Determining the threat of Pierce's disease to Virginia vineyards.

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Abstract

Pierce's disease (PD) is a vascular disease of grapevines caused by *Xylella fastidiosa* (*Xf*) which is transmitted by xylophagous insect vectors. PD infection in Virginia vineyards was thought to be isolated to southeastern portions of the state as there have been no reports of vine loss in western Virginia and cold winter temperatures experienced there limit the effects of the bacterium from year to year. Upward trends in winter temperatures have raised PD concern in the mid-Atlantic. My risk assessment study has not only found PD symptomatic vines beyond the modeled boundary for infection, confirmed *Xf*-positive with DAS-ELISA, but I also found vine loss in regions considered to be at moderate to low risk. Yellow sticky traps were used to survey Virginia vineyards throughout the 2006 and 2007 growing seasons to identify sharpshooter (Cicadellinae) species in six growing regions. *Graphocephala versuta* (Say) and *Oncometopia orbona* (Fabricius) (Hemiptera: Cicadellidae) were trapped in the greatest abundance and were both present in every region surveyed. This study uses geographical representation of climatological data to estimate risk for Pierce's disease.

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Table of Content **Page #**

List of Tables

Chapter One:

Chapter Two: A Survey of Sharpshooter Vectors of Pierce's Disease within Virginia Vineyards

Chapter Three: A Survey of *Xylella fastidiosa* in Virginia vineyards.

Chapter Four: Assessing risk of Pierce's disease to Virginia vineyards.

List of Figures

Chapter Two: A Survey of Sharpshooter Vectors of Pierce's Disease within Virginia Vineyards

Chapter Four: Assessing risk of Pierce’s disease to Virginia vineyards.

CHAPTER ONE

Pierce's Disease of Grapevines: Literature Review

Pierce's disease (PD) is a vascular disease of grapes caused by the xylem-limited bacterium *Xylella fastidiosa* (Wells et al.) (*Xf*) which is transmitted by xylophagous auchenorrhynchan vectors, primarily from the families Cicadellidae (Frazier and Freitag 1946) and Cercopidae (Severin 1950).

"The California vine disease" was first described by N.B. Pierce (1892), also known as mysterious disease, vine plague, Anaheim disease and ultimately Pierce's disease. *Xf* is difficult to grow on artificial media and early investigators were unable to isolate the causal bacterium. Because PD could be transmitted by grafting and by insect vectors, and because a bacterium could not be confirmed, the causal agent was assumed to be a virus (Hewitt et al. 1942). Not until the 1970's was the causal agent of PD identified as a bacterium (Wells et al. 1987).

Xylella fastidiosa is responsible for several diseases of economically important crops including PD in grape (Davis et al. 1978), citrus variegated chlorosis (CVC; Chang et al. 1993), almond leaf scorch (ALS; Mircetich et al. 1976), alfalfa dwarf (Goheen et al. 1973), phony peach, plum scald (Wells et al. 1981), and of several species of ornamental plants, e.g. oleander leaf scorch (OLS; Purcell et al. 1999), and bacterial leaf scorch (BLS) of *Ulmus* spp., *Quercus* spp. (Hearon et al. 1980), *Acer* spp. (Sherald et al. 1987), *Platanus* spp. (Sherald et al. 1982), etc. More than 100 host plant species for *Xf* have been reported (Hopkins and Purcell 2002), many of which show no symptoms of infection (Hopkins and Adlerz 1988).

Although described as a single species (Wells et al. 1987), there are several pathotypes of *Xf* that cause symptoms in distinct host ranges. Schaad et al. (2004) described three major subspecies: *X. fastidiosa* subsp. *fastidiosa* (syn. *piercei*) includes strains isolated from grape (*Vitis* spp.), almond (*Prunus dulcis*), alfalfa (*Medicago sativa*) and maple (*Acer* spp). *X. fastidiosa* subsp. *multiplex* includes isolates from almond (*Prunus dulcis*), peach (*P. persica*), plum (*P. salicina*), pigeon grape (*Vitis aestivalis*), and several shade trees including elm (*Ulmus* spp.) and sycamore (*Platanus* spp.). *X. fastidiosa* subsp. *pauca* includes strains isolated from citrus.

Symptoms of PD occur when bacteria proliferate within the xylem; both the bacteria (Newman et al. 2003) and host responses to infection (Stevenson et al. 2005) block the flow of xylem fluid to the shoots. Production of biofilms by the bacteria and such plant responses as formation of gums and tyloses can also contribute to occlusion of xylem vessels (Marques et al. 2002, Fritschi et al. 2008). Infection leads to vine decline, yield loss, and vine death within two to three years of infection if there are optimal temperatures (Gubler et al. 2006). Affected grapevines show symptoms related to water deficits, like interveinal chlorosis, marginal necrosis with marginal yellow or red line (Hopkins 1989). Water stress can intensify the symptoms of an infected plant; however, there are some visual symptoms that are only observed in PD infected plants, including green islands and matchstick petioles (Thorne et al. 2006). Uneven formation of the periderm leads to "green islands" of living epidermis that remain while the rest of the shoot turns brown as it goes dormant (Stevenson et al. 2005). Matchstick petioles remain on shoots once the leaf blade abscises from the petiole. This abscission occurs without the normal formation of a separation zone between leaf blade and petiole, allowing for

dehydration before the wound periderm can form, resulting in a necrotic tip that resembles a burnt matchstick (Stevenson et al. 2005).

Xylella fastidiosa has a patchy distribution within the grapevine and there is no clear relationship between bacterial population and marginal leaf scorch symptom development (Gambetta et al. 2007); however, sampling late in the season will ensure the highest possible titer within the plant and sampling matchstick petioles from portions of the plant closest to the cordon increases the probability of *Xf* detection (Krell et al. 2006).

Enzyme-Linked ImmunoSorbent Assay (ELISA) uses antibodies that bind to proteins on the outer wall of *Xf* to detect its presence or absence in a sample. Unfortunately, ELISA does not identify particular strains of *Xf*, as is possible when using polymerase chain reaction (PCR); however, commercial ELISA kits are available and this method of detection is easier, faster and cheaper than PCR. Also, ELISA has been found to be equally effective as PCR in detecting *Xf* in almond (Groves et al. 2005).

Xylella fastidiosa is transmitted to host plants by insect vectors. Although all xylophagous insects are theoretically potential vectors, efficiency of transmission in each species is variable (Purcell and Hopkins 1996). Members of the subfamily Cicadellinae (sharpshooters) are all xylem-feeders (Young 1968) and thirty-nine species in nineteen genera of Cicadellinae have been shown to be capable vectors of PD (Redak et al. 2004). Xylem fluid offers sharpshooters minimal risk from plant defenses but is a poor source of nutrition. Sharpshooters are extremely efficient at assimilating the nutrients of xylem fluid; nearly all organic compounds in the xylem fluid are utilized and sharpshooters have adapted to excretion of ammonia rather than urea (Andersen et al. 1989). In spite of the high efficiency of metabolic conversion, sharpshooters must take in a large amount of

fluids, up to 100 times that of their dry body weight (Brodbeck et al. 1993). The large amount of water may act to reduce toxicity of ammonia in excretia (Redak et al. 2004). A bulbous clypeus houses large cibarial muscles to feed on xylem fluids, under negative pressure due to plant evapotranspiration from stomata in leaf surfaces (Novotny and Wilson 1997). A steady excretion of honey dew is "flicked" off a feeding sharpshooter's abdomen, thus the name "sharpshooter" (Riley and Howard 1893).

Sharpshooters obtain bacterial infection by feeding on a plant infected with *Xf* and these bacteria colonize the inner linings of the insect's mouthparts (Timmer et al. 1983). As few as 10^4 colony forming units (CFU) per gram of plant tissue are necessary for acquisition of infection by the vector (Hill and Purcell 1995). Xylem fluids are drawn in through interlocked maxillary stylets and into the precibarium before the xylem fluid is pumped into the gut (Backus and McLean 1982, Leopold et al. 2003). Electrostatic attraction between bacterial surface proteins and the insect cuticle allow for bacteria suspended in the xylem fluid to stick to the inner linings of the insect's mouthparts (Osiro et al. 2004).

Xylella fastidiosa has both Type I and Type IV pili, filamentous bacterial surface structures. Type IV pili are involved in bacteria mobility while Type I are involved in adhesion of bacteria as well as production of an exopolysaccharide (EPS) biofilm (Fuente et al. 2007). Biofilms are produced both within the plant host and within the insect vector where it covers the monolayer of loosely attached and polarly arranged bacterial colonies (Brlansky et al. 1983). Biofilms protect microbial communities from antibiotics, dehydration, host defenses and contribute to adhesion and bacterial virulence (Stoodley et al. 2004).

The primary mode of *Xf* transmission to grapevines is through vector feeding, i.e. egestion from the foregut, specifically the precibarium, of an infected vector to the host plant xylem vessels (Purcell et al. 1979, Almeida and Purcell 2006). With this type of infection there is little to no latent period between the insect acquiring bacteria and being able to transmit it to a new plant (Severin 1949). As few as 2 hours are needed to pass infection in a very efficient vector species such as *Graphocephala atropunctata* (Signoret) (Purcell and Finlay 1979).

It is also possible for transmission to occur in cases of reduced or no feeding. Bextine et al. (2004) evaluated the effect of an anti-feedant (pymetrozine) on incidence of PD infection and found that, although excretia of GWSS was greatly reduced, incidence of infection was actually greater in plants treated with the anti-feedant than in control plants. In this case, the anti-feedant acts to inhibit phagostimulatory effects of the xylem fluid and there is an increase in number of probes of the xylem cells. It is likely that the increase in probing is the cause of an increase in PD incidence. Transmission of *Xf* by pruning shears is rare but possible (Krell et al. 2007).

Infectivity can be retained for several months in adult sharpshooters (Almeida and Purcell 2003). *Xf* is not transovarially transmitted (Freitag 1951), therefore offspring of an infective individual must feed on infected host plant tissue before becoming infective themselves. Also, because bacterial colonies are held in the foregut, infectivity is non-transtadial and is lost with each molt (Purcell and Finlay 1979).

Sharpshooters overwinter as adults in the southeastern U.S. (Turner and Pollard 1959). Infectivity obtained by adults in the previous growing season is retained in the first wave of sharpshooters entering a vineyard from overwintering habitat (Freitag and

Frazier 1954). However, the subsequent generations must acquire *Xf* from infected host plants; this inoculum source could be a vine within managed areas or a wild host species. Myers et al. (2007) found that proportions of infective sharpshooters trapped in the early months of the growing season in North Carolina (April - May) were higher than in sharpshooters trapped in the summer (July).

Early season transmission of *Xf* is of greatest concern. An early introduction of *Xf* allows for more time in the growing season for bacterial colonies to proliferate within the xylem vessels of the vine (Purcell 1981). Early season introduction also implies an introduction of bacteria closer to permanent portions of the plant (the cordon), and a better chance of chronic infection as infected tissue will not be removed with regular winter pruning; such infections will have the best chance of surviving subsequent winters. Incidence of PD infection in California vineyards is highest in edge rows and particularly those rows that border sharpshooter overwintering habitats like riparian vegetation (Purcell 1974). Adults emerging from over-wintering habitats in the spring are more likely to be infective than sharpshooters feeding later in the summer (Myers et al. 2007). In addition, *Xf* populations are more likely to persist in wild host plants that act as sources of incoculum for new vector infections (Baumgartner and Warren 2005).

The importance of summertime *Xf* transmission in Virginia vineyards is unclear. Early season spatial pattern of *G. atropunctata* resembles the spatial pattern of PD in northern California, while late season dispersal does not (Purcell 1975). Summer infections are not considered threatening in California (Feil et al. 2003). Late summer temperatures in this region are cool, which slows the rate of *Xf* multiplication (Feil and Purcell 2001). Late summer temperatures in Virginia are hotter than those of California;

mean temperature in the month of August in Napa Co., CA is 18^{o}C versus 24^{o}C in Albemarle Co., VA (1997-2007 weatherunderground.com). The assumption that summer transmission does not allow enough time for *Xf* to proliferate to dangerous levels may need to be tested in Virginia.

Because xylem fluid is a poor source of nutrition, sharpshooters may spend a limited amount of time feeding on *Vitis* in order to move on to better food sources. Movement of sharpshooter populations from host species to host species is directed by changes in xylem fluid nutrition and water tension within xylem vessels through the growing season (Mizell and French 1987). Levels of dietary nitrogen, available carbon and ratios of amino acids in xylem fluids act as phagostimulants that determine host plant acceptance and duration of feeding once the leafhopper has made a "test-probe" into the xylem (Brodbeck et al. 1995). In addition, drought stress increases the water tension within xylem vessels, encouraging sharpshooters to move on to alternative host plant species rather than exspend extra energy to extract xylem fluids (Andersen et al. 1992).

Periodic outbreaks of PD in California were attributed to imports of infected material, but this disease was manageable until introduction of the Glassy-winged sharpshooter (GWSS), *Homalodisca coagulata* (Say), from Florida in 1980 (Blua et al.1999). Until this species was introduced to California, there were several efficient, native vectors present, e.g. *G. atropunctata*, *Draeculacephala minerva* Ball, *Carneocephala fulgida* Nottingham (Severin 1949). However, these species rarely feed within managed vineyards because of their limited flight strength. In general, native Californian sharpshooters do not move great distances from riparian areas and have a preference for grass feeding; nonetheless PD infection does occur (Purcell and Frazier

1985). Moreover, the native California sharpshooters prefer to feed on more delicate plant tissue and PD infections introduced by cicadellini sharpshooters are typically distal to the cordon and removed with regular winter pruning. As GWSS, as well as other proconiines, are able to feed on tougher plant tissue and even on dormant plant tissue (Turner and Pollard 1959).

Xylella fastidiosa is endemic to the southeastern U.S. (Hewitt 1958) and is the limiting factor in *Vitis* production in the Gulf Coast states. Until now, inland Virginia was thought to be within the range of moderate risk of PD, as winter pruning and cold winter temperatures (lethal to the bacteria) keep infections from becoming chronic/systemic (Purcell 1980). As winter temperatures increase, concern has been raised for PD as a future problem in the mid-Atlantic. *Vinifera* and most French-American hybrid varieties are considered highly susceptible to PD (Raju and Goheen 1981) and account for the majority of commercial grape production in Virginia; Chardonnay, Cabernet Franc, Cabernet Sauvignon, Merlot and Vidal Blanc represented more than half of the 5,600 tons of commercial grapes produced in Virginia in 2007 (USDA 2008).

Several vector species have been recorded in Virginia, although little is known of their presence in commercial vineyards (Stearns 1927). *Oncometopia orbona* (in the same tribe as GWSS) and *Graphocephala versuta* were trapped in a previous collection in a vineyard on Virginia's Eastern Shore and have been shown to be capable PD vectors in transmission studies (Turner and Pollard 1959, Myers et al. 2007).

Pierce's disease infection in Virginia vineyards was thought to be isolated to southeastern portions of the state as cold winter temperatures experienced in inland

Virginia limit the effects of the bacterium from year to year. However, upward trends in winter temperatures have raised PD concern in the mid-Atlantic (Sutton 2005). Optimal temperature for *Xf* development falls between 25-32^{o}C, but temperatures below 12^{o}C and above 34^{o}C may affect survival in plants (Feil and Purcell 2001). Sutton (2005) predicts little PD incidence in regions that experience more than three days below -12.2^{o}C or more than five days below -9.4^{o}C over a winter season. See Table 1.1 for risk scores according to Sutton's standard.

Spread of PD infection through the vineyard can be slowed by control of the insect vector using a xylem-translocated insecticide such as the neonicotinoid, imidacloprid (Krewer et al. 2002). Soil drench application of imidacloprid at the high end of label rates achieves the recommended titer of 10 µg of active ingredient per liter of xylem fluid to control vectors (Castle et al. 2005). Although it takes several days to reach this titer, it is maintained for roughly three months which would protect vines for the period of high concern for *Xf* transmission by insects (Byrne and Toscano 2006).

There are several biological control agents under investigation. There are strains of *Beauveria bassiana* (Balsamo) virulent against GWSS (Dara et al. 2007) but not yet a commercially available product. There are several species of *Gonatocerus* (Hymenoptera: Mymaridae) that attack the egg stage of several sharpshooter species that are native to the southeast, including Virginia (Triapitsyn et al. 2003). *G. novifasciatus* Girault is a parasitoid of *G. versuta* (Huber 1988).

Alternative products such as kaolin and harpin show promise in reducing incidence of PD infection but both require multiple applications (Tubajika et al. 2007). Kaolin particle film barrier acts to inhibit insect host-plant finding ability and feeding,

and also triggers excessive grooming behavior as particles collect on insect body parts (Stanley 1998). Surround WP® (Engelhard Corporation, Iselin, New Jersey) is product with kaolin clay as its active ingredient and is listed as a product approved by the Organic Materials Review Institute (OMRI). Harpin protein, naturally produced by the bacterium *Erwinia amylovora* (Burrill) (Winslow et al. 1920), does not act on pest species directly but acts to activate natural plant defenses by promoting plant growth (Obradovic et al. 2004). Messenger® (Eden Bioscience Corp., Bothell, WA), a product containing harpin proteins, also contains ethylenediamine tetraacetic acid (EDTA). EDTA is a chelating agent, used in agriculture to improve availability of micronutrients, and has been found to inhibit biofilm formation in *Xf* (Toney and Koh 2006), which may also negatively affect virulence.

Hill and Purcell (1995, 1997) suggested that vector management in not a cure for PD, but rather the use of resistant grape varieties is the only reliable method of PD management. Although Krewer et al. (2002) showed that PD incidence was lower in insecticide treated vines than the untreated control; vineyard life was extended by only one year under high PD pressure. *Vitis* species native to areas of severe PD pressure, e.g. *V. rotundifolia* and *V. arizonica*, appear to be very resistant to PD infection (Ruel and Walker 2006). However, resistant varieties may not be acceptable substitutes in a wine grape industry, where varietal recognition is a crucial part of market value.

The mechanism for resistance is not well understood but is a function of the ability of the bacteria to survive within the plant rather than resistance to insect vector feeding. Bacterial invasion with xylem vessels induces invaginations of xylem walls called tyloses and production of gums, a possible mechanism of pathogen isolation and

therefore a mechanism for resistance; Fritschi et al. (2008) suggested that this response is actually detrimental to vine health as it contributes to xylem vessel blockage. Huang et al. (2007) investigated the pathways necessary to produce resveratrol, a phytoalexin or antibiotic produced by plants as part of their defense system. Resistant native *Vitis* species tend to produce higher concentrations of resveratrol than do European *Vitis vinifera*.

Pierce's disease management also includes removal of *Xf* host plants that act as sources of inoculum. Park et al. (2006) found that diseased vines in the Coachella Valley were spatially aggregated and suggested that one infected vine served as an inoculum source for each aggregation. University of California's Integrated Pest Management program suggests that, in areas of high PD pressure, vines that show PD symptoms should be severely pruned in the winter to just above the graft union and vines that show symptoms for more than one year should be removed entirely (Gubler et al. 2006). Several investigations have identified weed species as potential sources of inoculum (Freitag 1951, Raju et al. 1980, Hopkins and Adlerz 1988, Purcell and Saunders 1999, Costa et al. 2004) but it is unclear if removing these plants from areas surrounding vineyards helps to limit PD incidence.

Sharpshooters are effectively monitored using yellow sticky traps hung at a height of 0.6 to 1.8 meters above the ground (Ball 1979).

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1.3 Tables and Figures

Table 1.1: Estimated risk of PD infection according to Sutton's standard, number of days where daily minimum temperature falls below -12.2°C or -9.4°C.

	days below -12.2°C	days below -9.4°C
Low Risk	3 or more	5 or more
Moderate Risk	2	4
High Risk	1 or zero	3 or fewer

CHAPTER TWO

A Survey of Sharpshooter Vectors of Pierce's Disease within Virginia Vineyards

2.1 Introduction

Pierce's disease (PD) is a vascular disease of grapes caused by the xylem-limited bacterium *Xylella fastidiosa* (Wells et al.) (*Xf*) and is transmitted by xylophagous auchenorrhynchan vectors, primarily from the families Cicadellidae (Frazier and Freitag 1946) and Cercopidae (Severin 1950). PD is a limiting factor in *vinifera* production in the southeastern U.S. Virginia lies on the northern edge of range the range of PD because cold winter temperatures prevent chronic bacterial infection of vines (Feil and Purcell 2001). Since minimum winter temperatures appear to be increasing, concern has been raised for PD as a future problem in the mid-Atlantic states. *Vinifera* and most French-American hybrid varieties are considered highly susceptible to PD (Raju and Goheen 1981). These varieties account for the majority of commercial grape production in Virginia; Chardonnay, Cabernet Franc, Cabernet Sauvignon, Merlot and Vidal Blanc represented more than half of the 5,600 tons of commercial grapes produced in Virginia in 2007 (USDA 2008).

Although all xylophagous insects are theoretically potential vectors, efficiency of transmission among species is variable (Purcell and Hopkins 1996). Members of the subfamily Cicadellinae (sharpshooters) are all xylem-feeders (Young 1968) and thirty-nine species in nineteen genera of Cicadellinae have been shown to be capable vectors of PD (Redak et al. 2004). Spread of PD infection through the vineyard can be slowed by control of the insect vector (Krewer et al. 2002); therefore, knowledge of the major vector

species as well as the timing of their movement in and out of vineyards could aid in PD management.

Sharpshooters acquire bacterial infectivity by feeding on a plant infected with *Xf* and these bacteria colonize the inner linings of the insect's mouthparts (Timmer et al. 1983). Bacteria are transmitted from the foregut by egestion during feeding by infective sharpshooters (Purcell et al. 1979). With this type of infection there is little to no latent period between bacterial acquisition and the ability to transmit it to a new plant (Severin 1949); less than two hours are necessary to pass infection in an efficient vector species, *Graphocephala atropunctata* (Signoret) (Purcell and Finlay 1979).

Infectivity can be retained for several months in adult sharpshooters (Almeida and Purcell 2003). However, this infectivity is not transovarially transmitted (Freitag 1951), therefore the offspring of an infective individual must feed on infected host plant tissue before becoming infective themselves. Also, because bacterial colonies are held in the foregut, infectivity is lost with each molt (non-transtadial; Purcell and Finlay 1979).

Sharpshooters overwinter as adults in the southeastern U.S. (Turner and Pollard 1959). Infectivity obtained by adults in the previous growing season is retained in the first wave of sharpshooters entering the vineyard from overwintering habitats. Subsequent generations must acquire *Xf* from infected host plants (i.e. a vine within managed areas or a wild host species). Proportions of infective sharpshooters to non-infective sharpshooters trapped in early spring of the growing season in North Carolina (April-May) tend to be higher than in July (Myers et al. 2007).

Transmission of *Xf* in the early spring (April – June) is of greatest concern. Feeding sharpshooters are more likely to be infective at this time of year. Also, an early

introduction of *Xf* allows for more time in the growing season for bacterial colonies to proliferate within the xylem vessels of the vine. Early season transmission also implies an introduction of bacteria closer to permanent portions of the plant (the cordon) and a better chance of chronic infection as infected tissue will not be removed with regular winter pruning as would distal infections.

Movement of sharpshooter populations from host plant to host plant is directed by changes in xylem fluid nutritional content and water tension within xylem vessels of those plants throughout the growing season (Mizell and French 1987). Levels of dietary nitrogen, available carbon and ratios of amino acids in xylem fluids act as phagostimulants that determine host plant acceptability and duration of feeding once the sharpshooter has made a "test-probe" into the xylem (Brodbeck et al. 1995). As nutrition shifts from optimal, sharpshooters move to new host plants. Moreover, drought stress increases the water tension with xylem vessels, encouraging sharpshooters to move on to alternative host plant species (Andersen et al. 1992).

Periodic outbreaks of PD in California were attributed to imports of infected material, but this disease was manageable until the introduction of the Glassy winged sharpshooter (GWSS), *Homalodisca coagulata* (Say), from Florida in 1980 (Blua et al.1999). Before the introduction of GWSS, there were several efficient, native, Cicadellini vectors present, e.g. *G. atropunctata*, *Draeculacephala minerva* Ball, *Carneocephala fulgida* Nottingham (Severin 1949). However, these species rarely feed within managed vineyards because of their limited flight strength. In general, native Californian sharpshooters do not move great distances from riparian areas and have a preference for grass feeding; nonetheless PD infection does occur (Purcell and Frazier

1985). However, PD infections introduced by Cicadellini sharpshooters are typically distal to the cordon and removed with regular winter pruning. GWSS, as well as other proconiines, are able to feed on tougher plant tissue and even on dormant plant tissue (Turner and Pollard 1959), and this has exacerbated PD in California.

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Oncometopia orbona (Fabr.) and *Graphocephala versuta* (Say) were trapped in a previous collection in a vineyard on Virginia's Eastern Shore and have been shown to be capable PD vectors in transmission studies (Myers et al. 2007).

Oncometopia orbona belong to the Proconiini, the tribe containing the Glassy-winged sharpshooter (GWSS), *Homalodisca coagulata* (Say), the vector species of greatest concern in Florida and California (Purcell and Saunders 1999). Proconiines are strong fliers, able to cover a longer distance than other leafhoppers, and are able to feed on tougher, more basal, host plant tissue. Turner and Pollard (1959) observed two generations and a partial third in lab-reared *O. orbona*.

Graphocephala versuta is part of the Cicadellini. Several members of this tribe are known to be capable PD vectors (Nielson 1968) but they are smaller and not as strong fliers as proconiines and also prefer to feed on more delicate host plant tissue, like leaves. Turner and Pollard (1959) observed four generations of *G. versuta* lab-reared on cowpea.

This study seeks to identify the presence of capable PD vectors within Virginia vineyards, identify the most abundant sharpshooter species within Virginia vineyards. Also, this study seeks to gain temporal information on the movement of sharpshooters in and out of the vineyard and to compare insect activity on edge rows versus middle rows.

2.2 Materials and Methods

Ten commercial vineyards (V1-V10), each growing several varieties of European bunch grapes (*Vitis vinifera* L.), were monitored in 2006 and 2007. In both years, bud-break occurred approximately the first week of April at V1-V4, V7, V8 and V10 (Fig. 2.1, Table 2.1). A late frost in the first week of April 2007 damaged many vines at these sites. Timing of the second flush of growth in 2007 was variable as vine loss was not uniform even within each site. In 2006 and 2007, bud-break occurred approximately the second week of April at V7-V9 (Fig. 2.1, Table 2.1). Although Vineyards 9 and 10, in the Southern Piedmont, are within close proximity to each other, V9 is at a higher elevation than V10 so bud-break occurred a little later at V9 than V10. Little frost damage was seen at these sites where bud-break occurred after the late frost in April 2007. Coastal Plain sites (V3 and V4) experienced severe hail damage in July 2007. To maintain the plant health, vineyard managers removed that year's growth in the blocks monitored for sharpshooters.

American grape species are more tolerant to PD than European grapes; however, cultivated American varieties are considered susceptible to PD (Olmo 1979). One commercial vineyard (V11; Fig. 2.1, Table 2.1) growing American bunch grapes (*Vitis labruscana* L.) was monitored in 2006 and 2007. A second *V.* x *labruscana* vineyard (V12; Fig. 2.1, Table 2.1) was included in 2007. Resulting data from vineyards growing American varieties is reported separately from that of vineyards growing European varieties because of variation in timing of grapevine phenology and in vineyard management (American varieties generally require less maintenance than European varieties).

Yellow sticky traps (22.9 x 27.9 cm unbaited Pherocon AM; Trécé Inc., Adair, OK) were hung at each site April-October 2006 and March-October 2007. Trapping began one month earlier in 2007 since sharpshooters were captured in April 2006 traps. Six traps were hung at each site and replaced approximately every two weeks. Three rows were monitored through the season; two opposite edge rows and one middle row, which was located at least ten rows from any edge. Those traps hung on edge rows were oriented inward (Fig. 2.2) so as to avoid attracting insects from areas outside managed vines.

Yellow sticky traps were examined under a dissecting microscope in the lab. Cicadellids were counted and keyed to subfamily or species (when possible) and preserved in ethanol for systematic identification by the USDA Systematic Identification Lab (Beltsville, MD). Reference specimens are located in the Virginia Tech Insect Collection (Blacksburg, VA).

Because sharpshooter feeding behavior and movement are directed by vine phenology, trap collection numbers were associated with growing degree days accumulated by the beginning of each trapping period. Growing degree days after January 1st were calculated from daily minimum and maximum temperatures collected from the nearest NOAA weather station for each location. Growing Degree Days = $((T_{max} + T_{min})/2) - 10^{o}C$, $10^{o}C$ minimum threshold, 32 ^{o}C maximum threshold.

For comparison of trap captures *across the growing season within a site*, analysis of variance with repeated measures was carried out using JMP (SAS Institute, Cary, NC). For comparison of trap captures *during single trapping periods* or a comparison of *total number trapped*, a standard ANOVA was carried out using JMP (SAS Institute, Cary, NC).

Clear sticky interception traps (72.6 x 152.4 cm plexiglass™ coated with STP® gas treatment to act as an adhesive; Armor All/STP Products Co., Boca Raton, FL) were hung at two sites during the 2006 growing season in an attempt to monitor sharpshooter activity without the visual attraction of a yellow sticky trap. At one Southern Piedmont site (V10) two traps were hung 1m above ground level at center of trap in one edge row and two traps in one middle row. At an additional Eastern Shore site, two traps were hung at 1 m at center of trap within the managed vineyard and two outside the managed vineyard in a wooded habitat (i.e. a likely sharpshooter overwintering habitat). Traps were maintained every week, observed for sharpshooters, cleaned and painted with a fresh coat of gas treatment. This method was not repeated in 2007 as there were no sharpshooters captured in any of the clear sticky interception traps and because of the great labor required to maintain them.

Olson yellow sticky traps (15.2 x 30.5 cm; Olson Products, Medina, OH) were hung in three vineyards in 2007 in an attempt to compare trapping efficiency to that of Pherocon traps. Olson traps were found to be more effective in trapping western corn rootworm than Pherocon traps by Youngman et al. (1996). This type of trap is not practical for use in commercial vineyards as the force of output from air-blast sprayers is enough to break these plastic traps and knock them to the vineyard floor, and will not be discussed further.

2.3 Results and Discussion

Several species of known and potential PD vectors were trapped at every collection site in 2006 and 2007 (Tables 2.2-4). *G. versuta* and *O. orbona* were trapped in greatest abundance among all potential vector species in *V. vinifera* vineyards (Tables 2.2 and 2.3). Both species were trapped at every region in both years (Figs. 2.3 and 2.4).

The total number of *G. versuta* trapped within nearly all vineyards was significantly higher than the total number of any other species at that site (Figs. 2.3 and 2.4). Coastal Plain sites are exceptions because, even though the total number of *G. versuta* trapped was numerically higher than any other species in 2006 and 2007, this difference was not significant in either year. This is most likely because generally low numbers of all leafhoppers were trapped at those sites.

Although *O. orbona* was trapped in lower numbers than *G. versuta,* it occurs within the vineyard at a critical time in PD infection (i.e. early in the season), therefore *O. orbona* is of greater concern. Proconiini are conspicuous on yellow sticky traps and may

present an effective searching image for those vineyard managers wishing to monitor sharpshooter populations.

Graphocephala versuta was trapped in numbers significantly greater than any other species but, since Cicadelliini prefer to feed on leaves rather than the tougher shoots of grapevines, this species is of less concern as a PD vector. An infection introduced to the plant farther from the cordon and later in the season is more likely to be removed during winter pruning.

Graphocephala coccinea (Forster) is distributed throughout the Northeast U.S. (Lowry 1922, Stearns 1927) and was trapped at all sites in 2006 and 2007. *G. coccinea* is considered a potential PD vector as presence of *Xf* has been confirmed in the mouthparts of assayed individuals (Pooler et al. 1997). However, *G. coccinea*'s capability of transmission to grape is unknown.

Paraulacizes spp. was trapped in several vineyards and is considered a potential vector as it is of the same tribe as GWSS and *Oncometopia*, the Proconiini, but efficiency of transmission to grape is unknown. This species was recorded earlier in Virginia, under the synonym *Aulicizes* spp. (Stearns 1927). This insect was trapped only in the early season and only in colder climates of the Blue Ridge, Northern and Central Piedmont (Fig. 2.29).

Draeculacephala spp. are widely distributed from Alaska to Argentina (Wilson and Claridge 1991) and prefer to feed on grasses (Purcell and Frazier 1985). Several members of *Draeculacephala* spp. have been confirmed as capable PD vectors (Severin 1949, Purcell 1980). My surveys trapped very low numbers of Draeculacephala spp.

within Virginia vineyards (Tables 2.2 and 2.3) but were, nonetheless, a species of consideration.

Homalodisca insolita (Walker) was trapped in the Coastal Plain (V3) in 2007, the most northern record of this species, a congener to GWSS (*H. coagulata*). *H. insolita* is a capable vector (Nielson 1968) but its efficiency in comparison to native Virginia vectors is unknown. GWSS has also recently expanded its range northward within the coastal plain of North Carolina (T.B. Sutton, personal communication). The range expansion of either of these species could add to the vector complex in Virginia and could also be another indication of climate change in addition to detection of *Xf* in western portions of Virginia (Wallingford et al. 2007).

There was no significant difference ($\alpha = 0.05$) across the growing season between numbers of sharpshooters trapped in any row (Edge A, Edge B, Middle) in the majority of sites. Exceptions follow: At Vineyard 5, significantly more *G. versuta* were trapped in wooded edge row traps and middle row traps than in grassy edge row traps across the 2006 growing season (F = 45, Df = 2, 3, p-value = 0.0059), but there was no significant difference between rows in 2007. At Vineyard 9, significantly more *G. versuta* were trapped across the growing season in the wooded edge row and middle row than in the grassy edge row in 2006 (F = 11, Df = 2, 3, p-value = 0.0408) and 2007 (F = 17, Df = 2, 3, p-value = 0.0232). At Vineyard 3, significantly more *O. orbona* were trapped across the 2006 growing season in the wooded edge row and the middle row than in the row bordering mix of grass and trees (F = 6.5, Df = 2, 3, p-value = 0.0028) but there was no significant difference between rows in 2007.

At most sites the area monitored was small (1-2 hectares), which is reflective of a typical Virginia vineyard. Because of the short distance between monitored rows and the long trapping period (14 days), it is not surprising that the data did not reveal many differences in insect activity between rows. It does appear, however, that wooded environments support a larger population of sharpshooters than grassy environments. Forest debris adding to the suitability of an over-wintering site and a wider diversity of host plants in wooded environments are possible factors contributing to higher populations of sharpshooters trapped.

Oncometopia orbona were first trapped around bud break in early April (~100-200 DD) in 2006 and 2007. In both years, peak capture occurred mid-May to end of June (~300-900 DD), but *O. orbona* was rarely trapped after July (~1500 DD; Figs. 2.5, 2.6). As lab populations of *O. orbona* can complete at least two generations a year (Turner and Pollard 1959), it is likely that the subsequent generation migrates to other host plants, perhaps searching out optimal nutrition.

Also, insect pest management likely contributes to a decline in numbers of sharpshooters trapped in the later summer. For many Virginia vineyard managers, lepidopteran pests are the main concern in the early months of the growing season and there are a wide range of products utilized for control of these pests that are not lethal to leafhoppers. Later in the summer, when Japanese beetle (*Popillia japonica* Newman) control is a concern, managers will use broad-spectrum insecticides that may contribute to reduction of leafhopper populations. Vineyard 1, on the Eastern Shore, is the only site out of all ten that did target chemical sprays for sharpshooters in the spring. The effect of

management is reflected as sharpshooters trapped across the season were lower at this vineyard than other sites and there was no clear peak capture (Figs. 2.9, 2.10).

There were significantly more *O. orbona* were trapped across the 2006 growing season than the 2007 growing season at four sites: V1 (F = 7, Df = 1, 10, p-value = 0.0213; Fig. 2.9), V2 (F = 5, Df = 1, 8, p-value = 0.0434; Fig. 2.11), V8 (F = 12, Df = 1, 10, p-value = 0.0062; Fig. 2.23), and V9 (F = 11, Df = 1, 10, p-value = 0.0085; Fig. 2.25). The majority of *O. orbona* trapped within managed vineyards were trapped in the early months (over-wintered adults); the late frost that occurred in April 2007 could explain the difference in numbers trapped. At V10 (Fig. 2.27), a relatively new vineyard planted summer 2005, significantly more *O. orbona* were trapped across the 2007 growing season than the 2006 growing season (F = 5, Df = 1, 10, p-value = 0.0488). At all other sites (V3, V4, V5, and V6), there was no significant difference between growing seasons.

Peak capture of *G. versuta* was later than that of *O. orbona* in 2006 and 2007, June through August (~500-1500 DD). However, *G. versuta* were trapped throughout the growing season at all sites in both years. First capture usually occurred before bud break (~0-100 DD) in edge row traps, as early as the first week of March in some sites, and *G. versuta* were trapped on the last collection period of the year in nearly all sites in 2006 and 2007 (Figs. 2.7, 2.8).

There were significantly more *G. versuta* trapped across the 2007 growing season than the 2006 growing season at three locations: V1 (F = 14, Df = 1, 10, p-value = 0.0035; Fig. 2.10), V5 (F = 3, Df = 1,8, p-value = 0.0225; Fig. 2.18), and V10 (F = 9.5, Df = 1, 9, p-value = 0.0130; Fig. 2.28). The majority of *G. versuta* trapped within

managed vineyards were trapped in summer months (offspring of over-wintered adults). It was unexpected to see higher numbers of *G. versuta* across the 2007 growing season, following the late frost in April 2007; there is a possibility that natural enemies were also negatively affected, resulting in reduced parasitization or predation of those eggs laid by over-wintered adults. At V8 (Fig. 2.24) significantly more *G. versuta* were trapped across the 2006 growing season than across the 2007 growing season (F = 16, Df = 1, 10, p-value = 0.0330). At all other sites (V2, V3, V4, V6, V7, V9) analysis of variance with repeated measures revealed no significant different in *G. versuta* trapped in 2006 versus 2007.

Several species of potential and capable vectors were trapped in *V.* x *labruscana* vineyards. Like other sites, *G. versuta* was trapped in the greatest abundance, but *O. orbona* was ranked third after *G. coccinea* and the Coelidinae (Table 2.4).

2.4 Conclusion

There are capable PD vectors present in every grape growing region of Virginia. When cold temperatures (lethal to the bacterium) are not experienced, the early season is the time of greatest concern to protect vines from all sharpshooter species flying within the vineyard. Individuals feeding in early months (March-May) are more likely to carry the bacteria than those vectors flying in the summer and will introduce infection closer to the cordon, early in the season, allowing for maximal time for bacterial proliferation.

Oncometopia orbona and *G. versuta* are the species of greatest concern as they are both capable vectors of *Xf* and are both present in all growing regions. *O. orbona* occur in lower numbers than *G. versuta,* however, since *O. orbona* are captured within

the vineyard at a critical time for PD infection (i.e. early in the season) they are of greater concern. Proconiini are conspicuous on yellow sticky traps which should aid commercial managers wishing to monitor sharpshooter populations.

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2.6 Tables and Figures

Table 2.1: Sharpshooter collection sites (V1-V12), locations, elevation and description of edge habitats near traps.

Vineyard #	Region	County	Latitude	Longitude	Elevation (m)	edge A	edge B
V1	E. Shore	Northampton	37° 34.153'	75° 53.307'	4	field crops	field crops
V2		Accomack	37° 49.208'	75° 37.442'	9	field crops	field crops
V3	Coastal	Williamsburg	37° 14.112'	76° 43.240'	21	wooded	grass/trees
V4		Williamsburg	37° 14.112'	76° 43.240'	21	mown grass	mown grass
V5	N. Piedmont	Fauquier	38° 53.280'	78° 04.059'	404	wooded	mown grass
V6		Fauquier	38° 55.697'	78° 00.070'	240	wooded	mown grass
V7	C. Piedmont	Albemarle	38° 02.237'	78° 47.487'	257	pasture	wooded
V8		Albemarle	38° 00.694'	78° 51.457'	300	mown grass	wooded
V9	S. Piedmont	Patrick	36° 44.027'	80° 11.732'	494	pasture	wooded
V10		Patrick	36° 34.677'	80° 07.226'	332	pasture	pasture
V11	Blue Ridge	Floyd	36° 46.958'	80° 23.723'	942	pasture	wooded
V12		Augusta	38° 04.034'	75° 58.017'	451	wooded	wooded

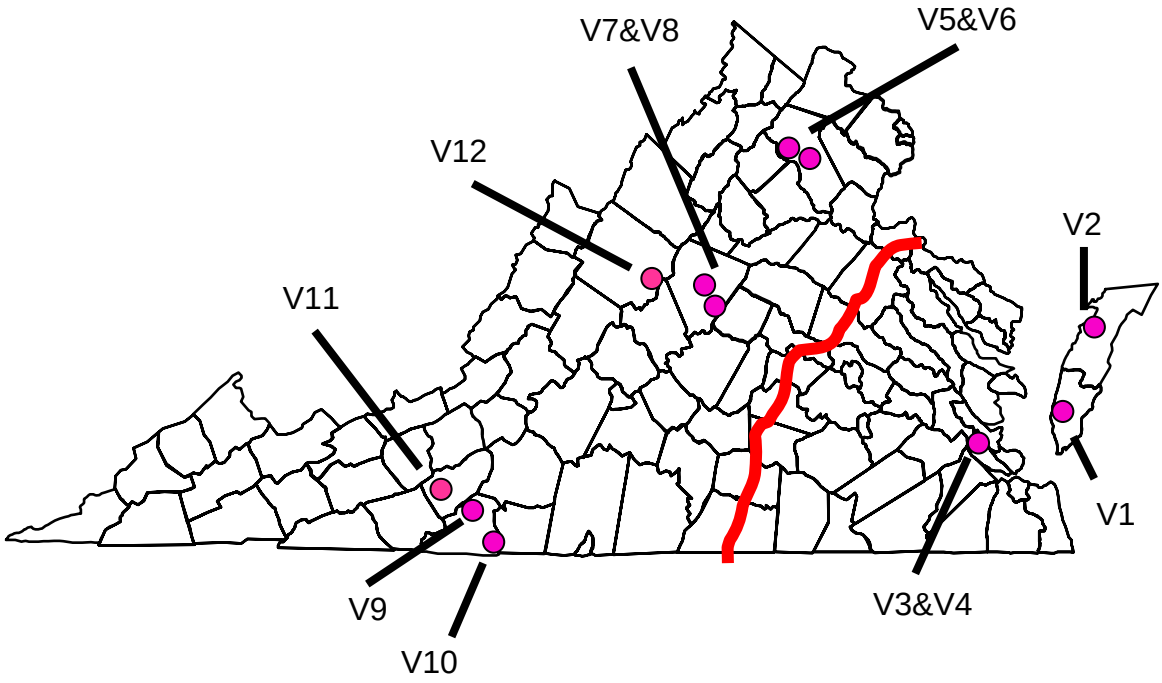

Figure 2.1: Sharpshooter collections sites (V1-V12) are indicated by pink dots. The red line indicates a risk isoline based on 30-year average of minimum winter temperatures (Feil and Purcell 2001); all regions west of this line are outside the historical boundary of clinical Pierce's disease.

Figure 2.2: Orientation of yellow sticky traps on edge rows in Virginia vineyards surveyed for Pierce's disease vectors. Image: A.K. Wallingford

Table 2.2: Leafhopper species (Cicadellidae) captured in *V. vinifera* vineyards (V1-V10) April-October 2006, using yellow sticky traps (Typhlocybinae excluded as they are mesophyll-feeders).

		2006	
Subfamily	Species	Total trapped in 10 vineyards	Percentage from all Cicadellidae trapped
Cicadellinae (potential vectors)	*Graphocephala versuta* (Say)**	9719	82.81%
	Oncometopia orbona (Fabr.)**	811	6.91%
	Graphocephala coccinea (Forster)*	72	0.61%
	Paraulacizes spp.	61	0.52%
	Draeculacephala spp.**	13	0.11%
	Sibovia spp.	2	0.02%
	Cuerna spp.**	1	0.01%
Agallinae	*Agallia* spp.	485	4.13%
Aphrodinae	*Aphrodes* spp.	2	0.02%
Deltocephalinae	*Paraphlepsius* spp.*	284	2.42%
	Scaphytopius spp.	147	1.25%
	Osbornellus spp.	20	0.17%
	Colladonus spp.	12	0.10%
	Penthimia americana (Fitch)	1	0.01%
Coelidinae	Unknown	69	0.59%
Gyponinae	Unknown	38	0.32%

** indicates those species that are documented as capable PD vectors

* indicates species documented to carry *Xf* in mouthparts but not a capable PD vector or capability is unknown

Table 2.3: Leafhopper species (Cicadellidae) captured in *V. vinifera* vineyards (V1-V10) March-October 2007, using yellow sticky traps (Typhlocybinae excluded as they are mesophyll-feeders).

2007			
Subfamily	Species	Total trapped in 10 vineyards	Percentage from all Cicadellidae trapped
Cicadellinae (potential vectors)	*Graphocephala versuta* (Say)**	12924	84.67%
	Oncometopia orbona (Fabri)**	401	2.63%
	Graphocephala coccinea (Forster)*	60	0.39%
	Paraulacizes spp.	36	0.24%
	Draeculacephala spp.**	9	0.06%
	Homolodisca insolita (Walker)**	3	0.02%
	Sibovia spp.	3	0.02%
Agallinae	*Agallia* spp.	698	4.57%
Aphrodinae	*Aphrodes* spp.	7	0.05%
Deltocephalinae	*Scaphytopius* spp.	589	3.86%
	Paraphlepsius spp.*	163	1.07%
	Osbornellus spp.	42	0.28%
	Colladonus spp.	8	0.05%
	Penthimia americana (Fitch)	4	0.03%
Gyponinae	Unknown	165	1.08%
Coelidinae	Unknown	152	1.00%

** indicates those species that are documented as capable PD vectors

* indicates species documented to carry *Xf* in mouthparts but not a capable PD vector or capability is unknown

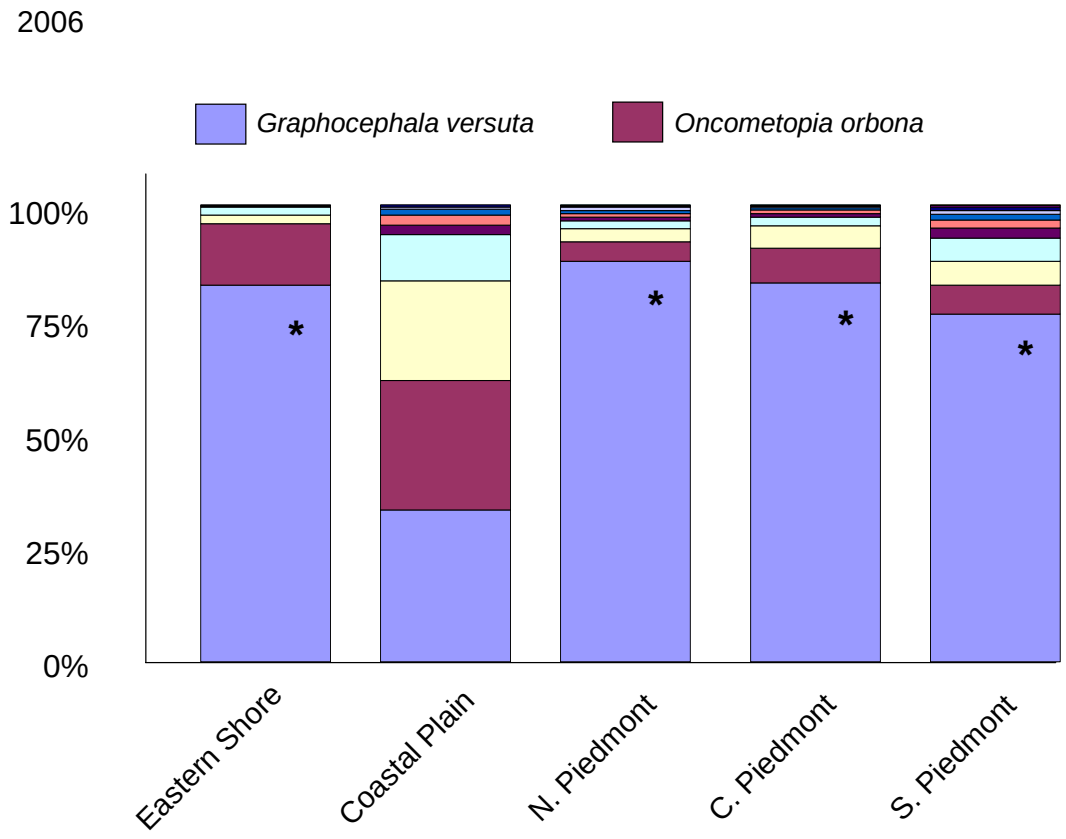

Figure 2.3: Total leafhoppers trapped April-October 2006, by region, as a percentage, in order of greatest to least abundant (mesophyll-feeding Typhlocybinae excluded).
* indicates significant difference ($\alpha = 0.05$).

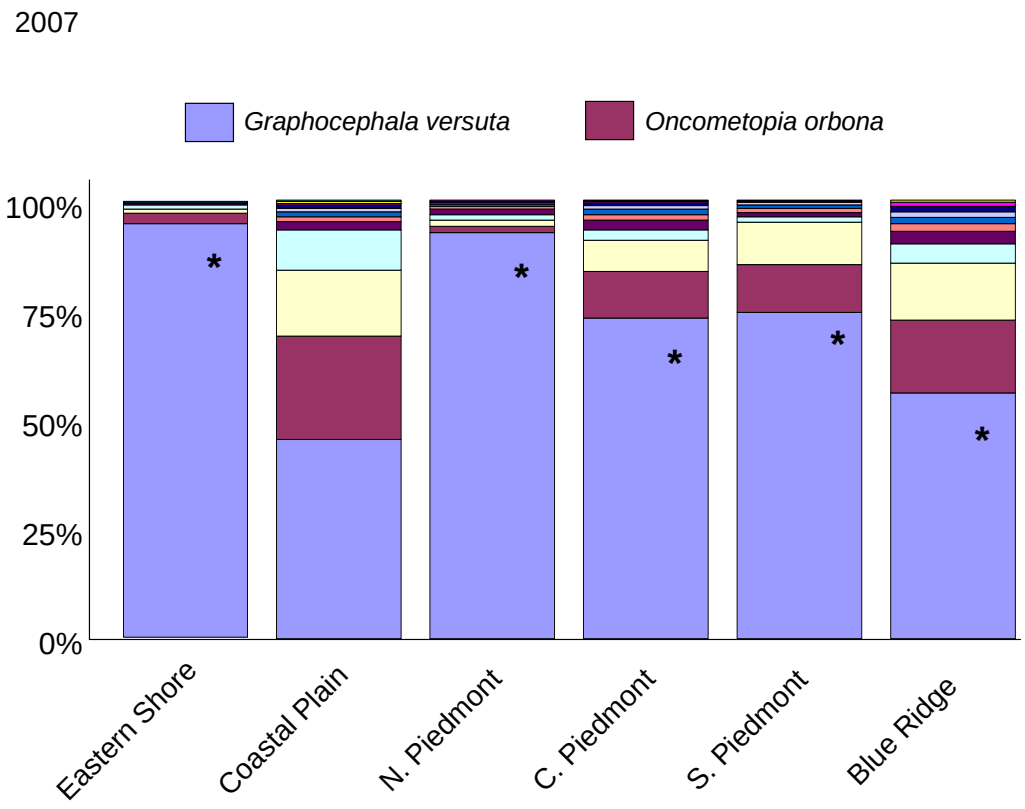

Figure 2.4: Total leafhoppers trapped March-October 2007, by region, as a percentage, in order of greatest to least abundant (mesophyll-feeding Typhlocybinae excluded).
* indicates significant difference ($\alpha = 0.05$).

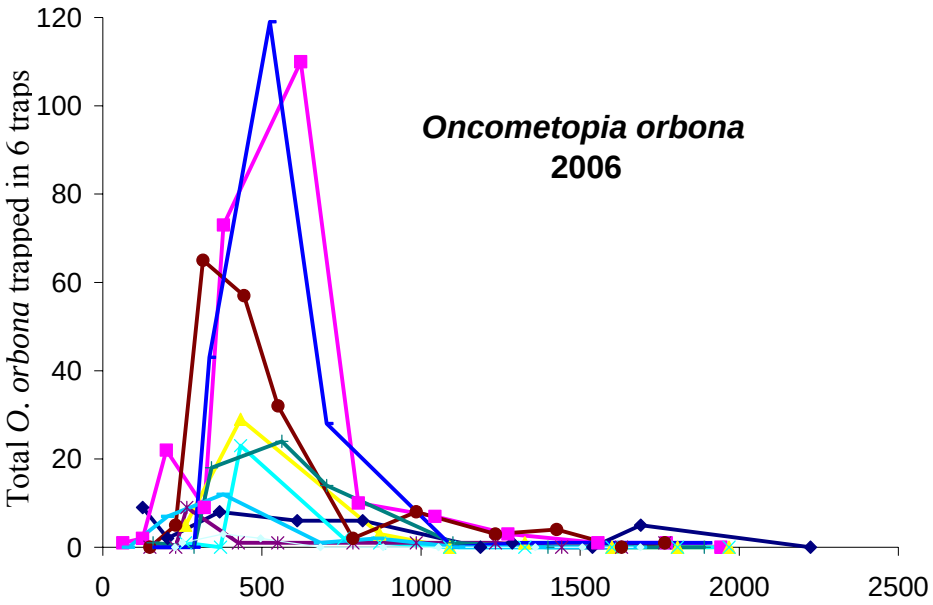

Figure 2.5: *Oncometopia orbona* trapped in all six traps at each *V. vinifera* site (April-October 2006, Vineyards 1-10). Growing degree days after January 1st = ((Tmax + Tmin)/2 – 10°).

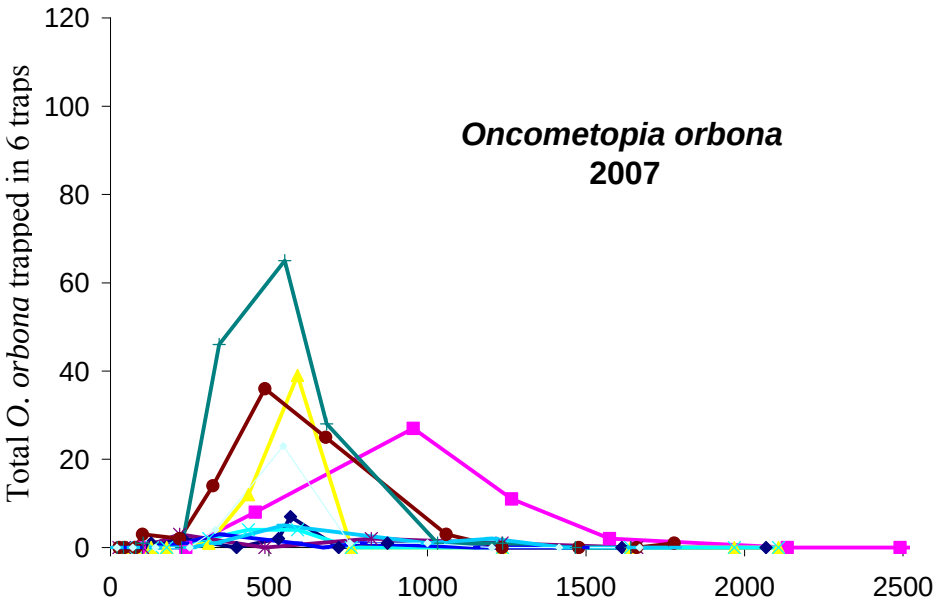

Figure 2.6: *Oncometopia orbona* trapped in all six traps at each *V. vinifera* site (March-October 2007, Vineyards 1-10). Growing degree days after January 1st = ((Tmax + Tmin)/2 – 10°C).

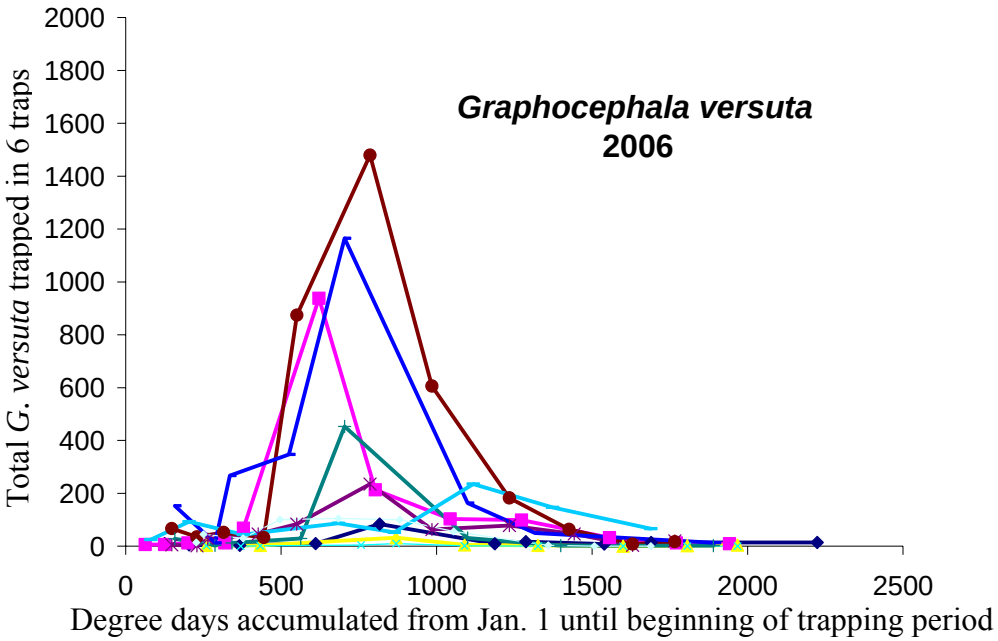

Figure 2.7: *Graphocephala versuta* trapped in all six traps at each *V. vinifera* site (April-October 2006, Vineyards 1-10). Growing degree days after January 1st = ((Tmax + Tmin)/2 – 10°C).

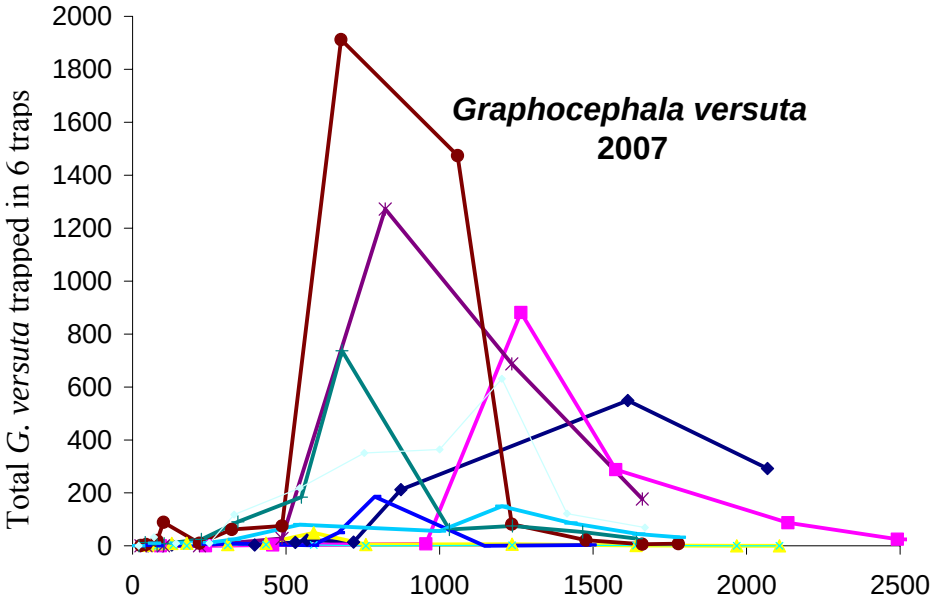

Figure 2.8: *Graphocephala versuta* trapped in all six traps at each *V. vinifera* site (April-October 2006, Vineyards 1-10). Growing degree days after January 1st = ((Tmax + Tmin)/2 – 10°C).

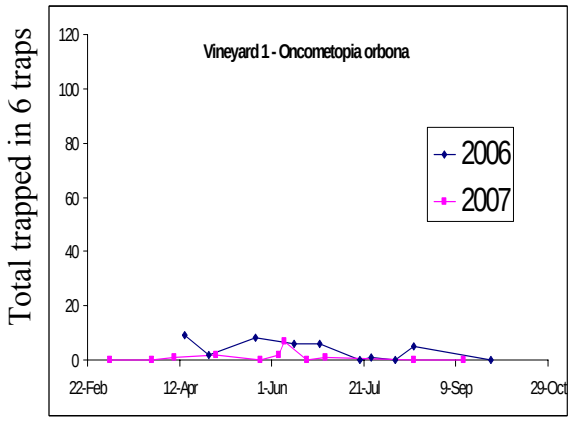

Figure 2.9: Vineyard 1, Eastern Shore Total *O. orbona* for each trapping period

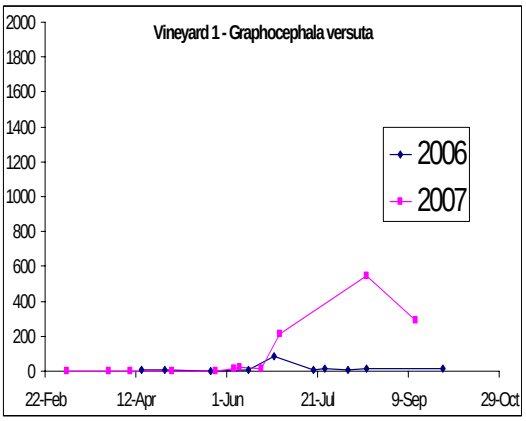

Figure 2.10: Vineyard 1, Eastern Shore Total *G. versuta* for each trapping period

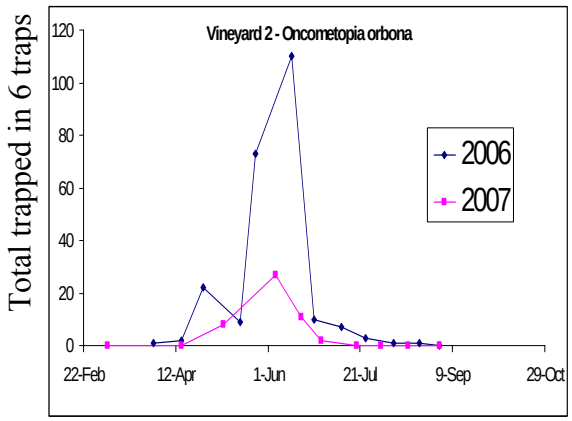

Figure 2.11: Vineyard 2, Eastern Shore Total *O. orbona* for each trapping period

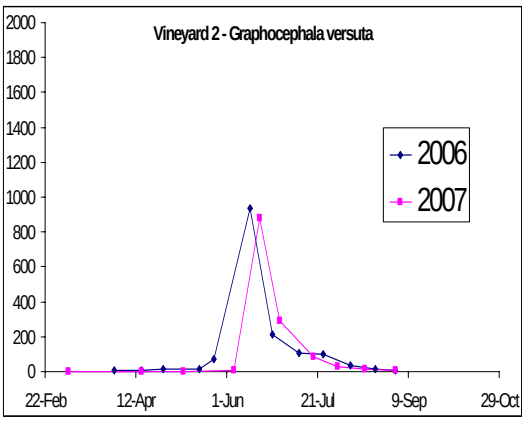

Figure 2.12: Vineyard 2, Eastern Shore Total *G. versuta* for each trapping period

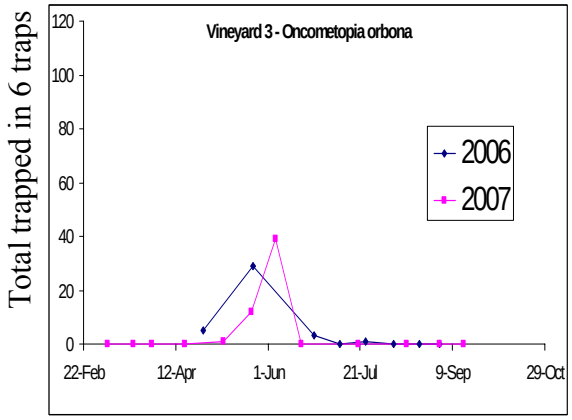

Figure 2.13: Vineyard 3, Coastal Plain Total *O. orbona* for each trapping period

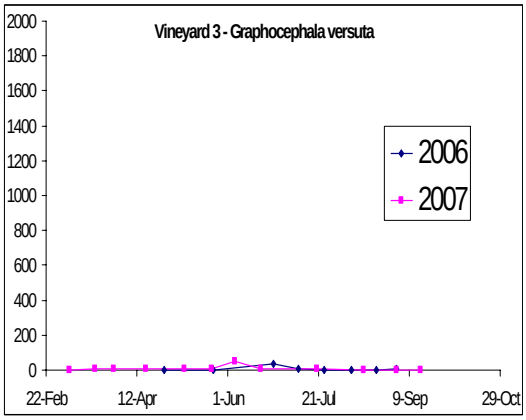

Figure 2.14: Vineyard 3, Coastal Plain Total *G. versuta* for each trapping period

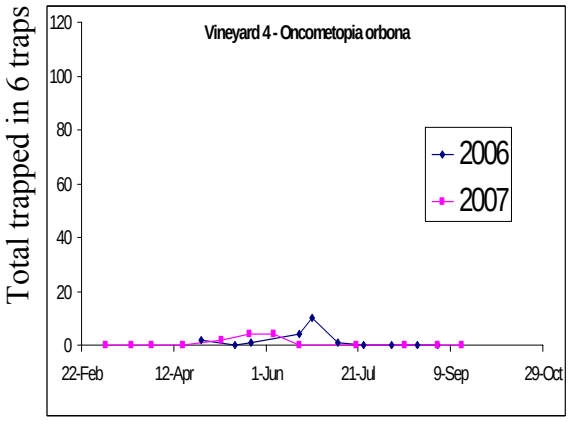

Figure: 2.15: Vineyard 4, Coastal Plain
Total *O. orbona* for each trapping period

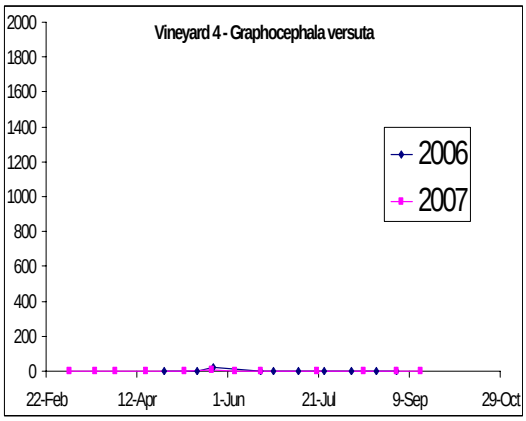

Figure 2.16: Vineyard 4, Coastal Plain
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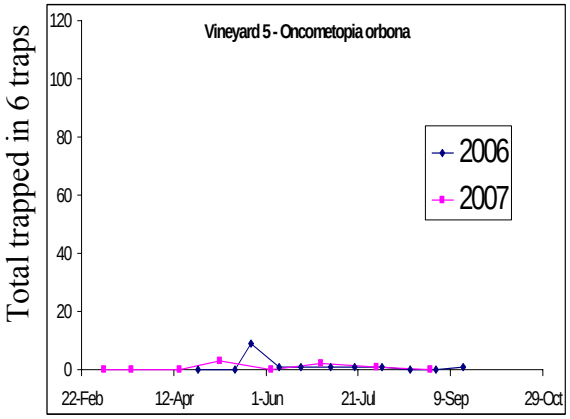

Figure 2.17: Vineyard 5, N. Piedmont
Total *O. orbona* for each trapping period

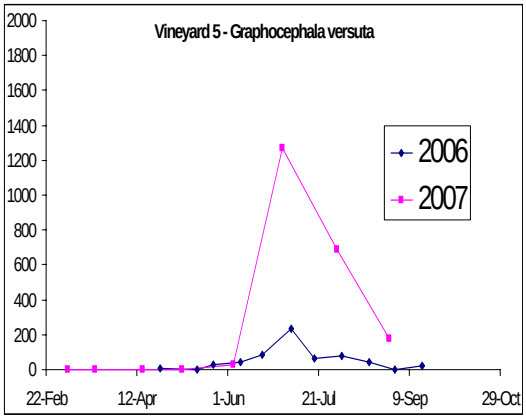

Figure 2.18: Vineyard 5, N. Piedmont
Total *G. versuta* for each trapping period

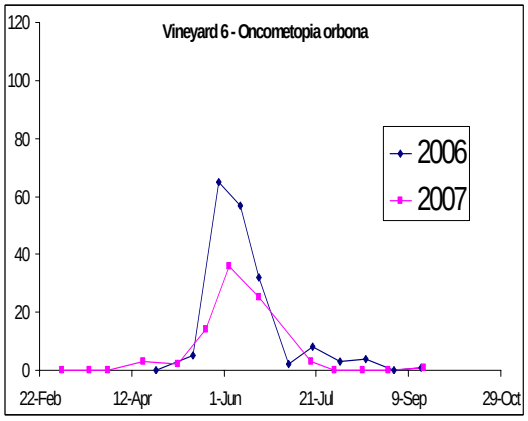

Figure 2.19: Vineyard 6, N. Piedmont
Total *O. orbona* for each trapping period

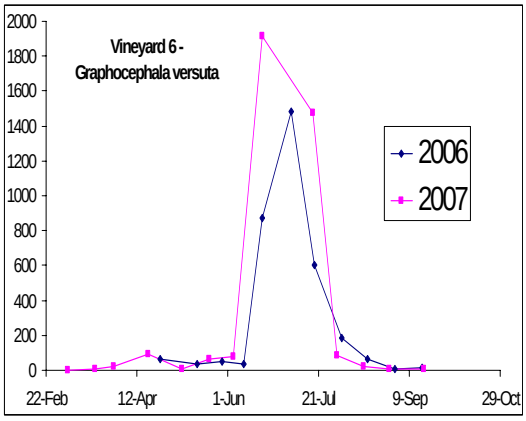

Figure 2.20: Vineyard 6, N. Piedmont
Total *G. versuta* for each trapping period

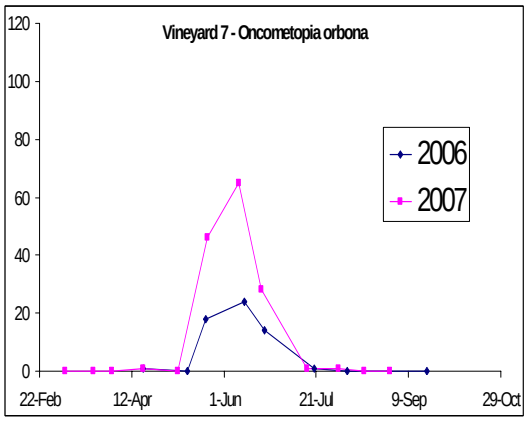

Figure 2.21: Vineyard 7, Central Piedmont Total *O. orbona* for each trapping period

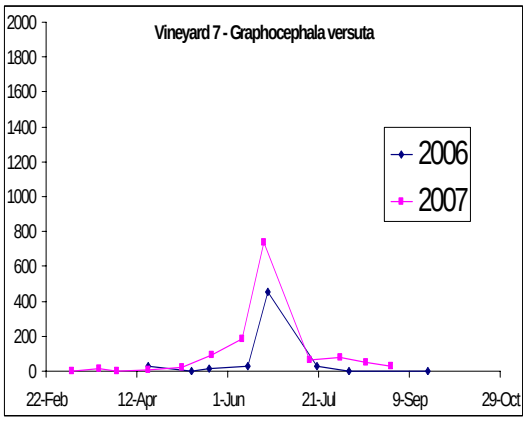

Figure 2.22: Vineyard 7, Central Piedmont Total *G. versuta* for each trapping period

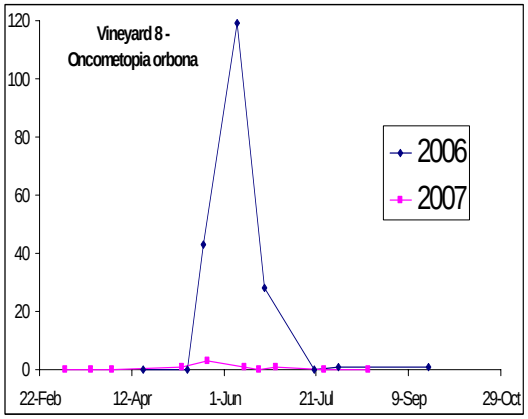

Figure 2.23: Vineyard 8, Central Piedmont Total *O. orbona* for each trapping period

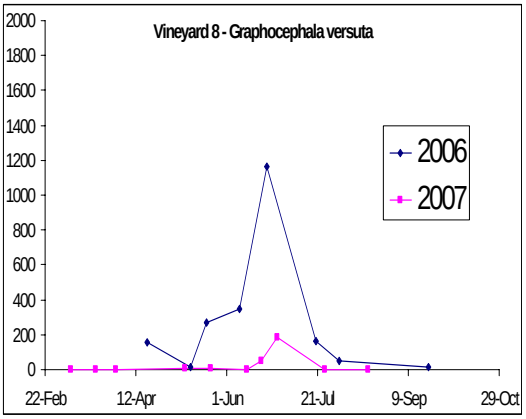

Figure 2.24: Vineyard 8, Central Piedmont Total *G. versuta* for each trapping period

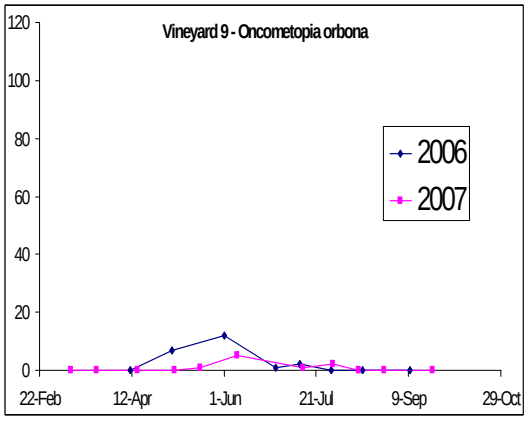

Figure 2.25: Vineyard 9, S. Piedmont Total *O. orbona* for each trapping period

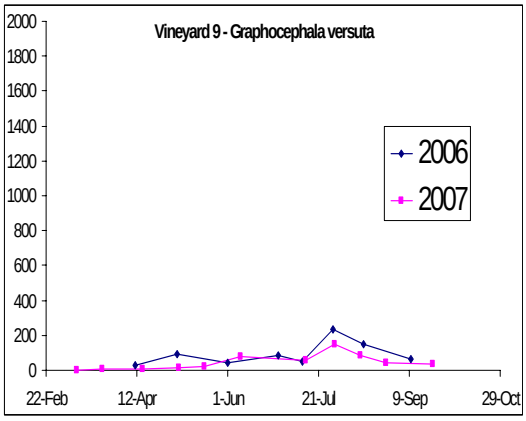

Figure 2.26: Vineyard 9, S. Piedmont Total *G. versuta* for each trapping period

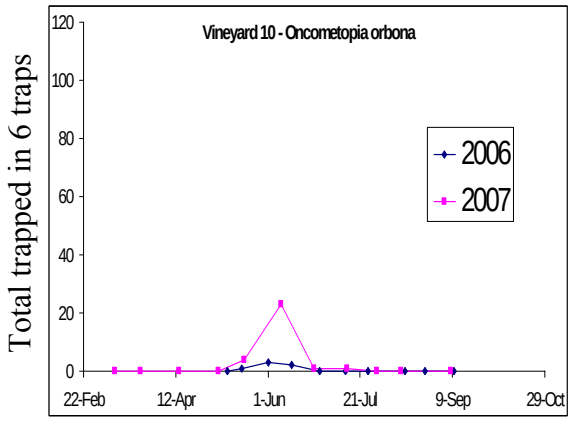

Figure 2.27: Vineyard 10, S. Piedmont Total *O. orbona* for each trapping period

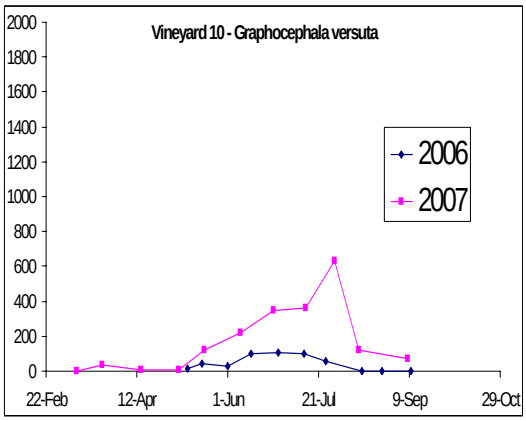

Figure 2.28: Vineyard 10, S. Piedmont Total *G. versuta* for each trapping period

Table 2.4: Leafhopper (Cicadellinae) species captured in *V. labrusca* vineyards (V11 and V12) March – October 2007, using yellow sticky traps (Typhlocybinae excluded).

		2007	
Subfamily	Species	Total trapped in 2 sites	Percentage of Cicadellidae
Cicadellinae	*Graphocephala versuta* (Say)**	1236	56.03%
	Graphocephala coccinea (Forster)*	367	16.64%
	Oncometopia orbona (Fabr.)**	39	1.77%
	Paraulacizes spp.	29	1.31%
Agallinae	*Agallia* spp.	62	2.81%
Deltocephalinae	*Scaphytopius* spp.	97	4.40%
	Paraphlepsius spp.*	31	1.41%
	Osbornellus spp.	23	1.04%
	Collanadus spp.	9	0.41%
	Penthimia Americana (Fitch)	1	0.05%
Coelidinae	Unknown	286	12.96%
Gyponinae	Unknown	26	1.18%

** indicates those species that are documented as capable PD vectors

* indicates species documented to carry *Xf* in mouthparts but not a capable PD vector or capability is unknown

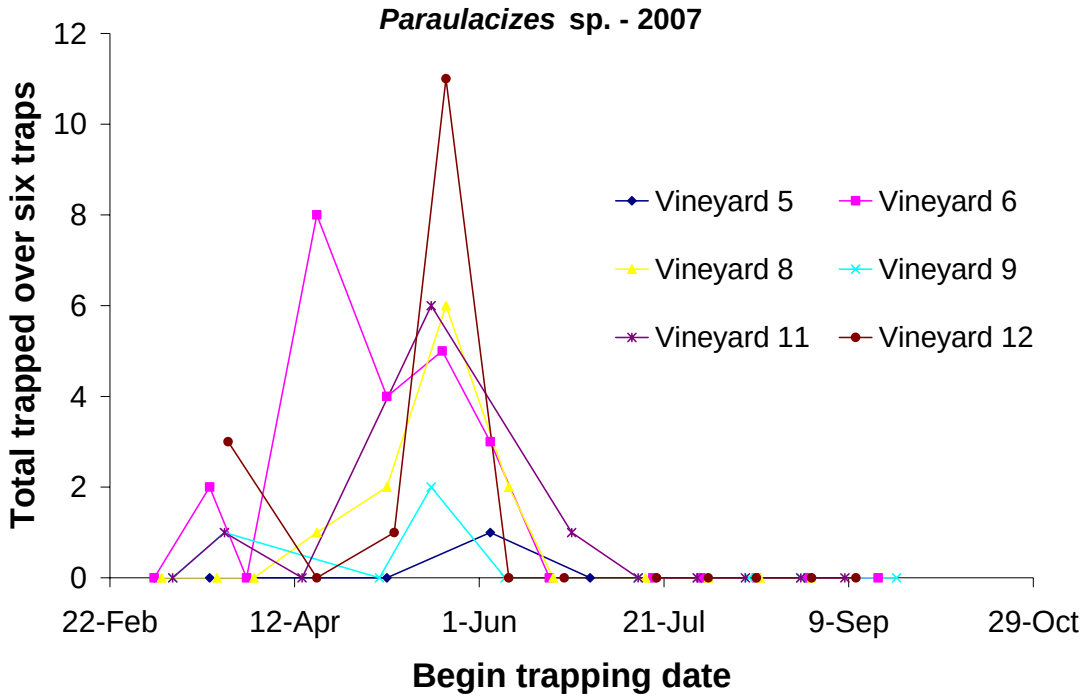

Figure 2.29: *Paraulacizes* spp. trapped in 2007 (March- October). Sites where *Paraulacizes* spp. were not trapped are excluded.

CHAPTER THREE

A Survey of *Xylella fastidiosa* in Virginia vineyards

3.1 Introduction

Pierce's disease (PD) is a vascular disease of grapes caused by the xylem-limited bacterium *Xylella fastidiosa* (Wells et al.) (*Xf*) and is transmitted by xylophagous auchenorrhynchan vectors, primarily from the families Cicadellidae (Frazier and Freitag 1946) and Cercopidae (Severin 1950). PD infection in Virginia vineyards was thought to be isolated to southeastern portions of the state. There have been no reports of vine loss in western Virginia and cold winter temperatures limit the effects of the bacterium from year to year (Hopkins and Purcell 2002). However, upward trends in winter temperatures have raised concerns over PD among grape growers in the mid-Atlantic region (Sutton 2005).

Symptoms of PD occur when bacteria proliferate within the xylem; both the bacteria (Newman et al. 2003) and host responses to infection (Stevenson et al. 2004) block the flow of xylem fluid to the shoots. Infection leads to vine decline, yield loss (Fig. 3.1), and, within two to three years, vine death (Gubler et al. 2006). Affected grapevines show symptoms related to water deficits, like interveinal chlorosis and marginal necrosis with marginal yellow or red lines (Fig.3.2; Hopkins 1989). Symptoms that are specific to *Xf* infected plants are green islands (Fig. 3.3) on shoot bark after normal tissue turns brown, and leaf abscission from the distal end of petioles, leaving characteristic "matchstick petioles" with necrotic tips (Fig. 3.2; Thorne et al. 2006).

Optimal temperature for *Xf* development falls between 25-32^{o}C and temperatures below 12^{o}C or above 34^{o}C may negatively affect survival in plants (Feil and Purcell

2001). Based on historical temperature data, Sutton (2005) found little PD incidence in regions that experience three or more days below -12.2^{o}C or five or more days below -9.4^{o}C, during the dormant period. According to Sutton's standard (Table 3.1), vineyards that consistently experience an adequate number of cold days, areas that have experience the "cold temperature requirement," are considered at low to no risk of PD. Figure 3.4 shows a geographic representation of general risk zones in Virginia according to this scale.

Xylella fastidiosa has a patchy distribution within the grapevine and there is no clear relationship between bacterial population and symptom development (Gambetta et al. 2007); however, sampling late in the season will ensure highest possible titer within the plant and sampling matchstick petioles from portions of the plant closest to the cordon will increase the probability of *Xf* detection (Krell et al. 2006).

Incidence of PD infection in California vineyards is highest in edge rows and particularly those rows that border sharpshooter overwintering habitats, i.e. riparian vegetation (Purcell 1974). Sharpshooters entering the vineyard in early spring (April/May) are more likely to be infective than sharpshooters feeding in the summer (Myers et al. 2007). Also *Xf* populations are more likely to persist in wild host plants that act as sources of inoculum for new vectors (Baumgartner and Warren 2005).

Enzyme-Linked ImmunoSorbent Assay (ELISA) uses antibodies that bind to proteins on the outer wall of *Xf* to detect its presence or absence in a sample. Unfortunately, ELISA does not separate individual strains of *Xf*, as is possible when using polymerase chain reaction (PCR), but it can be assumed that when *Xf* is detected in grape, the PD strain of *Xf* is involved. Commercial ELISA kits are available and this

method of detection is more economical and efficient than PCR. ELISA has been found to be equally effective as PCR in detecting *Xf* in almond (Groves et al. 2005).

This study seeks to survey grape-growing regions of Virginia for the presence or absence of the causal agent of PD, *X. fastidiosa*.

3.2 Materials and Methods

Ten commercial vineyards (V1-V10), each growing several varieties of European bunch grapes (*Vitis vinifera* L.), were monitored in 2006 and 2007. In both years, bud-break occurred approximately the first week of April at V1-V4, V7, V8 and V10 (Fig. 3.1, Table 3.2). A late frost in the first week of April 2007 damaged many vines at these sites. Timing of the second flush of growth in 2007 was variable as vine loss was not uniform even within each site. In 2006 and 2007, bud-break occurred approximately the second week of April at V7-V9 (Fig. 3.1, Table 3.2). Although Vineyards 9 and 10, in the Southern Piedmont, are within close proximity to each other, V9 is at a higher elevation than V10 so bud-break occurred a little later at V9 than V10. Little frost damage was seen at these sites where bud-break occurred after the late frost in April 2007. Coastal Plain sites (V3 and V4) experienced severe hail damage in July 2007. To maintain the plant health, vineyard managers removed that year's growth in the blocks I used for this survey.

American grape species are more tolerant to PD than European grapes; however, cultivated American varieties are considered susceptible to PD (Olmo 1979). One commercial vineyard (V11; Fig. 3.1, Table 3.2) growing American bunch grapes (*Vitis labruscana* L.) was monitored in 2006 and 2007. A second *V.* x *labruscana* vineyard (V12; Fig. 3.1, Table 3.2) was included in 2007. Resulting data from vineyards growing

American varieties is reported separately from that of vineyards growing European varieties because of variation in timing of grapevine phenology and in vineyard management (American varieties generally require less maintenance than European varieties).

Ten vines displaying matchstick petioles and leaves with marginal necrosis were selected (Chardonnay when available) from edge rows of each site in once in October 2006 and once again in 2007. Two petiole samples were taken from each vine; one from portions close to the cordon and one from portions distal to the cordon. Vineyards 3 & 4 were treated as one site because of their proximity. For the first two sites sampled in October 2006 (V1 and V2), any petiole from a symptomatic vine was used in ELISA tests, regardless of the condition of that tissue. For the rest of 2006 sampling and all of 2007 sampling, only symptomatic tissue (i.e. matchstick petioles) was used for ELISA tests as this procedure revealed more consistent results.

Vines confirmed to be *Xf* positive in October 2006 were revisited in April/June 2007 and again in October 2007, and were observed for PD symptoms and assayed for the presence of *Xf*.

At one location, grower concern was expressed over yellow sticky traps concentrating sharpshooter activity on neighboring plants, so the vines closest to each of the six yellow sticky traps hung within each vineyard were observed for PD symptoms in October 2007 and presence or absence of *Xf* was determined using ELISA. In addition, thirty vines were randomly selected at each site to act as checks, observed for PD symptoms and assayed for the presence of *Xf*.

Each sample, three petioles cut and weighed to 0.3-0.5 g (average total length ~2cm), was ground in a mesh grinding bag with 3 ml of general extract buffer (Agdia, Inc, Elkhart, IN.). Each sample was then placed into two test wells ((100 µL each) and assayed for *Xf* using a DAS-ELISA PathoScreen kit (Agdia, Inc.). Absorbance at 650 nm was recorded with a Spectramax Plus (Molecular Devices, Sunnyvale, CA). Samples giving values greater than the mean absorbance of known negatives plus three times the standard deviation of known negatives were considered positive.

3.3 Results and Discussion

Pierce's disease symptoms were observed at every site scouted in 2006 and 2007, and several of these sites were beyond the expected boundary of PD occurrence, based on historical winter temperatures (Fig. 3.5; Feil and Purcell 2001, Hoddle 2004).

In October 2006, at least one vine was confirmed *Xf* positive using ELISA at each of the ten *V. vinifera* vineyards described in Table 3.2. Of the varieties sampled, at least one vine tested positive in each of the following varieties: Chardonnay, Merlot, Cabernet Franc, Cabernet Sauvignon, Petit Verdot, Vidal.

None of the previously confirmed *Xf* positive vines tested positive for *Xf* the following spring (April/June 2007). ELISA may not be sensitive enough to detect *Xf* colonies at the low density to be expected following winter. Also, none of the plant tissue sampled in the spring of 2007 was symptomatic; therefore, even if bacterial colonies were present within the vine, the chances of selecting that tissue in sampling were low.

In October 2007, at least one vine was confirmed positive using ELISA for presence of *Xf* in each region, but not at every site. The causal agent was not confirmed present in V5 in the Northern Piedmont. Although symptoms were observed at this site in October 2007, no sample tested *Xf* positive using ELISA, including the three vines that tested positive the previous fall. Another exception was V8 in the Central Piedmont, where none of the samples tested positive for *Xf* including two that tested positive the previous fall. Both V5 and V8 had experienced the cold temperature requirement, five or more days below -9.4^{o}C, necessary for limiting PD.

There were several vines that tested positive in 2006, but negative in 2007 (V3, V5, V6, V7, V8 and V9, Figs. 3.8-13 respectively) but there is no clear explanation for all "recoveries." Northern Piedmont sites (V5 and V6, Figs. 3.9 and 3.10) experienced the cold temperature threshold, five or more days below -9.4^{o}C, and this was the likely cause of "recovery;" pruning may have removed infected tissue as well. At V3 (Fig. 3.8) in the Coastal Plain, there were two vines that tested positive in 2006 but not in 2007. However this region did not experience threshold temperatures between 2006 and 2007. That year's growth was removed from these vines after severe hail damage in July 2007 so it is likely that symptoms did not have enough time to develop by October 2007. There is also a possibility that the late frost in April 2007 may have negatively contributed to PD infection in October 2007 for similar reasons. V7 and 8 (Central Piedmont) were both considered to be at "moderate risk" according to historical temperature records, 2-4 days below -9.4^{o}C, and both experienced loss of vegetative growth after the late frost. Interestingly, at V7 (Fig. 3.11) the vines that did not "recover" from previous infection were those that did not experience loss of vegetative growth due to the late frost.

The winter prior to the 2006 growing season was milder than the winter prior to the 2007 growing season and therefore more of the state was considered "high risk" to PD (Figs. 3.17 and 3.18). There was a higher incidence of infection in the 2006 growing season. Symptoms were observed earlier in the 2006 growing season (matchstick petioles first observed in July) compared to the 2007 growing season (matchstick petioles first observed in August) and this may be the effect of a milder winter preceding the 2006 growing season. The late frost in April 2007 also resulted in growers removing some early vegetative growth and a reduction in the overwintered sharpshooters present at this time. In addition time for bacterial colonies to proliferate was reduced in 2007.

Ultimately, evaluating the effect of winter temperatures on PD infection is difficult with the data collected in the period of this study. Without reliable detection of *Xf* in the spring, it is unclear if positive *Xf* results in 2006 and 2007 were from the same infection retained over the winter, or if infection was re-introduced by sharpshooter feeding during the 2007 growing season. Also, on-site temperature records were not available to confirm that cold temperature requirements were experienced at any particular site.

There was little evidence that yellow sticky traps contributed to incidence of PD infection (Table 3.3) as almost all of the vines neighboring traps were asymptomatic. At only one site, V10 in the Southern piedmont, were any of the vines neighboring yellow sticky traps confirmed *Xf* positive. This site was very small (~1 hectare) and incidence of infection was higher here at any other site (Table 3.3). The contribution of visually attractive yellow sticky traps to incidence of PD infection may be a moot point, however; traps hung just outside of the vineyard can be just as useful for monitoring vector

populations as those hung within managed vines, especially if traps are located between overwintering sites and the managed vineyard.

Although several vines were observed with characteristic PD symptoms (Figs. 3.19 and 3.20) were observed in *V.* x *labruscana* vineyards (V11 and V12) in 2006 and 2007, none of the vines tested were confirmed *Xf* positive. It is possible that ELISA was not sensitive enough to detect titers necessary to cause symptoms in this species of grape. It is also possible that samples collected did not contain the bacterial colonies that caused the symptoms. *V.*x *labruscana* is considered more tolerant to PD than *V. vinifera* but not immune (Mortensen 1968).

The causal agent was not confirmed in grape vines at either V11 or V12; however, a wild bramble displaying marginal necrosis with accompanying red line, was collected from a wild border at V11 and tested positive for *Xf* in the fall of 2007. ELISA does not indicate whether or not bacteria in a sample is the PD strain of *Xf*; nonetheless, an effective method of sampling American varieties should be pursued if PD continues its northwestern spread because these varieties are considered susceptible.

3.4 Conclusion

In Virginia, *Xf* has been found in vineyards located beyond the expected geographic range for PD (Hoddle 2004). However, out of the eight sites located in low to moderate risk zones, at only one site were vines removed because of PD (grower comment) and this loss was one or two vines among the ~7 hectares observed in this study. Infections observed in areas of low to moderate risk are rare and often distal to the cordon.

3.5 Literature cited

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3.6 Figures and Tables

Table 3.1: Estimated risk of PD infection according to Sutton's standard, number of days where daily minimum temperature falls below -12.2°C or -9.4°C.

	days below -12.2°C	days below -9.4°C
Low Risk	3 or more	5 or more
Moderate Risk	2	4
High Risk	1 or zero	3 or fewer

Figure 3.1: *Vitis vinifera* vine severely infected by *Xylella fastidiosa* and exhibiting Pierce's disease symptoms, including shriveled fruit. Image: A.K. Wallingford

Figure 3.2: Marginal necrosis with accompanying red/yellow line and "matchstick petioles," characteristic symptoms of Pierce's disease. Image: A.K. Wallingford

Figure 3.3: "Green island," a characteristic symptom of Pierce's disease. Image A.K. Wallingford

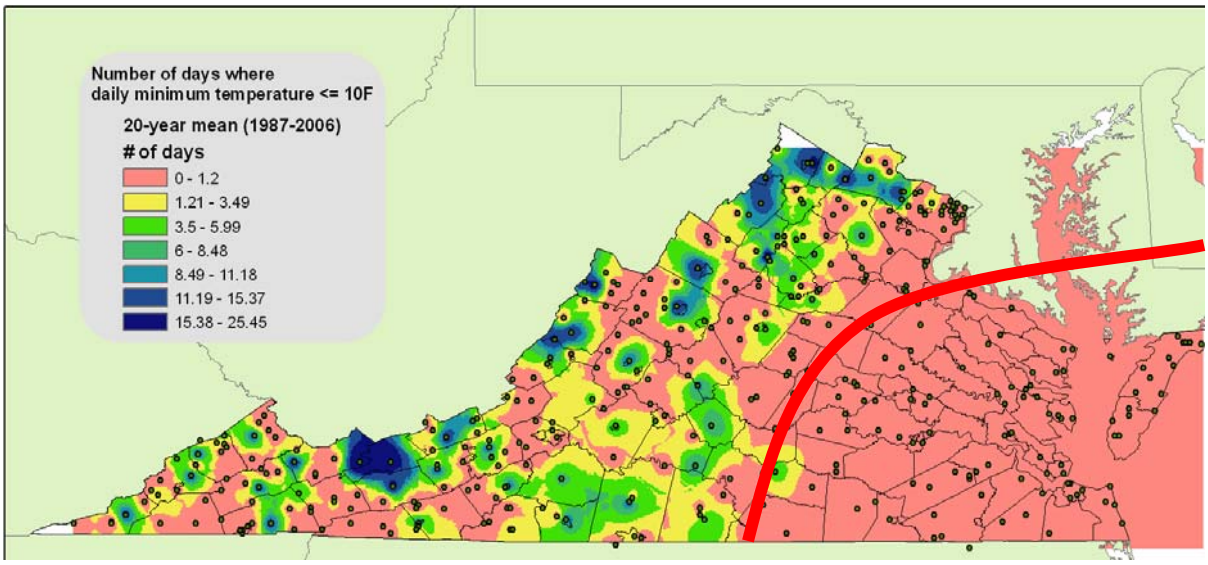

Figure 3.4: Risk zones according to temperature data from NOAA (collection sites shown as small dots). High risk areas are shown in pink, moderate risk in yellow, low to no risk in green to blue. Red line indicates modeled boundary based on climate for strains of *Xf* causing PD (Hoddle 2004). Map created by Peter Sforza, Virginia Tech Department of Geography.

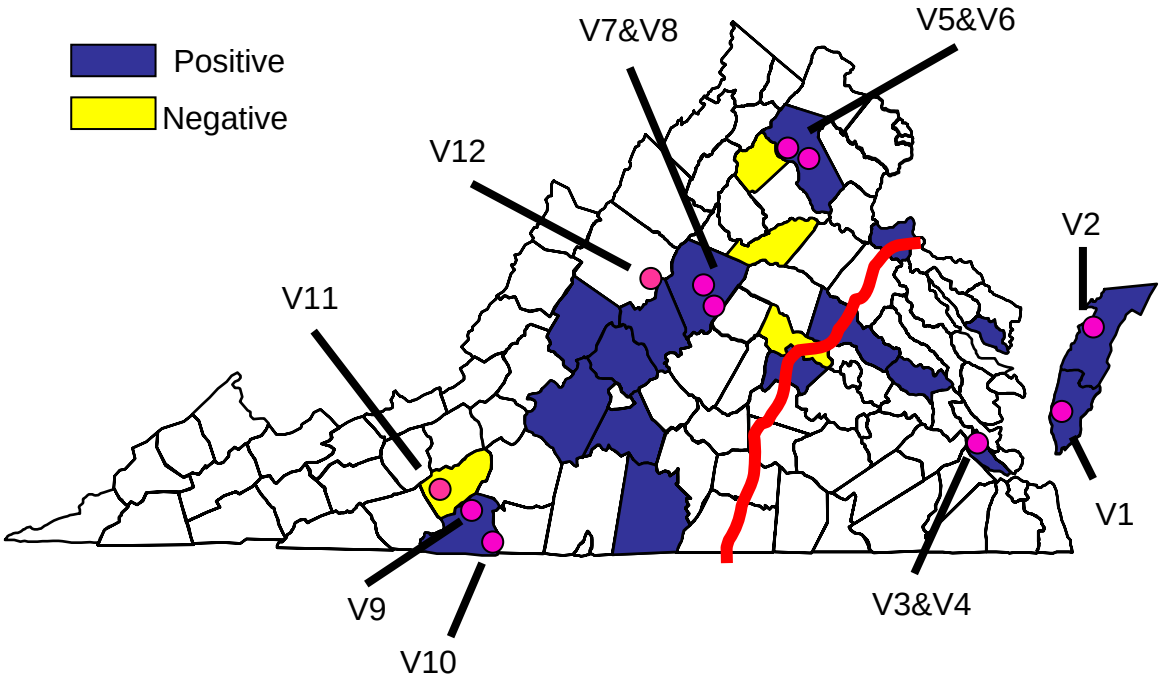

Figure 3.5: Collection sites (V1-V12) in survey for *Xylella fastidiosa* are indicated by pink dots. Counties in blue indicate locations where at least one symptomatic *Vitis vinifera* vine was confirmed positive for *X. fastidiosa* using ELISA. Counties in yellow indicate locations where vines were sampled but none were confirmed positive for *X. fastidiosa* using ELISA. Additional sites scouted are reported by Wallingford et al. (2007). The red line indicates a risk isoline based on a 30 year average of minimum winter temperatures; all regions west of this line were considered outside the boundary of clinical Pierce's disease (Feil and Purcell 2001).

Table 3.2: *Xylella fastidiosa* sampling sites in Virginia, 2006 and 2007: Locations, elevation and description of edge habitats near edge rows.

Vineyard #	Region	County	Latitude	Longitude	Elevation (m)	edge A	edge B
V1	E. Shore	Northampton	37° 34.153'	75° 53.307'	4	field crops	field crops
V2		Accomack	37° 49.208'	75° 37.442'	9	field crops	field crops
V3	Coastal	Williamsburg	37° 14.112'	76° 43.240'	21	Wooded	grass/trees
V4		Williamsburg	37° 14.112'	76° 43.240'	21	mown grass	mown grass
V5	N. Piedmont	Fauquier	38° 53.280'	78° 04.059'	404	Wooded	mown grass
V6		Fauquier	38° 55.697'	78° 00.070'	240	Wooded	mown grass
V7	C. Piedmont	Albemarle	38° 02.237'	78° 47.487'	257	Pasture	wooded
V8		Albemarle	38° 00.694'	78° 51.457'	300	mown grass	wooded
V9	S. Piedmont	Patrick	36° 44.027'	80° 11.732'	494	Pasture	wooded
V10		Patrick	36° 34.677'	80° 07.226'	332	Pasture	pasture
V11	Blue Ridge	Floyd	36° 46.958'	80° 23.723'	942	Pasture	wooded
V12		Augusta	38° 04.034'	75° 58.017'	451	Wooded	wooded

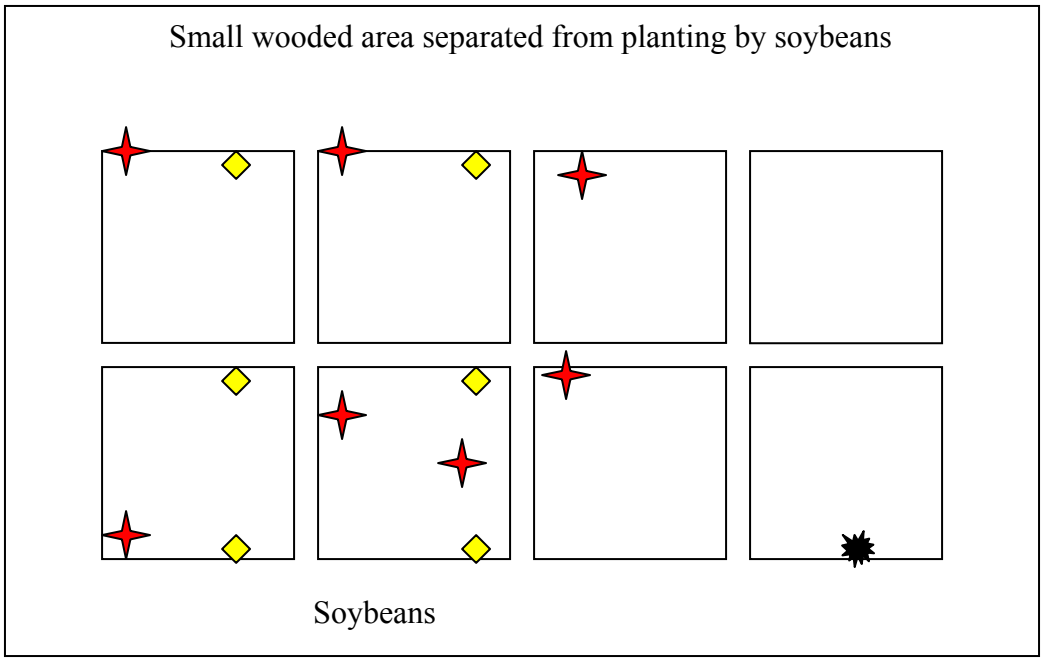

Figure 3.6: Vineyard 1 site map, Eastern Shore. Yellow diamonds indicate yellow sticky trap locations. Black splotch indicates location of a *Xf* positive vine in 10/2006 that was removed. Red 4-pointed stars indicate locations of *Xf* positive vines in 10/2007.

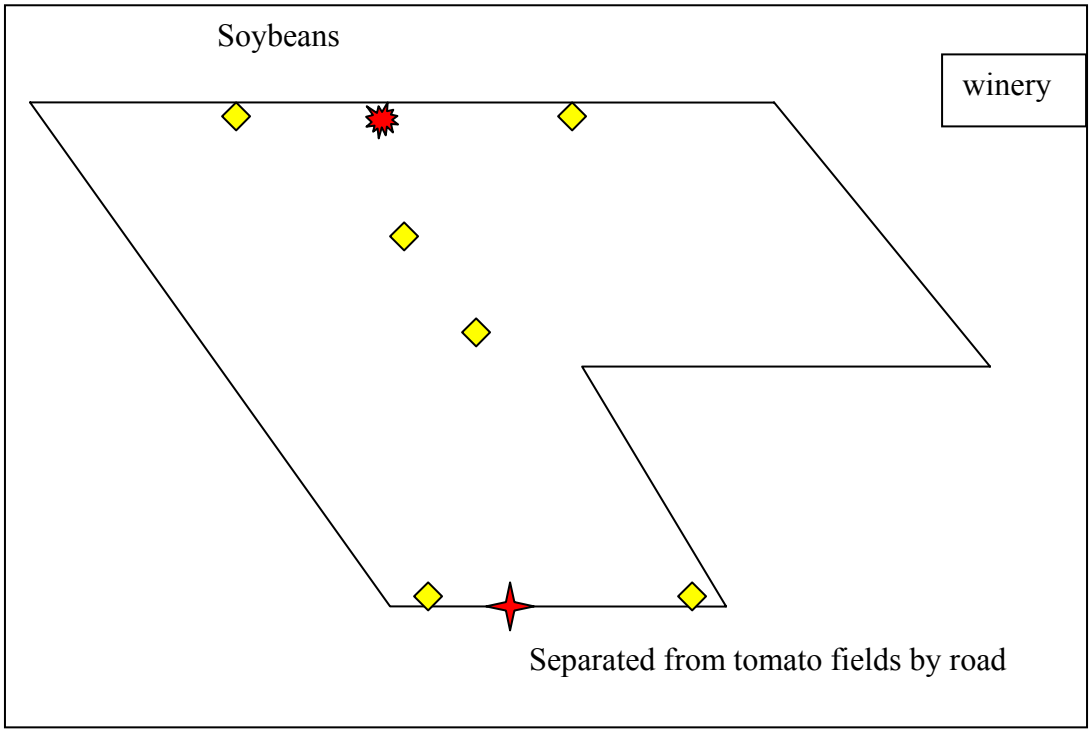

Figure 3.7: Vineyard 2 site map, Eastern Shore. Yellow diamonds indicate yellow sticky trap locations Red splotch indicate vine that tested *Xf* positive in 10/2006 and again in 10/2007. Red 4-pointed stars indicate locations of vine that tested Xf positive in 10/2007.

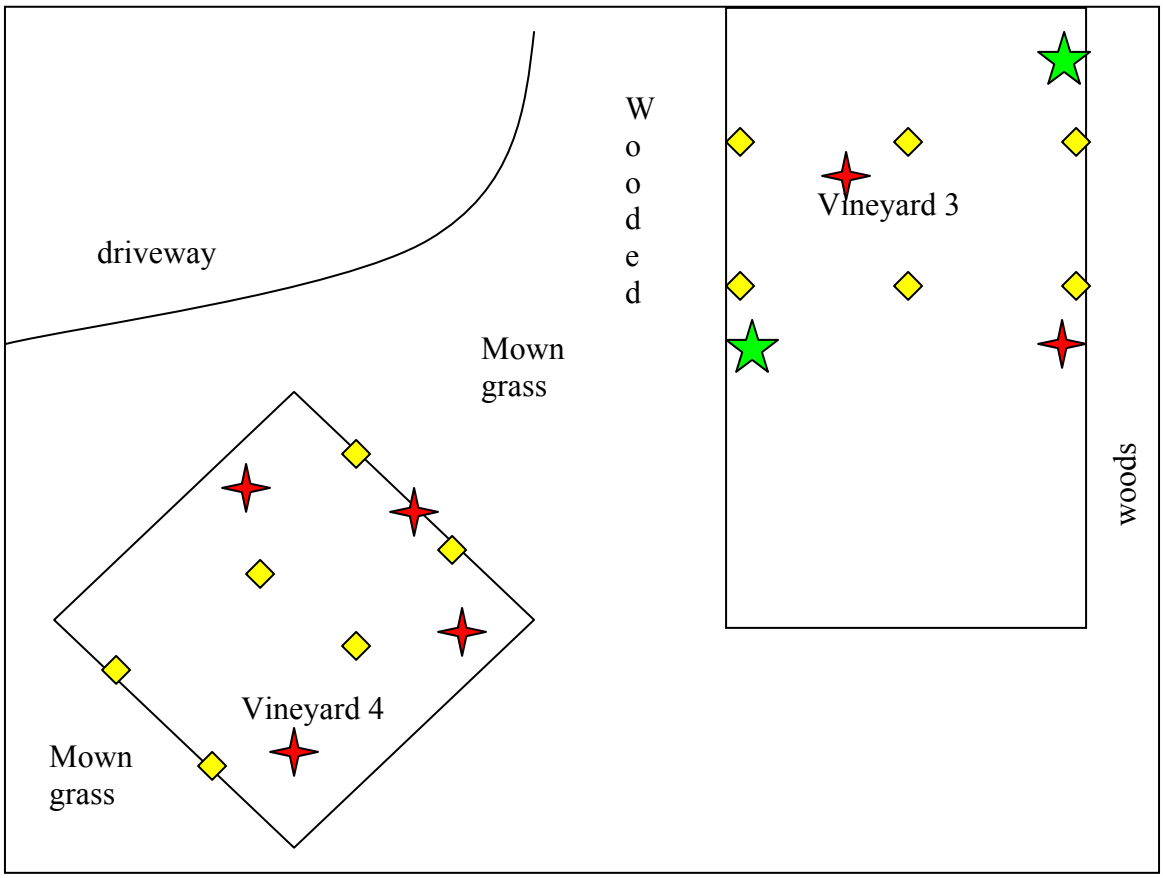

Figure 3.8: Vineyards 3 and 4 site map, Coastal Plain. Yellow diamonds indicate locations of yellow sticky traps. Green 5-pointed stars indicate locations of vines that tested *Xf* positive in 10/2006 but negative in 10/2007 (two other "recovered" vines are not shown here as they were removed between seasons). Red 4-pointed stars indicate locations of *Xf* positive vines in 10/2007.

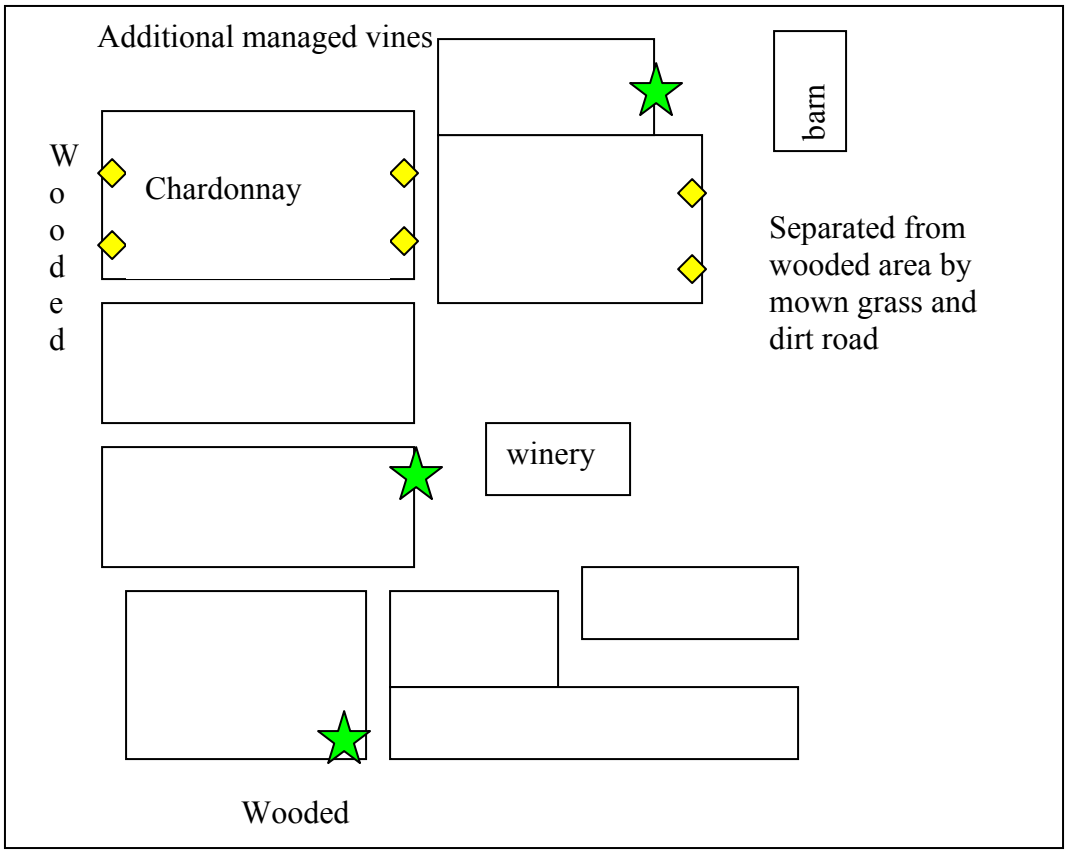

Figure 3.9: Vineyard 5 site map, Northern Piedmont. Yellow diamonds indicate locations of yellow sticky traps. Green 5-pointed stars indicate vines that tested positive for *Xf* in 10/2006 but tested negative in 10/2007, there were no positive vines in 10/2007.

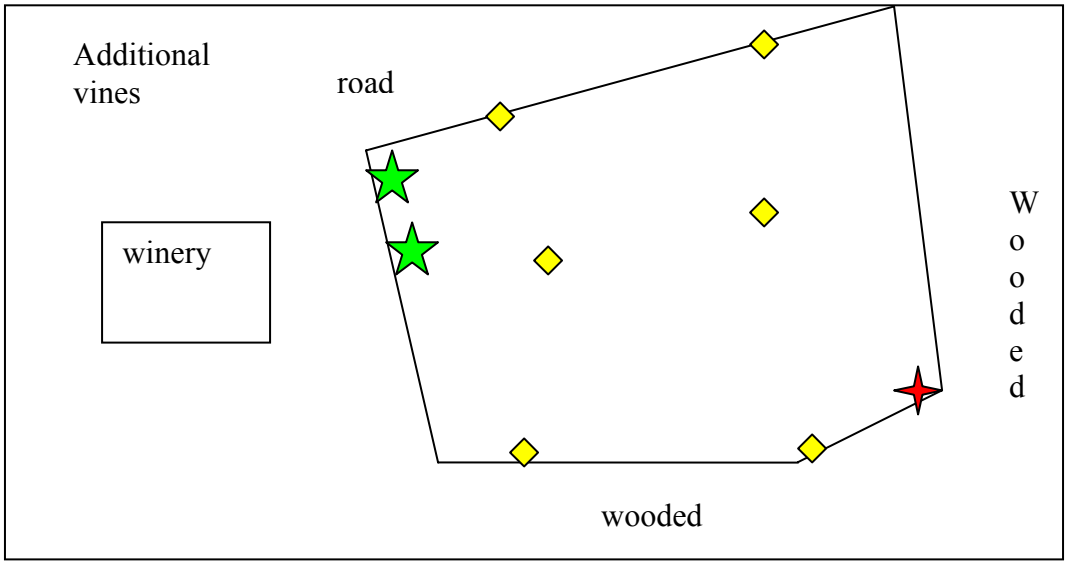

Figure 3.10: Vineyard 6 site map, Northern Piedmont. Yellow diamonds indicate locations of yellow sticky traps. Green 5-pointed stars indicate vines that tested positive for *Xf* in 10/2006 but tested negative in 10/2007. Red 4-pointed star indicates location of *Xf* positive vine in 10/2007.

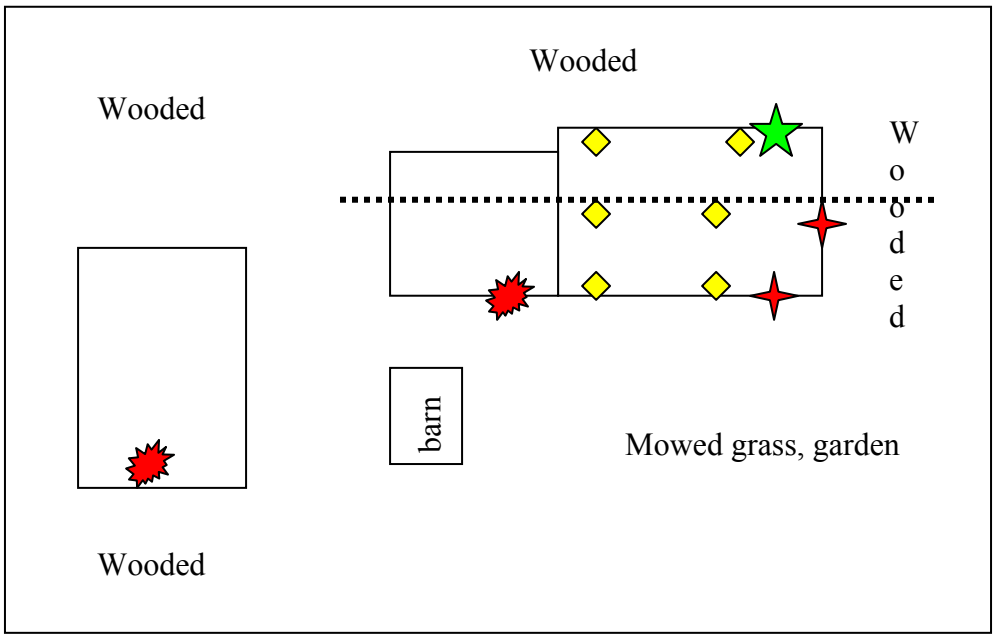

Figure 3.11: Vineyard 7 site map, Central Piedmont. Yellow diamonds indicate locations of yellow sticky traps. Green 5-pointed star indicates location of vine *Xf* positive vine in 10/2006 but tested negative in 10/2007. Red splotches indicate locations of vine that tested *Xf* positive in 10/2006 and 10/2007. Red 4-pointed star indicates locations of vines that tested *Xf* positive in 10/2007. Vines above dotted line experienced frost damage April 2007, while vines below line did not.

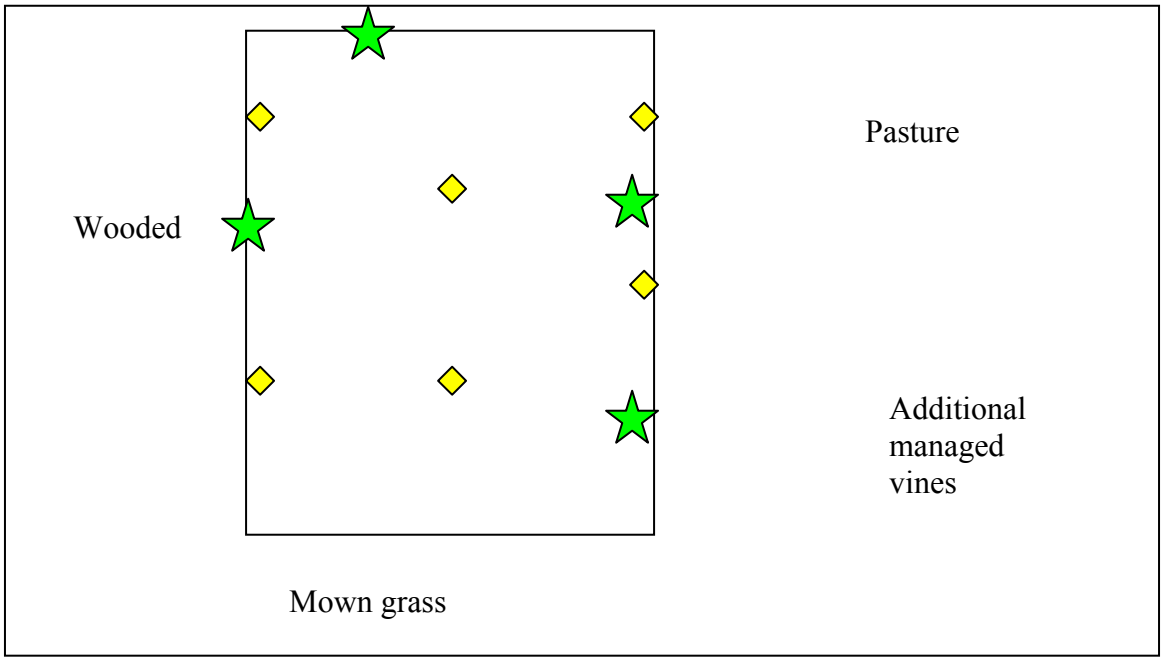

Figure 3.12: Vineyard 8 site map, Central Piedmont. Yellow diamonds indicate locations of yellow sticky traps. Green 5-pointed stars indicate locations of vines that tested *Xf* positive in 10/2006, but tested negative in 10/2007 (one other "recovered" vine is not pictured). There were no positive vines in 10/2007.

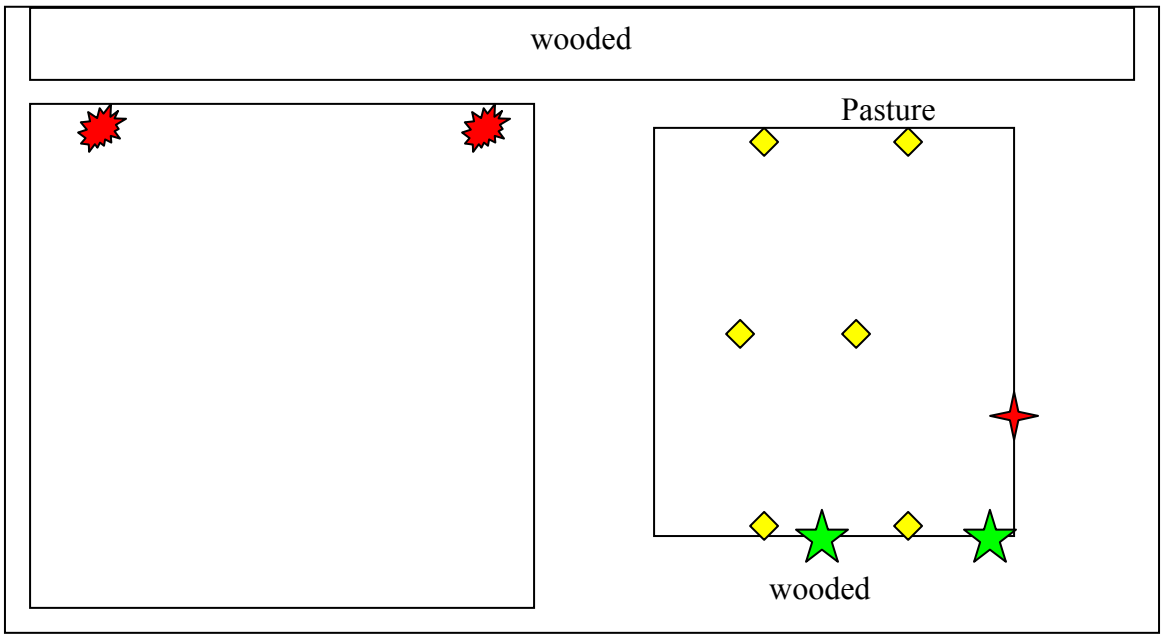

Figure 3.13: Vineyard 9 site map, Southern Piedmont. Yellow diamonds indicate locations of yellow sticky traps. Red splotches indicate vines that tested *Xf* positive in 10/2006 and 10/2007. Green 5-pointed stars indicate locations of vines that tested *Xf* positive in 10/2006 but tested negative 10/2007. Red 4-pointed star indicates location of vine that tested *Xf* positive 10/2007.

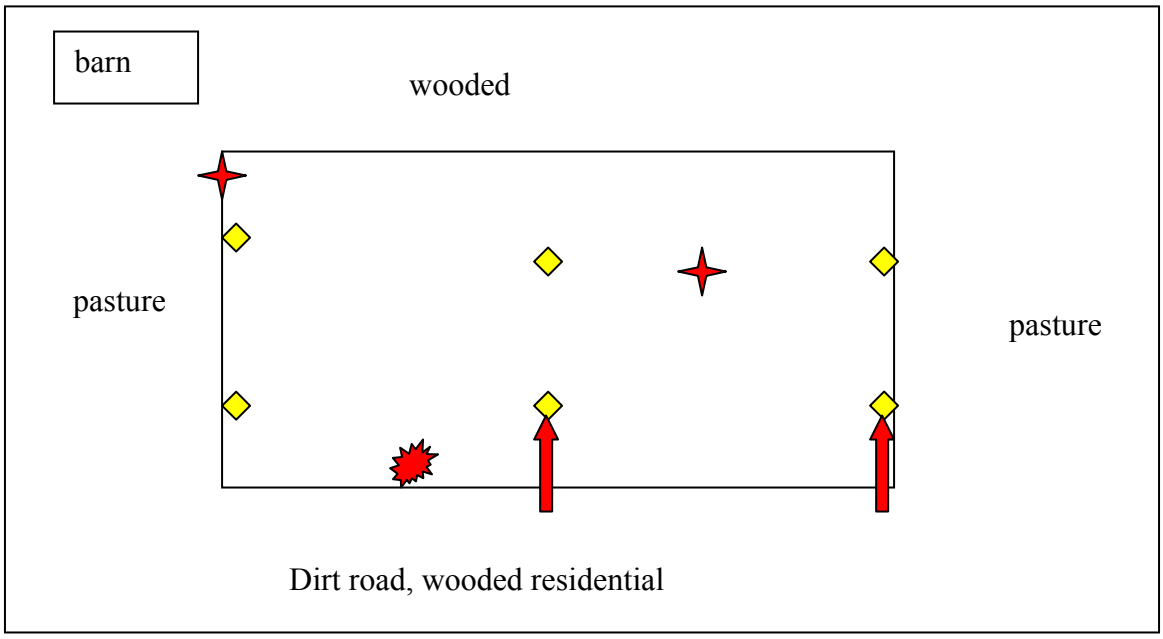

Figure 3.14: Vineyard 10 site map, Southern Piedmont. Yellow diamonds indicate locations of yellow sticky traps. Red splotch indicates location of vine that tested *Xf* positive in 10/2006 and 10/2007. Red 4-pointed stars indicate locations of vines that tested *Xf* positive in 10/2007. Red arrows indicate locations of vines neighboring yellow sticky traps that tested positive in 10/2007.

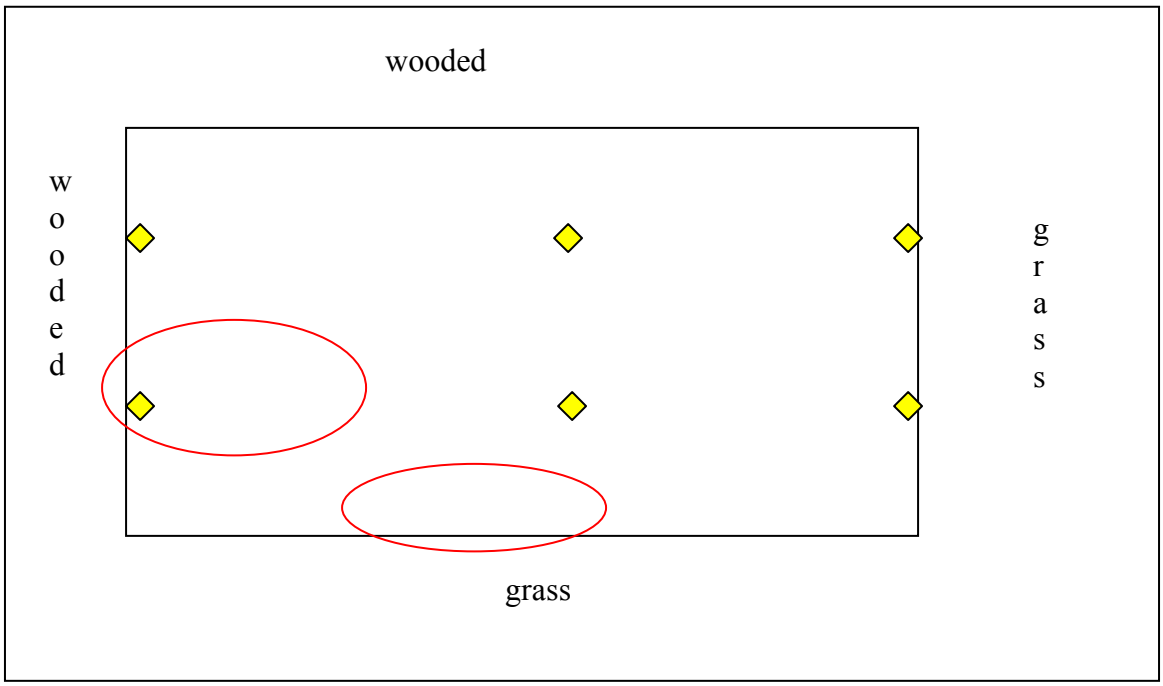

Figure 3.15: Vineyard 11 site map, Blue Ridge (Floyd Co.). Yellow diamonds indicate locations of yellow sticky traps. Red ovals indicate locations of symptomatic vines. There were no vines confirmed *Xf* positive in 10/2006 or 10/2007.

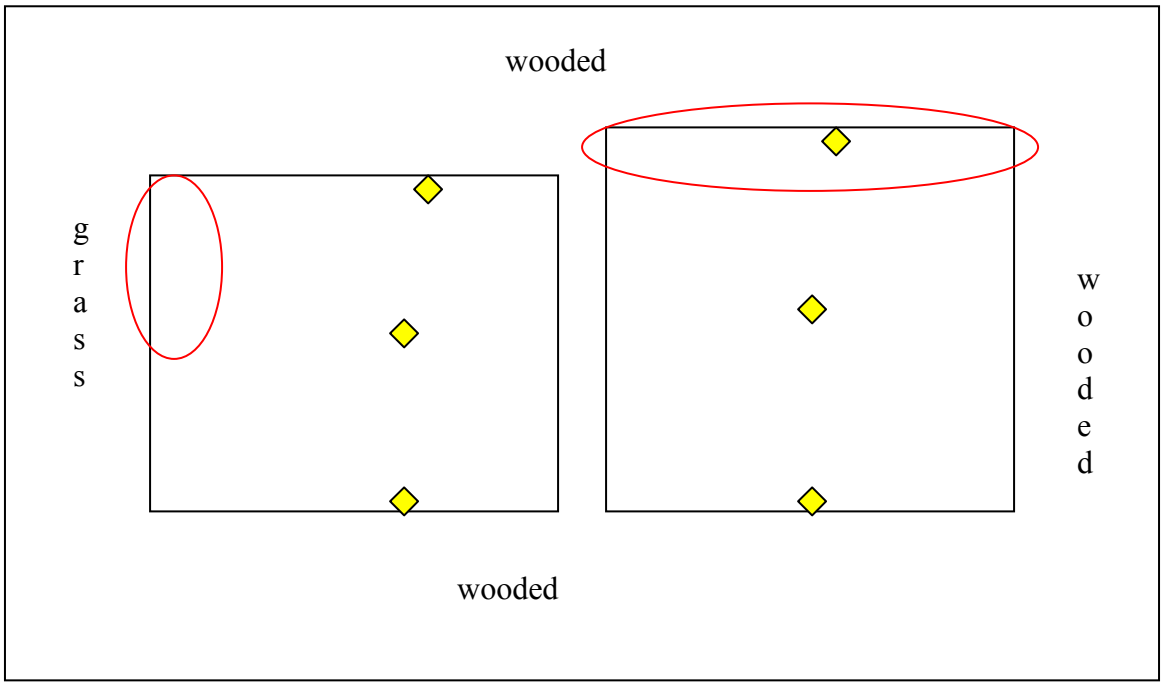

Figure 3.16: Vineyard 12 site map, Blue Ridge (Augusta Co.). Yellow diamonds indicate locations of yellow sticky traps. Red ovals indicate locations of symptomatic vines. There were no vines confirmed *Xf* positive in 10/2006 or 10/2007.

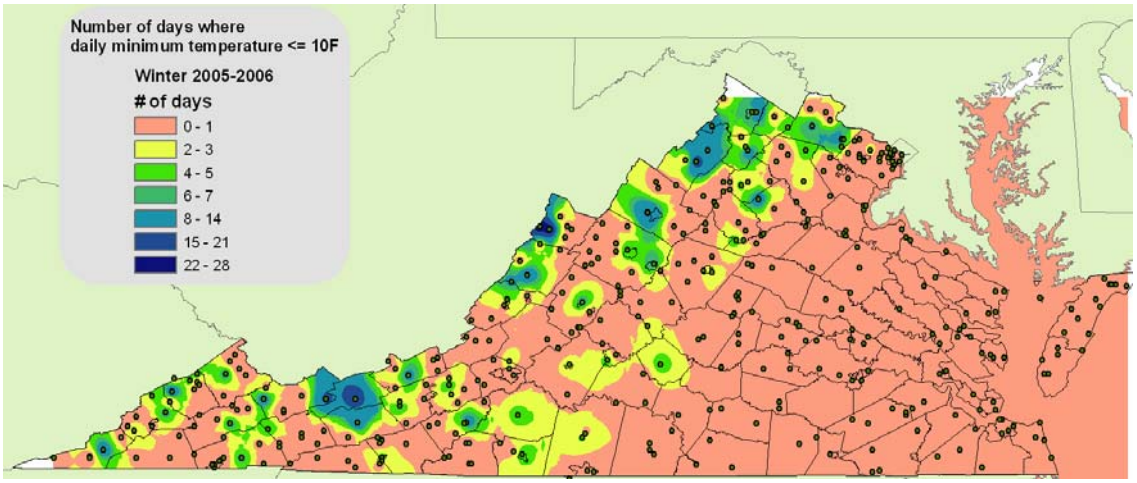

Figure 3.17: Risk scores for the 2006 growing season based on temperature records from NOAA during the winter of 2005/06. Areas in pink indicate high risk to PD infection, yellow areas are considered moderate risk, and green-blue areas are considered low to no risk of infection. Map created by Peter Sforza, Virginia Tech Department of Geography.

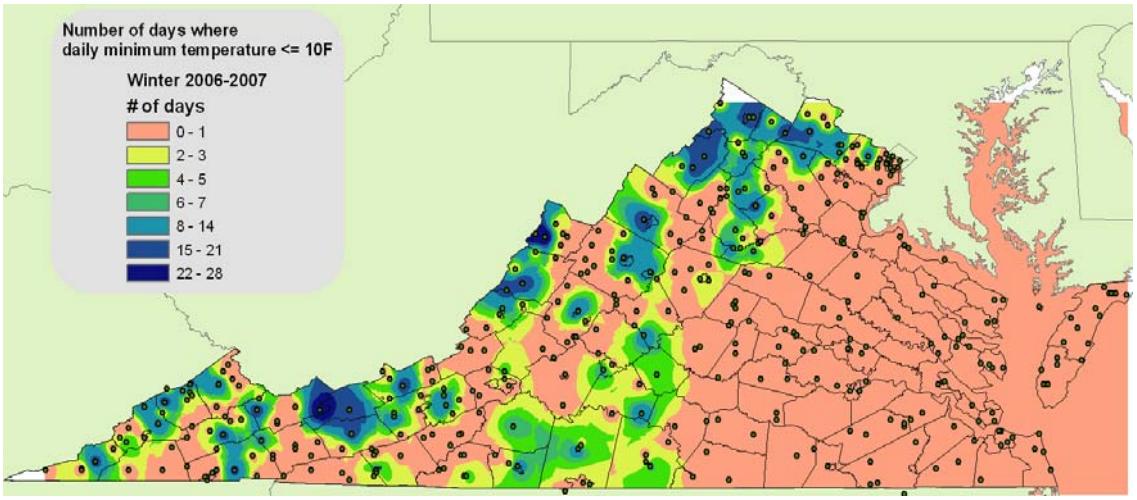

Figure 3.18: Risk scores for the 2007 growing season based on temperature records from NOAA during the winter of 2006/07. Areas in pink indicate high risk to PD infection, yellow areas are considered at moderate risk, and green-blue areas are considered low to no risk of infection. Map created by Peter Sforza, Virginia Tech Department of Geography.

Table 3.3: Percent confirmed *Xf* positive vines neighboring yellow sticky traps and percent confirmed *Xf* positive vines selected randomly. Temperature risk score based on preceding winter's temperature data from closest coop-ext. stations: high risk = 1 or fewer days below -9.4^{o}C, moderate risk = 2-3 days below -9.4 ^{o}C, low risk = 4 or more days below 9.4 ^{o}C.

Region	Site	Temperature risk score	Adjacent to Edge Traps (n=4)	Adjacent to Middle Traps (n=2)	Randomly selected (n=30)
E. Shore	Vineyard 1	high	0%	0%	3%
	Vineyard 2	high	0%	0%	3%
Coastal Plain	Vineyard 3	high	0%	0%	0%
	Vineyard 4	high	0%	0%	0%
Northern	Vineyard 5	low	0%	0%	0%
	Vineyard 6	low	0%	0%	0%
Central	Vineyard 7	low	0%	0%	0%
	Vineyard 8	moderate	0%	0%	3%
Southern	Vineyard 9	moderate	0%	0%	0%
	Vineyard 10	high	25%	50%	10%

Figure 3.19: *Vitis labruscana* 'Concord' (V11) with matchstick petioles, a characteristic symptom of Pierce's disease; ELISA tested negative for *Xf*. Image: A.K. Wallingford

Figure 3.20: *Vitis labruscana* 'Concord' (V11) with green island symptom, a characteristic symptom of Pierce's disease; ELISA tested negative for *Xf*. Image: A.K. Wallingford

CHAPTER FOUR

Assessing risk of Pierce's disease to Virginia vineyards

4.1 Introduction

Presence of *Xylella fastidiosa* (Wells et al. 1987) (*Xf*), the causal agent of Pierce's disease (PD), as well as several species of capable vectors (sharpshooters), has been recorded in every grape-growing region of Virginia. Infected grapevines have been observed and confirmed *Xf* positive in areas outside the expected boundary for PD (Wallingford et al. 2007). There is potential for a PD outbreak in Virginia vineyards given proper environmental conditions, i.e. warmer winters. However, vine loss due to PD is rare in Virginia. In only one site in my survey were vine losses attributed to PD (grower comment V1, Fig. 4.1). In one Central Piedmont site and one Southern Piedmont site (V8 and V10 respectively, Fig. 4.1) at least one vine was recommended for removal because of PD infection, but there was no yield loss attributed to PD and this recommendation was for removal of one or two sick vines out of hectares of apparently healthy vines. PD infected vines (with yield loss) were removed at the Coastal Plain site but these vines were not in our study area (V2 and V3, Fig. 4.1). Here we attempt to identify those factors that can be monitored by concerned parties in order to predict severe PD infection.

Reduction of vector populations has been shown to slow the spread of PD through the vineyard (Krewer et al. 2002). A greater abundance of sharpshooters would likely result in a greater probability of infection through the vineyard and therefore a higher

probability of severe infection. My survey of sharpshooters (Chapter Two) has revealed generally high numbers of sharpshooters trapped in vineyards surrounded by wooded areas than in vineyards surrounded by grass or agricultural land. It is unclear if these vineyards will be at greater risk of infection.

Early season transmission of *Xf* is of greatest concern. An early introduction of *Xf* allows for more time in the growing season for bacterial colonies to proliferate within the xylem vessels of the vine. Early season introduction also implies an introduction of bacteria closer to permanent portions of the plant (the cordon) and a better chance of chronic infection as infected tissue will not be removed with regular winter pruning. An earlier appearance of sharpshooters in the vineyard would likely result in a greater probability of chronic infection and therefore a higher probability of severe infection.

Cold winter temperatures limit the effects of the bacterium from year to year (Hopkins and Purcell 2002) and upward trends in winter temperatures have raised PD concern in the mid-Atlantic (Sutton 2005). If cold temperature threshold is not met for multiple years in succession, there is an increased likelihood of chronic infection and therefore a higher probability of severe infection.

4.2 Materials and Methods

Edge row vines and 20 randomly selected interior vines were scored at each site according to severity of infection in 2007 and ELISA was used to confirm presence of *Xf*. Photographic records and ELISA results were used to rank vines from 2006 scouting and sampling. The score from the highest ranking vine at each site was assigned as that

vineyard's PD severity score. Each vine examined was rated according to the following (modified from Myers 2005):

0 = no symptoms

1 = localized infection: Infection was distal to cordon and marginal necrosis was observed on <25% of leaves (Fig. 4.2).

2 = infection spread but not to entire vine: Marginal necrosis of leaves and bladeless petioles were observed on one or more entire shoots (Fig. 4.3).

3 = infection spread to whole vine: Bladeless petioles and marginal necrosis was observed on the majority of leaves (Fig. 4.4).

4 = vine defoliated and yield loss: Bladeless petioles and marginal necrosis was observed on the majority of leaves and fruit is shriveled (Fig. 4.5).

5 = vine dead

Sharpshooter abundance values for each site were taken from a survey of sharpshooters in Virginia vineyards (unpublished data). Total abundance = total number of sharpshooters (Cicadellinae) trapped throughout the growing season (April-October 2006, March- October 2007, Table 4.1); total *O. orbona* and total *G. versuta* trapped throughout the growing season were evaluated as well.

Timing of sharpshooter appearance was approximated by degree days accumulated at the Virginia Cooperative Extension weather station closest to each site during trapping periods when each of the two sharpshooter species of interest [*Oncometopia orbona* (Fabr.) and *Graphocephala versuta* (Say)] were (1) first captured, (2) during trapping periods when *O. orbona* was trapped in the greatest numbers (peak capture) and (3) during trapping periods when *G. versuta* was trapped in the greatest

numbers (peak capture, Table 4.2 and 4.3). However, in many cases, sharpshooter collection did not begin early enough for an accurate estimation of first flight. Because sharpshooter feeding behavior and movement is directed by vine phenology (Mizell and French 1987), trap numbers were associated with growing degree days degree days after January 1st = ((Tmax + Tmin)/2 – 10^{o}C, 10 ^{o}C minimum threshold, 32 ^{o}C maximum threshold.

Temperature risk score was assigned to each site according to the previous winter's weather data from the nearest NOAA weather station. Each location was rated as the following based on temperature risk scores from Sutton (2005; Table 4.1), using -9.4^{o}C as minimum temperature requirement.

Regression analysis, carried out using JMP (SAS Institute, Cary N.C.), was used to describe the relationship between PD symptom severity and (1) sharpshooter abundance, (2) timing of sharpshooter appearance in the vineyard and (3) temperature risk score in 2006 and 2007.

4.3 Results and Discussion

The non-linear regression shown in Fig. 4.6 is consistent with the hypothesis that more severe PD symptoms (Table 4.2) are seen in vineyards that have not, in the dormant period, met the cold temperature threshold of five or more days below -9.4^{o}C. This is a significant relationship (R^2 = 0.40, p-value = 0.0016), and the model accounts for 40% of the variation. Monitoring winter temperatures is an easy activity for growers. In areas where cold thresholds are met, regional temperature reports may suffice. All growers

should collect on-site temperature data, but this is especially critical for vineyards in areas where cold thresholds are reached only occasionally.

No relationship was found at any site between PD symptom severity and vector abundance, i.e. total sharpshooters, total *O. orbona* or total *G. versuta* (Table 4.2). No relationship was found at any site between PD symptom severity and time of vector appearance in the vineyard for either *O. orbona* or *G. versuta* (Table 4.3). As cold thresholds do play a role in symptom severity and there is variability in this factor between sites, this test may not be an accurate measure of the role of vector activity in symptom severity. Regardless, monitoring insect vectors is not considered a reliable way to predict PD severity in Virginia. Moreover, V1 (Fig. 4.1) managed insect vectors through chemical control and low numbers of sharpshooters were trapped in this site (Table 4.2), but this site also has severe PD symptoms.

4.4 Literature Cited

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Hopkins, D.L. and A.H. Purcell. 2002. *Xylella fastidiosa*: Cause of Pierce's disease of grapevine and other emergent diseases. Plant Disease 86: 1056-1066.

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

4.5 Tables and Figures

Table 4.1: Estimated risk of PD infection according to Sutton's standard, number of days where daily minimum temperature falls below -12.2°C or -9.4°C.

	days below -12.2°C	days below -9.4°C
Low Risk	3 or more	5 or more
Moderate Risk	2	4

High Risk	1 or zero	3 or fewer

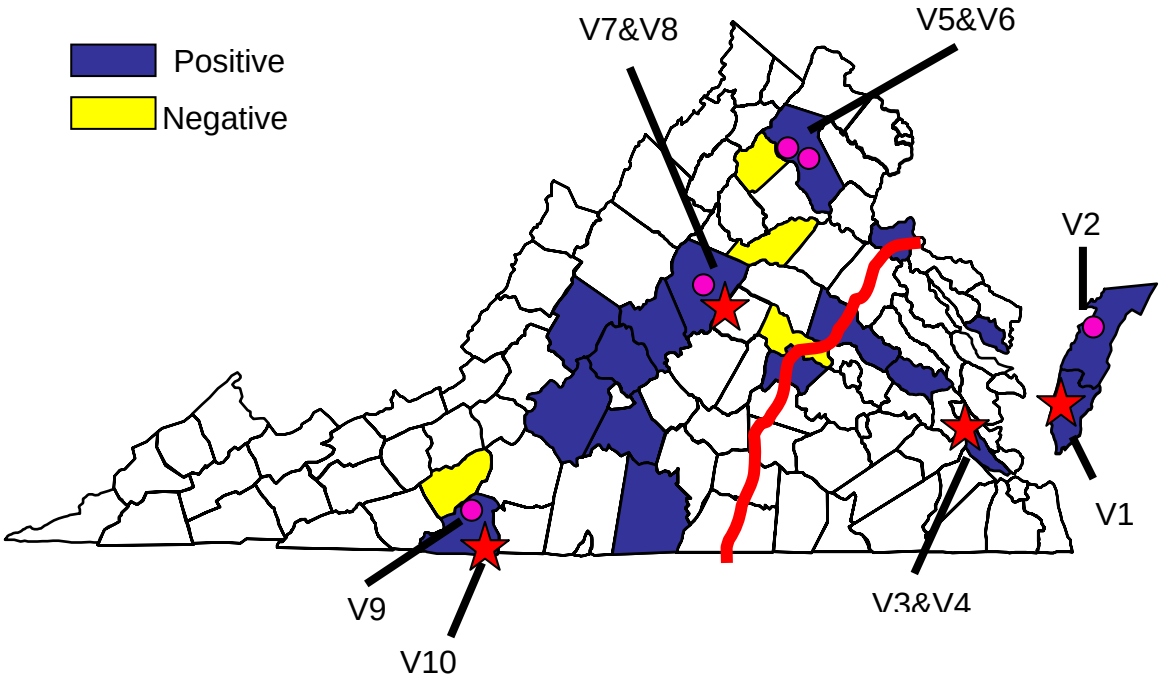

Figure 4.1: Collection sites (V1-V10) for sharpshooter and *Xf* survey are indicated by pink dots and red stars. Red stars indicate locations of sites with at least one vine lost to PD infection. Counties in blue indicate locations where at least one symptomatic *V. vinifera* vine was confirmed positive for *X. fastidiosa* using ELISA. Counties in yellow indicate locations where vines were sampled but none were confirmed positive for *X. fastidiosa* using ELISA. Additional sites scouted are reported in Wallingford et al. 2007. The red line indicates a risk isoline based on 30 year average of minimum winter temperatures (Feil and Purcell 2001); all regions west of this line were considered outside the boundary of clinical Pierce's disease.

Figure 4.2: PD severity score of 1. Symptoms are distal to cordon and observed on fewer than 25% of leaves. Image: A.K. Wallingford

Figure 4.3: PD severity score of 2. Symptoms on entire shoots. Image: A.K. Wallingford

Figure 4.4: PD severity score of 3. Entire vine is symptomatic. Image: A.K. Wallingford

Figure 4.5: PD severity score of 4. Entire vine is symptomatic and fruit is shriveled. Image: A.K. Wallingford

Table 4.2: PD severity score (0-5), Temperature risk score (1-3), total Cicadellinae trapped over the growing season, total *O. orbona* trapped over the growing season and total *G. versuta* trapped over the growing season.

Site	Year	PD severity score	Temp. risk score	Total Cicadellinae	Total *O. orbona*	Total *G. versuta*
V 1	2006	5	3	216	37	174
	2007	5	3	1113	15	1111
V 2	2006	3	3	1749	239	1510
	2007	3	3	1357	48	1309
V 3	2006	2	3	101	38	51
	2007*	---	3*	159*	52*	105*
V 4	2006	3	3	45	27	18
	2007*	---	3*	33*	10*	15*
V 5	2006	2	2	637	15	608
	2007	2	1	2178	6	2164
V 6	2006	3	2	3651	177	3418
	2007	2	1	3789	84	3669
V 7	2006	2	2	621	58	560
	2007	2	2	263	6	257
V 8	2006	4	3	2404	192	2170
	2007	3	3	1432	142	1275
V 9	2006	3	3	788	22	749
	2007	2	2	518	9	494
V 10	2006	3	3	502	6	454
	2007	3	3	2574	29	2525

* indicates data removed from analysis

PD severity score:
0 = no symptoms
1 = symptoms distal to cordon
2 = symptoms on entire shoot(s)
3 = entire vine symptomatic
4 = entire vine symptomatic, fruit shriveled/lost
5 = vine dead

Temp. Risk score:
1 = low risk
2 = moderate risk
3 = high risk

Table 4.3: PD severity score (0-5), Temperature risk score (1-3), growing degree days accumulated during first and peak trapping period for the two species of interest, *O. orbona* and *G. versuta*. No data available for first capture in locations where sharpshooters were trapped during the first trapping period.

Site	Year	PD severity score	Temp. risk score	GDD first *O. orbona*	GDD first *G. versuta*	GDD peak *O. orbona*	GDD peak *G. versuta*
V 1	2006	5	3	---	---	225	1471
	2007	5	3	230	230	1023	2985
V 2	2006	3	3	---	---	1120	1120
	2007	3	3	457	457	955	1265
V 3	2006	2	3	---	---	779	1566
	2007*	---	3*	558*	---	1064*	1064*
V 4	2006	3	3	---	---	779	1566
	2007*	---	3*	558*	239*	783*	783*
V 5	2006	2	2	476	---	476	1414
	2007	2	1	391	875	391	1481
V 6	2006	3	2	413	---	567	1414
	2007	2	1	181	---	875	1222
V 7	2006	2	2	---	---	1013	1267
	2007	2	2	183	73	990	1227
V 8	2006	4	3	603	---	119	1267
	2007	3	3	409	73	618	1429
V 9	2006	3	3	367	---	680	2072
	2007	2	2	596	129	981	2165
V 10	2006	3	3	450	---	680	1232
	2007	3	3	596	129	975	2165

* indicates data removed from analysis

PD severity score:
0 = no symptoms
1 = symptoms distal to cordon
2 = symptoms on entire shoot(s)
3 = entire vine symptomatic
4 = entire vine symptomatic, fruit shriveled/lost
5 = vine dead

Temp. Risk score:
1 = low risk
2 = moderate risk
3 = high risk

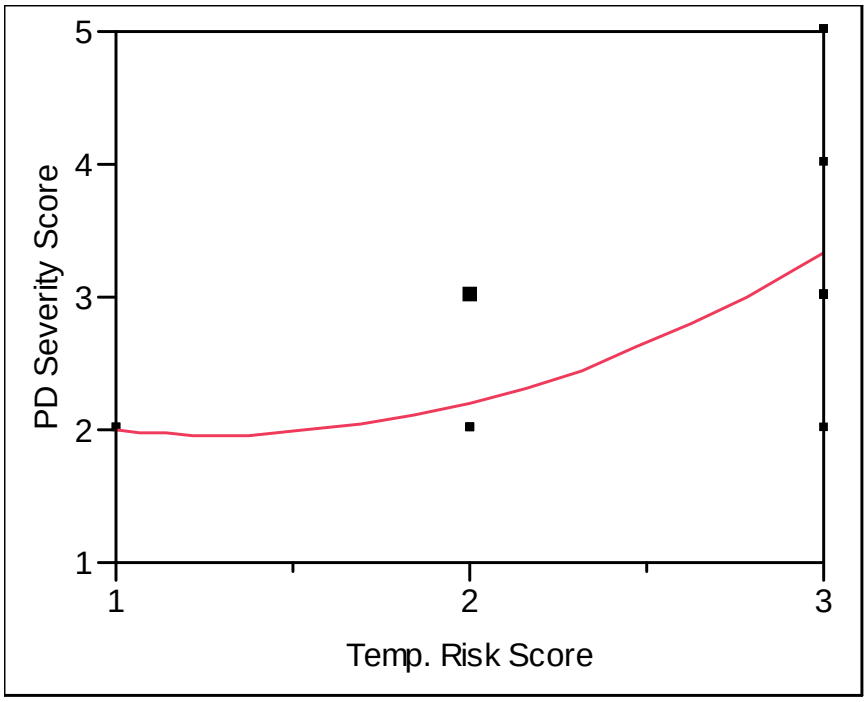

Figure 4.6: Non-linear regression showing significant relationship between PD Severity scores in Virginia vineyards (0-5) and Temperature Risk scores (1-3) in 2006 and 2007 ($R^2 = 0.4$, $\alpha = 0.5$).

Summary

In Virginia, *Xf* has been found in vineyards located beyond the expected geographic range for PD. However, out of the eight sites located in low to moderate risk zones, at only one site were vines removed because of PD (grower comment) and this loss was one or two vines among the ~7 hectares observed in this study. Infections observed in areas of low to moderate risk are rare and often distal to the cordon.

There are capable PD vectors present in every grape growing region of Virginia, within managed vineyards. When cold temperatures (lethal to the bacterium) are not experienced, the early season (April/May) is the time of greatest concern to protect vines from all sharpshooter species flying within the vineyard.

Oncometopia orbona and *G. versuta* are the species of greatest concern as they are both capable vectors of *Xf* and are both present in all growing regions. *O. orbona* occur in lower numbers than *G. versuta,* however, since *O. orbona* are captured within the vineyard at a critical time for PD infection (i.e. early in the season) they are of greater concern. *O. orbona* are also larger in size, making this species more conspicuous on yellow sticky traps which should aid commercial managers wishing to monitor sharpshooter populations.

Pierce's disease symptom severity is correlated to cold winter temperatures. Monitoring winter temperatures is an easy activity for growers. In areas where cold thresholds are met, regional temperature reports may suffice in indicating whether or not insect vectors should be controlled. All growers are urged to collect on-site temperature data, but this is especially critical for vineyards in areas where cold thresholds are reached only occasionally.