Across the United States from California to Florida and as far north as Virginia, grapevines are succumbing to Pierce’s Disease (PD) which currently infects more than 30% of grapevines and is a threat to the $30 billion wine industry. PD is caused by the bacterial pathogen, Xylella fastidiosa, which blocks water from flowing through the xylem of plants. Affected grapevines develop leaves that are discolored and dried out, and usually die within 2-5 years. X. fastidiosa is transmitted by xylem feeding insects. The most effective insect vector is a leafhopper, Glassy-Winged Sharpshooter (GWSS, Homalodisca vitripennis). GWSSs are able to survive in mild to scorching temperatures and can feed on the woody stems of plants, making them an optimal vector of the pathogen. In the summer, GWSS populations explode, creating the greatest period of X. fastidiosa infection of grapevines. During summer, 2010, we performed a survey on unsprayed grapevines on the UCR campus to determine patterns of GWSSs visiting the plants and their infection status. Insects were collected on yellow sticky traps from late June to late October. All insects were surface sterilized, the DNA was extracted, and real-time PCR was used to test for presence of X. fastidiosa. GWSS visitation to the plants was highest in midsummer. From 112 insects collected, 83 were tested for X. fastidiosa and approximately 76% were found to be positive. These results indicate that many of the GWSSs feeding on the unsprayed grapevines are infected with X. fastidiosa, placing the plants at high risk of infection.
INTRODUCTION

Pierce’s Disease is a bacterial disease of grapevines, first recognized by Newton Barry Pierce in the late 1800’s near Anaheim, California. To date, the disease has been documented in vineyards from California to Florida and along the East Coast as far north as Virginia (Hoddle, 2004; Wallingford et al., 2007). Affected grapevines develop leaves that are discolored and dried out, and they usually die within 2-5 years (Hopkins, 1989). The disease is caused by infection with the bacterial pathogen, *Xylella fastidiosa*, which multiplies in the xylem of plants, forming a gel-like biofilm that blocks water flow to the rest of the plant (Hopkins, 1989).

*X. fastidiosa* is an insect transmitted bacterium that lives only in the xylem of the infected plant. The xylem is responsible for transporting water throughout the plant. The insects feeding on the xylem transfer the pathogen from the infected plants to healthy plants. There are several pathogenic strains of *X. fastidiosa* each of which is associated with a specific host plant causing diseases in different plants from various families (Hopkins, 1989). *X. fastidiosa* infections are seen more often in areas with mild to high temperatures. These temperatures are also ideal for the insect vector, *Homalodisca vitripennis*, which is frost sensitive and thrives best in hot climates (Johnson et al., 2008).

*H. vitripennis*, most commonly known as the Glassy-Winged Sharpshooter (GWSS), is one of many leafhopper species, referred to as sharpshooters (Figure 1). These sharpshooters feed on the xylem fluid, thus transmitting *X. fastidiosa* to the plant. The GWSS appears to be a much more effective vector of the pathogen than most other sharpshooter species. This ability has been attributed to the insect’s high mobility, wide distribution, and ability to have many hosts (Almeida et al., 2005). Transmission of the pathogen can occur immediately after the GWSS has acquired the bacteria (Hopkins, 1989). This species has become well established and widely distributed in Southern California since its accidental introduction in early 1990’s, making it very difficult for wineries to control the disease. Although GWSS have many hosts, they mainly reside on citrus plants where egg laying occurs during the late winter and early spring (Redak et al., 2004). Insect emergence takes place within 2 weeks of egg laying, adults remain active throughout the summer, then the numbers decrease in early fall (Redak et al., 2004). The Pierce’s Disease pathogen is transmitted most during summer when GWSS populations are at their highest (Almeida et al., 2005).

Figure 1. Photo of adult *H. vitripennis* http://cisr.ucr.edu/ssp_director/albums/album-9/lg/glassy-winged_sharpshooter_gwss-6.jpg.

Pierce’s Disease is a persistent threat to the $30 billion wine industry encompassing 890,000 acres of vineyards across Southern California. In 2000, PD affected 30% of California grape crops and has likely increased since then (Toscano et al., 2006). As a result of this disease, the vine growers have turned to intense use of chemical insecticides for the containment of GWSS. This widespread use of insecticides often prevents us from surveying the natural patterns of *X. fastidiosa* occurrence in relation to GWSS and the grapevines. We obtained access to unsprayed grapevines in AgOps fields on the UCR campus, which gave us the opportunity to conduct a rare survey of GWSS visitations in a natural environment. The information collected allows us to examine visitation patterns as well as patterns of the natural infection of GWSS carrying the bacterium, *X. fastidiosa*. Such information will contribute to understanding the insect-pathogen-plant dynamics of the PD system and aid in the development of more efficient management skills in controlling the disease.
MATERIALS AND METHODS

Surveying and Collection

A plot of 9 unsprayed grapevine rows in the AgOps field on the UCR campus was used for this survey. Each row contained 25 plants, spaced approximately 8 feet apart with trellising wires at 40 in. and 52 in. to allow the plants to fill the spaces in between. We placed 8 insect traps evenly spaced throughout the top 6 rows of the plot. Traps were set on Sunday of each week and left to collect GWSS for 7 days. After 7 days, the number of GWSSs on the sticky traps was recorded and the insects were collected from each trap. All insects were then stored at -80°C for further analysis.

DNA Extraction

X. fastidiosa resides within its insect host. In order to detect the presence of the bacterium, DNA was extracted from the GWSSs, which included insect and bacterial DNA. Up to 10 GWSSs were randomly selected from the total collection each week for DNA extraction and analysis. When fewer than 10 insects were collected, all insects were processed. In total 83 insects were used for DNA extraction. The selected GWSSs were individually surfaced sterilized in 70% ethanol, 10% bleach, and water for 2 minutes in each. The GWSS heads were then carefully severed from the body and DNA was extracted as follows. Each head was macerated in a separate microcentrifuge tube containing 200 µl of PBS using a homogenizer. After the solution was fully homogenized, 180 µl of this solution was transferred to a new microcentrifuge tube. To this, 180 µl of ATL buffer and 20 µl of proteinase K were added to lyse the cell tissue. The solution was then incubated at 55°C for one hour to allow full cell lysing to take place. Lastly, 100 µl of AE buffer, elution buffer, was added and the samples were incubated for one minute at room temperature and then centrifuged. The column was discarded and the remaining DNA solution was labeled and stored at 4°C.

Real-Time PCR

To detect the presence of X. fastidiosa, the polymerase chain reaction was performed on extracted DNA using primers specific to the bacterium. If X. fastidiosa DNA is present in a sample, the bacterial DNA is amplified through this process, resulting in a clear positive result. If the bacterium is absent in the GWSS sample, no amplification occurs and a negative result is recorded. Due to its high sensitivity, real-time PCR was performed instead of the traditional PCR. The real-time PCR was run following protocol developed by Schaad et al. (2002). Each run was performed in duplicate and each run included a positive and a non-template control. The following primers and probe, selected to amplify the ITS sequence of X. fastidiosa, were used for the PCR run: forward primer (5'AAAAATCGCCAACATAAACCCA3'), reverse primer (5'CCAGGCGTCCTCAAAAGTTAC3'), and probe (5'6-FAM CCTATGCCAACATCAAACCCTGAATGCA BHQ-1 3') (Schaad et al., 2002). We modified the technique outlined in Arora (2010) to perform real-time PCR reactions in 50 µl units using 5 µl of template DNA. The real-time PCR cycle was run using the following conditions: 3 minutes at 95°C for enzyme activation, followed by 40 cycles of denaturing at 95°C for 15 seconds and annealing and extension at 62°C for 1 minute.

RESULTS

A total of 103 GWSSs were collected from July to October, 2010, the duration of this survey. The number of GWSSs collected in a single week ranged from a low of 0 insects during one week in October to a high of 25 GWSSs in August. The months of October and August also showed the lowest and highest monthly collections with 14 and 59 insects, respectively. We observed two population peaks, one in August followed by a decline and then another peak in late September to early October. The second population peak was also followed by a decline
in the number of GWSS visiting the plants. After the population peak in August of 25 GWSSs collected, GWSS visitation declined steadily, reaching a low of 3 insects collected. After the second, smaller peak in early October, with a maximum count of 9 GWSSs, the population dropped to 0 then increased to a few GWSSs for the remainder of the season.

Real-time PCR analysis successfully detected the presence or absence of *X. fastidiosa* in collected samples (Figure 2), revealing an overall frequency of infection of 76 percent. We tested 83 of the 103 GWSSs collected for the *X. fastidiosa* bacterium. Of those tested, 63 GWSSs showed positive results for *X. fastidiosa*. We saw two peaks in infection rates, the first from July 23rd to September 9th and the second peak from September 17th to October 15th. There were also two dramatic decreases in infection rate with 0 percent infection on dates September 10th and October 22nd. The weekly infection rates during the survey ranged from 50% to 100% positive per date, except those of September 10th and October 22nd.

**DISCUSSION**

Based on the multiple population peaks seen in the survey results, we believe there are three generations of GWSS during the active season from July to October. In this population survey, two population peaks can be clearly seen, the first peak occurring on August 20th and the second peak occurring on October 1st. The population survey shows rapid declines in GWSS visitation after each population peak (Figure 3). These dramatic decreases in population are believed to correspond to the intermediate points between each generation. A slight decrease in population numbers can also be seen from July 23rd to July 30th, which may correspond to a decline from the previous population peak. Therefore, we believe our survey shows generations 2 and 3 out of a 3 generation active season. The 1st generation of three was likely missed in this survey since collection did not occur until almost mid-season, in late July. As temperatures reached their highest in August, so did the GWSS population. This is expected since GWSS thrive best in hot temperatures. Further, the first generation would be expected to be smaller than the second generation which appeared in our August and early September collections.

![GWSS Collected in Ag Ops Grape Plot](image)

**Figure 3.** Weekly insect collections from July 23rd to October 29th.

The rate of infection among collected GWSSs corresponds to the visitation pattern seen in the population survey with infection rates increasing and decreasing with population generations (Figure 4). Just as there are two visible population peaks in the GWSS visitation survey, there are two clear peaks in...
**X. fastidiosa** infection. Infections rates are relatively high from July 23rd to September 3rd, the period corresponding to what we believe is the 2nd active season generation, and rates are high again from September 17th to October 15th, the period that corresponds to the 3rd generation. However, the weeks in which the infection rate was at 0 percent may be due to the fact that very few to no GWSSs were collected during those weeks and may not accurately show the infection rates at the lowest points of each generation.

**CONCLUSION**

This population survey consisted of a 15 week collection of GWSS visiting a local, untreated grapevine plot from July to October. The number of GWSS visiting the grapevines was recorded weekly and the insect samples were analyzed using real-time PCR and *X. fastidiosa*-specific primers to detect infection with the pathogen responsible for causing Pierce’s Disease in grapevines. The GWSS population survey presented here demonstrates the presence of two GWSS population generations at the AgOps grape plots. Based on our knowledge of GWSS biology and the timing of the survey, we conclude these are most likely the 2nd and 3rd generations of 3 generation active season. The overall rate of infection of *X. fastidiosa* among GWSS visiting the AgOps plot was measured at 76%. This rate appears uncharacteristically high compared to those of previous population and infection rate studies (Hail et al., 2010) but can be explained by the use of the more sensitive real-time PCR technique for bacterial DNA detection. As GWSS visitation appears cyclical, so does the *X. fastidiosa* infection rate. *X. fastidiosa* infection rates correspond to the visitation pattern seen in the population survey with infection rates increasing and decreasing with population generations. These low infection rates may represent an actual fluctuation in infection frequency or they may due to low numbers of GWSS collected on the corresponding collection dates. Further studies will need to be performed to confirm the cause of the cyclical infection rates.

This single season survey gives researchers information about GWSS visitation patterns on local grapevines and the rate of infection of *X. fastidiosa* in local GWSS populations. This valuable data offers insight into the local threat of Pierce’s disease and will be helpful in determining control methods for the pest and bacterial vector, Glassy-Winged Sharpshooter. This survey provides a foundation for additional studies investigating GWSS visitation and *X. fastidiosa* infection patterns. Continuation of this annual survey will be helpful in understanding the dynamics of Pierce’s disease and developing control measures for GWSS and *X. fastidiosa*.

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