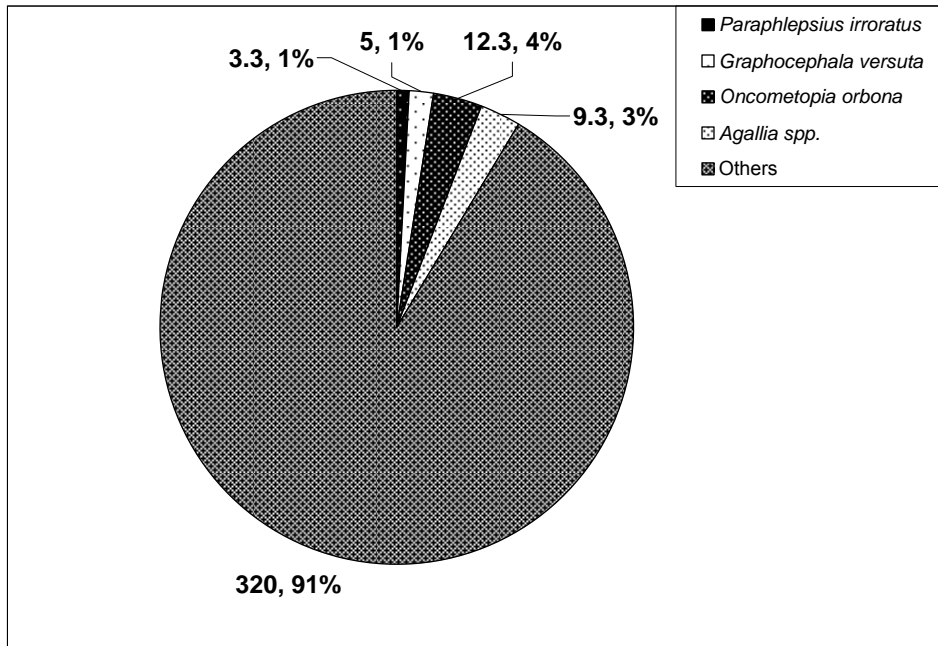
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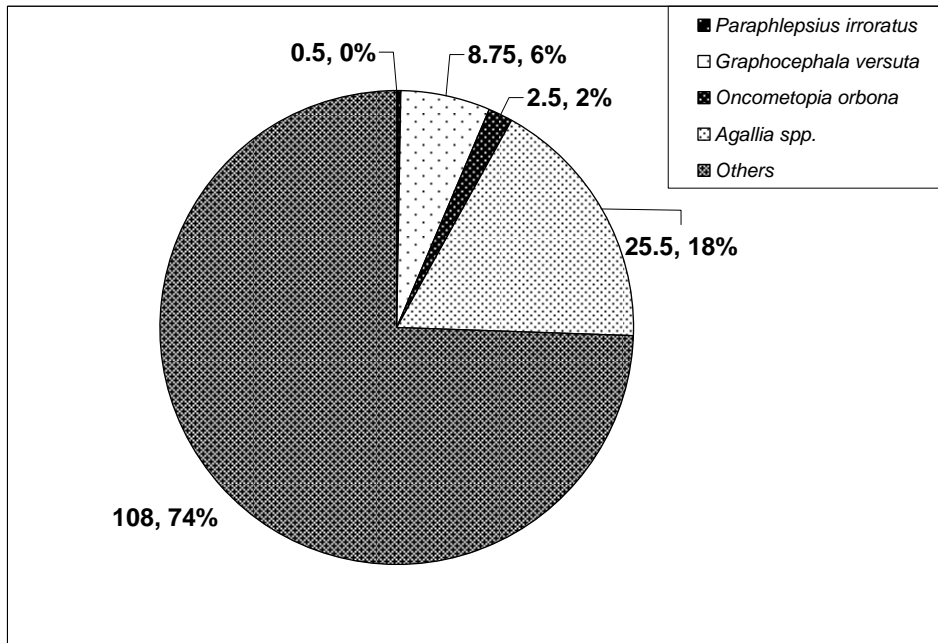
Figure 6. Proportion of leafhoppers found in Currituck Co. NC vineyard # 2 in (A) 2006 and (B) 2007 showing average number of leafhoppers per trap, followed by percentage. Category "others" refers to all other members of the Cicadellidae and the Membracidae.

A
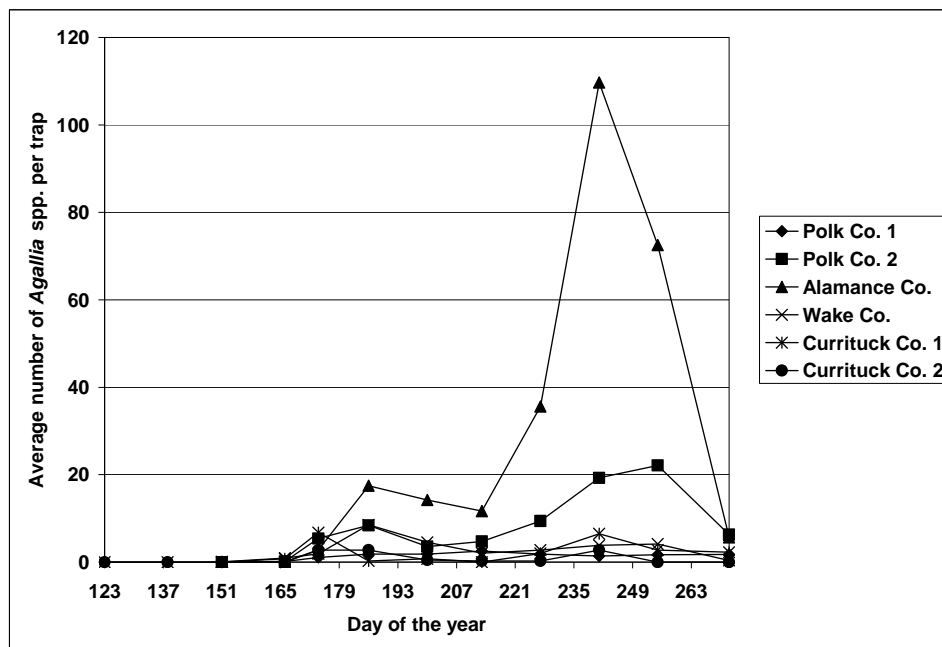
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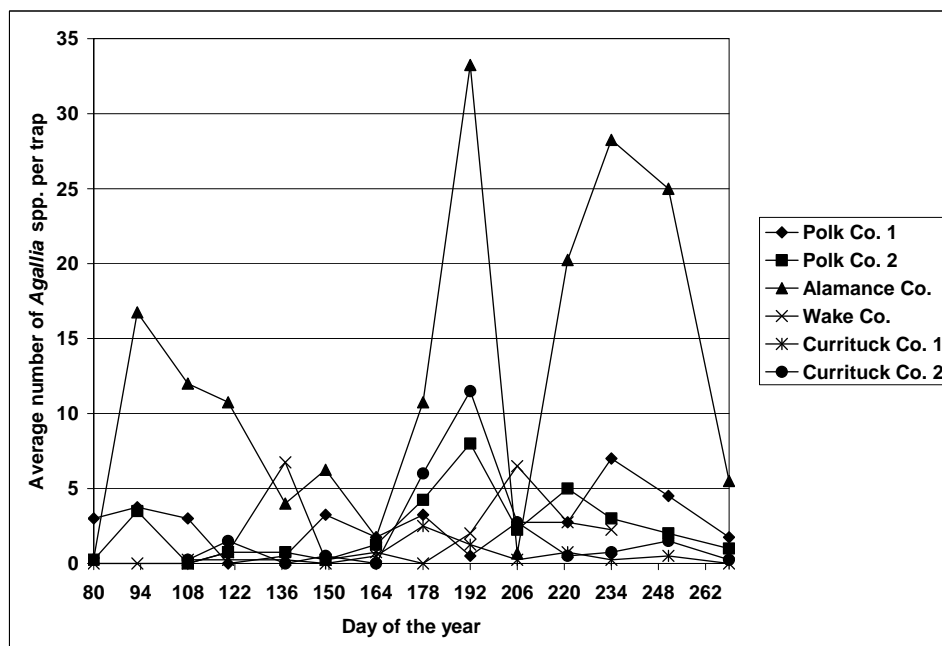
Figure 7. Average number of *Agallia* spp. per trap across all vineyards in (A) 2006 and (B) 2007. Note the differences in the scales of the x- and y-axes.

A
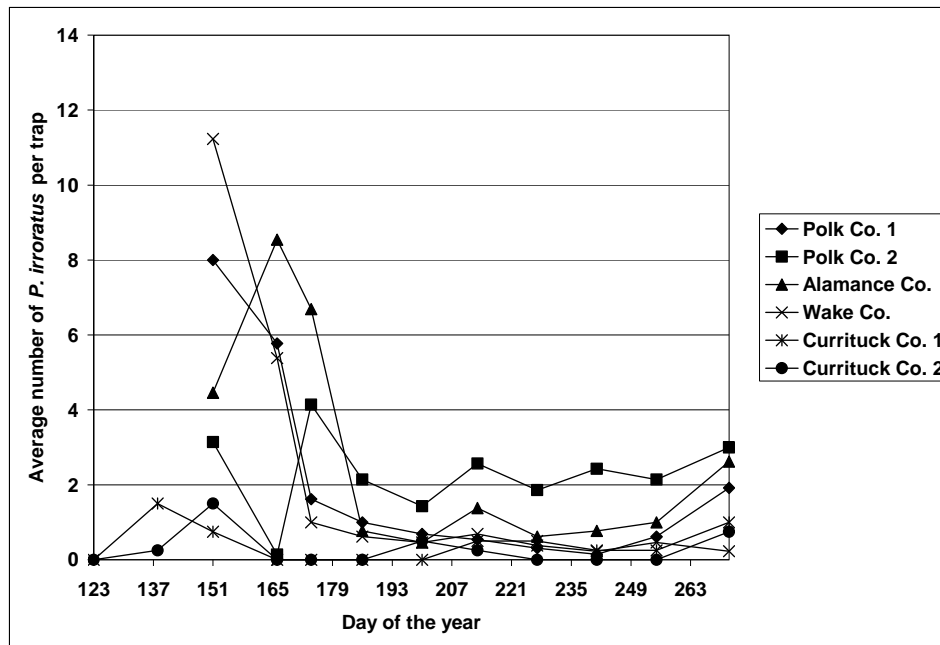
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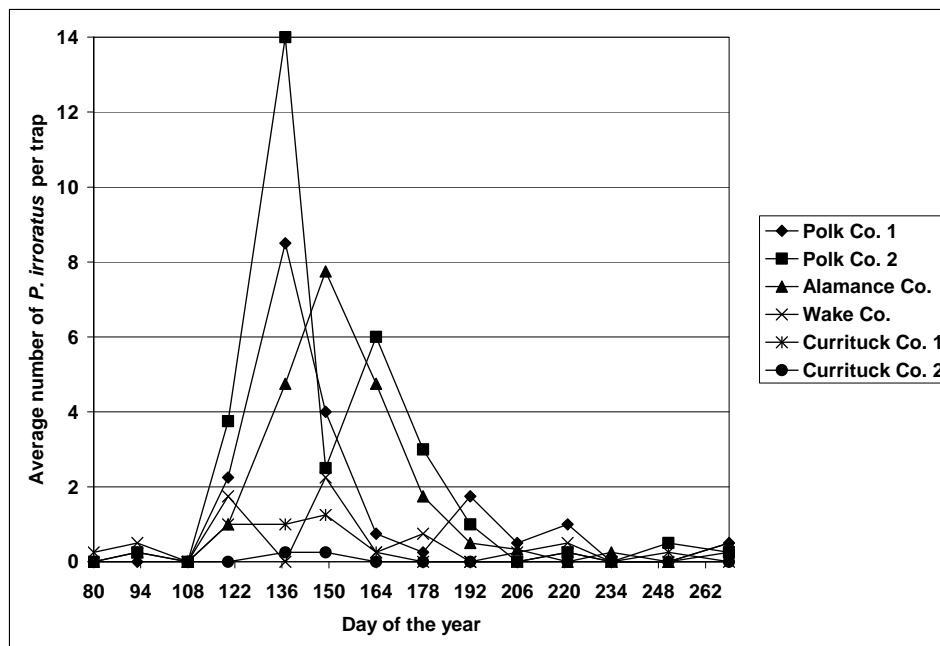
Figure 8. Average number of *Paraphlepsius irroratus* per trap across all vineyards in (A) 2006 and (B) 2007. Note the differences in the scales of the x-axes.

A
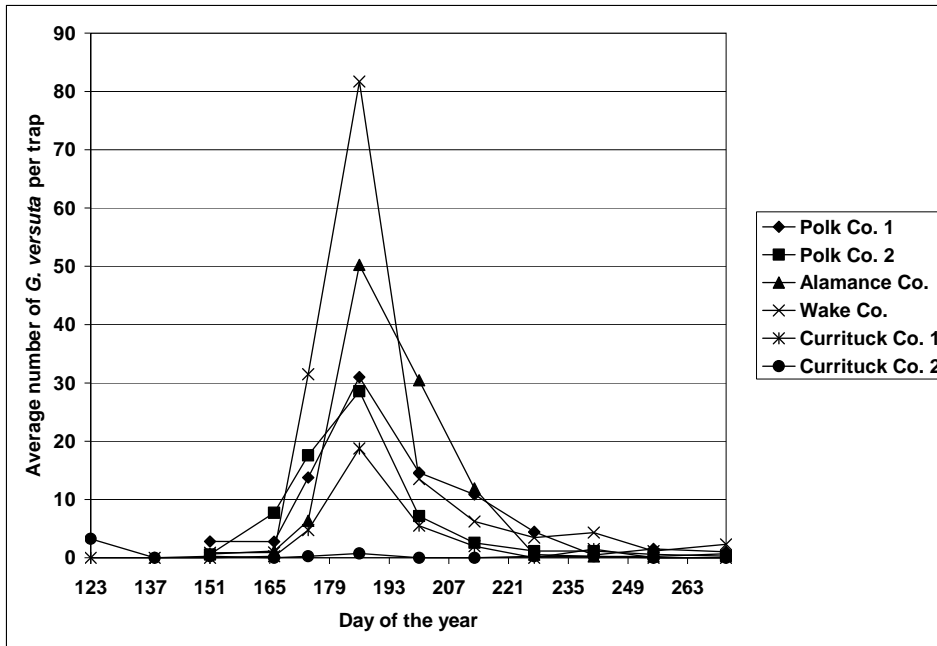
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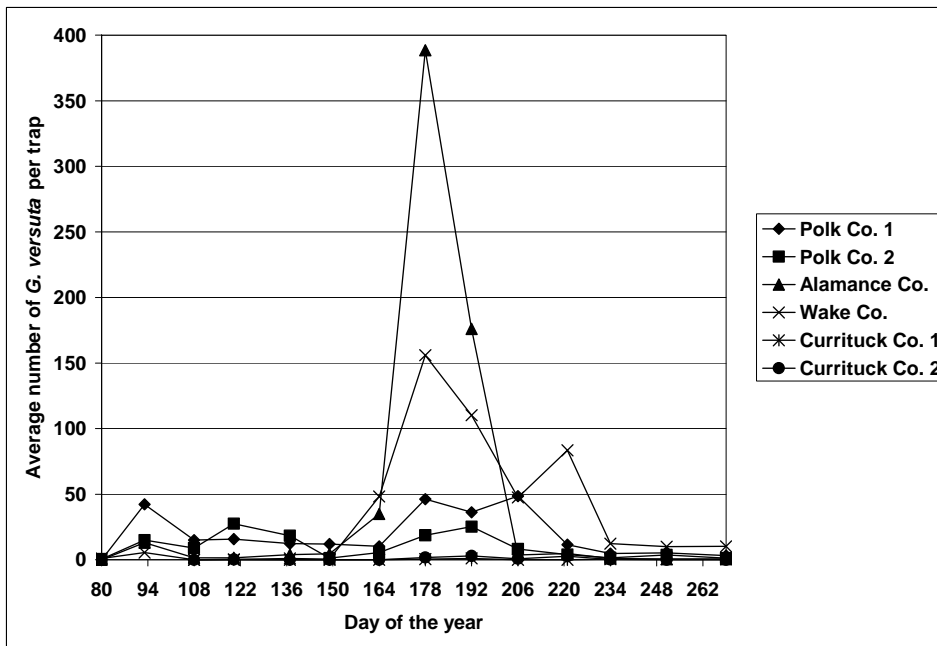
Figure 9. Average number of *Graphocephala versuta* per trap across all vineyards in (A) 2006 and (B) 2007. Note the differences in the scales of the x- and y-axes.

A
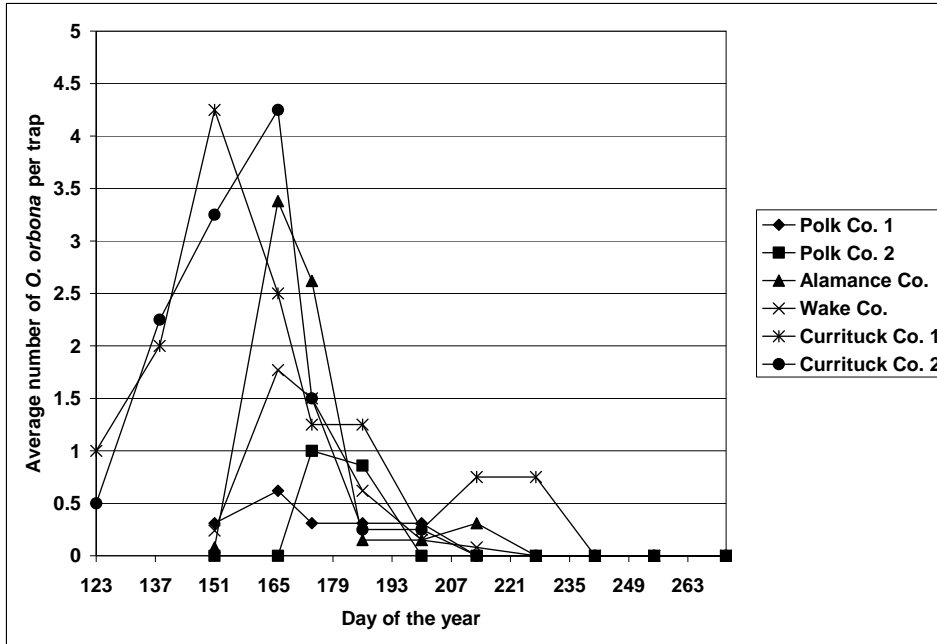
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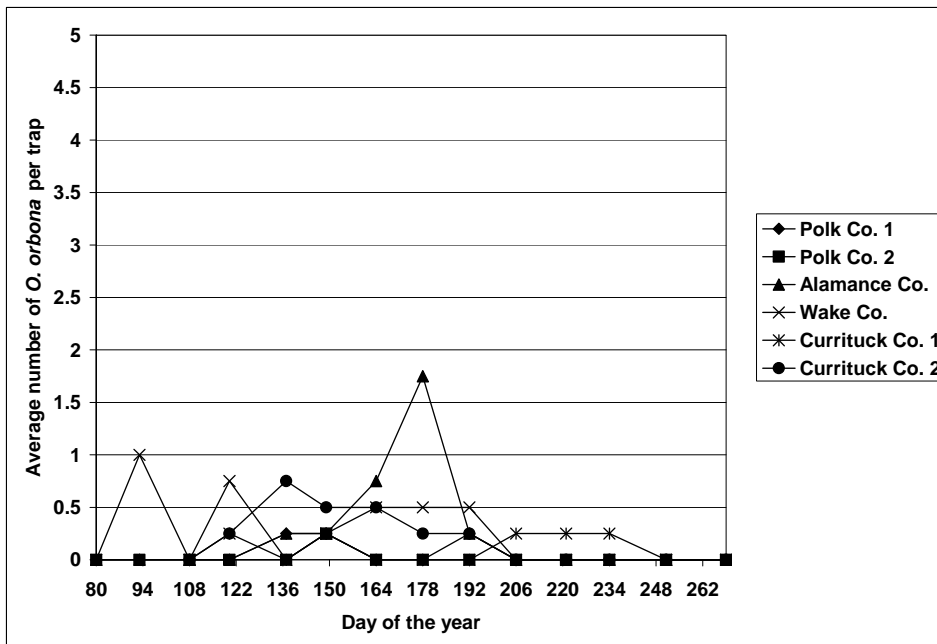
Figure 10. Average number of *Oncometopia orbona* per trap across all vineyards in (A) 2006 and (B) 2007. Note the differences in the scales of the x-axes.

44

A
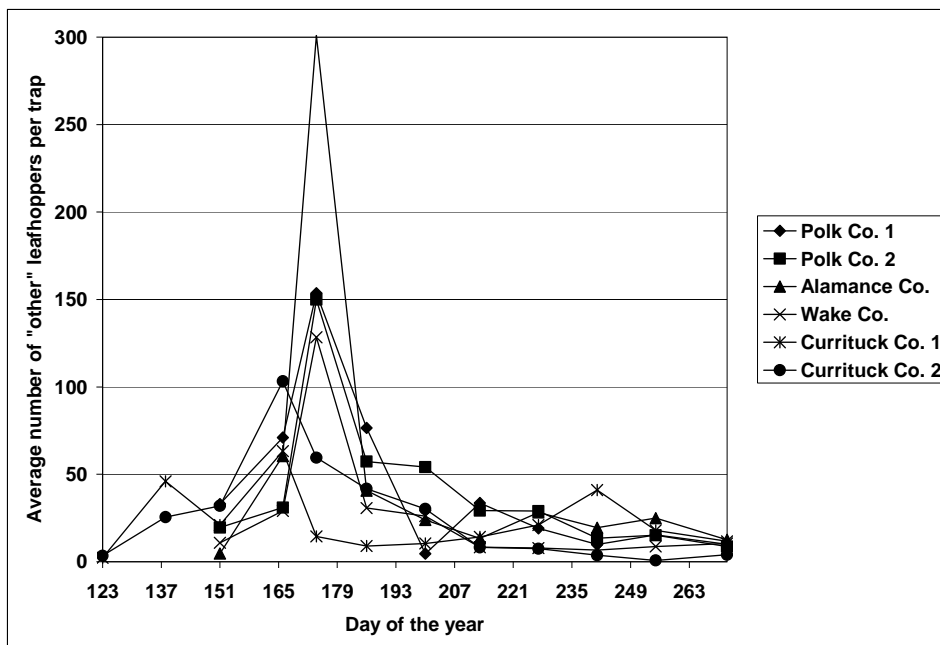
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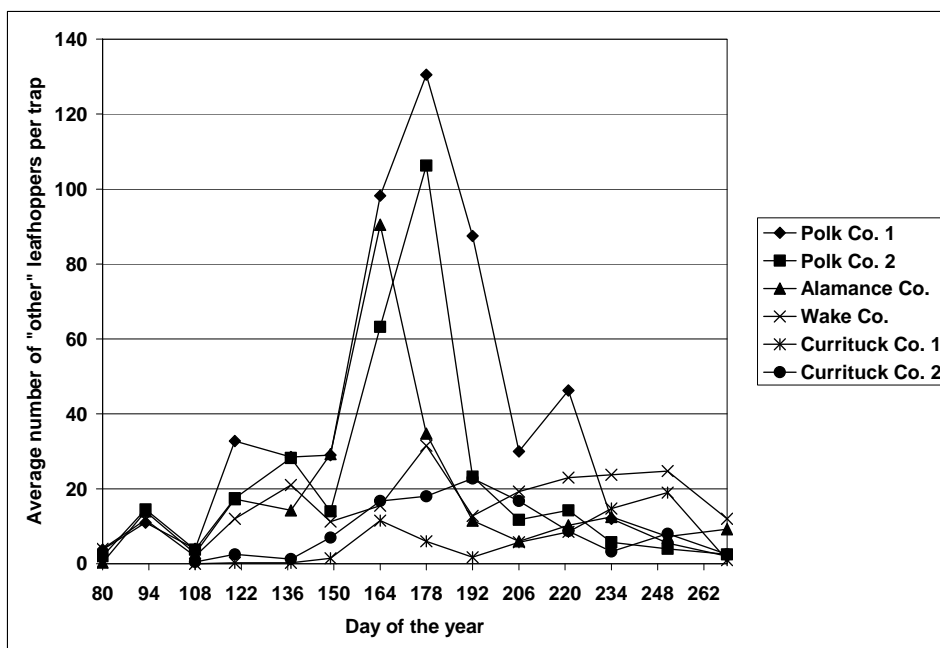
Figure 11. Average number of "other" leafhoppers per trap across all vineyards in (A) 2006 and (B) 2007. Note the differences in the scales of the x- and y-axes.

Table 1. Vineyard floor vegetative composition in 2007 in (A) Polk, (B) Yadkin and (C) Guilford counties, NC.

A

Botanical Family	Scientific Name	Common Name	% Vineyard Floor	
			Spring	Fall
Asteraceae	*Gamochaeta claviceps*	cudweed	5	---
Fabaceae	*Trifolium* spp.	hop clover	2.3	---
Fabaceae	*Trifolium repens*	white clover	23	1.6
Poaceae	*Festuca* sp.	hard fescue	14	---
Poaceae	*Digitaria* sp.	crabgrass	---	15.8
Poaceae	*Eleusine indica*	goosegrass	---	3.2
Poaceae	------	sterile grasses	---	2.3
Rubiaceae	*Galium divaricatum*	Lamarck's bedstraw	2.3	---
------	------	other[1]	0.4	1.3
------	------	barren	53	75.8

B

| Botanical Family	Scientific Name	Common Name	% Vineyard Floor	
			Spring	Fall
Asteraceae	*Ambrosia artemisiifolia*	ragweed	2	---
Euphorbiaceae	*Euphorbia maculata*	prostrate spurge	---	4
Fabaceae	*Trifolium arvense*	rabbitfoot clover	---	6.8
Fabaceae	*Trifolium repens*	white clover	12	7.8
Poaceae	*Cynodon dactylon*	bermudagrass	---	5.4
Poaceae	*Digitaria* sp.	crabgrass	---	9.6
Poaceae	*Paspalum dilatatum*	dallisgrass	2	---
Poaceae	*Festuca* spp.	fescue	9	---
Poaceae	*Setaria* sp.	foxtail	---	5.6
Poaceae	*Dactylis glomerata*	orchardgrass	3	---
Poaceae	------	sterile grasses	3	34.2
Rosaceae	*Duchesnea indica*	wild strawberry	4	---
------	------	sterile broadleaf	4	---
------	------	other	19	5.9
------	------	barren	42	20.7

Table 1 continued.

C

Botanical Family	Scientific Name	Common Name	% Vineyard Floor	
			Spring	Fall
Asteraceae	*Taraxacum officinale*	common dandelion	2	---[2]
Poaceae	*Paspalum notatum*	bahiagrass	2	---
Poaceae	*Digitaria* sp.	crabgrass	---	30
Poaceae	*Lolium multiflorum*	Italian (annual) ryegrass	8	---
Poaceae	*Sorghum halepense*	johnsongrass	7	---
Poaceae	------[3]	sterile grasses	4	4
------	------	sterile broadleaf	2	---
------	------	other	9	3
------	------	barren	66	63

[1]Plant species comprising ≤1 % of the vineyard floor are grouped into the category "Other."
[2]Plant species not observed for a sampling date or location are denoted with "---."
[3]Plants whose botanical family and/or scientific name could not be determined are denoted with "------."

Table 2. Vineyard floor vegetative composition in 2008 in (A) Polk, (B) Yadkin and (C) Guilford counties, NC.

A

Botanical Family	Scientific Name	Common Name	% Vineyard Floor	
			Spring	Fall
Caryophyllaceae	*Scleranthus annuus*	knawel	2.2	---
Fabaceae	*Trifolium repens*	white clover	21.2	17.1
Geraniaceae	*Geranium carolinianum*	Carolina geranium	1.3	---
Poaceae	*Cynodon dactylon*	bermudagrass	9.2	17.3
Poaceae	*Digitaria* sp.	crabgrass	---	6.5
Poaceae	------	sterile grasses	23.7	13.5
------	------	unknown broadleaf	---	1.3
------	------	other[1]	6.4	2.4
------	------	barren	36	41.9

B

Botanical Family	Scientific Name	Common Name	% Vineyard Floor	
			Spring	Fall
Campanulaceae	*Triodanis perfoliata*	Venus' looking glass	---	2.5
Fabaceae	*Trifolium repens*	white clover	13.8	13.9
Poaceae	*Cynodon dactylon*	bermudagrass	1.5	---
Poaceae	*Digitaria* sp.	crabgrass	---	5.8
Poaceae	*Paspalum dilatatum*	dallisgrass	---	24.4
Poaceae	*Festuca* sp.	fescue	5.4	---
Poaceae	*Trifolium* spp.	hop clover	1.4	4.3
Poaceae	*Hordeum pusillum*	little barley	5.3	---
Poaceae	------	sterile grasses	26.9	21.4
------	------	other	9	5.8
------	------	barren	36.7	21.9

Table 2 continued.

C

Botanical Family	Scientific Name	Common Name	% Vineyard Floor	
			Spring	Fall
Asteraceae	*Taraxacum officinale*	common dandelion	---[2]	2.9
Fabaceae	*Vicia sativa*	common vetch	1.4	---
Fabaceae	*Trifolium* spp.	hop clover	12.7	---
Fabaceae	*Trifolium repens*	white clover	1.5	4
Geraniaceae	*Geranium carolinianum*	Carolina geranium	1.5	---
Oxalidaceae	*Oxalis stricta*	oxalis, yellow Woodsorrel	---	3.5
Poaceae	*Digitaria* sp.	crabgrass	---	5.4
Poaceae	*Paspalum dilatatum*	dallisgrass	---	1.2
Poaceae	*Festuca* sp.	fescue	4.2	---
Poaceae	*Lolium multiflorum*	Italian (annual) ryegrass	3.3	---
Poaceae	*Sorghum halepense*	johnsongrass	3.4	---
Poaceae	*Hordeum pusillum*	little barley	6.5	---
Poaceae	------[3]	sterile grasses	2.5	33.5
Polygonaceae	*Rumex crispus*	curly dock	4.1	3.2
Ranunculaceae	*Ranunculus* sp.	buttercup	3.5	---
------	------	unknown broadleaf	---	1.2
------	------	other	10.2	7
------	------	barren	45.2	38.1

[1]Plant species comprising ≤1 % of the vineyard floor are grouped into the category "Other."
[2]Plant species not observed for a sampling date or location are denoted with "---."
[3]Plants whose botanical family and/or scientific name could not be determined are denoted with "------."

Table 3. Plant species tested for *Xf* with ELISA, locations and dates from which samples were taken, and frequency with which species tested positive.

Botanical Family	Scientific Name	2007						2008					
		Spring			Fall			Spring			Fall		
		G[2]	Y[3]	P[4]	G	Y	P	G	Y	P	G	Y	P
Asteraceae	*Ambrosia artemisiifolia*	---[5]	---	---	---	---	---	---	0/2	---	---	0/1	---
Asteraceae	*Gamochaeta claviceps*	---	---	0/10	---	---	---	---	---	---	---	---	---
Asteraceae	*Taraxacum officinale*	0/8	---	---	---	---	---	0/2	0/1	---	0/9	---	---
Asteraceae	*Conyza canadensis*	---	0/5	---	---	---	---	0/1	0/2	---	---	---	---
Asteraceae	*Erigeron strigosus*	0/1	0/1	---	0/1	---	---	---	---	---	---	---	---
Asteraceae	*Crepis capillaris*	0/2	---	---	---	---	---	---	---	---	---	---	---
Brassicaceae	*Lepidium virginicum*	---	---	---	---	---	---	---	0/2	---	---	---	---
Campanulaceae	*Triodanis perfoliata*	---	---	---	---	---	---	0/2	---	---	---	0/5	---
Convolvulaceae	*Ipomoea* sp.	---	---	---	---	0/1	---	---	---	---	---	0/2	---
Euphorbiaceae[1]	***Chamaesyce maculata***	---	0/3	---	---	0/3	---	---	0/2	---	---	---	2/5
Euphorbiaceae	*Acalypha rhomboidea*	---	---	---	---	0/1	---	---	---	---	---	---	---
Fabaceae	*Vicia sativa*	---	---	---	---	---	---	---	---	---	---	---	---
Fabaceae	***Trifolium* spp.**	---	---	1/4	---	---	---	0/15	---	0/2	---	0/9	---
Fabaceae	***Trifolium arvense***	---	0/2	---	---	3/4	---	---	---	---	---	---	---
Fabaceae	*Trifolium pratense*	---	0/2	---	---	---	---	---	---	---	---	0/1	---
Fabaceae	***Trifolium repens***	---	0/2	1/18	---	1/11	0/8	---	1/18	0/27	0/6	0/19	4/23
Geraniaceae	***Geranium carolinianum***	---	---	---	---	---	---	1/7	0/3	2/3	---	---	---
Oxalidaceae	***Oxalis stricta***	---	---	---	---	---	---	0/1	---	1/1	6/8	---	---
Plantaginaceae	***Plantago lanceolata***	---	---	---	---	---	---	0/1	1/2	---	---	---	---
Poaceae	*Paspalum notatum*	0/4	---	---	---	---	---	---	---	---	---	---	---
Poaceae	*Echinochloa crus-galli*	---	0/1	---	---	---	---	---	---	---	---	---	---
Poaceae	***Digitaria sp.***	---	---	---	0/16	4/7	0/23	---	---	---	0/14	0/4	0/8
Poaceae	*Paspalum dilatatum*	---	0/4	---	0/2	---	---	---	---	---	0/3	0/12	---
Poaceae	***Festuca sp.***	---	1/4	---	0/3	---	0/3	0/6	3/10	---	---	---	---
Poaceae	*Festuca* spp.	---	0/1	---	---	---	---	---	---	---	---	---	---
Poaceae	***Setaria* sp.**	---	---	---	---	2/6	---	---	---	---	---	0/1	---
Poaceae	*Eleusine indica*	---	---	---	---	0/1	0/11	---	---	---	---	---	0/3
Poaceae	*Festuca trachyphylla*	---	---	0/8	---	---	---	---	---	---	---	---	---
Poaceae	*Lolium multiflorum*	0/9	---	---	---	---	---	0/9	---	---	---	---	---
Poaceae	*Sorghum halepense*	0/10	---	---	0/1	---	---	0/8	---	---	---	---	---

Table 3 continued.

Poaceae	***Hordeum pusillum***	**1/1**	---	**---**	---	---	---	**0/15**	**1/11**	---	---	---	---
Poaceae	*Dactylis glomerata*	---	0/4	---	---	---	---	---	0/1	---	---	---	---
Poaceae	***Poa trivialis***	**1/1**	**---**	**---**	**---**	**---**	**---**	**---**	**---**	**---**	**---**	**---**	**---**
Poaceae	***Cynodon dactylon***	**0/1**	**1/1**	**---**	**---**	**2/2**	**0/4**	**---**	**0/2**	**0/9**	**0/8**	**---**	**---**
Polygonaceae	*Rumex crispus*	---	0/1	---	---	---	---	0/9	---	---	0/10	---	---
Ranunculaceae	***Ranunculus* sp.**	**---**	**---**	**---**	**---**	**---**	**---**	**0/8**	**1/1**	**---**	**---**	**---**	**---**
Rosaceae	*Duchesnea indica*	---	0/3	---	---	---	---	---	---	---	---	---	---
Rubiaceae	*Galium divaricatum*	---	---	0/4	---	---	---	---	---	---	---	---	---
Smilacaceae	*Smilax rotundifolia*	---	0/4	---	---	---	---	---	---	---	---	---	---
Solanaceae	*Solanum carolinense*	---	---	---	0/1	---	---	---	---	---	---	---	---

[1] Bold font indicates plant species testing positive for *Xf* with ELISA.
[2] "G" indicates vineyard located in Guilford County, NC.
[3] "Y" indicates vineyard located in Yadkin County, NC.
[4] "P" indicates vineyard located in Polk County, NC.
[5] Plant species not observed for a sampling date or location are denoted with "---."
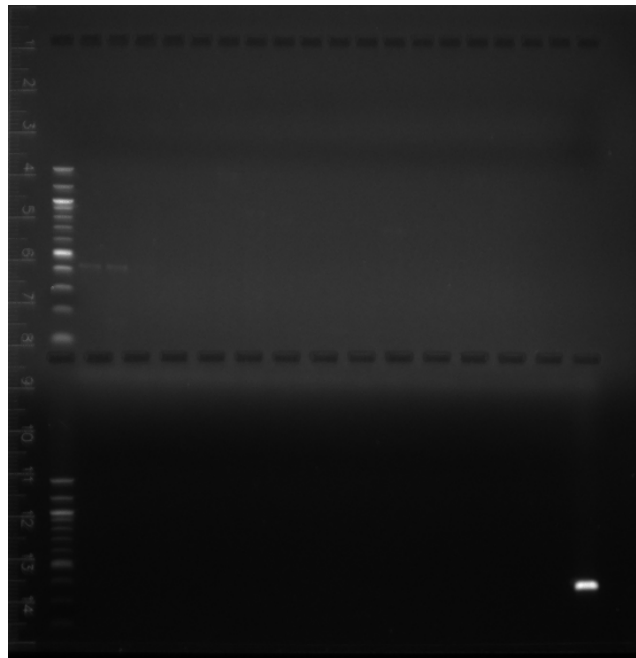
Figure 1. Agarose gel electrophoresis showing two ~400 base pair bands indicating positive PCR reactions for *Xf* from *Hordeum pusillum* (lane 2) and *Ranunculus* sp. (lane 3) (top row). Lane 15 (bottom row) shows positive control. Lanes 1 (top row) and 1 (bottom row) are DNA ladders.

A
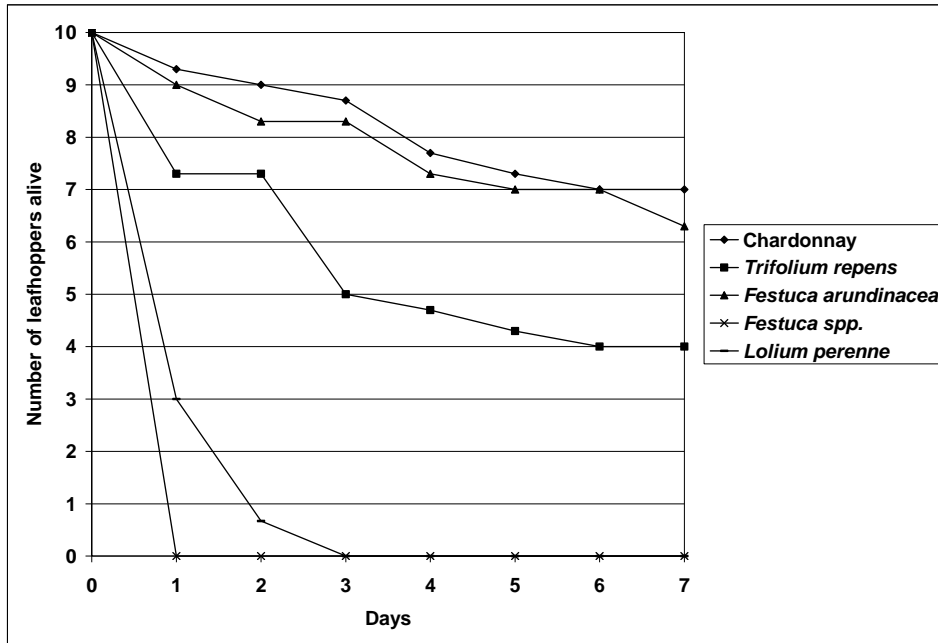
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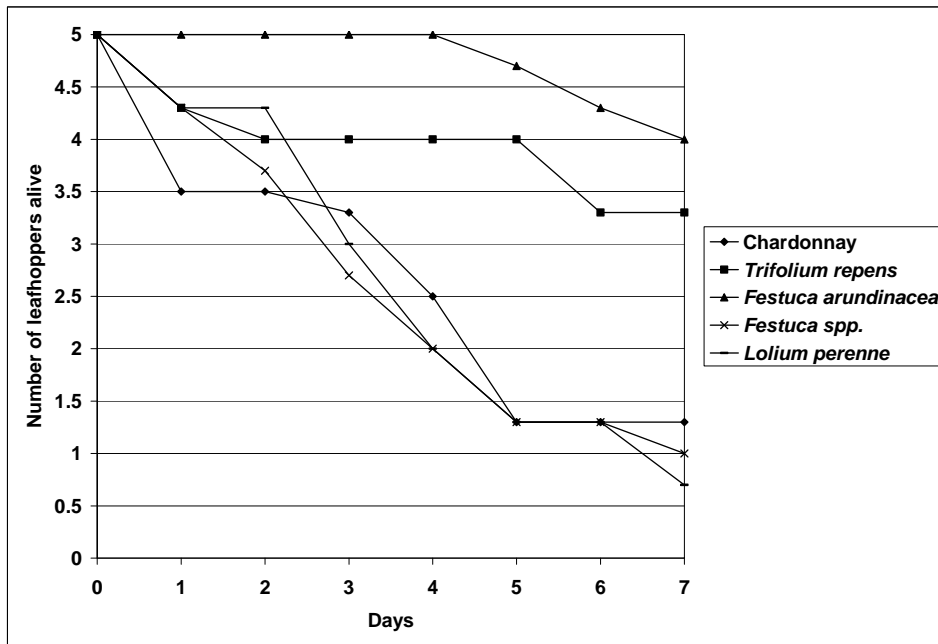
Figure 2. Survival of *Graphocephala versuta* when feeding exclusively on either *Trifolium repens*, *Festuca arundinacea*, fine fescue, *Festuca* spp., *Lolium perenne* or Chardonnay grapevines over 7 days in (A) 2007 and (B) 2008.

A
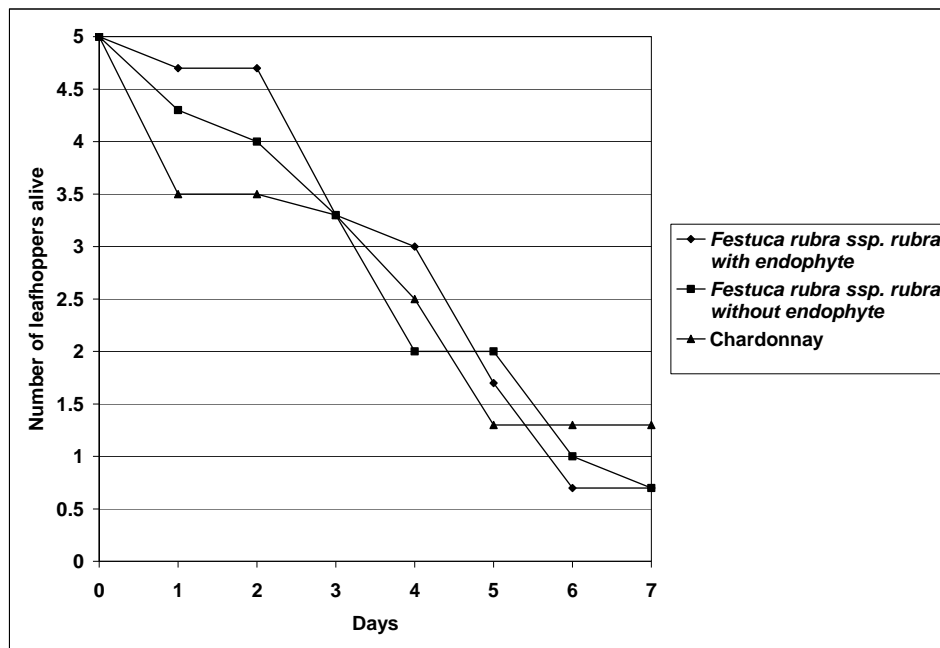
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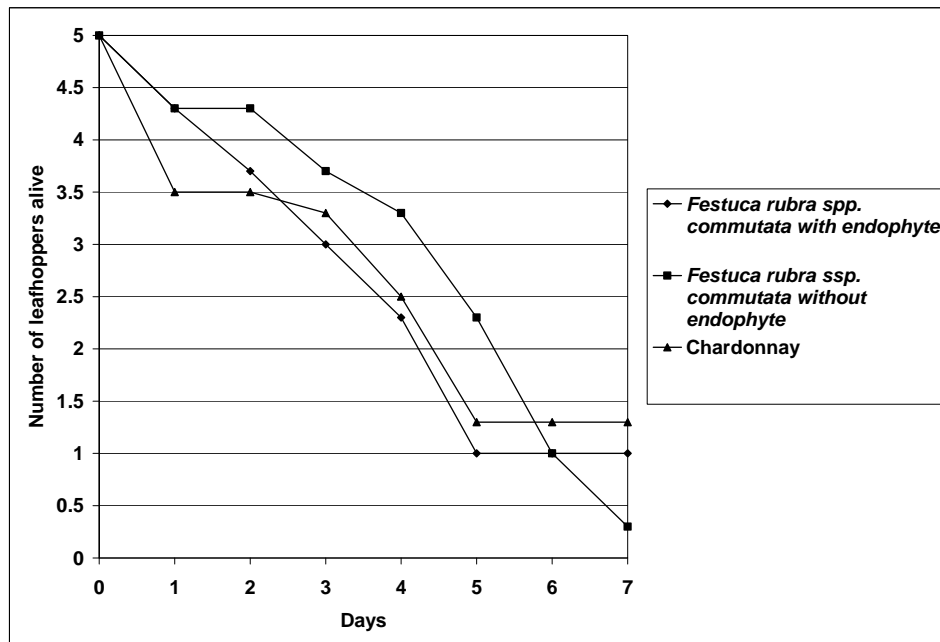
Figure 3. Survival of leafhoppers when feeding exclusively on (A) creeping red fescue, *Festuca rubra* ssp. *rubra*, or (B) Chewings fescue, *Festuca rubra* ssp. *commutata*, with and without endophyte for 7 days.

APPENDIX
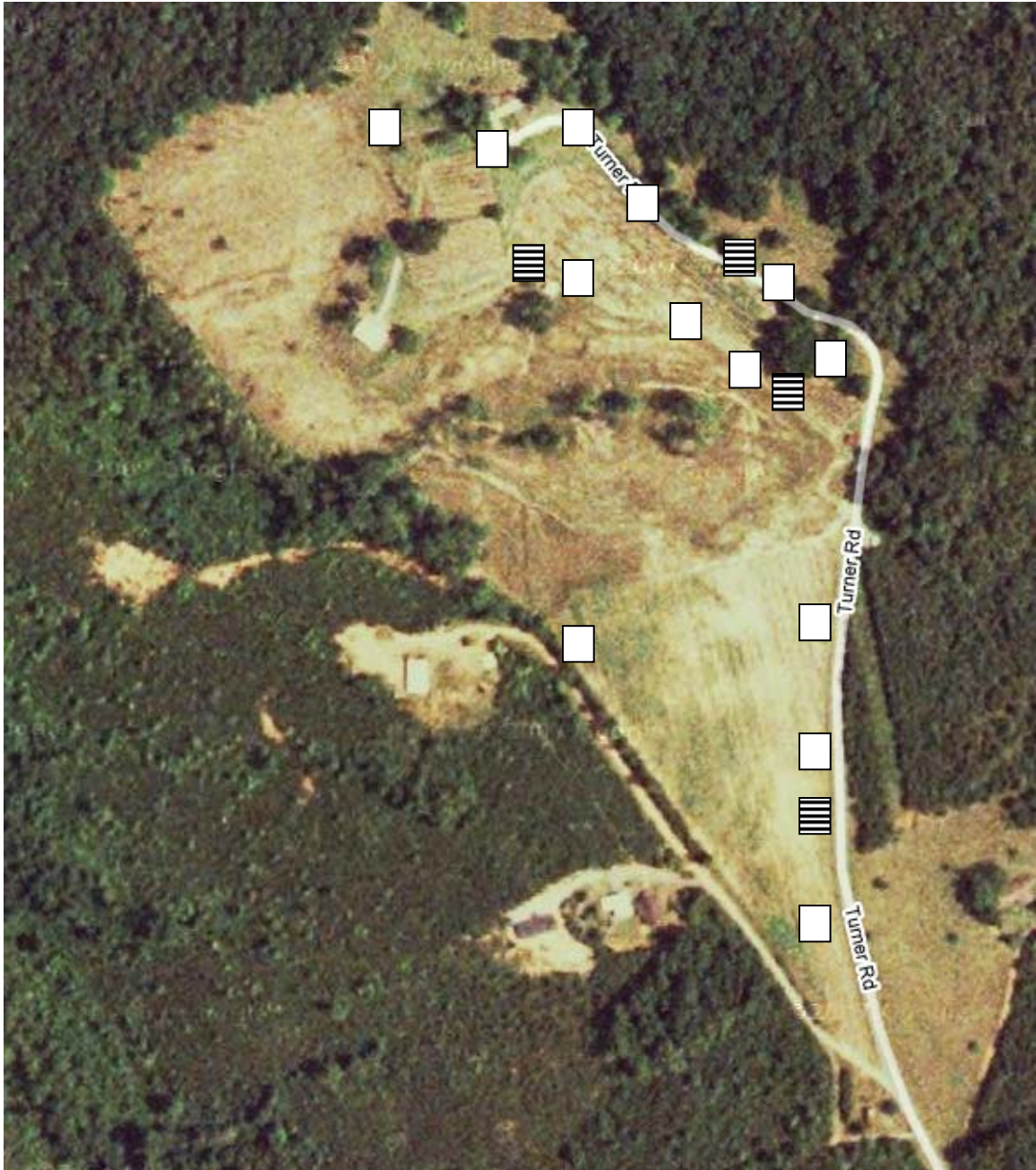
Figure 1. Placement of yellow sticky traps in Polk Co. NC vineyard 1 in 2006 (white boxes) and 2007 (striped boxes). Image from Google Earth.

Figure 2. Placement of yellow sticky traps in Polk Co. NC vineyard 2 in 2006 (white boxes) and 2007 (striped boxes). Image from Google Earth.

Figure 3. Placement of yellow sticky traps in Alamance Co. NC vineyard in 2006 (white boxes) and 2007 (striped boxes). Image from Google Earth.

Figure 4. Placement of yellow sticky traps in Wake Co. NC vineyard in 2006 (white boxes) and 2007 (striped boxes). Image from Google Earth.

Figure 5. Placement of yellow sticky traps (white boxes) in Currituck Co. NC vineyard 1 in 2006 and 2007. Image from Google Earth.

Figure 6. Placement of yellow sticky traps (white boxes) in Currituck Co. NC vineyard 2 in 2006 and 2007. Image from Google Earth.

Pierce's disease severity in three vineyards included in vegetation surveys in Guilford, Yadkin and Polk counties, NC, in 2008.

INTRODUCTION

Vegetation surveys were conducted in three vineyards in the spring and fall of 2007 and 2008 to determine which plant species comprise the typical North Carolina vineyard floor. The incidence and severity of PD ranges considerably across these vineyards.

MATERIALS AND METHODS

In September and October of 2008, during the fall vegetation surveys, PD severity was rated on a per vine basis, in the blocks where our surveys were conducted. Disease was rated on a 0 to 5 scale based on symptom development, where 0 = asymptomatic, 1 = <25% of leaves necrotic, 2 = 25-50% of leaves necrotic, 3 = 50-75% of leaves necrotic, 4 = >75% of leaves necrotic and 5 = dead vine. In Polk Co., samples of all vines rated 2-5 were collected and tested for *Xf* with ELISA. In Yadkin Co. all vines had a uniform marginal leaf necrosis, possibly caused by downy mildew, throughout the entire vineyard. Therefore, samples were collected arbitrarily and tested for *Xf* with ELISA. In Guilford Co. samples of all vines rated 1-5 were collected and tested for *Xf* with ELISA. Maps were created for each vineyard using shaded boxes to represent the severity rating. Missing vines were denoted with an "X", but were not noted in Yadkin Co. Boxes representing vines testing positive for *Xf* with ELISA are surrounded by bold borders.

0	0	0	1	0	0	1	1	3	
0	0	0	0	0	0	0	0	0	
0	1	0	0	0	0	0	0	0	
0	0	0	0	0	0	1	0	1	
4	0	0	0	0	0	3	3	0	
0	5	0	0	0	0	1	0	0	
0	0	0	0	0	1	0	1	0	
0	0	0	1	0	0	0	0	0	0
0	4	0	0	0	0	0	0	0	0
3	0	0	3	0	X	0	1	0	0
2	0	3	0	0	0	0	0	0	0
0	3	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	1	0	0
0	0	4	0	0	0	0	0	0	0
0	1	0	0	0	0	0	0	0	0
0	1	0	1	0	0	5	1	0	0
0	0	0	0	0	0	1	0	0	0
0	0	0	0	1	1	0	0	0	0
0	0	0	0	0	0	0	1	0	0
0	1	0	0	0	1	0	0	0	0
0	0	0	0	0	0	0	1	0	0
0	0	0	0	0	0	0	0	0	0
0	1	1	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0		
0	0	0	0	0	X				

Figure 7. Map of PD severity in Polk Co. vineyard in fall 2008. Each box represents a vine and the number within the box refers to the PD severity rating where 0 = asymptomatic, 1 = <25% of leaves necrotic, 2 = 25-50% of leaves necrotic, 3 = 50-75% of leaves necrotic, 4 = >75% of leaves necrotic and 5 = dead vine. Missing vines are denoted with an "X." Boxes representing vines testing positive for *Xf* with ELISA are surrounded by bold borders.

Figure 8. Map of Yadkin Co. vineyard in fall 2008. Note: All vines had >75% necrotic leaves, likely due to downy mildew, and therefore were not rated for PD. Boxes representing vines testing positive for *Xf* with ELISA are surrounded by bold borders.

0	0	0	0	0	0	0	0	5	0	X	0	0
0	0	0	4	0	0	0	0		1	0	0	0
0	0	0	0	0	0	0	0	X	4	1	0	0
0	0	0	0	0	0	1	0	0	X	X	0	0
0	0	0	0	0	X	0	0	0	0	2	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	X	0
0	0	0	0	0	0	2	0	0	0	0	0	0
0	3	0	0	0	0	0	0	0	0	X	X	0
0	0	0	0	0	0	0	0	4	4	X	X	0
0	0	0	0	0	0	0	3	2	0	0	0	0
0	0	1	0	0	1	0	0	4	0	X	0	0
0	0	0	0	0	0	0	0	4	0	0	0	0
0	0	0	0	X	0	0	0	2	0	0	0	0
0	0	0	0	0	0	0	0	X	0	0	X	0
0	0	0	0	0	0	1	0	0	0	5	0	0
4	0	0	0	0	0	0	0	0	0	0	4	3
0	0	0	X	0	0	0	0	X	3	4	4	0
0	0	0	0	0	0	0	0	X	0	3	4	0
1	0	0	0	0	0	0	0	0	0	0	1	4

Figure 9. Map of PD severity in Guilford Co. vineyard in fall 2008. Each box represents a vine and the number within the box refers to the PD severity rating where 0 = asymptomatic, 1 = <25% of leaves necrotic, 2 = 25-50% of leaves necrotic, 3 = 50-75% of leaves necrotic, 4 = >75% of leaves necrotic and 5 = dead vine. Missing vines are denoted with an "X." Boxes representing vines testing positive for *Xf* with ELISA are surrounded by bold borders.