

**The Occurrence of *Xylella fastidiosa* and Its Sharpshooter Vectors  
in Selected Alabama Orchards and Vineyards**

by

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## Abstract

Although the bacterium *Xylella fastidiosa* (*Xf*) has been confirmed to cause economic losses to numerous fruit crop species in the southeastern U.S. since 1933, no science-based information is available on the occurrence of infection in economic fruit crops grown in Alabama, as well as the presence of effective *Xf* vectors in the state. Our investigation aimed to determine the spread of *Xf* infection in selected major fruit crops, and to identify the sharpshooter (Hemiptera: Cicadellidae) fauna in selected orchards and vineyards grown at three distinct chilling zones in Alabama. Our study confirmed the occurrence of *Xf* in all six fruit crops grown throughout the three chilling zones in Alabama, and the highest disease pressure was found in the Gulf Coast area. The following sharpshooter species were recorded in Alabama: *Homalodisca vitripennis* (Germar), *H. insolita* (Walker), *Oncometopia orbona* (Fabricius), *Paraulacizes irrorata* (Fabricius), *Graphocephala coccinea* (Forster), *G. versuta* (Say), and *Draeculacephala* spp. *H. vitripennis* was the most abundant species in the Gulf Coast area, whereas *G. versuta* was the dominant species in Central and North Alabama. The preliminary data obtained from our study could be further utilized to develop a degree-day model to predict sharpshooter emergence in various Alabama locations and aid fruit growers in their management practices.

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## Table of Contents

|  |     |
|--|-----|
| Abstract.....  | ii  |
| Acknowledgments.....   | iii |
| List of Tables .....   | v   |
| List of Figures.....   | vii |
| Chapter One: <i>Xylella Fastidiosa</i> Affected Fruit Crops and Its Efficient Vector Species: Literature Review.....     | 1   |
| 1.1 Introduction .....   | 1   |
| 1.2 Literature Cited .....   | 16  |
| 1.3 Tables.....  | 31  |
| Chapter Two: Investigation into <i>Xylella Fastidiosa</i> Occurrence in Selected Alabama Orchards and Vineyards.....     | 32  |
| 2.1 Introduction .....   | 32  |
| 2.2 Materials and Methods .....  | 41  |
| 2.3 Results .....  | 43  |
| 2.4 Discussion .....   | 47  |
| 2.5 Literature Cited .....   | 48  |
| 2.6 Tables and Figures .....   | 57  |
| Chapter Three: Occurrence of <i>Xylella Fastidiosa</i> Sharpshooter Vectors in Alabama and Their Seasonal Phenology..... | 65  |
| 3.1 Introduction .....   | 65  |
| 3.2 Materials and Methods .....  | 72  |
| 3.3 Results .....  | 74  |
| 3.4 Discussion .....   | 80  |
| 3.5 Literature Cited .....   | 82  |
| 3.6 Tables and Figures .....   | 90  |

## List of Tables

### Chapter One

|   |    |
|---|----|
| Table 1. Total acreage of selected fruit crops in Alabama in 2007, according to the National Agricultural Statistics Service .....                          | 31 |
| Table 2. Estimated risk of PD infection according to Sutton’s standard – number of days when daily minimum temperature falls below -12.2°C, or -9.4°C ..... | 31 |

### Chapter Two

|  |    |
|--|----|
| Table 1. <i>Xylella fastidiosa</i> sampling sites in Alabama, 2008-2009: locations, elevation and edge habitats description.....   | 57 |
| Table 2. Occurrence of <i>Xylella fastidiosa</i> infected five fruit crops grown at six Alabama locations tested three times during the growing season in 2008 and 2009..... | 58 |
| Table 3. Occurrence of <i>Xylella fastidiosa</i> infected peach trees grown at four Alabama locations tested three times during the growing season in 2008 and 2009 .....    | 59 |
| Table 4. Occurrence of <i>Xylella fastidiosa</i> infected peach trees grown at three Alabama locations tested three times during the growing season in 2008 and 2009 .....   | 60 |

### Chapter Three

|  |    |
|--|----|
| Table 1. <i>Xylella fastidiosa</i> sampling sites in Alabama, 2008-2009: locations, elevation and edge habitats description.....   | 90 |
| Table 2. Insect management in selected orchards and vineyards surveyed at three Alabama locations, 2008 and 2009 .....   | 91 |
| Table 3. Type and total number of sharpshooter species captured on yellow sticky traps in commercial Alabama orchards and vineyards during 2008 and 2009.....              | 92 |
| Table 4. Seasonal mean capture of <i>Homalodisca vitripennis</i> trapped bi-weekly on five fruit crops using yellow sticky traps from three Alabama locations in 2008..... | 92 |
| Table 5. Seasonal mean capture of <i>Homalodisca vitripennis</i> trapped bi-weekly on six fruit crops using yellow sticky traps from three Alabama locations in 2009 ..... | 93 |
| Table 6. Seasonal mean capture of <i>Graphocephala versuta</i> trapped bi-weekly on six fruit crops using yellow sticky traps from three Alabama locations in 2009 .....   | 93 |
| Table 7. Seasonal mean capture of <i>Homalodisca insolita</i> trapped bi-weekly on six fruit crops using yellow sticky traps from three Alabama locations in 2009 .....    | 94 |

|   |    |
|---|----|
| Table 8. Seasonal mean capture of <i>Oncometopia orbona</i> trapped bi-weekly on six fruit crops using yellow sticky traps from three Alabama locations in 2009 .....     | 94 |
| Table 9. Seasonal mean capture of <i>Draeculacephala</i> spp. trapped bi-weekly on six fruit crops using yellow sticky traps from three Alabama locations in 2009 .....   | 95 |
| Table 10. Seasonal mean capture of <i>Graphocephala coccinea</i> trapped bi-weekly on six fruit crops using yellow sticky traps from three Alabama locations in 2009..... | 95 |
| Table 11. Seasonal mean capture of <i>Paraulacizes irrorata</i> trapped bi-weekly on six fruit crops using yellow sticky traps from three Alabama locations in 2009 ..... | 96 |

## List of Figures

### Chapter Two

- Figure 1. Sampling locations for *Xylella fastidiosa* in selected fruit crops in Alabama, 2008 and 2009.. .....61
- Figure 2. Symptoms of Pierce’s disease on bunch grape found in Chilton Horticultural Station: (A) Marginal leaf necrosis with chlorotic border (Image Courtesy of Elina Coneva); (B) Match stick petiole; and (C) green island symptoms on grape shoots. Image: Xing Ma.....62
- Figure 3. Marginal leaf necrosis symptoms caused by Plum leaf scald disease observed in the Chilton Horticultural Research and Extension Center, Clanton. Image: Xing Ma .....63
- Figure 4. Peach tree showing flat top and dark green canopy – typical phony peach disease symptoms (left) compared with a healthy peach tree (right). Image: Xing Ma.....64
- Figure 5. Interveinal chlorosis symptom on Satsuma mandarin leaf tested positive for *Xylella fastidiosa* infection. Image courtesy of Elina Coneva.....64

### Chapter Three

- Figure 1. Insect monitoring sites for selected fruit crops in Alabama, 2008 and 2009.....97
- Figure 2. Installation of yellow sticky traps: (A) Placed on the outer limb of a peach tree; (B) Attached to the trellis system wire in a muscadine vineyard. Image: Xing Ma.....98
- Figure 3. A *Homalodisca vitripennis*, also known as glassy-winged sharpshooter, captured during 2009 growing season in Alabama. Each grade on the ruler corresponds to 1 mm. The white secretions on wings are the brochosomes. Image: Xing Ma .....99
- Figure 4. A *Homalodisca insolita* captured during 2009 growing season in Alabama. Each grade on the ruler corresponds to 1 mm. Image: Xing Ma.....100
- Figure 5. An *Oncometopia orbona*, also known as broad-headed sharpshooter, captured during 2009 growing season in Alabama. Each grade on the ruler corresponds to 1 mm. Image: Xing Ma.....101
- Figure 6. A *Paraulacizes irrorata*, also known as speckled sharpshooter captured during 2009 growing season in Alabama. Each grade on the ruler corresponds to 1 mm. Image: Xing Ma.....102
- Figure 7. A *Graphocephala coccinea*, also known as red-banded sharpshooter captured during 2009 growing season in Alabama. Each grade on the ruler corresponds to 1 mm. Image: Xing Ma.....103
- Figure 8. A *Graphocephala versuta* captured during 2009 growing season in Alabama. Each grade on the ruler corresponds to 1 mm. Image: Xing Ma.....104

Figure 9. A *Draeculacephala* spp. captured during 2009 growing season in Alabama. Three species were identified: *D. balli*, *D. bradleyi*, *D. mollipes*. Each grade on the ruler corresponds to 1 mm. Image: Xing Ma.....105

Figure 10. Two-year seasonal abundance of *Homalodisca vitripennis* in three Alabama locations in 2009. Each number represents the average sharpshooter capture of four traps per crop on a given sampling cycle.....106

Figure 11. Seasonal abundance of seven sharpshooter species in Mobile, Alabama in 2009. Each number represents the average sharpshooter capture of four traps per crop on six crop species. Insect species name: HV= *Homalodisca vitripennis*; HI= *Homalodisca insolita*; GC= *Graphocephala coccinea*; GV= *Graphocephala versuta*; OO= *Oncometopia orbona*; PI= *Paraulacizes irrorata*; DS= *Draeculacephala* spp. ....107

Figure 12. Seasonal abundance of seven sharpshooter species in Clanton, Alabama in 2009. Each number represents the average sharpshooter capture of four traps per crop. Insect species name: HV= *Homalodisca vitripennis*; HI= *Homalodisca insolita*; GC= *Graphocephala coccinea*; GV= *Graphocephala versuta*; OO= *Oncometopia orbona*; PI= *Paraulacizes irrorata*; DS= *Draeculacephala* spp. ....108

Figure 13. Seasonal abundance of seven sharpshooter species in Athens, Alabama in 2009. Each number represents the average sharpshooter capture of four traps per crop. Insect species name: HV= *Homalodisca vitripennis*; HI= *Homalodisca insolita*; GC= *Graphocephala coccinea*; GV= *Graphocephala versuta*; OO= *Oncometopia orbona*; PI= *Paraulacizes irrorata*; DS= *Draeculacephala* spp. ....109

Figure 14 Relationship between cumulative captured *Homalodisca vitripennis* adults and degree days accumulation based on 10°C after January in two Alabama locations during 2008-2009 .....110

Figure 15. Relationship between cumulative captured *Graphocephala versuta* adults and degree days accumulation after January in three Alabama locations during 2009.....111



## CHAPTER ONE

### *Xylella Fastidiosa* Affected Fruit Crops and Its Efficient Vector Species:

#### Literature Review

##### 1.1 Introduction

Recently the xylem-limited bacterium *Xylella fastidiosa* (*Xf*) has emerged as one of the most significant new disease threats in the Americas (Hopkins and Prucell, 2002). This pathogen has a relatively wide host range (Freitag, 1951), and has caused economic losses to several agricultural industries (Redak et al., 2004; Ameida and Purcell, 2003). The bacterium causes a variety of plant diseases in numerous fruit and nut crops, such as bunch grape (Davis et al., 1978; Wells et al., 1983), peach, plum (Boyhan et al., 1997), citrus (Chang et al., 1993), avocado (Montero-Astúa et al., 2008), pecan (Sanderlin and Heyderich-Alger, 2000), and almond (Mircetich et al., 1976). Most recently, a new disorder was observed to affect southern highbush blueberry cultivars in the southeastern region of the U.S. and subsequently proved to be caused by the bacterium *Xf*. Chang et al. (2009), named the disease bacterial leaf scorch of blueberries. Other crops with considerable economical importance such as coffee (de Lima et al. 1998), and alfalfa (Thomson et al., 1978), were also confirmed to be susceptible to *Xf*. This pathogen is also known to cause numerous diseases on ornamental plants, such as oleander leaf scorch (Purcell et al., 1999), and bacterial leaf scorches in *Ulmus* spp. (Sherald, 1993), *Quercus* spp. (Chang and Walker, 1988), *Acer* spp. (Sherald et al., 1987), and in *Platanus* spp. (Sherald et al., 1983) .

*Xf* is transmitted by xylophagous vectors, mainly from the families Cicadellidae (Frazier and Freitag, 1946) and Cercopidae (Severin, 1950). Xylem-feeding sharpshooter (Cicadellidae: Cicadellinae) are the major group of *Xf* vectors.

### ***Disease Pathogenicity***

Water stress was considered as the cause of leaf necrotic symptoms induced by the bacterial occlusion of xylem-vessels in different plant species (Stevenson et al., 2005). The leaf necrosis and stunted plant growth symptoms occur when *Xf* bacteria proliferate within the xylem vessels and block the flow of xylem fluid to the shoots (Newmen et al., 2003). In response to the infection, plants produce tyloses and gums, which can cause water-conducting dysfunction and induce water stress in diseased plants (Mollenhauer and Hopkins, 1974; Mollenhauer and Hopkins, 1976). Studies demonstrated that water deficit can accelerate the expression of Pierce's Disease (PD) symptoms on infected grapevines (Thorn et al., 2006). Examination of the petiole and leaf vein sections by electron microscopy demonstrated that different vessels were blocked at different cross sections (Hopkins, 1981). Although a single cross section of PD diseased grape stem did not show high occlusion percentage among all the vessels, 5 mm long serial sectioning along the vessels showed that five times more xylem vessels were blocked than a single cross section (Hopkins, 1981).

In addition to the water stress, two more hypotheses - the phytotoxin genesis and the gibberellic acid inhibition - were established to explain the symptom expression in different *Xf* infected plants (Hopkins, 1989). A study demonstrated that phytotoxins isolated from grapevine with PD symptoms, almond with almond leaf scorch symptoms, and alfalfa with alfalfa dwarf symptoms can induce typical leaf scorch symptoms after re-inoculation on bunch grape (Lee et al., 1982). This hypothesis, however, cannot explain the lack of symptoms expressed on phony peach diseased trees, because phony peach does not show either leaf scorch or necrosis symptoms as other trees infected by *Xf*.

The abnormal growth and physiological change of phony peach diseased trees were examined by Andersen and French (1987). The authors suggested that root metabolism change could explain the symptoms expressed on phony peach diseased trees. Subsequent studies by French and Stassi (1978) showed that two consecutive applications of 0.246 ai g/L gibberellic acid can reduce phony peach dwarf symptoms on infected peach trees by allowing the terminal buds to resume growth. On the other hand, the study revealed that the application of gibberellic acid biosynthesis inhibitor can induce dwarf shoot growth, earlier blooming and higher leaf chlorophyll content. These responses are similar

to phony peach disease symptoms. It is possible that plant regulator imbalance accounts for the abnormal growth of the infected peach trees (DeJong and Doyle, 1984; Erez, 1984; Wood, 1984).

### ***Xf Strains***

*Xylella fastidiosa* was firstly named and described by classifying 25 phenotypically and genotypically related strains from ten host plants into one bacterial species (Wells et al., 1987). Subsequently, more strains with phenotypical and genotypical similarity were discovered and added to this species. During the past decades, differences among these strains were further studied (Chen et al., 1992; Pooler and Hartung, 1995; Coletta-Filho et al., 2001; Hernandez-Martinez et al., 2006) and several differentiation methodologies were developed based on bacterial host range, nutritional fastidiousness and DNA homology. DNA-DNA relatedness (Stackebrandt and Goebel, 1994) was used to determine phylogenetic relationships of 26 known strains, which were consequently classified into three subspecies: *Xylella fastidiosa* subsp. *piercei*, subsp. nov., *X. fastidiosa* subsp. *multiplex* subsp. nov., and *X. fastidiosa* subsp. *pauca*, subsp. nov., each of which comprises strains from one or more hosts (Schaad et al., 2004). This study determined that bacterial strains of *Xf* subsp. *piercei* are causing diseases in European bunch grape (*Vitis vinifera*), alfalfa (*Medicago sativa*), maple (*Acer* spp.), and almond (*Prunus dulcis*). Known hosts of strains of *X. fastidiosa* subsp. *multiplex* include peach (*Prunus persica*), plum (*P. domestica*), almond (*P. dulcis*), elm (*Ulmus* spp.), pigeon grape (*Vitis aestivalis*), sycamore (*Platanus* spp.), and other shade trees. Only strains of *X. fastidiosa* subsp. *pauca* are causal agent of citrus variegated chlorosis (Schaad et al., 2004).

### ***Disease Hosts and Symptoms***

*Xf* is a Gram-negative, non-motile, nonflagellate, rod-shaped bacterium (Wells et al., 1987), known to induce economically important diseases on various types of fruit crops including Pierce's disease (PD) in grape (Davis et al., 1978, Wells et al., 1983), phony peach disease, plum leaf scald (Boyhan et al., 1997), citrus variegated chlorosis (Chang et al., 1993), bacterial leaf scorch of blueberries (Chang et al., 2009, Brannen et al., 2007), leaf scorch in avocado (Montero-Astúa et al., 2008), pecan bacterial leaf scorch (Sanderlin and Heyderich-Alger, 2000), and almond leaf scorch (Davis et al., 1980). Other economically important crop species such as coffee (de Lima et al., 1998), alfalfa (Thomson et al., 1978) were also confirmed to be infected by *Xf*. To date, numerous weed

species are also known to host *Xf*. In a survey to determine the *Xf* infection of ground vegetation that consisted of major weed species surrounding an almond orchard in California, eleven out of 38 species were determined to be *Xf* positive (Shapland et al., 2006). Hundreds of plant species, considered potential hosts of *Xf*, are listed on the website of the College of Natural Resource, University of California at Berkeley, (<http://www.cnr.berkeley.edu/xylella/control/hosts.htm>). It was also found that some of the host plants remain symptomless, and according to Hopkins and Purcell (2002), the range of symptomless and symptomatic hosts may continue to expand.

***Pierce's Disease (PD)***: PD was first reported as an emerging disease in the 1880's in California, and in a short period of time, PD decimated grapevines from Los Angeles Basin of California (Pierce, 1882). Later studies demonstrated that PD was associated with the alfalfa fields infected with alfalfa dwarf virus: the incidence of PD decreased with the increase of the distance from the adjacent alfalfa fields (Hewitt and Houston, 1941). This observation led to the conclusion that the transmission of PD is mainly due to the xylem-feeding vectors like the leafhoppers and spittlebugs migrating from inoculum sources outside the vineyard. The causal agent of PD on grapes was not determined to be a bacterial organism until 1978 (Davis et al., 1978). In the southeastern U.S., the European grape, *Vitis vinifera*, has not been established since early Spanish settlement in the sixteenth century due to the interaction of PD and leafhopper vectors (Hopkins and Purcell, 2002). Stoner (1953) suggested that *Xf* has been endemic in this region for a long time. However, epidemiological studies of PD have not been conducted in southeastern states due to the limited vineyards size and the high abundance of sharpshooter vectors (Hopkins and Purcell, 2002).

On PD infected grapevines, the deposition of gums within xylem vessels occurred prior to any visible external symptoms (Esau, 1948). The typical external symptoms associated with the PD infection of grapevines appears as interveinal chlorosis; leaf necrosis then develops from leaf margin toward the base of the leaf blade with yellowish or reddish outline (Hopkins, 1989). Petioles in the late stage of the infection usually remain on the shoot when the leaf blade abscises from the petiole. This unique symptom is caused by a fracture at the most distal end of the petiole, because of the natural structural weakness produced by leaf necrosis, followed by dehydration of the petiole distal end. Remaining petioles are also known as "matchstick" symptoms (Stevenson et al., 2005). Another

typical symptom of PD infection is produced on the vine stems and is associated with the uneven formation of the periderm that leads to the formation of so-called “green islands” on the epidermis: central part of the vine stem remains green while the surrounding epidermis turns brown as it matures (Stevenson et al., 2005). Generally, PD infection leads to vine decline, yield loss, and vine death typically occurs within two to three years of infection under optimal temperatures (Gubler et al., 2006).

Microscopic examination of the number of the occlusion vessels in petioles from PD infected grapes over the growing season revealed the first presence of occlusion on April 21st, and was highest on June 7th. On October 10th, the observed occlusion number was greatly reduced, partially because most of the old leaves with PD symptoms had abscised (Hopkins, 1981). In this study, the bacterial infestation from leaf vein was highly correlated to the leaf necrosis symptom.

Grape species that are native to Gulf Coastal Plain of the U.S. are known to be resistant or tolerant to PD (Hewitt, 1958). Among those native grapes, muscadine grape - *Vitis rotundifolia* (Michx.) is the most popular in southeastern states because of its vigor and longevity under the stress of PD (Loomis, 1958). Despite its tolerance or resistance to PD, mild symptoms were observed on some muscadine grapes (Hewitt, 1961). Hopkins et al. (1974) determined PD tolerance levels among 20 muscadine grape cultivars. The authors concluded that Lucida, Scuppernong and Pride were the most susceptible varieties. They also noted some severe PD symptoms on muscadine grapes resembling those seen on bunch grape. Using electron microscopy, Hopkins et al. (1974) confirmed the occurrence of *Xf* in xylem tissues from symptomatic muscadine grapes.

***Phony Peach Disease (PPD)***: Since the 1890's, phony peach disease has been found to affect peach crop in the southeastern U.S. (Hutchins, 1933). Surveys conducted between 1929 and 1952 showed the disease was present in Alabama, Georgia, northern Florida, Louisiana, Mississippi, South Carolina, southern Arkansas, and eastern Texas. At the same time, two million unprofitable PPD infected peach trees were identified and destroyed (Cavanagh and Rothe, 1953). Boyhan et al. (1997) studied the incidence of *Xf* on plum and peach orchards in Alabama and indicated 14% of collected peach samples were PPD infected.

The symptomatic peach trees show stunted growth attributed to the shortened shoot internodes. Flatter and darker green canopies are also observed on infected peach trees, when compared to the healthy peach trees (Cavanagh and Rothe, 1953). Earlier blooming, delayed autumn leaf senescence and smaller-sized fruit than normal trees are other typical symptoms of diseased peach trees (Cavanagh and Rothe, 1953). However, using colorimeter measurements, it was found that the leaf color of the diseased tree does not turn darker green. The reason for the darker green canopy appearance is that the flatter canopy shape of the phony peach tree limits the sunshine distribution on the lower part of the canopy (Evert and Smittle, 1989).

***Plum Leaf Scald (PLS)***: First record of plum leaf scald originated from Argentina in 1954 (Fernandez-Valiela and Bakarcic, 1954). By 1978, the disease spread to Paraguay and Brazil (French and Kitajima, 1978). PLS infected plums were reported for the first time in the U.S. by French et al. (1977), but infected trees have expressed leaf scald with marginal area necrosis symptoms since 1970. In Alabama, plum leaf scald symptoms were firstly noted in the Chilton Horticultural Station, Clanton in 1971 (Latham and Norton, 1978). In a 1997 survey in Alabama, 12% of plum samples tested positive, using serological methods (Boyhan et al., 1997).

PLS symptom expression in Japanese plums was noted by Latham et al. (1980) from mid June until July with slight necrosis on the leaf margin or the leaf tip. With the season progression, the leaf necrosis expanded gradually and covered three quarters of the whole leaf area before the leaf abscission occurred. The study revealed that a severe defoliation and dieback can affect either several branches, or the entire diseased plum tree as disease develops.

***Citrus Variegated Chlorosis (CVC)***: Citrus variegated chlorosis is a relatively new devastating disease caused by the *Xf*. CVC was first reported in Brazil (Rossetti et al., 1990), and since then it has become a serious threat to the citrus industry in the Americas (Lee et al., 1991). During the following five years, over two million citrus trees had been infected by CVC (Laranjeira, 1997). In a 2009 survey on 1,659 citrus plots in the world's largest citrus growing region, Sao Paulo State, Brazil, CVC symptoms were found on 39.19% of the plots. (Fundecitrus, <http://www.fundecitrus.com.br/Pagina/Default.aspx?IDPagina=115>).

Early symptoms of CVC are associated with light brownish lesions on the lower surface of the mature citrus leaves. Gradually, the light brownish lesions develop into dark brownish lesions, or even necrosis (Beretta et al., 1993). Small and unmarketable citrus fruits produced by the infected tree usually mature earlier than normal fruits (Hopkins and Purcell, 2002).

***Bacterial Leaf Scorch in Blueberry:*** Disorders induced by *Xf* emerged most recently on highbush blueberry plants grown in the southeastern U.S. In 2004, symptoms of bacterial leaf scorch were first reported on southern highbush blueberry cultivars (Chang et al., 2009). According to these first observations the symptoms were initially associated with blueberry leaf marginal scorch, followed by an early leaf abscission. Consequently, notable yellowish stems and twigs on blueberry plants were observed. Further research has confirmed the presence of *Xf* pathogen within symptomatic blueberry tissues using serological methods. Since the discovery of the bacterial leaf scorch in blueberry is very recent, the impact of this disease on blueberry crop has not yet been assessed.

#### ***Economical Importance of Xf Associated Fruit Crops in Alabama***

According to the incidence report of diseases associated with *Xf* in California (Pierce, 1882), Florida (Stoner, 1953), Alabama (Boyhan et al, 1997), Georgia (Chang et al., 2009) and Brazil (Laranjeira, 1997; <http://www.fundecitrus.com.br/Pagina/Default.aspx?IDPagina=115>), bunch grape, plum, peach, blueberry, and citrus trees are the major fruit crops that are exposed to the threat of infection by *Xf*. Fruit growing acreage data for the state of Alabama (Department of Agriculture, National Agricultural Statistics Service <http://quickstats.nass.usda.gov/>) showed that peach production has been the largest fruit tree industry in Alabama for the last decade. Peach production area reached up to 1,013 ha in 2007 in Alabama (Table 1), of which 913 ha were bearing peach orchards. Blueberry production ranks second among fruit crops in terms of acreage, with a total of 249 ha. The big proportion of blueberry acreage still not in production shows the expected increase in production within the next few years. Grape production including bunch and muscadine grape ranks third with 189 hectares. When compared to the grape acreage one decade ago, there is a 41% increase representing a considerable growth. The category of plum and prune ranks next to the grape, and shows a slight increase for the last decade. One possible reason is the losses associated with plum leaf scald and the associated short longevity of plum orchards in Alabama, where the PLS disease pressure

is high. The citrus industry currently accounts for 44 ha. The production of Satsuma mandarin in Mobile and Baldwin County of Alabama has been growing since the early 1990's (Fadamiro et al., 2008). Recent introduction of cold hardy rootstocks (Campbell, et al., 2004), has contributed considerably to the expansion of the Satsuma mandarin production.

### ***Xf Induced Disease Transmission***

Mechanical transmission, root grafting and insect vectors are the known transmission routes of *Xf*. Mechanical transmission by shears and pruners are traditionally considered to be one of the primary routes of transmission in vineyards and orchards. Nevertheless, studies by Krell et al. (2007) on grapevines demonstrated a relatively low transmission rate of 4.5%, using shearing tools between infected and uninfected vines.

Phony Peach Disease strain of *Xf* was successfully transmitted via artificial root grafts between peaches (Hutchins, 1933). Cross-transmission studies by Wells et al. (1981) proved that *Xf* transmission also occurred reciprocally in root grafts between peach and plum. Natural root grafting takes place when the roots or branches of two nearby trees grow to contact each other for a long period (Graham and Bormann, 1966). Hence, translocation of nutrients and plant pathogenic organisms can take place after natural grafting (Hartmann and Kester, 1975). Hutchins and Rue (1939) stated that natural root grafting poses a threat to the spread of PPD strains of *Xf* from infected *Prunus* species to adjacent healthy congeners in orchards.

### ***Xf Detection***

Enzyme linked immunosorbant assay (ELISA), invented in 1960 (Yalow and Berson, 1960) is used in plant pathogen diagnosis since 1976 (Voller et al., 1976). ELISA uses antibodies which bind to proteins on the outer wall of bacteria, to detect its presence or absence in a sample. This method was considered to be optimal to detect plant pathogen which is difficult to identify by microscopy, or needs to be processed with a large number of samples (Clark and Adams, 1977). Nome et al. (1980) firstly adapted ELISA for detection of pathogens inducing PD and ALS. However, ELISA has some limitations: the assay does not distinguish between different strains of the *Xf* and cannot detect a low pathogenic concentration (Minsavage et al., 1994). Polymerase chain reaction (PCR) technique was introduced for plant disease detection (Henson and French, 1993), and determined to be 100 fold more



sensitive than ELISA (Minsavage et al., 1994). However, PCR is more difficult to handle, more time consuming and less economical on large quantities of plant tissue samples (Wallingford, 2008), whereas ELISA has been found to be equally effective as PCR in detecting *Xf* in almond (Groves et al., 2005).

### ***Sharpshooter Vectors of Xf***

Since *Xf* is strictly confined in xylem vessels of the plant, xylem fluid-feeding insects from the sharpshooters subfamily (Cicadellidae: Cicadellinae) (Houston et al., 1947; Severin, 1949), and the spittlebugs family (Cercopidae) (Severin, 1950) are considered the major vectors transmitting *Xf*.

Members of the Cicadellinae subfamily belonging to order Hemiptera, family Cicadellidae, are commonly known as sharpshooters (Riley and Howard, 1893). Insects in this subfamily usually have an inflated clypeus which encloses the musculature connected to the diaphragm, which is the organ that facilitates suction of xylem sap, and their xylophagous mouthpart is closely associated with the transmission of *Xf* (Frazier, 1943; Turner and Pollard, 1955). Twenty-eight species from the sharpshooter subfamily have been confirmed to transmit *Xf* to cause Pierce's disease or Phony peach disease (Nielson, 1968; Nielson, 1979). Among those *Xf* vectors, *Xyphon fulgida*, *Draeculacephala minerva*, *Homalodisca vitripennis*, *Graphocephala atropunctata*, and *Oncometopia* spp. are found to be abundant in diseased orchards or in adjacent fields in North America (Nielson 1968, Purcell and Frazier 1985, Turner and Pollard, 1959). Myers et al. (2007) determined that the most abundant sharpshooter vectors in North Carolina State were *Oncometopia orbona*, *G. versuta*, *Paraphlepsius irroratus*, and *Agalliota constricta*. Specimens of those sharpshooters are confirmed to be associated with the transmission of *Xf*. The identity of sharpshooter vectors in Alabama has not been reported before and science based information on this economically important vector is lacking.

The transmission efficiency of *Xf* is dependent on the combination of host, vectors and pathogen strains (Redak et al., 2004). The combination varies and only a small proportion of the combinations have been studied. The *H. vitripennis* mediated 32% PD transmission rate from grape to grape as observed by Almeida and Purcell (2003), while *H. vitripennis* mediated PPD transmission rate from peach to peach can reach only 15% (Turner and Pollard, 1959). Existing data of *Xf* transmission by *G. versuata* between peach trees indicated 29% transmission rate (Turner and Pollard, 1959). *H. insolita*

has the ability to transmit peach strains between peach trees with 48% efficiency rate, while in the same study, 33 out of 100 peach strains inoculations by *O. nigricans* were successfully attained. The transmission tests have been conducted on more combinations of *D. minerva*. Its transmission efficiency rate of almond leaf scorch strains between grapes is up to 92% (Hill and Purcell, 1995). In contrast to almond leaf scorch strains, PD strains were transmitted by *D. minerva* with a much lower efficiency rate of only 3% from almond to grape. 0-1% efficiency rate from alfalfa to grape were also recorded (Hill and Purcell, 1995; Severin, 1949).

The action of bacterial transmission by insects consists of three steps: acquisition, retention and inoculation (Chatterjee et al., 2008). The acquisition efficiency of *Xf* from a plant is associated with the bacterial density. The acquisition is only triggered when the bacteria within the vessel are above the  $10^4$  viable cells, whereas the established threshold from grapevines to vectors is up to  $10^{6-7}$  cells (Hill and Purcell, 1997). On the contrary, the transmission from insects to plants requires less than 200 viable cells within the vector's foregut (Almeida and Purcell, 2003). The lack of latent period from acquisition to transmission and the discontinuity of infectivity after molting (Purcell and Finlay, 1979) indicate the bacteria has noncirculative movement within the xylem feeder.

The importance of the *Xf* vector was considered to be related to its occurrence on, or near *Xf* infected orchards or vineyards (Almeida et al., 2005). Thus, *H. vitripennis* was thought to be the most economically important vector within the sharpshooter subfamily due to its abundance in disease threatened vineyards (Redak et al., 2004). *G. atropunctata* (Signoret) was observed to be abundant in riparian vineyards and irrigated landscapes in California (Hopkins and Purcell, 2002). The distribution of *D. minerva* and *X. fulgida* are associated with the *Xf* transmission in vineyards adjacent to irrigated pastures and alfalfa fields in the Central Valley, California (Hewitt et al., 1942). Compared with other species, *H. vitripennis* was of most interest, and its behavior was well documented.

Oviposition Behavior: According to Tipping et al., (2006), *Homalodisca* spp. and *Oncometopia* spp. deposit their eggs on the lower side of the leaves, with whitish brochosomes covering the egg masses, while *P. irrorata* deposits eggs on woody twigs, stem and lignified petioles without covering brochosomes.

### *Homalodisca vitripennis*

*H. vitripennis* (Hemiptera: Cicadellidae: Cicadellinae, Glassy-winged sharpshooter) which used to be limited to the southeastern U.S. (Turner and Pollard, 1959), invaded California in the 1990's (Redak et al. 2004). It is considered that the introduction of *H. vitripennis* triggered an outbreak of PD in Temecula Valley, California (Blua et al. 1999; Hopkins and Purcell, 2002). In comparison with native vectors in California, which usually have a limited host range, or a preferred habitat, *H. vitripennis* are capable of feeding on more than 100 plant species (Turner and Pollard, 1959; Mizell and French, 1987; Hoddle et al., 2003). A study in Florida indicated that *H. vitripennis* were captured from 72 plant species belonging to 37 families (Hoddle et al., 2003). The strong flying capability of *H. vitripennis* adults enables them to search a large area for the suitable feeding location, thus, posing more threat of *Xf* infection than other sharpshooter vectors (Blua et al., 2001; Purcell et al., 1979; Blackmer et al., 2003).

*H. vitripennis* adult and fourth, fifth instar require amides affluent xylem fluid, and adult females are generally inclined to oviposit on those plants. However, nymphs in early stages prefer fluid with a balanced amino-acid profile and may die if they stay on plants with high amides (Brodbeck et al., 1995). Greenhouse studies also concur that *H. vitripennis* adults and nymphs rarely remain on the same tree for feeding (Brodbeck et al., 2007). For instance, crape myrtle (*Lagerstroemia indica*) is a common native plant host for *H. vitripennis* in the U.S., but eggs and early stage nymphs are rarely found on it. In contrast, some host species such as *Euonymus japonica* are more favored by eggs and young nymphs (Brodbeck et al., 1995; 2004).

Feeding on a wide selection of plant xylem fluids can meet the requirement of *H. vitripennis* adults and nymphs. Generally, xylem fluid consists of more than 98% water (Raven, 1983) and remainder consisting monomers such as 19 different protein amino acids, five to seven organic acids, and three major sugars (Andersen et al. 1989; Andersen et al. 1992). The ratios of those monomeric components vary among different plant species and different parts of the plant (Andersen et al. 1992; Andersen et al., 1995). Furthermore, the ratios are also dependent on environmental variation (Andersen and Brodbeck, 1988; Andersen and Brodbeck, 1991; Andersen et al., 1989; Andersen et al., 1992; Andersen et al., 1995). Nadel et al. (2008) found that under controlled conditions, the

abundance, feeding and oviposition of *H. vitripennis* are greater on well-irrigated citrus and avocado trees, than on water-stressed controls.

High feeding rates are essential for xylem-feeders like *H. vitripennis* to compensate for high negative fluid tension and low nutritional density in xylem fluid (Andersen et al., 1992; Mizell et al., 2008). The energy obtained from xylem fluid is slightly higher than the energy used for sap extraction with exception of xylem fluid from peach. The net energy surplus obtained from feeding on peach crop by *H. vitripennis* is even slightly negative by theoretical calculation (Andersen et al., 1989). The hourly feeding rate of sharpshooters ranges from 0.21-0.70 ml, dependent on different host plants and is peaking on midday (Andersen et al., 1989; 1992; 2003; 2005; Brodbeck et al., 1995; 1996; 1999; 2007; Mizell et al., 2008).

High nutritional assimilation efficiency is another key characteristic in sharpshooter's survival strategy. They absorb more than 99% of the key amino acids, organic acids and sugars when xylem fluids travel through their complex long midgut (Andersen et al., 1989; Ammar, 1985).

Besides feeding on the young tissues like other sharpshooters, *H. vitripennis* has the tendency to feed on the base part, even woody parts of the grapevine shoot (Hopkins and Purcell, 2002). Overwintering as an adult, *H. vitripennis* has the ability to feed on dormant vines in the winter. Both parts of the vine they feed on, the dormant cane during the winter, and the basal part of a shoot in a growing season, have less chance of removal from orchard during winter pruning (Almeida et al., 2005). These feeding behaviors of *H. vitripennis* increase the possibility of chronic PD infections in vineyards.

#### ***Temperature Restriction to Xf Distribution***

Diseases caused by *Xf* are generally limited to the tropics and the subtropical belt. Freezing temperatures during the dormant season are observed to cure the plant from PD (Purcell, 1980). In vitro experiments revealed that *Xf* grows fast at 28°C, but does not grow at 12°C. *Xf* inoculated grape seedling subjected to temperature less than 5°C for 2 weeks resulted in bacterial multiplication decreased within plant tissues by 230-fold, but the population of bacteria did not decrease significantly over time (Feil and Purcell, 2001).

Several temperature-related methods were used to determine the disease potential to infect various fruit crops. From the relationship between temperature and insect mortality, Sutton (2005), defined three levels of PD infection risk according to the winter temperature. Locations where winter has less than one day when the temperature is  $-12.2^{\circ}\text{C}$  or less, or with less than three consecutive days the temperature is  $-9.4^{\circ}\text{C}$  or less, is defined as a very high PD infection risk area (Table 2).

Sharpshooters are considered to overwinter as adults in surrounding grass debris. Temperature is an important factor affecting the survival of sharpshooters and development of their eggs within the adult insects. Johnson et al. (2006) have determined that  $10^{\circ}\text{C}$  is a critical threshold for the survival of *H. vitripennis*. It was observed that sharpshooter individual was hardly feeding when the temperature was near or below  $10^{\circ}\text{C}$ , and will die when the temperature held below  $10^{\circ}\text{C}$  for more than 15 days. From this discovery, Johnson (2008) constructed a cooling degree-day (CDD) model to quantify the cold impact on the *H. vitripennis* mortality. He has accumulated the absolute value of the difference between daily mean temperature and  $10^{\circ}\text{C}$ , only when daily mean temperature is lower than the thermal activation threshold ( $10^{\circ}\text{C}$ ). Based on his curvilinear regression, an accumulated CDD that exceeds  $178^{\circ}\text{C}$  contributes to a 0% survival rate of *H. vitripennis*.

To study the temperature effect on egg production capability, egg loads of female adult *H. vitripennis* captured from July 2005 to October 2008 were examined in Texas (Lauziere, 2008). Egg loads demonstrated significant difference between months with a maximum mean number of 13.8 eggs per female in March. Furthermore, the insect sizes of female adults which are represented by the left hind tibia length also varied significantly with the largest size caught in May, June, and July, while the smallest insects were caught in December. In a set of greenhouse studies (Lauziere, 2008), pre-oviposition period of summer *H. vitripennis* females averaged 13 days, which was 5.6 times shorter than the pre-oviposition period of winter individuals. Despite the difference in the pre-oviposition period, the study concluded that the oviposition period, post-oviposition period and the laid egg numbers did not vary significantly between different months.

### ***Management Strategies for Pathogen and Vector***

***Monitoring Methods:*** Yellow sticky traps are widely used in field monitoring of sharpshooter species (Myers et al., 2007; Hall and Hunter, 2008; Wallingford, 2008). In a previous research studied

by Castle and Naranjo (2008) the efficacy of four different methods were compared to the efficacy of yellow sticky traps to monitor *H. vitripennis* populations, and a strong correlation was found between the adult capture numbers of all the methods tested and sharpshooter numbers captured from the yellow sticky traps.

**Cultivar Resistance:** Almost all European grapes (*Vitis vinifera*) and American bunch grapes (*V. labrusca*) are known to be susceptible to PD (Hopkins and Purcell, 2002). In California, different tolerances among *V. vinifera* grape cultivars were found (Raju and Goheen, 1981). Most of the *Vitis* species, native to areas of severe PD pressure such as *Vitis rotundifolia*, and *Vitis arizonica* appear to be very resistant to PD (Loomis, 1958; Ruel and Walker, 2006), but some are still expressing mild to severe symptoms (Hewitt, 1961; Hopkins et al., 1974). According to Hopkins and Purcell (2002) variety selection in PD infested areas should be based on resistance. Although Walker and Tenschler (2008) recently utilized marker-assisted selection technique to accelerate the introgression of the PdR1 allele from resistant grape species into *V. vinifera* grapes, currently there are no PD resistant *V. vinifera* based cultivars available on the market.

**Chemical and Physical Control:** Imidacloprid is a neuro-active chemical of neonicotinoid used to control *H. vitripennis*. Imidacloprid has long residual activity (Krewer et al., 2002) and xylem-translocation characteristics (Wang et al., 1999), and is able to hamper the spread of PD in field studies (Krewer et al., 2002). Research conducted by Villanueva et al. (2008) showed that fewer sharpshooter leafhoppers were captured in North Carolina vineyards after implementing a combination of neonicotinoid and pyrethroid insecticides. However, a study by Krewer et al. (2002) showed imidacloprid was not effective to slow the development of PD in areas where the vector species were abundant.

Kaolin (Surround WP®, Engelhard Co. Iselin, NJ) is thought to be an alternative pest management agent. Kaolin forms a mineral film surrounding plant tissues to prevent pest insects from feeding or ovipositioning on the plant organs (Glenn et al., 1999). In a field experiment conducted near Bakersfield, California, 6% of the kaolin treated grapevines tested positive for PD, while the infection rate for the untreated grapevines was 8% greater than the kaolin treated plants (Tubajika et al., 2007). The dust application of particle films used in Glenn et al.'s research was not considered

practical due to the lack of particle adhesion to the plant surfaces. A later study developed liquid formulations and improved the practicality (Glenn et al., 1999).

Harpin proteins are known to activate natural plant defenses by promoting vigorous growth (Alarcon et al., 1998; Tubajika et al., 2007). The protection efficacy is dependent on the treatment rate: A treatment rate of 460 g/ha has restricted the PD incidence to 6%, comparing with 19% PD incidence of the control treatment. Studies showed that plants treated with harpin had a lower incidence of the symptoms of PD and were less likely to be infected with *Xf*.

**Colonization Disruption by Benign Strains:** The PD control efficacies of several weakly virulent and avirulent strains of *Xf* were tested in greenhouse and vineyards in Florida (Hopkins, 2005). *Xf* strains isolated from sycamore and elderberry were the biological agents used to reduce the PD incidence. The study found that the latter strains have contributed to a good control of PD when *V. vinefera* grape seedlings were inoculated in the field. More experimental work involving different grape cultivars is needed to test the feasibility of this management (Hopkins, 2005).

**Biological and Natural Control Agents:** Host-specific egg parasitoid, *Gonatocerus ashmeadi* (Girault) (Hymenoptera: Cicadellidae), was introduced to reduce the *H. vitripennis* population in Tahiti and Mooresea of French Polynesia. A 90% reduction of *H. vitripennis* population was achieved with low infestation on non-target eggs after a release of 13,786 *Gonatocerus ashmeadi* individuals (Grandgirard et al., 2007; Grandgirard et al., 2008). Besides the high control efficiency, *G. ashmeadi* also has demonstrated a rapid spread and even self-introduction to the neighboring islands where it was not implemented. *G. fasciatus* (Girault) (Hymenoptera: Mymaridae) was also observed to parasitoid on eggs of *Homalodisca vitripennis* and *Oncometopia orbona* (Triapitsyn et al., 2003). Coincidentally, naturally occurring parasitoids on *P. irrorata* eggs was reared to arise from host eggs and identified as *G. fasciatus* (Tipping et al., 2006). Adult and nymph of *H. vitripennis* was also a prey to spiders from families Salticidae, Agelenidae, Oxyopidae and Lycosidae (Lopez et al., 2003). Mizell et al. (2008) also observed that insects of Odonata order seized adult *H. vitripennis* on the wing in flight.

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### 1.3 Tables

Table 1. Total acreage of selected fruit crops in Alabama in 2007, according to the National Agricultural Statistics Service

| Fruit Crop                | Total acreage (ha) | Acreage in Production (ha) |
|---------------------------|--------------------|----------------------------|
| Peach                     | 1013               | 914                        |
| Blueberry                 | 249                | 140                        |
| Bunch and muscadine grape | 189                | 140                        |
| Plum and Prune            | 45                 | 35                         |
| Satsuma mandarin          | 44                 | 30                         |

Table 2. Estimated risk of PD infection according to Sutton's standard – number of day when daily minimum temperature falls below -12.2°C, or -9.4°C

□

| Risk level      | No. of days under -12.2°C | No. of days under -9.4°C |
|-----------------|---------------------------|--------------------------|
| Very high       | 1 or less                 | 3 or less                |
| High            | 2                         | 4                        |
| Moderate to low | 3 or more                 | 5 or more                |

## CHAPTER TWO

### Investigation into *Xylella Fastidiosa* Occurrence in Selected Alabama

#### Orchards and Vineyards

##### 2.1 Introduction

*Xylella fastidiosa* (*Xf*) is a Gram-negative, non-motile, nonflagellate, rod-shaped bacterium (Wells et al., 1987). It has a relatively wide host range (Freitag, 1951), and has caused economic losses to several agricultural industries (Redak et al., 2004; Almeida and Purcell, 2003). The bacterium is known to induce economically important diseases on various fruit crops including Pierce's disease (PD) in grape (Davis et al., 1978, Wells et al., 1983), phony peach disease, plum leaf scald (Boyhan et al., 1997), citrus variegated chlorosis (Chang et al., 1993), leaf symptoms in avocado (Montero-Astúa et al., 2008), pecan bacterial leaf scorch (Sanderlin and Heyderich-Alger, 2000), and almond leaf scorch (Davis et al., 1980). Other economically important crop species such as coffee (de Lima et al., 1998) and alfalfa (Thomson et al., 1978) were also confirmed to be susceptible to *Xf*. Most recently, a new disorder was observed that affects highbush blueberry cultivars in the southeastern U.S. and subsequently proved to be caused by the bacterium, *Xf*. Chang et al. (2009) named the disease bacterial leaf scorch of blueberries. This pathogen is also known to cause numerous diseases on ornamental plants, such as oleander leaf scorch (Purcell et al., 1999), and bacterial leaf scorch in *Ulmus* spp. (Sherald, 1993), *Quercus* spp. (Chang and Walker, 1988), *Acer* spp. (Sherald et al., 1987), and in *Platanus* spp. (Sherald et al., 1983). Hundreds of plant species considered potential hosts of *Xf*, are listed on the website of the College of Natural Resource, University of California, (<http://www.cnr.berkeley.edu/xylella/control/hosts.htm>). It was shown that some of the infected plants remained symptomless, and according to Hopkins and Purcell (2002), the range of symptomless and symptomatic hosts may continue to expand.



### ***Disease Pathogenicity***

Both the bacteria (Newmen et al., 2003) and tyloses produced by the plant in response to the bacterial infection (Mollenhauer and Hopkins, 1974; Mollenhauer and Hopkins, 1976) can block xylem vessels (Stevenson et al., 2005) and cause water conducting dysfunction on host plants. Studies demonstrated water deficit can accelerate the expression of PD symptoms on infected grapevines (Thorn et al., 2006). In addition to the water stress, two more hypotheses – the phytotoxin genesis and the gibberellic acid inhibition were established to explain the symptom expression in different *Xf* infected plants (Hopkins, 1989). A study demonstrated that a phytotoxin isolated from grapevine with PD symptoms, almond with almond leaf scorch symptoms, and alfalfa with alfalfa dwarf symptoms can induce typical leaf scorch symptoms after re-inoculation on bunch grape (Lee et al., 1982). The abnormal growth and physiological change of phony peach diseased trees implicates root metabolism change could explain the symptoms expressed on phony peach infected peaches (Andersen and French, 1987). Subsequent studies by French and Stassi (1978) have shown gibberellic acid can reduce phony peach dwarf symptoms on infected peach trees by allowing the terminal buds to resume growth. On the other hand, the study has revealed the application of gibberellic acid biosynthesis inhibitor can induce phony peach symptoms.

### ***Pierce's Disease (PD):***

PD was first reported as an emerging disease in the 1880's in California, and in a short period of time, PD decimated grapevines from Los Angeles Basin of California (Pierce, 1882). Later studies demonstrated that PD was associated with the alfalfa fields infected with alfalfa dwarf virus: the incidence of PD decreased with the increase of the distance from the adjacent alfalfa fields (Hewitt and Houston, 1941). This observation led to the conclusion that the transmission of PD is mainly due to the xylem-feeding vectors like the leafhoppers and spittlebugs migrating from sources outside the vineyard. The causal agent of PD on grapes was not determined to be a bacterial organism until 1978 (Davis et al., 1978). In the southeastern U.S., the European grape, *Vitis vinifera*, has not become established since early Spanish settlement in sixteenth century due to the interaction of PD and leafhopper vectors (Hopkins and Purcell, 2002). Stoner (1953) suggested that *Xf* has been endemic in

the region for a long time. However, epidemiological studies of PD have not been conducted in southeastern states due to limited vineyard's size and the high abundance of sharpshooter vectors (Hopkins and Purcell, 2002).

On PD infected grapevines, the deposition of gums within xylem vessels occurred prior to any visible external symptoms (Esau, 1948). The typical external symptoms associated with the PD infection of grapevines appears as interveinal chlorosis; leaf necrosis then develops from leaf margin toward the base of the leaf blade with yellowish or reddish outline (Hopkins, 1989). Petioles in the late stage of the infection usually remain on the shoot when the leaf blade abscises from the petiole. This unique symptom is caused by a fracture at the most distal end of the petiole, because of the natural structural weakness produced by leaf necrosis, followed by dehydration of the petiole distal end. Remaining petioles are also known as “matchstick” symptoms (Stevenson et al., 2005). Another typical symptom of PD infection is produced on the vine stems and is associated with the uneven formation of the periderm that leads to the formation of so-called “green islands” on the epidermis: central part of the vine stem remains green while the surrounding epidermis turns brown as it matures (Stevenson et al., 2005). Generally, PD infection leads to vine decline, yield loss, and vine death typically occurs within two to three years of infection if there are optimal temperatures (Gubler et al., 2006).

Microscopic examination of the number of the occlusion vessels in petioles from PD infected grapes over the growing season revealed first presence of occlusion on April 21<sup>st</sup>, and highest on June 7<sup>th</sup>. On October 10<sup>th</sup>, the observed occlusion number was greatly reduced, partially because most of the old leaves with PD symptoms had abscised (Hopkins, 1981). In this study, the bacterial infestation from leaf vein was highly correlated to the leaf necrosis symptom.

Grape species that are native to the Gulf Coastal Plain of the U.S. are known to be resistant or tolerant to PD (Hewitt, 1958). Among those native grapes, muscadine grape - *Vitis rotundifolia* (Michx.) is the most popular in southeastern states because of its vigor and longevity under the stress of PD (Loomis, 1958). Despite its tolerance or resistance to PD, mild symptoms were observed on some muscadine grapes (Hewitt, 1961). Hopkins et al. (1974) determined PD tolerance levels among 20 muscadine grape cultivars. The authors concluded that Lucida, Scuppernong and Pride were the

most susceptible varieties. They also noted some severe PD symptoms on muscadine grapes resembling those seen on bunch grape. Using electron microscopy, Hopkins et al. (1974) confirmed the occurrence of *Xf* in xylem tissues from symptomatic muscadine grapes. Generally, bunch grapes are not recommended for growing to the south of Birmingham, Alabama due to the high pressure of *Xf* infection. However, several PD resistant hybrid bunch grape varieties recommended by Hilmerick and Dozier, (1996) are grown in central and northern locations of the state.

***Phony Peach Disease (PPD):***

Since the 1890's, phony peach disease has been found to affect peach crops in the southeastern U.S. (Hutchins, 1933). Surveys conducted between 1929 and 1952 showed the disease was present in Alabama, Georgia, northern Florida, Louisiana, Mississippi, South Carolina, southern Arkansas, and eastern Texas. At the same time, two million unprofitable PPD infected peach trees were identified and destroyed (Cavanagh and Rothe, 1953). Boyhan et al. (1997) studied the incidence of *Xf* on plum and peach orchards in Alabama and indicated 14% of collected peach samples were PPD infected.

The symptomatic peach trees show stunted growth attributed to the shortened shoot internodes. Flatter and darker green canopies are also observed on infected peach trees, when compared to the healthy peach trees (Cavanagh and Rothe, 1953). Earlier blooming, delayed autumn leaf senescence and smaller-sized fruit than normal trees are other typical symptoms of diseased peach trees (Cavanagh and Rothe, 1953). However, using colorimeter measurements, it was found that the leaf color of the diseased tree does not turn darker green. The reason for the darker green canopy appearance is that the flatter canopy shape of the diseased peach tree is limiting the sunshine distribution on the lower part of the canopy (Evert and Smittle, 1989).

***Plum Leaf Scald (PLS):***

First record of plum leaf scald originated from Argentina in 1954 (Fernandez-Valiela and Bakarcic, 1954). By 1978, the disease had spread to Paraguay and Brazil (French and Kitajima, 1978). PLS infected plums were reported for the first time in the U.S. by French et al. (1977), but infected trees have been showing leaf scald with marginal area necrosis symptoms since 1970. In Alabama, plum leaf scald symptoms were first noted in the Chilton Horticultural Station, Clanton in 1971

(Latham and Norton, 1978). In a 1997 survey in Alabama, 12% of plum samples tested positive, using serological methods (Boyhan et al., 1997).

PLS symptom expression in Japanese plums was noted by Latham et al. (1980) from mid June until July with slight necrosis on the leaf margin or the leaf tip. With season progression, the leaf necrosis did expand gradually and covered three quarters of the whole leaf area before leaf abscission occurred. The study revealed that a severe defoliation and dieback can affect either several braches, or the entire diseased plum tree as disease develops.

***Citrus Variegated Chlorosis (CVC):***

Citrus variegated chlorosis is a relatively new devastating disease caused by *Xf*. CVC was first reported in Brazil (Rossetti et al., 1990), and since then it has become a serious threat to the citrus industry in the Americas (Lee et al., 1991). During the following five years, over two million trees of citrus had been infected by CVC (Laranjeira, 1997). In a 2009 survey on 1,659 citrus plots, CVC symptoms were found on 39.19% of the orange plots in the world's largest citrus growing region, Sao Paulo State, Brazil (<http://www.fundecitrus.com.br/Pagina/Default.aspx?IDPagina=115>).

Early symptoms of CVC are associated with light brownish lesions on the lower side of the mature citrus leaves. Gradually, the light brownish lesions develop into dark brownish lesions, or even necrosis (Beretta et al., 1993). Small and unmarketable citrus fruits produced by the infected tree usually mature earlier than normal fruits (Hopkins and Purcell, 2002). Currently CVC is not reported to be present in the U.S.A.

***Bacterial Leaf Scorch in Blueberry:***

Disorders induced by *Xf* emerged most recently on blueberry plants grown in the southeastern U.S. In 2004, symptoms of bacterial leaf scorch were first reported on southern highbush blueberry cultivars (Chang et al., 2009). According to these first observations the symptoms were initially associated with blueberry leaf marginal scorch, followed by an early leaf abscission. Consequently, notable yellowish stems and twigs on blueberry plants were observed. Further research has confirmed the presence of *Xf* within symptomatic blueberry tissues using serological methods. Since the discovery of the bacterial leaf scorch in blueberry is very recent, the impact of this disease on blueberry crop has not yet been assessed.

### ***Economical Importance of Xf Associated Fruit Crops in Alabama:***

Peach acreage reached up to 2,503 in 2007 in Alabama, of which 2,259 acres were bearing peach orchards (<http://quickstats.nass.usda.gov/>). Blueberry production ranks second among fruit crops in terms of acreage, with a total of 249 ha. The big proportion of blueberry acreage still not in production shows the expected increase in production within the next few years. Grape production including bunch and muscadine grape ranks third with 189 ha. When compared to the grape acreage a decade ago, there is a 41% increase, which is a considerable growth. The category of plum and prune ranks next to the grape, and shows a slight increase for the last decade. One possible reason is the damage caused by the plum leaf scald and the associated short longevity of plum orchards in Alabama, where the PLS disease pressure is high. The citrus industry currently accounts for 44 ha. The production of Satsuma mandarin in Mobile and Baldwin County of Alabama has been growing since the early 1990's (Fadamiro et al., 2008). Recent introduction of cold hardy rootstocks (Campbell, et al., 2004), has contributed considerably to the expansion of the Satsuma mandarin production.

### ***Xf Transmission***

Mechanical transmission, root grafting and insect vectors are the known transmission pathways of *Xf*. Mechanical transmission by shears and pruners are traditionally considered to be one of the primary route of transmission in vineyards and orchards. Nevertheless, studies by Krell et al. (2007) on grapevines demonstrated a relative low transmission rate (one out of 21 attempts successful rate) by using shearing tools between infected and uninfected vines.

Phony Peach Disease strain of *Xf* was successfully transmitted via artificial root graft between peaches (Hutchins, 1933). Cross-transmission studies by Wells et al. (1981) proved that *Xf* transmission also occurred reciprocally in root grafting between peach and plum. Natural root grafting takes place when the roots or branches of two nearby trees grow to contact each other for a long period (Graham and Bormann, 1966). Hence, translocation of nutrients and plant pathogenic organisms can take place after natural grafting (Hartmann and Kester, 1975). Hutchins and Rue (1949) stated that natural root grafting poses a threat to the spread of PPD strains of *Xf* from infected *Prunus* species to adjacent healthy congeners in orchards.

Since *Xf* is strictly confined in xylem vessels of the plant, xylem fluid-feeding insects from the sharpshooters subfamily (Cicadellidae: Cicadellinae) (Houston et al., 1947; Severin, 1949), and the spittlebugs family (Cercopidae) (Severin, 1950) are considered the major vectors transmitting *Xf*. Among those *Xf* vectors, *Xyphon fulgida*, *Draeculacephala minerva*, *Homalodisca vitripennis*, *Graphocephala* spp., and *Oncometopia* spp. are found to be abundant in fields with infection, or in adjacent fields in North America (Nielson 1968; Purcell and Frazier, 1985; Turner and Pollard, 1959; Myers et al., 2007). Sharpshooter vectors are thought to overwinter as adults in surrounding overwinter habitats. Temperature is an important factor affecting the survival of overwintering sharpshooters and development of their eggs within the adult insects. Johnson et al. (2006) have determined that 10°C is a critical threshold for the survival of *H. vitripennis* (Lauziere, 2008).

### ***Xf* Detection**

Enzyme linked immunosorbant assay (ELISA) is used in plant pathogen diagnosis since 1976 (Voller et al., 1976). ELISA uses antibodies which bind to proteins on the outer wall of bacteria to detect its presence or absence in a sample. This method was considered to be optimal to detect plant pathogen which is difficult to identify by microscopy, or needs to be processed with a large number of samples (Clark and Adams, 1977). Nome et al. (1980) first adapted ELISA for detection of pathogens inducing PD and ALS. However, ELISA has some limitations: the assay does not distinguish between different strains of the *Xf* and cannot detect a low pathogen concentration (Minsavage et al., 1994). Polymerase chain reaction (PCR) technique was introduced for plant disease detection (Henson and French, 1993), and determined to be 100 fold more sensitive than ELISA (Minsavage et al., 1994). PCR is more difficult to handle, more time consuming and less economical on large quantities of plant tissue samples (Wallingford, 2008), while ELISA has been found to be equally effective as PCR in detecting *Xf* in almond (Groves et al., 2005).

### ***Temperature Restriction to Xf Distribution***

Diseases caused by *Xf* are generally limited to the tropics and the subtropical belt. Freezing temperatures during the dormant season are observed to cure the plant from Pierce's disease (Purcell, 1980). In vitro experiments revealed that *Xf* grows fast at 28°C, but doesn't grow at 12°C. *Xf* inoculated grape seedlings treated under 5°C for 2 weeks decreased bacterial multiplication within

plant tissues by 230-fold, but the population of bacteria didn't decrease significantly over time (Feil and Purcell, 2001).

Several temperature-related methods were used to determine the disease potential to infect various fruit crops. From the relationship between temperature and insect mortality, Sutton (2005), defined three levels of PD infection risk according to the winter temperature. Locations where winter has less than one day when the temperature is less than -12.2°C or with less than three consecutive days the temperature is less than -9.4°C, is defined as a very high PD infection risk area.

### ***Management Strategies for Pathogen and Vector.***

***Cultivar Resistance:*** Almost all European grapes (*Vitis vinifera*) are known to be susceptible to Pierce's disease (Hopkins and Purcell, 2002). In California, different tolerances of different *V. vinifera* grape cultivars were distinguished (Raju and Goheen, 1981). Most of the *Vitis* species, native to areas of severe PD pressure such as *Vitis rotundifolia*, and *Vitis arizonica* appear to be very resistant to PD (Loomis, 1958; Ruel and Walker, 2006), but some express mild to severe symptoms (Hewitt, 1961; Hopkins et al., 1974). According to Hopkins and Purcell (2002) variety selection in PD infested areas should be based on resistant varieties. A list of hybrid bunch grape cultivars reported to have resistance to PD is recommended for planting in Alabama (Hilmerick and Dozier, 1996). Although recently Walker and Tenschler (2008) utilized marker-assisted selection technique to accelerate the introgression of the *PdR1* allele from resistant grape species into *V. vinifera* grapes; currently there is no PD resistant *V. vinifera* based cultivars available on the market.

***Chemical and Physical Control:*** Imidacloprid is a neuro-active chemical of neonicotinoid used to control *H. vitripennis*. Having long residual activity (Krewer et al., 2002), and xylem-translocation characteristics (Wang et al., 1999), imidacloprid is able to hamper the spread of PD in field studies (Krewer et al., 2002). Research conducted by Villanueva et al. (2008) showed that fewer sharpshooter leafhoppers were captured in North Carolina vineyards after implementing a combination of neonicotinoid and pyrethroid insecticides. However, a study by Krewer et al. (2002) showed imidacloprid was not effective to slow the development of PD in areas where the vector species were abundant.

Kaolin (Surround WP®, Engelhard Co. Iselin, NJ), is thought to be an alternative pest management agent instead of conventional insecticides. Kaolin forms a mineral film surrounding plant tissues to prevent pest insects from feeding or ovipositioning on the plant organs (Glenn et al., 1999). In a field experiment conducted in California, 6% of the kaolin treated grapevines tested PD infected, while the infection rate for the untreated grapevines was 8% or greater (Tubajika et al., 2007). The dust application of particle films used in Glenn's research was not considered practical due to the lack of particle adhesion to the plant surfaces. A later study developed liquid formulations and improved the practicality (Glenn et al., 1999).

Harpin proteins are known to activate natural plant defenses by promoting a vigorous growth (Alarcon et al., 1998; Tubajika et al., 2007). The protection efficacy is dependent on the treatment rate: A treatment rate of 460 g/ha has restricted the PD incidence to 6%, compared with 19% PD incidence of the control treatment. Studies showed that plants treated with harpin had a lower incidence of the symptoms of Pierce's disease and less likely to be infected with *Xf*.

**Colonization Disruption by Benign Strains:** The PD control effectiveness of several weakly virulent and avirulent strains of *Xf* was tested in greenhouse and vineyards in Florida (Hopkins, 2005). *Xf* strains isolated from sycamore and elderberry were the biological agents used to reduce the PD incidence. The study found that the latter strains contributed to a good control of PD when *V. vinefera* grape seedlings were inoculated in the field. More experimental work involving different grape cultivars is needed to test the feasibility of this management (Hopkins, 2005).

**Biological and Natural Control Agents:** Host-specific egg parasitoid, *Gonatocerus ashmeadi* (Girault) (Hymenoptera: Cicadellidae), was introduced to reduce the *H. vitripennis* population in Tahiti and Moorea of French Polynesia. A 90% reduction of *H. vitripennis* population was achieved with low infestation on non-target eggs after a release of 13,786 *Gonatocerus ashmeadi* individuals (Grandgirard et al., 2007; Grandgirard et al., 2008). Besides the high control efficiency, *G. ashmeadi* also demonstrated a rapid spread and even self-introduction to the neighboring islands where it was not implemented. *Gonatocerus fasciatus* (Girault) (Hymenoptera: Mymaridae) was also observed to arise from eggs of *Homalodisca vitripennis* and *Oncometopia orbona* (Triapitsyn et al., 2003). Coincidentally, a naturally occurring parasitoid on *P. irrorata* eggs was reared to arise from host eggs



and identified as *G. fasciatus* (Tipping et al., 2006). Adults and nymphs of *H. vitripennis* were also prey to spiders from families Salticidae, Agelenidae, Oxyopidae and Lycosidae (Lopez et al., 2003). Mizell et al. (2008) observed that insects of Odonata order seized adult *H. vitripennis* on the wing.

Although *Xf* infected peaches and plums have been confirmed in the southeastern U.S. since 1933 (Hutchins, 1933), the current occurrence of *Xf* induced diseases in Alabama-grown fruit crops and grapes is not documented. Previous research conducted by Boyhan et al. (1997), reported the incidence of *Xf* induced diseases in plum and peach groves only about one decade ago. No literature exists on the occurrence of PD in hybrid bunch grapes and muscadine grapes grown in the state. Recently, the spread of the newly emerged blueberry leaf scorch infection in blueberry plantings in Alabama is also unknown. The objective of our study was to investigate the occurrence of *Xf* induced diseases in selected fruit crops and vineyards under the threat of *Xf* infection grown in three distinct chilling zones in Alabama. This investigation can help us to better understand the spread of *Xf* in Alabama-grown fruit crops and its relationship with local climatic differences. The newly acquired knowledge will help us to adjust the IPM practices in fruit orchards and vineyards.

## 2.2 Materials and Methods

### *Sites and Fruit Species Selection*

Orchards and vineyards were selected from six locations in Alabama (Figure 1) to represent fruit species grown in three distinct chilling zones in Alabama (<http://www.aces.edu/pubs/docs/A/ANR-0053-D/>), where the Gulf Coast area represented the extreme southern and southern chilling zone: Mobile (30°26.4', 88°13.1' and Dothan (31°21.2', 85°19.4'), Central Alabama was represented by: Clanton (32°55.2', 88°40.3'), and Alexander City (32°51.2', 86°1.7'), and North Central and North Alabama were represented by: Athens (34°46.1', 86°54.0'), and Hayden (33°57.3', 86°40.9') (Table 1). In Mobile and Dothan, experimental orchards or vineyards were provided by multiple site owners.

The combinations of location and fruit tree species sampled are stated in Table 1. In 2008, PD resistant hybrid bunch grapes, muscadine grape (*V. rotundifolia*), peach (*P. persica*), plum (*P. salicina*), and Satsuma mandarin, (*Citrus unshiu*) were investigated and tissue samples were collected

on June 18, September 1 and October 5. In 2009, sampling occurred on June 7, August 2 and September 24, and the study was expanded to include rabbiteye blueberry species (*Vaccinium ashei*).

### ***Sampling Methods***

Since *Xf* induced infections could cause symptom expression or remain symptomless (Hopkins and Purcell, 2002), ten experimental trees or vines were randomly selected and marked at each experimental site. During the first sample collection early in the growing season in 2008, only PPD symptomatic peach trees were possible to distinguish due to the nature of symptom expression (Cavanagh and Rothe, 1953). Thus, five symptomatic and five asymptomatic peach trees were chosen and labeled for sampling in Mobile and Dothan locations at the beginning of the investigation. All other crops in the study were symptomless at this point of time and the experimental trees and vines were randomly selected. Each tissue sample represented a single experimental tree. The number of leaves comprising a sample depended on the type of crop. For peaches, plums and blueberries, a tissues sample consisted of ten leaves per tree. The muscadine and bunch grape, as well as the Satsuma mandarin samples consisted of six leaves with their petioles. Whole leaves, including petiole, were collected from multiple branches of the experimental trees for adequate representation (Boyhan et al., 1997) and (Hutchins et al., 1953). On muscadine and bunch grape vines, leaf tissues were collected from the basal nodes of the cane, where the chances to detect the bacteria were greater (Krell et al., 2006). As for other fruit species investigated, mature leaves located at mid stem were sampled for *Xf* detection. Tissue samples collected from trees and vines were loosely sealed in a plastic bag, transported from the field and stored in a cooler at 4°C prior to analysis.

In 2009, similar sampling procedures were used as in 2008. However, modifications in the peach sampling method were applied in Mobile and Clanton locations. Due to the high PPD pressure at the Mobile site, it was difficult to find five asymptomatic peach trees for tissue sampling. Thus, seven symptomatic and three symptomless trees were studied from Mobile location in 2009. In Clanton, at the beginning of 2009 growing season, three

symptomatic peach trees were noted that were not showing symptoms in the 2008 season and included them in the survey. Seven asymptomatic trees were also included for a total number of ten experimental trees in Clanton. In Dothan, bacterial spot disease caused severe early defoliation in the experimental peach orchard which made the screening for symptomatic and asymptomatic trees difficult. Therefore, samples were collected in a random manner in Dothan during the second year of the survey.

### ***Xf* Detection**

Enzyme-linked immunosorbent assay (ELISA) kits (Agdia, Inc., Elkhart, IN) was used for *Xf* detection from collected leaf tissues. The ELISA procedure for detection of *Xf* was performed according to the manufacturer's recommendations (Agdia, Inc.). Leaf samples were processed using a motorized leaf squeezing apparatus (Piedmont Machine and Tool, Six Mile, SC). All samples were ground in the extraction buffer (3 ml per sample) recommended by the manufacturer (Agdia, Inc.). The antibody coating and the antigen extract steps involved incubation in a moist chamber at 4°C for at least 12 hr. The conjugated antibody step involved incubation at 37°C for 2 hrs. Substrate solution (TMB peroxidase) was added and reactions were recorded using a Sunrise microtiter plate reader (Phoenix Research Product, Hayward, CA) at 620 nm, which is close enough to the wave length recommended by Agdia to produce the reliable results (Randall et al., 2009). A sample was considered positive for the presence of *Xf* if the ELISA absorbance value was greater than twice the average absorbance reading of three negative control samples added to each microtiter plate (Randall et al., 2009). Negative control samples consisted of tissue extracts of *Xf*-free peach, bunch grape and citrus purchased from Agdia, Inc.

## **2.3 Results**

### **Hybrid Bunch Grapes:**

Tissue samples of bunch grape were collected from Clanton (Central AL) and Athens (North AL) locations only in both years of the study. In general, PD pressure for the region south of Birmingham is very high and newly planted bunch grapes usually die within the first or second season in the field.

In the first year, tissue samples were collected three times during the growing season. Bunch grape samples collected in Clanton on June 18 tested negative for *Xf* infection. Thirty percent of the samples on September 1<sup>st</sup>, tested positive for *Xf*, while late in the season, on October 5<sup>th</sup>, only ten percent of the samples tested positive for *Xf*. The percentage of *Xf* infected bunch grape samples collected from Athens, showed gradual increase throughout the growing season, from 10% in June to 50% in October. In 2009, no *Xf* infection was found in bunch grapes from Clanton early in the season (Table 2). Highest infection rate was found on August 2, when 20% of bunch grape samples tested *Xf* positive. During the last sampling cycle in September, two of the grapevines that previously tested positive for *Xf* in the August sampling cycle and had expressed severe Pierce's disease symptoms (Figure 2) were dead, therefore, no samples were collected. As a result, only eight samples were tested, one of which was infected with *Xf*. In Athens, no *Xf* infections were evident on bunch grapevines during the entire 2009 growing season.

During the period of our study, leaf symptoms (Figure 2A) of Pierce's disease infection on bunch grapevines usually appeared during July and August, or late in the growing season. Most of the grapevines expressing PD symptoms were found on the margins and corners of the vineyards. The two dead vines at the Clanton location in 2009 were located on the outward row adjacent to a grass field.

### **Muscadine Grape**

In 2008, tissue samples of muscadine grape were collected from Mobile, Clanton and Alexander City locations on June 18, September 1, and October 5 respectively (Table 2). No positive *Xf* reactions were registered for muscadine grapes collected from Mobile and Clanton throughout the growing season. The only positive infection on muscadine grape in 2008 occurred on a sample collected from Alexander City, on September 1. Two PD infections were detected in 2009 on muscadine grapes. One positive sample was found in Mobile on August 2 and another on September 24 in Alexander City. The infection rate during two seasons varied over time. The absorbance reading of positive reactions showed five times higher values than the positive-negative threshold, which implicates that the possibility of false positive reaction was low.

## **Plum**

A total number of four plum orchards located in Central and North Alabama were selected for our study (Table 2). In 2008, two plum trees grown in Mobile site of the Gulf Coast were surveyed; both trees in Mobile expressed severe PLS symptoms and *Xf* infection was confirmed throughout the entire growing season. By the end of 2008 these two plum trees were dead, and the Mobile site was excluded from the 2009 survey. Sixty percent of the samples tested from Alexander City were positive for *Xf* infection (Table 2), even though no disease symptoms were obvious during the first sampling cycle. The disease occurrence in both Central Alabama locations was relatively high and increased through the growing season from 80% in September to 100% in the October sampling cycle. Levels of PLS disease occurrence were relatively high in Central Alabama in 2009, with a slight decrease of positive infections observed among plum trees sampled from the Clanton location late in the growing season. No PLS infection was found in both years of our survey in plum trees grown in Athens, one of the North Alabama locations studied (Table 2). In Hayden, the highest *Xf* infection was registered on September 1, 2008, whereas no positive reactions were evident in 2009. In our experiment, PLS leaf marginal necrosis symptoms as described by Latham et al. (1980) started to appear later in the growing season, and were well-expressed on the leaves of infected plum trees during August and September (Figure 3).

## **Satsuma mandarin**

Satsuma mandarin orchards from four locations: Mobile, Dothan, Clanton, and Alexander City were included in this survey to represent Central Alabama and Gulf Coast areas (Table 2). In 2008, no positive *Xf* infections were found on Satsuma trees from the surveyed locations. In 2009, positive *Xf* reactions were observed from all of the four locations tested. In the Mobile location of the Gulf Coast region, 50% of Satsuma mandarin samples tested on August 2<sup>nd</sup> were positive for *Xf*. No positive samples were found late in the season, on September 24<sup>th</sup>. In Dothan location, one positive infection was found on June 7, and no positive reactions were seen later in the season. In Central Alabama, one positive infection was found in Clanton on August 2<sup>nd</sup> and one in Alex City on September 24<sup>th</sup>. Leaf

symptoms resembling CVC symptoms described in the literature were noticed on Satsuma mandarin trees late in the growing season (Figure 4).

### **Blueberry**

Samples from five rabbiteye blueberry (*V. ashei*) orchards representing all three major areas of the state were collected only in 2009 (Table 2) after the initial *Xf* positive blueberry sample was detected in Dothan, Alabama (Coneva et al., 2009, Mullen et al., 2009). One of the ten samples tested from Mobile location on August 2<sup>nd</sup> showed positive *Xf* reaction. Samples from all other locations showed negative *Xf* reaction during the entire growing season. We did not observe the yellow twigs symptoms as described on southern highbush blueberries (Chang et al., 2009), in the blueberry sites surveyed, but some leaf marginal necrosis was seen in rabbiteye blueberry fields.

### **Peach**

In 2008, no *Xf* infection was found from the tissue samples collected from both Central Alabama locations tested (Table 3), as well as from Athens, one of the two North Alabama sites surveyed. An increase in *Xf* infection was observed late in the season among samples tested from Hayden, the second North Alabama location. However, the occurrence of *Xf* infections was very high in symptomatic peach samples from the Mobile and Dothan sites (Table 4). The percentage of positive *Xf* infections was also very high among asymptomatic peach samples collected from the Gulf Coast area and ranged between 60 and 80%.

During the 2009 season, our results revealed the highest percentage *Xf* infected peach trees were found in the Gulf Coast area. Random sampling in the Dothan site resulted in 80 to 100% *Xf* positive results throughout the growing season (Table 3), while no positive samples were found in both North Alabama locations studied. The highest percentage of *Xf* positive reactions for symptomatic peach samples was recorded on August 2<sup>nd</sup> for samples collected from Mobile and Clanton locations (Table 4). One out of three asymptomatic samples from Mobile tested *Xf* positive on August 2<sup>nd</sup> tissue collection, while no positive *Xf* reactions were evident for the rest of the asymptomatic samples tested.

In 2008, we observed that most of the peach trees in our experimental plot in the Mobile location were expressing severe phony peach disease symptoms (Figure 5). At the Clanton location, we did not find symptomatic trees in 2008, but in 2009 three stunted peach trees were observed and included in

the study. Phony peach diseased trees had shortened nodes, stunted growth and flat, dark green canopy, similar to that described by Cavanagh and Rothe (1953).

## 2.4 Discussion

Our study confirmed the occurrence of *Xf* in all six fruit crops grown throughout the three chilling zones in the state of Alabama. This fact is in agreement with a study by Sutton (2005) ranking Alabama as a high PD risk zone. Although positive PD infections were found in hybrid bunch grapes reported to have resistance to this pathogen, no chronic infections were detected in muscadine grapevines tested during the course of our investigation. Our results suggest that it is possible to find occasional infection on shoots of some muscadine varieties. This observation supports the conclusion made by Hopkins and Purcell (2002), that muscadine grapes are usually highly resistant, but not immune to the Pierce's disease. Phony peach disease in peaches and plum leaf scald infection in plums were confirmed for samples collected at the Gulf Coast, Central Alabama, and as far North as Hayden in North Alabama. The highest bacterial pressure was found in the Gulf Coast area of the state which is in agreement with findings of Boyhan et al., (1997). Our study also revealed *Xf* infected peach trees may remain symptomless for certain period of time as stated by Wells et al. (1980) thus, visual symptoms of infection are not a reliable PPD detection method. Although positive *Xf* reactions were found in our study among Satsuma mandarin groves in Alabama, no official reports document the presence of CVC in the U.S. to date. More sensitive detection methods such as PCR techniques need to be used to confirm the presence of the bacterium in the Satsuma mandarin crop, to identify the strain, and to determine its pathogenicity by inoculating extracted pathogen to healthy plants. Very little scientific information is currently available on different aspects of blueberry leaf scorch disease caused by *Xf*. Although the type of symptoms caused by *Xf* on southern highbush varieties have been documented (Brannen et al, 2007), no reliable information exists to describe BLS symptoms on rabbiteye blueberry. Further research is also needed to evaluate the incidence of BLS in rabbiteye blueberry, since no data exist on cultivar resistance to *Xf*.

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## 2.6 Tables and Figures

Table 1. *Xylella fastidiosa* sampling sites in Alabama, 2008-2009: locations, elevation and edge habitats description.

| Region             | Location | County  | Latitude | Longitude | Elevation<br>(m) | Crop  | North edge  | South edge   | West edge  | East edge  |   |  |  |   |  |   |                                 |                                  |                                     |                                       |
|--------------------|----------|---------|----------|-----------|------------------|---|---|--|--|--|---|--|--|---|--|---|---------------------------------|----------------------------------|-------------------------------------|---------------------------------------|
| Gulf Coast         | Mobile   | Mobile  | 30°26.4' | 88°13.1'  | 23               | peach<br>Satsuma mandarin   | grass<br>woods                                    | road<br>woods  | grass<br>woods   | road<br>persimen                                       |   |  |  |   |  |   |                                 |                                  |                                     |                                       |
|                    |          | Baldwin | 30°32.6' | 87°44.5'  | 41               | Plum<br>muscadine grape<br>Blueberry                                    | grass<br>woods<br>house                           | grass<br>road<br>woods                                 | apple<br>road<br>grass   | road<br>grass<br>apple                                 |   |  |  |   |  |   |                                 |                                  |                                     |                                       |
|                    | Dothan   | Henry   | 31°21.2' | 85°19.4'  | 118              | Satsuma<br>Blueberry  | pin<br>grass                                      | grass<br>pin   | pin<br>grass   | grass<br>grass   |   |  |  |   |  |   |                                 |                                  |                                     |                                       |
|                    |          | Coffee  | 31°20.5' | 85°52.2'  | 122              | peach   | road  | grass  | grass  | road   |   |  |  |   |  |   |                                 |                                  |                                     |                                       |
| Central<br>Alabama | Clanton  | Chilton | 32°55.2' | 88°40.3'  | 207              | peach<br>plum<br>Satsuma<br>muscadine grape<br>bunch grape<br>blueberry | road<br>road<br>tunnel<br>grass<br>grass<br>woods | grass<br>grass<br>kiwifruit<br>house<br>house<br>grass | grass<br>walnut<br>blackberry<br>kiwifruit<br>road<br>blackberry | grass<br>tunnel<br>grass<br>road<br>kiwifruits<br>pond |   |  |  |   |  |   |                                 |                                  |                                     |                                       |
|                    |          |         |          |           |                  | Alex City   | Tallapoosa  | 32°51.2'   | 86°1.7'  | 211  | peach<br>plum<br>Satsuma mandarin<br>muscadine grape<br>blueberry | woods<br>woods<br>road<br>woods<br>woods | road<br>road<br>tunnel<br>road<br>nucery | plums<br>woods<br>woods<br>peaches<br>woods | muscadine grape<br>peaches<br>road<br>woods<br>woods |   |                                 |                                  |                                     |                                       |
|                    |          |         |          |           |                  |   |   |  |  |  | Athens  | Limestone                                | 34°6.1'                                  | 86°54.0'                                    | 207  | peach<br>plum<br>muscadine grape<br>blueberry | road<br>grass<br>house<br>grass | woods<br>peach<br>woods<br>woods | apple<br>pond<br>blueberry<br>grass | grass<br>peach<br>road<br>bunch grape |
|                    |          |         |          |           |                  |   |   |  |  |  |   |  |  |   |  | Hayden  | Blount                          | 33°57.3'                         | 86°40.9'                            | 213                                   |

Table 2. Occurrence of *Xylella fastidiosa* infected five fruit crops grown at six Alabama locations tested three times during the growing season in 2008 and 2009.

| Fruit crop         | Region     | Location   | Total No. of samples tested | No. of positive samples | Percent positive (%) | 2008     |              |           | Total No. of samples tested | No. of positive samples | Percent positive (%) |    |
|--------------------|------------|------------|-----------------------------|-------------------------|----------------------|----------|--------------|-----------|-----------------------------|-------------------------|----------------------|----|
|                    |            |            |                             |                         |                      | June 18  | September 1  | October 5 |                             |                         |                      |    |
| Hybrid bunch grape | N. Alabama | Athens     | 10                          | 1                       | 10                   | 10       | 2            | 20        | 10                          | 5                       | 50                   |    |
|                    |            | C. Alabama | Clanton                     | 10                      | 0                    | 0        | 10           | 3         | 30                          | 10                      | 1                    | 10 |
|                    | C. Alabama |            |                             |                         | 2009                 |          |              |           |                             |                         |                      |    |
|                    |            |            |                             |                         | June 7               | August 2 | September 24 |           |                             |                         |                      |    |
|                    |            | N. Alabama | Athens                      | 10                      | 0                    | 0        | 10           | 0         | 0                           | 10                      | 0                    | 0  |
|                    |            | C. Alabama | Clanton                     | 10                      | 0                    | 0        | 10           | 2         | 20                          | 8                       | 1                    | 13 |
| Muscadine grape    | C. Alabama | Clanton    | 10                          | 0                       | 0                    | 10       | 0            | 0         | 10                          | 0                       | 0                    |    |
|                    |            | Alex City  | 10                          | 0                       | 0                    | 8        | 1            | 13        | 10                          | 0                       | 0                    |    |
|                    |            | Gulf Coast | Mobile                      | 10                      | 0                    | 0        | 10           | 0         | 0                           | 10                      | 0                    | 0  |
|                    | C. Alabama |            |                             |                         | 2009                 |          |              |           |                             |                         |                      |    |
|                    |            |            |                             |                         | June 7               | August 2 | September 24 |           |                             |                         |                      |    |
|                    |            | Clanton    | Alex City                   | 10                      | 0                    | 0        | 10           | 0         | 0                           | 10                      | 0                    | 0  |
| Gulf Coast         | Mobile     | 10         | 0                           | 0                       | 10                   | 1        | 10           | 10        | 1                           | 10                      |                      |    |
| Plum               | N. Alabama | Athens     | 10                          | 0                       | 0                    | 10       | 0            | 0         | 10                          | 0                       | 0                    |    |
|                    |            | Hayden     | 10                          | 0                       | 0                    | 10       | 2            | 20        | 10                          | 1                       | 10                   |    |
|                    | C. Alabama | Clanton    | .                           | .                       | .                    | 10       | 8            | 80        | 9                           | 9                       | 100                  |    |
|                    |            | Alex City  | 10                          | 6                       | 60                   | 10       | 8            | 80        | 10                          | 10                      | 100                  |    |
|                    | Gulf Coast | Mobile     | 2                           | 2                       | 100                  | 2        | 2            | 100       | 2                           | 2                       | 100                  |    |
|                    | C. Alabama |            |                             |                         | 2009                 |          |              |           |                             |                         |                      |    |
|                    |            |            | June 7                      | August 2                | September 24         |          |              |           |                             |                         |                      |    |
| N. Alabama         |            | Athens     | 10                          | 0                       | 0                    | 10       | 0            | 0         | 10                          | 0                       | 0                    |    |
| Hayden             |            | 10         | 0                           | 0                       | 10                   | 0        | 0            | 10        | 0                           | 0                       |                      |    |
| C. Alabama         | Clanton    | 10         | 5                           | 50                      | 10                   | 8        | 80           | 10        | 6                           | 60                      |                      |    |
|                    | Alex City  | 10         | 8                           | 80                      | 10                   | 9        | 90           | 10        | 9                           | 90                      |                      |    |

Table 2. (Continued)

| Fruit crop       | Region     | Location  | Total No. of samples tested | No. of positive samples | Percent positive (%) | Total No. of samples tested | No. of positive samples | Percent positive (%) | Total No. of samples tested | No. of positive samples | Percent positive (%) |
|------------------|------------|-----------|-----------------------------|-------------------------|----------------------|-----------------------------|-------------------------|----------------------|-----------------------------|-------------------------|----------------------|
|                  |            |           |                             |                         |                      |                             |                         |                      |                             |                         |                      |
| Satsuma mandarin |            |           | 2008                        |                         |                      |                             |                         |                      |                             |                         |                      |
|                  |            |           | June 18                     |                         |                      | September 1                 |                         |                      | October 5                   |                         |                      |
|                  | C. Alabama | Clanton   | .                           | .                       | .                    | 10                          | 0                       | 0                    | 10                          | 0                       | 0                    |
|                  |            | Alex City | 10                          | 0                       | 0                    | 10                          | 0                       | 0                    | 10                          | 0                       | 0                    |
|                  | Gulf Coast | Mobile    | 10                          | 0                       | 0                    | 10                          | 0                       | 0                    | 10                          | 0                       | 0                    |
|                  |            | Dothan    | .                           | .                       | .                    | 10                          | 0                       | 0                    | 10                          | 0                       | 0                    |
|                  |            |           | 2009                        |                         |                      |                             |                         |                      |                             |                         |                      |
|                  |            |           | June 7                      |                         |                      | August 2                    |                         |                      | September 24                |                         |                      |
|                  | C. Alabama | Clanton   | 10                          | 0                       | 0                    | 10                          | 1                       | 10                   | 10                          | 0                       | 0                    |
|                  |            | Alex City | 10                          | 0                       | 0                    | 10                          | 0                       | 0                    | 10                          | 1                       | 10                   |
|                  | Gulf Coast | Mobile    | 10                          | 0                       | 0                    | 10                          | 5                       | 50                   | 10                          | 0                       | 0                    |
|                  |            | Dothan    | 10                          | 1                       | 10                   | 10                          | 0                       | 0                    | 10                          | 0                       | 0                    |
| Blueberry        |            |           | 2009                        |                         |                      |                             |                         |                      |                             |                         |                      |
|                  |            |           | June 7                      |                         |                      | August 2                    |                         |                      | September 24                |                         |                      |
|                  | N. Alabama | Athens    | 10                          | 0                       | 0                    | 10                          | 0                       | 0                    | 10                          | 0                       | 0                    |
|                  | C. Alabama | Clanton   | 10                          | 0                       | 0                    | 10                          | 0                       | 0                    | 10                          | 0                       | 0                    |
|                  |            | Alex City | .                           | .                       | .                    | 10                          | 0                       | 0                    | 10                          | 0                       | 0                    |
|                  | Gulf Coast | Mobile    | 10                          | 0                       | 0                    | 10                          | 1                       | 10                   | 10                          | 0                       | 0                    |
|                  |            | Dothan    | .                           | .                       | .                    | 10                          | 0                       | 0                    | 10                          | 0                       | 0                    |

Table 3. Occurrence of *Xylella fastidiosa* infected peach trees grown at four Alabama locations tested three times during the growing season in 2008 and 2009.

| Region     | Location  | Total No. of samples tested | No. of positive samples | Percent positive (%) | Total No. of samples tested | No. of positive samples | Percent positive (%) | Total No. of samples tested | No. of positive samples | Percent positive (%) |  |
|------------|-----------|-----------------------------|-------------------------|----------------------|-----------------------------|-------------------------|----------------------|-----------------------------|-------------------------|----------------------|--|
|            |           |                             |                         |                      |                             |                         |                      |                             |                         |                      |  |
| 2008       |           |                             |                         |                      |                             |                         |                      |                             |                         |                      |  |
|            |           |                             | June 18                 |                      |                             | September 1             |                      |                             | October 5               |                      |  |
| N. Alabama | Athens    | 10                          | 0                       | 0                    | 10                          | 0                       | 0                    | 10                          | 0                       | 0                    |  |
|            | Hayden    | 10                          | 0                       | 0                    | 10                          | 0                       | 0                    | 10                          | 1                       | 10                   |  |
| C. Alabama | Clanton   | .                           | .                       | .                    | 10                          | 0                       | 0                    | 10                          | 0                       | 0                    |  |
|            | Alex City | 10                          | 0                       | 0                    | 10                          | 0                       | 0                    | 10                          | 0                       | 0                    |  |
| 2009       |           |                             |                         |                      |                             |                         |                      |                             |                         |                      |  |
|            |           |                             | June 7                  |                      |                             | August 2                |                      |                             | September 24            |                      |  |
| N. Alabama | Athens    | 10                          | 0                       | 0                    | 10                          | 0                       | 0                    | 10                          | 0                       | 0                    |  |
|            | Hayden    | 10                          | 0                       | 0                    | 10                          | 0                       | 0                    | 10                          | 0                       | 0                    |  |
| C. Alabama | Alex City | 10                          | 0                       | 0                    | 10                          | 0                       | 0                    | 10                          | 1                       | 10                   |  |
| Gulf Coast | Dothan    | 10                          | 9                       | 90                   | 10                          | 10                      | 100                  | 10                          | 8                       | 80                   |  |

Table 4. Occurrence of *Xylella fastidiosa* infected peach trees grown at three Alabama locations tested three times during the growing season in 2008 and 2009.

| Region          | Location | June 18                     |                             |                      | September 1                 |                             |                      | September 24                |                             |                      | October 5                   |                             |                      |
|-----------------|----------|-----------------------------|-----------------------------|----------------------|-----------------------------|-----------------------------|----------------------|-----------------------------|-----------------------------|----------------------|-----------------------------|-----------------------------|----------------------|
|                 |          | Total No. of samples tested | No. of positive samples (%) | Percent positive (%) | Total No. of samples tested | No. of positive samples (%) | Percent positive (%) | Total No. of samples tested | No. of positive samples (%) | Percent positive (%) | Total No. of samples tested | No. of positive samples (%) | Percent positive (%) |
| 2008            |          |                             |                             |                      |                             |                             |                      |                             |                             |                      |                             |                             |                      |
|                 |          | Symptomatic                 |                             |                      | Asymptomatic                |                             |                      | Symptomatic                 |                             |                      | Asymptomatic                |                             |                      |
| Gulf Coast      | Mobile   | 5                           | 5                           | 100                  | 5                           | 4                           | 80                   | 5                           | 5                           | 100                  | 5                           | 4                           | 80                   |
|                 | Dothan   | .                           | .                           | .                    | 5                           | 5                           | 100                  | 5                           | 3                           | 60                   | 5                           | 5                           | 100                  |
| 2009            |          |                             |                             |                      |                             |                             |                      |                             |                             |                      |                             |                             |                      |
|                 |          | Symptomatic                 |                             |                      | Asymptomatic                |                             |                      | Symptomatic                 |                             |                      | Asymptomatic                |                             |                      |
|                 |          | Symptomatic                 |                             |                      | Asymptomatic                |                             |                      | Symptomatic                 |                             |                      | Asymptomatic                |                             |                      |
| Central Alabama | Clanton  | 3                           | 1                           | 33                   | 7                           | 0                           | 0                    | 3                           | 3                           | 100                  | 7                           | 0                           | 0                    |
| Gulf Coast      | Mobile   | 7                           | 6                           | 86                   | 3                           | 0                           | 0                    | 7                           | 7                           | 100                  | 3                           | 1                           | 33                   |
|                 |          | Symptomatic                 |                             |                      | Asymptomatic                |                             |                      | Symptomatic                 |                             |                      | Asymptomatic                |                             |                      |
|                 |          | Symptomatic                 |                             |                      | Asymptomatic                |                             |                      | Symptomatic                 |                             |                      | Asymptomatic                |                             |                      |
|                 |          | Symptomatic                 |                             |                      | Asymptomatic                |                             |                      | Symptomatic                 |                             |                      | Asymptomatic                |                             |                      |

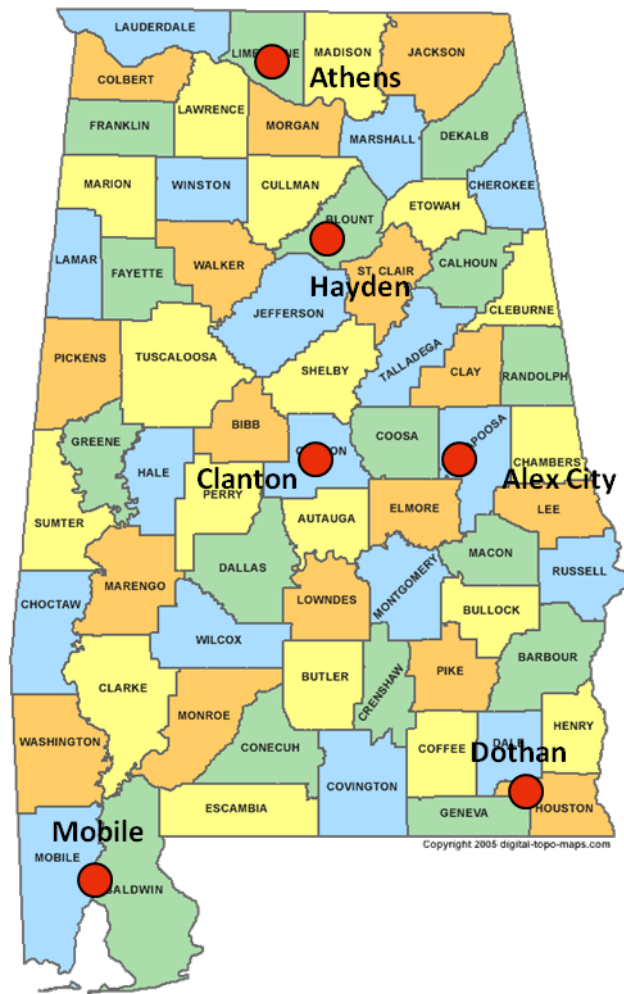


Figure 1. Sampling locations for *Xylella fastidiosa* in selected fruit crops in Alabama, 2008 and 2009.

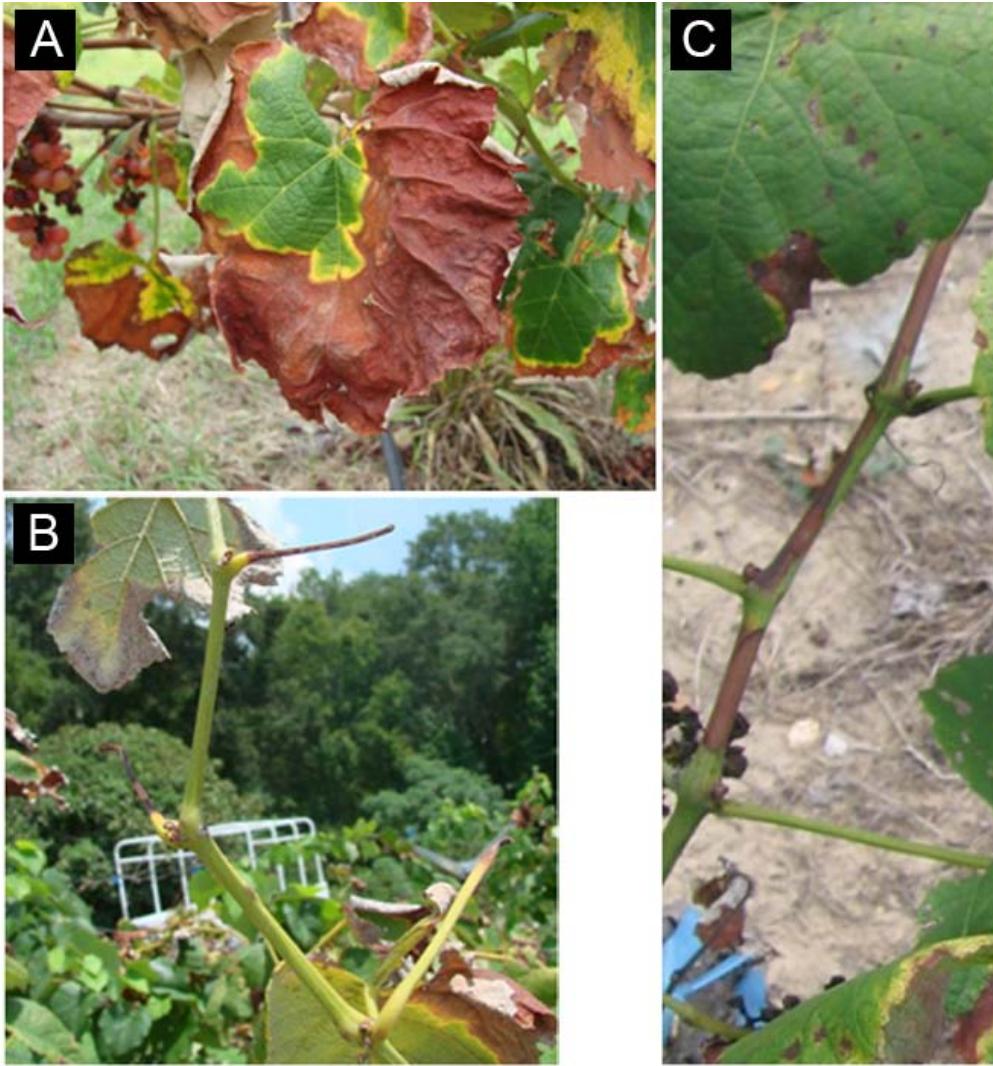


Figure 2. Symptoms of Pierce's disease on bunch grape found in Chilton Horticultural Station: (A) Marginal leaf necrosis with chlorotic border (Courtesy of Elina Coneva); (B) Match stick petiole; and (C) green island symptoms on grape shoots. Image: Xing Ma



Figure 3. Marginal leaf necrosis symptoms caused by Plum leaf scald disease observed in the Chilton Horticultural Research and Extension Center, Clanton. Image: Xing Ma.



Figure 4. Interveinal chlorosis symptom on Satsuma mandarin leaf tested positive for *Xylella fastidiosa* infection. Image courtesy of Elina Coneva.



Figure 5. Peach tree showing flat top and dark green canopy – typical phony peach disease symptoms (left) compared with a healthy peach tree (right). Image: Xing Ma.



## CHAPTER THREE

### Occurrence of *Xylella Fastidiosa* Sharpshooter Vectors in Alabama and Their Seasonal Phenology

#### 3.1 Introduction

Recently the xylem-limited bacterium, *Xylella fastidiosa* (*Xf*), emerged as one of the most significant new disease threats in the Americas (Hopkins and Prucell, 2002). This pathogen has a relatively wide host range (Freitag, 1951), and induces a variety of plant diseases in numerous fruit and nut crops, such as bunch grape (Davis et al., 1978; Wells et al., 1983), peach, plum (Boyhan et al., 1997), citrus (Chang et al., 1993), avocado (Montero-Astúa et al., 2008), pecan (Sanderlin and Heyderich-Alger, 2000) and almond (Mircetich et al., 1976). Most recently, a new disorder was observed to affect southern highbush blueberry cultivars in the southeastern U.S. and subsequently proved to be caused by *Xf* and named bacterial leaf scorch of blueberries (Chang et al., 2009). Other crops with considerable economical importance such as coffee (de Lima et al., 1998) and alfalfa (Thomson et al., 1978) were also confirmed to be hosts of *Xf*. This pathogen is also known to cause numerous diseases in ornamental plants, such as oleander leaf scorch (Purcell et al., 1999), and bacterial leaf scorch in *Ulmus* spp. (Sherald, 1993), *Quercus* spp. (Chang and Walker, 1988), *Acer* spp. (Sherald et al., 1987) and in *Platanus* spp. (Sherald et al., 1983).

In Alabama, fruit crops of economic importance threatened by the spread of *Xf* include peach (*Prunus persica*), plum (*Prunus domestica*), blueberry (*Vaccinium ashei*), hybrid bunch grapes (*Vitis* spp.) and Satsuma mandarin (*Citrus unshiu*). According to the National Agricultural Statistics Service, United States Department of Agriculture (<http://quickstats.nass.usda.gov/>), peach production has been the largest fruit tree industry in Alabama for the last decade. Peach acreage reached 1,013 ha in 2007 in Alabama, of which 913 ha were bearing peach orchards. Blueberry production ranks second among fruit crops in terms of acreage, with a total of 249 ha. Grape production, including

bunch and muscadine grape, ranks third with 189 hectares. When compared to grape production one decade ago, there is a 41% increase representing considerable growth. The category of plum and prune ranks next to the grape, and shows a slight increase for the last decade. One possible reason are the losses associated with plum leaf scald and the associated short longevity of plum orchards in Alabama, where the PLS disease pressure is high. The citrus industry currently accounts for 44 ha. The production of Satsuma mandarin in Mobile and Baldwin County of Alabama has been growing since the early 1990's (Fadamiro et al., 2008). Recent introduction of cold hardy rootstocks (Campbell, et al., 2004) has contributed considerably to the expansion of the Satsuma mandarin production.

Mechanical transmission, root grafting and insect vectors are the known transmission pathways of *Xf* (Krell et al., 2007; Wells et al., 1981; Houston et al., 1947). Since *Xf* is strictly confined in xylem vessels of the plant, xylem-feeding insects from the sharpshooters subfamily (Cicadellidae: Cicadellinae) (Houston et al., 1947; Severin, 1949) and the spittlebugs family (Cercopidae) (Severin, 1950) are considered the major vectors transmitting *Xf* induced diseases.

The Cicadellinae subfamily, belongs to order Hemiptera, family Cecadellidae, commonly known as sharpshooters (Riley and Howard, 1893). Insects in this subfamily usually have an inflated clypeus which encloses the musculature connected to the diaphragm, which is the organ that facilitates suction of xylem sap, and their xylophagous mouthpart is closely associated with the transmission of *Xf* (Frazier, 1943; Turner and Pollard, 1955). Twenty-eight species from the sharpshooter subfamily have been confirmed to transmit *Xf* to induce Pierce's disease or Phony peach disease (Nielson, 1968; Nielson, 1979). Among those *Xf* vectors, *Xyphon fulgida*, *Draeculacephala minerva*, *Homalodisca vitripennis*, *Graphocephla atropunctata*, and *Oncometopia* spp. were found to be abundant in field with *Xf* infection or in adjacent field in North America (Nielson, 1968; Purcell and Frazier, 1985; Turner and Pollard, 1959). Myers et al. (2007) determined that the most abundant sharpshooter vectors in North Carolina were *Oncometopia orbona*, *G. versuta*, *Paraphlepsius irroratus*, and *Agalliota constricta*. Specimens of those sharpshooters were confirmed to be associated with the transmission of *Xf*. The identity of sharpshooter vectors in Alabama has not yet been reported and science based information on this economically important vector is lacking.

The transmission efficiency of *Xf* is dependent on the combination of host, vectors and pathogen strains (Redak et al., 2004). The combination varies and only a small proportion of the combinations were studied. The *H. vitripennis* mediated 32% PD transmission rate from grape to grape as observed by Almeida and Purcell (2003), while *H. vitripennis* mediated PPD transmission rate from peach to peach can reach only 15% (Turner and Pollard, 1959). Existing data of *Xf* transmission by *G. versuata* between peach trees indicated 29% transmission rate (Turner and Pollard, 1959). *H. insolita* has the ability to transmit peach strains between peach trees with 48% efficiency rate, while in the same study, 33 out of 100 peach strain inoculations were successfully attained by *Oncometopia nigricans*. The transmission tests have been conducted on more combinations of *D. minerva*. Its transmission efficiency of almond leaf scorch strains between grapes is up to 92% (Hill and Purcell, 1995). In contrast to ALS strains, PD strains were transmitted with a much lower efficiency of only 3% from almond to grape, and 0-1% efficiency rate from alfalfa to grape (Hill and Purcell, 1995; Severin, 1949).

The action of bacterial transmission by insects consists of three steps: acquisition, retention and inoculation (Chatterjee et al., 2008). The acquisition efficiency of *Xf* from a plant is associated with bacterial density. The acquisition is only triggered when the bacteria within the vessel is above the concentration of  $10^4$  viable cells, whereas the established threshold on grapevines is up to  $10^{6-7}$  cells (Hill and Purcell, 1997). In contrast, the transmission from insects to plants requires less than 200 viable cells within the vector's foregut (Almeida and Purcell, 2003). The lack of latent period from acquisition to transmission and the discontinuity of infectivity after molting (Purcell and Finlay, 1979) indicate the bacteria has noncirculative movement within the xylem feeder.

The importance of the *Xf* vector was considered to be related to its presence in, or near *Xf* infected orchards or vineyards (Almeida et al., 2005). Thus, *H. vitripennis* was thought to be the most economically important vector within the sharpshooter subfamily due to its abundance in vineyards (Redak et al., 2004). *Graphocephala atropunctata* (Signoret) was abundant in riparian vineyards and irrigated landscapes in California (Hopkins and Purcell, 2002). The distribution of *D. minerva* and *X. fulgida* were associated with the *Xf* transmission in vineyards adjacent to irrigated pastures and alfalfa fields in the Central Valley, California (Hewitt et al., 1942). Compared with other species, *H.*

*vitripennis* is of great research interest and its behavior is well documented.

Oviposition behavior was studied by Tipping et al. (2006). *Homalodisca* spp. and *Oncometopia* spp. deposit their eggs on the lower side of the leaves, with whitish brochosomes covering the egg masses, while *P. irrorata* deposits eggs on woody twigs, stem and lignified petioles without covering brochosomes.

### ***H. Vitripennis (Glassy-winged sharpshooter)***

*H. vitripennis* (Glassy-winged sharpshooter, GWSS) (Hemiptera: Cecadellidae: Cicadellinae, *Homalodisca vitripennis*), which used to be limited to the southeastern U.S. (Turner and Pollard, 1959) invaded California in the 1990's (Redak et al. 2004). The introduction of *H. vitripennis* triggered an outbreak of PD in Temecula Valley, California (Blua et al. 1999; Hopkins and Purcell, 2002). In comparison with the native vectors in California, which usually have a limited host range or a preferred habitat, *H. vitripennis* are capable of feeding on more than 100 plant species (Turner and Pollard, 1959; Mizell and French, 1987; Hoddle et al., 2003). A study in Florida indicated that *H. vitripennis* were captured from 72 plant species belonging to 37 families (Hoddle et al., 2003). The strong flying capability of *H. vitripennis* adults enables them to search a large area for the suitable feeding location, thus, posing more threat of *Xf* infection than other sharpshooter vectors (Blua et al., 2001; Purcell et al., 1979; Blackmer et al., 2003).

*H. vitripennis* adults and fourth and fifth instars require the amides affluent xylem fluid, and adult females are generally inclined to oviposit on those plants. However, nymphs in early stages prefer fluid with a balanced amino-acid profile and may die if they stay on plants with high amide levels (Brodbeck et al., 1995). Greenhouse studies concur that *H. vitripennis* adults and nymphs rarely remain on the same tree for feeding (Brodbeck et al., 2007). For instance, crape myrtle (*Lagerstroemia indica*) is a common native plant host for *H. vitripennis* in the U.S., but eggs and early stage nymphs are rarely found on it. In contrast, some host species, such as *Euonymus japonica*, are more favored by ovipositioning adults and young nymphs (Brodbeck et al., 1995; 2004).

Feeding on a wide selection of plant xylem fluids can meet the requirement of *H. vitripennis* adults and nymphs. Generally, xylem fluid consists of more than 98% water (Raven, 1983) and remainder consisting of components such as 19 different amino acids, five to seven organic acids and

three major sugars (Andersen et al. 1989; Andersen et al., 1992). The ratios of those monomeric components vary among different plant species and different parts of the plant (Andersen et al. 1992; Andersen et al., 1995). Furthermore, the ratios are also dependent on environmental variation (Andersen and Brodbeck, 1988; Andersen and Brodbeck, 1991; Andersen et al., 1989; Andersen et al., 1992; Andersen et al., 1995). Nadel et al. (2008) found that under controlled conditions, the abundance, feeding and oviposition of *H. vitripennis* are greater on well-irrigated citrus and avocado trees, than on water-stressed controls.

High feeding rates are essential for xylem-feeders to compensate for high negative fluid tension and low nutritional density in xylem fluid (Andersen et al., 1992; Mizell et al., 2008). The energy obtained from xylem fluid is slightly higher than the energy used for sap extraction. However, the net energy surplus obtained from feeding on a peach crop by *H. vitripennis* is even slightly negative by theoretical calculation (Andersen et al., 1989). The hourly feeding rate of sharpshooters ranges from 0.21-0.70 ml, dependent on different host plants and peaks on midday (Andersen et al., 1989; 1992; 2003; 2005; Brodbeck et al., 1995; 1996; 1999; 2007; Mizell et al., 2008).

High nutritional assimilation efficiency is another key characteristic in sharpshooter's survival strategy. They absorb more than 99% of the key amino acids, organic acids and sugars when xylem fluids travel through their complex long midgut (Andersen et al., 1989; Ammar, 1985).

Besides feeding on the young tissues like other sharpshooters, *H. vitripennis* has the tendency to feed on the basal parts, even woody parts of the grapevine shoot (Hopkins and Purcell, 2002). Overwintering as an adult, *H. vitripennis* has the ability to feed on dormant vines in winter. Both parts of the vine they feed on: the dormant cane during the winter and the basal part of a shoot in a growing season are less likely to be removed from orchard during winter pruning (Almeida et al., 2005). The feeding behavior of *H. vitripennis* increases the possibility of chronic PD infections in vineyards.

### ***Temperature Restriction to Xf Distribution***

Diseases caused by *Xf* are generally limited to the tropics and the subtropical belt. Freezing temperatures during the dormant season are observed to cure the plant from Pierce's disease (Purcell, 1980). In vitro experiments revealed that *Xf* grows fast at 28°C, but doesn't grow at 12°C. *Xf* inoculated grape seedling treated under 5°C for 2 weeks resulted in the bacterial multiplication

decrease within plant tissues by 230-folds, but the population of bacteria didn't decrease significantly over time (Feil and Purcell, 2001).

Several temperature-related methods were used to determine the disease potential to infect various fruit crops. From the relationship between temperature and insect mortality, Sutton (2005), defined three levels of PD infection risk according to the winter temperature. Locations where winter has less than one day when the temperature is less than  $-12.2^{\circ}\text{C}$ , or with less than three consecutive days the temperature is less than  $-9.4^{\circ}\text{C}$ , is defined as a very high PD infection risk area.

Sharpshooters are thought to overwinter as adults in surrounding grass debris. Temperature is an important factor affecting the survival of sharpshooters and development of their eggs within the adult insects. Johnson et al. (2006) have determined that  $10^{\circ}\text{C}$  is a critical threshold for the survival of *H. vitripennis*. It was observed that sharpshooter feeding was greatly reduced when the temperature was near or below  $10^{\circ}\text{C}$ , and died when the temperature held below  $10^{\circ}\text{C}$  for more than 15 days. From this discovery, Johnson et al. (2008) constructed a Cooling Degree Days (CDD) model to quantify the cold impact on the *H. vitripennis* mortality. He accumulated the absolute value of the difference between daily mean temperature and  $10^{\circ}\text{C}$ , only when daily mean temperature is lower than the thermal activation threshold ( $10^{\circ}\text{C}$ ). Based on his curvilinear regression, an accumulated CDD that exceeds  $178^{\circ}\text{C}$  contributes to a 0% survival rate of *H. vitripennis*.

To study the temperature effect on egg production capability, egg loads of female adult *H. vitripennis* captured from July 2005 to October 2008 were examined in Texas (Lauziere, 2008). Egg loads demonstrated significant difference between months with a maximum mean number of 13.8 eggs per female in March. Furthermore, the insect sizes of female adults which are represented by the left hind tibia length also varied significantly with the largest size caught in May, June, and July, while the smallest size insects were caught in December. In a set of greenhouse studies (Lauziere, 2008), pre-oviposition period of summer *H. vitripennis* females averaged 13 days, which was 5.6 times shorter than the pre-oviposition period of winter individuals. Despite the difference in the pre-oviposition period, the study concluded that the oviposition period, post-oviposition period and the laid egg numbers did not vary significantly between different months.

## **Vector Management**

**Monitoring Methods:** Yellow sticky traps are widely used in field monitoring of sharpshooter species (Myers et al., 2007; Hall and Hunter, 2008; Wallingford, 2008). In studies by Castle and Naranjo (2008) the efficacy of four different methods were compared to the efficacy of yellow sticky traps to monitor *H. vitripennis* population. A strong correlation was found between the adult capture numbers of all the methods tested and sharpshooter numbers captured from the yellow sticky traps.

**Chemical and Physical Control:** Imidacloprid is a neuro-active chemical of neonicotinoid used to control *H. vitripennis*. It has long residual activity (Krewer et al., 2002), and xylem-translocation characteristics (Wang et al., 1999), and is able to hamper the spread of PD in field studies (Krewer et al., 2002). Research conducted by Villanueva et al. (2008) showed that fewer sharpshooter leafhoppers were captured in North Carolina vineyards after implementing a combination of neonicotinoid and pyrethroid insecticides. However, a study by Krewer et al. (2002) showed imidacloprid was not effective to slow the development of PD in areas where the vector species were abundant.

Kaolin (Surround WP®, Engelhard Co. Iselin, NJ), is an alternative pest management agent. Kaolin forms a mineral film surrounding plant tissues to prevent pest insects from feeding or ovipositioning on the plant organs (Glenn et al., 1999). In a field experiment conducted in California, 6% of the kaolin treated grapevines tested PD infected, while the infection rate for the untreated grapevines was 8% greater (Tubajika et al., 2007). The dust application of particle films used in Glenn's research was not considered practical due to the lack of particle adhesion to the plant surfaces. A later study developed liquid formulations and improved the practicality (Glenn et al., 1999).

Harpin proteins are known to activate natural plant defenses by promoting a vigorous growth (Alarcon et al., 1998; Tubajika et al., 2007). The protection efficacy is dependent on the treatment rate: A treatment rate of 460 g/ha has restricted the PD incidence to 6%, comparing with 19% PD incidence of the control treatment. Studies showed that plants treated with harpin had a lower incidence of the symptoms of PD and were less likely to be infected with *X. fastidiosa*.

**Biological and Natural Control Agents:** Host-specific egg parasitoid, *Gonatocerus ashmeadi*

(Girault) (Hymenoptera: Cicadellidae), was introduced to reduce the *H. vitripennis* population in Tahiti and Moorea of French Polynesia. A 90% reduction of *H. vitripennis* population was achieved with low infestation on non-target eggs after a release of 13786 *Gonatocerus ashmeadi* individuals (Grandgirard et al., 2007; Grandgirard et al., 2008). Besides the high control efficiency, *G. ashmeadi* also has demonstrated a rapid spread and even self-introduction to the neighboring islands where it was not implemented. *Gonatocerus fasciatus* (Girault) (Hymenoptera: Mymaridae) was also observed to parasitoid on eggs of *Homalodisca vitripennis* and *Oncometopia orbona* (Triapitsyn et al., 2003). Coincidentally, naturally occurring parasitoid on *P. irrorata* eggs was reared to arise from host eggs and identified as *G. fasciatus* (Tipping et al., 2006). Adults and nymphs of *H. vitripennis* were also a prey to spiders from families Salticidae, Agelenidae, Oxyopidae and Lycosidae (Lopez et al., 2003). Mizell et al. (2008) also observed that insects of Odonata order seized adult *H. vitripennis* on the wing in flight.

Although in the southeastern U.S., *Draeculacephala* spp., *Homalodisca vitripennis*, *Graphocephala* spp., and *Oncometopia* spp. (Mizell et al., 2003; Myers et al., 2007) vector species are considered to be prevalent in orchards and vineyards, no scientifically based records exist of the prevailing species' identity and abundance in Alabama orchards and vineyards. The main objective of our study was to: (1) identify the major species of sharpshooters occurring in Alabama orchards located at three different climatic zones, (2) study the phenology of each sharpshooter species, and (3) compare the vector's abundance on six different fruit crops as an attempt to determine sharpshooter adult's crop preference.

## **3.2 Materials and Methods**

### ***Sites Selection***

Various growing regions were selected in Alabama (Figure 1, Table 1) to represent fruit species grown in three distinct chilling zones (Powell et al., 2002), where the Gulf Coast area represented the extreme southern and southern Alabama: Mobile (30°26.4', 88°13.1'), Central Alabama was represented by Clanton (32°55.2', 88°40.3'), and North Central and North Alabama were represented by Athens (34°46.1', 86°54.0').



### ***Sharpshooter Trapping***

Double sided 7.62 cm X 15.24 cm yellow sticky traps (Great Lakes IPM Inc., Vestaburg, MI) (Hall and Hunter, 2008) were deployed on five fruit species, including hybrid bunch grape, muscadine grape, peach, plum and Satsuma mandarin in the 2008 survey, whereas rabbiteye blueberry crops were added to the 2009 survey. In all cases, traps were deployed on each fruit crop studied for a presence of *Xf* infection. Studies by Chen et al. (2004a, 2004b) have shown yellow sticky traps are one of the most effective and nontoxic ways to attract leafhoppers and monitor their abundance. The experimental fruit orchards or vineyards included in our investigation were maintained by the landowners or the experiment station personnel and the common commercial IPM practices were applied. Specific insect management information provided by the site managers and pertaining to each crop under investigation is presented in Table 2.

In 2008, starting on May 21 until October 10, four traps per crop per site were used to capture insect present in the orchards. The number of glassy-winged sharpshooter (*H. vitripennis*) captured on each trap was recorded bi-weekly. In 2009, trapping at the experimental sites began on April 1 and continued until September 18.

For each fruit species, traps were placed on the outer limbs of the fruit trees located on the edge of the orchard periphery (Figure 2). Each trap was strengthened by clear tape on the top side of the trap. Every other week, used traps were removed from trees, enclosed in 1.25 L plastic bags for transportation to the lab and subsequently stored at a room temperature prior to insect identification and counting.

### ***Sharpshooter Identification and Counting***

Traps were processed in the lab by using HistoClear II to remove vector species. Different species were identified by comparison to a published key developed by the Texas A&M University (<http://beaumont.tamu.edu/research/agroecosystems/grapes/KeyToLeafhoppers.htm#Introduction>) and using sharpshooter pictures confirmed by the Plant Disease Diagnostic lab at Auburn University. The total number of insects of each confirmed sharpshooter species were counted and recorded after identification. Individual species representatives were preserved in ethanol and subsequently

presented as a voucher specimen. Specimens of collected sharpshooters are deposited in the Auburn University Entomological Museum.

The cooling degree-days (CDD) were computed according to the method of Johnson et al. (2006) to estimate the impact of winter temperature on sharpshooter mortality. Cooling degree-days were defined as the accumulation of the absolute value of daily average temperature minus 10°C, only when daily average temperature was lower than 10°C.

Growing degree-day accumulation was used to correlate the insect emergence. The percentage of insect emergence was determined as the proportion of accumulative bi-weekly captive adult number versus total seasonal capture number of insect adults. Degree-days accumulation was recorded after January 1<sup>st</sup> based on average daily temperature above 10°C. Meteorological data were compiled from Alabama Mesonet Weather Database (AWIS Weather Service, Inc. Auburn, AL).

### 3.3 Results

#### *Sharpshooter Identification*

During the initial investigation in 2008, several insects that are potential vectors of *Xf* were found. In 2008, a total number of 5,289 *H. vitripennis* adults from 432 yellow sticky traps deployed at three Alabama locations were captured (Table 3). Nine sharpshooter species from five genera were identified in 2009. The total number of all sharpshooter insects captured during the second year of our study was 6,534. Four large size species (body length > 10 mm) were identified in Alabama orchards and vineyards, including: *Homalodisca vitripennis* (Germar) - (glassy-winged sharpshooter) (Figure 3), *H. insolita* (Walker) (Figure 4), *Oncometopia orbona* (Fabricius), (broad-headed sharpshooter) (Figure 5), and *Paraulacizes irrorata* (Fabricius) (speckled sharpshooter), (Figure 6). Small size species (body length < 10 mm) included *Graphocephala coccinea* (Forster) (red-banded leafhopper), (Figure 7), *G. versuta* (Say), (Figure 8), and *Draeculacephala* spp. (Figure 9). Due to the only slight color pattern differences, the various *Draeculacephala* species were considered as a group in this study. Identification of *Draeculacephala* species suggested that *Draeculacephala* species consisted of *D. balli*, *D. bradleyi*, and *D. mollipes*.

Our results revealed that *H. vitripennis* and *G. versuta* were the most prevalent sharpshooter species captured in Alabama orchards and vineyards, consisting of 51.4 and 38.8%, respectively (Table 3). All other sharpshooter species captured in our traps accounted for a small percentage of total sharpshooter populations and varied from 0.7% for *P. irrorata* to 3.7% for *H. insolita*.

Individual representatives from all sharpshooter species were preserved in 75% alcohol. Voucher specimens of sharpshooter taxa were made from collected insects and deposited in the Auburn University Entomological Museum.

### ***Sharpshooter Phenology***

The two year data for the seasonal average number of *H. vitripennis* captured per trap at three distinct chilling zone locations in Alabama suggest this species has only one generation per year (Figure 10) and the emergent period of *H. vitripennis* occurred in mid- to late May in both years throughout the state. At the Mobile location, *H. vitripennis* had peak abundance in June, followed by a sharp decrease in July, and a gradual decrease after August 1<sup>st</sup>. In Clanton, *H. vitripennis* emerged in the beginning of June and its population peak was observed in late July to early August in both years of the study. In 2008, the insect population increased gradually, while in 2009, population increase was observed in early July, and *H. vitripennis* numbers dropped in late July. A second peak in insect population was evident at the Clanton location in August. In the Athens site, low *H. vitripennis* numbers were captured during July to September in 2008 (less than one per trap on average at its peak), and no *H. vitripennis* individuals were captured during the 2009 season.

*H. vitripennis* captured on six crops was the most abundant sharpshooter species at the Mobile location, as evident by the seasonal mean number of captured individuals, with a peak in mid June, when the maximum mean number of *H. vitripennis* was nearly 40 insects per trap (Figure 11). A decrease in population numbers was observed in July and by the beginning of August the mean number of insects captured per trap was 5. All other sharpshooter species present at the Mobile location had a relatively low abundance throughout the entire season. *H. insolita* were in high abundance in two periods: early April and late July to early August. In early April, average number of *H. insolita* captured per trap reached 1.3, and increased to 4.7 individuals per trap in July to early August. The first emergence of *G. versuta* was in mid April and persisted throughout the growing

season with a low average number per trap. Its population peaked in late June with an average number of 2.2 captures per trap. No *Draculacephala* spp. were trapped until late May. In late June, 0.3 individuals per trap were recorded. *G. coccinea*, *O. orbona* and *P. irrorata* were occasionally absent from yellow sticky traps deployed at the Mobile location. Their average number never exceeded one per trap in the growing season.

In Clanton (Figure 12), *H. vitripennis* and *G. versuta* were the most abundant sharpshooter species. The peak abundance for *H. vitripennis* was recorded in late June, when the average number of insects per trap reached 34. In early August, a second peak of *H. vitripennis* population was observed when an average of 16 insects per trap was captured. *G. versuta* sharpshooters were captured for the first time in Clanton in mid-June during the 2009 season. In late June, a peak abundance of *G. versuta* populations was recorded, when the average number of captures reached 18 per trap. Numbers of captured *G. versuta* remained greater than 10 insects per trap throughout the rest of the season in 2009. *O. orbona* was also observed on traps in Clanton after mid-June. Its peak abundance occurred in late June and early August when 2 insects per trap were recorded. *Draeculacephala* spp. emerged in Clanton at the same period as *G. versuta* and *O. orbona* species. Its abundance was low throughout the season, with average number of captures less than 0.3 per trap. Individuals from *H. insolita* species were captured in nine out of ten sampling cycles. The only cycle in which it did not appear on traps was in mid April. *H. insolita* had its peak abundance of 1.2 insects per trap in early April. *P. irrorata* was captured only in early April and mid June throughout the 2009 season and the number of captured individuals was very low.

At the Athens location, no *H. vitripennis* were captured during the 2009 sampling season (Figure 13). *H. insolita* was found on traps only in the last two sampling cycles in September. *G. versuta* was the most abundant sharpshooter species in Athens, where 12 insects per trap were recorded in late June. Our results suggest that the *G. versuta* population emerged in late May in Athens and its abundance gradually decreased to 0.1 insect per trap in mid-September. *G. coccinea* population appeared on yellow sticky traps in mid June and the mean number of insect capture was low (0.3 per trap). *O. orbona* emerged in mid May and persisted until late August in Athens with a peak in insect abundance observed in late July (1.3 individuals per trap). *Draeculacephala* spp. appeared between

mid-June and early September with a highest abundance of 2 insects per trap in late August. *P. irrorata* was captured in mid-April and July and had low numbers of insects trapped.

Since CDD accumulation of 178°C is considered as a threshold causing a 100% mortality rate, our data showing 298 and 532 CDD accumulation in Clanton and Athens, respectively, in 2008 could explain the higher overwintering *H. vitripennis* mortality rate than in Mobile. Although the 178°C CDD is considered a threshold causing 100% mortality, the experiment by Johnson et al. (2008) was conducted using a temperature chamber to simulate fluctuating winter temperatures, it is reasonable to expect slightly different results in an open field investigation. The difference between *H. vitripennis* numbers observed at the Central Alabama location and North Alabama could be explained by the various field habitats that may have protected *H. vitripennis* from a 100% mortality rate.

The relationship between percentage of *H. vitripennis* adults' emergence and cumulative heating degree-days was attempted to establish for each location in both years (Figure 14). Our results suggest the population of *H. vitripennis* emerged when cumulative degree-days reached approximately 750 to 850, and 50% emergence of *H. vitripennis* occurred at approximately 1500 °C-day.

The population of *G. versuta* from the Mobile, Clanton and Athens locations emerged at approximately 500 °C-day (Figure 15), and 50% emergence rate was reached at about 1500, 1700 and 1000 °C-day from Mobile, Clanton and Athens, respectively. This suggests other variables, such as orchard maintenance, elevation, crop species, surrounding habitat, are probably affecting the development of *G. versuta* population.

The preliminary data obtained from our study could be utilized to develop a degree-day model to predict sharpshooter emergence in various Alabama locations.

### ***Fruit Crop Preference***

***H. vitripennis*:** In 2008, *H. vitripennis* was found in all three locations studied in our survey. Significant differences were found between the average numbers of *H. vitripennis* captured per trap during the entire season based on the type of crop species grown in Clanton (F=8.2354, P<0.0001); and Mobile, (F=3.845, P=0.0021) locations (Table 4). The greatest number of *H. vitripennis* in Clanton location was found on hybrid bunch grape (17.2 insects per trap). High number of *H.*

*vitripennis* was also found on Satsuma mandarin and muscadine grape in the same location, while significantly lower mean number of *H. vitripennis* were observed on peaches and plums. In the Mobile location, the highest number of *H. vitripennis* was observed on muscadine grape (52.3 insects per trap), and was not significantly different from the mean number of *H. vitripennis* found on plums (42.8 insects per trap). In both locations, significantly low *H. vitripennis* populations were found on peach trees. The abundance of *H. vitripennis* was very low throughout the growing season on all of the crops studied in Athens location. In 2009, no *H. vitripennis* were captured on yellow sticky traps in Athens (Table 5). In Clanton and Mobile, significant differences between the insect abundance were found depending on crop species ( $F=8.909$ ,  $P<0.0001$ ). The highest average populations of *H. vitripennis* in Clanton were found on hybrid bunch grape (21.5 insects per trap) followed by muscadine grape (13.8 insects per trap), and Satsuma mandarin (9.5 insects per trap). In Mobile location, *H. vitripennis* abundance differed significantly between the studied crops, where the insect populations had the highest density on Satsuma mandarin, followed by the populations on muscadine grape.

***Graphocephala versuta*:** Within each location included in our study, significant differences were found in the mean number of *G. versuta* between different fruit crop species during 2009 season. In Athens location, the highest seasonal abundance of *G. versuta* was found on blueberry (3.4 insects per trap) ( $F=4.9384$ ,  $P=0.0025$ ), which was not significantly different from the insect abundance on hybrid bunch grape (2.6 insects per trap) (Table 6). Significant differences of seasonal *G. versuta* mean number captured per trap were found between crops in Clanton location ( $F=5.965$ ,  $P<0.0001$ ) (Table 6). The average *G. versuta* captures from muscadine grape, hybrid bunch grape and Satsuma mandarin were high - 20.3 insects per trap, 17.8/trap and 8.5 insects per trap respectively. However, blueberry and peach species were not preferred by *G. versuta* in Clanton, where an average of 4.3 insects per trap and 3.8 insects per trap were observed respectively. In Mobile, a significant difference was found in the seasonal abundance of *G. versuta* based on the fruit species studied ( $F=12.0401$ ,  $P<0.0001$ ) (Table 6), with the highest seasonal mean number of *G. versuta* captured on Satsuma mandarin - 2.1 insects per trap, which is significantly greater than the average capture number from the other three fruit crops studied.

***H. insolita*:** *H. insolita* were found in all three locations included in our survey. Generally, the abundance of *H. insolita* remained very low through the season to show crop preference in Athens and Clanton locations (Table 7). Only the average *H. insolita* number captured on Satsuma mandarin in the Mobile location exceeded one insect per trap. Significant difference of seasonal mean number of *H. insolita* between crops was found in Mobile (Table 7). Satsuma mandarin from Mobile location had the significantly higher seasonal mean number of *H. insolita* than muscadine and peach.

***Oncometopia orbona*:** In 2009, significant differences between the seasonal means of *O. orbona* were found dependent on the crops species in Athens (F=3.646, P=0.0137), Clanton (F=3.9995, P=0.0017) and Mobile (F=4.3848, P=0.0052) locations (Table 8). In Athens location, the highest seasonal mean of *O. orbona* adults captured were found on blueberry (0.5 insects per trap) and peach (0.3 insects per trap). The abundance of *O. orbona* on hybrid bunch grape and plum species was relatively low - 0.1 insect/trap. The highest seasonal mean of *O. orbona* captured on yellow sticky traps in Clanton location was observed on Satsuma mamdarin (1.2 insects per trap), followed by the insect densities on hybrid bunch grape (0.9 insects per trap) and on muscadine grape (0.8 insects per trap) (Table 8). *O. orbona* had significantly lower abundance in peach, plum and blueberry crop. Among the four crop species studied in the Mobile location, the seasonal *O. orbona* mean observed in Satsuma mandarin grove was 0.5 insects per trap, which was significantly higher than the insect abundance on blueberry and muscadine grape (Table 8).

***Draeculacephala* spp.:** In 2009, significant differences of seasonal average number of *Draeculacephala* species between crops were observed only from Athens (F=6.6959, P=0.0003) and Mobile location (F=3.287, P=0.0218) (Table 9). In Athens location, the highest abundance of *Draeculacephala* spp. occurred on blueberry crop averaging 1.1 insects per trap. Seasonal means of *Draeculacephala* spp. number for hybrid bunch grape, peach and plum did not differ significantly. In the Mobile location, the highest abundance of *Draeculacephala* spp. was observed on muscadine grape at 0.4 insects per trap, followed by blueberry at 0.1 insects per trap.

***Graphocephala coccinea*:** Significant differences of seasonal mean *G. coccinea* numbers among different crop species were only evident in the Mobile location (F=5.1214, P=0.002), where the highest abundance of *G. coccinea* was found in Satsuma mandarin orchards (Table 10). The mean

numbers of *G. coccinea* captured from blueberry and muscadine grape crops in Mobile averaged less than 0.1 insects per trap.

***Paraulacizes irrorata*:** Significant differences of *P. irrorata* abundance between the studied fruit crop species was found in Mobile location (Table 11), where the highest *P. irrorata* abundance was registered on Satsuma mandarin crop (0.5 insects per trap), and peach (0.3 insects per trap) crops.

### 3.4 Discussion

Seven sharpshooter species were identified for the first time in orchards and vineyards representing three different chilling zones in Alabama. *H. vitripennis* and *G. versuta* were determined to be the primary sharpshooter species in Alabama orchards and vineyards due to their high population. *H. vitripennis* are known to be endemic to the southeast U.S. (Turner and Pollard, 1959), and recent research has confirmed their prevalence in Florida (Hunter and Hall, 2008). Our results confirmed *H. vitripennis* prevalence in Central and South Alabama, where *Xf* occurrence is high. *Xf* was ubiquitously available among wild plants (Purcell and Hopkins, 2002) in the southeastern U.S., which coincides with the fact that *H. vitripennis* are feeding on various types of host plants (Hoddle et al., 2003). The high population densities of *H. vitripennis* found in Central and South Alabama, where the disease pressure is high, might contribute to the spread of *Xf* infection to economic fruit crops in the state.

Our results suggest *G. versuta* is the second abundant sharpshooter in Alabama considering the overall capture number. It could be the major vector of *Xf* in Limestone County and Blount County, which rank second and third in peach production (Alabama Agricultural Statistical Bulletin, 2009), where *H. vitripennis* population was low. All other sharpshooter species found in orchards and vineyards appear to have low population densities, but since most of them are effective *Xf* vectors and appear to be highly involved in *Xf* epidemics, further research is needed to determine their significance as *Xf* vectors in Alabama.

Most of the sharpshooter species were found on traps on all of the six fruit crops included in our study. This observation is in agreement with Turner & Pollard (1959), describing the sharpshooter species as polyphagous insects. Sharpshooter population densities showed a significant difference



between various fruit crop species studied in most of the sampled locations. Our observations suggest that hybrid bunch grapes, muscadine grapes and Satsuma mandarins are preferred to plum and peach trees for most of the sharpshooter species found in Alabama. However, *G. versuta*, *H. insolita*, *O. orbona* and *Draeculacephala* spp. were also found in high densities on blueberry crop. Although our literature search did not return information on sharpshooter fruit crop preference, Hoddle et al. (2003) stated that *H. vitripennis* were most commonly found on citrus than other plants in a Florida survey. Danne et al. (2004) reported that a significantly higher density of *H. vitripennis* was found on grape crops than on citrus plants. Our results also agreed with the previous finding that peach crop was not preferred by sharpshooter species (Mizell and French, 1987).

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### 3.6 Tables and Figures

Table 1. Sharpshooter trapping sites in Alabama, 2008-2009: locations, elevation, crops, and edge habitats description.

| Region          | Location | County    | Latitude | Longitude | Elevation (m) | Crop            | North edge  | South edge | West edge  | East edge   |
|-----------------|----------|-----------|----------|-----------|---------------|-----------------|-------------|------------|------------|-------------|
| Gulf Coast      | Mobile   | Mobile    | 30°26.4' | 88°13.1'  | 23            | peach           | grass       | road       | grass      | road        |
|                 |          |           |          |           |               | Satsuma         | woods       | woods      | woods      | persimen    |
|                 |          |           |          |           |               | mandarin        |             |            |            |             |
| Central Alabama | Clanton  | Baldwin   | 30°32.6' | 87°44.5'  | 41            | Plum            | grass       | grass      | apple      | road        |
|                 |          |           |          |           |               | muscadine grape | woods       | road       | road       | grass       |
|                 |          |           |          |           |               | Blueberry       | house       | woods      | grass      | apple       |
|                 |          |           |          |           |               |                 |             |            |            |             |
|                 |          |           |          |           |               |                 |             |            |            |             |
| Central Alabama | Chilton  | Chilton   | 32°55.2' | 88°40.3'  | 207           | peach           | road        | grass      | grass      | grass       |
|                 |          |           |          |           |               | plum            | road        | grass      | walnut     | high tunnel |
|                 |          |           |          |           |               |                 |             |            |            |             |
|                 |          |           |          |           |               | Satsuma         | high tunnel | kiwifruit  | blackberry | grass       |
|                 |          |           |          |           |               | muscadine grape | grass       | house      | kiwifruit  | road        |
| North Alabama   | Athens   | Limestone | 34°6.1'  | 86°54.0'  | 207           | peach           | road        | woods      | apple      | grass       |
|                 |          |           |          |           |               | plum            | grass       | peach      | pond       | peach       |
|                 |          |           |          |           |               | muscadine grape | house       | woods      | blueberry  | road        |
|                 |          |           |          |           |               | blueberry       | woods       | grass      | blackberry | kiwifruits  |
|                 |          |           |          |           |               |                 |             |            |            | pond        |

Table 2. Insect management in selected orchards and vineyards surveyed for presence of GWSS at six Alabama locations, 2008 and 2009.

| Location         | Crop                        | Insecticide                    | Timing                         |
|------------------|-----------------------------|--------------------------------|--------------------------------|
| Athens           | Bunch grape                 | Dormant Oil and Lorsban        | Late winter                    |
|                  |                             | Ambush                         | Petal fall                     |
|                  | Peach and plum<br>Blueberry | Imidan<br>No insecticide       | Every 10 days after petal fall |
| Clanton          | Bunch grape                 | Admire                         | Late winter                    |
|                  |                             | Lannate                        | Late winter, summer            |
|                  |                             | Malathion                      | Weekly after bud break         |
|                  | Muscadine grape             | Sevin                          | When needed in summer          |
|                  |                             | Ambush                         | When needed in summer          |
|                  |                             | Lannate                        | Late winter                    |
|                  | Peach and plum              | Dormant oil                    | Dormant spray                  |
|                  |                             | Lorsban                        | Dormant spray                  |
|                  |                             | Imidan                         | Spring                         |
|                  |                             | Sevin                          | When needed in summer          |
| Satsuma mandarin | Micromite                   | As needed in summer            |                                |
| Blueberry        | No insecticide              |                                |                                |
| Mobile           | Peach                       | Thytan, Endosulfan and Lannate | Weekly, Alternatively          |
|                  | Satsuma mandarin            | No insecticide                 |                                |
|                  | Muscadine                   | No insecticide                 |                                |
|                  | Blueberry                   | No insecticide                 |                                |

Table 3. Type and total number of sharpshooter species captured on yellow sticky traps in commercial Alabama orchards and vineyards during 2008 and 2009.

| Year  | Species                        | Total trapped in all orchards and vineyards | Percentage of all trapped (%) | Total No. of traps |
|-------|--------------------------------|---|-------------------------------|--------------------|
| 2008  | <i>Homalodisca vitripennis</i> | 5289  | 100                           | 432                |
| ----- |                                |   |                               |                    |
| 2009  | <i>Homalodisca vitripennis</i> | 3358  | 51.4                          |                    |
|       | <i>Graphocaphala versuta</i>   | 2532  | 38.8                          |                    |
|       | <i>Homalodisca insolita</i>    | 241   | 3.7                           |                    |
|       | <i>Oncometopia orbona</i>      | 195   | 3.0                           |                    |
|       | <i>Draeculacephala</i> spp.    | 90  | 1.4                           |                    |
|       | <i>Graphocephala coccinea</i>  | 69  | 1.1                           |                    |
|       | <i>Paraulacizes irrorata</i>   | 49  | 0.7                           |                    |
|       | Total                          | 6534  | 100                           | 618                |

Table 4. Seasonal mean capture of *Homalodisca vitripennis* trapped bi-weekly on five fruit crops using yellow sticky traps from three Alabama locations in 2008

| Crop                      | Location  |     |             |     |               |     |
|---------------------------|-----------|-----|-------------|-----|---------------|-----|
|                           | Athens    |     | Clanton     |     | Mobile        |     |
|                           | Mean      | SEM | Mean        | SEM | Mean          | SEM |
| Hybrid bunch grape        | 0.0 ± 0.0 |     | 17.2 ± 5.0a |     | -             |     |
| Peach                     | 0.2 ± 0.1 |     | 0.4 ± 0.1c  |     | 10.1 ± 2.6b   |     |
| Plum                      | 0.3 ± 0.2 |     | 1.5 ± 0.4bc |     | 42.8 ± 15.4ab |     |
| Satsuma mandarin          | -         |     | 8.9 ± 2.5ab |     | 14.4 ± 3.6b   |     |
| Muscadine grape           | -         |     | 7.1 ± 1.8ab |     | 52.3 ± 13.3a  |     |
| F-value                   | 2.2830    |     | 8.2354      |     | 5.1558        |     |
| P-value                   | 0.1061    |     | <0.0001     |     | 0.0021        |     |
| Significance <sup>z</sup> | NS        |     | ***         |     | **            |     |

<sup>z</sup>NS indicates not significant differences at P=0.05; \*, P<0.05; \*\*, P<0.01; \*\*\*, P<0.001

Table 5. Seasonal mean capture of *Homalodisca vitripennis* trapped bi-weekly on six fruit crops using yellow sticky traps from three Alabama locations in 2009

| Crop                      | Location         |     |             |     |             |     |
|---------------------------|------------------|-----|-------------|-----|-------------|-----|
|                           | Athens           |     | Clanton     |     | Mobile      |     |
|                           | Mean             | SEM | Mean        | SEM | Mean        | SEM |
| Blueberry                 | 0.0 ± 0.0        |     | 0.2 ± 0.1c  |     | 4.2 ± 1.8b  |     |
| Hybrid bunch grape        | 0.0 ± 0.0        |     | 21.5 ± 7.1a |     | -           |     |
| Peach                     | 0.0 ± 0.0        |     | 1.3 ± 0.5bc |     | 3.7 ± 1.0b  |     |
| Plum                      | 0.0 ± 0.0        |     | 1.8 ± 0.5bc |     | -           |     |
| Satsuma mandarin          | -                |     | 9.5 ± 3.4ab |     | 15.0 ± 5.1a |     |
| Muscadine grape           | -                |     | 13.8 ± 3.5a |     | 12.5 ± 3.1a |     |
| F-value                   | N/A <sup>y</sup> |     | 8.9090      |     | 3.8454      |     |
| P-value                   | N/A              |     | <0.0001     |     | 0.0105      |     |
| Significance <sup>z</sup> | N/A              |     | ***         |     | *           |     |

<sup>y</sup>No *H. vitripennis* was captured in Athens, 2009

<sup>z</sup>NS indicates not significant differences at P=0.05; \*, P<0.05; \*\*, P<0.01; \*\*\*, P<0.001

Table 6. Seasonal mean capture of *Graphocephala versuta* trapped bi-weekly on six fruit crops using yellow sticky traps from three Alabama locations in 2009

| Crop                      | Location    |     |              |     |            |     |
|---------------------------|-------------|-----|--------------|-----|------------|-----|
|                           | Athens      |     | Clanton      |     | Mobile     |     |
|                           | Mean        | SEM | Mean         | SEM | Mean       | SEM |
| Blueberry                 | 3.4 ± 1.0a  |     | 4.3 ± 1.3c   |     | 0.3 ± 0.1b |     |
| Hybrid bunch grape        | 2.6 ± 1.0ab |     | 17.8 ± 4.7ab |     | -          |     |
| Peach                     | 0.9 ± 0.3b  |     | 3.8 ± 1.4c   |     | 0.4 ± 0.1b |     |
| Plum                      | 0.6 ± 0.2b  |     | 7.6 ± 2.0bc  |     | -          |     |
| Satsuma mandarin          | -           |     | 8.5 ± 1.9abc |     | 2.1 ± 0.5a |     |
| Muscadine grape           | -           |     | 20.3 ± 3.5a  |     | 0.2 ± 0.1b |     |
| F-value                   | 4.9384      |     | 5.9650       |     | 12.0401    |     |
| P-value                   | 0.0025      |     | <0.0001      |     | <0.0001    |     |
| Significance <sup>z</sup> | **          |     | ***          |     | ***        |     |

<sup>z</sup>NS indicates not significant differences at P=0.05; \*, P<0.05; \*\*, P<0.01; \*\*\*, P<0.001

Table 7. Seasonal mean capture of *Homalodisca insolita* trapped bi-weekly on six fruit crops using yellow sticky traps from three Alabama locations in 2009

| Crop                      | Location  |     |            |     |             |     |
|---------------------------|-----------|-----|------------|-----|-------------|-----|
|                           | Athens    |     | Clanton    |     | Mobile      |     |
|                           | Mean      | SEM | Mean       | SEM | Mean        | SEM |
| Blueberry                 | 0.1 ± 0.1 |     | 0.8 ± 0.3a |     | 0.6 ± 0.2ab |     |
| Hybrid bunch grape        | 0.0 ± 0.0 |     | 0.3 ± 0.1a |     | -           |     |
| Peach                     | 0.0 ± 0.0 |     | 0.1 ± 0.1a |     | 0.3 ± 0.1b  |     |
| Plum                      | 0.0 ± 0.0 |     | 0.1 ± 0.1a |     | -           |     |
| Satsuma mandarin          | -         |     | 0.5 ± 0.2a |     | 2.1 ± 0.1a  |     |
| Muscadine grape           | -         |     | 0.3 ± 0.1a |     | 0.3 ± 0.1b  |     |
| F-value                   | 0.7846    |     | 2.5322     |     | 4.0413      |     |
| P-value                   | 0.5038    |     | 0.0299     |     | 0.0081      |     |
| Significance <sup>z</sup> | NS        |     | *          |     | **          |     |

<sup>z</sup>NS indicates not significant differences at P=0.05; \*, P<0.05; \*\*, P<0.01; \*\*\*, P<0.001

Table 8. Seasonal mean capture of *Oncometopia orbona* trapped bi-weekly on six fruit crops using yellow sticky traps from three Alabama locations in 2009

| Crop                      | Location    |     |            |     |             |     |
|---------------------------|-------------|-----|------------|-----|-------------|-----|
|                           | Athens      |     | Clanton    |     | Mobile      |     |
|                           | Mean        | SEM | Mean       | SEM | Mean        | SEM |
| Blueberry                 | 0.5 ± 0.2a  |     | 0.3 ± 0.1b |     | 0.1 ± 0.1ab |     |
| Hybrid bunch grape        | 0.1 ± 0.0b  |     | 0.9 ± 0.2a |     | -           |     |
| Peach                     | 0.3 ± 0.1ab |     | 0.1 ± 0.1b |     | 0.1 ± 0.0b  |     |
| Plum                      | 0.1 ± 0.0b  |     | 0.2 ± 0.1b |     | -           |     |
| Satsuma mandarin          | -           |     | 1.2 ± 0.4a |     | 0.4 ± 0.1a  |     |
| Muscadine grape           | -           |     | 0.8 ± 0.3a |     | 0.0 ± 0.0b  |     |
| F-value                   | 3.6460      |     | 3.9995     |     | 4.3848      |     |
| P-value                   | 0.0137      |     | 0.0017     |     | 0.0052      |     |
| Significance <sup>z</sup> | *           |     | **         |     | **          |     |

<sup>z</sup>NS indicates not significant differences at P=0.05; \*, P<0.05; \*\*, P<0.01; \*\*\*, P<0.001

Table 9. Seasonal mean capture of *Draeculacephala* spp. trapped bi-weekly on six fruit crops using yellow sticky traps from three Alabama locations in 2009

| Crop                      | Location   |     |           |     |             |     |
|---------------------------|------------|-----|-----------|-----|-------------|-----|
|                           | Athens     |     | Clanton   |     | Mobile      |     |
|                           | Mean       | SEM | Mean      | SEM | Mean        | SEM |
| Blueberry                 | 1.1 ± 0.6a |     | 0.3 ± 0.1 |     | 0.1 ± 0.1ab |     |
| Hybrid bunch grape        | 0.0 ± 0.0b |     | 0.0 ± 0.0 |     | -           |     |
| Peach                     | 0.0 ± 0.0b |     | 0.0 ± 0.0 |     | 0.0 ± 0.0b  |     |
| Plum                      | 0.0 ± 0.0b |     | 0.0 ± 0.0 |     | -           |     |
| Satsuma mandarin          | -          |     | 0.2 ± 0.1 |     | 0.0 ± 0.0b  |     |
| Muscadine grape           | -          |     | 0.1 ± 0.1 |     | 0.4 ± 0.2a  |     |
| F-value                   | 6.6959     |     | 2.1195    |     | 3.2870      |     |
| P-value                   | 0.0003     |     | 0.0643    |     | 0.0218      |     |
| Significance <sup>z</sup> | ***        |     | NS        |     | *           |     |

<sup>z</sup>NS indicates not significant differences at P=0.05; \*, P<0.05; \*\*, P<0.01; \*\*\*, P<0.001

Table 10. Seasonal mean capture of *Graphocephala cocinea* trapped bi-weekly on six fruit crops using yellow sticky traps from three Alabama locations in 2009

| Crop                      | Location  |     |           |     |             |     |
|---------------------------|-----------|-----|-----------|-----|-------------|-----|
|                           | Athens    |     | Clanton   |     | Mobile      |     |
|                           | Mean      | SEM | Mean      | SEM | Mean        | SEM |
| Blueberry                 | 0.1 ± 0.1 |     | 0.1 ± 0.1 |     | 0.0 ± 0.0b  |     |
| Hybrid bunch grape        | 0.1 ± 0.0 |     | 0.0 ± 0.0 |     | -           |     |
| Peach                     | 0.1 ± 0.0 |     | 0.2 ± 0.1 |     | 0.2 ± 0.1ab |     |
| Plum                      | 0.0 ± 0.0 |     | 0.2 ± 0.2 |     | -           |     |
| Satsuma mandarin          | -         |     | 0.0 ± 0.0 |     | 0.3 ± 0.1a  |     |
| Muscadine grape           | -         |     | 0.3 ± 0.3 |     | 0.0 ± 0.0b  |     |
| F-value                   | 0.7846    |     | 0.8050    |     | 5.1214      |     |
| P-value                   | 0.5038    |     | 0.5427    |     | 0.0020      |     |
| Significance <sup>z</sup> | NS        |     | NS        |     | **          |     |

<sup>z</sup>NS indicates not significant differences at P=0.05; \*, P<0.05; \*\*, P<0.01; \*\*\*, P<0.001

Table 11. Seasonal mean capture of *Paraulacizes irrorata* trapped bi-weekly on six fruit crops using yellow sticky traps from three Alabama locations in 2009

| Crop                      | Location  |     |           |     |             |     |
|---------------------------|-----------|-----|-----------|-----|-------------|-----|
|                           | Athens    |     | Clanton   |     | Mobile      |     |
|                           | Mean      | SEM | Mean      | SEM | Mean        | SEM |
| Blueberry                 | 0.0 ± 0.0 |     | 0.1 ± 0.0 |     | 0.0 ± 0.0b  |     |
| Hybrid bunch grape        | 0.0 ± 0.0 |     | 0.0 ± 0.0 |     | -           |     |
| Peach                     | 0.0 ± 0.0 |     | 0.0 ± 0.0 |     | 0.3 ± 0.1ab |     |
| Plum                      | 0.0 ± 0.0 |     | 0.0 ± 0.0 |     | -           |     |
| Satsuma mandarin          | -         |     | 0.0 ± 0.0 |     | 0.5 ± 0.1a  |     |
| Muscadine grape           | -         |     | 0.0 ± 0.0 |     | 0.0 ± 0.0b  |     |
| F-value                   | 0.0128    |     | 0.4097    |     | 8.1037      |     |
| P-value                   | 0.9980    |     | 0.8418    |     | <0.0001     |     |
| Significance <sup>z</sup> | NS        |     | NS        |     | ***         |     |

<sup>z</sup>NS indicates not significant differences at P=0.05; \*, P<0.05; \*\*, P<0.01; \*\*\*, P<0.001





Figure 1. Insect monitoring sites for selected fruit crops in Alabama, 2008 and 2009.

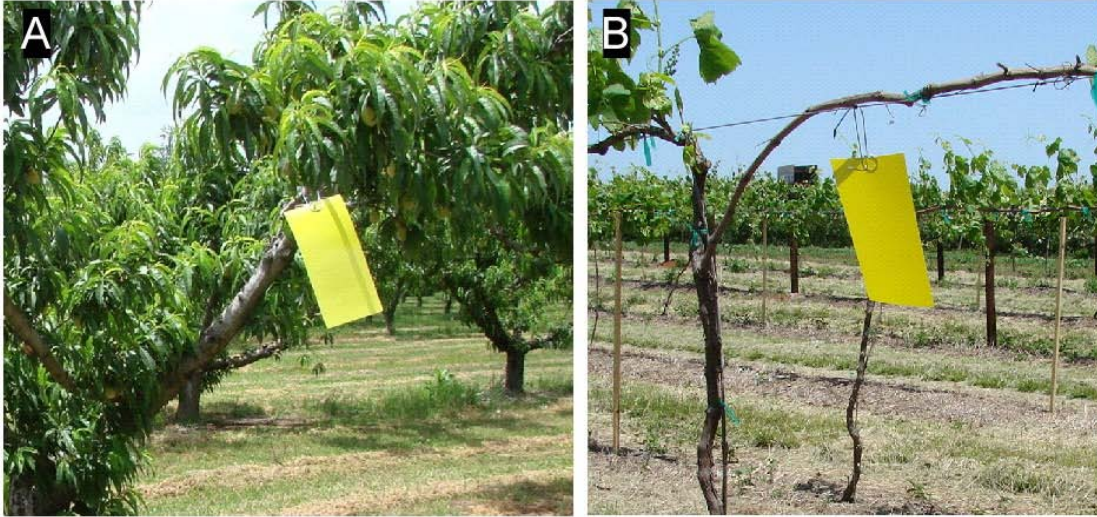


Figure 2. Installation of yellow sticky traps: (A) Placed on the outer limb of a peach tree; (B) Attached to the trellis system wire in a muscadine vineyard. Image: Xing Ma.



Figure 3. A *Homalodisca vitripennis*, also known as glassy-winged sharpshooter, captured during 2009 growing season in Alabama. Each grade on the the ruler corresponds to 1 mm. The white secretions on wings are the brochosomes. Image: Xing Ma.



Figure 4. A *Homalodisca insolita* captured during 2009 growing season in Alabama. Each grade on the ruler corresponds to 1 mm. Image: Xing Ma.



Figure 5. An *Oncometopia orbona*, also known as broad-headed sharpshooter, captured during 2009 growing season in Alabama. Each grade on the ruler corresponds to 1 mm. Image: Xing Ma.



Figure 6. A *Paraulacizes irrorata*, also known as speckled sharpshooter captured during 2009 growing season in Alabama. each grade on the ruler corresponds to 1 mm. Image: Xing Ma



Figure 7. A *Graphocephala coccinea*, also known as red-banded sharpshooter captured during 2009 growing season in Alabama. Each grade on the ruler corresponds to 1 mm. Image: Xing Ma.



Figure 8. A *Graphocephala versuta* captured during 2009 growing season in Alabama. Each grade on the ruler corresponds to 1 mm. Image: Xing Ma.





Figure 9. A *Draeculacephala* spp. captured during 2009 growing season in Alabama. Three species were identified: *D. balli*, *D. bradleyi*, *D. mollipes*. Each grade on the ruler corresponds to 1 mm.

Image: Xing Ma.

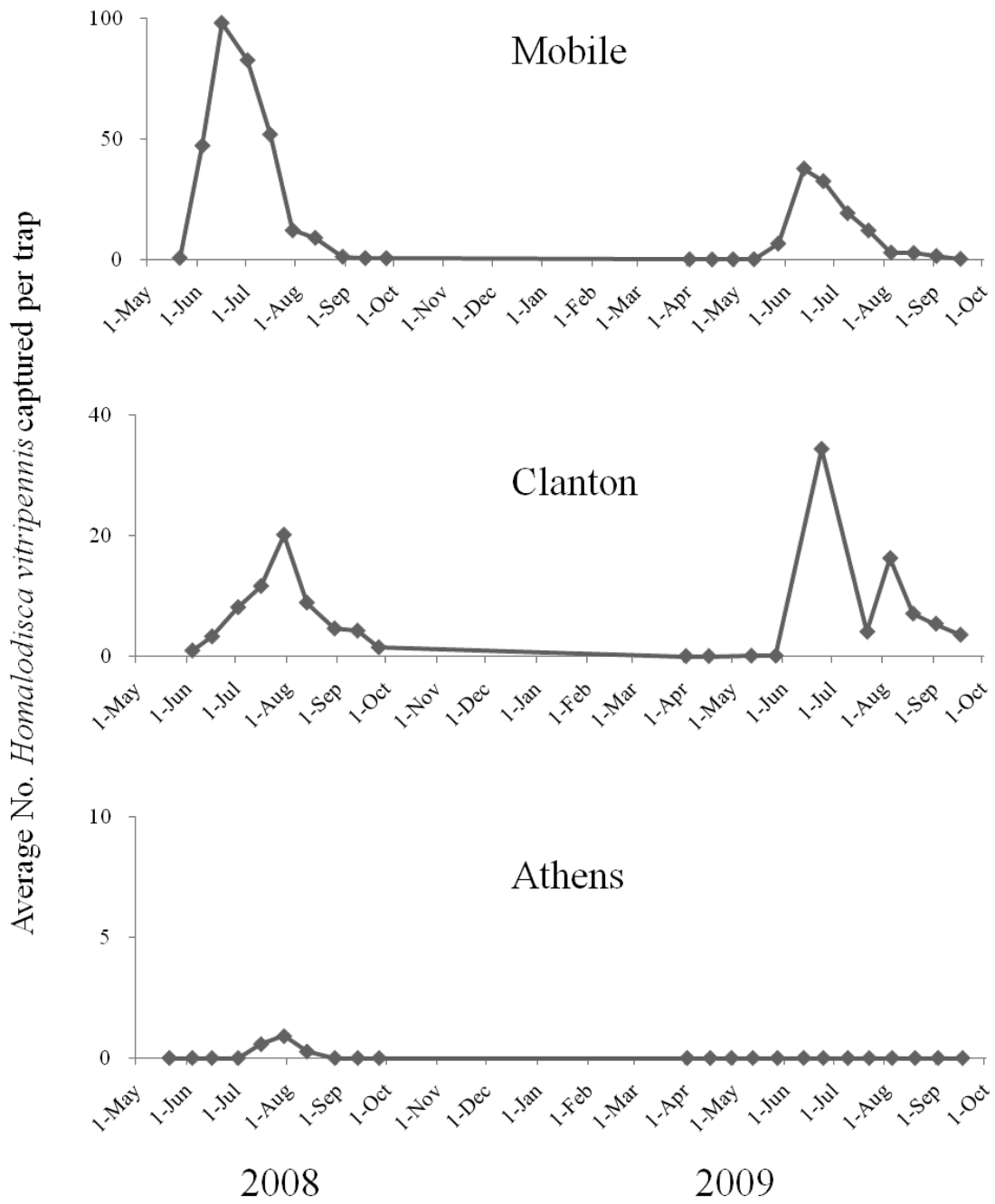


Figure 10. Two-year seasonal abundance of *Homalodisca vitripennis* in three Alabama locations in 2009. Each number represents the average sharpshooter capture of four traps per crop on a given sampling cycle.

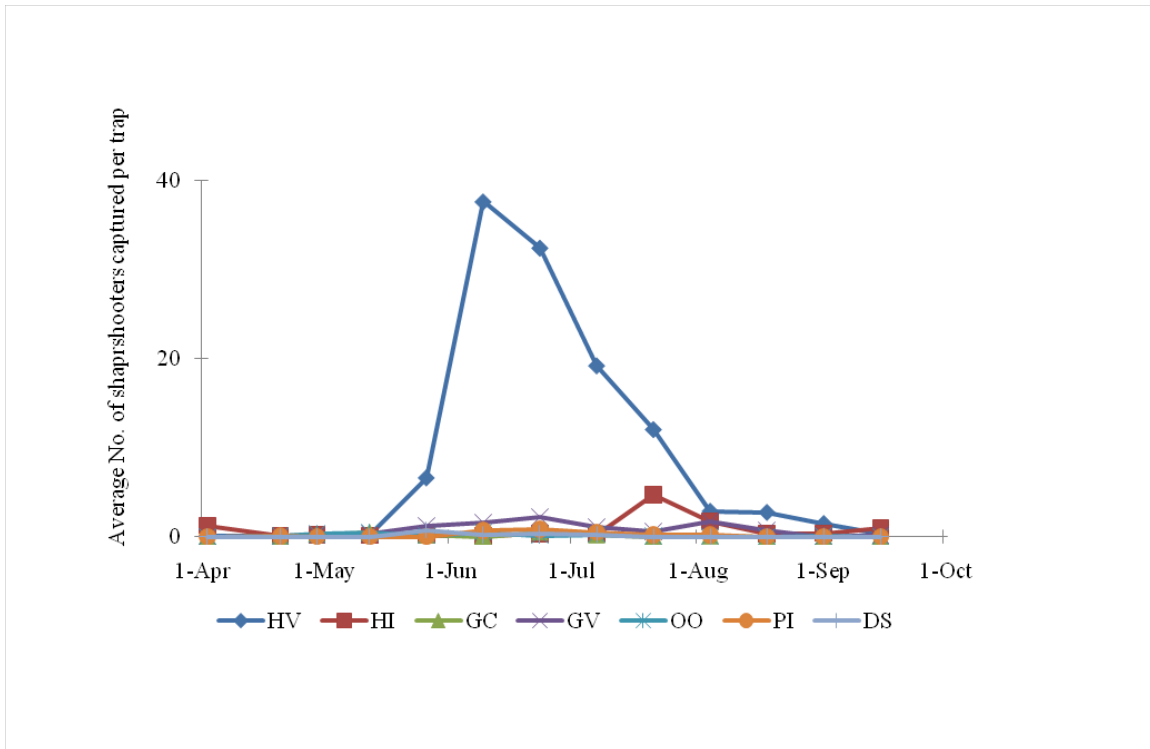


Figure 11. Seasonal abundance of seven sharpshooter species in Mobile, Alabama in 2009. Each number represents the average sharpshooter capture of four traps per crop on six crop species. Insect species name: HV= *Homalodisca vitripennis*; HI= *Homalodisca insolita*; GC= *Graphocephala coccinea*; GV= *Graphocephala versuta*; OO= *Oncometopia orbona*; PI= *Paraulacizes irrorata*; DS= *Draeculacephala* spp.

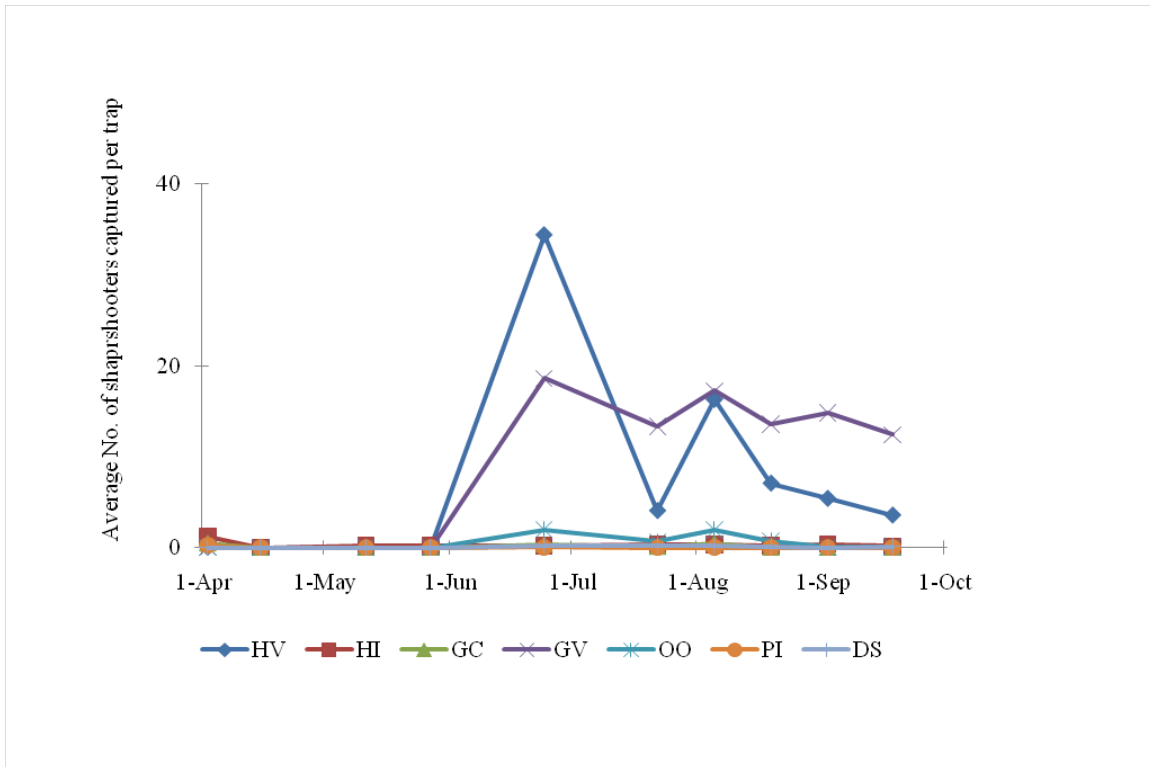


Figure 12. Seasonal abundance of seven sharpshooter species in Clanton, Alabama in 2009. Each number represents the average sharpshooter capture of four traps per crop. Insect species name: HV= *Homalodisca vitripennis*; HI= *Homalodisca insolita*; GC= *Graphocephala coccinea*; GV= *Graphocephala versuta*; OO= *Oncometopia orbona*; PI= *Paraulacizes irrorata*; DS= *Draeculacephala* spp.

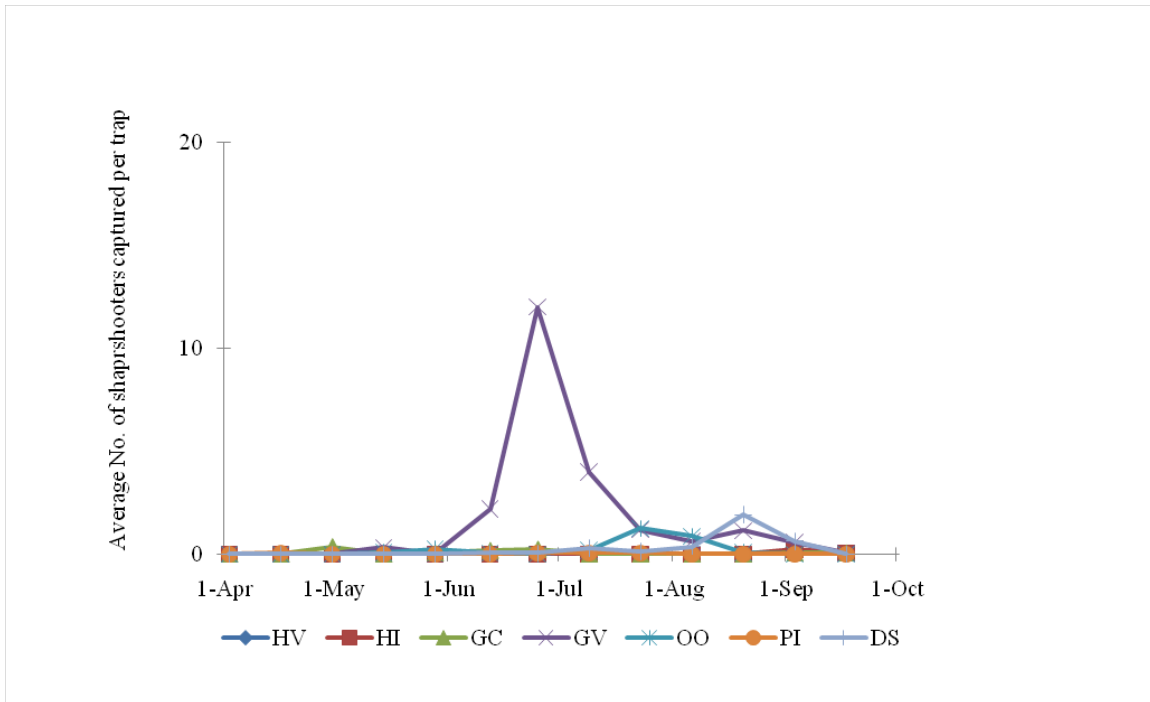


Figure 13. Seasonal abundance of seven sharpshooter species in Athens, Alabama in 2009. Each number represents the average sharpshooter capture of four traps per crop. Insect species name: HV= *Homalodisca vitripennis*; HI= *Homalodisca insolita*; GC= *Graphocephala coccinea*; GV= *Graphocephala versuta*; OO= *Oncometopia orbona*; PI= *Paraulacizes irrorata*; DS= *Draeculacephala* spp.

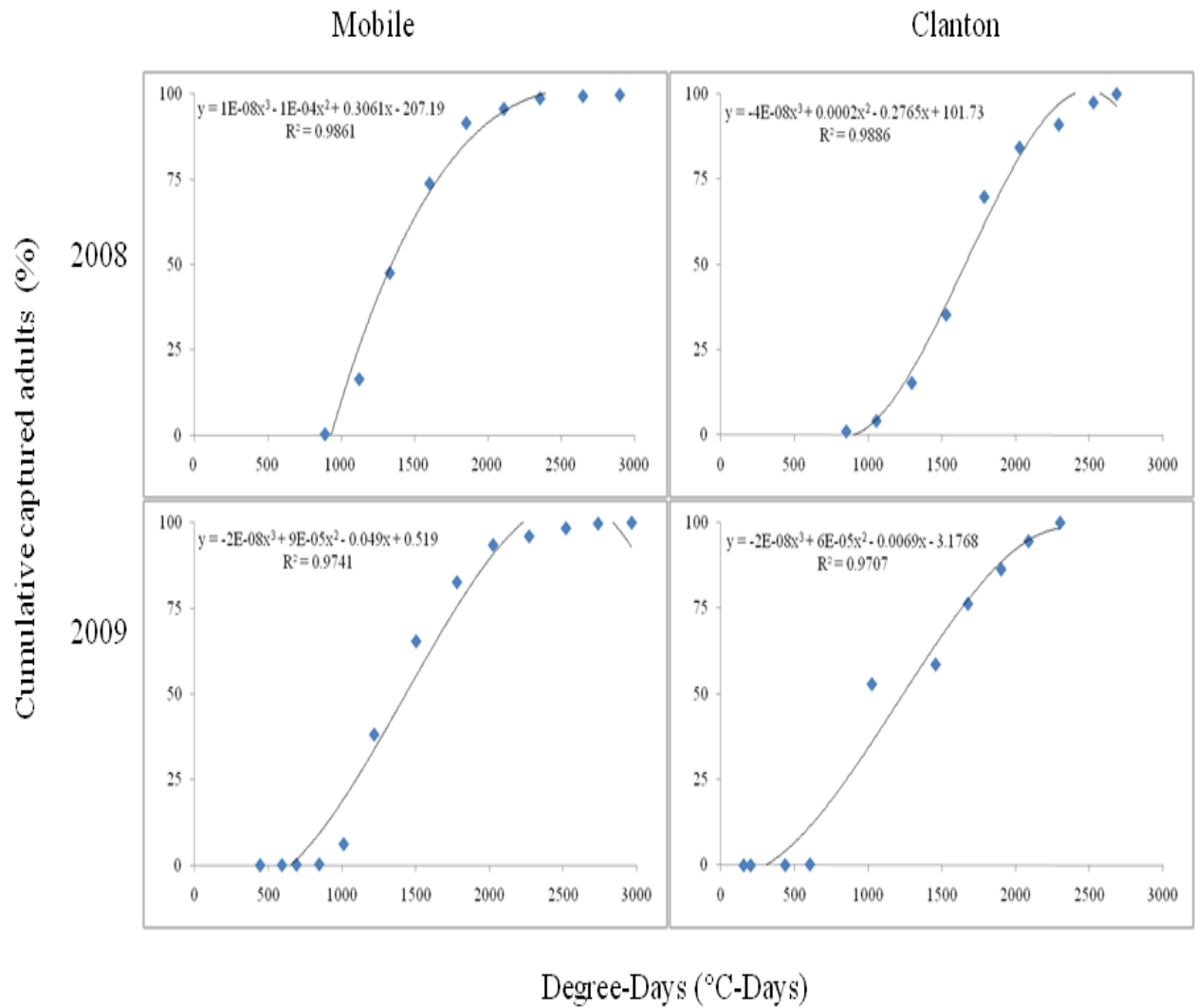


Figure 14. Relationship between cumulative captured *Homalodisca vitripennis* adults and Growing Degree Days accumulation based on 10°C after January in two Alabama locations during 2008-2009.

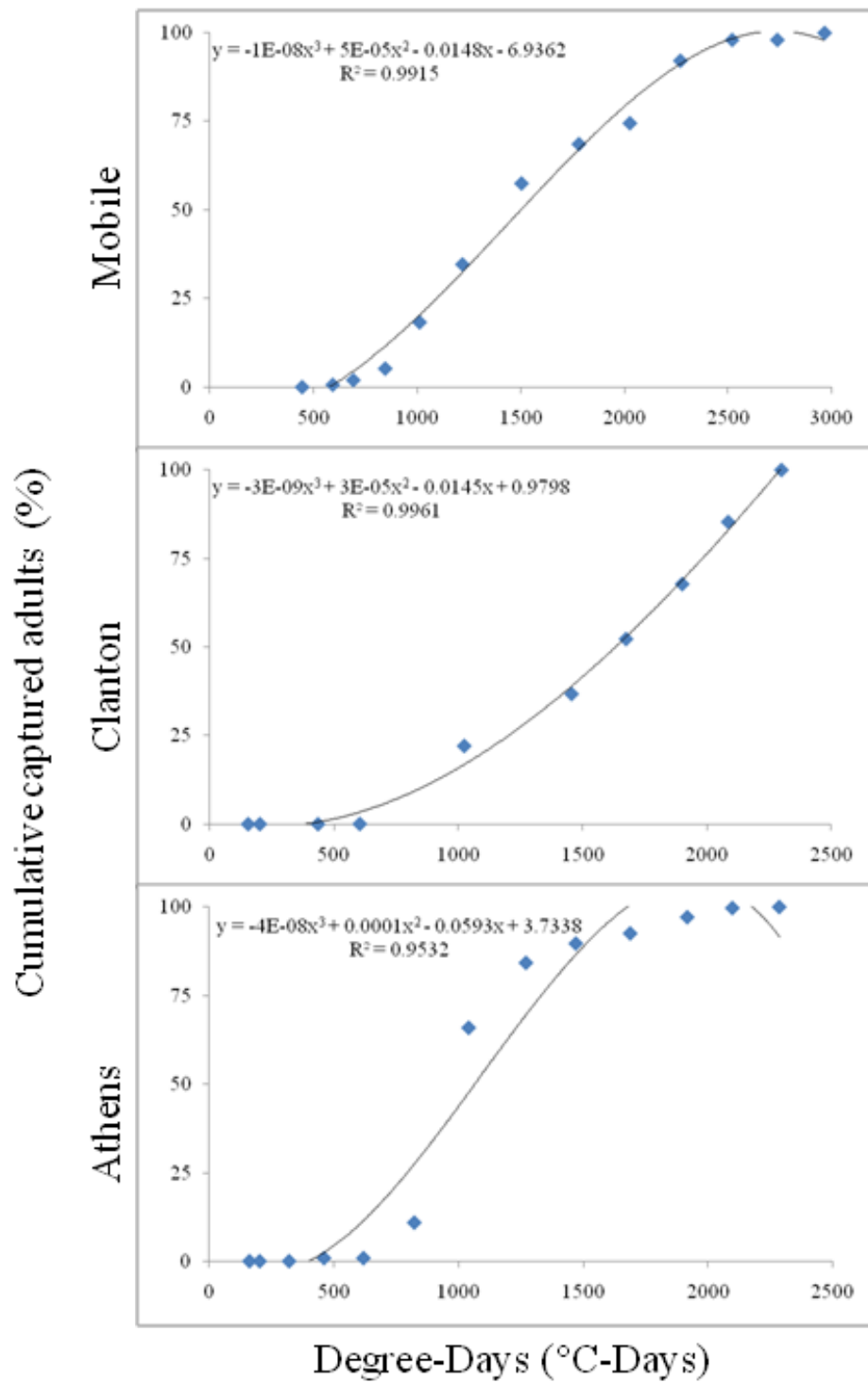


Figure 15. Relationship between cumulative captured *Graphocephala versuta* adults and Growing Degree Days accumulation after January in three Alabama locations during 2009.