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Seasonal Abundance and Diversity of Potential *Xylella* (Xanthomonadales: Xanthomonadaceae) Vectoring Leafhoppers in Mississippi Muscadine Vineyards

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ABSTRACT

Pierce's disease remains an important economic threat to American wine, table and raisin grape production. Although muscadine grapes exhibit resistance, when stressed or weakened the vines can become susceptible. Vector management may be a useful tool in limiting the pathogen spread within a vineyard, but knowledge of leafhopper species composition and their *Xylella*-vectoring potential remains scant. Sticky traps placed among five muscadine cultivars (Alachua, Carlos, Nesbit, Noble and Southern Home) in two vineyards in southern Mississippi were used to identify potential leafhopper vectors. While the glassy-winged sharpshooter was predictably abundant, other species also were prominent including the invasive *Sophonia orientalis* (Matsumura). Gaining knowledge on species of potential *Xylella* vectors, their relative abundance and seasonal distribution within the vineyards provides data on potential disease pressure in this under-studied region and will facilitate future efforts to quantify *X. fastidiosa* infection of these species.

Additional index words: Cicadellidae, Pierce's disease, glassy-winged sharpshooter, invasive, sticky trap

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Xylella fastidiosa Wells [(Xanthomonadales: Xanthomonadaceae) *Xf*] is a bacterial pathogen that causes leaf scorch symptoms in a wide range of host plants including Pierce's disease in grape, phony peach disease and citrus variegated chlorosis, as well as symptoms in alfalfa, almond, pecan, olive, oleander and ornamental shade trees (Overall and Rebek 2015). Valued at over $3 billion annually, American wine, table and raisin grape production is the highest value American fruit crop (Tumber et al., 2014). Pierce's disease is a serious constraint to American grape production, and is expected to become increasingly problematic with the warming climate (Purcell 1997). Crop loss, inspection activities and vector management programs lead to economic loss estimates of over $100 million per year from Pierce's disease in California (Tumber et al., 2014).

In contrast with widely-cultivated *Vitis vinifera* (L.) and *V. labrusca* (L.), some cultivars of the muscadine grape (*V. rotundifolia* Michx.) are tolerant of *Xf* infection (Hopkins et al., 1974; Fry and Milholland 1990). Native to the southeastern U.S., muscadines are well-adapted to this region and its disease pressures, requiring relatively few chemical inputs when grown with good cultural practices (Hickey et al., 2019). Muscadines have an increasing economic importance, bringing in >$5 million in annual farmgate value for Georgia and North Carolina combined (Cline and Fisk 2006; Jagdale et al., 2019). While true resistance has not been demonstrated, it is suggested that by co-evolving in a region native to both *Xf* and its primary vector, *Homalodisca vitripennis* Germar (Hemiptera: Cicadellidae), muscadine grapes have developed a tolerance to this bacterial infection, possibly through a larger xylem cell structure that is less prone to bacterial clogging and freeze injury. Still, disease symptoms can lead to stunting, marginal leaf burn and dieback, with associated loss in fruit yields (Hopkins et al., 1974).

Vector management strategies often are centered on *H. vitripennis,* commonly known as the glassy-winged sharpshooter, but all members of Cicadellidae are xylem-feeders and likely capable of transmitting *Xf (*Overall and Rebek 2015; Redak et al., 2004*).* Along with some species of the distantly related Cercopidae and Cicadidae*,* at least 39 species of Cicadellidae have been reported as *Xf* vectors (Redak et al., 2004; Myers et al., 2007). Colonizing two distinct habitats, plant xylem tissues and the Hemipteran vector foregut, *Xf* does not require an incubation period, but can persistently be transmitted immediately after acquisition by both nymph and adult vectors alike (Almeida et al., 2005). *Xf* cells do not systemically colonize the plant or insect and cannot be transmitted transovarially to progeny, but they typically are organized as a single-layer biofilm in the foregut, cibarium and precibarium

(Newman et al.,, 2003; Backus and Morgan, 2011). Nymphs may present less of a vectoring threat as the foregut is renewed with each molting, and they only reacquire the bacterium through subsequent feeding on infected vines (Backus and Morgan, 2011).

Since winged adults are the primary means for spread, vector management has become an important method of *Xf* management (Krewer et al., 2002; Fierro et al., 2019). Yet while populations of xylem-feeding leafhoppers have been studied in neighboring states including Alabama, Louisiana, North Carolina and Texas, to date there has not been a comprehensive assessment of leafhopper populations inhabiting muscadine vineyards in Mississippi (Ma et al., 2010; Sanderlin and Melanson 2010; Myers et al., 2007; Lauziere et al., 2008). Furthermore, considering the outsized impact of exotic-invasive species in agricultural and natural ecosystems, early detection of these populations can provide necessary time for responding with containment or even eradication programs (Reaser et al., 2020).

MATERIALS AND METHODS

Field plots: Leafhopper community composition was examined at two research vineyards (Pearl River County and Stone County, Mississippi) using yellow sticky cards (Trece, Inc., Adair, OK) distributed among five cultivars of muscadine grape (Alachua, Noble, Nesbit, Carlos and Southern Home). The Pearl River County vineyard encompasses 2.75 hectares and is part of a larger reclaimed cattle pasture since converted to vineyards and blueberry orchards, as well as open fields and meadows, and surrounded by suburban mixed residential/commercial development (Fig. 1a). The Stone County vineyard encompasses 0.3 hectares and is part of a research farm composed primarily of blueberry orchards and meadows, and surrounded by a large mixed pine/hardwood forest (Fig. 1b). Each vineyard is rectangular in shape with cultivars distributed in a randomized complete block design, and with regularly mowed surrounding fields.

Vector monitoring: Four individual plants per cultivar at each vineyard were sampled, yielding a total of 20 experimental plants per site. Lateral vine shoots were clipped off of the main vine arm for approximately 60cm, allowing for the unimpeded attachment of sticky cards with a wire twist tie directly underneath the main arm, approximately 1.2 – 1.5 m above the ground and in partial shade to full sun, depending on the plant. Sampling occurred from April – October in both 2019 and 2020, with cards left attached to vines for a two-week sample period, and then replaced and brought back to the lab for analysis.

Insect identification: All leafhopper specimens were identified to species, or to morpho-type, using taxonomic keys (Dietrich 2005) and voucher specimens located at the Louisiana State Arthropod Museum.

Statistical analysis: Comparisons of leafhopper species richness and evenness were made between the two research vineyards from each year using the Shannon-Wiener diversity index. Further analysis was focused on three leafhopper species of primary interest: *H. vitripennis, Graphocephala versuta* Say and *Sophonia orientalis* (Matsumura) (Hemiptera: Cicadellidae). These species were chosen due to their relative abundance in the vineyards, and either their status as known *Xf*-vectors (the first two species) or as an invasive species (the third species). Data from the dependent variable of trap capture of leafhoppers were log (x+1) transformed prior to analysis of variance (Proc GLM, SAS) using the independent variables of year, sample period, site, and plant cultivar, as well as their interactions, based on $\alpha = 0.05$.

RESULTS

Nearly 20,000 leafhopper specimens representing 37 species or morpho-types were identified over both years (Table 1). Populations at the Stone County vineyard had greater diversity and evenness in each year, with both sites having greater diversity and evenness during the first year as compared with the second (Table 2). While there were significant differences in trap capture from both year ($F= 12.73$; $P = 0.0004$) and site ($F= 111.11$; $P < 0.0001$) variables, there was not a year*site interaction ($F= 0.15$; $P = 0.6991$) (Fig. 2a-b). In each year at least 90% of captures were represented by three species: *H. vitripennis, G. versuta* and the exotic *S. orientalis,* with *H. vitripennis* (48%) being the predominant species in 2019 and *S. orientalis* (40%) in 2020.

Fig. 1a - b. Satellite image of the Pearl River (a) and Stone County (b) research farms, with muscadine vineyards outlined in red.

Capture by sample period also was significant ($F= 177.76$; $P < 0.0001$), starting relatively low at the beginning of each season, from April – early May (Figure 3a-b). But by the middle of May, especially for the three primary species, captures increased significantly and climbed to a peak in mid-June. While

Table 1. Diversity and abundance of leafhopper captures at muscadine grape vineyards in South Mississippi, 2019-20.

Species	**2019**		**2020**	
	Pearl River	**Stone**	**Pearl River**	**Stone**
Agallia constricta	15	15	41	24
Chlorotettix sp.	1	2	2	2
Cuerna costalis	0	6	2	1
Draeculacephala sp.	7	9	5	22
Empoasca sp.	2	7	0	0
Erasmoneura nigra	1	0	0	0
Erasmoneura vulnerata	2	6	5	17
Erythroneura diva	6	41	0	4
Erythroneura rubra	4	106	14	115
Erythroneura tricincta	1	17	7	16
Graminella nigrifrons	15	17	1	7
Graminella villica	6	35	11	14
Graphocephala coccinea	2	2	9	2
Graphocephala versuta	1321	369	1313	439
Homalodisca insolita	2	1	2	1
Homalodisca vitripennis	3061	1532	1730	1929
Illingina illinoensis	0	3	0	8
Macrosteles sp.	3	16	3	5
Menosoma cincta	0	1	0	0
Oncometopia orbona	153	23	95	21
Osbornellus rotundus	0	0	0	1

Osbornellus clarus	1	5	0	0
Paraphlepsius sp.	66	37	87	49
Paraulacizes irrorata	2	6	0	5
Penestregania robusta	2	1	4	5
Planicephalus sp.	3	0	1	1
Prairiana sp.	108	40	88	8
Sanctanus cruciatus	0	1	0	0
Scaphoideus sp.	5	0	6	1
Scaphytopius cinereus	0	1	6	10
Scaphytopius sp.	5	10	9	10
Sophonia orientalis	1788	395	3460	937
Tylozygus fasciatus	3	7	0	0
Tylozygus geometricus	1	0	0	0
Xestocephalus sp.	2	1	0	0
Xyphon reticulatum	2	3	9	14
Sum	6603	2716	6910	3668

Table 2. Species richness and evenness values of leafhopper captures at two muscadine grape vineyards in South Mississippi, 2019-20, as calculated using the Shannon-Wiener diversity index, where H = diversity and E = evenness.

	2019		**2020**	
	Pearl River	**Stone**	**Pearl River**	**Stone**
H	1.34	1.571	1.239	1.37
H_{max}	3.401	3.434	8.841	8.207
E	0.394	0.457	0.140	0.167

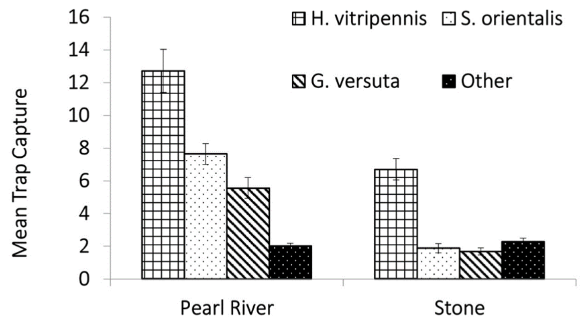

Fig. 2a. Mean trap captures (+/- SE) of the three predominant species of leafhoppers, and all other species, from two research vineyards in Stone County and Pearl River County, MS, 2019.

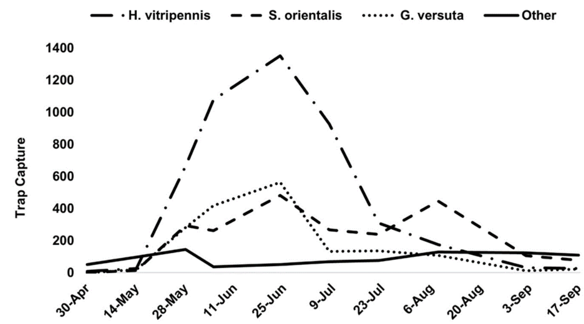

Fig. 3a. Total biweekly captures of three primary species of leafhoppers, and of all other species, from two research vineyards in Stone County and Pearl River County, MS, over ten sample periods in 2019.

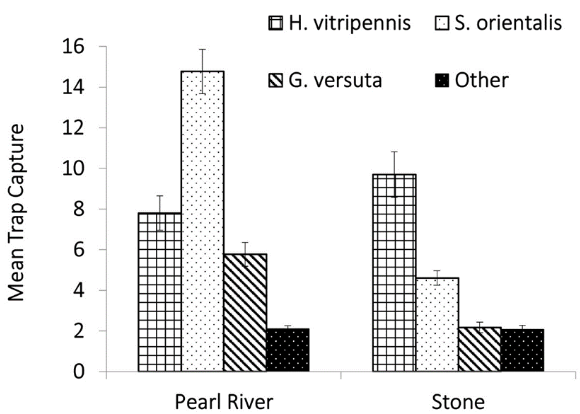

Fig. 2b. Mean trap captures (+/- SE) of the three predominant species of leafhoppers, and all other species, from two research vineyards in Stone County and Pearl River County, MS, 2020.

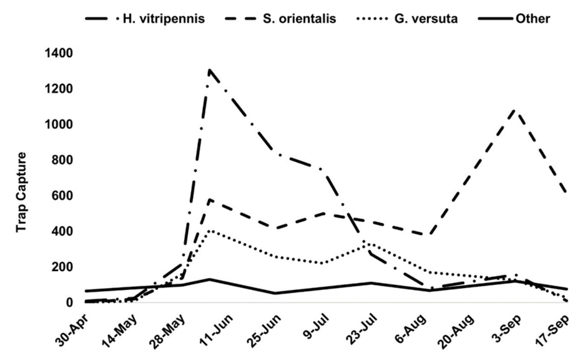

Fig. 3b. Total biweekly captures of three primary species of leafhoppers, and of all other species, from two research vineyards in Stone County and Pearl River County, MS, over ten sample periods in 2020.

captures of *H. vitripennis* and *G. versuta* dropped off quickly from mid-June through the end of each season, those of *S. orientalis* saw a smaller second peak in August.

Distribution of trap captures among the five muscadine cultivars were significant (F= 5.51; *P* = 0.0002), with more leafhoppers collected from Southern Home and Carlos than from Nesbit and Alachua, and there was not a significant year*cultivar interaction (F= 1.38; *P* = 0.2382) (Figure 4a-b).

DISCUSSION

In both years, the leafhopper population at the Stone County vineyard had greater species diversity and evenness as compared to the Pearl River vineyard. Differences in the surrounding habitats (suburban development and meadows at Pearl River vs. mixed forest at Stone County) may account for this greater number and more even distribution of leafhopper species at the Stone County vineyard, but future research could include documentation of surrounding vegetation to gain a better understanding of populations.

The most abundant leafhopper species in 2019 was *H. vitripennis,* accounting for nearly half of all captured specimens. Due to previously reported correlations between high vector populations and disease prevalence, our findings corroborate the status of *H. vitripennis* as a primary vector of Pierce's disease (Almeida et al., 2005, Overall and Rebek 2017). *Graphocephala versuta* also is a known *Xylella* vector and represented nearly 20% of our leafhopper captures in 2019, while the exotic invasive leafhopper *S. orientalis* was the second most abundant species collected after *H. vitripennis* (Overall and Rebek 2015).

Sophonia orientalis, which is occasionally referred to by its pseudonym *S. rufofascia,* has become widely invasive and considered a major tropical pest of both food crops and endangered plants alike (Howarth et al., 2001; Aguin-Pombo et al., 2007). First reported in California in 1996, *S. orientalis* is extremely polyphagous and is reported from >300 plant species in 83 families, including *V. vinifera (*Garrison 1996; Fukada

1996; Aguin-Pombo 2007). Plant damage such as chlorosis and stunting result from both feeding and oviposition injuries (Jones et al., 2000).

The large increase in captures of *S. orientalis,* from 24% of total captures in 2019 to 40% in 2020, likely represents a further population expansion of this exotic -invasive insect. While the Stone County site saw a modest increase in mean capture of *S. orientalis* in 2020, community composition remained similar from 2019 – 2020. It was the Pearl River site that accounted for the major increase in the *S. orientalis* population, nearly doubling from 8 insects per trap in 2019 to 15 insects per trap in 2020. Proximity of this site to a suburban environment may facilitate a more rapid invasion by *S. orientalis*, which is readily transported with infested plant materials, but a more comprehensive survey of the surrounding environment would facilitate a greater understanding of the population biology (Avenasyan et al., 2019). Although *S. orientalis* was demonstrated to not transmit the important virus yellows disease of papaya and guava, further research is needed to determine its potential as a *Xylella* vector, and the threat posed by its rapidly growing population to profitable muscadine production (Jones et al., 1998).

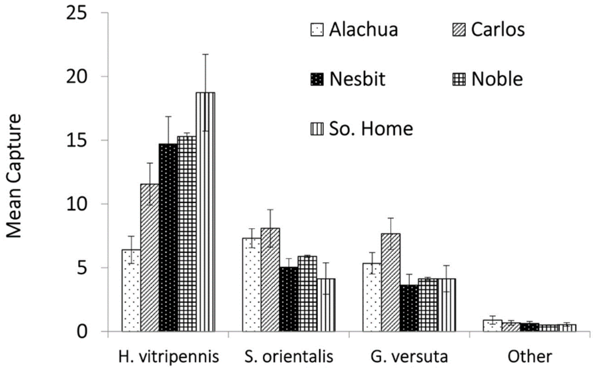

Fig. 4a. Mean captures (+/- SE) of the three primary species, and of all other species of leafhoppers, from each of the five muscadine cultivars at two vineyards in Stone County and Pearl River County, MS, in 2019.

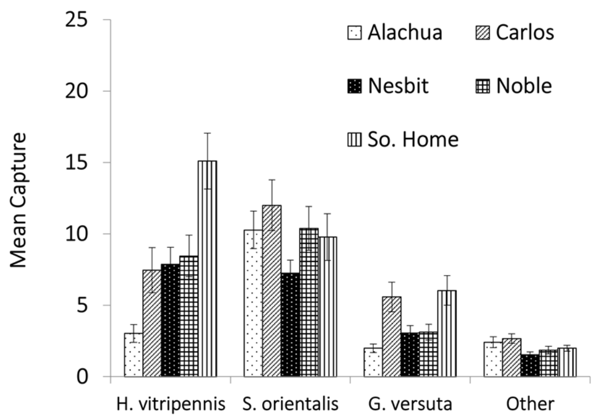

Fig. 4b. Mean captures (+/- SE) of the three primary species, and of all other species of leafhoppers, from each of the five muscadine cultivars at two vineyards in Stone County and Pearl River County, MS, in 2020.

With *H. vitripennis* captures rising sharply in mid-May and captures of other known and suspected Xylella vectors shortly thereafter, we can recommend insecticidal applications just prior to this population spike. Managing *Xf* infection through vector control has been effective in other crop systems including olive and wine grape (Almeida et al., 2005; Fierro et al., 2019). Even with some cultivars of muscadine grape exhibiting resistance or tolerance to *Xf*-infection, this pathogen still would likely play the role of an additive stressor on vines experiencing abiotic stress such as adverse weather conditions. Managing leafhopper populations prior to the May outbreaks could improve vineyard production and vine longevity, and this hypothesis will be tested going forward.

Trap captures among the five cultivars of muscadines remained consistent from 2019-20, especially when considering the primary *Xylella vector, H. vitripennis.* In each year the glassy-winged sharpshooter was most abundant on the Southern Home cultivar, which shares parentage with both *V. rotundifolia and V. vinifera* and retains some interesting differences in growth pattern from the other cultivars. The cut leaf pattern offers an attractive ornamental appearance, but it also sprouts vines in a dense, bushy pattern that offers greater shelter from heat and predators alike, and could account for this preference by *H. vitripennis.*

Winter curing is a phenomenon where cold temperatures can reduce *Xf* survival, limiting secondary disease spread in regions environmentally suboptimal to *Xf*. Dormant grapevines with Pierce's Disease have had their *Xf* bacteria eliminated from overwintering tissues, provided sufficient duration and severity of low temperatures (Feil and Purcell 2001; Purcell 1980). Under the warming climate scenario that we will face in the coming decades, there may be less opportunity for winter curing of grape vines. Furthermore, because *H. vitripennis* overwinters as active adults and can feed on dormant vines during winter months, potentially after pruning has occurred, this may allow inoculations in the fall or winter months to establish chronic disease. Even though leafhopper populations are less abundant in late summer, vector management during the fall may make sense for some vineyard owners hoping to reduce disease incidence in dormant vines.

These findings justify future research into quantifying *Xf* infection in various muscadine cultivars at several points throughout the growing season, allowing us to determine if and when disease management would be most useful (Faino *et al., 2019)*. We also plan to quantify *Xf* infection of the primary leafhopper species at the same seasonal intervals to determine their vectoring potential, with special emphasis on the invasive *S. orientalis (*Sisterson et al., 2020). Identifying the local *X. fastidiosa* strain and describing the genome of various vector species will provide valuable contributions to the body of scientific literature for entomologists and horticulturists alike. Enhancing our knowledge of the insect vectors of *Xf*, including their seasonal activity and abundance and their infection status throughout the year, will help IPM professionals to improve their recommendations for management of a disease that is likely to become increasingly important.

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