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Title: Evaluation of *Grapevine red blotch virus* Epidemiology with Reference to Potential Insect Vectors

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Grapevine red blotch virus (GRBV), the causal agent of red blotch disease (RBD) in grapevine, *Vitis vinifera* L., is an emerging pathogen of significance to the wine grape industry of Oregon, USA. To address knowledge gaps of GRBV epidemiology, spread of GRBV was evaluated in Oregon vineyards over four years. Insect populations that could potentially transmit GRBV were assessed for biological attributes, and their ability to transmit GRBV was tested under controlled conditions.

Virus spread occurred in all study sites where GRBV was initially present. Field surveys for potential vector species yielded treehopper (Hemiptera: Membracidae) species *Spissistilus festinus* (Say), *Stictocephala basalis* (Walker), *Stictocephala bisonia* (Kopp and Yonke), and *Tortistilus albidosparsus* (Stål).

In the Willamette Valley, *St. basalis* immature stages appeared in late May, and adult emergence began in July. Suitable host plants were identified for all life stages. Collections in southern Oregon showed appearance of *T. albidosparsus* immature stages in April. Adults emerged in late June in Willamette Valley and southern Oregon sites. An edge effect of treehopper feeding damage was apparent at two vineyards.

Populations of *St. basalis* and *T. albidosparsus* were maintained in the laboratory and greenhouse. Eggs of *St. basalis* were deposited behind resting buds of woody plants, while *T. albidosparsus* deposited eggs along stems of woody plants. Adult feeding and oviposition occurred near apical tips of shoots. Late-instar nymphs of *St. basalis* emerged as adults on four of five herbaceous plant species in a growth chamber.

Controlled GRBV transmission bioassays on potted grapevines were conducted in 2016–2018 using adults of *Sp. festinus*, *St. basalis* and *T. albidosparsus*. Immature *St. basalis* were additionally used. No evidence of GRBV transmission was found using *St. basalis* or *T. albidosparsus*. A single vine was positive following infestation by *Sp. festinus*. GRBV was acquired in all species.

Voucher specimens of four treehopper species were deposited in the Oregon State Arthropod Collection, Corvallis, Oregon, USA and at the Smithsonian National Museum of Natural History, Washington, D.C., USA.

Evaluation of *Grapevine red blotch virus* Epidemiology with Reference to Potential
Insect Vectors

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Dedicated to the lasting memory of Dr. Chad Finn.

January 9, 1962 – December 17, 2019

CHAPTER 1

Introduction

Daniel T. Dalton

***Grapevine red blotch virus* in Oregon.** *Grapevine leafroll-associated virus 3* (*Closteroviridae*) (GLRaV-3) is the most important etiological agent of grapevine leafroll disease (GLD) and is a primary concern in Oregon wine grape production systems (Martin et al. 2005, Maree et al. 2013). Symptoms of infection include interveinal reddening of leaves in red-fruited cultivars, frequently with inward curling of leaf margins. In a study examining relationships of GLRaV-3 with the primary vector species *Pseudococcus maritimus* (Ehrhorn) (Hemiptera: Pseudococcidae), grape mealybug (Walton et al. 2013), researchers noted widespread, severe GLD-like symptoms at study sites. However, most samples tested negative for GLRaV-3 (Walton, *personal communication*). Near the conclusion of the study period, researchers at Cornell University, New York published findings of a newly discovered single-stranded DNA (ssDNA) virus in grapevine materials from a University of California research vineyard, determined through rolling circle amplification, that shared genomic similarities with known viruses of the *Geminiviridae* (Krenz et al. 2012). Further work showed the widespread distribution of the virus in the United States (Krenz et al. 2014, Sudarshana et al. 2015). The new virus was tentatively named Grapevine red blotch-associated virus due to its association with red blotch disease (RBD) (Al Rwahnih et al. 2013). Agroinoculation on *Vitis vinifera* L. using *Agrobacterium tumefaciens* allowed completion of Koch's postulates, and the virus species was named *Grapevine red blotch virus* (GRBV) (Yepes et al. 2018). Testing of samples from Oregon vineyards that had apparent GLD-like symptoms showed that much of the diseased material was instead affected by RBD (Dalton et al. 2019).

Taxonomy, structure, and replication of *Grapevine red blotch virus*. *Geminiviridae* comprise the most diverse family of plant viruses, containing nine recognized genera and nearly 450 described species (Rojas et al. 2018). A recent revision to the framework of virus taxonomy was executed, aligning *Geminiviridae* with six other ssDNA virus families characterized by circular, Rep-encoding ssDNA viruses forming phylum *Cressdnaviricota* (Krupovic et al. 2020). Most geminivirus genera (*Becurtovirus*, *Capulavirus*, *Curtovirus*, *Eragrovirus*, *Grablovirus*, *Mastrevirus*, *Topocuvirus* and *Turncurtovirus*) are monopartite, while *Begomovirus* are mono- or bipartite in twinned

incomplete icosahedral particles. DNA-A and DNA-B genomes of bipartite begomoviruses share a common region of the long intergenic region that includes the Rep origin site (Zerbini et al. 2017). For all geminiviruses, the highly conserved nonanucleotide sequence TAATATTAC is at the origin stem loop (Ori) that is nicked by the Rep protein to initiate genome replication.

Differences in the genome structure and the limited sequence similarity to other geminiviruses led to GRBV classification as the type specimen within the new genus *Grablovirus* (Varsani et al. 2017). Additional *Grablovirus* species include *Wild Vitis latent virus* (common name wild Vitis virus 1, WVV1) and *Prunus latent virus* (Perry et al. 2016, Al Rwahnih et al. 2018). The genome of GRBV, at 3,206 nt in length, is somewhat longer than other geminiviruses. Two clades of GRBV have been identified (Krenz et al. 2014).

Replication of geminiviruses is accomplished within the host plant in three stages using a suite of viral and host factors (Gutierrez 1999). In Stage A, virus particles penetrate the host cell nucleus through the nuclear pore complex. Virus-encoded superfamily 3 helicase Rep protein binds to plant recombination proteins, thereby providing viral regulation of the plant cell cycle (Singleton et al. 2007, Ramesh et al. 2017). Through binding of host DNA dependent DNA polymerase, the DNA replication process is modified (Hull 2009). Virus ssDNA becomes a template for synthesis of the negative strand, generating double-stranded DNA (dsDNA). Transcription via rolling circle replication is initiated in Stage B through nicking of Ori by activity of HUH endonuclease within Rep (Chandler et al. 2013, Krupovic et al. 2020). Transcription occurs in a bidirectional manner. The GRBV genome contains four open reading frames (ORFs) in the virion sense (clockwise from Ori: V0, V1, V2, V3) and three ORFs in the complimentary sense (counterclockwise from Ori: C1, C2, C3) (Vargas-Ascensio et al. 2019) (Fig.1.1). For GRBV, an incomplete picture of translational products is understood; however, V1 encodes coat protein (CP), while C1 encodes RepA, and a spliced transcript of C1:C2 encodes Rep (Krenz et al. 2014). It is believed that products from other ORFs are involved in tropism of the virus (Cieniewicz et al. 2017a). This tropism may account for differential diagnostic

capability of molecular testing according to timing of sampling and selection of appropriate tissue for analysis (Setiono et al. 2018). In Stage C, viral proteins may then be encapsidated, although *Grablovirus* have not been confirmed to date to be encapsidated into geminate virions (Rojas et al. 2018). Virus particles are released from the cell through budding, or ssDNA may be converted again to dsDNA for further replication, or ssDNA may be transported out of the nucleus and transferred to other plant cells through plasmodesmata (Rojas et al. 2005, Hulo et al. 2010).

Detection of *Grapevine red blotch virus*. Symptoms of RBD have been recognized in field-grown *V. vinifera* since 2008 (Calvi 2011, Sudarshana et al. 2015). However, existence of GRBV can be traced back to a latest date of 1940. That year, a grape leaf sample was collected by H. Olmo, Department of Enology and Viticulture, University of California, Davis (UCD) that, when tested for GRBV 74 years later, shared up to 99% sequence similarity to voucher GRBV specimens (Al Rwahnih et al. 2015). It appears that GRBV has been present for many decades in California vineyards.

In the United States, reports have shown distribution of GRBV in commercial vineyards of California (Al Rwahnih et al. 2013, Cieniewicz et al. 2018a, Cieniewicz et al. 2019), Oregon (Dalton et al. 2019), Washington (Poojari et al. 2013, Adiputra et al. 2018), Idaho (Thompson et al. 2019), Texas and Arkansas (Sudarshana et al. 2015), Ohio (Yao et al. 2018), Missouri (Schoelz et al. 2019), Georgia (Brannen et al. 2018), North Carolina (Hoffmann et al. 2020), Virginia (Jones and Nita 2019), Maryland, Pennsylvania, New Jersey, and New York (Krenz et al. 2014). GRBV has also appeared on *Vitis* L. species in germplasm collections of California, including *V. aestivalis* Michx., *V. amurensis* Rupr., *V. biformis* Rose, *V. blancoii* Munson, *V. bloodworthiana* Comeaux, *V. monticola* Buckley, and *V. nesbittiana* Comeaux (Thompson et al. 2018). Findings of GRBV have been made on grapevine in other countries, including India (Marwal et al. 2018), Switzerland (Reynard et al. 2017), South Korea (Lim et al. 2016), Argentina (Luna et al. 2019), Mexico (Gasperin-Bulbarela and Licea-Navarro 2019), and in Ontario (Xiao et al. 2018), British Columbia (Poojari et al. 2017), and Nova Scotia, Canada (Poojari et al. 2020). In Switzerland, GRBV is currently believed to be isolated to a small set of grapevines on a research vineyard. These materials were

obtained from California in 1985 (Reynard et al. 2017). GRBV particles do not appear to replicate on woody or herbaceous plants other than *Vitis* spp. (Bahder et al. 2016a, Cieniewicz et al. 2019). Spread of RBD has been documented in California and Oregon (Cieniewicz et al. 2017b, Dalton et al. 2019). Vines that were located away from vineyards in California were found to be infected with GRBV and WVV1, with evidence of intraspecific recombination between clades (Perry et al. 2016, Cieniewicz et al. 2018a). Seedling grapevines adjacent to an infected vineyard block in southern Oregon also tested positive for GRBV (Dalton et al. 2019).

Several techniques using polymerase chain reaction (PCR) have been used to identify GRBV in sample materials, including end-point multiplex PCR (e.g. Krenz et al. 2014, Al Rwahnih et al. 2015), quantitative PCR (Sudarshana et al. 2015, Setiono et al. 2018, Dalton et al. 2019), and droplet digital PCR (Bahder et al. 2016b). Virus DNA extraction from plants using the RNeasy Mini Kit or DNeasy Mini Kit (Qiagen, Hilden, Germany) (Al Rwahnih et al. 2015, Bahder et al. 2016b), H. P. Plant DNA kit (OMEGA Biotek, Norcross, GA, USA) (Cieniewicz et al. 2019), or custom-made guanidine thiocyanate extraction buffers followed by a nucleic acid concentration processes (Dalton et al. 2019) have been used effectively. For virus DNA extraction from insect specimens, both the E.Z.N.A. Insect Kit (OMEGA Biotek, Norcross, GA, USA) (Cieniewicz et al. 2019) and Qiagen Blood and Tissue Kit (Bahder et al. 2016b) have been used. Various primer sets amplifying specific virus proteins or genomic regions are available (Al Rwahnih et al. 2013, Sudarshana et al. 2015, Setiono et al. 2018, Gasperin-Bulbarela and Licea-Navarro 2019). Recently, loop-mediated isothermal amplification (LAMP) has been developed to identify GRBV in plant samples (Romero et al. 2019). This technique may represent a breakthrough in diagnostic GRBV discovery because LAMP is far more sensitive than PCR techniques, is inexpensive with lesser use of laboratory consumables, and can be completed faster than PCR assays.

Impacts on grapevine metabolism. Effects of GRBV on *V. vinifera* have been investigated by multiple research groups. In one study, on *V. vinifera* 'Chardonnay', volatile compounds in wine made from berries of infected grapevines were impacted,

changing objective measurements of alcohol content and pH and leading to a perceived decrease of wine quality (Girardello et al. 2019). Detrimental effects on the berry ripening process are evident, as highlighted by impacts of RBD on leaf chemistry and transcriptional analysis. In a study of *V. vinifera* 'Cabernet Franc' (CF) and *V. vinifera* 'Cabernet Sauvignon' (CS), the level of the host defense amino acid proline, among other foliar amino acids, was elevated in GRBV-infected vines (Wallis and Sudarshana 2016). Proline is associated with increased flavonoid content in plants undergoing oxidative stress (Chutipaijit et al. 2009). Flavonoid content was greater, constituting a more severe defense reaction, in infected CF compared to infected CS (Wallis and Sudarshana 2016). In a separate study on CS, standardized levels of total soluble solids (TSS), as measured in °Brix, indicated that berries of infected vines had lower pH and anthocyanin concentrations relative to those of healthy vines (Martínez-Lüscher et al. 2019). However, berries of field-grown GRBV-infected vines were found to have lower TSS as compared to non-infected vines (Calvi 2011), further compounding the negative effects. The changes of berry and leaf chemistry characteristics of grapevines affected by RBD coincide with the phenological onset of véraison (Blanco-Ulate et al. 2017) and mark the earliest time point in which leaf collections could reliably indicate increased titer of GRBV (Setiono et al. 2018). In sum, effects on wine grapes are caused by inhibition of ripening pathways, leading to changes in color, flavor, or the aroma profile of finished wine (Blanco-Ulate et al. 2017).

Vectors of *Grapevine red blotch virus*. A greenhouse study initially linked infection of GRBV with infestation by putatively viruliferous *Erythroneura ziczac* Walsh (Hemiptera: Cicadellidae), Virginia creeper leafhopper (Poojari et al. 2013). Insects were introduced onto GRBV-positive inoculum source vines for a 72h acquisition access period (AAP), then transferred onto virus-free grapevines for a 72h inoculation access period (IAP). Insects and freshly emerging leaves were tested for presence of GRBV. However, infection could not be repeated in independent verification assays (Bahder et al. 2016b). Greenhouse tests conducted at UCD using *Spissistilus festinus* (Say) (Hemiptera: Membracidae: Smiliinae), threecornered alfalfa hopper, indicated its role in transmission of GRBV (Bahder et al. 2016b). In a California vineyard with high

prevalence of GRBV, individuals of *Sp. festinus*, *Colladonus montanus reductus* (Van Duzee) (Hemiptera: Cicadellidae), *Osbornellus borealis* DeLong and Mohr (Hemiptera: Cicadellidae), and *Melanoliarus* Fennah spp. (Hemiptera: Cixiidae) were found to have ingested GRBV particles, but only viruliferous *Sp. festinus* were spatially associated with infected vines (Cieniewicz et al. 2018b). Virus transmission was previously shown in the case of *Micrutalis malleifera* Fowler (Hemiptera: Membracidae: Smiliinae), vector of *Tomato pseudo-curlytop virus* (TPCTV, *Geminiviridae*: *Topocuvirus*), on solanaceous plants (Simons and Coe 1958). The discovery of *Sp. festinus* as vector of GRBV, if confirmed, marks the second known case of geminivirus transmission by a smiliine treehopper.

Plant viruses exploit insect vector species through several possible mechanisms of transmission (Ammar and Nault 2002). Four primary modes are described as nonpersistent stylet-borne, semipersistent foregut-borne, persistent circulative, and persistent propagative transmission (Hogenhout et al. 2008). For phloem-limited viruses such as GRBV, a persistent circulative strategy is used. Persistent transmission occurs where virus particles enter the body of the vector insect and accumulate in the hemolymph and salivary gland. Circulative transmission requires multiple steps; 1) insect ingestion of virus particles; 2) passage of particles through the gut lumen; 3) filtering of particles into the hemocoel; 4) transfer of particles to the salivary gland; 5) injection of particles into a host plant through the salivary canal (Fereres and Raccah 2015). Molecular barriers to transmission are found throughout the pathway, and the ability to cross these barriers is virus-specific (Ng and Falk 2006). Multiple examples implicate geminivirus coat protein as the primary determinant allowing virus particles to cross from hemolymph to the accessory salivary gland (Briddon et al. 1990, Fereres and Raccah 2015, Hogenhout et al. 2008).

Geminiviruses are transmitted by diverse taxa of Hemiptera, and different genera share a vector type (Whitfield et al. 2015). Furthermore, virus-vector specificity factors could result in differential abilities of insect populations to transmit disease (Burrows et al. 2007). Recent molecular diagnostic assays of mitochondrial and nuclear genes showed that populations of *Sp. festinus* from the southwestern United States are

genetically distinct from southeastern populations (Cieniewicz et al. 2020). While it remains to be seen if population differences have an effect on transmission of GRBV, this finding may potentially explain variable rates of disease transmission in different geographic regions. No reports are published examining the capacity of other Membracidae to transmit GRBV.

Management actions in affected vineyards. Prevalence of GRBV may affect profit margins of a commercial vineyard (Sudarshana et al. 2015). Little information is available on the economic impact of RBD, but one economic analysis showed potentially extreme effects in high-value production areas such as Sonoma and Napa Counties, California (Ricketts et al. 2017). Incidence of GRBV appears to be most likely associated with primary inoculum, or infected planting stock, with limited secondary spread (Cieniewicz et al. 2019). The first line of defense against RBD is planting of certified GRBV-free stock. A management approach has been suggested in which vines infected with GRBV be rogued and replaced if overall virus incidence of a vineyard block is less than 30%. Whole block removal should be considered if incidence is greater than 30%, due to likely price penalties (Ricketts et al. 2017). If the presence of a vector insect is confirmed, rogueing of infected plants coupled with systemic insecticide application could be a viable approach to eliminate GRBV in an affected vineyard (Rojas et al. 2018). Insecticides are effective in leguminous crops to control populations of *Sp. festinus* (Beyer et al. 2017). While mowing may provide a level of control, this management approach will not likely eliminate all *Sp. festinus* from affected alfalfa fields because insects are multivoltine, and eggs are often found at the hypocotyl near the base of a plant (Preto et al. 2019).

Phylogeny of treehoppers and relation to plant diseases. The true hoppers (Hemiptera: Auchenorrhyncha) are a large group of phloem-feeding insects, many of which are important in the transmission of plant disease (Bartlett et al. 2018). Fulgoroid species (Hemiptera: Auchenorrhyncha: Fulgoroidea: Cixiidae and Delphacidae) have long been recognized as important vectors of diverse families of plant viruses, while at least two membracoid families (Hemiptera: Auchenorrhyncha: Membracoidea: Cicadellidae and Membracidae) also contain insects that are known to transmit plant

viruses (Ammar and Nault 2002). Within the Membracoidea, cicadellids are common vectors of phytoplasmas (Weintraub and Beanland 2006).

Treehoppers are among the most diverse members of the superfamily Membracoidea, containing more than 430 genera and 3,400 species worldwide (Bartlett et al. 2018). Regions across the world are home to Membracidae, and the Smiliinae represent the most species-rich subfamily in North and South America. Physical characteristics, including morphology of the pronotum and male genitalia, were traditionally used to determine treehopper phylogeny (Van Duzee 1908, Funkhouser 1917, Caldwell 1949). The enlarged membracid pronotum appears to have been gained or lost at least two times over evolutionary time (Dietrich et al. 2001). Wing venation is another reliable physical characteristic separating families and tribes of the Membracidae, except for the smiliine tribes Acutalini and Micrutalini that share similar wing venation to members of the Darniinae (Dietrich et al. 2001). Today, development of molecular tools provides a basis to reevaluate previous understanding of membracid phylogeny (Cryan et al. 2000, Cryan and Urban 2012). The Membracoidea likely arose from a monophyletic lineage that today is represented by the families Membracidae, Aetalionidae, and Melizoderidae (Cryan and Urban 2012). Assessment of mitochondrial and nuclear genes indicates that Smiliinae share a common ancestor with Membracinae but are recognized as a paraphyletic group, whereas membracine species are monophyletic (Lin et al. 2004).

Membracidae share a common ancestor with the Cicadellidae. While many cicadellids are considered plant pests, inflicting indirect damage on hosts due to their broad capacity to vector bacteria, phytoplasmas, spiroplasmas, and viruses, membracids are known primarily as occasional direct pests causing girdling of host plant materials (Bartlett et al. 2018, Beyer et al. 2017, Yothers 1934). In limited instances, smiliine insects have been found to transmit microbial agents causing plant disease. The species *M. malleifera* is recognized as a vector of TPCTV, a phloem-limited geminivirus (Tsai and Brown 1991, Simons and Coe 1958, Simons 1962, D'Arcy and Nault 1982). Notably, the coat protein of TPCTV contains features more similar to leafhopper-transmitted geminiviruses, rather than those transmitted by the

whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) (Briddon et al. 1996). *Spissistilus festinus* are believed to transmit GRBV into grapevines in North America (Bahder et al. 2016b, Cieniewicz et al. 2018b, Thompson et al. 2018). Additionally, *Sp. festinus* are carriers of the ArAWB phytoplasma in subtropical regions of South America (Grosso et al. 2016) and are direct pests of leguminous crops (Beyer et al. 2017). The smiliine treehopper *Ophiderma definita* Woodruff was one of many treehopper species found on oak to contain *Xylella fastidiosa* Wells et al., illustrating their potential to vector bacterial leaf scorch of oak (Zhang et al. 2011). A study investigating potential transmission of *X. fastidiosa* biotypes in citrus found that a single individual of the treehopper *Cyphonia claviger* (Fab.) successfully transmitted bacteria to uninfected plants (Dellapé et al. 2016). While the broad capacity for transmission of phytoplasmas and other microbes by membracids remains uninvestigated, such a finding would be unsurprising due to their associations with diverse woody perennial plant species (Weintraub and Beanland 2006).

Smiliine treehoppers are represented by seven tribes, including the Ceresini which contain species that are native across the United States (Caldwell 1949, Kopp and Yonke 1973, 1979). Species include *Sp. festinus* native to the US southeast (Caldwell 1949, Newsom et al. 1983), *Stictocephala bisonia* (Kopp and Yonke) native to the Midwest (Kopp and Yonke 1979), *Stictocephala basalis* (Walker) native to areas of the Northeast, and *Tortistilus* Caldwell spp. from regions of California (Van Duzee 1908). *Stictocephala* Stål and *Tortistilus* cannot be easily distinguished from one another based on the form of the male genitalia, differing only by divergent styles on the lateral valves (Caldwell 1949). Species belonging to these two genera also share key life cycle characteristics, with the eggs borne on woody host plants and immature stages completing their development on herbaceous plant species. Despite these similarities, *Tortistilus* can be differentiated from *Spissistilus* based on size, and also by the shape of the pronotum, which is comparatively flattened in *Spissistilus*. (Caldwell 1949). *Stictocephala bisonia* is an exotic species in Europe that has been present for over 100 years in ruderal habitats of Austria, the Czech Republic, and Poland (Schedl 1991, Lauterer et al. 2011, Świerczewski and Stroiński 2011, Walczak et al. 2018). Given the

remarkable diversity of New World membracids, and in particular the Smiliinae, the potential exists that state-of-the-art molecular diagnostic procedures will identify increasing numbers of plant pathogens that are naturally associated with smiliine treehoppers (Ammar and Nault 2002).

Behavior of Membracidae. Membracids have remarkable diversity in sociobiological characteristics including differences in maternal care, ant-nymph mutualisms, and vibrational communication for defense or mating (Bartlett et al. 2018). Many of these differences occur at generic or tribal classifications (Lin et al. 2004). Many pre-social treehopper species use an alarm pheromone to alert conspecifics of active predation. Upon piercing of the insect body, the pheromone is released, repelling other nymphs away from the predator (Nault et al. 1974). Pheromone emissions may increase the antennation of ants, thereby providing a second mechanism of protection against natural enemies (Nault and Phelan 1984). More frequently, vibrational communication is used to modulate treehopper behavior (Cocroft and Rodríguez 2005). To mate, the male emits a spontaneous call, and a receptive female responds, initiating a back-and-forth vibrational duet in which the male fine-tunes its signal as it wanders in search of the mate (Hunt 1993, Miranda 2006, Rodríguez et al. 2012). The insects will copulate to complete the mating ritual. Specialized calls are emitted synchronously by competing males in order to mask the original male call or female response (Legendre et al. 2012). The efficacy of masking is the basis for mating disruption against *Scaphoideus titanus* Ball (Hemiptera: Cicadellidae) and other species that use artificial vibrational cues (Eriksson et al. 2012, Mazzoni et al. 2017). Physical interactions may additionally occur between rivals (Sullivan-Beckers and Cocroft 2010). Vibration is further used for communication between a female and its offspring, and in gregarious species for communication within an aggregation. When nymphs of the gregarious species *Umbonia crassicornis* Amyot & Serville are attacked by a parasitoid, brief vibrational pulses are emitted by individuals. The resultant chain of signals will rapidly alert the female to the attack, and it may successfully defend the colony up to 75% of the time (Cocroft 1999, 2001). By contrast, solitary species may have cryptic coloration to match their host plants and hide from natural enemies (Lin 2006). Ant attendance

and parental care of treehopper nymph colonies are common forms of protection for many species of the membracid subfamily Endoiastinae and in the Aetalionidae (Dietrich et al. 2001, Reithel and Campbell 2008).

Life history characteristics are known for diverse species of Membracidae. Treehoppers are oviparous organisms that undergo ecdysis as they develop through multiple instar stages. Species can be univoltine (Yothers 1934, Ebel and Kormanik 1965) or multivoltine (Wood 1976; Nixon and Thompson 1987). Nymphs and adults may use separate plant species as feeding hosts. They use their piercing-sucking mouthparts to penetrate host plant phloem tissue, feeding circumferentially around leaf petioles or tender plant stems, resulting in partial or complete girdling of vegetative stem (Fig. 1.2a) or leaf petiole tissue (Fig. 1.2b) (Andersen et al. 2002, Grosso et al. 2016, Beyer et al. 2017). Seasonal dynamics of *Sp. festinus* at vineyard field sites were found to include their capacity to overwinter as adults on leguminous plant hosts, as well as completion of at least two generations per year (Preto et al. 2019). Females of *Sp. festinus* migrate from oviposition sites earlier in the season than males (Mitchell and Newsom 1984a). Signs of treehopper feeding activity manifest as visible flagging or reddening above the feeding site (Bahder et al. 2016b). In a replicated trial of *Sp. festinus* behavior, all tested legume species were found to be reproductive hosts, while few other species supported reproduction (Preto et al. 2018a). A polyphagous feeding habit is also seen in other species, including *Micrutalis calva* (Say) (Hemiptera: Membracidae: Smiliinae), and diverse smiliine oak specialists (Nixon and Thompson 1987, Wallace and Troyano 2006).

Most economic damage attributed to infestations of *Stictocephala* spp. and *Tortistilus* spp. is a consequence of ovipositional activity on woody shoots (Yothers 1934, Caldwell 1949, Bartlett et al. 2018). Ovipositional behavior may vary by treehoppers species. *Stictocephala militaris* (Gibson and Wells) was documented to deposit eggs at the base of resting buds in groups of 1-12 eggs per bud (Ebel and Kormanik 1965). *Stictocephala* spp. and *Tortistilus* spp. similarly deposited eggs behind buds (Fig. 1.3a), but some biotypes instead laid eggs longitudinally or in stacked

aggregations along stem tissue (Fig. 1.3b) (Yothers 1934). Species of Aetalionidae and certain membracids lay eggs in large masses on leaf tissues (Bartlett et al. 2018).

Economic damage from RBD in United States Pacific Coast vineyards led to investigations revealing that GRBV was the causative agent of the disease (Sudarshana et al. 2015, Yepes et al. 2018). Thus, in addition to recognizing the host and environments in which RBD could be found, knowledge of the pathogen itself allowed completion of the disease triangle (Fig. 1.4). Insect species that could potentially transmit the disease to unaffected vines were found to include *Sp. festinus* and *E. ziczac* (Bahder et al. 2016b, Poojari et al. 2013). Given the phloem-limited nature of GRBV (Bahder et al. 2016b), feeding strategies of mesophyll-feeding leafhoppers compared to phloem-feeding treehoppers (Bartlett et al. 2018), and the historical occurrence in Oregon of *Sp. festinus* (Appendix A, Dalton et al. 2020), efforts were dedicated to assessing the potential for smiliine treehopper species to transmit GRBV to uninfected vines. Secondary spread of GRBV was assessed, and greenhouse transmission trials occurred concurrently with investigations on behavioral aspects of smiliine treehopper species *Sp. festinus*, *St. basalis*, and *T. albidosparsus*.

Logic model of research project. This dissertation serves as a major contribution of the Oregon State University (OSU) Horticultural Entomology Laboratory research to better understand causes of red-leaf symptoms in Oregon vineyards (Fig. 1.5). Beginning in 2010, efforts were undertaken to investigate spatial correlations between distribution of *P. maritimus* and vines exhibiting symptoms of GLD. In 2013, after GRBV was identified as the main causal agent of red-leaf symptoms at vineyard study sites, the trajectory of the research program shifted to identify other potential vectors of disease. Funding sources included grants from the United States Department of Agriculture – National Institute of Food and Agriculture – Specialty Crops Research Initiative, the Oregon Wine Board, and California Department of Food and Agriculture. Entomologists, plant pathologists, and assistants from OSU and University of California contributed to the project through myriad activities ranging in scope from short- to long-term impact. It is expected that the findings of this research project will be disseminated, and relevant information on treehopper biology and epidemiological

interactions will provide a strong basis for future research. These efforts thus aim to serve the needs of growers to address the issue of GRBV epidemiology in wine grape production systems.
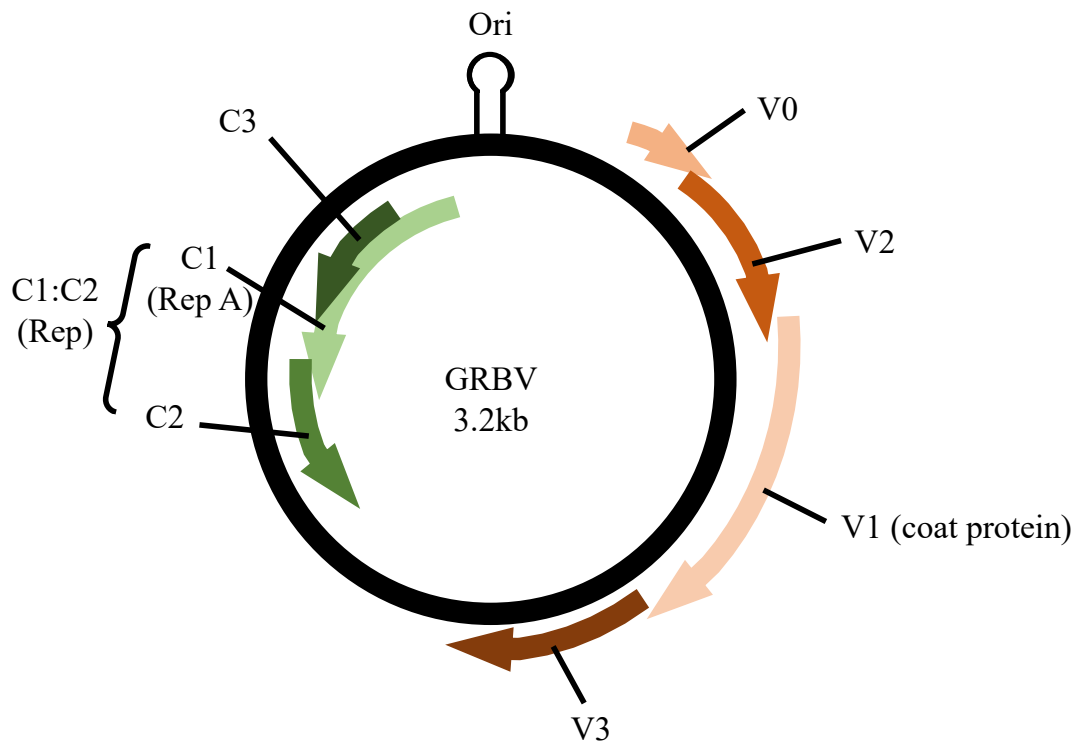
Figure 1.1 Arrangement of Grapevine red blotch virus genome containing seven open reading frames and known protein translation products. Ori is the origin stem loop structure where replication begins.
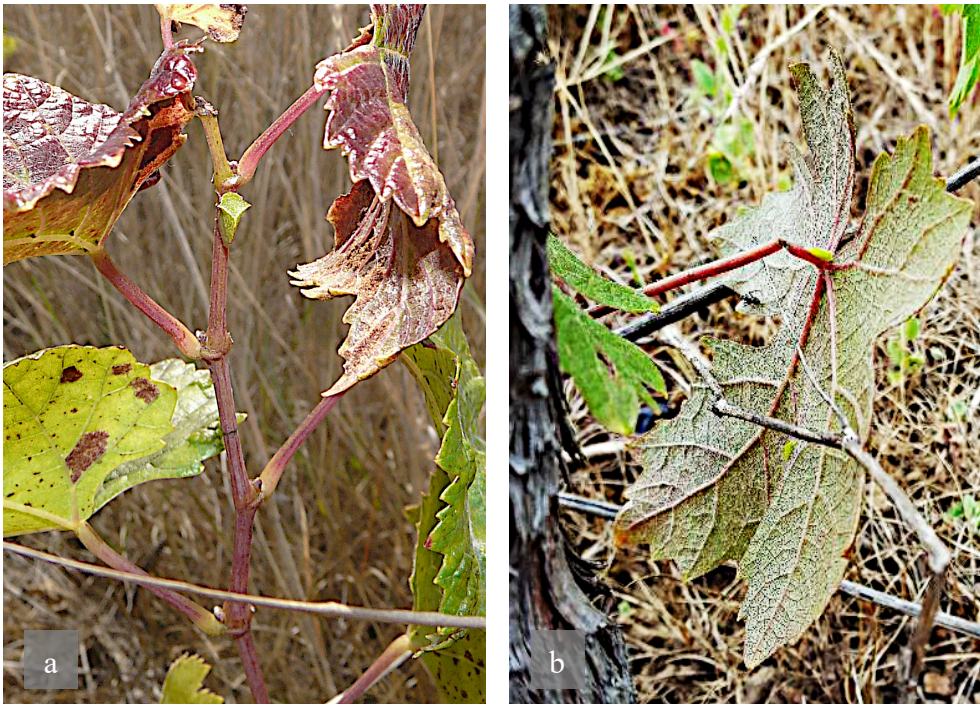
Figure 1.2 *Vitis vinifera* stem (a); and leaf petiole (b) girdles induced by *Stictocephala basalis* feeding activity.

Figure 1.3 Egg of (a) *Stictocephala basalis*; (b) *Tortistilus albidosparsus* deposited on woody host plants.
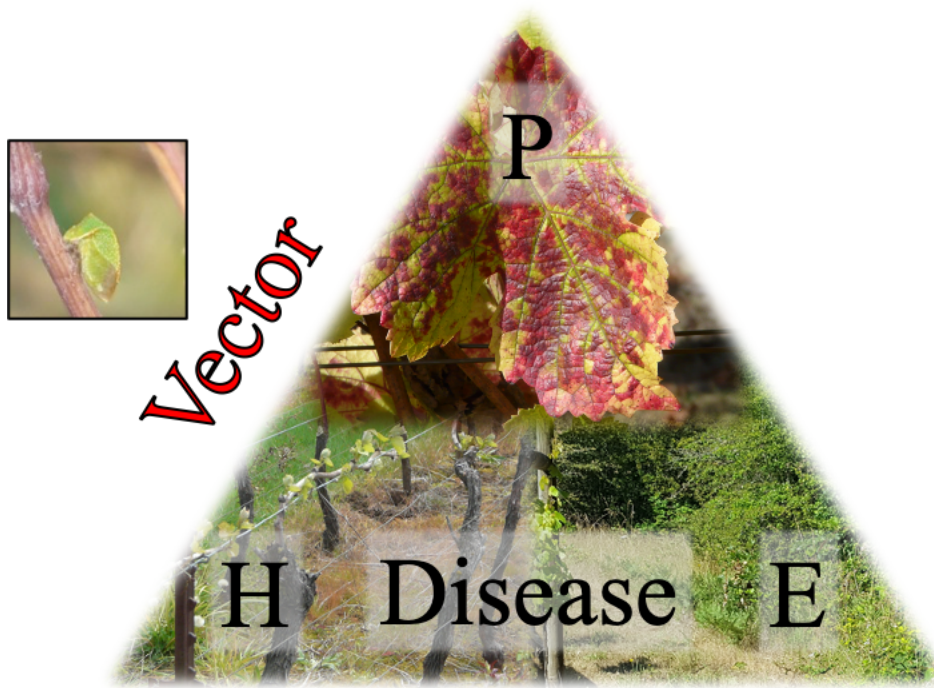
Figure 1.4 Disease triangle of red blotch disease, involving presence of a pathogen (P) and a susceptible host (H) in a conducive environment (E). A treehopper (Hemiptera: Membracidae) is the assumed vector linking P and H.

Inputs	**Grants**
	2010-2012 USDA-NIFA-SCRI
	• Mealybugs and GLRaV-3
	2014-2016 OWB
	• Spatial patterns of virus-symptomatic vines; preliminary vector investigations
	2017-2019 CDFA
	• Treehopper biology as potential vectors of GRBV

Resources

People involved:
- Entomologists
- Plant pathologists
- Research assistants and Post-doctoral scholars
- Graduate and undergraduate students

Specialized materials:
- Greenhouse, hoop house, growth chamber, dedicated laboratory materials
- Biological agents – plants and insects
- Field site access

Outputs

Participants

Growers, scientists, students

Activities

Workshops   Research Symposia   Reports & Publications

Outcomes & Impacts

Short-term	Medium-term	Long-term
Maintain laboratory research presence in viticulture community	Disseminate knowledge of treehopper field behavior	Develop recommendations for integrated pest management of treehoppers and GRBV
Improve knowledge about treehopper biology	Extend expertise about treehopper biology to academic community	Invite collaboration across programs for future projects
Stimulate interest in treehopper-GRBV vector ecology	Disseminate findings to research community	Lay groundwork for career in entomology

Short-term ⟹ *Medium-term* ⟹ *Long-term*

Figure 1.5 Logic model for dissertation, "Evaluation of *Grapevine red blotch virus* Epidemiology with Reference to Potential Insect Vectors".

CHAPTER 2

Spatial associations of vines infected with grapevine red blotch virus in Oregon vineyards

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ABSTRACT

Spread and in-field spatial patterns of vines infected with grapevine red blotch virus (GRBV) were documented in Oregon vineyards using field sampling, molecular diagnostics, and spatial analysis. Grapevine petiole tissue collected from 2013–2016 was tested using quantitative PCR for GRBV. At Jacksonville in southern Oregon, 3.1% of vines were infected with GRBV in 2014, and GRBV incidence reached 58.5% of study vines by 2016. GRBV-infected plants and GRBV-uninfected plants were spatially aggregated at this site in 2015, and infected plants were spatially associated between years 2015 and 2016. In a southern Oregon vineyard near Talent, 10.4% of vines were infected with GRBV in 2014, and infection increased annually to 21.5% in 2016. At Talent, distribution of infected vines was spatially associated across all years. GRBV infection was highest at Yamhill, in the Willamette Valley, where 31.7% of the tested vines had GRBV infection in 2014. By 2016, 59.2% of the vines tested positive for GRBV. Areas of aggregation increased and were spatially associated across all years. From 2013–2015, GRBV was not detected at Milton-Freewater in eastern Oregon. Spatial patterns of GRBV infection support evidence of spread by a mobile insect vector. GRBV is a significant threat to Oregon wine grape production due to its drastic year-over-year spread in affected vineyards.

Key words: vineyard pathogens, vineyard diseases, virus epidemiology, Membracidae

INTRODUCTION

The epidemiology of grapevine viruses is a subject of particular interest to the grape industry because of perceived negative impacts of viruses on fruit yield and wine quality. To date, more than 80 viruses or virus-like agents are known to infect commercial cultivars of grapevine (*Vitis* spp.) (Maliogka et al. 2015; Martelli 2014). Grapevine leafroll-associated viruses (GLRaVs) (*Closteroviridae*: *Ampelo- Clostero- and Velarivirus*) are among the grapevine viruses of primary economic concern worldwide (Almeida et al. 2013; Maliogka et al. 2015; Naidu et al. 2015). Infection by GLRaVs leads to symptoms of grapevine leafroll disease (GLD): interveinal reddening

or chlorosis, cupping of the leaf blade, uneven ripening of grape clusters, reduced crop quality, and declining plant vigor (Almeida et al. 2013; Naidu et al. 2015).

Some Oregon vineyards showing apparent symptoms of GLD tested negative for strains of GLRaV. In 2012, a virus damaging to grapevine was revealed, and its presence helped explain apparently GLD-symptomatic vines that tested negative for GLRaVs. This new-to-science virus is in the family *Geminiviridae*, and was initially named Grapevine cabernet franc-associated virus (Krenz et al. 2012), Grapevine red blotch-associated virus (Al Rwahnih et al. 2013), Grapevine red leaf-associated virus (Poojari et al. 2013), and Grapevine geminivirus (Seguin et al. 2014), but is now universally described as grapevine red blotch virus (GRBV) (Varsani et al. 2017). GRBV is the causal agent of red blotch disease (RBD) and is the type species of the genus *Grablovirus* (Varsani et al. 2017; Yepes et al. 2018). Additional proposed species of *Grablovirus*, tentatively named Wild Vitis virus 1 and Prunus geminivirus A, have been further identified (Perry et al. 2018; Al Rwahnih et al. 2018). Isolates of these newly described geminivirus species have circular monopartite ssDNA genomes that are slightly larger than those of most other *Geminiviridae* at 3.2 kb (Al Rwahnih et al. 2013; Varsani et al. 2017). RBD can compromise the ripening process of grapes post-véraison (Blanco-Ulate et al. 2017), and symptoms expressed in leaf tissue can be similar to those of GLD (Sudarshana et al. 2015).

Spread of GRBV was documented from 2014–2016 at a vineyard in Napa Valley, California (Cieniewicz et al. 2017b). Insects collected from that site and representing at least three families within the order Hemiptera tested positive for presence of the virus, indicating successful virion uptake by diverse insect taxa (Cieniewicz et al. 2018b). The threecornered alfalfa hopper, *Spissistilus festinus* (Say) (Hemiptera: Membracidae), was found to transmit GRBV under greenhouse conditions (Bahder et al. 2016b), making GRBV only the second known geminivirus to be transmitted by a member of Membracidae, following the discovery that the treehopper *Micrutalis malleifera* (Fowler) transmits tomato pseudo-curly top virus (TPCTV) in tomatoes (Simons and Coe 1958). The field distribution of *S. festinus* was spatially associated with GRBV-infected grapevines in a vineyard block in California where GRBV was spreading (Cieniewicz et al. 2018b). Treehopper species including *S. festinus* and

numerous species belonging to the genus *Tortistilus* occur as incidental species across the US, including in wine grape production regions of Oregon and California (Kopp and Yonke 1979). Host plants for these insects are common in the vegetative communities surrounding vineyards in western North America (Goeden and Ricken 1985; Swiecki and Bernhardt 2006; Valenti et al. 1997). Despite the presence of the vector *S. festinus* and closely related species in Oregon, no study has so far reported the patterns of GRBV distribution in Oregon vineyards. The objectives of this study were to document the infection patterns and spread of GRBV in Oregon vineyards.

MATERIALS AND METHODS

Site selection and description. Vineyards were initially selected based on grower concerns about the presence or apparent spread of grapevine viruses. Previously, vineyard blocks were surveyed for infestations of *Pseudococcus maritimus* (Ehrhorn) (Walton et al. 2013) and presence of GLD-like symptoms post-véraison. Vines representing the vineyard study blocks were assessed using real-time qPCR from 2013–2016 for infection by GRBV. Study blocks were located in three Oregon wine grape regions (Jones et al. 2003) and are hereafter identified by proximity to the nearest town: in eastern Oregon, Milton-Freewater was planted in 2003 and was surveyed for GRBV from 2013–2015; in southern Oregon, a vineyard near Jacksonville was established in 2010, and a vineyard near Talent was planted in 1990. The southern Oregon sites were surveyed for GRBV from 2014–2016. In the Willamette Valley, the Yamhill site was planted in 1990 and surveyed for GRBV from 2013–2016.

The Milton-Freewater block was in a heavily managed area formerly occupied by an apple orchard with no surrounding wild habitat. The Jacksonville site was previously a pear orchard block with riparian vegetation dominated by Oregon white oak (*Quercus garryana*) to the north and east. Seedling grapevines were found at Jacksonville along the riparian habitat and nearby fence lines. Testing in 2015 indicated that GRBV was present in grapevine seedlings in the habitat immediately surrounding the vineyard block (data not shown). The area to the south and west was planted to wine grapes. The Talent block was bordered by a diversified organic vegetable and berry farm to the north and wine grapes to the east, south, and west. The Yamhill block was bordered to the north and east by a stand of Oregon white oak, to the east and west by wine grapes,

and to the south by unmanaged riparian habitat dominated by ash (*Fraxinus* spp.), blackberry (*Rubus armeniacus*), hazelnut (*Corylus* spp.) and Oregon white oak.

The sampling schemes differed at all sites based on the layout of study blocks. The Milton-Freewater study block was composed of the cultivars Petit Verdot, Malbec, Merlot, and Cabernet franc. In 2013, leaves from every eighth vine within each of 20 consecutive rows were collected, providing material for GRBV laboratory tests of approximately 10 vines in each row. In 2014, leaves from vines alternating with those selected in 2013 were collected from within the same rows. Leaves from vines previously tested in 2013 and 2014 were collected in 2015 for qPCR analysis of presence of GRBV. At Jacksonville, approximately 20 vines of cultivar Pinot noir were examined in each of 10 even-numbered rows in 2014 and 2015. Vines were spaced 1.8 m apart within rows. Vines in even-numbered rows were examined, producing observations in rows that were spaced 5.5 m apart. The eastern-most row bordering the field edge of the Jacksonville block was Row 14, and the block extended to the west past Row 32. Leaves from every fourth vine were collected in 2014, and collections in 2015 were taken from the same vines. Due to the grower's aggressive removal of virus-symptomatic vines, sampling at Jacksonville in 2016 was restricted to a subset of vines that were tested in 2014–2015. At Talent, approximately 10 vines were sampled in 20 consecutive rows, beginning at Row 19, of a block containing cultivars Cabernet franc and Pinot gris. Leaves were collected from every fifth vine each season from 2014–2016. In this block, rows were spaced 3 m apart, with in-row spacing of 1.8 m between individual vines. At Yamhill, surveyed rows of Pinot noir were coupled with 1 m between coupled rows, and 1.6 m between non-coupled rows. Plants were spaced 1 m apart within Rows 44–63. In 2013, leaves from every seventh vine beginning from the 28th vine from the northern edge of a row were assayed with qPCR in 20 rows to assess approximately 10 vines per row for presence of GRBV. In 2014, leaves were collected from every seventh vine beginning from the 31st vine from the northern edge of a row to combine with the previous season's collections to assay a total number of approximately 20 individual vines per row. In 2015, leaves from previously assayed vines were collected for analysis. In 2016 fresh tissues from previously virus-negative vines were assayed for presence of GRBV.

Leaf tissue samples. Leaf petiole tissue was collected in all sites using the described pre-determined grid pattern of each block. At each site and collection date (Table 2.1), 2–5 leaves were collected from basal and middle areas of the current season's growth by inverting a zippered plastic bag and pulling the leaves from the vine without touching the material to the collector's hand. Leaf petioles from sampled regions of GRBV-infected vines have sufficient titer during the late season to reliably capture GRBV particles for diagnostic assays (Setiono et al. 2018). Tissues were stored in cooled insulated containers during transport to the laboratory and were processed fresh for nucleic acid extraction or stored at -80°C until they could be processed. Positive controls consisted of diluted GRBV DNA or leaf petiole materials that were deep-frozen at -80°C and had previously tested positive for GRBV, and negative controls consisted of leaf petiole materials that had previously tested negative for GRBV. Materials from control vines were handled in an identical manner as the field-collected materials, and buffer controls were added to all runs of the thermocycler. Cross-sections (1 mm) of fresh or frozen grape petioles were removed using sterilized tools. Approximately 0.2 g per sample of homogenized petiole tissue was used. In 2013, samples were homogenized using a Precellys 24 Tissue Homogenizer (Bertin Instruments, Montigny-le-Bretonneux, France) at 6,500 Hz for two 10-s cycles with a 30-s intermission between cycles. From 2014–2016, samples were homogenized using a Qiagen TissueLyser II (Qiagen, Hilden, Germany). Petiole material was subsequently incubated in a guanidine thiocyanate buffer (pH 5.0) to extract total plant DNA. Sample DNA was further processed using a MagMAX (2014-2016) or KingFisher ™ Flex Purification System (2016) (Thermo Scientific, Waltham, MA, USA) using the unmodified MagMAX protocol Am 1836_DW_50_V2 to generate purified nucleic acid extracts for use in qPCR reactions (Osman et al. 2012).

qPCR assays for GRBV. An Applied Biosystems qPCR machine with 7500 Fast System SDS Software was used to test all 389 samples for presence of GRBV in 2013, for 79 samples in 2016 (17.8% of the season total), and to retest material from 2015 from 19 vines (1.6% of the season total) at the end of the study period. A QuantStudio 6 Flex Real-Time PCR machine (Thermo Scientific, Waltham, MA, USA) was used to test all other samples for presence of GRBV. For the latter thermocycler, each 20-µl

reaction was prepared to contain 10.0 µl 2X SsoFast EvaGreen Master Mix (Bio-Rad Laboratories, Inc., Hercules, CA, USA), 2 µl PVP-40 (10% w/v), 0.3 µl 1:500 concentration ROX reference dye, 0.3 µL 10 µM primer GVGF1 and 0.3 µL 10 µM primer GVGR1 (Al Rwahnih et al. 2013), 1.0 µl DNA extract, and 6.1 µl Millipore water. Analysis using the 7500 Fast System SDS in 2016 was performed on 20-µl samples containing 10.0 µl 2X Fast SYBR Green Master Mix (Thermo Scientific), 2 µl PVP-40 (10% concentration by weight), 0.3 µL 10uM primer GVGF1, 0.3 µL 10 µM primer GVGR1, 1.0 µl DNA extract, and 6.4 µl Millipore water. A Ct value of 30.0 was selected as the upper cutoff for a positive result on condition that the melting point (Tm) was within ± 2°C compared to the positive control of the plate on which it was run.

Spatial analysis of virus infection patterns.

SADIE. To analyze patterns of GRBV infection, the program Spatial Analysis by Distance IndicEs (SADIE) was used (Perry 1995, Perry et al. 1999). An extension of the SADIE program was used for further analysis of association or dissociation of distributional patterns of infected vines within a site across years (Winder et al. 2001). SADIE was not conducted at Milton-Freewater or in Jacksonville in 2014 because the incidence of infected vines was below 5% (Pethybridge and Turechek 2003).

The SADIE program integrates spatial coordinates along with count or incidence data and creates random permutations of the data. By comparing the distance to regularity (D) of actual versus simulated data, permutations produce a value against which the actual data are compared to provide a measure of significance of aggregation, P_a ($\alpha < 0.05$), as well as outputs of the index of aggregation, I_a, and degrees of clustering, v_{ij}. When $I_a > 1$ clustering of counts is aggregated; $I_a < 1$ indicates regular arrangement of counts, and $I_a = 1$ indicates a random arrangement of counts (Madden et al. 2007). Degree of clustering is statistically significant when $v_i > 1.5$, indicating higher than average concentration of virus-infected vines, or $v_j < -1.5$, indicating lower than average concentration of virus-infected vines. As values of v_{ij} increase or decrease above or below these values, the strength of the patch or gap, respectively, likewise increases (Perry et al. 1999).

To assess possible association or dissociation of GRBV-infected vines between years, the infection status of sampled vines was compared between years. Between-year comparisons were made for the coupled years 2015–2016 in all sites that contained vines infected with GRBV, and additionally for the coupled years 2014–2015 and 2014–2016 for Yamhill and Talent. Within a given association analysis between two seasons, the parameter X represented the measure of spatial association between the two cluster indices. Dutilleul-corrected N represented the effective sample size, accounting for autocorrelation within a vineyard but distinct from the actual N used by the statistical program (Dutilleul et al. 1993). The significance of association was determined by interpretation of P_X where $P_X \leq 0.025$ indicated that infection status of vines was significantly spatiotemporally associated, $P_X \geq 0.975$ indicated a significant spatiotemporal dissociation of infected vines, and intermediate values $0.025 < P_X < 0.975$ indicated non-significant random association of incidence of infection across years.

During the study, diagnostic tests resulted in unexpected differential results for a small proportion of assayed vines (2.6%). In these cases, a vine tested positive for GRBV in an earlier season, but tested negative during a later season. Frozen material from these vines was retested at the conclusion of the study, resulting in similar findings. All vines testing negative for GRBV following a positive result from previous seasons were counted as GRBV-positive in subsequent seasons for the purpose of spatial analysis.

Quadrat analysis. To make further inference on the in-vineyard spatial patterns of GRBV infection, binomial distribution (BD) and β-binomial distribution (βBD) analyses were compared on quadrats within study sites as described by Madden et al. (2007). Analyzed sites were assessed for the cumulative infection of vines at the end of the three seasons (2014, 2015, and 2016). Vineyard blocks with GRBV infection were divided into site-specific quadrats of relevant sizes. At Jacksonville, the vineyard block was divided into 50 quadrats measuring 8 vines × 4 rows in size. Because every fourth vine and only even-numbered rows were surveyed at this site, four surveyed vines were analyzed at the quadrat scale. At Talent, 25 quadrats measuring 10 vines × 4 rows were evaluated and contained eight surveyed vines per quadrat. At Yamhill, 25 quadrats measuring 14 vines × 4 rows were evaluated. Due to the closer spacing of the

rows, this site had 16 surveyed vines per quadrat. In limited instances, fewer surveyed vines were contained within a quadrat than is schematically listed.

BD and βBD analyses were conducted using the BBD program developed by Madden and Hughes (1994). BD is an appropriate analytical tool when infection is distributed randomly within a study block. However, in cases where the estimate of unbiased variance of distribution of infection is large, a clustered pattern is evident. In such cases of overdispersion, the βBD may have an explanative function. βBD analysis produces the intra-cluster correlation coefficient ρ, and an index of aggregation θ. In quadrat analysis, positive values where $0<\rho<1$ indicate the tendency for plants within a quadrat to have the same infection status as other plants. As θ increases, ρ approaches its asymptote =1 (Madden et al. 2007). The BBD program calculates the parameter log likelihood for both BD and βBD, providing a χ^2 statistic against which to compare BD to βBD (Madden and Hughes 1994). This is expressed as the Likelihood Ratio Test Probability that, when significant at $\alpha<0.05$, indicates a better fit of the βBD model over the BD model.

RESULTS

The year-by-year outcomes of GRBV detection using qPCR analysis from 2013–2016 are summarized in Table 2.1. Samples collected from 2013–2015 at the Milton-Freewater site did not reveal the presence of GRBV. At all other sites, GRBV was present and cumulative incidence of infection increased annually (Fig. 2.1, Table 2.2).

Leaf material from Talent revealed that the cumulative proportion of vines infected by GRBV was 10.4% in 2014, 19.0% in 2015, and 21.5% in 2016. Grapevines at Talent displayed significant patterns of overdispersion within the block, as represented by virus distribution mapping over the course of the study (Fig. 2.2). Spatiotemporal analysis indicated strong association of GRBV-infected vines at Talent across all years of the study (Table 2.3). Aggregation of infected vines, as indicated by increasing values of θ, increased annually (Table 2.4).

Epidemiological studies began at Jacksonville in 2014. Of the material collected in 2014, 3.1% of sampled vines tested positive for GRBV, compared to 31.8% of cumulatively positive vines in 2015 and 58.5% of cumulatively positive vines in 2016 (Fig. 2.1). SADIE was not possible in 2014 due to low (<5%) incidence of infection,

and spatiotemporal association analysis could only be conducted between years 2015 and 2016. Plants infected with GRBV were spatially associated between these two seasons (Table 2.3). βBD analysis showed that the βBD model was a superior fit to the BD model in 2015 and in 2016 (Table 2.4). The spatiotemporal distribution of GRBV at Jacksonville is depicted in Fig. 2.3.

At Yamhill in 2013 and 2014, two distinct sets of vines were examined within the same vineyard rows but were combined for analytical purposes and expressed as combined data in year 2014. These assayed vines had a combined incidence of 31.1% GRBV infection. However, GRBV incidence of infection had increased in the same vines to 49.3% by 2015. Additional vines that tested positive in 2016 but negative in previous seasons brought the overall proportion of GRBV-infected vines at Yamhill to 59.2% (Fig. 2.1). Locations of GRBV-infected and uninfected vines were associated across all years (Table 2.3). Areas of aggregation, as represented by the βBD parameter θ, increased and were spatially associated across all years (see Table 2.4). The spatiotemporal distribution of GRBV at the conclusion of the study is presented for Yamhill in Fig. 2.4.

Over the course of the study, the average Tm of positive controls generated from the 7500 Fast System was 76.79°C, and for the QuantStudio 6 Flex the average Tm of positive controls was 81.81°C. Median Tm was 59.85°C with no measurable Ct value for 94.2% of all samples that were scored as GRBV-negative (data not shown).

DISCUSSION

The current study is the first to report spatial patterns and spread of GRBV in Oregon vineyards. Researchers have verified the spread of the virus in California from highly aggregated sections of a vineyard originating at the edge and extending toward the center of the block (Cieniewicz et al. 2017b). Our assessment of the infection status of tested vines showed a significant trend of increase of GRBV incidence in three of the four Oregon vineyard study sites. The annual increase of virus incidence was rapid, with observed doubling to 10-fold increases recorded in sites from 2014–2016. In a survey conducted in 2016 by the Oregon Department of Agriculture, GRBV was found in all wine grape production regions of Oregon, including in the Columbia Basin of eastern Oregon (D. Poudyal, *pers. comm.*). Thus, the findings of the current study do

not represent a complete picture of GRBV in Oregon, but rather confirm that spread of GRBV is taking place in at least two geographical regions of Oregon.

We analyzed the association or dissociation of spatial patterns of GRBV-infected vines between years. In all vineyards and between years, the spatial pattern of virus incidence was associated with the previous time period (see Table 2.3). The rapid increase of GRBV recorded in the vineyard in Jacksonville is unique, as demonstrated by qPCR assays over the three years of the study. In 2014, incidence of infected vines was 3.1% and spatial analysis by SADIE was not possible. By 2016 most vines were infected with GRBV, and uninfected vines were randomly distributed throughout the block. These observations explain why there was a statistically clumped distribution of GRBV-positive vines only in 2015. The overall proportion of infected vines in Jacksonville is likely a conservative figure because many vines that tested negative for GRBV in 2015 were removed by the grower before the 2016 field collections. Most of the previously surveyed vines at Jacksonville that remained in the study block in 2016 tested positive for GRBV (see Table 2.1).

The observed in-field spatial pattern of GRBV infection supports spread by an insect vector. Evidence exists for vector transmission of both GLRaVs (Almeida et al. 2013) and GRBV (Bahder et al. 2016b; Cieniewicz et al. 2018b). Spread of GLRaV occurs slowly from established foci of infection. Female mealybug and scale insect vectors of GLRaVs are wingless and migrate slowly along the vine during the growing season (Bahder et al. 2013), while winged adult males do not feed (Borges da Silva et al. 2009). The historical appearance of TPCTV in Florida tomato fields, transmitted by the membracid *M. malleifera*, rarely spread more than 30–60 m from the field edge, with minimal secondary spread, likely due to low in-field movement of insect vector (Simons 1962). Transmission of TPCTV was further associated with annual weeds within the field and along a bordering drainage ditch, with decreasing rates of infection as distance from the field margin increased (Simons and Coe 1958). The confirmed treehopper vector of GRBV is alate and has the potential to rapidly move throughout the vineyard as an adult. Thus, while GLRaV spread occurs in close proximity to previously infected vines (Almeida et al. 2013), new infections of GRBV were sometimes recorded in areas of the vineyard study blocks that had not been previously

afflicted with virus. The rapid spread of GRBV as observed in Jacksonville has not been recorded previously but strongly indicates the presence of an insect vector.

Within the subfamily Smiliinae, the membracid tribe Ceresini contains closely related genera *Spissistilus* and *Tortistilus* (Cryan et al. 2000). Populations of *S. festinus* were observed in a California vineyard where the spatiotemporal spread of GRBV was examined (Cieniewicz et al. 2017b). This species, together with other species in the genus *Tortistilus*, was found in the Jacksonville vineyard that was part of the current study (Hilton unpubl. data). In contrast, only *Tortistilus* was observed at Talent and Yamhill study sites. Given the observed spread of GRBV in multiple locations in Oregon, including in locations that do not apparently have the confirmed presence of the vector *S. festinus*, it is plausible that multiple species of membracids may transmit GRBV. It is noteworthy that vines in vineyards where GRBV spread was documented in this study exhibited feeding damage consistent with feeding by resident populations of treehoppers (Walton, unpubl. data). It is possible that these insect species may have contributed to the spread of the virus in the study vineyards. Currently, however, there is no conclusive evidence to confirm that *Tortistilus* insects are vectors of GRBV. Given the observed spread reported in the current study, the next logical step is to conduct controlled transmission bioassays using suspected vector species from study sites (Bahder et al. 2016b). Detailed spatiotemporal studies on the biology and ecology of potential insect vectors will help determine population dynamics and target biological weaknesses of vector populations for management.

It is challenging to effectively manage GRBV without a strong understanding of the epidemiology of the virus. It is clear that RBD has a significant economic impact on vineyards (Ricketts et al. 2017). It is important that growers implement a focused monitoring effort to identify and remove infected vines in such areas as a first step to minimize the spread of the virus (Cieniewicz et al. 2017b). This work provides advancement to the understanding of the epidemiology of RBD. Multiple aspects of the epidemiology of red blotch disease are still poorly understood and knowledge gaps remain. Some of these gaps include a better understanding of the underlying reason for the significant differences in the rate of spread, as was recorded in this study, identification of possible additional insect vectors, and advances in transmission

biology. Continued focus on these and other gaps will aid to more optimally manage the spread of RBD.

ACKNOWLEDGEMENTS

Table 2.1 Summary of vineyard leaf petiole samples used in the current study. N indicates the number of vines sampled each season from 2013–2016 and assayed for the presence of grapevine red blotch virus (GRBV) using qPCR.

Region	Site	Petiole collection date	N	GRBV +	GRBV -	% Infected
Eastern Oregon	Milton-Freewater	15 Oct 2013	199	0	199	0.0
		22 Sep 2014	196	0	196	0.0
		25 Sep 2015	395	0	395	0.0
Southern Oregon	Jacksonville	22 Sep 2014	194	6	188	3.1
		17 Sep 2015	195	61	134	31.3
		24 Aug 2016	72	55	17	76.4
Southern Oregon	Talent	22 Sep 2014	193	20	173	10.4
		17 Sep 2015	200	34	166	17.0
		12 Sep 2016	196	40	156	20.4
Willamette Valley	Yamhill	10 Oct 2013	190	61	129	32.1
		13 Oct 2014	173	54	119	31.2
		8 Oct 2015	375	172	203	45.9
		28 Sep 2016	176	37	139	21.0

Table 2.2 Cumulative incidence of grapevine red blotch virus (GRBV) in four Oregon vineyards from 2013–2016, as determined using qPCR. The indices presented using the program Spatial Analysis by Distance IndicEs (SADIE, Perry 1995) are: I_a=index of aggregation; $\overline{V}j$=mean index of gap strength; $\overline{V}i$=mean index of patch strength; D=distance to regularity. Values are significant when P_a<0.05. Site-years in which infection was <5% or >95% were not analyzed using SADIE.

Site	Year	N	% Infected	I_a	$\overline{V}j$	$\overline{V}i$	D	P_a
Milton-Freewater	2013–2014	395	0.0	n/a	n/a	n/a	n/a	n/a
	2015	395	0.0	n/a	n/a	n/a	n/a	n/a
	2014	194	3.1	n/a	n/a	n/a	n/a	n/a
Jacksonville	2015	195	31.8	4.30	-4.48	4.28	957.5	<0.001
	2016	195	58.5	1.17	-1.21	1.17	276.1	0.189
	2014	193	10.4	1.99	-1.95	2.01	195.9	0.002
Talent	2015	200	19.0	2.30	-2.23	2.41	299.9	<0.001
	2016	200	21.5	2.17	-2.11	2.24	298.8	0.001
	2013–2014	363	31.7	3.78	-4.03	3.64	1034.9	<0.001
Yamhill	2015	375	49.3	4.37	-4.80	4.16	1316.4	<0.001
	2016	375	59.2	4.46	-4.95	4.24	1318.2	<0.001

Table 2.3 Spatial Analysis by Distance IndicEs (SADIE, Perry 1995) association analysis between vines infected with grapevine red blotch virus (GRBV) across years in three Oregon vineyards. N is the Dutilleul-corrected sample size to account for autocorrelation. χ is the χ^2 statistic. Cumulative incidence of GRBV is statistically associated when $P_\chi<0.025$, statistically dissociated when $P_\chi>0.975$, and random when $0.025<P_\chi<0.975$.

Site	Year 1	Year 2	N	χ	P_χ
Jacksonville	2015	2016	189.8	0.576	<0.001
	2014	2015	168.6	0.687	<0.001
Talent	2014	2016	161.5	0.635	<0.001
	2015	2016	141.0	0.925	<0.001
	2014	2015	348.5	0.668	<0.001
Yamhill	2014	2016	355.2	0.552	<0.001
	2015	2016	319.3	0.819	<0.001

Table 2.4 Comparison of binomial distribution (BD) and β-binomial distribution (βBD) analyses. *pBD* and *pβBD* indicate the estimated likelihood that a plant will be infected with grapevine red blotch virus using binomial distribution and β-binomial distribution analysis, respectively. In β-binomial distribution analysis, θ is the index of aggregation and ρ is a measure of departure from binomial distribution. Likelihood ratio test probability (LRTP) is a comparison of log likelihood metrics between binomial and β-binomial distributional analyses. β-binomial distribution is the preferred test when LRTP<0.05.

| Site | Year | Quadrat size (vines) | BD Analysis | | βBD Analysis | | | | |
			p_{BD}	log likelihood	$p_{\beta BD}$	θ	ρ	log likelihood	LRTP
Jacksonville	2014	8×4	0.031	38.9	0.031	0.102	0.093	37.6	0.107
	2015	8×4	0.313	161.1	0.316	0.395	0.283	144.8	<0.001
	2016	8×4	0.585	152.7	0.585	0.135	0.119	149.1	0.007
Talent	2014	10×4	0.104	65.5	0.105	0.089	0.082	61.9	0.007
	2015	10×4	0.170	87.8	0.170	0.171	0.146	79.4	<0.001
	2016	10×4	0.215	102.1	0.215	0.232	0.188	87.9	<0.001
Yamhill	2014	14×4	0.307	188.3	0.301	0.319	0.242	126.6	<0.001
	2015	14×4	0.459	286.3	0.470	0.714	0.417	136.3	<0.001
	2016	14×4	0.592	247.1	0.600	0.519	0.342	135.5	<0.001
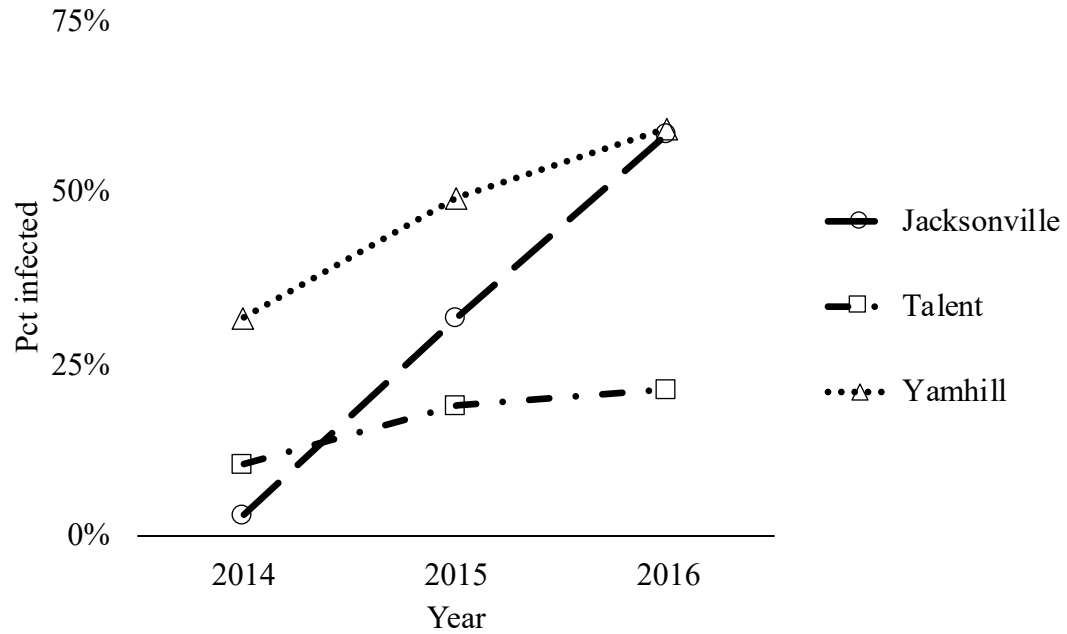
Figure 2.1 Change of incidence of cumulative infection by grapevine red blotch virus (GRBV) as determined by qPCR on vines repeatedly assayed from 2013–2016 in three Oregon vineyard sites. Assays of vines from Milton-Freewater revealed no incidence of GRBV (data not shown).
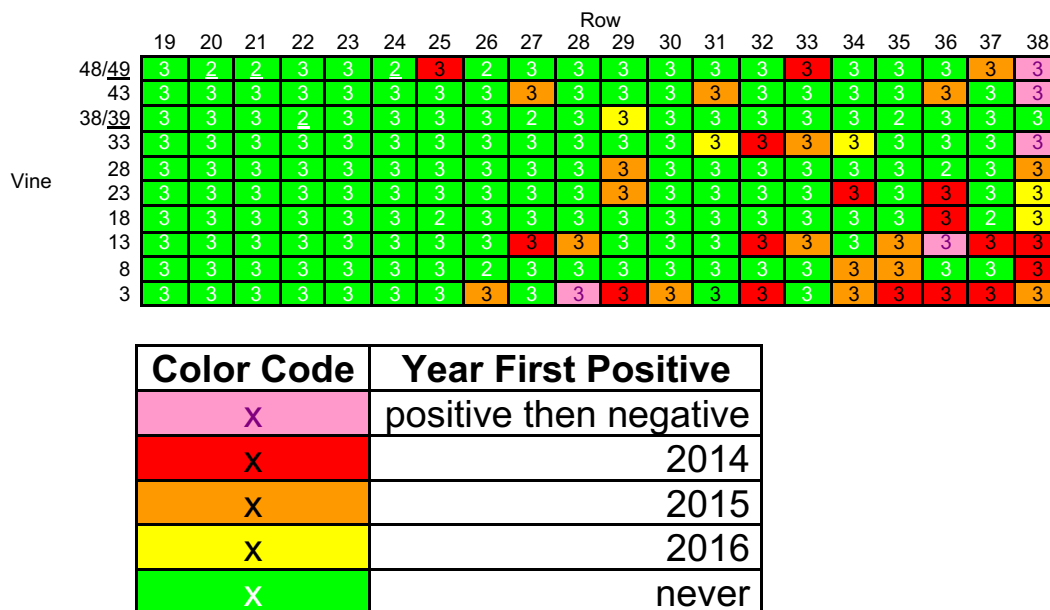
Figure 2.2 Distribution of grapevine red blotch virus (GRBV) at Talent (southern Oregon). Cells represent individual vines that were repeatedly assayed using qPCR from 2014–2016. Legend provides color code indicating first year in which a vine first tested positive for GRBV. Number within cell indicates how many times a vine was tested. Underlined number indicates location of vine sample that deviated from pre-determined grid pattern. Figure not drawn to scale.

Vine \ Row	32	31	30	29	28	27	26	25	24	23	22	21	20	19	18	17	16	15	14
4/5	2		3		3		3		3		3		3		3		2		2
8/9	3		3		3		3		3		3		3		3		2		2
12/13	3		3		3		3		3		3		3		3		2		2
16/17	3		3		3		3		3		3		3		3		2		2
20/21	3		3		3		3		3		3		3		3		2		2
24/25	3		3		3		2		3		3		3		3		2		2
28/29	3		2		3		3		2		3		3		2		2		2
32/33	3				3		3		2		3		2		3		2		2
36/37	3		3		3		2		3		2		3		2		2		2
40/41	2		3		2		3		2		3		2		2		2		2
44/45	3		2		3		2		2		2		2		2		2		2
48/49	3		3				2		2		2				2		2		2
52/53	2		3		2		2		2		2		2		2		2		2
56/57	3		2		3		2		2		2		2		2		2		2
60/61	2		2		2		2		2		2		2		2		2		2
64/65	2		2		2		2		2		2		2				2		2
68/69	3		2		2		2		2		2		2		2		2		2
72/73	2		2		2		2		2		2		2		2		2		2
76/77	2		2		2		2		2		2		2		2		2		2
80/81	2		2				2		2		2		2		2		2		2

Color Code	Year First Positive
X (pink)	positive then negative
X (red)	2014
X (orange)	2015
X (yellow)	2016
X (green)	never

Figure 2.3 Distribution of grapevine red blotch virus (GRBV) at Jacksonville (southern Oregon). Cells represent individual vines that were repeatedly assayed using qPCR from 2014–2016. Legend provides color code indicating first year in which a vine first tested positive for GRBV. Number within cell indicates how many times a vine was tested. Underlined number indicates location of vine sample that deviated from pre-determined grid pattern. Empty cells indicate vines that were sampled less than two times during the study period. Figure not drawn to scale.

Row

Vine	63	62	61	60	59	58	57	56	55	54	53	52	51	50	49	48	47	46	45
28	3	3	3	3	3	3	3	3	3	3	3	3	2	3	3	3	3	3	3
31	2	3	3	3	2	2	2	3	3	3	2	3	2	3	3	3	3	3	3
35	3	3	3	3	3	3	3	3	2	3	3	3	2	3	3	3	3	3	3
38	2	3	2	3	2	3	2	3	3	2	3	3	2	3	3	2	3	3	3
42	3	3	3	3	3	3	2	2	2	2	2	2	3	3	3	3	3	3	
45	2	2	2	2	2	3	2	3	3	2	3	2	2	3	3	3	3	3	3
49	2	3	3	3	3	2	3	3	2	3	2	3	3	3	3	3	3	3	3
52	3	2	2	2	2	3		3	2	2	2	2	3	2	3	2	2	2	3
56	2	2	2	2	2	2	2	2	2	2	2	2	2	3	2	2	3	2	3
59	2	2	3	2	2	2	3	2	2	2	2	2	2	3	3	2	2	2	3
63	2	2	2	2	2	2	2	2	2	2	2	3	3	2	2	3	3	3	
66	2	2	2	2	2	2	2	2	2	3	2	2	3	2	2	3	2		
70	2	2	2	2	2	2	2	2	2	2	2	2	2	2	3	3	3	3	
73	2	2	2	2	2	2	2	2	2	2		2	2	2	2	3	2	3	
77	2	2	2	2	2	2	2	2	2	3	2	2	3	3	3	2	3		
80	2	2	2	2	2	2	2	2	2	2	2	3	2	3	2	3	3	2	
84	2	2	2	2	2	2	2	2	2	2	3	2	3	2	3	3	3	3	
87	2	2	2	2	2	2	2	2	2	3	2	3	2	3	3	3	3	2	
90/91	2	2	2	3	2	2	3	3	3	3	3	2	3	3	3	3	3	3	
94	2	2	3	2	2	2	2		3	3	2	3	3	3	3	3	3	3	

Color Code	Year First Positive
X (pink)	positive then negative
X (red)	2013-2014
X (orange)	2015
X (yellow)	2016
X (green)	never
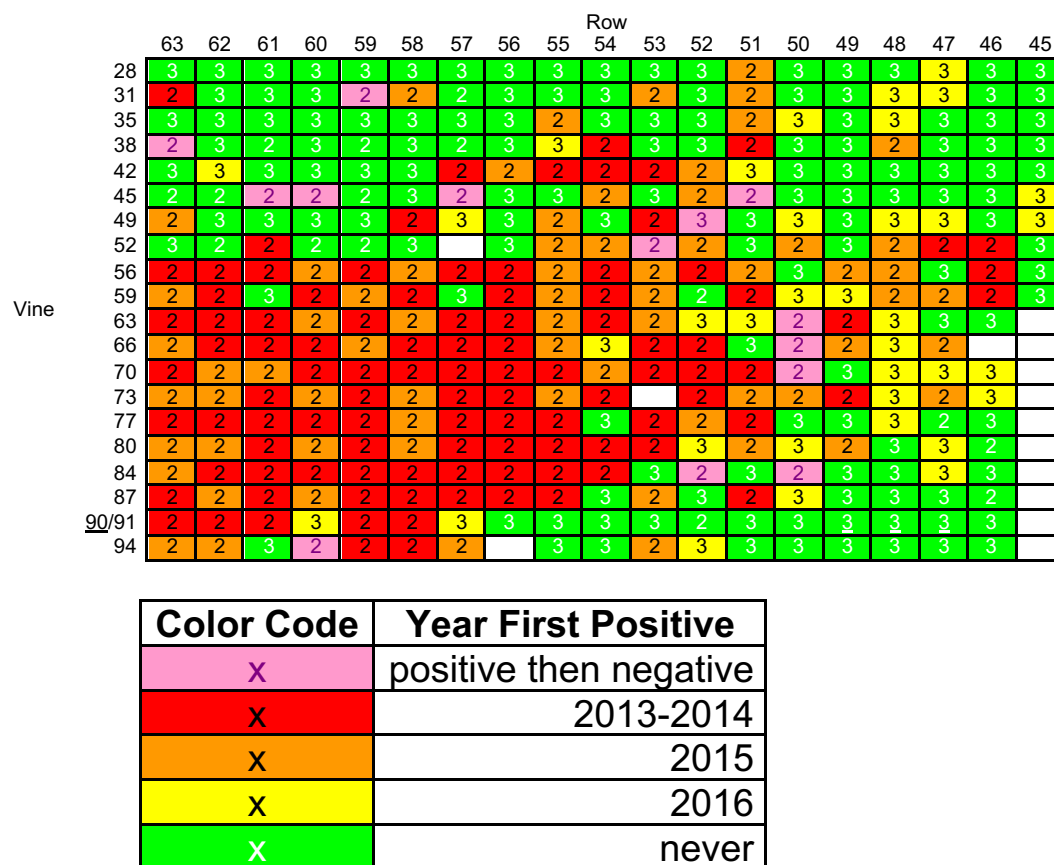
Figure 2.4 Distribution of grapevine red blotch virus (GRBV) at Yamhill (Willamette Valley). Cells represent individual vines that were repeatedly assayed using qPCR from 2013–2016. Legend provides color code indicating first year in which a vine first tested positive for GRBV. Number within cell indicates how many times a vine was tested. Underlined number indicates location of vine sample that deviated from pre-determined grid pattern. Empty cells indicate vines that were sampled less than two times during the study period. Figure not drawn to scale.

CHAPTER 3

Effects of temperature and host plant on treehopper species (Hemiptera: Membracidae: Smiliinae) collected in Oregon

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ABSTRACT

Spread of *Grapevine red blotch virus* in wine grape (*Vitis vinifera* L.) vineyards of Oregon, USA led to investigations of treehopper (Hempitera: Membracidae) species biology. *Stictocephala basalis* (Walker) and *Tortistilus albidosparsus* (Stål) were sampled from vineyard sites and reared under controlled environmental conditions. In the growth chamber, *St. basalis* 4th instar insects developed more rapidly than *T. albidosparsus* 4th instar insects, but other life stages had similar thermal requirements. *Stictocephala basalis* 5th instar nymphs successfully emerged as adults on *Trifolium alexandrinum* L., *T. pratense* L., *Lolium multiflorum* Lamarck, and *Pisum sativum* L., but no adults emerged on *Brassica rapa* L. var. *silvestris*. The cumulative mean duration of successive instar stages was 451.7 degree-days (DD) and 460.2 DD for *St. basalis* and *T. albidosparsus*, respectively. In a greenhouse, feeding by adult *St. basalis* rarely induced tissue girdling in *V. vinifera*, and the highest incidence of girdling was on tissue approximately 2mm in diameter. Most adults were observed within 15cm of apical tips of infested canes. Both *St. basalis* and *T. albidosparsus* deposited eggs and successfully overwintered on *V. vinifera*. *Pyrus communis* L. and *Crataegus douglasii* Lindl. were suitable overwintering hosts of *St. basalis*. Oviposition occurred on average about 8.25cm from shoot tips of rosaceous hosts. More eggs were laid per oviposition site by *St. basalis* than *T. albidosparsus*. Information from controlled behavioral studies provides a foundation for monitoring of treehopper species as a first step to mitigate potentially damaging populations in vineyard agroecosystems.

INTRODUCTION

Smiliine treehoppers (Membracidae: Smiliinae) utilize a diversity of plant hosts to complete their life cycles, and many species switch from herbaceous plants to perennial hosts as they develop to the adult stage (Caldwell 1949, Nixon and Thompson 1987, Yothers 1934). Treehoppers typically pass through five immature instar stages over the course of their development (Yothers 1934, Kopp and Yonke 1973, Preto 2018a). According to investigations by Yothers (1934), body length of *Stictocephala basalis* (Walker), ranged from 1.65-1.85mm, 2.35-2.75mm, 3.25-4.0mm, 5.0-5.75mm, and

6.0-6.75mm as 1st, 2nd, 3rd, 4th, and 5th instars, respectively, while the adult form measured approximately 7.5mm in length.

To aid in the development of an integrated pest management program applicable to a crop production system, a robust knowledge of interactions between insects and their host plants is required (Williams 1984). *Spissistilus festinus* (Say) inflicts considerable economic damage on crop species *Arachis hypogaea* (peanut), *Glycine max* (soybean), and *Medicago sativa* (alfalfa) (Mack et al. 1987, Mueller and Jones 1983, Moellenbeck and Quisenberry 1991). Adults and nymphs of *Sp. festinus* insert their stylets into vascular tissues of host plants, feeding circumferentially along host petiole or stem tissue and thereby induce formation of girdles (Andersen et al. 2002, Kopp and Yonke 1973, Mitchell and Newsom 1984b). The physical effects of girdling compromise the translocation of photosynthates, resulting in a temporary increase of sugars and amino acids above the girdling point that is attenuated upon callusing of damaged tissues (Hicks et al. 1984, Andersen et al. 2002). Early-season feeding by *Sp. festinus* on young soybean plants decreases a plant's capacity to fix nitrogen, resulting in induction of plant mortality in some cases (Hicks et al. 1984). Cumulative effects of treehopper-induced girdling starve the root system of carbohydrates in perennial legumes such as alfalfa, with potential significant effects on crop yield and overwintering capacity of host plants (Mitchell and Newsom 1984b, Grosso et al. 2016).

Aside from *Sp. festinus*, membracids are not typically considered significant direct pests of crops. Most direct damage attributed to treehopper infestation is a consequence of ovipositional activity on host shoots (Bartlett et al. 2018). Treehopper oviposition damage was reported by Yothers (1934) to cause mortality of small caliper woody stems. Adults of *St. basalis* were reported to utilize rosaceous hosts including apple, pear, peach, and prune for reproduction, while immature stages were documented to utilize herbaceous legumes (Caldwell 1949, Yothers 1934). Funkhouser (1917) reported that species of smiliine genera *Ceresa* (=*Tortistilus*) and *Stictocephala* have a host range of more than a dozen plant families. Notably absent from lists of known treehopper herbaceous hosts are plants of the family Brassicaceae (Funkhouser 1917). This family of plants characteristically produces sulfur-rich glucosinolate compounds (Avato et al. 2013). Such secondary plant metabolites are associated with toxicant or

repellent effects on insects, requiring trophic specialization on the part of coevolved herbivores (Ahuja et al. 2010).

Ovipositional behavior by treehoppers varies by species. Whereas *St. basalis* were observed to deposit eggs within long slits caused by its ovipositor (Yothers 1934), *Stictocephala militaris* (Gibson and Wells) (Membracidae: Smiliinae) deposited eggs at the base of dormant buds in groups of 1–12 eggs per bud (Ebel and Kormanik 1965). Certain species, including *Sp. festinus* and hoppers of the family Aetalionidae, lay eggs in masses on leaf tissues or new growth of the stem (Bartlett et al. 2018, Daigle et al. 1988, Preto et al. 2018b). Damage from oviposition occurs through the cutting activity of the ovipositor through the cambium tissues, causing oozing of sap, providing opportunities for secondary infestation by other pests or pathogenic decay, and resulting in potential decline of host plants (Yothers 1934). Stone fruit species are particularly susceptible to ovipositional damage, although pear and apple trees are subjected to similar effects of lesser severity (Sorenson 1928). Young trees and those growing in proximity to herbaceous legumes are at particular risk for damage caused by oviposition (Yothers 1934).

Monitoring of hemipteran behavioral activities is undertaken by a variety of techniques. Under controlled laboratory conditions, electropenetrography (EPG) has emerged as the primary tool to quantify behaviors including feeding and oviposition (Backus et al. 2019, Walker and Perring 1994). Upon penetration of the proboscis or ovipositor into plant tissues, insect species create unique and reproducible waveforms that are specific to the affected tissue type or a particular insect behavior (Walker 2000). One drawback of EPG is the dedicated work required to correlate behavior with EPG output. Such work may involve the use of stylectomy and histological techniques, coupled with EPG monitoring, to track the progress of stylet ingress into plant tissues (Tjallingii and Hogen Esch 1993, Walker 2000). Time lapse video can be used to establish correlations of frequency or duration of contact events between the insect and its host (Jackson et al. 2008). Field observational studies can give further insight into insect behavior (Dennis 1964, Nixon and Thompson 1987), allowing empirical descriptions that accurately portray relevant insect behaviors (Lehner 1998). Past observational studies on *Sp. festinus* included monitoring of immature stage girdling

activity on soybean in the greenhouse, followed by frequent monitoring of plants for documentation of girdle characteristics, while adult field behaviors were studied to allow quantification of eggs and location of oviposition sites in soybean plants (Mitchell and Newsom 1984a). Laboratory-reared F_1 adults of *Ceresa nigripectus* Remes Lenicov (Hemiptera: Membracidae) were placed in male-female pairs onto alfalfa to document oviposition and development of emergent nymphs (Grosso et al. 2014).

True hoppers (Hemiptera: Auchenorrhyncha) comprise a highly species-diverse classification of insects found across the world. They use their piercing-sucking mouthparts to access nutrients contained within plant vascular tissues. Phloem-feeding hoppers are particularly suited to transmit plant pathogenic agents, including phytoplasmas and viruses, because many plant pathogens are phloem-limited (Ammar and Nault 2002, Weintraub and Beanland 2006). Yet, because most investigations on hopper-transmitted microbial agents focus on known pathogens in agroecosystems, the true scope of hopper-borne transmission of disease-causing agents is likely severely underestimated (Bartlett et al. 2018). In South America, *C. nigripectus* is a known carrier of the ArAWB phytoplasma (Grosso et al. 2016). The species *Ophiderma definita* Woodruff (Hemiptera: Membracidae) was implicated in the transmission of *Xylella fastidiosa* Wells et al. into oak, potentially acting as a vector of bacterial leaf scorch (Zhang et al. 2011). Yet perhaps the most well-known example of plant disease transmission by a membracid vector is the case of *Micrutalis malleifera* Fowler (Hemiptera: Membracidae: Smiliinae), vector of *Tomato pseudo-curlytop virus* (*Geminiviridae*, *Topocuvirus*) in tomato (Ammar and Nault 2002, Simons 1962). Because hoppers have been investigated primarily as a component of the agroecosystem, as opposed to natural systems, the capacity of most species as vectors of plant disease is obscure (Bartlett et al. 2018).

In recent years *Grapevine red blotch virus* (GRBV) (*Geminiviridae*, *Grablovirus*), was identified as the causal agent of red blotch disease (RBD) in wine grape vineyards across the United States (Krenz et al. 2014, Yepes et al. 2018). Economic effects of RBD are potentially extreme, as infections of wine grape cultivars detrimentally affect berry chemistry and wine quality (Blanco-Ulate et al. 2017, Ricketts et al. 2017).

GRBV was transmitted under greenhouse conditions by *Sp. festinus* (Bahder et al. 2016b), and in a separate study GRBV was transmitted by the leafhopper *Erythroneura ziczac* (Hemiptera: Cicadellidae) (Poojari et al. 2013). Genera of geminivirus are transmitted by vectors that are taxonomically related (Whitfield et al. 2015). The observed spread of RBD in vineyards of southwestern and northwestern Oregon could not be linked to known distributions of *Sp. festinus* or *E. ziczac* (Dalton, unpublished data), but multiple smiliine treehopper species were present in proximity to affected vineyards (Dalton et al. 2020, Appendix B, Stowasser et al. 2020).

To investigate the missing link between the observed spread of RBD in the absence of the presumed vector *Sp. festinus*, studies on the developmental biology of locally abundant treehopper species found in Oregon vineyard agroecosystems were undertaken in controlled environments. Ovipositional and feeding behaviors were examined through greenhouse infestations of plant species and relatives that had been previously documented as hosts of smiliine treehoppers (Yothers et al. 1934, Caldwell 1949). Groundcovers of previously known host plant families were examined for their capacity to support the 5th instar stage of *St. basalis* (Funkhouser 1917), and *Brassica rapa* var. *silvestris* (field mustard) was included to test for potential repellent, deterrent, or stimulant effects (Ahuja et al. 2010). In the case that smiliine treehoppers are found to play a significant epidemiological role in the field transmission of GRBV, or otherwise become significant direct pests in agroecosystems of Oregon, knowledge of biological attributes of locally abundant treehopper populations will assist in the development of integrated pest management strategies.

MATERIALS AND METHODS

Selection of plants and insects. In 2017 and 2018, seedlings of *Pisum sativum* L. 'Oregon Trail' (sweet pea), a highly suitable host plant for immature stages of *Sp. festinus*, were used in a growth chamber as hosts of treehopper nymphs (Beyer et al. 2017, Preto et al. 2018a). Additional leguminous species *Trifolium alexandrinum* L. (berseem clover) and *T. pratense* L. (red clover), as well as *Lolium multiflorum* Lamarck (annual ryegrass) and *Brassica rapa* L. var. *silvestris* (field mustard) were grown to test their suitability as immature stage hosts of *St. basalis*. Seedlings were grown in 3cm diameter × 20cm deep cells and trellised onto 2–4mm diameter bamboo

shoots. Potted perennial plants were acquired for treehopper infestation in the greenhouse. *Vitis vinifera* L. 'Pinot noir' clone 828 on Schwarzmann rootstock was selected due to the varietal susceptibility to GRBV and its economic importance to the Oregon wine grape industry (Seguin et al. 2014). Pome fruit species (Rosaceae: Maloideae) *Malus pumila* Mill. 'Liberty', *Pyrus communis* L. 'Red d'Anjou', and *Crataegus douglasii* Lindl. were selected due to the known status of Rosaceae as reproductive hosts of smiliine treehoppers (Yothers 1934, Hummer and Janick 2009).

Adult and immature treehoppers were collected in 2017–2018 in Oregon wine grape production regions for use in growth chamber or greenhouse infestation trials. Individuals of *St. basalis* were collected from vineyards near Carlton (CV), Lafayette (LV2), or Yamhill (YV), and individuals of *Tortistilus albidosparsus* (Stål) originated from a vineyard near Cave Junction (CJV) or CV (Dalton et al. 2020).

Environmental monitoring. Studies of treehopper behavior and development occurred in controlled growth chamber and greenhouse environments. Air temperatures were measured hourly in the growth chamber using portable temperature sensors (Onset Computer Corp., Bourne, MA, USA) and in the greenhouse using an automated environmental monitoring system. For degree-day (DD) calculations, lower and upper developmental thresholds of 10 °C and 30 °C, respectively, were selected (Pruess 1983). Temperatures that were below the lower developmental threshold or above the upper developmental threshold were assigned values equal to the nearest threshold. Daily DD were determined by subtracting the lower developmental threshold temperature from all readings of a given day, then adding all values and dividing by the number of readings for that day. Cumulative DD were obtained by sequentially adding daily DD to the previous day's cumulative sum. Missing daily DD values were interpolated using environmental set point parameters, as calculated below.

In the growth chamber in 2017, a 14h photophase at 21 °C and 10h scotophase at 17 °C were programmed to provide 9.33 DD day^{-1}. In 2018, two separate programs were set to an identical 14:10 diurnal photoperiod. From 29 March to 15 April, and again from 3 July to 6 August, the photophase was set to 26 °C, and the scotophase was set to 15 °C, equaling 11.42 DD day^{-1}. From 16 April to 2 July, the photophase was adjusted to 20 °C, and the scotophase was maintained at 15 °C, resulting in 7.92 DD

day^{-1}. Humidity was not regulated in the growth chamber, but in 2018 relative humidity was monitored with a hygrometer (Onset Computer Corp., Bourne, MA).

Greenhouse temperature set points were programmed on a diurnal cycle and were changed seasonally to mimic outdoor conditions. Supplemental heating was activated when temperatures dropped below the heating set point, and circulating fans were activated when temperatures exceeded the cooling set point.

Treehopper growth chamber behavior. Immature treehoppers were placed in a climate-controlled growth chamber for periods of their development in 2017 and 2018. In 2017, field-collected early instar nymphs of *St. basalis* from YV were reared on *P. sativum* in the laboratory. Beginning on 17 July, insects of the 2nd – 4th instar stages were introduced individually onto *P. sativum* seedlings in the growth chamber. Nymphs were additionally placed onto *P. sativum* seedlings in the growth chamber within 24h of collection from the field. Instar stages of *St. basalis* were estimated by size (Fig. 3.1a) provided by Yothers (1934). In 2018, early instar nymphs of *St. basalis* (Fig. 3.1b) and *T. albidosparsus* (Fig. 3.1c) that had emerged from greenhouse-grown overwintering hosts were placed onto *P. sativum* seedlings in the growth chamber. Field collected late instar stages from YV, LV2, and CV were additionally introduced. In both years, instar stage was documented at least two times per week, and duration of instar stage was calculated as cumulative DD for all insects. Data encompassing the complete duration of an instar stage were used for analysis; partial instar stage data were excluded. One-way analysis of variance (ANOVA) was employed using the JMP statistical package (JMP Pro version 14.1.0, SAS Institute Inc. 2018, Cary, NC, USA). Year and species effects were evaluated separately because *T. albidosparsus* nymphs were not available in 2017, and only a single treehopper could be tracked from 1st instar to adult emergence. Normality of variance was assessed using Levene's test, and residuals were examined using Shapiro-Wilk goodness-of-fit test. In instances where assumptions of normality were not met, Welch's F-statistic was used to interpret significance at $\alpha=0.05$, followed by pairwise comparison using Games-Howell post-hoc test (Lee and Lee 2018).

Growth chamber cover crop bioassay. In 2018, *St. basalis* 5th instar nymphs that had passed the previous instar stage on *V. vinifera* 'Pinot noir' were experimentally placed

onto host plant species representing potential ground cover species in Oregon vineyards. Herbaceous plants included *T. alexandrinum*, *B. rapa* var. *silvestris* 'Carlinda', *T. pratense* 'GO-MOB', *L. multiflorum* 'Lonestar', and *P. sativum* 'Oregon Trail'. Seeds were sown in 10.2 cm diameter containers and were thinned to 2–5 seedlings per container prior to introduction of insects. Five nymphs were introduced into each container, and containers were bagged within organza netting to prevent insect escape. Three replicates were provided for all herbaceous species. Containers were removed from the growth chamber for observation in the early afternoon inside a $60 \times 60 \times 120$ cm insect tent (Mega View, Taichung City, Taiwan) for up to 5 minutes per date, depending on ease of locating insects. Adult emergence was evidenced by the appearance of winged insects and presence of late-instar exuviae; feeding or probing activities were determined by visible contact of insect stylets onto host tissues; non-feeding stationary behavior was recorded in instances where mouthparts were not visibly in contact with host tissues, but no insect movement was apparent during the observation period; and active wandering activity of individual insects was recorded. Insects were placed onto host materials beginning on 11 July, with observations occurring at 2-day intervals until 6 August, with the exception of 21 July in which no observations were performed, and 4 August when only *L. multiflorum* replicates were observed because all treehoppers on other host plants had died or had previously emerged as adults.

Wilcoxon signed rank matched pair analysis was employed using JMP (JMP Pro version 14.1.0, SAS Institute Inc. 2018, Cary, NC, USA) to separate mean numbers of cumulatively emerged adults across observation periods. Using this procedure, numbers of adults were determined for each observation date, values from all dates summed, and the sum divided by the number of observation dates. While only containers of *L. multiflorum* were observed on 4 August, insect life stages from the other four host species were assumed to be the same as the previous observation date. The resulting averages were subjected to pairwise comparison using a 2-tailed t-test. Pairwise differences in numbers of emerged adults were obtained to allow correlation between host species of emergence by Julian date. Following the trial period, numbers

of girdles were assessed using ANOVA for all replicates. Comparison of mean numbers of girdles on all plant species was performed using Tukey-Kramer HSD.

Adult greenhouse behavioral activity. In 2017, 30 *St. basalis* adults that were collected in the field or that had emerged under controlled environmental conditions were individually secured in the greenhouse onto distal ends of *V. vinifera* 'Pinot noir' canes using organza netting. In limited instances, more than one adult was placed on the same vine, but insects were given unique markings to distinguish individuals from one another. Grapevines hosting adults were examined in the late morning 2–4 times per week from 18 July to 16 August for insect activity. Care was taken to leave insects undisturbed; if an adult could not be found through a visual search within 30 seconds, records of its activity were not taken on that day. Girdling, as manifested by the appearance of necrotic or deformed tissues around stem or leaf petiole material and coupled with reddening of tissues distal to damaged areas, was visually identified (Bahder et al. 2016b). Locations of girdles relative to apical shoot tips were recorded, and feeding-induced girdles were measured using digital calipers 1 mm above deformed tissue. Locations of insects from shoot tips were measured using a ruler or string. Daily movement was quantified by recording the distance between an insect's location and its previous location, then dividing by the number of days that had elapsed between observational records. Frequency analysis was employed to determine the number of times that insects were observed on a vine location, feeding, and for proportions of girdles that were observed. Numbers of girdles per individual were also recorded. Feeding was designated as behavior in which the stylets of the insect could be seen touching or piercing plant tissues.

Oviposition on perennial plants in the greenhouse. In 2017, separate collections of *St. basalis* adults were procured from YV in late July-early August and placed onto grapevines in the greenhouse within 24h of collection. Adults of *T. albidosparsus* were field-collected at CJV in early-mid August and maintained on-site on *V. vinifera* 'Pinot noir' Dijon clone 115 or at OSU Southern Oregon Research and Extension Center on *P. communis* shoots until transfer to the greenhouse. Cohorts of 5 individual *St. basalis* or *T. albidosparsus* insects containing females and males were placed onto GRBV-positive field-collected cuttings of *V. vinifera* 'Pinot noir' clone 667 on 16 August prior

to being rotated onto potted GRBV-negative *V. vinifera* 'Pinot noir' for 1-week intervals from 22 August–26 September. Surviving insects were transferred in conspecific groups containing both female and male insects onto greenhouse-grown plants of *C. douglasii*, *M. pumila*, and *P. communis* until 30 October 2017. Insects were then removed from all plants, and organza netting (30.5 × 35.6 cm) was secured over the previously infested areas. During the dormant period, grapevines and rosaceous host plants were observed for evidence of ovipositional activity. Recorded metrics for *St. basalis* included distance of eggs from the distal end of the shoot, number of visible eggs per bud, number of buds containing eggs, and total number of buds examined. For *T. albidosparsus*, the number, location, length of oviposition scars, and numbers of visible eggs per scar were recorded. For both species and all available cohorts, number of eggs per surviving female per week were obtained from *V. vinifera* host plants. Oviposition site searches were made on greenhouse-grown plants during the dormant season. For non-*Vitis* host plants, which received a single rotation of treehopper female-male cohorts, relative timing of oviposition events was not determined. Plants were monitored in spring 2018 for emergence of treehopper nymphs. To determine differences in ovipositional site characteristics, ANOVA was conducted, followed by separation of means using Tukey-Kramer HSD analysis.

RESULTS

Environmental monitoring. Grapevines and treehoppers were maintained in a greenhouse in 2017–2018, and greenhouse heating and cooling set point temperatures were periodically modified to represent seasonal outdoor changes. Actual temperatures deviated from set point temperatures when solar intensity was high. In limited instances in fall 2018, ambient temperatures briefly fell below heating set point temperatures. Humidity readings in 2018 showed average daily humidity readings of 78.9–96.6% (data not shown).

Treehopper growth chamber behavior. Field-collected *St. basalis* of varying instar stages were placed onto sweet pea seedlings in the growth chamber in 2017 beginning on 17 July, and the last insects were removed on 22 August. Forty-one *St. basalis* immature insects completed at least one full instar stage in the growth chamber, allowing calculation of number of DD to pass through specific instar stages to the adult

form. In 2018, 25 immature *St. basalis* and 20 immature *T. albidosparsus* were documented for at least one complete instar stage occurring from 29 March–12 July in the growth chamber (Fig. 3.2).

One-way ANOVA revealed a species difference in the number of DD required to pass through the 4th instar stage ($F_{1,49}$=10.904, P=0.002). As determined by Tukey-Kramer HSD, *T. albidosparsus* 4th instar insects required 44.5 ± 13.5 DD (mean ± SEM, 95% CI=17.4–71.5 DD) more than *St. basalis* to reach the 5th instar stage. No species effect on development of other instar stages was apparent (P>0.05 for all other analyses) (Table 3.1). One-way ANOVA further revealed differential DD accumulation by year for passage of *St. basalis* 3rd–4th ($F_{1,35}$=23.131, P<0.001) and 4th–5th ($F_{1,49}$=20.930, P<0.001) instar stages, as well as 5th instar stage-adult ($F_{1,58}$=11.541, P=0.001). Insects developed more rapidly through the 3rd–4th (78.2 ± 16.3 less DD, 95% CI=45.2–111.2 DD) and 4th–5th (48.0 ± 10.5 less mean DD ± SEM, 95% CI=26.9–69.1 DD) instar stages in 2017 compared to 2018, whereas insects developed more rapidly from the 5th instar stage-adult (50.2 ± 14.8 less mean DD ± SEM, 95% CI=20.6–79.9 DD) in 2018 compared to 2017.

Growth chamber cover crop bioassay. Overall adult emergence was high on herbaceous species *T. alexandrinum*, *T. pratense*, *L. multiflorum*, and *P. sativum* (Fig. 3.3). Wilcoxon signed rank matched pair analysis revealed that most species had distinct emergence patterns in comparison to other species. Only mean numbers across observation dates of emerged adults of pairs *T. alexandrinum*/*T. pratense* and *P. sativum*/*L. multiflorum* were not statistically different (Table 3.2). Adults of *T. alexandrinum* and *T. pratense* first emerged on 15 July. First emergence of an adult was observed on 13 July within two days of placement on *L. multiflorum*, and the latest-emerging adult also occurred on *L. multiflorum* on 6 August. On *P. sativum*, first emergence occurred relatively later, on 23 July. Numbers of observed host plant girdles differed between species (Table 3.3), with the highest numbers of girdles on *L. multiflorum* and the lowest numbers of girdles on *B. rapa* var. *silvestris*.

Nymphs and adults exhibited feeding behaviors on *T. alexandrinum* for the majority of observation periods until the final eight days of the trial. Non-feeding stationary

behavior made up the balance of observations. During the final 12 days of the trial, adults increasingly wandered off of plant materials (Fig. 3.4).

No adults emerged following placement on *B. rapa* var. *silvestris*, and nymphs rarely exhibited feeding behavior (Fig. 3.5). Most nymphs disappeared from host plants and were found on the soil surface upon careful examination following the experimental period. The latest observation of a living nymph on *B. rapa* var. *silvestris* occurred 8 days after placement (19 July).

Behavioral activity of *St. basalis* on *T. pratense* was similar to behavior on *T. alexandrinum*. Nymphs and adults were found to be feeding during the majority of observational periods in which they were present (Fig. 3.6), and adults were observed to be feeding or stationary until about halfway through the trial. During the second half of the trial, adults wandered off of host plant material.

On *L. multiflorum*, immature stages of *St. basalis* were present until 6 August. Frequency of feeding observations was generally lower than for the leguminous species in the trial (Fig. 3.7). The balance of observations during the first half of the trial was comprised of stationary, non-feeding behaviors. Wandering was observed for nymphs and adults during the second half of the trial. Emergence was significantly delayed on *L. multiflorum*, compared to *T. alexandrinum* and *T. pratense*, with half of surviving insects emerging as adults within the last six days of the trial.

Emergence of adults on *P. sativum* was delayed, compared to emergence on *T. alexandrinum* and *T. pratense*, but followed a similar pattern to the other leguminous species in the trial (Fig. 3.8). Feeding activity was frequently observed through most of the trial. Adult non-feeding stationary activity occurred toward the end of the trial. Insects exhibited low frequency of wandering, even at the end of the period.

Adult greenhouse behavioral activity. Adult *St. basalis* were observed in the greenhouse on grapevine from two to 10 times per individual from 18 July to 16 August 2017 for behavioral characteristics. Insects were present on grapevines from 2 to 29 days. Frequency analysis showed incidence of feeding activity on 41.7% of the observations. Girdling of plant materials was found on 14.6% of the observations. A marginally significant relationship was found using ANOVA between the proportion of times of insect feeding and proportion of times girdles were observed ($F_{1,28}$=3.438,

P=0.07) (Fig. 3.9a). Total movement ranged from 0.5–5.4cm per day with movement of 2.8 ± 0.3 cm per day (Fig. 3.9b). Most insects did not cause apparent girdling of grapevine tissue. Of the 11 insects that induced girdling, most individuals induced multiple girdles. The caliper of girdles ranged from 1.0–3.7mm. More than half (52%) of all girdles were 2.0mm diameter or less in caliper (Fig. 3.10). Most observations (66.2%) showed that adults were within 15cm of the shoot tip at the time of observation (Fig. 3.11).

Oviposition on perennial plants in the greenhouse. Nymphs of *St. basalis* and *T. albidosparsus* emerged in 2018 from woody host plants that were infested with adults in fall 2017 (Table 3.4). Both species completed the overwintering stage on *V. vinifera*. Successful emergence additionally occurred for *St. basalis* on *P. communis* and *C. douglasii*.

Multiple ANOVA indicated no difference ($F_{3,37}=0.191$, P=0.902) in distance of oviposition site from the shoot tip between *St. basalis* or *T. albidosparsus* on host species *C. douglasii*, *P. communis*, or *M. pumila* (Fig. 3.12). Mean ovipositional distance from the shoot tip was 8.25 cm (95% CI=5.9–10.6 cm).

Welch's ANOVA ($F_{1,195.9}=33.631$, *P<0.001*) showed a highly significant difference in the number of eggs per oviposition site deposited by *St. basalis* and *T. albidosparsus* on *V. vinifera*. The Games-Howell pairwise comparison test indicated that *St. basalis* deposited 1.83 ± 0.22 (95% CI of the mean=1.12–2.54) more eggs per oviposition site than *T. albidosparsus*. By contrast, Welch's ANOVA ($F_{1,200}=0.565$, *P=0.442*) did not show a difference in the distance of oviposition site from the apical shoot tip between *St. basalis* (Fig. 3.13a) and *T. albidosparsus* (Fig. 3.13b).

Mean length of *T. albidosparsus* oviposition scars on grapevine measured 5.77mm $\pm$ 0.17mm (95% CI of the mean=5.43–6.11mm). The median scar length was 5.08mm, and more than 90% of scars were less than 11mm in length (Fig. 3.14). Mean numbers of eggs deposited per female were highest for *St. basalis* during the week of 5 September, whereas mean numbers of eggs per female were highest for *T. albidosparsus* during the week of 29 August (Table 3.5).

DISCUSSION

The treehopper species *Sp. festinus* is the first reported treehopper capable of transmitting GRBV (Bahder et al. 2016b). In field surveys, RBD was found to be spreading in Oregon vineyards areas where *Sp. festinus* was apparently absent; however, *St. basalis* and *T. albidosparsus* were found in multiple field sites (Dalton et al. 2019, Dalton et al. 2020, Stowasser et al. 2020). Given the apparent absence of *Sp. festinus* in certain vineyard sites where RBD was found to be spreading, we thus considered that other smiliine treehopper species may have an epidemiological role in the spread of RBD (Bahder et al. 2016b, Cieniewicz et al. 2017b).

In 2017–2018, nymphs of *St. basalis* and *T. albidosparsus* were maintained in the growth chamber on *P. sativum*. Immature stage development was tracked, and DD were calculated for fully completed instar stages; however, a clear picture of immature development could not be shown. Only one insect was present from the day of eclosion as a 1st instar nymph until emergence as an adult. Due to mortality factors and the constraint to use insects that had completed part of their development in the field, all other measurements comprised only a portion of individual insect development. Differences occurred between years for *St. basalis* instar stages, and between species in the year 2018, further contributing to ambiguity of the thermal requirements for treehopper development. The initial temperature regime implemented in 2017 was not repeated in 2018. Developmental rates of insects have non-linear relationships to temperature (Buckley et al. 2017); likewise, treehopper insect life stages could have progressed at different rates under slightly different temperature regimes, even when cumulative DD were similar. Nymphs of *T. albidosparsus* could not be obtained in 2017, thereby prohibiting investigation of interactive effects of species and year. Ideally, future study on development of smiliine immature stages would include large enough numbers of nymphs to allow simultaneous developmental trials conducted under multiple regimes of constant temperature. Such refined studies will be necessary to formulate phenological DD models of treehopper development. Additional information to create an exceptional model would include frequent observations of adult ovipositional activity in controlled environments. The availability of an EPG monitor would further allow observation of waveforms that could be correlated to

particular behaviors including oviposition and probing or feeding activity (Backus et al. 2019).

Stictocephala basalis are polyphagous and, like many smiliine treehopper species, may feed on a broad range of host plants, including annual or perennial species (Nixon and Thompson 1987, Preto et al. 2018a, Preto et al. 2018b, Wallace and Troyano 2006, Yothers 1934). Individuals were placed on legumes and other herbaceous seedlings in a growth chamber study to track their development and behavior. Host species *T. alexandrinum* and *T. pratense* had the highest mean values of adults per observation period due to their early and rapid emergence. Species *P. sativum* and *L. multiflorum* were also suitable hosts for *St. basalis* development, although emergence of adults was delayed on *P. sativum*. The observed extended period of late instar stage development in *L. multiflorum* resulted in a lower mean number of adults per observation date and a protracted period of feeding. Longer periods of feeding by nymphs may have contributed to the greater number of girdles found on seedlings of *L. multiflorum* at the end of the study period. In contrast, *B. rapa* var. *silvestris* had a strong repellent effect. Nymphs abandoned the host plants and died in the potting medium.

Adults of *St. basalis* emerging from plants in laboratory and growth chamber colonies were caged using organza bags onto greenhouse-grown grapevines, and behaviors were monitored. The proportion of observations that an insect was found to be feeding was not linked to its daily movement. Insects moved little from one day to the next, although movement was limited by the presence of the bag. However, low rates of movement are consistent with field observations of treehoppers at rest, in which undisturbed individuals remained in place with little to no movement for hours at a time (Dennis 1964), and limited late instar movement by *Sp. festinus* was found following girdling activity (Andersen et al. 2002). In our studies, most adult *St. basalis* did not induce tissue girdles, while few individuals induced multiple girdles. A weak positive relationship was found in which treehoppers that more frequently fed on host tissues induced a greater number of girdles. Girdling activity induces a concentration of carbohydrates and other nutrients in the host, as manifested by discoloration of tissues above the girdling point (Andersen et al. 2002). This presents a positive

feedback mechanism that could stimulate more feeding and limit movement along host plant stems.

Most greenhouse observations (66.2%) of insect location indicated that adult treehoppers remained within 15cm of the shoot tip. Distal ends of woody stems are generally smaller in caliper than proximal regions and thus more closely match the caliper of tissue that was likely to be girdled. This insight could be used in field surveys for treehopper adults because sampling efforts could be efficiently concentrated toward shoot tips and leaves.

In 2017, cohorts of adult *St. basalis* or *T. albidosparsus* containing females and males were rotated weekly onto previously uninfested potted *V. vinifera* in the greenhouse. Groups were then placed on woody perennial species *M. pumila*, *P. communis*, and *C. douglasii* in the greenhouse. More eggs per oviposition site were deposited by *St. basalis*. Adults of *T. albidosparsus* laid eggs on *P. communis* and *C. douglasii*, while *St. basalis* laid eggs on all species. For both species, most eggs were deposited within 10cm of the apical shoot tip, with 9.8% of observations at a distance of 15 cm or greater from the tip. The concentrated zone of ovipositional activity aligns with locations where treehoppers were found to be feeding. Ovipositional scars of *T. albidosparsus* on woody stems were similar in appearance to those left by *Sp. festinus* on tender grapevine tissue (Preto et al. 2018b). In contrast to the detailed observations made by Yothers (1934), eggs were deposited by *St. basalis* behind dormant buds, while eggs of *T. albidosparsus* were laid under the bark in slits made by the female ovipositor. This inconsistency might be attributable to nomenclatural changes that have occurred through taxonomic revisions over the past century. State-of-the-art genomic barcoding should receive priority for future study to elucidate genetic relationships of the Smiliinae.

On grapevine, nymphs of *T. albidosparsus* emerged earlier than nymphs of *St. basalis*; however, on *C. douglasii* and *P. communis*, only *St. basalis* emerged (see Table 3.4). Few branches of *C. douglasii*, *M. pumila*, or *P. communis* were offered to *T. albidosparsus* due to small numbers of available female/male groupings. Given their exhibited capacity to oviposit on multiple host plants it seems probable that *T.*

albidosparsus would oviposit and successfully emerge from many host species if higher numbers of mated females were available.

The findings reported in these studies have implications for management of treehopper populations because they provide biological information to wine grape growers that may allow targeted approaches for monitoring of immature and adult stages of local treehopper species. As a next step, field studies on cover crop plantings will be necessary to guide recommendations for vineyard interrow species composition. Information from this work can act as a building block upon which a management program could be devised if smiliine treehoppers become pests of concern in viticultural production systems.

ACKNOWLEDGEMENTS

Table 3.1 Mean developmental time ± SEM in degree-days (DD) for passage of instar stages of two treehopper species (Hemiptera: Membracidae) in a growth chamber. A lower developmental threshold of 10 °C was applied for DD calculations.

Year	1st Instar	2nd Instar	3rd Instar	4th Instar	5th Instar–Adult	5th Instar–Female	5th Instar–Male	5th Instar–Unknown gender
Stictocephala basalis								
2017			*n*=6	*n*=20	*n*=38	*n*=12	*n*=12	*n*=14
			21.8 ± 5.7	38.3 ± 5.0	148.5 ± 9.8	172.6 ± 9.1	102.6 ± 12.9	167.3 ± 18.8
2018	*n*=4	*n*=5	*n*=21	*n*=20	*n*=15	*n*=7	*n*=7	*n*=1
	82.4 ± 29.1	89.2 ± 19.8	105.3 ± 9.2	77.5 ± 8.0	102.1 ± 11.0	84.9 ± 10.1	114.3 ± 19.8	137.3
Tortistilus albidosparsus								
2018	*n*=3	*n*=4	*n*=10	*n*=11	*n*=7	*n*=4	*n*=3	*n*=0
	26.6 ± 10.1	152.5 ± 32.2	88.7 ± 9.7	102.3 ± 15.8	90.1 ± 20.0	100.1 ± 28.6	76.8 ± 31.7	

Table 3.2 Wilcoxon signed rank matched pair analysis for plant species infested in the growth chamber by 5th instar nymphs of *Stictocephala basalis*. Values in parentheses indicate mean cumulative emergence of adults ± SEM across observational periods. Lower left-hand values represent mean differences ± SEM of species pairs; upper right-hand values are correlation values. Significance of two-tailed t-test at α=0.01 is indicated by *.

	Trifolium alexandrinum	*Trifolium pratense*	*Pisum sativum*	*Lolium multiflorum*	*Brassica rapa* **var.** *silvestris*
T. alexandrinum (8.15±1.80)		0.99	0.99	0.90	0.00
T. pratense (8.08±1.77)	0.08±0.29		0.99	0.90	0.00
P. sativum (7.15±1.85)	1.00±0.32*	0.92±0.29*		0.89	0.00
L. multiflorum (5.38±1.25)	2.77±0.87*	2.69±0.85*	1.77±0.96		0.00
B. rapa var. *silvestris* (0.00±0.00)	8.15±1.80*	8.08±1.77*	7.15±1.85*	5.38±1.25*	

Table 3.3 Girdling of herbaceous host plant stems by *Stictocephala basalis* 5th instar nymphs in a growth chamber. Different letters indicate significant differences at α=0.05 using Tukey-Kramer HSD.

Species	Mean Girdles ± SEM	
Lolium multiflorum	14.0 ± 1.5	A
Trifolium pratense	5.7 ± 1.5	B
Trifolium alexandrinum	4.0 ± 0.6	BC
Pisum sativum	2.3 ± 0.9	BC
Brassica rapa var. *silvestris*	0.0 ± 0.0	C

Table 3.4 Dates of emergence, cumulative degree-days (DD), plant genera hosting eggs, and numbers of emergent nymphs of two treehopper (Hemiptera: Membracidae) species in 2018 in the greenhouse. DD were calculated by applying a 10 °C low base temperature and a 30 °C high cutoff temperature (Pruess 1983).

		Stictocephala basalis				*Tortistilus albidosparsus*		
		Vitis	*Crataegus*	*Pyrus*	*Malus*	*Vitis*	*Crataegus*	*Pyrus*
Plants with emergence		6	2	3	0	15	0	0
Total plants infested		62	3	3	3	53	3	3
Emergence Date	**DD**							
29-Mar	368	0	0	0	0	4	0	0
30-Mar	377	0	0	0	0	7	0	0
2-Apr	396	0	0	0	0	2	0	0
4-Apr	408	0	0	0	0	1	0	0
5-Apr	414	0	0	0	0	1	0	0
12-Apr	459	0	0	0	0	1	0	0
16-Apr	476	5	0	0	0	3	0	0
18-Apr	487	0	0	0	0	1	0	0
30-Apr	578	0	0	0	0	1	0	0
9-May	643	1	0	0	0	0	0	0
17-May	703	0	2	14	0	0	0	0
22-May	743	0	1	0	0	0	0	0

Table 3.5 Mean number of eggs per female ± SEM deposited by two treehopper species at weekly intervals in 2017 on potted *Vitis vinifera* L. 'Pinot noir' in a greenhouse.

Species	22–29 Aug.	29 Aug.–5 Sep.	5–12 Sep.	12–19 Sep.	19–26 Sep.
Stictocephala basalis					
		n=12	n=12	n=11	n=10
		3.44 ± 0.92	3.65 ± 0.60	1.41 ± 0.79	1.63 ± 0.58
Tortistilus albidosparsus					
	n=13	n=12	n=10	n=3	n=2
	0.75 ± 0.46	7.61 ± 2.57	2.86 ± 1.69	5.57 ± 2.94	2.40
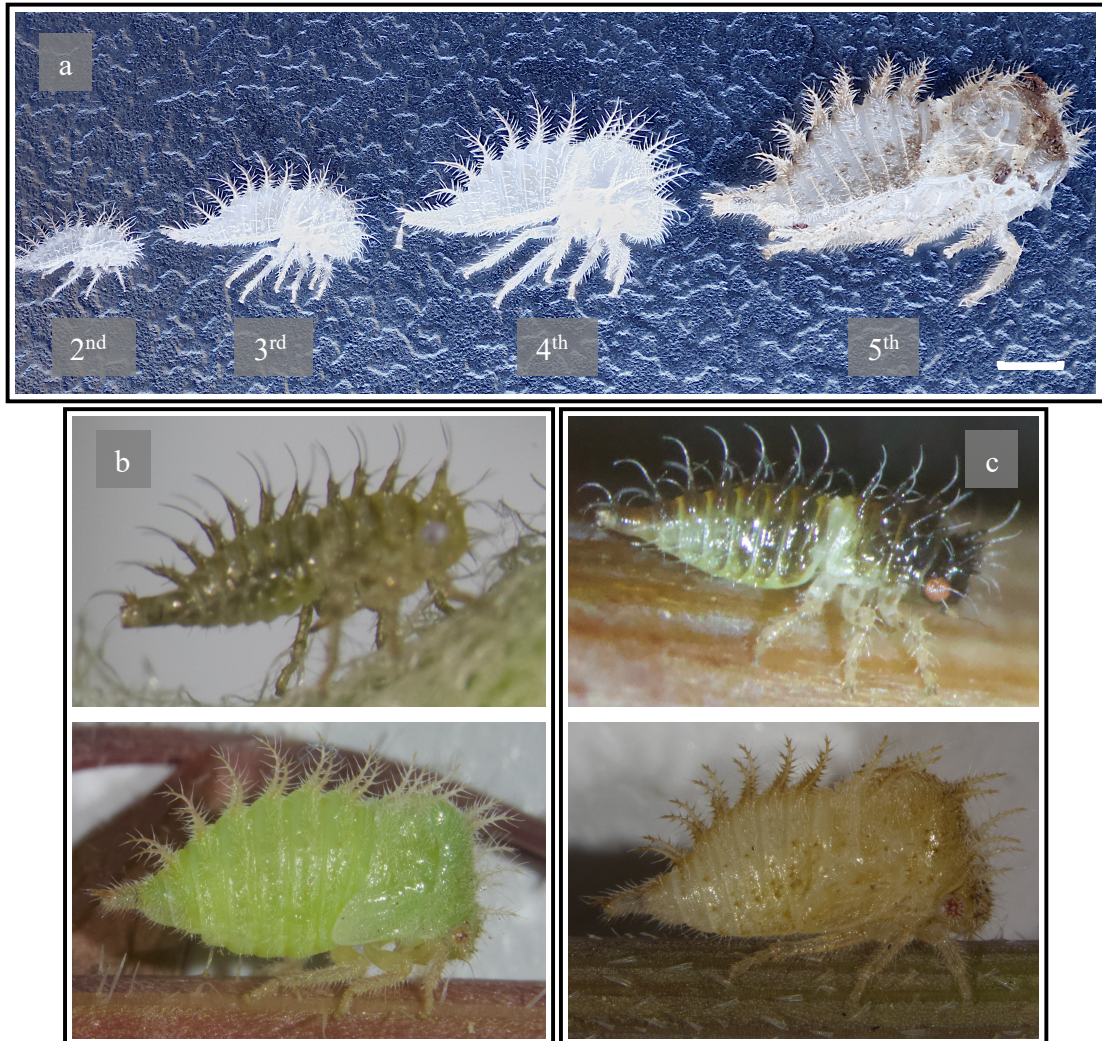
Figure 3.1 Life stages of (a) *Stictocephala basalis* 2nd–5th instar exuviae. Scalebar=1 mm as approximated by sizes reported by Yothers (1934); (b) *St. basalis* 2nd (top) and 5th (bottom) instar stages; (c) *Tortistilus albidosparsus* 2nd (top) and 5th (bottom) instar stages.
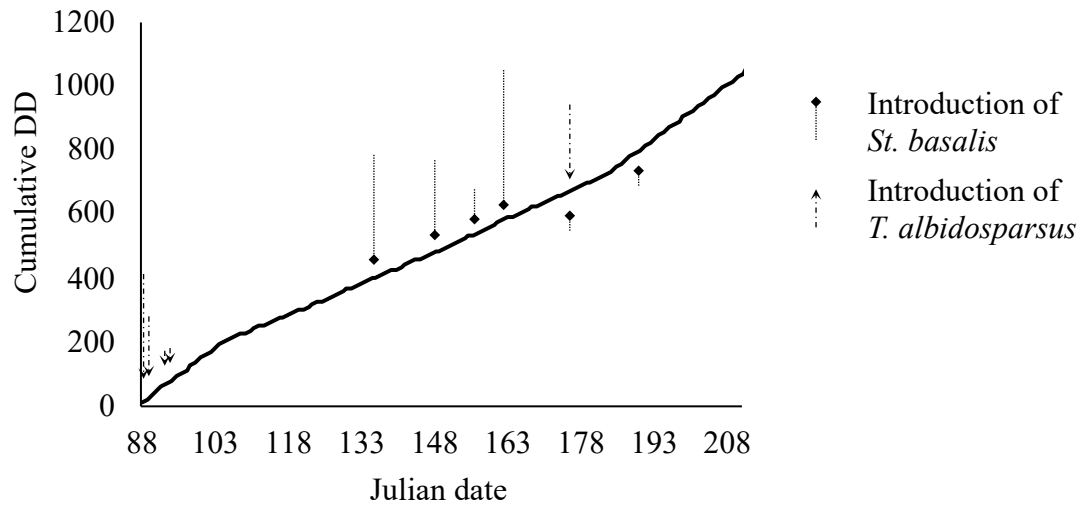
Figure 3.2 Growth chamber heat accumulation in 2018 and timing of introduction of *Stictocephala basalis* and *Tortistilus albidosparsus* immature stages. Size of bars indicates relative number of insects introduced on the corresponding date. DD were calculated using a 10 °C base temperature and upper cutoff threshold of 30 °C (Pruess 1983).
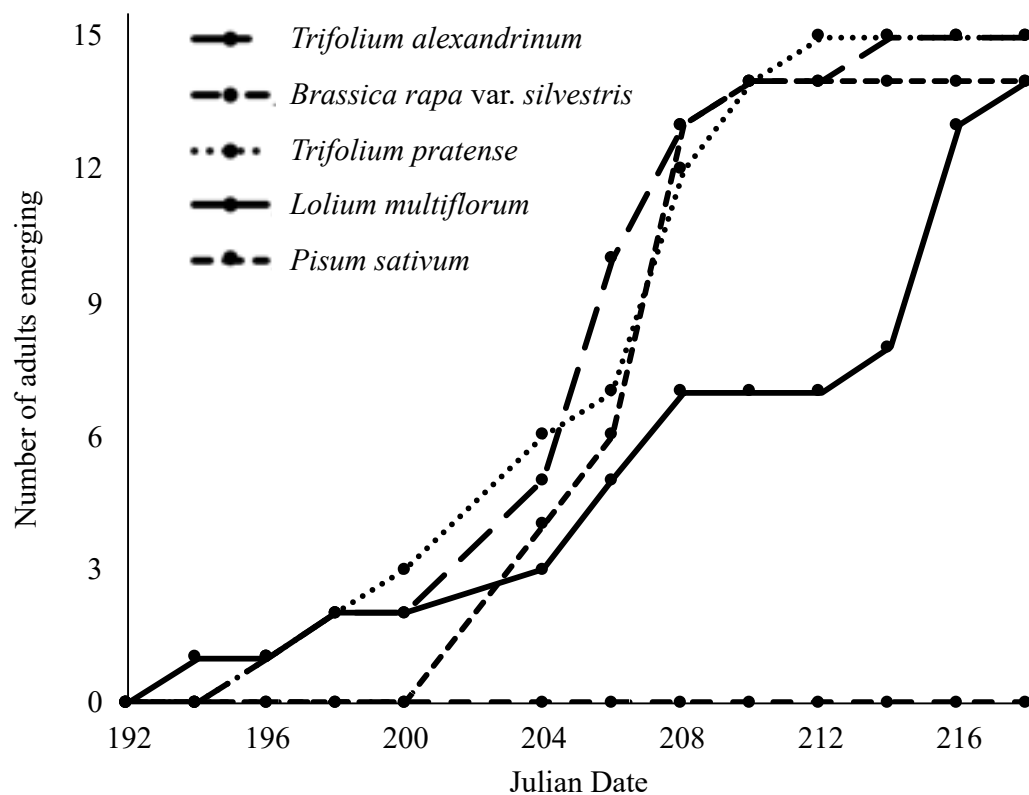
Figure 3.3 Cumulative emergence of *Stictocephala basalis* adults on herbaceous plant species in a growth chamber from 11 July–6 August 2018.
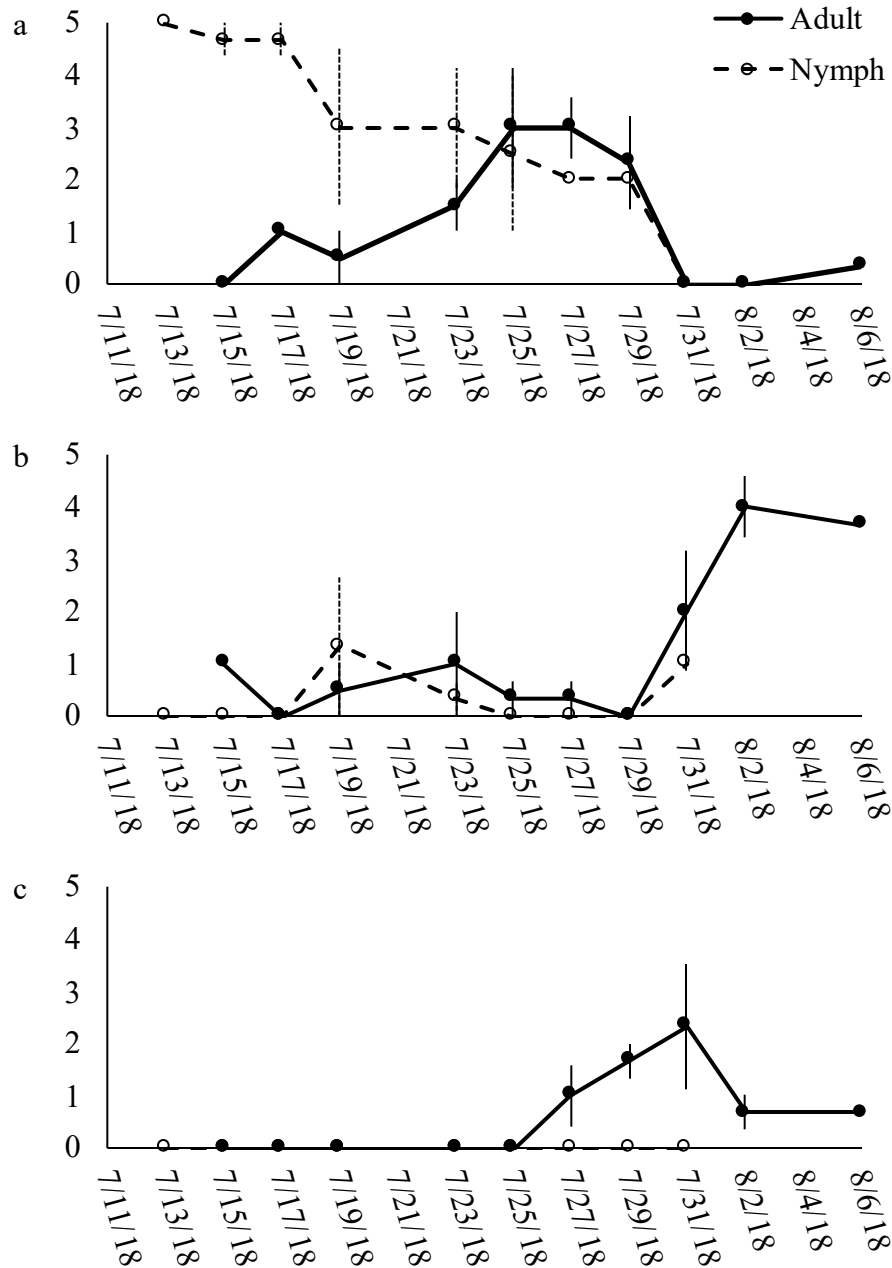
Figure 3.4 Mean counts ± SEM of (a) feeding activity; (b) non-feeding stationary activity; and (c) wandering activity of *Stictocephala basalis* 5th instar nymphs and adults on *Trifolium alexandrinum* in a growth chamber.
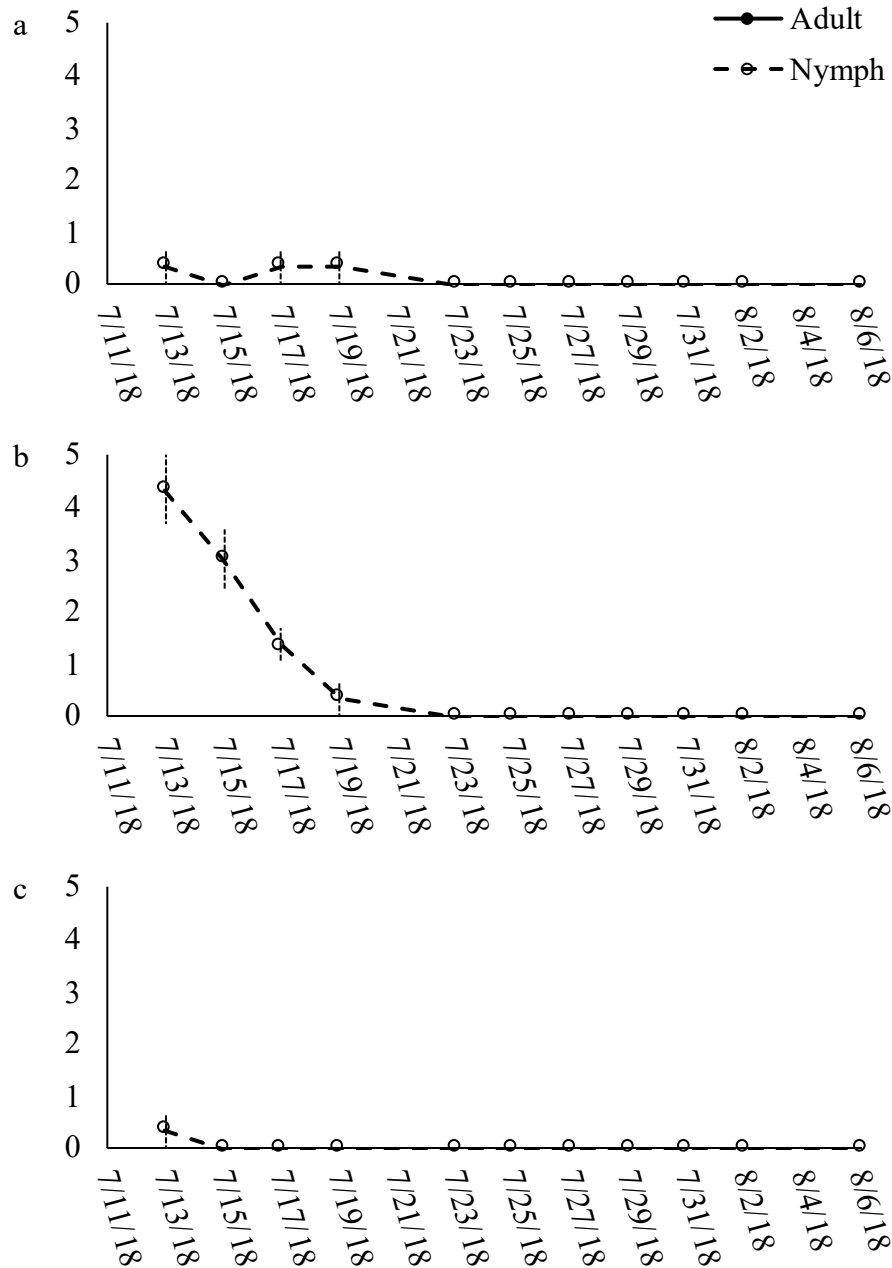
Figure 3.5 Mean counts ± SEM of (a) feeding activity; (b) non-feeding stationary activity; and (c) wandering activity of *Stictocephala basalis* 5th instar nymphs and adults on *Brassica rapa* var. *silvestris* in a growth chamber.
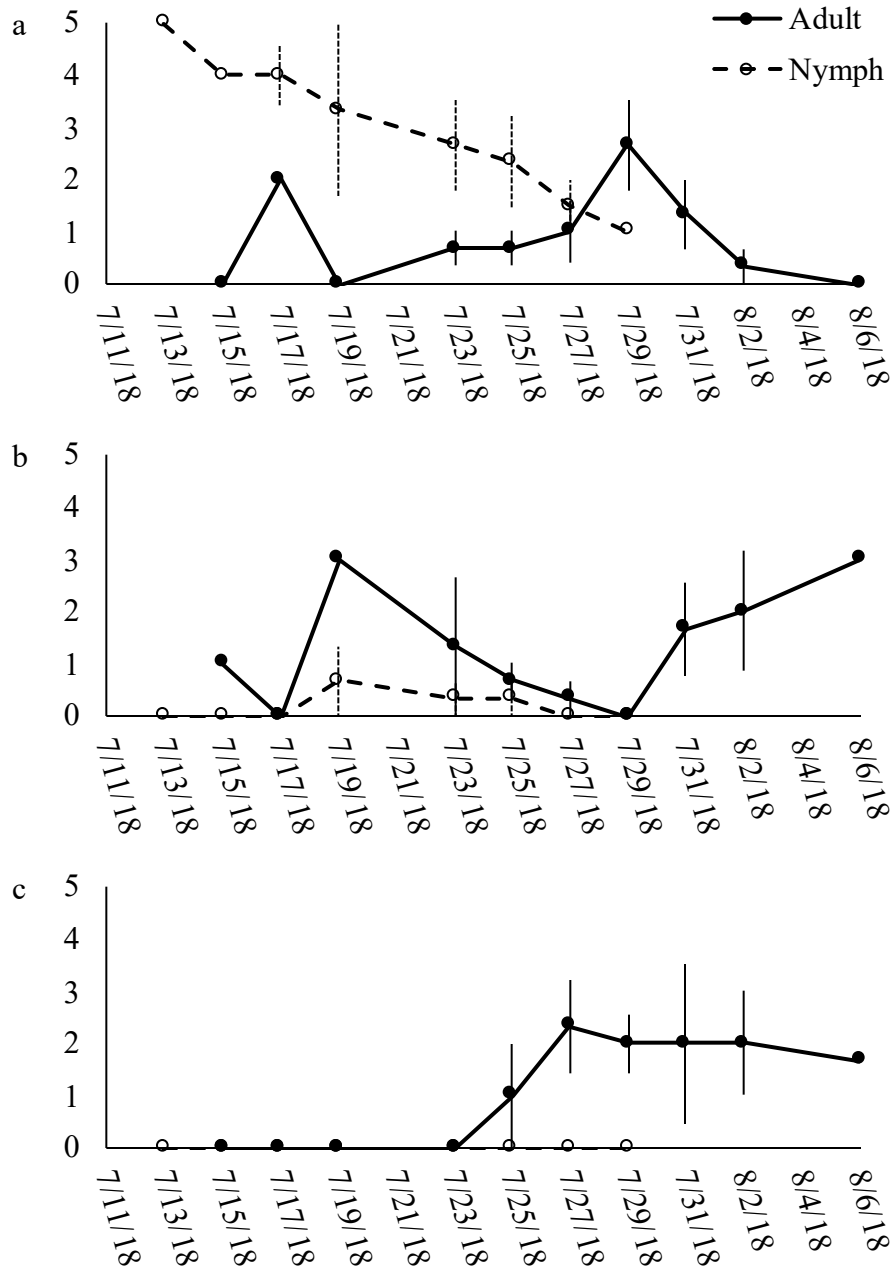
Figure 3.6 Mean counts ± SEM of (a) feeding activity; (b) non-feeding stationary activity; and (c) wandering activity of *Stictocephala basalis* 5th instar nymphs and adults on *Trifolium pratense* in a growth chamber.
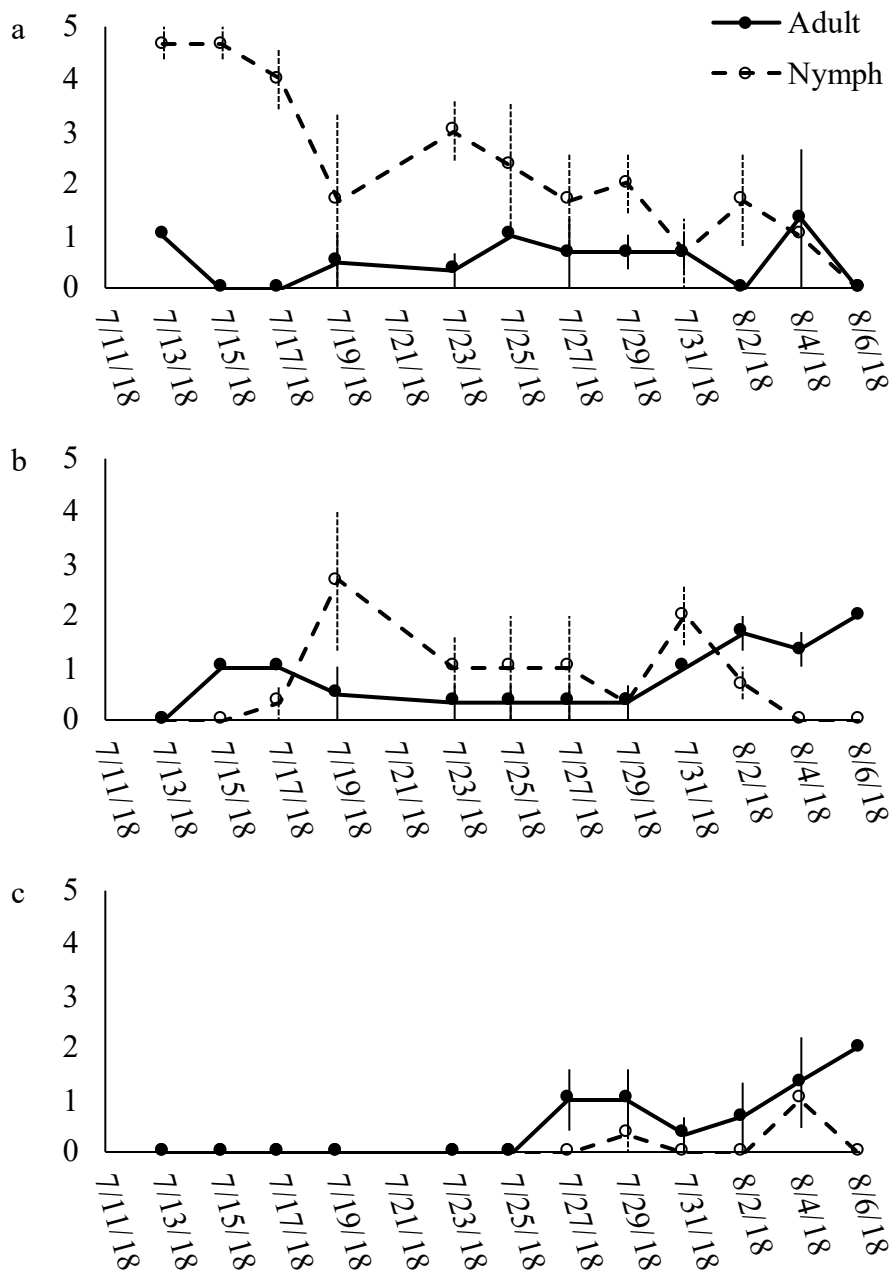
Figure 3.7 Mean counts ± SEM of (a) feeding activity; (b) non-feeding stationary activity; and (c) wandering activity of *Stictocephala basalis* 5th instar nymphs and adults on *Lolium multiflorum* in a growth chamber.
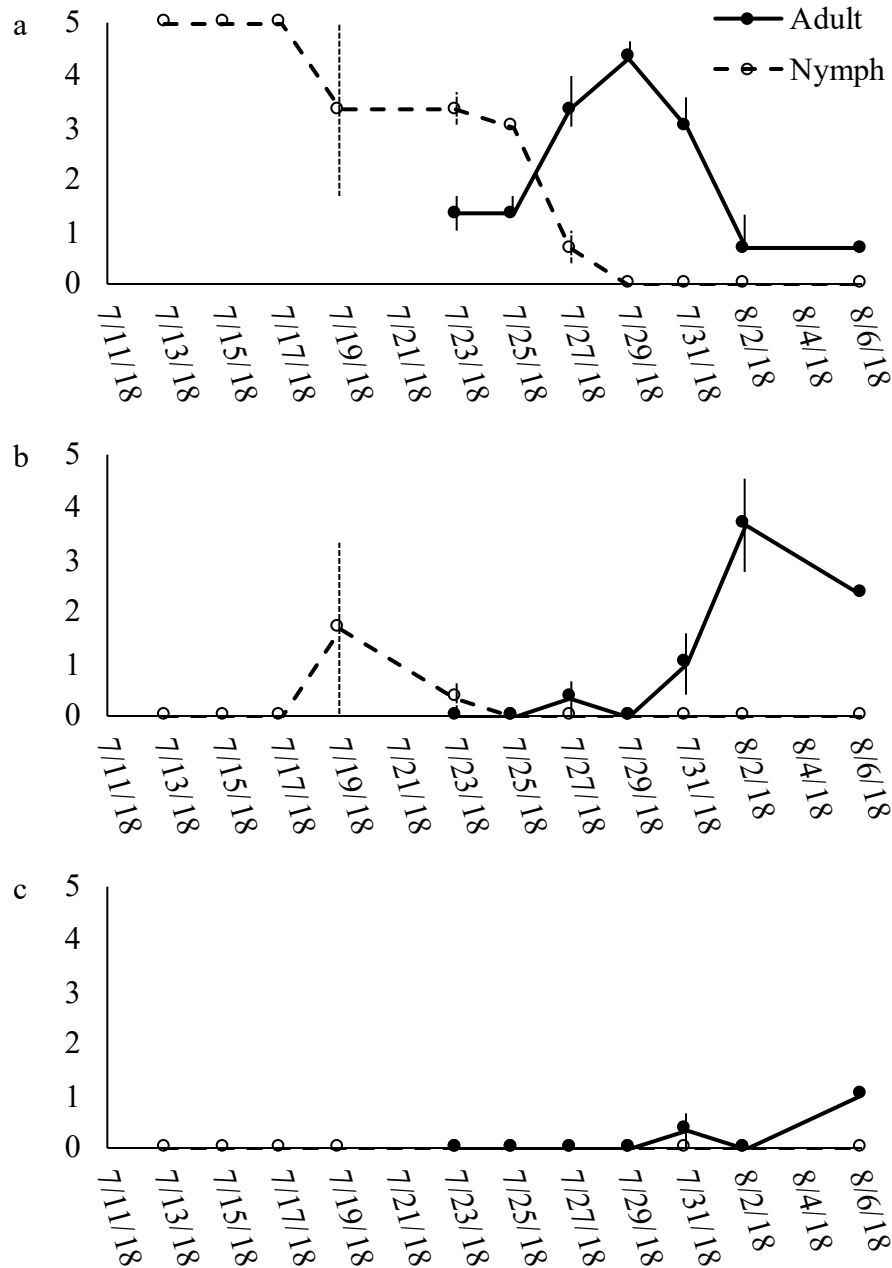
Figure 3.8 Mean counts ± SEM of (a) feeding activity; (b) non-feeding stationary activity; and (c) wandering activity of *Stictocephala basalis* 5th instar nymphs and adults on *Pisum sativum* in a growth chamber.
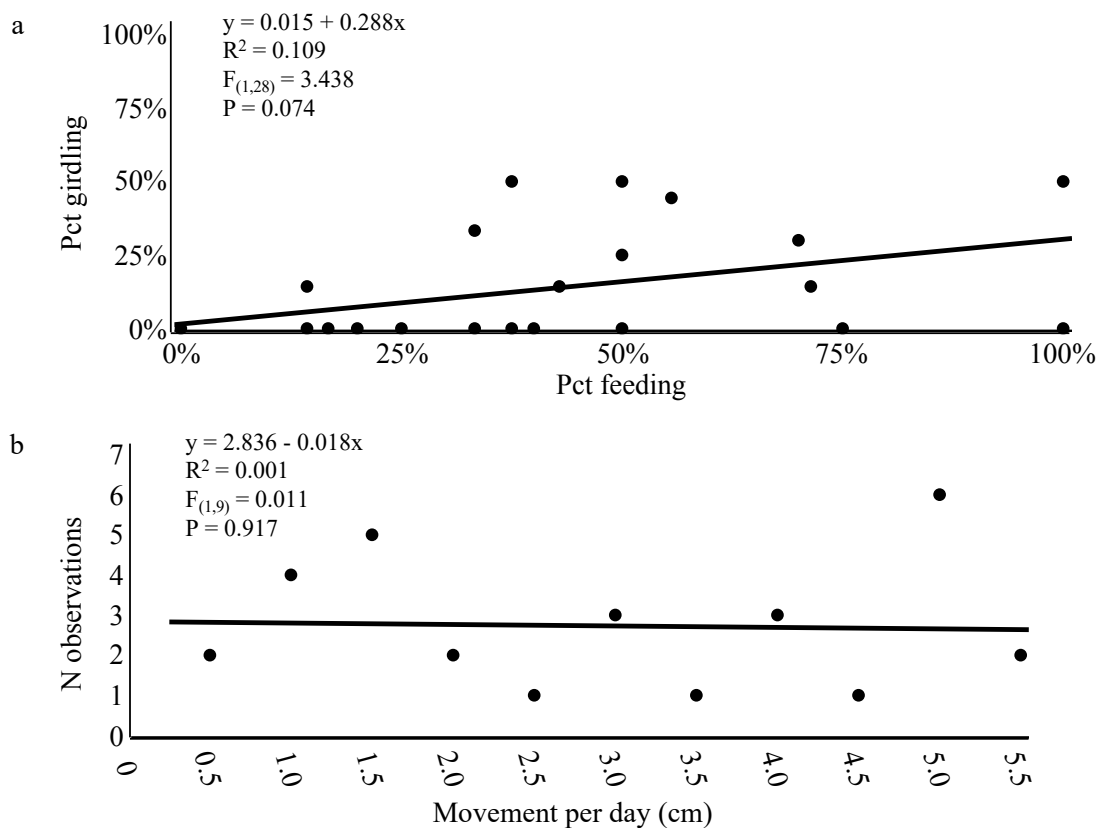
Figure 3.9 Linear regression of (a) frequency of girdling of greenhouse-grown potted *Vitis vinifera* 'Pinot noir' tissue by *Stictocephala basalis* as a response of its feeding activity; (b) movement per day of *St. basalis* adults that were caged on *V. vinifera* 'Pinot noir'.
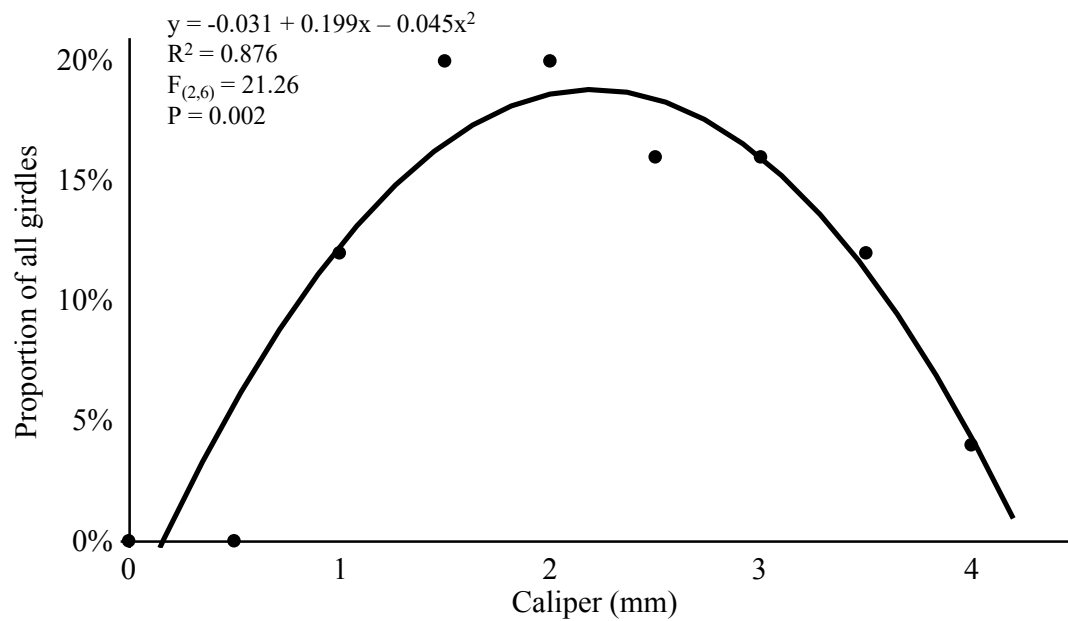
Figure 3.10 Caliper classifications of observed girdles induced by *Stictocephala basalis* in a greenhouse.

$$y = 1/(\pi*1.631*(1+((x-3.208)/1.631)^2))*191.8$$
$$R^2 = 0.790$$
$$F_{(1,10)} = 25.55$$
$$P < 0.001$$
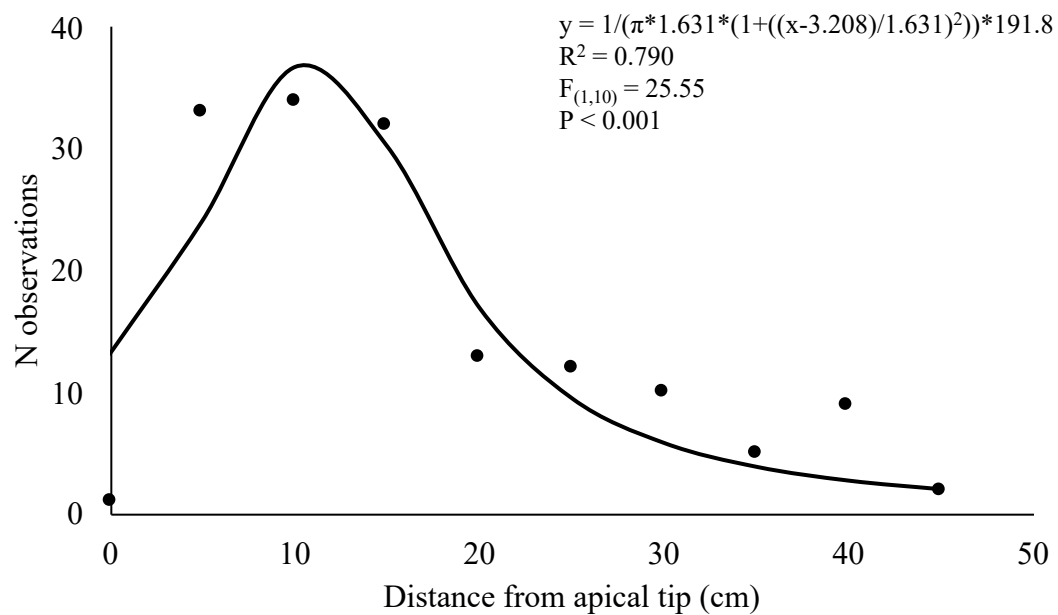
Figure 3.11 Location classifications where *Stictocephala basalis* adults were observed in greenhouse trials.
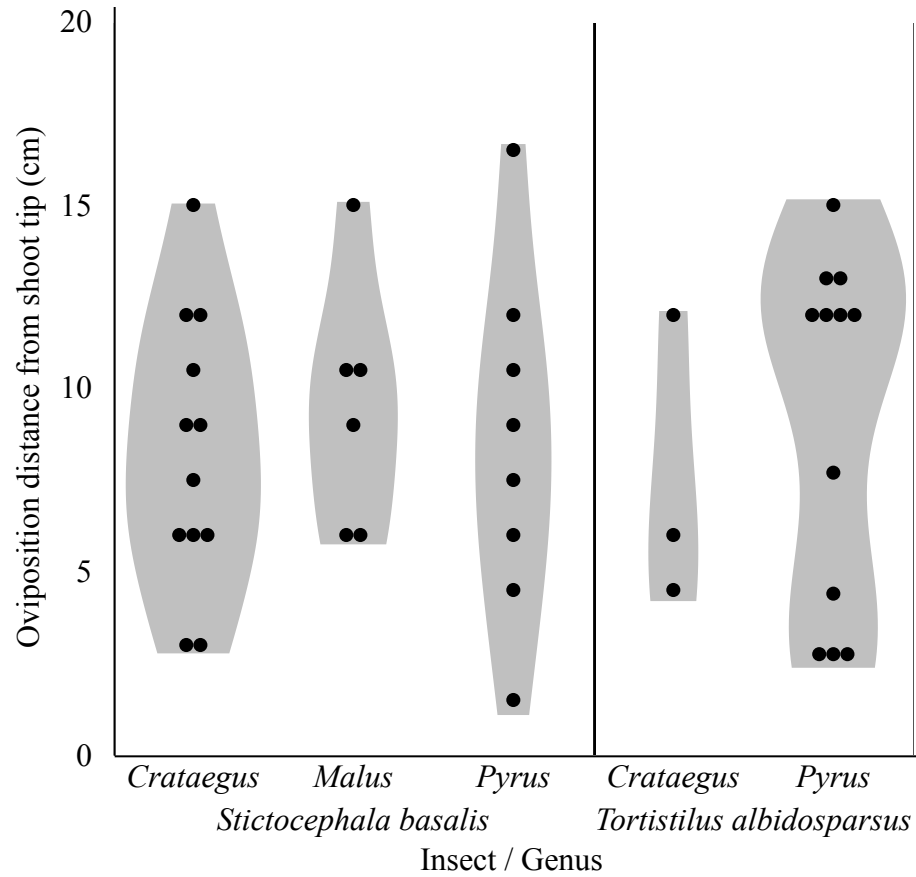
Figure 3.12 Distance of *Stictocephala basalis* and *Tortistilus albidosparsus* oviposition sites from shoot tips of woody host plants in a greenhouse. Width of shaded areas indicates relative abundance of eggs deposited at the corresponding distance. Black dots indicate raw data measurements.

a

$y = 1/(\pi*1.112*(1+((x-2.464)/1.112)^2))*126.4$
$R^2 = 0.967$
$F_{(1,5)} = 75.66$
$P = 0.013$

N observations

Distance from shoot tip (cm)

b

$y = 44.87 - 1.497x$
$R^2 = 0.981$
$F_{(1,4)} = 209.7$
$P < 0.001$

N observations

Distance from shoot tip (cm)
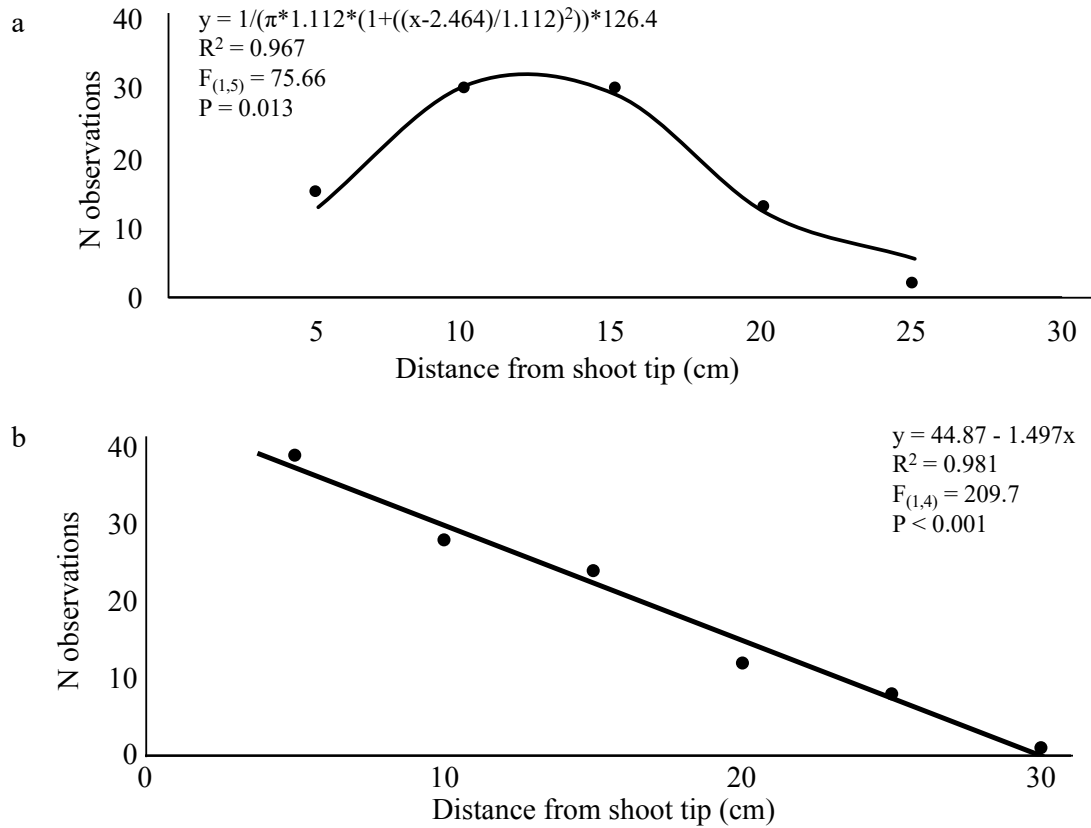
Figure 3.13 Oviposition sites of (a) *Stictocephala basalis*; (b) *Tortistilus albidosparsus* on artificially-infested grapevines in a greenhouse.

$y = 133.7 - 7.562x$
$R^2 = 0.972$
$F_{(1,4)} = 138.0$
$P < 0.001$
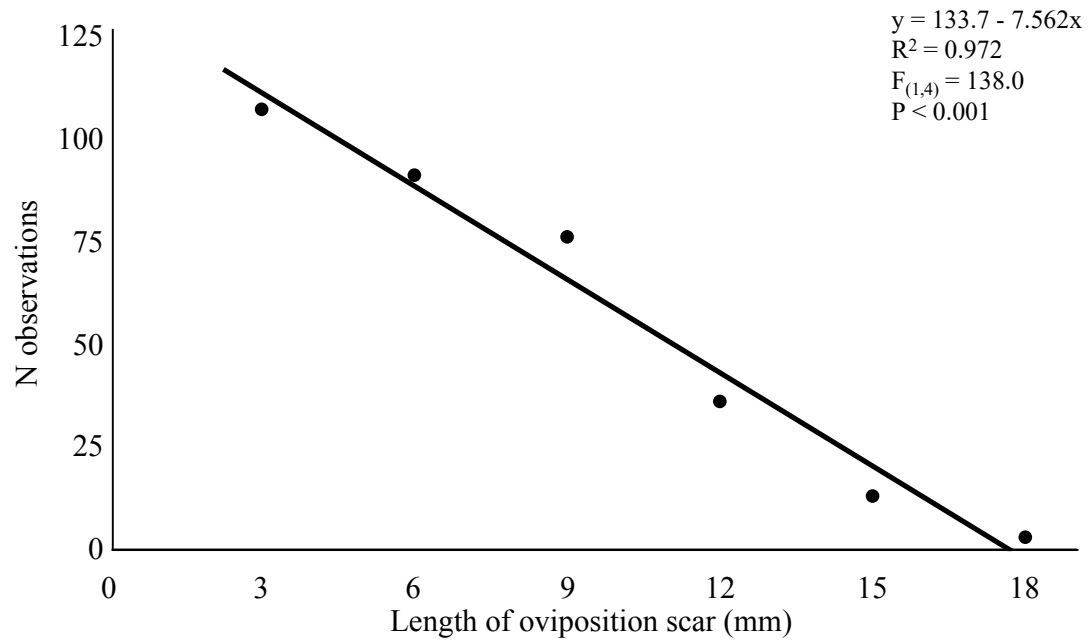
Figure 3.14 Length of oviposition scars caused by *Tortistilus albidosparsus* on grapevines in a greenhouse.

CHAPTER 4

Seasonal phenology of treehopper species (Hemiptera: Membracidae: Smiliinae) associated with Oregon vineyards

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ABSTRACT

Phenological progression of life stages of treehopper species (Hemiptera: Membracidae: Smiliinae) *Stictocephala basalis* (Walker) and *Tortistilus albidosparsus* (Stål) was tracked in 2017 and 2018 at vineyard sites in the Willamette Valley and southern Oregon. Adults of *Spissistilus festinus* (Say) and *Stictocephala bisonia* (Kopp and Yonke) were additionally discovered in a previously removed vineyard where *Grapevine red blotch virus* had been present. Host genera of *St. basalis* life stages were determined. *Quercus* L. hosted the highest proportion of eggs. Early instar stages were most commonly found on *Vicia* L., while middle and late instar stages were most often found on *Daucus* L. All life stages were found on *Vitis vinifera* L. Eggs of *St. basalis* hatched from April–June, and immature insects underwent five molts. Emergence of adults began in July, and fresh eggs were discovered in August. Hand collections were the most efficient technique to monitor treehopper development, while sticky cards effectively trapped *T. albidosparsus* adults in southern Oregon. Feeding damage manifested as petiole and stem girdles on grapevine, occurring at the treehopper 5th instar stage and continuing until October. A strong edge effect of feeding activity was found at two vineyard sites. Petiole girdles were more common and smaller in caliper than stem girdles. Distance of a deposited egg from the apical shoot tip differed between host species. Most eggs of *St. basalis* were found singularly within a dormant bud. Assessment of treehopper phenology will aid in population mitigation techniques where direct or indirect damage is a concern to vineyard managers.

Key words: degree-days, *Vitis vinifera*, *Stictocephala basalis*, *Tortistilus albidosparsus*, host plants.

INTRODUCTION

Treehoppers (Hemiptera: Membracidae) comprise a highly diverse insect family, with nearly 4,000 species worldwide (Bartlett et al. 2018). Members of subfamily Smiliinae are native to North, Central, and South America and are represented by at least seven tribes (Deitz et al. 2012), including the Ceresini containing the genera *Spissistilus*,

Stictocephala, and *Tortistilus* that share key morphological traits as late instar insects (Quisenberry et al. 1978). Many smiliine treehopper species are considered oak specialists (Wallace and Troyano 2006). In the southeastern United States, *Spissistilus festinus* (Say) feed on leguminous crops including peanut, soybean, and alfalfa (Beyer et al. 2017). The polyphagous feeding behavior of *Stictocephala bisonia* (Kopp and Yonke) likely enabled the species to establish in the western United States and in Europe following introduction from midwestern North America (Kopp and Yonke 1979). In Central Europe, *St. bisonia* have been present for more than 100 years (Lauterer et al. 2011, Walczak et al. 2018, Schedl 1991). In the western United States, *Tortistilus* spp. (Hemiptera: Membracidae) have long been recognized as pests of minor concern in tree fruit production due to ovipositional activity (Yothers 1934).

Treehoppers use their piercing-sucking mouthparts to feed circumferentially around stems and leaf petioles, manifesting in the formation of girdles (Grosso et al. 2016). Feeding damage induces accumulation of carbohydrates and certain amino acids above the girdling point, creating a positive feedback in which insects will migrate apically to continue feeding on nutrient-rich tissue (Andersen et al. 2002, Mitchell and Newsom 1984b). Multiple shoot girdles can cause mortality of affected stems (Grosso et al. 2016). Oviposition occurs in woody species on internodal regions of plant stems (Sorenson 1928, Yothers 1934) or under bud scales (Ebel and Kormanik 1965). Certain treehopper species may deposit eggs on annual growth (Rice and Drees 1985, Preto et al. 2018a). Some species may utilize a single host, while other species may utilize multiple hosts to complete their life cycle (Yothers 1934, Dietrich et al. 1999, Grosso et al. 2014).

Temperature has significant influence on insect development, and closely related species have similar thermal requirements for development (Jarošík et al. 2011, Ikemoto 2005). Developmental rate curves can explain insect and plant development, where at the lower range of the curve development is minimal and survival of the organism is compromised, the middle range of the curve is approximated by a linear relationship of optimal development, and at temperatures above the optimum, developmental rate slows and survival decreases (Damos and Savopoulu-Soultani 2011, Wagner et al. 1984). In plants, this is explained by enzyme catalytic activity; at

below-optimal temperatures, an enzymatic reaction occurs inefficiently due to rigidity of the enzyme, at above-optimal temperature the enzyme may coagulate, and the optimal temperature is found somewhere in between (Bonhomme 2000). In insects, it has been shown that phenology is influenced by two main factors: lower developmental temperature and degree-day (DD) accumulations (Buckley et al. 2017).

Lower developmental temperature of temperate species shows a general decrease with increasing latitude for all insect life stages (Honěk 1996), and populations from high-latitude origins are particularly sensitive to changes in the number of generations per season (Buckley et al. 2017). Effects of elevation on insect development generally depend on the ability of individuals to relocate temporally to avoid extreme abiotic conditions (Hodkinson 2005). DD effects on insect development are complex processes; as such, models are often imprecise and development can be characterized equally well by use of calendar dates (Pruess 1983). Yet, temperature is fundamental to explaining developmental rates of an insect (Tochen et al. 2014, Hough-Goldstein et al. 2016).

Treehopper feeding activity can cause a decline in plant health through induction of vascular tissue necrosis and may increase plant susceptibility to stem lodging (Grosso et al. 2016). In a study of soybean using foliar-applied radioisotope ^{14}C, translocation was reduced following girdling by *Sp. festinus*, with concomitant reductions in root nodulation (Hicks et al. 1984). Depending on the timing of girdling, plants may compensate for feeding damage through reallocation of plant resources or generation of adventitious roots above the girdling point (Herzog et al. 1975). In soybean, a study using a systemic herbicide revealed resumption of normal translocation within 7 days of girdling by *Sp. festinus* (Spurgeon and Mueller 1991).

A more serious effect of treehopper feeding may be the potential for insects to transmit plant pathogenic microbes (Daane et al. 2018). Treehoppers were found to carry the ArAWB phytoplasma of alfalfa in Argentina, threatening the economic feasibility of crop production (Grosso et al. 2014). Based on their characteristic phloem-feeding habits, the capacity of membracids to transmit phytoplasmas into woody hosts appears likely; however, further study on individual pathosystems is needed for confirmation (Weintraub and Beanland 2006). In a study conducted in New

Jersey, USA, more than half of the membracid species collected from oak were carriers of *Xylella fastidiosa* Wells et al., the causal agent of oak bacterial leaf scorch (Zhang et al. 2011). While Cicadellidae are well-known for their ability to transmit geminiviruses, virus transmission is well established for only one membracid species (Ammar and Nault 2002). The treehopper *Micrutalis malleifera* Fowler is a competent vector of *Tomato pseudo-curly top virus* (TPCTV) (*Geminiviridae*, *Topocuvirus*) on solanaceous plants (Simons and Coe 1958, Simons 1962). Specificity of TPCTV transmission was found to be imparted by the coat protein nucleotide sequence (Briddon et al. 1996).

Insect species *Erythroneura ziczac* Walsh (Hemiptera: Cicadellidae) and *Sp. festinus* (Hemiptera: Membracidae) were implicated as potential vectors of *Grapevine red blotch virus* (GRBV) (*Geminiviridae*, *Grablovirus*), causative agent of red blotch disease (RBD), in greenhouse experiments (Poojari et al. 2013, Bahder et al. 2016b, Yepes et al. 2018). Symptoms of RBD were first observed on grapevine, *Vitis vinifera* L., in California in 2008 (Sudarshana et al. 2015). Expression of RBD results in physiological changes of berry chemistry and leaf coloration that are similar to symptoms caused by grapevine leafroll-associated viruses (Al Rwahnih et al. 2013, Blanco-Ulate et al. 2017, Martínez-Lüscher et al. 2019), with potentially severe economic impacts on wine grape production (Ricketts et al. 2017).

Field transmission of GRBV remains to be shown for either *E. ziczac* or *Sp. festinus*, although individuals of *Sp. festinus* were carriers of GRBV in a California vineyard (Cieniewicz et al. 2018b). GRBV infects a wide range of *Vitis* spp. hosts (Thompson et al. 2018) but was not found to replicate in *Rubus armeniacus* Focke (Bahder et al. 2016b) or in leguminous weeds (Cieniewicz et al. 2019) that are common to North American Pacific Coast wine grape production regions. Infections of GRBV are distributed in vineyards across the United States (Krenz et al. 2014, Sudarshana et al. 2015). Secondary spread was found in California (Cieniewicz et al. 2017b) and Oregon (Dalton et al. 2019), suggesting involvement of a mobile vector species. Coat protein sequence analysis placed GRBV in close phylogenetic relation with TPCTV, while leafhopper- and whitefly-transmitted geminiviruses were more distantly related (Bahder et al. 2016b). The close phylogenetic alignment of GRBV and TPCTV

indirectly supports the likelihood that they are transmitted by closely related insect species (Whitfield et al. 2015).

Spread of RBD in Oregon vineyards prompted investigations into potential vectors of the disease. Smiliine treehoppers were found in all study regions (Dalton et al. 2020), whereas *Erythroneura* spp. were found in few vineyard sites (Walton, *personal communication*). Field observational studies were conducted to determine phenological attributes and species composition of Membracidae in Oregon vineyard sites.

MATERIALS AND METHODS

Site selection and treehopper collections. Sites in Oregon, USA were observed from 2016–2018 for insights into the behavior and life history of locally abundant populations of Membracidae. A vineyard, hereafter referred to as YV, located in Yamhill County near the town of Yamhill, served as the primary field site for collections, phenological, and behavioral observations of *St. basalis*. Additional survey sites in Yamhill County included two vineyards near Lafayette (LV1 and LV2); and one vineyard near Carlton (CV). In Polk County, a vineyard near the community of Kings Valley (KVV) was visited to search for membracid eggs on a single date. In Benton County, a park located in Corvallis (CP) and a research vineyard in the Coast Range near Alpine (CRV) were infrequently surveyed. Southern Oregon survey sites included a vineyard near the city of Cave Junction, Josephine County (CJV), serving as the primary field site for observations of *T. albidosparsus*, as well as vineyards in Jackson County near Eagle Point (EPV) and Jacksonville (JV). Detailed site descriptions are provided in Appendix C.

Degree-day calculations. A DD calculator was used to approximate the amount of heat units that occurred in the field from 2016 to 2018 (Coop 2019). Developmental thresholds of 10 °C (lower) and 30 °C (upper), in which temperature values below and above thresholds were assigned a value equal to the nearest threshold, were applied to all temperature data (Pruess 1983). Models were set to run from 1 January through 31 December of each year. For sites CV, LV1, LV2, and YV, weather data from McMinnville Municipal Airport (FAA Identifier MMV, 45.195° N, 123.136° W, elev. 50m), McMinnville, Oregon were used. For sites CP, CRV, and KVV, weather data from Corvallis Municipal Airport (FAA Identifier CVO, 44.497° N, 123.290° W, elev.

76m), Corvallis, Oregon were used. For site CJV, weather data from Illinois Valley Airport (FAA Identifier 3S4, 42.104° N, 123.682° W, elev. 425m), Cave Junction, Oregon were used. For sites EPV and JV, weather data from Rogue Valley International Airport (FAA Identifier MFR, 42.374° N, 122.874° W, elev. 407m), Medford, Oregon were used.

Seasonal observations of Stictocephala basalis. Life stages of *St. basalis* were observed at five field sites from April through October of 2017 and 2018. CRV was surveyed on 5 October 2017 and in 2018 on 10 April and 7 June. YV was surveyed beginning in April approximately once every two weeks in 2017 and once every 2–3 weeks in 2018. Additional field collection trips at CP, CV, and LV2 occurred at irregular intervals in 2018.

Assorted surveying techniques were used in order to maximize collection efficiency of different insect life stages. Eggs, which overwinter within woody tissues of host plants (Yothers 1934), were collected through acquisition of woody cuttings in the late-dormant period, early growing season, or after terminal buds had set. Bud dissections were performed in the laboratory using sharp probes under 10× magnification. When eggs were found, infested wood was sealed inside a Petri dish containing fresh leaves and observed three times per week in the laboratory for early instar nymph emergence. Emergent insects were transferred to *Pisum sativum* L. 'Oregon Trail' seedlings using a fine-bristled paintbrush or by placing infested material on top of seedlings. Early instar insects were located through intensive searches of herbaceous vegetation using a hand loupe at 10× magnification. Insects were collected by clipping infested materials into ventilated plastic containers. Mid and late instar insects were readily visible to the naked eye, and infested plant tissues were collected with secateurs or a vacuum sampler (D-vac model 122, Rincon Vitova Insectaries, Inc., Ventura, CA, USA). Vacuum sampling was employed infrequently to quickly collect large numbers of immature insects but for safety reasons could not be used during hot weather. In the laboratory, collected immature treehoppers were placed onto *P. sativum* seedlings using a fine-bristled paintbrush and allowed to progress through subsequent life stages. Adults were collected via aspiration and subsequently transferred into ventilated containers. When possible, host plant associations were recorded upon insect collection in the field.

Seasonal observations of Tortistilus albidosparsus Routine field surveys occurred at CJV in 2017 and 2018 in order to identify seasonality of *T. albidosparsus* life stages. Surveys occurred every 1 to 3 weeks from May through September of both years. To collect immature stages, vacuum sampling using a Vortis insect suction sampler (Patent No. 9207468.1, Burkard Manufacturing Company Limited, Hertfordshire, England, UK) occurred in May and June. Samples were returned to the laboratory, numbers of nymphs were counted, and surviving nymphs were placed on seedlings of thistle (*Cirsium arvense* L.) or alfalfa (*Medicago sativa* L.) to allow life stage progression. Adult insects were collected primarily by hand. Sweep netting was conducted on a single date in 2017 but was not effective. Yellow sticky cards (7.6 × 12.7cm, Olson Products, Inc., Medina, OH, USA) were systematically deployed in the vineyard beginning on 13 June and 15 June in 2017 and 2018, respectively. Adults were counted on each survey date and removed from cards. Sticky cards were replaced if found to be excessively soiled.

At CV, hand collections of *T. albidosparsus* were made in 2018 on six dates from 27 June through 29 August. To collect nymphs, wild-growing vegetation outside of the vineyard blocks was visually scouted for known host plants. Herbaceous vegetation was collected by clipping infested plant material into a ventilated plastic container. Adults were located through systematic visual surveys of vineyard rows and sampled by aspiration. All surviving insects were placed onto host plants in the laboratory or greenhouse.

Treehopper field feeding damage and ovipositional characteristics. Surveys for leaf petiole girdles (GP) and stem girdles (GS) took place at CJV and at YV by walking slowly down an interrow and scanning the vine canopy for discolored tissue and damage consistent with treehopper feeding. In 2016, a girdling survey occurred at CJV during the first week of November, but no materials were collected. In 2017, girdled materials were collected from CJV on 15 August during a field search for treehopper adults. Systematic surveys occurred at YV in the west block and southwest block from 2016 to 2018. In 2016, all vines were assessed once from 26 September to 6 October for treehopper feeding activity, but no tissues were collected. In 2018, the southwest block was revisited on 9 October, and girdled plant tissues were collected. Vines in

rows 45–46 of the west block were repeatedly observed in 2017 and 2018 for girdling activity. Fully girdled materials were taken to the laboratory for measurement on the dates of collection. Partially-girdled materials were removed from plants but were not measured or collected. When multiple girdles were present on a sample, the diameter of each girdle was individually recorded. Locations 1mm above deformed tissue were measured using digital calipers.

To analyze spatial distribution patterns of treehopper feeding damage, the statistical program Spatial Analysis by Distance IndicEs (SADIE) was used (Perry 1995). SADIE is a computer program that interpolates spatiotemporal distribution patterns of data. X, Y coordinates of an area are paired with corresponding integer count data and entered into the program interface. The data are then randomly permuted, resulting in a metric of distance to regularity (D). This simulated value provides a test of the actual data, thereby allowing interpretation of significance of aggregation (I_a) at $\alpha < 0.05$. When $I_a > 1$, counts are statistically aggregated, when $I_a < 1$, counts are regularly distributed, and when $I_a = 1$, counts are randomly distributed (Madden et al. 2007). Degree of clustering (v_{ij}) provides a value to inform the strength of aggregation compared to the plot average. When $v_i > 1.5$, degree of positive clustering is indicated, and when $v_j < -1.5$, degree of negative clustering is indicated. Intermediate values indicate non-significant aggregation compared to the plot average counts. More extreme values indicate stronger clustering (Perry et al. 1999). SADIE outputs were processed into contour maps using a surface map interface computer program (Surfer® version 17.1.288, Golden Software, Golden, Colorado, USA).

Because of limitations to the size of the data set that can be handled with SADIE, at CJV the average number of girdles per group of four consecutive vines along the vineyard rows was calculated. The resulting fraction was multiplied by 12 to produce integer values for all groups. Due to the layout of the block, groups rarely contained less than four vines each. At YV, west block rows 45–46, southwest block rows 0–2, and southwest block rows 3–21 were analyzed separately for each year that girdling surveys took place. YV blocks were small enough to be inputted into SADIE using raw integer counts.

To investigate a potential edge effect of incidence of girdling, row and vine locations at all distances from the vineyard edge were assessed at CJV and YV using the proportion of girdles per location divided by the proportion of vines in each location. The resulting fractions were then divided by the factor that was found at edge locations, allowing a comparison of the likelihood of girdling at locations away from the edge compared to the standardized edge average of 1. Locations where no girdles were observed thus resulted in a value of 0. Proportions were then used in Chi-square analysis to test whether occurrence of girdling was random across locations.

Ovipositional behavior was quantified through assessment of the number of eggs per bud, status of field-collected eggs (hatched or unhatched), and location of infested buds relative to host shoot tips. When an infested bud was discovered in the laboratory, a ruler was used to measure the distance of the bud (mm) from the shoot tip. Findings of healthy, unhatched eggs were used to determine timing of field emergence. To confirm species composition, collected eggs were held in the laboratory to allow emergence and subsequent development of instar stages.

RESULTS

Site selection and treehopper collections. Smiliine treehopper species were collected from 2016–2018 in eight of ten sampling sites (Table 4.1). Willamette Valley sites CP, CRV, LV2, and YV yielded only *St. basalis*, as confirmed by examination of the physical characteristics of voucher specimens (Dalton et al. 2020, Stowasser et al. 2020). At CV, *T. albidosparsus* was the most abundant species, while *St. basalis* was collected in small numbers. Collections in southern Oregon sites CJV and EPV yielded only *T. albidosparsus*, while sampling at JV revealed the presence of *Sp. festinus*, *St. bisonia*, and *T. albidosparsus*. No treehopper life stages or evidence of treehopper damage were observed in 2018 at KVV or LV1.

Degree-day calculations. Patterns of DD accumulation in the field followed nearly identical trends from 2016 through 2018. Data from all weather stations were assessed from 2016 to 2018. At CVO, MFR and MMV, by year, the greatest DD accumulation occurred in 2016 (Fig. 4.1a), followed by 2017 (Fig. 4.1b), and the least DD accumulation occurred in 2018 (Fig. 4.1c). Weather station 3S4 experienced the greatest cumulative DD in 2017, followed by 2016, and lastly 2018. Overall, the

greatest DD accumulation was observed at MFR, while CVO, MMV, and 3S4 had equivalent heat accumulation.

Seasonal observations of Stictocephala basalis. Through field collections, phenological development of *St. basalis* was determined (Table 4.2). Unhatched, viable treehopper eggs were procured from collections of woody cuttings containing dormant buds of woody perennial plants at CRV and YV in October 2017, and from April-June 2018. At YV, early instar stages overlapped with the last occurrence of unhatched eggs in 2017, and late instar stages occurred until the end of July of both years. Emergence of the first adults occurred on 20 July and 10 July in 2017 and 2018, respectively. In both years, successive instar stages overlapped with the previous stages, resulting in an overall synchronous pattern of immature stage development (Fig. 4.2). Intermediate and late instar nymphs of *St. basalis* were collected at CV and LV2 in late June and early July 2018, respectively, while adults were collected at CV on 24 July 2018. Fresh, unhatched eggs were obtained at YV in August 2018 following terminal bud formation on woody species. The complete seasonal progression of the *St. basalis* lifecycle was thus recorded (Fig. 4.3). Nymphs of all instar stages collected at YV developed into adult *St. basalis* in the laboratory. Adults were present through the final sampling date of both years.

Seasonal observations of Tortistilus albidosparsus. Immature and adult stages of *T. albidosparsus* were collected at CJV in 2017 and 2018 (Table 4.3). In 2017, nymphs were collected beginning on 18 May, and the last finding of nymphs occurred on 27 June. Adults were collected beginning on 6 July and were present through the remainder of the growing season. In 2018 nymphs were collected beginning on 1 May and were present until 15 June. Emergence of adults was first documented on 28 June. In 2018, first appearance of immature stages and adults was earlier and with fewer cumulative DD, compared to 2017. Sticky cards and hand collection both effectively captured treehopper adults at CJV. In the Willamette Valley, a population of *T. albidosparsus* was discovered at CV in 2018. Late-instar nymphs were collected on 27 June, and some nymphs emerged as adults in the laboratory within one day of collection. No immature stages were found on subsequent dates. Adults were captured on all visits from 10 July to 29 August.

Treehopper field feeding damage and ovipositional characteristics. Detailed collections of leaf petiole and apical stem girdles occurred at YV once in 2016, on six dates in 2017, and on two dates in 2018. Overall, 65 of 66 vines in Row 46, directly adjacent to unmanaged habitat, experienced feeding damage (98.5%), and 49 of 58 vines in the adjacent Row 45 presented at least one girdle (84.5%) over the three-year period (Fig. 4.4). The highest incidence of feeding damage was recorded in 2017. In the southwest block in 2016, the highest incidence of girdling occurred in row 3, the eastern-most row nearest unmanaged wild habitat, and generally decreased toward the center rows. The western-most rows of the southwest block had low incidence of girdling (Fig. 4.5a). In 2018, there was no clear trend in southwest block rows 3–21, except that girdling was lower overall with 58 observed girdles in 2018 compared to 84 observed girdles in the same rows in 2016. In 2018, girdling was observed in rows 11–15 on re-sprouting grapevines that had been previously cut to ground level (Fig. 4.5b). Row 2, the southeastern-most row directly adjacent to unmanaged habitat, displayed higher girdling in 2016 compared to rows 0 and 1 (Fig. 4.5c). In 2018, row 2 had numerically more girdles (n=5) compared to rows 0 and 1 (n=2 and n=3, respectively), although overall incidence of girdling was low (Fig. 4.5d). Significant clustering of girdles (P_a<0.05) was observed at YV in 2016 in west block rows 45 and 46 and southwest block rows 3–21. Significant clustering was observed in 2018 in southwest block rows 0–2. Marginally significant clustering (P_a=0.059) occurred in 2018 in southwest block rows 3–21 (Table 4.4). Spatial analysis displayed significant clustering of girdles (P_a <0.05) at CJV in 2016 (see Table 4.4). Girdles were aggregated at points along the edges of the block (Fig. 4.6).

A factor relating the field average number of girdles to distances from the vineyard edge revealed that edge locations at CJV and YV had the highest rate of girdling. At CJV, incidence of girdles per location dropped sharply below the edge average by location 2 away from the edge, approximating a highly significant loss function (Fig. 4.7a). A significant loss function was likewise observed at YV in which proportions of girdles at all locations away from the edge were lower than the proportion at the edge (Fig. 4.7b). Chi-square analysis confirmed that there was a highly significant difference

in girdling incidence by location relative to the distance from the block edge at both CJV ($\chi^2_{144,27}$=193.3, P<0.001) and YV ($\chi^2_{613,8}$=510.0, P<0.001).

The size of grapevine tissue that was susceptible to feeding damage by *St. basalis* at YV and *T. albidosparsus* at CJV was assessed using ANOVA. At YV, GP (mean caliper 1.81 ± 0.03mm) and GS (mean 2.14 ± 0.04mm) were non-normally distributed (W=0.985, P=0.004; W=0.985, P=0.046, respectively) but had equal variance. GP (mean 1.70 ± 0.11mm) and GS (mean 1.73 ± 0.09mm) tissues from CJV showed normal distribution and equal variance. Significant effects of tissue type and species were found, but with no interaction ($F_{2,511}$=28.27, P<0.001). Tissues girdled by *St. basalis* ranged in caliper from 1.0mm to 4.0mm, and tissues girdled by *T. albidosparsus* ranged in caliper from 1.0mm to 3.0mm. At YV, GP were found more frequently than GS, and at lesser caliper groupings (Fig. 4.8a). The most frequent caliper size class was 1.51–2.0mm (Fig. 4.8b). Girdling induced by *T. albidosparsus* at CJV occurred more frequently on GS (Fig. 4.8c), and the most frequent caliper size class was 1.51–2.0mm (Fig. 4.8d).

Occurrence of eggs of *St. basalis* was discovered on woody perennial plants in unmanaged habitat at CRV and YV in 2017 and 2018 (Table 4.5). In all cases, eggs were observed behind resting buds or under bud scales. At both sites, eggs were found primarily on oak branches, while at YV sizeable numbers were also found on hawthorn and apple wood. Egg distance from the apical tip of collected wood was recorded on *Malus* spp., *Crataegus* spp., and *Quercus* spp. (Fig. 4.9). Using ANOVA, a highly significant difference was found at YV locations of oviposition sites when comparing host genus ($F_{2,115}$=11.65, *P*<0.001). Distances of oviposition sites of oak were 66.9mm (CI 30.0–103.9mm) nearer the shoot tip than oviposition sites of apple, and oviposition sites on hawthorn were 46.6mm (CI 2.8–137.8mm) nearer the shoot tip than on seedling plum. Oviposition site locations of hawthorn were not significantly different from oviposition site locations of oak.

Numbers of eggs per infested bud (mean 1.21±0.05 eggs per bud, 95% CI of the mean 1.12–1.31 eggs per bud) were recorded for the host genera *Crataegus*, *Malus*, *Prunus*, and *Quercus*. One-way ANOVA found no significant difference in the mean

number of eggs per bud between genera ($F_{3,117}$=2.050, P=0.111). The majority (82.6%) of infested buds contained a single egg.

DISCUSSION

Field observations of treehopper populations inhabiting vegetation in proximity to wine grape vineyards were conducted in the Willamette Valley and in southern Oregon in 2017 and 2018. All life stages of *St. basalis* were found in a vineyard with high incidence of GRBV infection. Nymphs and adults of *T. albidosparsus* were found in both regions. Resident populations of *Sp. festinus* and *St. bisonia* were additionally discovered at a southern Oregon vineyard block that had been previously removed due to an alarming rate of infection by GRBV (Dalton et al. 2019). The close taxonomic connections between these species must be carefully considered in the context of GRBV epidemiology due to the putative ability of *Sp. festinus* to transmit RBD. While life stages of *Sp. festinus* were not able to persist on *V. vinifera* alone (Preto et al. 2018b), all life stages of *St. basalis* were recorded on *V. vinifera* in the presence of infected plants.

In the current study, oak trees appeared to be the predominant overwintering hosts of *St. basalis*. Approximately 5% of *Q. garryana* and *Q. rubra* buds contained eggs. Smiliine treehoppers are known to be generally associated with oak species (Wallace 2014, Deitz et al. 2012), not only in temperate areas of North America, but also extending to Central America (McKamey 2008). Thus, considering their reproductive capacity on oak, it is probable that *St. basalis* are oak specialists. At YV, eggs were found on apple in about 3% of examined buds, and in hawthorn and re-sprouting grapevine canes at about 1% frequency. Current season eggs were found only on 1st or 2nd year woody growth, while old, hatched egg remnants were occasionally found on older wood. It is clear that *St. basalis* are capable of reproducing on many woody species. Feeding of immature insects was primarily observed on herbaceous *Vicia* spp. in late spring to early summer and *Daucus* spp. during mid-summer. Both wild carrot and vetch are natural components of oak woodland habitats in Oregon (Franklin and Dyrness 1973) and are widely distributed across the region.

Scouting of known host vegetation for nymphs through hand collection of host plants was efficient. Surveys for adult insects were concentrated on the vineyard

canopy. While adults were occasionally found on woody plants outside the vineyard blocks, the heterogeneous structure of unmanaged habitat made systematic collections difficult. However, their presence in the landscape is undeniable, as eggs were found on *M. pumila* and *Q. garryana* in August. Systematic deployment of yellow sticky cards in unmanaged habitat or edge rows may be an effective approach to determine whether a site has a local population of treehoppers (Wallace and Troyano 2006). In the current work, we report the results of a systematic survey using sticky cards to indicate adult flight of *T. albidosparsus*.

Treehopper feeding damage, as manifested through leaf or shoot discoloration distal to girdling points, was observed at YV on woody and herbaceous hosts. In this study, reported field girdling incidence should be considered a conservative estimate, since only complete girdles were considered (Preto et al. 2019). The highest incidence of feeding damage occurred at YV in 2017. This could be an artifact of sampling intensity, as the greatest number of girdling surveys occurred during that season. Girdled leaves, which are in a state of senescence, will fall from an affected vine (Grosso et al. 2016). Infrequent surveys represent a snapshot of feeding damage because tissue that was previously damaged may have already dropped. As was shown in our girdling surveys, and in accordance with findings of treehopper oviposition in tree fruit crops (Sorenson 1928), most host plants near habitat containing treehoppers are subject to feeding activity. However, consistent with findings of Grosso et al. (2016), girdling activity at our sites was not apparent until late instar or adult stages appeared. Early instar treehoppers may not fully girdle host materials or may quickly fall from the overwintering host and encounter another suitable feeding host (Yothers 1934). The relative immobility of young instar stages suggests limited risk of early migration into the vineyard. Adult treehoppers that immigrate from surrounding habitat will not likely disperse great distances into a vineyard. This may be due to the structure of trellised grapevines, as adult treehoppers tend to stay on suitable host plants with little movement if left undisturbed (Dennis 1964). Dormant season pruning of the previous season's grapevine cane growth or hedging the vineyard rows in late summer could further eliminate treehopper eggs within vineyard blocks.

Various approaches may be available to manage treehopper populations in a vineyard. The juvenile stage is likely the weak link of the treehopper life cycle, as mobility of the insect is restricted. Controlling herbaceous vegetation near production blocks could be a key management tactic to controlling populations of treehoppers, but precise measures remain to be tested. Such strategies may include limitation of habitat for insect juvenile stages. It is possible that management of treehopper populations could include chemical control of weeds (Knowles et al. 1999) or mowing, although the latter technique may not effectively control multivoltine species such as *Sp. festinus* (Preto et al. 2019). Timing and implementation of these approaches should be evaluated as a possible next step to controlling treehopper populations. Adult treehopper reproductive activity, which is mediated by vibrational communication, could be interrupted through playback of vibrational mating signals (Hunt 1993). This approach has been applied to discourage mating of leafhoppers in vineyards, providing indirect control against phytoplasmas (Polajnar et al. 2016). To date, the mating call of *St. basalis* has been identified (R. Nieri, *personal communication*), and further work should be conducted to evaluate a mass-trapping or vibrational mating disruption system.

ACKNOWLEDGEMENTS

Table 4.1 Counts of *Spissistilus festinus*, *Stictocephala basalis*, *Stictocephala bisonia*, and *Tortistilus albidosparsus* collected from Oregon study sites from 2016–2018. Life stages are: E=egg; N=nymph; A=adult. Weather stations are: CVO=Corvallis Municipal Airport; MMV=McMinnville Municipal Airport; 3S4=Illinois Valley Airport; MFR=Rogue Valley International Airport.

Site	County	Weather station	Type	Species	Life stage	2016	2017	2018	Total
CJV	Josephine	3S4	commercial vineyard	*T. albidosparsus*	E	0	0	0	0
					N	0	14	10	24
					A	19	180	92	291
CP	Benton	CVO	natural area	*St. basalis*	E	-	-	0	0
					N	-	-	59	59
					A	-	-	1	1
CRV	Benton	CVO	research vineyard	*St. basalis*	E	-	55	21	76
					N	-	0	8	8
					A	-	0	0	0
CV	Yamhill	MMV	commercial vineyard	*St. basalis*	E	-	-	0	0
					N	-	-	7	7
					A	-	-	2	2
CV	Yamhill	MMV	commercial vineyard	*T. albidosparsus*	E	-	-	0	0
					N	-	-	14	14
					A	-	-	85	85
EPV	Jackson	MFR	commercial vineyard	*T. albidosparsus*	E	-	0	0	0
					N	-	0	1	1
					A	-	65	253	318
JV	Jackson	MFR	commercial vineyard	*Sp. festinus*	E	0	0	0	0
					N	0	0	0	0
					A	1	11	33	45
JV	Jackson	MFR	commercial vineyard	*St. bisonia*	E	0	0	0	0
					N	0	0	0	0
					A	1	0	3	4
JV	Jackson	MFR	commercial vineyard	*T. albidosparsus*	E	0	0	0	0
					N	0	0	0	0
					A	0	1	6	7
LV2	Yamhill	MMV	commercial vineyard	*St. basalis*	E	-	-	0	0
					N	-	-	5	5
					A	-	-	0	0
YV	Yamhill	MMV	commercial vineyard	*St. basalis*	E	0	134	147	281
					N	0	205	285	490
					A	20	70	23	113

Table 4.2 Field sampling dates of *Stictocephala basalis* in Willamette Valley, Oregon study sites. CP=natural area in Corvallis; CRV=vineyard near Alpine; CV=vineyard near Carlton; KVV=vineyard near Kings Valley; LV1 and LV2=vineyards near Lafayette; and YV=vineyard near Yamhill. Collected life stages are indicated with an X. Degree-days (DD) were calculated by applying a 10 °C low base temperature and a 30 °C high cutoff temperature to weather data (Pruess 1983).

Table 4.2

Year	Site	Week	Julian Date	Cumulative DD (°C)	Eggs	Immatures	Adults	Collection method
2017	YV	16	111	30.7	X			cuttings
	YV	18	125	65.3	X			cuttings
	YV	20	139	97.8	X			cuttings
	YV	22	153	198.3	X	X		cuttings
	YV	25	173	320.7		X		hand
	YV	27	187	458.3		X		vacuum
	YV	29	201	582.7		X	X	vacuum
	YV	30	209	668.5		X	X	vacuum; hand
	YV	32	222	817.0		X	X	hand
	YV	34	236	954.4			X	hand
	YV	36	250	1107.1			X	hand
	YV	38	264	1203.1			X	hand
	YV	40	276	1271.2	X		X	cuttings
	CRV	40	278	1322.9	X			cuttings
2018	YV	14	92	15.8	X			cuttings
	CRV	15	100	22.2	X			cuttings
	KVV	17	113	32.3				cuttings
	YV	17	114	41.4	X			cuttings
	YV	20	135	126.2	X			cuttings
	YV	22	149	198.8	X			cuttings
	CRV	23	158	241.3		X		hand
	YV	24	163	255.1		X		hand
	YV	26	177	362.4		X		hand
	CV	26	178	370.7		X		hand
	LV1	26	178	370.7				hand
	CP	28	190	482.0		X		hand
	CV	28	191	474.8		X	X	hand
	YV	28	191	474.8		X	X	vacuum; hand
	LV2	28	193	495.8		X		hand
	CP	28	194	527.1		X		hand
	CV	30	205	615.2			X	hand
	YV	30	205	615.2		X	X	vacuum; hand
	CP	30	206	656.5		X	X	hand
	CV	32	221	787.6			X	hand
	CV	34	235	925.3			X	cuttings; hand
	YV	34	235	925.3	X		X	cuttings; hand
	CV	35	241	970.1			X	hand
	YV	36	247	1013.3			X	hand
	YV	39	268	1120.8			X	hand
	YV	41	282	1179.4			X	hand

Table 4.3 Field sampling dates of *Tortistilus albidosparsus* at a Josephine County, Oregon vineyard in 2017–2018. Collected life stages are indicated with an X. Degree-days (DD) were calculated by applying a 10 °C low base temperature and a 30 °C high cutoff temperature to weather data (Pruess 1983).

Year	Week	Julian Date	Cumulative DD (°C)	Eggs	Immatures	Adults	Collection method
2017	20	138	103.6		X		vacuum
	22	152	214.4		X		vacuum
	26	178	410.8		X		vacuum
	27	187	500.6			X	beat sheet
	28	195	584.2			X	hand
	29	202	648.9			X	hand, sticky card
	30	208	726.7			X	sticky card
	31	215	806.9			X	hand, sticky card
	33	227	941.7			X	hand, sticky card
	34	235	1026.4			X	sticky card
	35	243	1101.4			X	sticky card
	36	250	1173.9			X	sticky card
	37	257	1247.2			X	sticky card
	39	272	1319.3			X	sticky card
2018	18	121	49.4		X		vacuum
	20	135	119.4		X		vacuum
	24	166	302.8		X		vacuum
	26	179	421.7			X	hand; sweep net
	28	193	559.2			X	hand, sticky card
	29	197	609.2			X	hand, sticky card
	30	204	683.6			X	hand, sticky card
	32	220	847.8			X	hand, sticky card
	34	236	1002.5			X	hand, sticky card
	39	271	1236.9			X	sticky card

Table 4.4 Spatial analysis of girdling activity at a Josephine County, Oregon vineyard (CJV) and a Yamhill County, Oregon vineyard southwest block rows 0–2 (SW 0–2), 3–21 (SW 3–21), and west block rows 45–46 (West). Indices generated using Spatial Analysis by Distance IndicEs (Perry 1995) are: I_a=index of aggregation; $\bar{V}j$=mean index of gap strength; $\bar{V}i$=mean index of patch strength; D=distance to regularity. Values are significant when $P_a < 0.05$.

Block	Year	N	Mean ± Var	I_a	$\bar{V}j$	$\bar{V}i$	D	P_a
CJV	2016	1184	0.579 ± 5.255	2.643	-2.645	2.792	5999.92	<0.001
SW 0–2	2016	98	0.265 ± 0.259	0.789	-0.818	0.8	44	0.662
SW 0–2	2018	98	0.102 ± 0.093	1.879	-1.917	2.015	62.07	0.03
SW 3–21	2016	860	0.098 ± 0.093	3.316	-3.431	3.223	929.3	<0.001
SW 3–21	2018	894	0.066 ± 0.084	1.451	-1.503	1.322	383	0.059
West	2016	124	0.492 ± 0.252	1.903	-1.878	1.892	223.1	0.036
West	2017	124	2.218 ± 5.684	0.713	-0.736	0.641	391.5	0.736
West	2018	124	0.750 ± 1.116	0.733	-0.72	0.659	244	0.718

Table 4.5 Life stages of *Stictocephala basalis* collected in 2017–2018 from host plant genera in Willamette Valley study sites. 1st–5th indicate collected instar stages.

Host genus	Buds	Buds with unhatched eggs	Eggs	1st	2nd	3rd	4th	5th	Adults
Amelanchier	51	1	1	0	0	0	0	0	0
Cirsium	8	0	0	0	0	1	0	0	0
Crataegus	2,227	20	20	0	0	0	0	0	6
Daucus	0	0	0	5	39	50	87	94	4
Geranium	0	0	0	0	1	2	1	0	0
Hypericum	0	0	0	0	1	5	1	0	0
Hypochaera	0	0	0	1	0	0	0	0	0
Malus	2,821	85	110	3	0	0	0	0	2
multiple[†]	0	0	0	1	18	42	33	40	10
Oemleria	51	0	0	0	0	0	0	0	0
Philadelphus	45	3	3	0	0	0	0	0	0
Prunus	2,934	7	7	0	0	0	0	0	3
Quercus	3,079	159	213	0	3	0	0	0	0
Rosa	664	7	8	1	0	0	0	0	0
Rubus	88	0	0	2	2	1	4	1	14
Toxicodendron	0	0	0	0	0	0	0	1	0
Vicia	0	0	0	13	47	14	1	3	0
Vitis	669	7	8	2	7	16	10	16	57

[†] unable to determine due to bulk collection method
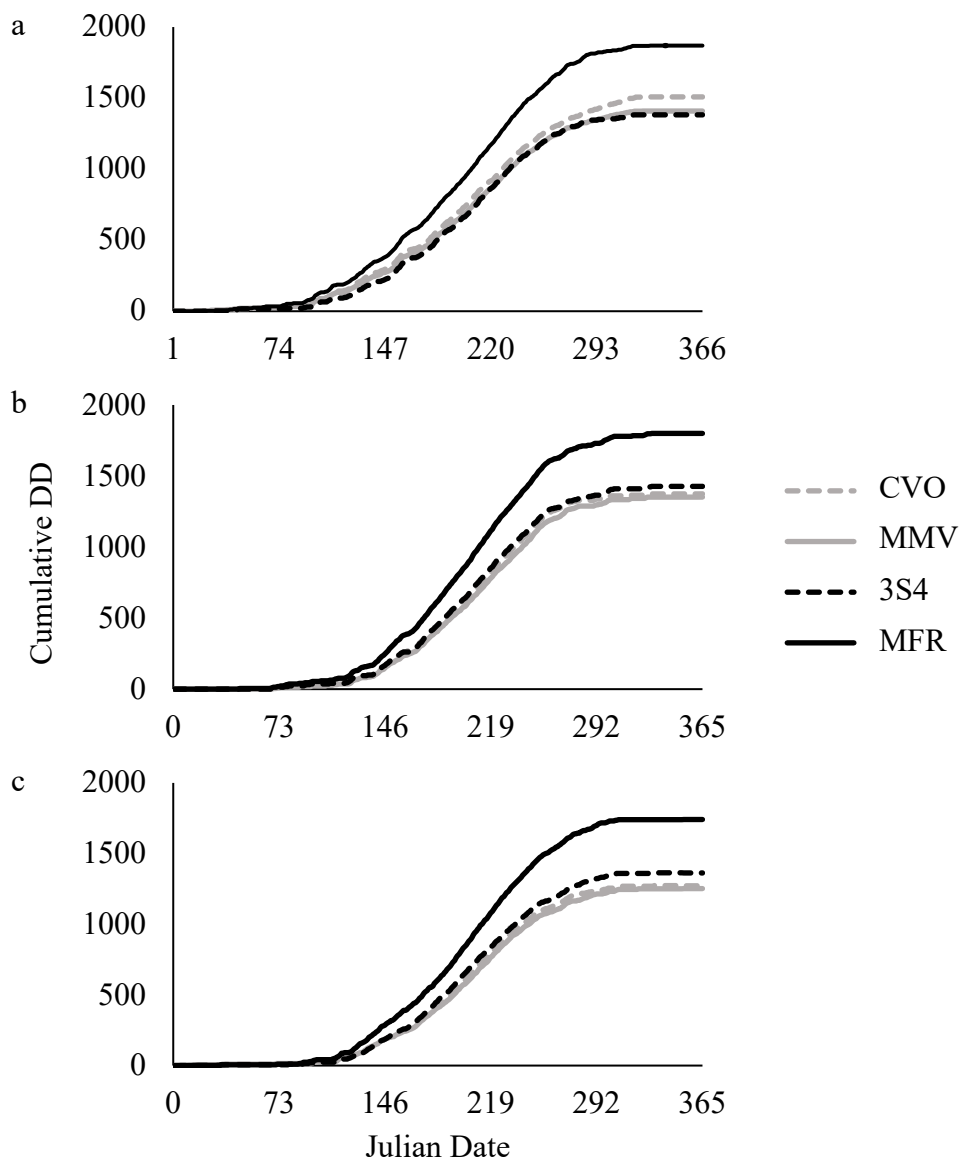
Figure 4.1 Field heat accumulation at weather stations in proximity to sampling sites in (a) 2016; (b) 2017; (c) 2018. Degree-days (DD) were calculated using a 10 °C base temperature and upper cutoff threshold of 30 °C (Pruess 1983). Weather stations abbreviated as follows: CVO=Corvallis Municipal Airport; MMV=McMinnville Municipal Airport; 3S4=Illinois Valley Airport; MFR=Rogue Valley International Airport.
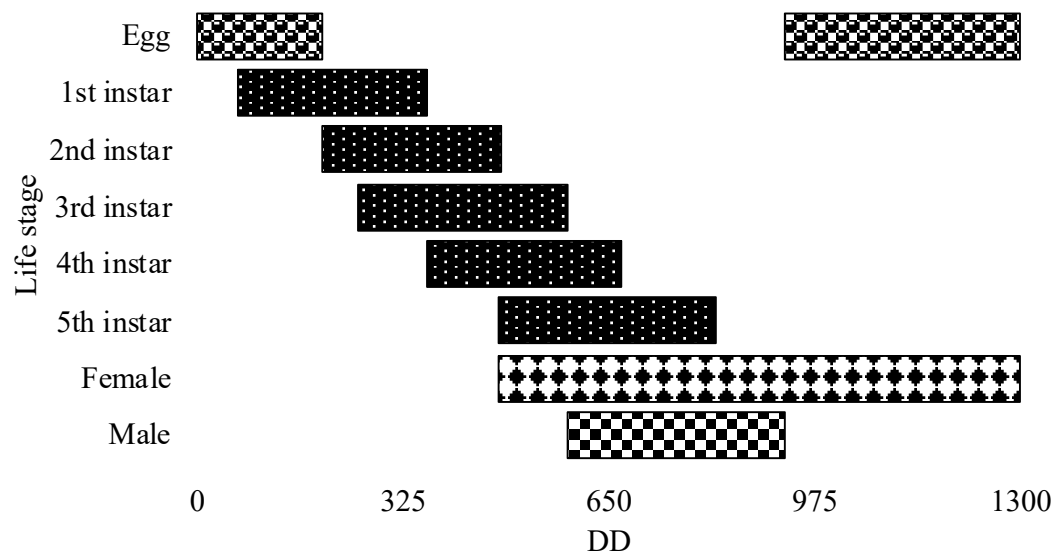
Figure 4.2 Phenological progression of *Stictocephala basalis* life stages at a vineyard near Yamhill, Oregon in 2017 and 2018. Cumulative degree-day (DD) calculations began on 1 January using a 10 °C low base temperature and a 30 °C high cutoff temperature (Pruess 1983).
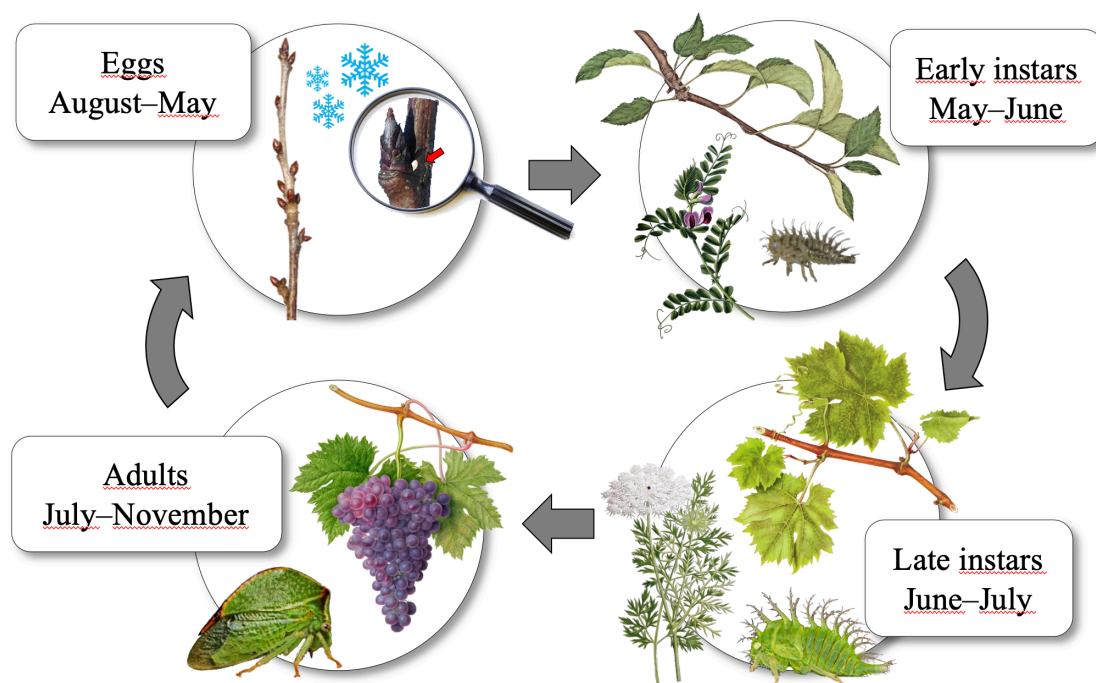
Figure 4.3 Current understanding of the life cycle of *Stictocephala basalis* based on insect collections from a vineyard near Yamhill, Oregon. Eggs overwinter behind dormant buds of woody hosts. Early instar stages drop onto succulent understory vegetation. Late instar stages migrate to drought-hardy vegetation. Adults emerge and fly onto woody hosts for reproduction after hardening of the current season's growth.
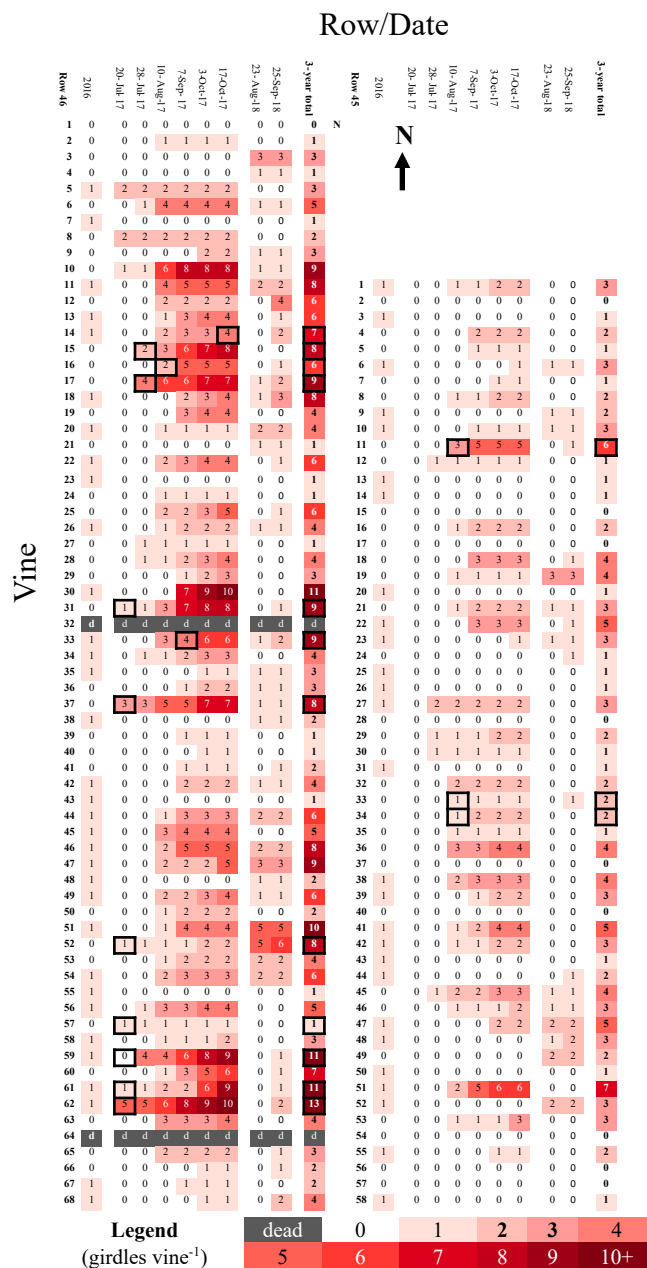
Figure 4.4 Spatial distribution and incidence of girdled tissues of *Vitis vinifera* 'Pinot noir' clone 667 in west block rows 46 and 45 at a Yamhill County, Oregon vineyard. 2016 data indicate presence (=1) or absence (=0) of girdled tissues. Boxes show vines from which adult or immature treehoppers were collected. Map not drawn to scale.
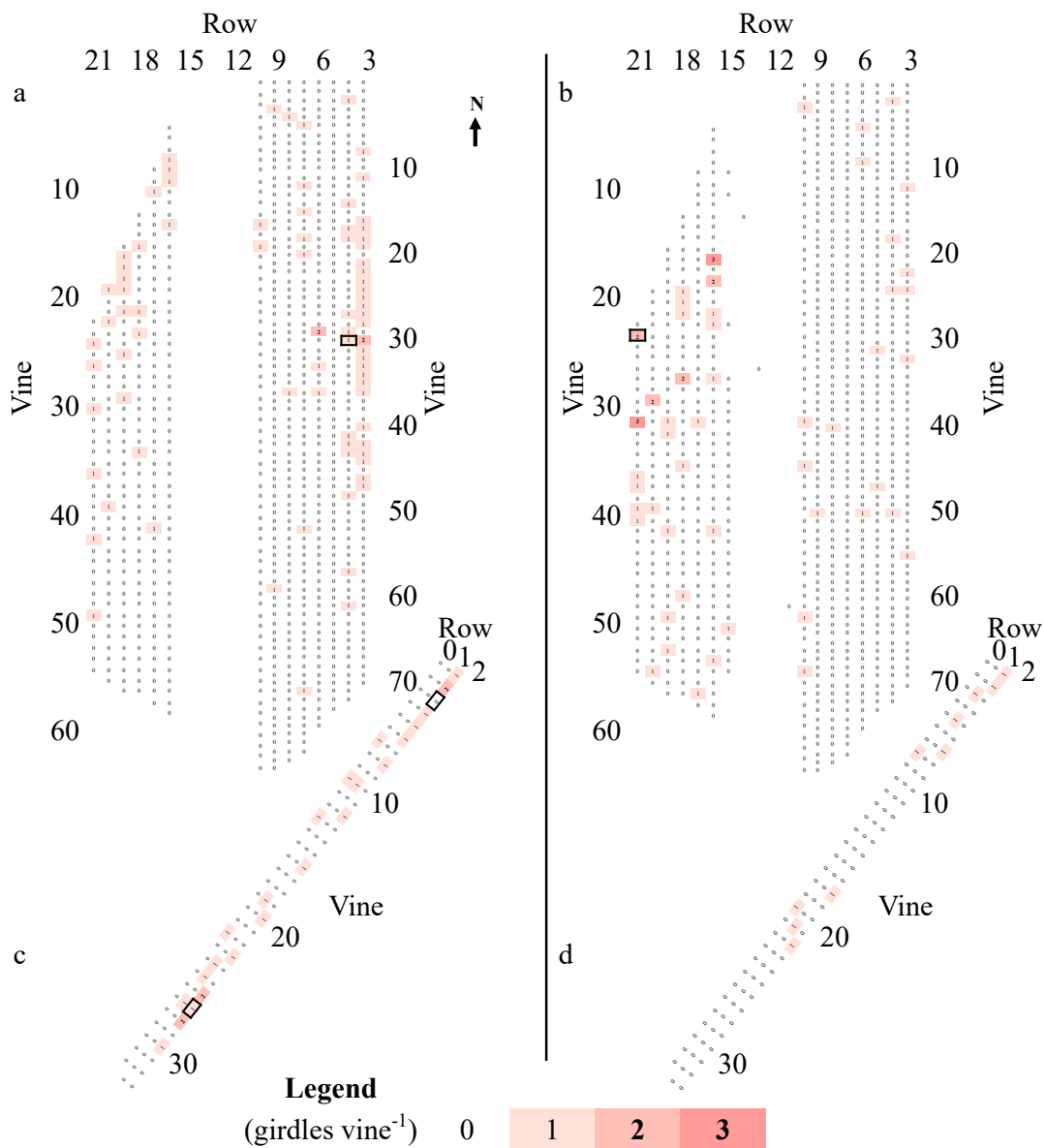
Figure 4.5 Spatial distribution and incidence of girdled tissues of *Vitis vinifera* 'Pinot noir' clone 667 at a Yamhill County, Oregon vineyard in (a) southwest block rows 3–21 in 2016; (b) in 2018; and (c) southwest block rows 0–2 in 2016; (d) in 2018. Values show total number of girdles per vine for the respective year. Boxes show vines from which adult or immature treehoppers were collected. Map not drawn to scale.
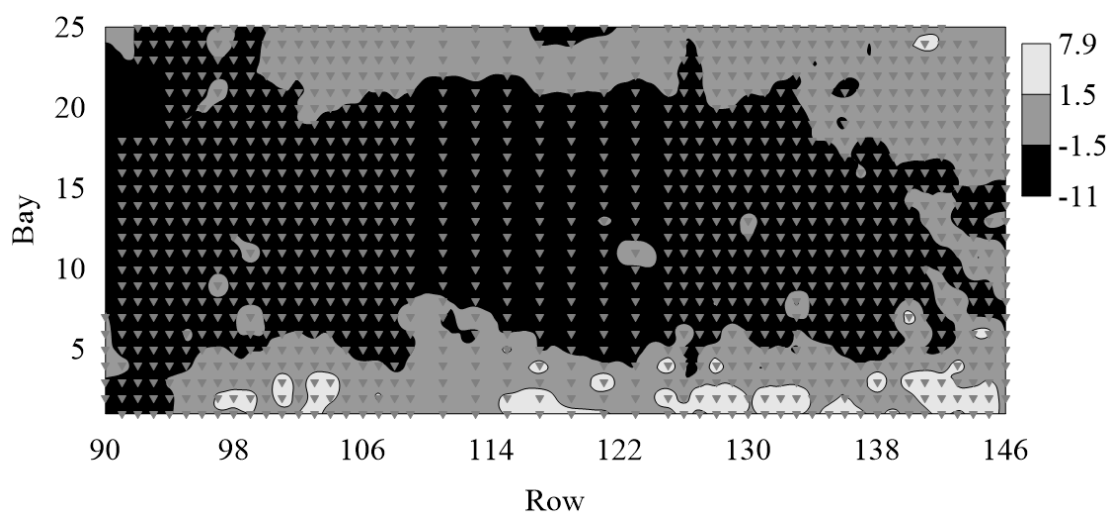
Figure 4.6 Spatial Analysis by Distance IndicEs (Perry 1995) of girdle distribution at a Josephine County, Oregon vineyard in 2016. Values >1.5 indicate significant patches of higher than average aggregations of girdles, relative to field total. Values <-1.5 indicate significant gaps of lower than average aggregations of girdles. Triangles show relative location of bays. Map not drawn to scale.

a

$y = 1/(1+\exp(0.393x-1.528))$
$R^2 = 0.898$
$F_{(1,28)} = 170.6$
$P < 0.001$

b
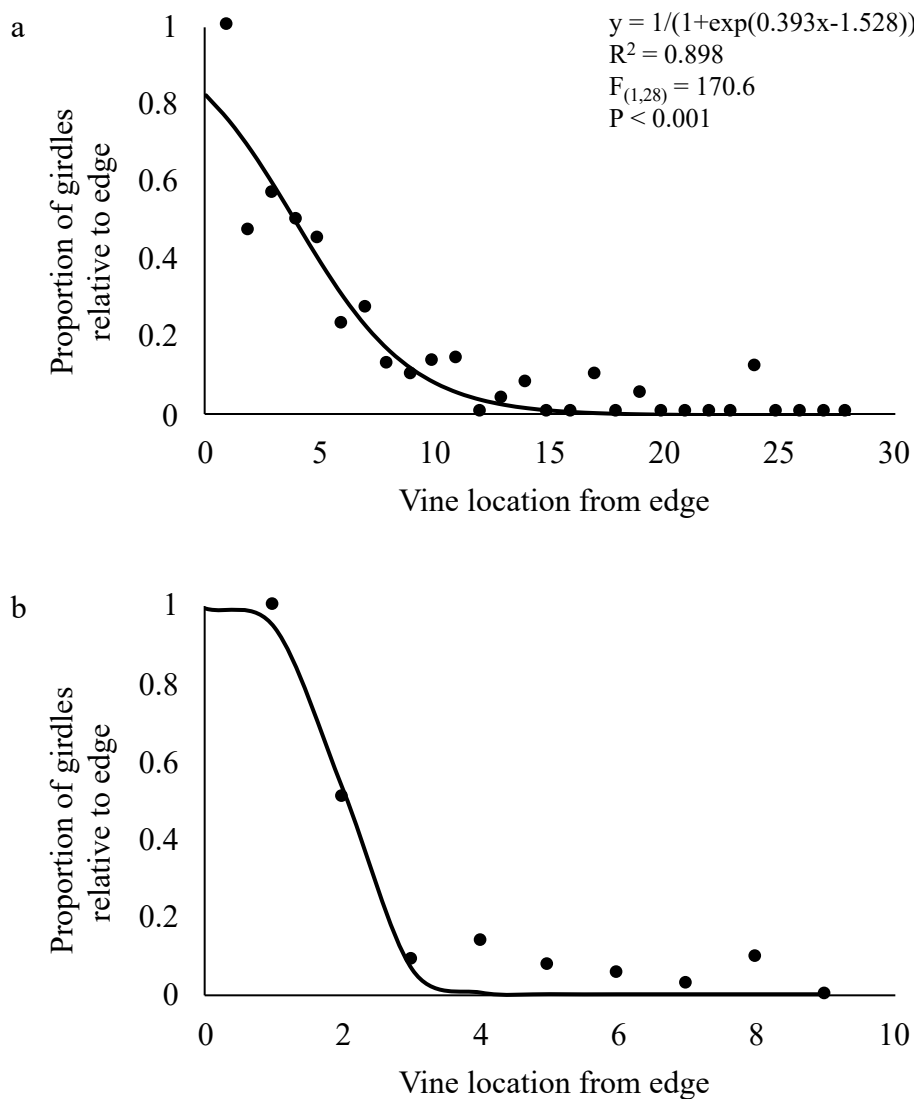
Figure 4.7 Proportion of girdles found on *Vitis vinifera* relative to edge locations of (a) a vineyard block at a Josephine County, Oregon vineyard in 2016; (b) a Yamhill County, Oregon vineyard from 2016–2018. Value of 1 is the standardized proportion of girdles found at the edge of the block.
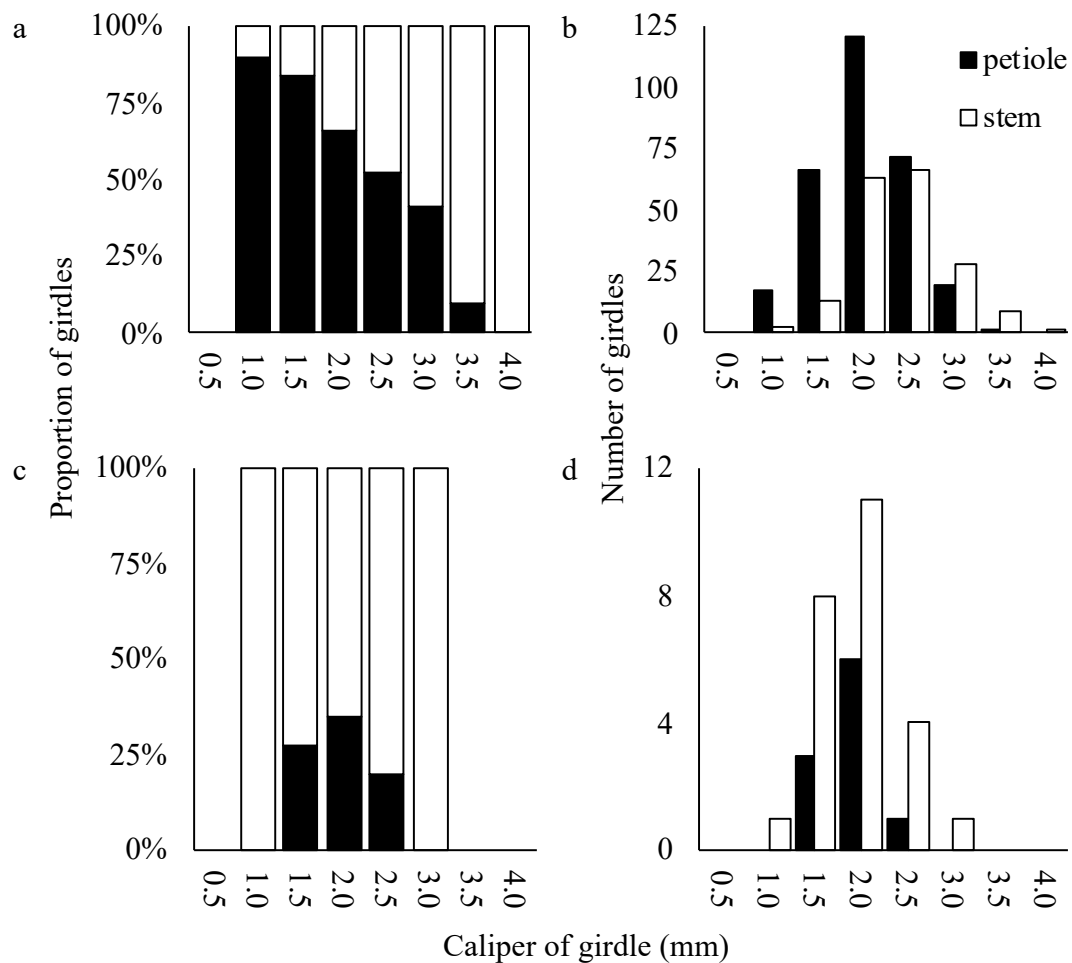
Figure 4.8 Caliper of girdles collected from a Yamhill County, Oregon vineyard as (a) proportion of total girdles; (b) total girdles; from a Josephine County, Oregon vineyard as (c) proportion of total girdles; (d) total girdles.
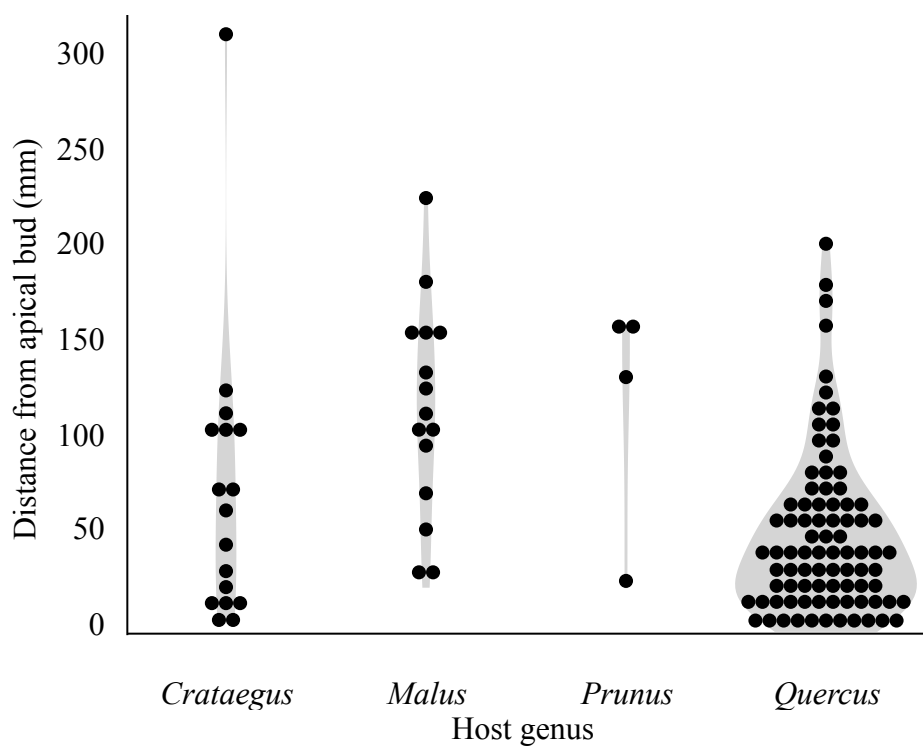
Figure 4.9 Distance from shoot tip of eggs deposited by *Stictocephala basalis* onto woody hosts at a Yamhill County, Oregon vineyard. Gray areas indicate distribution and relative quantities of eggs found at corresponding distances. Black dots within shaded areas are raw data measurements.

CHAPTER 5

Evaluation of Grapevine red blotch virus transmission using populations of Oregon treehoppers (Hemiptera: Membracidae)

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ABSTRACT

Controlled greenhouse and laboratory infestation trials were conducted to determine the ability of treehopper populations to transmit grapevine red blotch virus (GRBV). Collections of live *Spissistilus festinus* (Say), *Stictocephala basalis* (Walker), and *Tortistilus albidosparsus* (Stål) (Hemiptera: Membracidae) were made in proximity to commercial vineyards of *Vitis vinifera* L. in Oregon, USA. Adult insects were used in GRBV transmission trials conducted from 2016–2018, and immature insects of *St. basalis* were additionally used in 2018. Insects were caged individually or in groups of 5 individuals per plant onto GRBV-infected inoculum source materials for acquisition access periods ranging from 2d–6d, then transferred to uninfected *V. vinifera* 'Pinot noir' clones for inoculation access periods (IAP) of 1d–7d. Species *St. basalis* and *T. albidosparsus* were not observed to transmit GRBV to uninfected vines. One male *Sp. festinus* successfully transmitted GRBV, as determined by quantitative PCR (qPCR) and droplet digital PCR, to a single vine. A positive test of petiole tissue from a leaf basal to the feeding leaf occurred following a 24-hour IAP. Testing of individual insects using qPCR revealed that GRBV can persist inside the bodies of all three treehopper species; however, a greater proportion of *Sp. festinus* successfully tested positive for GRBV compared to *St. basalis* or *T. albidosparsus*. Nymphs of *St. basalis* were capable of ingesting virus. Results showed no evidence following successive years of testing that GRBV can be transmitted by source populations of *St. basalis* or *T. albidosparsus*. A single case of transmission of GRBV by *Sp. festinus* was observed.

Key words: wine grape, grapevine red blotch virus, droplet digital PCR, threecornered alfalfa hopper

INTRODUCTION

Disease and pest management programs are integral components of grapevine (*Vitis vinifera* L., Vitales: Vitaceae) cultivation in the United States. Several viruses infecting grapevine are of economic importance, many of which are transmitted by insects or nematodes (Maliogka et al. 2015). Insect species *Planococcus ficus* (Signoret), *Pseudococcus maritimus* (Ehrhorn), and *Pseudococcus longispinus* (Targioni-Tozzetti) (Hemiptera: Pseudococcidae) are indirect pests that are capable of transmitting

grapevine leafroll-associated viruses (GLRaV), the putative causative agents of grapevine leafroll disease (GLD) (Walton et al. 2004, Tsai et al. 2010). Perhaps the most serious viral disease affecting wine grape production to date, GLD causes considerable changes to fruit characteristics from infected vines (Atallah et al. 2012, Naidu et al. 2014). Pathogens including *Xylella fastidiosa*, transmitted by sap-feeding leafhoppers (Hemiptera: Cicadellidae), or root infestations by *Daktulosphaira vitifoliae* (Fitch) (Hemiptera: Phylloxeridae) cause decline of grapevines, eventually resulting in vine death in the absence of intervention measures (Baldi and La Porta 2017, Fisher et al. 2003). Direct damage is inflicted on grapevines through infestation of leaves, stems, and fruits by insects and mites (Fisher et al. 2003, Duso et al. 2012, Pfeiffer et al. 2012). Additional insect species have been traditionally considered as incidental or occasional pests to wine grape production, including treehopper species *Spissistilus festinus* (Say) (Hemiptera: Membracidae: Smiliinae) (Fisher et al. 2003).

In 2008, grapevines affected with GLD-like symptoms, but testing negative for strains of GLRaV, were discovered at a University of California research vineyard (Calvi 2011, Sudarshana et al. 2015). Genomic sequencing of grapevine tissues from California and New York vineyards indicated that a sequence of single-stranded circular DNA most closely aligned with the virus family *Geminiviridae* was present in samples from both locations (Al Rwahnih et al. 2013, Krenz et al. 2014). *Grapevine red blotch virus* (GRBV) was subsequently classified under the new genus *Grablovirus* (Varsani et al. 2017) and was revealed to be the causative agent of red blotch disease (RBD) (Yepes et al. 2018). Secondary spread of RBD occurred in Oregon and California vineyards, but not in New York (Dalton et al. 2019, Cieniewicz et al. 2017b, 2019), indicating the potential presence of one or more species of insects present along the Pacific Coast of North America that could transmit GRBV.

Secondary spread of RBD in California and Oregon raises important epidemiological questions. While leafhopper and treehopper species were implicated as vectors of GRBV in greenhouse studies (Poojari et al. 2013, Bahder et al. 2016b), the role of an insect vector has not been confirmed in the field. However, the epidemiology of *Tomato pseudo-curlytop virus* (*Geminiviridae*, *Topocuvirus*) was found to include feeding on solanaceous plant species by *Micrutalis malleifera* Fowler

(Hemiptera: Membracidae: Smiliinae) (Simons 1962). Several insects, including *Sp. festinus*, were positively associated with the spread of GRBV in at least one commercial vineyard in California (Cieniewicz et al. 2018b). A field study on potential herbaceous and woody hosts of GRBV in California revealed that only wild grape, *Vitis californica* Benth. × *V. vinifera* was a natural host, despite transient presence of GRBV in *Rubus armeniacus* Focke (Bahder et al. 2016a). A laboratory study subsequently confirmed that understory plant species commonly found in commercial vineyards, including all tested legumes and certain herbaceous forbs, are suitable reproductive hosts for *Sp. festinus* (Preto et al. 2018a). Young, green grapevine tissues were further found to support *Sp. festinus* up to the second instar stage (Preto et al. 2018b). These findings hint at complex plant-insect dynamics that may drive epidemiology of RBD.

Results from previous greenhouse and field studies implicated a membracid insect as the most likely vector of GRBV (Bahder et al. 2016b, Cieniewicz et al. 2018b). From 2016–2018, multiple smiliine treehopper species were found in a natural area and in vineyards of southern Oregon and the Willamette Valley, Oregon, including *Stictocephala bisonia* (Kopp & Yonke), *Stictocephala basalis* (Walker), *Sp. festinus*, and *Tortistilus albidosparsus* (Stål) (Dalton et al. 2020, Stowasser et al. 2020). Some vineyard sites were dramatically affected by GRBV (Dalton et al. 2019). While *Sp. festinus* were found in low numbers in southern Oregon, their apparent absence in the Willamette Valley suggested that one or more related treehopper species may transmit GRBV.

The goal of the current study was to test whether treehopper populations sourced from viticultural regions of Oregon have the capacity to transmit GRBV from GRBV-infected inoculum source materials to GRBV-free grapevines. Controlled transmission biology experiments were conducted from 2016–2018 in the greenhouse or laboratory. Treatment grapevines were tested annually to determine whether Oregon populations of membracid species *Sp. festinus*, *St. basalis*, and *T. albidosparsus* could transmit GRBV.

MATERIALS AND METHODS

Greenhouse and laboratory-based transmission bioassays were conducted from 2016–2018. The 2016 greenhouse GRBV transmission bioassay utilized adults of *St. basalis*

and *T. albidosparsus*. A similar test was conducted in the greenhouse in 2017 using adults of *St. basalis* and *T. albidosparsus*, but with modified methodology (2017 greenhouse GRBV transmission bioassay). Immature 3rd- or 4th-instar *St. basalis* nymphs were used in 2018 for a greenhouse GRBV transmission bioassay (2018 greenhouse GRBV transmission bioassay). Laboratory tests investigating the immediate migration of GRBV within *V. vinifera* occurred in 2018 using adults of *Sp. festinus*, *St. basalis* and *T. albidosparsus* (2018 laboratory GRBV transmission bioassay).

Plant sources. Plant materials were obtained from commercial nurseries for the greenhouse and laboratory tests. In 2016, *V. vinifera* 'Pinot noir' clone Pommard on 3309 rootstock (hereafter Pommard vines) were purchased, and in 2017, *V. vinifera* 'Pinot noir' clone 828 on Schwarzmann rootstock (Schwarzmann vines) were obtained through donation. Self-rooted *V. vinifera* 'Pinot noir' clone Wädenswil (Wädenswil vines) were additionally donated in 2018. All commercially-sourced vine materials tested negative for GRBV prior to use in transmission bioassays. Inoculum source materials were obtained from vines at a vineyard near Yamhill (YV), Yamhill County, that had previously tested positive for GRBV infection and expressed symptoms of RBD.

All grapevines were provided approximately 10g of 19-6-12 slow-release fertilizer (Osmocote® Smart-Release® Plant Food Plus, The Scotts Company LLC, Marysville, OH, USA) as necessary. All vines were maintained in the greenhouse, except for Pommard treatment vines and unused Schwarzmann vines that were maintained in an outdoor hoop house from 2018–2019. Vines were provided irrigation 3–5 times per week. No insecticides were applied to grapevines prior to the introduction of insects.

Collection of plant samples. Plant leaf tissues were collected from inoculum source materials, controls, and treatment vines from 2016–2019 following previously established methodologies (Dalton et al. 2019). Roots of vines from the 2016 greenhouse GRBV transmission bioassay and roots of treatment vines in the 2018 laboratory GRBV transmission bioassay were additionally collected. To sample roots, a plant was lifted from its container, and material was ripped from the soil medium by hand using an inverted plastic zippered bag. In the laboratory, the roots were submerged

into a 2L Pyrex beaker filled halfway with de-ionized water (dH$_2$O). The sample was shaken to dislodge particles of the soil medium, and roots were transferred to a second 2L Pyrex beaker to dislodge remaining soil. Beakers were decanted and rinsed with a jet of dH$_2$O for 45–60 seconds prior to taking the next sample. Washed roots were then stored in a new zippered plastic bag until DNA extraction using identical methodologies as leaf samples (Dalton et al. 2019). Fresh laboratory gloves were donned between processing of each sample. Data were not obtained for vines that were unavailable during periods of leaf collection.

Insect sources. Treehopper species were field-collected from multiple sites in Oregon for use in GRBV transmission bioassays. Adults of *St. basalis* were field-collected in 2016–2018 from YV, where spread of GRBV had been previously documented (Dalton et al. 2019). Immature *St. basalis* were collected from YV in 2017 and 2018 and maintained on *Pisum sativum* L. 'Oregon Trail' until emergence as adults or use in the 2018 greenhouse GRBV transmission bioassay. Eclosed adults were transferred to Schwarzmann vines prior to bioassays. A population of *T. albidosparsus*, collected as adults in 2016 and 2017 from a vineyard near Cave Junction (CJV), Josephine County, with no history of GRBV infection, was used in greenhouse GRBV transmission bioassays. Adults of *T. albidosparsus* were additionally collected from a vineyard with unknown GRBV infection history near Carlton (CV), Yamhill County, for use in the 2018 laboratory GRBV transmission bioassay. Live adults of *Sp. festinus* were collected in Jackson County vineyards and an alfalfa field for the 2018 laboratory GRBV transmission bioassay. One Jackson County site had a documented GRBV infection history (Dalton et al. 2019). Frozen individuals of *Sp. festinus* were additionally provided for testing in 2017 from a laboratory colony that had fed on a GRBV-infected *V. vinifera* 'Pinot noir' plant at University of California, Davis (UCD).

Collection of insect samples. All treehoppers from Oregon populations that were collected for molecular analysis were aspirated into scintillation vials containing 70% ethanol. In the 2017 greenhouse GRBV transmission bioassay, cohorts of 3–5 insects of *St. basalis* and *T. albidosparsus* were collected at random for analysis following the 1st–4th inoculation access periods (IAP), and two days after the conclusion of the 5th IAP. For comparison with the putative vector of GRBV, adults of *Sp. festinus* were

freeze-killed after an acquisition access period (AAP) of 6 days on a GRBV-infected plant at UCD. At the conclusion of the 2018 greenhouse GRBV transmission bioassay, multiple nymphs and one freshly emerged female *St. basalis* were collected for qPCR analysis. Adults and immature life stages of *St. basalis* that had survived the 2018 greenhouse GRBV transmission bioassay but later died on seedlings of herbaceous plants were individually collected. Adult *St. basalis* and *T. albidosparsus* that died during the AAP of the 2018 laboratory GRBV transmission bioassay were collected, and adult *Sp. festinus*, *St. basalis*, and *T. albidosparsus* were collected individually following the IAP of the 2018 laboratory GRBV transmission bioassay.

Insect weights. To weigh and surface-sterilize specimens collected in 2018, treehopper adults and nymphs were individually transferred using toothpicks from collection vials into microcentrifuge tubes containing a 1:4 bleach (10% v/v): dH$_2$O mixture. Specimens were subjected to intense vortexing for five seconds, tubes emptied and replenished with dH$_2$O immediately afterward, and specimens again vortexed for five seconds. Specimens were removed from the microcentrifuge tubes with fresh toothpicks, allowed to dry completely on fresh Kimwipe sheets, and transferred using toothpicks into new microcentrifuge tubes for homogenization. Weights of treehoppers were obtained using an analytical scale balance (Pioneer™ PA64, Ohaus Corporation, Parsippany, NJ, USA). Each microcentrifuge tube containing an individual dried specimen was weighed. After removal of specimens, all tubes were washed with dH$_2$O and allowed to dry completely in a fume hood. Each microcentrifuge tube was then reweighed, and the difference was taken as the weight of the dehydrated specimen. One specimen returned a negative weight value and was excluded from comparative analysis. Weights of control specimens of *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) that were never exposed to GRBV-infected plants were also excluded.

DNA extraction and analysis using quantitative PCR and droplet digital PCR. To prepare plant samples for laboratory analysis, petiole and root materials were diced into 1-mm thick segments, leaf blades were minced into approximately 4mm^2 pieces using ethanol- and flame-sterilized sharp scalpels and forceps, and DNA extraction occurred in guanidine thiocyanate buffer with an electric homogenizer (TissueLyser II, Qiagen, Hilden, Germany), using previously established methodologies (Dalton et al. 2019).

Nucleic acid extracts were additionally obtained in 2017 and 2018 from collected treehopper specimens.

To non-destructively extract DNA from treehopper specimens collected during the 2017 greenhouse GRBV transmission bioassay, a modified protocol of the extended proteinase and extended detergent (EPED) procedure (Bahder et al. 2015) was conducted. Adult treehoppers were placed individually into 2mL microcentrifuge tubes with 180μL ATL buffer and 20μL proteinase K. Tubes were placed into an orbital shaking incubator set at 25 rotations min^{-1} and 56 °C for 72 hours. DNA from the supernatant of each sample was quantified with a spectrophotometer (NANODROP ND-1000 v. 3.8, ThermoFisher Scientific, Waltham, MA, USA) and diluted with dH$_2$O into standardized concentrations of 4ng μL^{-1}. Because collections of additional voucher specimens from CJV and YV were easily obtained (Dalton et al. 2020), the fat body clearing step of the EPED protocol (Bahder et al. 2015) was not performed, and insect exoskeletons were discarded. No attempt to surface-sterilize specimens collected in 2017 was made. In 2018, insect tissue DNA was extracted using a commercial package (Blood and Tissue Kit, Qiagen, Hilden, Germany), employing the supplementary protocol for DNA extraction from insects. Insects were homogenized in phosphate buffered saline using an electric homogenizer (TissueLyser II, Qiagen, Hilden, Germany).

Most molecular tests for GRBV in plants and insects (~3,000 samples) were conducted using an Applied Biosystems 7500 Fast Real-Time PCR System quantitative PCR (qPCR) instrument (ThermoFisher Scientific, Waltham, MA, USA); however, in 2016, 101 samples were assessed using a QuantStudio 6 Flex Real-Time PCR machine (Thermo Scientific, Waltham, MA, USA). Primers GVGF1 and GVGR1, designed to amplify a novel sequence within the V2 region of the GRBV genome (Sudarshana et al. 2015, Al Rwahnih et al. 2013), were used to test all collected plant and insect tissues. Fast SYBR™ Green Master Mix (Applied Biosystems, Waltham, MA, USA) was used as the binding dye in all reactions for insect and plant sample analysis.

Criteria were applied to all qPCR assays to diagnose a GRBV-positive result. Assays resulting in cycling threshold (Ct) values exceeding 35, and samples in which the Ct value exceeded the negative control, were considered negative for GRBV. The

mean melting point value (Tm) was calculated from all positive control samples that were tested using the Applied Biosystems 7500 Fast Real-Time PCR System. Samples that were outside of 1 standard deviation of the mean Tm value (76.97 ± 0.67 °C) were considered GRBV-negative. Plates in which the negative controls did not perform as expected were re-tested, and the mean Tm value of each sample was then used to determine the qPCR result. An exception to these criteria was made for the 2018 greenhouse GRBV transmission bioassay, which occurred before the phenological onset of véraison of field-grown grape vines. In-plant distribution of GRBV is variable in early summer, resulting in a high rate of false negative tests (Setiono et al. 2018). For the early-summer transmission bioassay, all qPCR results in which there was a detectable Ct and matching Tm were considered positive for GRBV.

To confirm results of qPCR assays, treatment samples showing potentially low titer levels were additionally subjected to analysis using a QX200 droplet digital PCR (ddPCR) system (Bio-Rad Laboratories, Ltd., Hercules, CA, USA), following manufacturer instructions. Two dilutions of the DNA extract were employed: 1×10^0 (undiluted) and 1×10^{-2} (1% dilution). Three positive controls, three negative controls, and individual treatment plant samples were run in duplicate at each dilution in the ddPCR assay.

Statistical analysis. All statistical analyses comparing insect population differences were conducted using JMP (JMP Pro version 14.1.0, SAS Institute Inc. 2018, Cary, NC, USA). Goodness of fit was conducted using the Shapiro-Wilk W test for all data. Homogeneity of variance was assessed using Levene's test, followed by Welch's F-test in cases of unequal variance. Significance was determined for all statistical analyses at α=0.05. For the 2016 greenhouse GRBV transmission bioassay, one-way analysis of variance (ANOVA) was conducted to compare the effect of insect species on formation of tissue girdles. Longevity and number of girdles caused by *St. basalis* and *T. albidosparsus* were additionally assessed. For the 2017 greenhouse GRBV transmission bioassay, differences in mortality by species and by gender were determined by Chi-square analysis. Multi-factorial ANOVA was conducted comparing characteristics of girdles by plant tissue type and by insect species. In the 2018 greenhouse and laboratory GRBV transmission bioassays, weights of immature instar

stages and weights of treehopper species adults, respectively, were assessed using one-way ANOVA. Means ± SEM are reported unless otherwise specified.

2016 greenhouse GRBV transmission bioassay. In 2016, *T. albidosparsus* adults were collected at CJV in July and maintained on-site on *V. vinifera* 'Pinot noir' clone Dijon, or on *Pyrus communis* L. shoots at Oregon State University Southern Oregon Research and Extension Center (SOREC). Cuttings of inoculum source materials were placed in water to prevent desiccation of vascular tissue and enclosed inside a $60 \times 60 \times 60$ cm insect tent (Mega View, Taichung City, Taiwan). Insects (n=19) were placed individually onto the cuttings on 1 August and provided an AAP of 48 hours. Individuals were then transferred onto shoots of previously uninfested Pommard vines and provided an IAP of 48 hours. Insects were subsequently rotated individually onto previously uninfested Pommard vines for IAP of 7 days per plant until all insects had died. Pommard vines that never received insects were maintained as GRBV-negative controls. Organza netting was used to confine insects onto all host materials.

At YV, adult *St. basalis* were collected in August and September 2016 and were maintained prior to the experiment within an insect tent (Mega View, Taichung City, Taiwan) containing *P. communis* and *V. vinifera* 'Pinot noir' cuttings. Infestations of inoculum source material began with 4 adults on 2 September, 9 more adults were added on 12 September, and 7 additional adults were added on 15 September. All insects (n=20) were provided an AAP of 48 hours on fresh field-collected cuttings of *V. vinifera* 'Pinot noir' and then provided IAP rotations in a similar manner as described for *T. albidosparsus*.

Grapevines infested with either *T. albidosparsus* or *St. basalis* were observed three times per week for treehopper mortality and girdling activity until December 2016. Following transfer of insects, organza netting was replaced over the previously infested material. In 2016, leaves were collected for molecular analysis approximately 1-3 days prior to treatment dates and, when possible, prior to plant dormancy. Root materials were procured from all vines in February 2017, and leaf petiole samples were taken from available vines in August-October 2017-2019.

2017 greenhouse GRBV transmission bioassay. In August 2017, adults of *T. albidosparsus* collected from CJV were maintained on canes of *V. vinifera* 'Pinot noir'

clone Dijon at CJV or on young *P. communis* shoots at SOREC. The day prior to the AAP, *T. albidosparsus* adults (n=65) were delivered to the greenhouse on cuttings of host material. Immature and adult stages of *St. basalis* were collected from YV in spring and summer 2017, and nymphs were reared to the adult stage for use in the experiment. For the trial, 70 adults of *St. basalis* were used.

On 16 August, all insects were transferred inside a $60 \times 60 \times 120$ cm insect tent (Mega View, Taichung City, Taiwan) onto rooted cuttings of inoculum source material. Insects were provided a 6-day AAP in groups of 8–12 individuals of the same population before being transferred in groups of 5 insects per cohort onto previously uninfested Schwarzmann vines. Cohorts were rotated onto previously uninfested Schwarzmann vines at 7-day IAP intervals for five weeks. One cohort of each species per week was collected at random for qPCR analysis. For all other rotations, dead insects were transferred along with living specimens of a cohort onto previously uninfested Schwarzmann vines. Following the fifth IAP, surviving insects were placed with conspecifics onto previously uninfested Schwarzmann vines for two days, after which 4 *St. basalis* and 3 *T. albidosparsus* were collected at random for molecular analysis.

Plant tissue samples were collected from inoculum source, treatment, and negative control vines prior to the first IAP and from August–October in 2018 and 2019. In limited instances, the GRBV infection status of a potentially positive vine, as determined by qPCR analysis, was verified using ddPCR.

2018 greenhouse GRBV transmission bioassay. To compliment the greenhouse GRBV transmission bioassays conducted in 2016 and 2017, a greenhouse GRBV transmission bioassay was performed in early summer 2018 to test the ability of field-collected nymphs of *St. basalis* to transmit GRBV. On 28 June, nymphs were placed onto branches of self-rooted inoculum source vines in the greenhouse and were provided a 6-day AAP. Instar stages were estimated at the conclusion of the AAP according to Yothers (1934). Insects of the 3rd or 4th instar stages (n=150) were transferred using a fine-bristled paintbrush in cohorts of five insects per plant onto previously uninfested Schwarzmann or Wädenswil vines and provided an IAP of 7 days. In total, 30 vines were infested with *St. basalis*, and 30 uninfested negative

control vines were maintained. Leaves from treatment and Schwarzmann negative control vines were collected at the end of the AAP. Petioles of Wädenswil negative control vines were collected in September 2018. Leaf petioles were collected from inoculum source vines at the beginning and at the conclusion of the AAP, and survival of nymphs was quantified at the end of the IAP. Nymphs that had died and emergent adults were collected into 70% ethanol to test for GRBV using qPCR.

2018 laboratory GRBV transmission bioassay. To investigate the fate of GRBV in grapevines following treehopper feeding activity, laboratory tests using three treehopper species were conducted in fall 2018. Adult *St. basalis* and *T. albidosparsus* were placed in clip cages onto leaf petioles of field-grown GRBV-infected vines at YV. Clip cages were constructed of two 10mm lengths of 40mm diameter PVC pipe, each covered with storm window screening on one side and fitted with a foam ring on the other side, that were affixed to each other by using adjustable cable bands (Velcro USA Inc, Manchester, NH USA). The treatment leaf petiole was sandwiched between the foam, thereby creating an escape-proof breathable chamber for insects. In Willamette Valley locations, *Sp. festinus* had not been documented since 1980 (Dalton et al. 2020). To eliminate the possibility of introducing southern Oregon biotypes of *Sp. festinus* into Yamhill County vineyard sites, insects were secured in clip cages on fresh cuttings of GRBV-infected *V. vinifera* 'Pinot noir' in the laboratory.

All species were provided a 6-day AAP on infected inoculum source materials. Following the AAP, insects were transferred onto treatment vines individually within clip cages and provided a 24-hour or 72-hour IAP in the laboratory on Wädenswil or Schwarzmann vines. Trials using *St. basalis* and *T. albidosparsus* began on 29 August and ended on 5 September or 7 September, depending on IAP treatment, while the trial using *Sp. festinus* began on 26 September and ended on 3 October or 5 October.

At the conclusion of the IAP, plant tissues from seven areas of each test vine were harvested into zippered plastic bags within 30 minutes of removal of insects. The petiole section that was directly exposed to the insect was obtained by cutting with a double-sterilized scalpel where it entered and exited the clip cage. The petiole was pulled from the clip cage with sterile forceps, and the insect was aspirated from inside the chamber. The remainder of the feeding leaf petiole that was not exposed to the

insect was bulk-collected with the feeding leaf blade and constituted a single sample. The petioles of leaves directly above and below the feeding leaf were individually collected. The apical leaf petiole or top 2cm of shoot tip growth were collected, and lastly a root sample was taken. All tissue samples were analyzed using qPCR for a total of six tests per plant. DNA extracts from vine tissues that tested positive for GRBV, as assessed by qPCR analysis, were retested using ddPCR. Tissues from uninfested Schwarzmann and Wädenswil control vines were harvested at the same time as tissues from treatment vines, but only one leaf petiole was collected per vine. Due to limited numbers of available vines, four control vines from the August translocation bioassay were used as treatment vines in the September bioassay.

RESULTS

2016 greenhouse GRBV transmission bioassay. While most grapevine inoculum source materials tested positive for GRBV infection, no treatment or negative control vines developed a diagnostic GRBV infection from 2016–2019, as determined by qPCR analysis (Table 5.1). Of the inoculum source materials that were exposed to *St. basalis*, 11 out of 15 tests of leaf petiole tissue (73.3%) resulted in a positive reading. Of the inoculum source materials provided to *T. albidosparsus*, tests of petiole tissues resulted in a positive reading in 11 out of 19 assays (57.9% of vines).

In the 2016 greenhouse GRBV transmission bioassay, longevity of *St. basalis* averaged 32.7 ± 4.52 days, and longevity of *T. albidosparsus* averaged 39.0 ± 6.88 days. Assumptions of normality and equal variance were met for *St. basalis*; however, normality (W=0.875, P=0.018) and homogeneity of variance ($F_{1,37}$=4.875, P=0.034) of *T. albidosparsus* longevity were not met. As determined through a Welch's F-test, no significant difference in longevity was found between the two species ($F_{1,37}$=0.596, P=0.446).

2017 greenhouse GRBV transmission bioassay. Inoculum source material vines hosting only *St. basalis* (n=6), only *T. albidosparsus* (n=5), and both species concurrently (n=3) during the AAP were tested using qPCR for presence of GRBV. GRBV infection was identified in 12 out of 14 inoculum source material vines in 2017, but no treatment or negative control vines tested positive for GRBV from 2017–2019 (Table 5.2).

Of the 135 insects used in the 2017 greenhouse GRBV transmission bioassay, 10 insects were collected dead and 29 insects were collected alive for qPCR analysis, 51 insects died and were not tested using qPCR, 1 insect could not be found, and 44 insects survived the six-week study period. Seven surviving insects were collected for qPCR analysis two days after the conclusion of the experiment. Nine *Sp. festinus* adults from UCD were additionally subjected to qPCR analysis. Mortality of insects that were not collected for qPCR analysis was significantly different between species, with higher mortality of *T. albidosparsus* and lower mortality of *St. basalis*, compared to the overall average (Pearson's $X^2_{1,95}$=5.796, P=0.016; Fig. 5.1). No evidence of a difference in survival was found between males and females (Pearson's $X^2_{1,95}$=0.154, P=0.695).

Girdling of petioles and stems was observed on vines treated with *St. basalis* (Fig. 5.2a) and *T. albidosparsus* (Fig. 5.2b). Tissue type and insect species significantly affected girdling characteristics, but no interaction occurred between these two factors ($F_{2,112}$=39.182, P<0.001). Stem tissues affected by treehopper-induced girdles were 0.82mm larger in diameter than petiole tissues with girdles (95% confidence interval of the mean 0.63 to 1.02mm), and girdled tissues affected by *T. albidosparsus* were 0.36mm larger than tissues with girdles caused by *St. basalis* (95% confidence interval of the mean 0.11 to 0.57mm).

2018 greenhouse GRBV transmission bioassay. Virus assays conducted in 2018 and 2019 on grapevines that had been infested by *St. basalis* nymphs failed to identify GRBV infection in negative control vines or vines infested with *St. basalis* nymphs. Of the inoculum source material, 3 of 4 vines subjected to qPCR assays returned at least two samples with measurable Ct and a Tm value in line with the expected values (Fig. 5.3) By the conclusion of the IAP, 5 insects had died on Schwarzmann vines (6.7%), and 7 insects had died on Wädenswil vines (9.3%). Two adult male insects emerged on Wädenswil vines during the IAP.

Weights of *St. basalis* 3rd, 4th, and 5th instar stages were collected from nymphs that were used in the 2018 greenhouse GRBV transmission bioassay. Weights of all immature stages had approximately normal distributions and equal variance. One-way ANOVA indicated that there was no significant difference in the weights of 4th and 5th instar nymphs, and 3rd instar nymphs were significantly lighter than later instar stages

($F_{2,27}$=8.112, P=0.002). Mean weight of 3rd instar nymphs was 1.52±0.16 mg; mean weight of 4th instar nymphs was 4.95±0.32 mg; and mean weight of 5th instar nymphs was 3.63±0.55 mg.

2018 laboratory GRBV transmission bioassay. Vegetative tissues were harvested in 2018 from uninfested negative control vines and from seven areas of Schwarzmann and Wädenswil vines that had been infested with individual treehopper adults immediately before sampling (Fig. 5.4). Of all tissues, only two results from qPCR assays suggested potential transmission of GRBV from the insect to its host grapevine (Table 5.3). Nucleic acid samples from potentially affected tissues were subjected to confirmation using ddPCR. Results showed that the well containing petiole material from below a feeding leaf that was infested for 24 hours with a male *Sp. festinus* (sample e of vine 18663) contained droplets containing GRBV on the same order of magnitude as positive controls. Root tissue of a vine that was infested for 72 hours with a male *St. basalis* (sample g of vine 18670) tested negative for GRBV using ddPCR. Positive and negative controls performed as expected (Table 5.4).

Weights of adults collected in 2018 were compared by species. Weights of adult *St. basalis* approximated a normal distribution, while weights of adult *Sp. festinus* (n=6, W=0.733, P=0.014) and *T. albidosparsus* (n=20, W=0.874, P=0.014) were non-normally distributed. Homogeneity of variance was not violated. One-way ANOVA revealed that no significant differences in weight were observed across the three species in the adult stage ($F_{2,81}$=0.964, P=0.386). Mean adult insect weight was 12.90 ± 0.555 mg.

Insect weights. In 2017 and 2018, insects were collected following infestation of grapevine materials in the greenhouse, field, and laboratory to determine the persistence of GRBV as assayed by qPCR (Table 5.5). In 2017, 12 insects (21.8%) tested positive for the presence of GRBV. The virus could be detected up to three weeks following the AAP. Equal numbers (n=3) of *St. basalis* and *T. albidosparsus* carried GRBV, and most *Sp. festinus* carried GRBV. Thirty-five treehoppers (30.4%) tested positive for the presence of GRBV in 2018. In both years, *Sp. festinus* had the highest rate of positive results. Immature life stages of *St. basalis* appeared to uptake GRBV,

with 40% of specimens testing positive. Specimens of *H. halys* that were never exposed to GRBV did not return positive results.

DISCUSSION

RBD is an emerging viral disease of economic importance in Oregon wine grape production systems, and observed spread in Oregon vineyards suggested field transmission by a mobile vector (Dalton et al. 2019). Multiple species of insects have shown the ability to uptake GRBV, but in-depth studies have yielded inconsistent and even contradictory results (Poojari et al. 2013, Bahder et al. 2016b, Cieniewicz et al. 2018b). The *Geminiviridae* represent a diverse group of plant viruses containing at least nine genera, many of which are transmitted by specific insect vectors (Zerbini et al. 2017). The treehopper *M. malleifera* is the only known vector of *Tomato pseudo-curlytop virus*. Given the similarities between the genome organization of genera *Topocuvirus* and *Grablovirus*, of which the type species GRBV is purportedly transmitted by *Sp. festinus*, the likelihood exists that at least one treehopper species is responsible for field spread of GRBV (Bahder et al. 2016b). Archived voucher specimens of *Sp. festinus* collected from sites in western Oregon suggest the historical presence of the species; however, specimens were decades old, and the prevalence of GRBV in viticultural regions cannot be explained by the known distribution of *Sp. festinus* as determined through recent collections (Dalton et al. 2020). On the other hand, the widespread potential distribution of *Sp. festinus* in western Oregon cannot be discounted. Dozens of adults were collected from 2016–2018 in Jackson County within or directly adjacent to a vineyard block that was recently removed due to high incidence of GRBV infection (Stowasser et al. 2020, Dalton et al. 2019). Moreover, in the Willamette Valley, a single adult *Sp. festinus* was collected in August 2019 in a vineyard in close proximity to YV and CV study sites (J. Lee, *personal communication*).

While our GRBV transmission bioassays were mostly ineffective, it is possible that the biotype of the potential vector might impact transmission efficiency. In a seminal study on transmission of whitefly-transmitted geminiviruses, it was found that populations of *Bemisia tabaci* (Gennadius) differentially transmitted viruses from diverse geographic regions, and with variable efficiency on different plant hosts

(Bedford et al. 1994). Biotypes of *Sp. festinus* from distinct geographic regions of the southern United States were recently differentiated using DNA metabarcoding of the insect *mt-CO1* gene and the nuclear internal transcribed spacer 2 region (Cieniewicz et al. 2020). While in some cases there may be no considerable differences of virus transmission in vector populations, as was found in an assessment of vector-pathogen specificity of the GLRaV complex (Tsai et al. 2010), it remains to be seen whether any differences of epidemiological importance exist between Oregon and California populations of *Sp. festinus*. Furthermore, plant host tissue could have an effect on the ability of an insect to transmit a virus. When caged on whole plants, *Sp. festinus* successfully transmitted GRBV to test plants (Bahder et al. 2016b), whereas in our 2018 laboratory bioassay, treehoppers were restricted to leaf petioles of treatment vines. This is an important distinction because feeding-induced girdling of a leaf petiole might inhibit the further translocation of GRBV particles (Andersen et al. 2002). Partial girdles of petioles, or alternatively stem girdles, may allow more efficient translocation of GRBV, but this hypothesis needs verification. The girdling status of the plant that tested positive in the 2018 laboratory GRBV transmission bioassay is unknown because the leaf petiole was harvested before girdling could develop.

In the 2016 greenhouse GRBV transmission bioassay, the 2-day AAP could partially explain the apparent lack of transmission of GRBV. A latent period inside vector insects must be satisfied prior to transmission of geminiviruses (Gray et al. 2014). Potentially, insects in the 2016 greenhouse GRBV transmission bioassay did not feed on inoculum source materials, and the possibility thus exists that no uptake of virions could have occurred. To maximize the likelihood of feeding, subsequent infestation trials used AAP of 6 days. In the current work, inoculum source materials and treehoppers of all source populations tested positive for GRBV. If treehoppers truly are capable of transmitting GRBV, it is likely that test plants were challenged with GRBV during the IAP. Notably, the highest incidence of GRBV in insects following bioassays was found in *Sp. festinus*, lending further support to its potential role as a vector of GRBV. While transmission of GRBV by *Sp. festinus* was observed in one instance in the laboratory bioassays, field transmission remains to be shown. The comprehensive suite of greenhouse and laboratory GRBV transmission bioassays

conducted in the current study was unable to show transmission by most populations of smiliine treehoppers collected in Oregon.

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Table 5.1 Adult treehoppers used in 2016 for a greenhouse Grapevine red blotch virus (GRBV) transmission bioassay. Source vine indicates whether inoculum source material tested positive (=1) or negative (=0) for GRBV. Samples indicated with † were destroyed and could not be tested. Negative control indicates vines that were not infested with insects.

Species	Insect	Days alive	Source vine	N infested vines	Positive assays	Total assays
Tortistilus	19	7	1	2	0	11
albidosparsus	4	11	1	2	0	13
	8	11	0	2	0	11
	12	11	0	2	0	13
	14	11	1	2	0	13
	11	15	0	3	0	16
	5	16	0	3	0	18
	1	18	1	3	0	16
	16	18	1	3	0	15
	7	28	1	5	0	24
	13	38	0	6	0	28
	18	42	1	6	0	27
	9	56	0	9	0	42
	6	58	0	9	0	40
	2	70	1	10	0	40
	17	70	0	11	0	41
	15	77	1	12	0	50
	10	84	1	13	0	52
	3	102	1	15	0	51
Stictocephala	20	4	0	1	0	6
basalis	26	4	†	1	0	7
	32	7	1	2	0	11
	23	10	0	2	0	11
	36	11	1	2	0	10
	34	13	1	3	0	15
	28	21	†	4	0	18
	38	25	1	4	0	14
	22	26	1	4	0	19
	31	32	†	5	0	21
	33	34	1	6	0	25
	30	42	1	7	0	33
	25	46	†	7	0	28
	35	46	1	7	0	29
	37	46	1	7	0	32
	24	51	0	8	0	31
	21	52	1	8	0	42
	39	53	1	8	0	34
	29	63	†	11	0	43
	27	67	0	10	0	40
Negative control				0	0	420

Table 5.2 Grapevine red blotch virus (GRBV) transmission bioassay on 'Pinot noir' clone 828 on Schwarzmann rootstock infested in 2017 with treehopper species *Stictocephala basalis* and *Tortistilus albidosparsus*. Start of acquisition access period (AAP), inoculation access periods (IAP), and date of insect collection are indicated by Julian date.

Event	Julian date	Insect	Vines	Positive assays	Total assays	Insects collected
AAP	228	*St. basalis*	9	8	12	
		T. albidosparsus	8	7	13	
IAP 1	234	Control	14	0	39	
		St. basalis	14	0	39	
		T. albidosparsus	13	0	38	
IAP 2	241	Control	13	0	33	
		St. basalis	13	0	33	5
		T. albidosparsus	12	0	29	4
IAP 3	248	Control	12	0	29	
		St. basalis	12	0	34	5
		T. albidosparsus	11	0	31	5
IAP 4	255	Control	11	0	27	
		St. basalis	11	0	29	5
		T. albidosparsus	10	0	24	5
IAP 5	262	Control	10	0	24	
		St. basalis	10	0	26	5
		T. albidosparsus	6	0	17	5
Post-trial	269	*St. basalis*	2	0	5	
		T. albidosparsus	1	0	2	
	271	*St. basalis*				4
		T. albidosparsus				3
	299	*Sp. festinus*				9

Table 5.3 Results of quantitative polymerase chain reaction assays of 'Pinot noir' clone 828 on Schwarzmann rootstock and self-rooted 'Pinot noir' clone Wädenswil. Oregon populations of treehopper insects were provided inoculation access periods (IAP) of either 24 or 72 hours in the laboratory. Bolded numbers indicate vines showing a potentially positive finding.

| Insect species | 24-hour IAP | | | | | | 72-hour IAP | | | | | |
| | Schwarzmann | | | Wädenswil | | | Schwarzmann | | | Wädenswil | | |
	Vine	(+)	N	Vine	(+)	N	Vine	(+)	N	Vine	(+)	N
Tortistilus albidosparsus	18678	0	6									
	18681	0	6									
	18682	0	6									
	18688	0	6									
Spissistilus festinus	18702	0	6	18650	0	6	18704	0	6	18645	0	6
	18703	0	6	18655	0	6	18705	0	6	18648	0	6
	18707	0	6	**18663**	**1**	**6**	18706	0	6	18656	0	6
	18710	0	6	18672	0	6	18708	0	6	18668	0	6
	18711	0	6	18696	0	6	18709	0	6	18669	0	6
Stictocephala basalis	18677	0	6	18640	0	6	18679	0	6	18644	0	6
	18683	0	6	18643	0	6	18680	0	6	18660	0	6
	18686	0	6	18658	0	6	18684	0	6	18667	0	6
	18687	0	6	18659	0	6	18685	0	6	**18670**	**1**	**6**
	18690	0	6	18665	0	6	18689	0	6	18671	0	6
uninfested control	18697	0	1	18642	0	1	18691	0	1	18661	0	1
	18698	0	1	18646	0	1	18692	0	1	18655	0	1
	18699	0	1	18649	0	1	18693	0	1	18656	0	1
	18700	0	1	18664	0	1	18694	0	1	18663	0	1
	18701	0	1	18666	0	1	18695	0	1	18696	0	1

Table 5.4 Results of droplet digital polymerase chain reaction assays conducted in 2018 on 'Pinot noir' grapevine. Two dilutions (1% and 100%) of DNA were tested. Previously tested Grapevine red blotch virus (GRBV)-positive and GRBV-negative (+ and -) control vines are indicated. Percent values indicate the fraction of positive droplets from duplicate runs of each sample.

Vine	1% dilution		100% dilution		Determination
	Pct (+) droplets	total droplets	Pct (+) droplets	total droplets	
17357	0.1%	36,909	<0.1%	37,019	negative
17360	0.1%	35,571	0.0%	34,114	negative
17395	0.1%	35,305	<0.1%	35,921	negative
17400	0.1%	34,947	0.1%	35,986	negative
18663e	4.3%	36,594	91.6%	33,895	positive
18670g	<0.1%	38,620	0.0%	36,072	negative
990 (+)	2.7%	33,358	95.4%	30,753	positive
1027 (+)	16.0%	34,967	53.7%	25,081	positive
1049 (+)	4.0%	34,485	99.3%	30,951	positive
651 (-)	0.0%	32,748	0.0%	37,562	negative
687 (-)	0.2%	31,077	0.1%	35,954	negative
698 (-)	0.1%	31,563	0.1%	32,548	negative

Table 5.5 Grapevine red blotch virus (GRBV) infection status of treehopper species *Stictocephala basalis*, *Tortistilus albidosparsus*, and *Spissistilus festinus*, after placement on infected 'Pinot noir' inoculum source materials, and *Halyomorpha halys* that were never exposed to GRBV.

Year	GRBV transmission trial	Species	Life stage	Positive	N
2017	greenhouse bioassay T1	*St. basalis*	adult	2	5
		T. albidosparsus	adult	0	4
	greenhouse bioassay T2	*St. basalis*	adult	0	5
		T. albidosparsus	adult	3	5
	greenhouse bioassay T3	*St. basalis*	adult	1	5
		T. albidosparsus	adult	0	5
	greenhouse bioassay T4	*St. basalis*	adult	0	5
		T. albidosparsus	adult	0	5
	greenhouse bioassay post-assay	*St. basalis*	adult	0	4
		T. albidosparsus	adult	0	3
	greenhouse bioassay greenhouse AAP	*Sp. festinus*	adult	6	9
2018	greenhouse bioassay greenhouse AAP	*St. basalis*	immature	2	5
	greenhouse bioassay	*St. basalis*	immature	5	12
		St. basalis	adult	0	1
	cover crop growth chamber bioassay	*St. basalis*	immature	5	13
		St. basalis	adult	2	7
	grapevine growth chamber bioassay	*St. basalis*	adult	2	9
	laboratory bioassay field AAP	*St. basalis*	adult	5	19
		T. albidosparsus	adult	0	16
	laboratory bioassay	*Sp. festinus*	adult	4	6
		St. basalis	adult	9	23
		T. albidosparsus	adult	1	4
	negative control	*H. halys*	adult	0	4
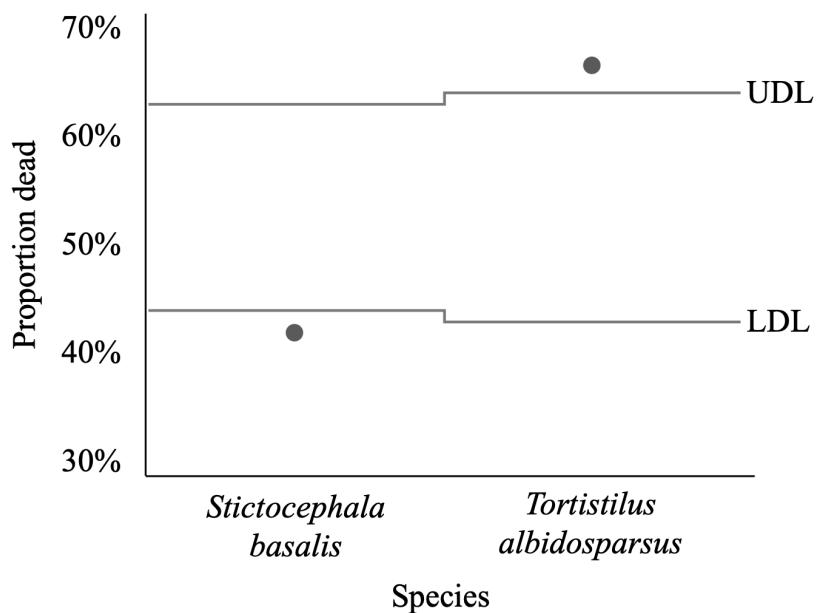
Figure 5.1 Percent mortality of treehopper species (Hemiptera: Membracidae) in 2017 at the conclusion of a greenhouse Grapevine red blotch virus transmission bioassay. Lower decision limit (LDL) and upper decision limit (UDL) are indicated by horizontal lines and are significant at $\alpha=0.05$.
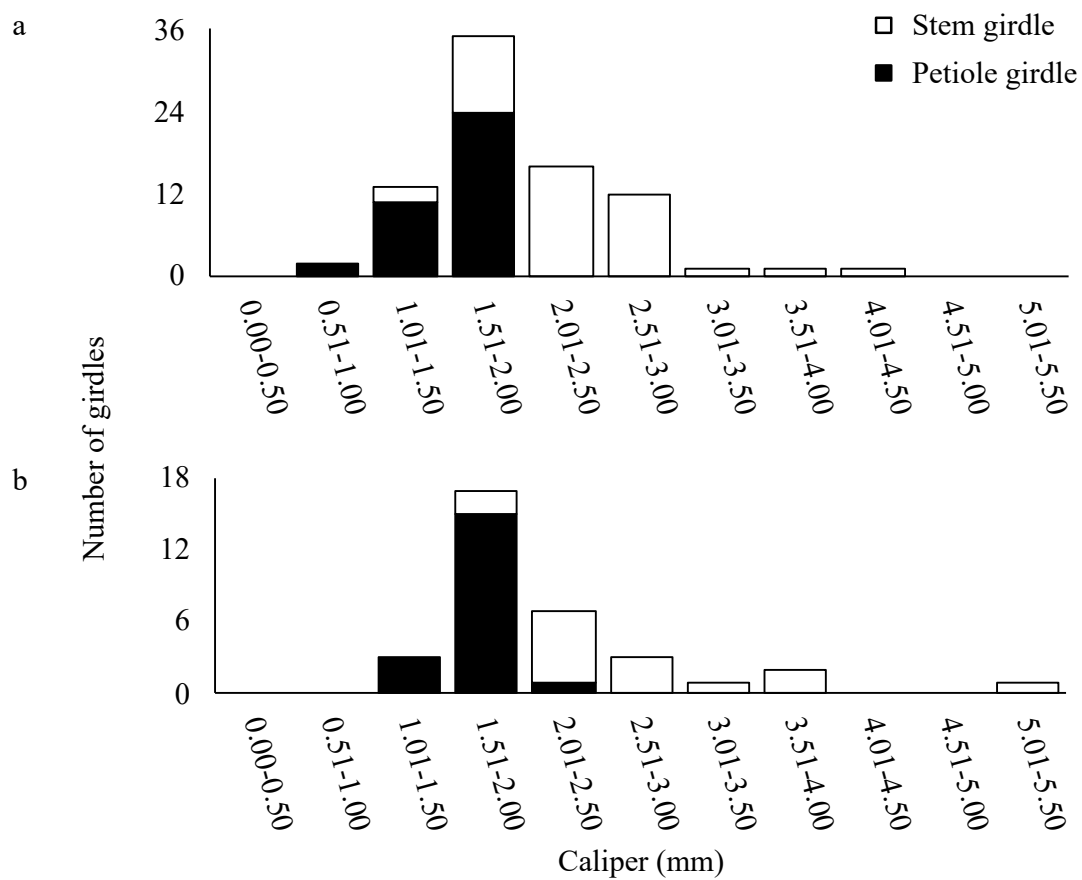
Figure 5.2 Caliper of tissues in a 2017 greenhouse Grapevine red blotch virus transmission bioassay affected by feeding-induced girdle by (a) *Stictocephala basalis*; (b) *Tortistilus albidosparsus*.
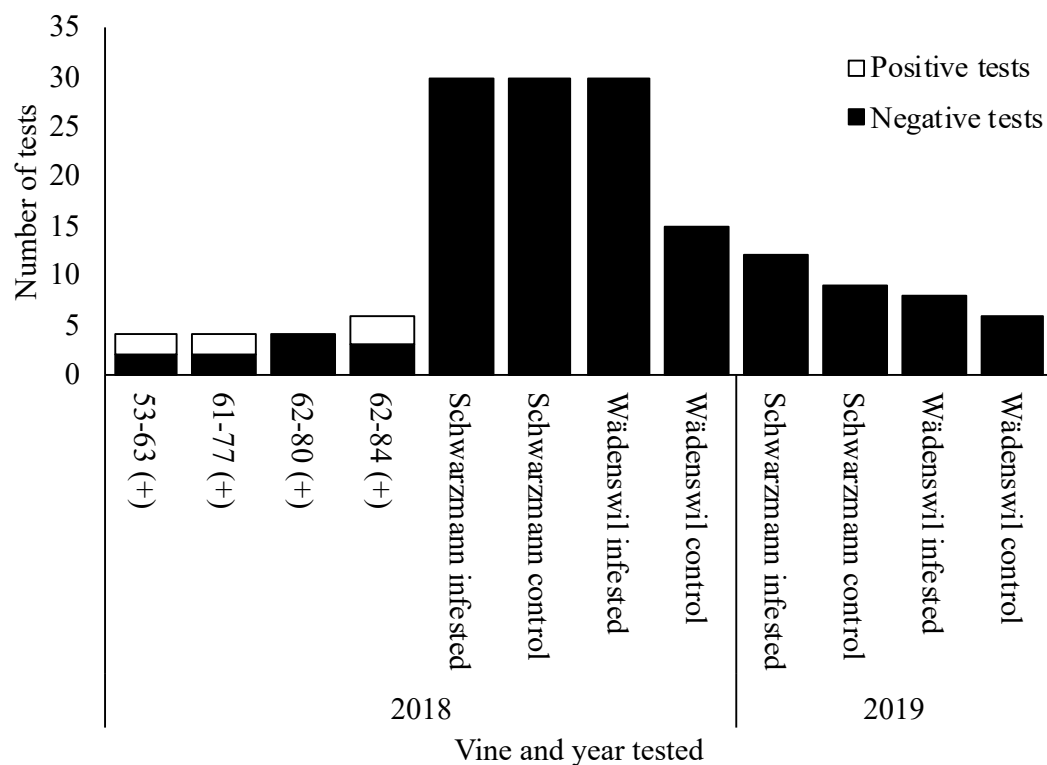
Figure 5.3 Grapevine red blotch virus (GRBV) molecular test results from *Vitis vinifera* 'Pinot noir' clone 828 on Schwarzmann rootstock or self-rooted clone Wädenswil that were infested in a greenhouse with *Stictocephala basalis* nymphs, and GRBV-positive and negative control *V. vinifera*. GRBV inoculum source branches denoted with (+).
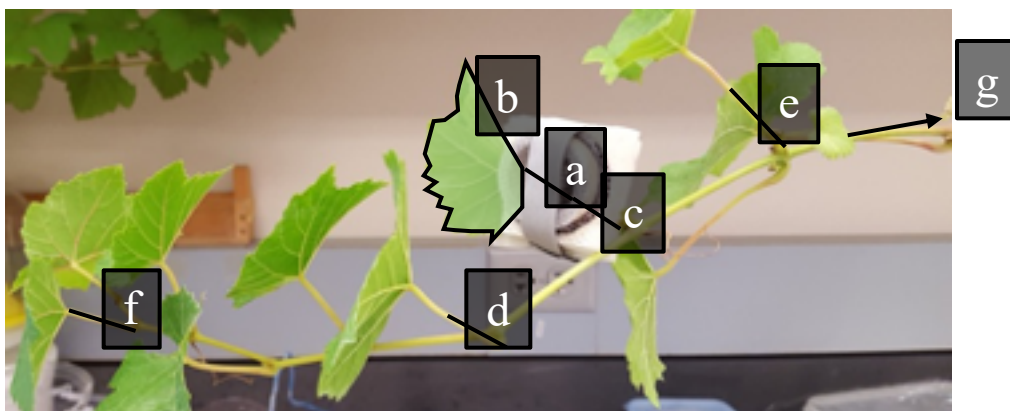
Figure 5.4 Location of tissue samples harvested from vines infested in a laboratory with three species of treehoppers from Oregon. Lines and corresponding letters indicate the plant tissues collected for genetic assays: (a) petiole within clip cage; (b) leaf blade above clip cage; (c) petiole below clip cage; (d) petiole of leaf above infested leaf; (e) petiole of leaf below infested leaf; (f) stem tip or apical leaf petiole; (g) roots (arrow).

CHAPTER 6

Conclusion

Daniel T. Dalton

The geminivirus *Grapevine red blotch virus* is the causative agent of red blotch disease (RBD), which negatively affects the quality of fruit produced for winemaking. The work presented in this dissertation was conducted in an effort to determine insect species contributing to the epidemiology of RBD in wine grape (*Vitis vinifera* L.) production regions of Oregon, USA. The first objective was to verify whether GRBV was spreading in the field. Year-by-year spread of GRBV was confirmed in three of four vineyard study sites. Number of infected vines doubled at a vineyard in Yamhill County, Oregon, rising from 31.7 % in 2013–2014 to 59.2% in 2016. GRBV incidence doubled at a vineyard in Jackson County, Oregon, from 10.4% infection in 2014 to 21.5% infection in 2016. At a second Jackson County site, incidence of GRBV increased nearly 20-fold, from 3.1% infection in 2014 to 58.5% infection in 2016. No GRBV-infected vines were found at a vineyard in Umatilla County.

The treehopper (Hemiptera: Membracidae: Smiliinae) *Spissistilus festinus* (Say) is known to transmit GRBV under greenhouse conditions. Intensive visual searches for *Sp. festinus* and related species revealed differential presence of smiliine treehoppers in Oregon viticultural regions. In the Willamette Valley, *Stictocephala basalis* (Walker) were found at vineyard sites and a natural area, and *Tortistilus albidosparsus* (Stål) were found at one vineyard. In southern Oregon, *Sp. festinus*, *Stictocephala bisonia* Kopp and Yonke, and *T. albidosparsus* were discovered in Jackson County, and *T. albidosparsus* were additionally found in Josephine County. Voucher specimens of all species were deposited in arthropod collections at Oregon State University, Corvallis, Oregon, and the National Museum of Natural History in Washington, D.C.

In 2017 immature stages of *St. basalis* were field-collected and placed on *Pisum sativum* L. 'Oregon Trail' to document development inside a growth chamber. Upon field collection or adult emergence in 2017, *St. basalis* were placed onto potted *V. vinifera*, *Pyrus communis* L., *Crataegus douglasii* Lindl., and *Malus pumila* Mill. Adult movement, girdling activity, and feeding behaviors were documented. Surviving adults of *St. basalis* and *T. albidosparsus* from a greenhouse GRBV transmission trial were additionally placed on woody hosts. Females deposited eggs on stem tissues, and ovipositional characteristics were recorded. Nymphs emerging from overwintered eggs of both species were placed on *P. sativum* in spring 2018 to further characterize

phenological development. In a replicated trial, a population of late-instar *St. basalis* was further introduced onto herbaceous seedlings of *P. sativum*, *Trifolium alexandrinum* L., *Trifolium pratense* L., *Lolium multiflorum* Lamarck, and *Brassica rapa* L. var. *silvestris*. Behavioral and feeding characteristics were documented for immatures and emergent adults, and survival to the adult stage was assessed. Where comparison between species was possible, few differences were found; however, *St. basalis* produced more eggs per oviposition site and emerged from a greater number of host species. Survival of *St. basalis* nymphs was equivalent on herbaceous seedlings, except for *B. rapa* var. *silvestris*, on which no nymphs survived to the adult stage.

All life stages of *St. basalis* were discovered in Willamette Valley sites. Phenological progression of life stages was clear. Eggs were found to be deposited behind or underneath the scales of dormant buds of woody perennial species and hatched in May–June. Early instar nymphs fed on green succulent vegetation, primarily *Vicia* L. spp., while late instar nymphs were commonly found on drought-hardy species such as *Daucus carota* L. Instar stages were also found in relative abundance on *Rubus armeniacus* Focke. Adults emerged in mid-July, and oviposition on overwintering hosts occurred in August. All life stages of *St. basalis* were found on *V. vinifera*. Ovipositional characteristics were documented, including percentage of infested buds on woody hosts, number of deposited eggs per infested bud, and distance of eggs from shoot tips. In Josephine County, immature stages and adults of *T. albidosparsus* were found earlier in the season, indicating egg hatch in April–May. Adults of *T. albidosparsus* emerged beginning in late June or early July from the Josephine County site. Timing of *T. albidosparsus* adult emergence in a Willamette Valley site was identical, although only one season of emergence was observed. For both *St. basalis* and *T. albidosparsus*, feeding damage was characterized by target tissue and number of girdles per plant. A strong edge effect of feeding damage was apparent at field sites in Josephine County and in the Willamette Valley.

Greenhouse bioassays were conducted to determine capacity of populations of *St. basalis* and *T. albidosparsus* to transmit GRBV under controlled greenhouse conditions. In 2016, adults were placed individually on GRBV-infected *V. vinifera* 'Pinot noir' cuttings and provided a 2-day acquisition access period (AAP). Insects

were then transferred to GRBV-negative 'Pinot noir' for inoculation access periods (IAPs) ranging from 2–7 days. A trial occurred in 2017 in which cohorts of 5 adults of *St. basalis* or *T. albidosparsus* were provided a 6-day AAP on GRBV-infected *V. vinifera*, followed by weekly rotations onto GRBV-negative vines, providing 7-day IAPs. Cohorts were randomly collected during the 2017 study for molecular analysis. Immature insects of *St. basalis* were provided a 6-day AAP on infected grapevines, followed by a 7-day IAP on 'Pinot noir' clone 828 on Schwarzmann rootstock or self-rooted clone Wädenswil, in a 2018 transmission bioassay. Lastly, near harvest in 2018, *Sp. festinus*, *St. basalis*, and *T. albidosparsus* were provided a 6-day AAP on GRBV-infected plant materials, followed by a 24h or 72h IAP in the laboratory on Schwarzmann or Wädenswil. Insects used in the 2018 trials were analyzed using molecular techniques for presence of GRBV. Available plant materials from all transmission bioassays were sampled in all years for molecular analysis. No tests of materials infested with *St. basalis* or *T. albidosparsus* indicated transmission of GRBV. A single leaf petiole sample tested positive for GRBV following infestation with *Sp. festinus*. Virus acquisition occurred in all insect species and was highest for *Sp. festinus*.

Insect-transmitted microbes causing diseases of grapevine are among the primary challenges facing wine grape producers. Following a series of GRBV transmission bioassays, no evidence of GRBV transmission by two Oregon treehopper populations was established, and transmission by the previously identified vector species, *Sp. festinus*, was low. Detailed investigations of GRBV transport within the alimentary canal of *Sp. festinus* remain to be performed, both to confirm the level of vector competence and as a model for treehopper-mediated transmission of plant viruses. While transmission of GRBV by *St. basalis* and *T. albidosparsus* was not achieved, the possibility remains that these species may have a role in the epidemiology of RBD. Environmental factors enabling transmission need to be further investigated in relation to treehopper infestation. Such factors should include interactive effects of water and heat stress, along with treehopper population-level effects. Alternately, different insect taxa may be responsible for GRBV transmission in the field. To serve growers, consideration of the ability of transmission by other phloem-feeding insects, including locally abundant cixiid species, is warranted.

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APPENDICES

APPENDIX A

Vouchers for treehoppers (Hemiptera: Membracidae) collected in Benton, Josephine, and Yamhill Counties, Oregon

Daniel T. Dalton, Richard J. Hilton, Dennis D. Kopp, and Vaughn M. Walton

ABSTRACT

Treehopper insect populations (Hemiptera: Membracidae) were surveyed in 2018 in Benton, Josephine, and Yamhill Counties, Oregon to determine their potential roles in the epidemiology of Grapevine red blotch virus. *Stictocephala basalis* and *Tortistilus albidosparsus* were identified through a taxonomic assessment of samples collected by hand near vineyards and in a natural area. Historical presence of *Spissistilus festinus* in the Willamette Valley is discussed. Voucher specimens were deposited in the Oregon State Arthropod Collection and at the United States National Museum of Natural History.

INTRODUCTION

Investigations into potential insect vectors of Grapevine red blotch virus (GRBV) began at Oregon State University in 2016 as a result of scientific breakthroughs in the identification and spatial dynamics of the virus (Al Rwahnih et al. 2013, Dalton et al. 2019). Viruses that are vectored by insects are typically transmitted by a narrow taxonomic group (Whitfield et al. 2015). The insect species *Spissistilus festinus* (Say) (Hemiptera: Membracidae: Smiliinae: Ceresini) was determined to be a competent vector of GRBV under greenhouse conditions (Bahder et al. 2016b).

MUSEUM SPECIMENS/RECORDS

Historical records showed the presence of *Sp. festinus* in viticultural regions of northwest Oregon, but the most recent finding documented in the Oregon State Arthropod Collection (OSAC) occurred in 1980, and specimens of *Sp. festinus* from southwest Oregon were also decades old (Appendix Table A.1).

FIELD SURVEYS

Insect trapping surveys were conducted in regions of the Willamette Valley and the Illinois Valley, Oregon, in 2018. The goal was to determine whether populations of potential insect vectors of GRBV are currently present in areas of commercial wine grape production. Treehoppers were collected in four Willamette Valley sites and one site near Cave Junction, Oregon. Representative specimens were sent to Dr. Dennis Kopp, Volunteer Curator of Hemiptera at the Smithsonian National Museum of Natural History (USNM), Washington D.C., USA, who identified them to species based on external morphology and characters on the male genitalia. In the Willamette Valley,

individuals of *Stictocephala basalis* (Walker) (Appendix Fig. A.1) were collected by hand as late-instar nymphs in a natural area near Corvallis, Benton County, and reared to the adult stage for species identification. In Yamhill County, specimens of *St. basalis* were collected as nymphs and adults in three commercial vineyards using hand and vacuum sampling techniques. Examples of *Tortistilus albidosparsus* (Stål) (Appendix Fig. A.2) were collected as nymphs and adults at one of the Yamhill County vineyards. In Josephine County, adult examples of *T. albidosparsus* were found and were the only species collected. No populations of *Sp. festinus* were discovered at these sites in 2018.

This paper serves to document the deposition of exemplar specimens for these species in a public research collection. Specimens were assigned unique identifiers that were then printed as human-readable and 2D matrix codes on acid free labels affixed to the specimens. In total 34 specimens were deposited in the OSAC on 3 December 2019 (Accession OSAC_AC-2019-12-18-01-001) and 8 specimens were deposited at the USNM (Appendix Table A.2).

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APPENDIX B

Vouchers for treehoppers (Hemiptera: Membracidae) collected in 2018 from Jackson County, Oregon

Mariana Stowasser, Richard J. Hilton, Dennis D. Kopp, Daniel T. Dalton, and Vaughn M. Walton

In 2016, research scientists at University of California-Davis found that the insect species *Spissistilus festinus* (Say) (Hemiptera: Membracidae: Smiliinae: Ceresini), a treehopper native to southeast North America and now found across the continent, is a competent vector of Grapevine red blotch virus (GRBV) under greenhouse conditions (Bahder et al. 2016b). Subsequently, Oregon State University researchers determined that GRBV was present in viticultural regions of Oregon and was found to be spreading in multiple locations (Dalton et al. 2019). It is known more generally in vector ecology that a narrow taxonomic group is typically capable of transmitting a particular virus (Whitfield et al. 2015). Taken together, these observations gave credence to the possibility of a smiliine treehopper acting as the agent responsible for the observed spread of GRBV in Oregon wine grape vineyards.

Insect trapping surveys were conducted in southern Oregon to determine the composition of treehopper species in areas of commercial wine grape production. Multiple morphotypes were discovered in an alfalfa field near Central Point, Oregon, on two dates in late July 2018. Voucher specimens were sent to Dr. Dennis Kopp, Volunteer Curator of Hemiptera at the Smithsonian National Museum of Natural History in Washington, D.C., who identified them to species based on external morphology and characters on the male genitalia. Three specimens of *S. festinus* (Appendix Fig. B.1) were collected by Mariana Stowasser using a sweep net on 21 July 2018. Sweep net samples by the same collector on 27 July 2018 revealed the presence of *Stictocephala bisonia* (Kopp & Yonke) (Appendix Fig. B.2) and *Tortistilus albidosparsus* (Stål) (Appendix Fig. B.3).

This publication documents the deposition of exemplar specimens for these species in a public research collection. All of the specimens were assigned unique identifiers that were then printed as human-readable and a 2D matrix codes on acid free labels affixed to the specimens and deposited in the Oregon State Arthropod Collection on 6 November 2019 (Appendix Table B.1).

ACKNOWLEDGEMENTS

APPENDIX C

Description of field sites

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Detailed field site descriptions. Field sites were visited in 2016, 2017 and 2018 by technicians trained to identify immature and adult treehopper (Hemiptera: Membracidae) life stages, as well as ovipositional and feeding damage consistent with treehopper infestation. Collection dates were converted to sequential Julian date notation (Appendix Table C.1, Appendix Table C.2).

A small area (0.75 ha) of YV was routinely visited from spring through fall of 2017 and 2018 for detailed phenological observations of *Stictocephala basalis* (Walker) (Appendix Fig. C.1). Treehoppers were collected primarily along the western edge of the vineyard on bordering hedge rows of wild vegetation and in two blocks of grapevines of multiple cultivars, the west block and the southwest block. In the following description, except as otherwise specified, vines of *Vitis vinifera* L. 'Dolcetto' and 'Pinot noir' clone 667 were grown on 101-14 rootstock and were planted in a north-south orientation. Within-row vine spacing was approximately 1m for rows of 'Pinot noir' and 1.25m for 'Dolcetto'. Interrow spacing was approximately 1.6m for all vineyard rows. All rows of 'Pinot noir' were planted in 1996, and all rows of 'Dolcetto' were planted in 2001. Vineyard row descriptions are given from east to west, and south to north, as follows.

The southwest block was composed of rows 0–21. Rows 0–2 were planted to 'Dolcetto' on the eastern edge of the block and oriented in a southwest-northeast orientation. To the west and south were rows 3–9 planted to 'Pinot noir', and row 10 planted to 'Dolcetto'. Vines and trellising infrastructure in rows 11–15 had been previously removed, leaving an open, grassy area approximately 9m wide populated with seedling wild blackberry, Oregon white oak, and few re-sprouting grapevine crowns. West of the open area were rows 16–21 planted to 'Pinot noir'.

The west block contained rows 22–46. Rows 22–37 were planted to mixed clones of 'Pinot noir' grapevines and were not assessed for presence of treehoppers. West of these rows was an open, grassy area about 13m wide from which seven rows of grapevines (rows 38–44) had been previously removed, and to the west of the open area were rows 45–46 planted to 'Dolcetto' and bordering unmanaged habitat. Alleys between grapevine rows and open within-vineyard areas were maintained as wild-growing vegetation dominated by grasses, with low densities of *Rubus armeniacus*

Focke (wild blackberry) plants, seedlings of *Quercus garryana* Douglas ex Hook. (Oregon white oak), and re-sprouting grapevine crowns. Infrequent mowing and herbicide applications were used to control weeds in 2016–17, but routine mowing in 2018 effectively minimized interrow vegetation. No irrigation or insecticide sprays were applied to the vineyard blocks during the study period, contributing to moderate-to high-stress vineyard growing conditions.

Spread of GRBV was previously documented at YV (elev. 80–100m) (Dalton et al. 2019), and a resident population of *St. basalis* was discovered at the site in August 2016. Several vineyard blocks ranging in age and size were in production at YV. The vineyard was situated on a shallow Willakenzie soil profile with a moderate 10–20% south-facing slope. Adjacent unmanaged areas included a mix of riparian habitat at the bottom of steep, heavily vegetated slopes. *Fraxinus latifolia* Bentham (Oregon ash) and wild blackberry constituted the dominant perennial species in the riparian habitat. Primary species above the riparian areas included *Malus* Mill. spp. (seedling apple), Oregon white oak, *Prunus domestica* L. (seedling plum), wild blackberry, *Acer macrophyllum* Pursh (bigleaf maple) and *Crataegus* L. spp. (hawthorn). Minor deciduous species included *Rosa* L. spp. (wild rose), *Toxicodendron diversilobum* Greene (poison oak), *Amelanchier alnifolia* Nutt. (serviceberry), and *Corylus cornuta* Marshall (wild hazelnut). Understory vegetation included herbaceous species *Daucus carota* L. (wild carrot), *Vicia* L. spp. (vetch), *Geranium dissectum* L. (cut-leafed cranesbill), *Hypericum perforatum* L. (St. John's wort), *Hypochaeris radicata* L. (cat's ear), and unidentified grasses.

In Yamhill County, surveys in 2018 additionally included LV1 (elev. 85–125m) that was surveyed one time on 27 June, LV2 (elev. 85–135m) that was surveyed one time on 12 July, and CV (elev. 110–150m) that was repeatedly surveyed. LV1 was a medium-sized vineyard (~20 ha) planted to multiple clones of *V. vinifera* 'Pinot noir' on 101-14 or 16-16 rootstock. Blocks were planted in a north-south orientation with a vine spacing of 1.5m and row spacing of 2.1m. The majority of the vineyard was on Goodin silty clay loam or Melbourne-Goodin silt loam soils. Habitats surrounding the vineyard included large open areas of grass with patches of wild blackberry and closed-canopy Oregon white oak forest with native understory vegetation (Franklin and

Dyrness 1973). A closed-canopy deciduous forest containing Oregon white oak, *Populus* L. spp. (poplar), big-leaf maple, and native understory vegetation separated LV1 and LV2 by about 400m. LV2 was a medium-sized vineyard (~20 ha) on a moderate south-facing slope. About half of the vineyard was on Steiwer-Chehulpum complex soils, and Wellsdale-Willakenzie-Dupee soils constituted the remaining portion of the vineyard. The southern and eastern edges of the vineyard were bordered by grass seed fields, and the western margin was bordered by unmanaged riparian habitat. Collection efforts were focused on a patch of unmanaged grassy vegetation populated with scrubby hawthorn and Oregon white oak trees at the top of a hill. CV was a small vineyard (0.5 ha) planted about 1000m to the west of LV2 to *V. vinifera* 'Pinot noir' clones Pommard or 115 on 3309 rootstock. Most rows were planted in a north-south orientation, while some rows were planted in a SW-NE or SE-NW orientation. The moderate to steep south-facing slope was on Steiwer-Chehulpum complex or Witzel-Ritner complex stony soils and bordered on all sides by a perimeter of mature Oregon white oak. Dominant understory plants included wild blackberry, rose, poison oak, and unidentified grasses.

Grower-reported girdling activity at KVV (elev. 120–150m) in 2017 led to collections of woody cuttings from the surrounding habitat in April 2018 to determine potential presence of membracid eggs. The vineyard was situated on McAlpin silty clay loam soils, with a small fraction of the site on Abiqua silty clay loam. Vegetation to the south and east was a closed-canopy deciduous oak forest. To the north was a mix of prairie and deciduous forest containing apple, ash, blackberry, hawthorn, Oregon white oak, and *Pyrus communis* L. (European pear). To the west was scrubby vegetation on an exposed east-facing hillside, and to the southwest was a grass seed field. A heavily vegetated canal flowing north dominated by *Salix* L. spp. (willow) bisected the vineyard near the western edge, and a sparsely vegetated canal flowing northwest bisected most of the vineyard area to the east.

A population of *St. basalis* was examined in 2018 at CP (elev. 105m), Benton County. The site was located at the base of a hill in a very small clearing (0.05 ha) on Bashaw clay soil. Oak forest surrounded the site and contained dominant plant species Oregon white oak, hawthorn, blackberry, and poison oak. Understory vegetation

included wild carrot, cut-leafed cranesbill, cat's ear, *Bellis perennis* L. (daisy) and unidentified grasses. Site visits occurred on 9, 13, and 25 July.

CRV (elev. 170–190m) was a research vineyard operated by Oregon State University near Alpine, Benton County. The vineyard was surveyed for presence of treehopper populations on 5 October 2017 and on 10 April and 7 June 2018. The study area was a small section of unmanaged habitat on Willakenzie loam soil to the southeast of a 0.75-ha planting of *V. vinifera* 'Pinot noir' clone 115 on 101-14 rootstock that had unexplained leaf reddening symptoms. The vineyard block was planted in 2015 in a north-south orientation. Dominant species included Oregon white oak, *Quercus rubra* L. (red oak), wild blackberry, *Pseudotsuga menziesii* (Mirb.) Franco (Douglas fir), with occurrences of *Oemleria cerasiformis* (Torr. & A. Gray) J. W. Landon (osoberry), *Philadelphus lewisii* Pursh (mock orange), and unidentified grasses. The survey site extended about 30m under a sparse canopy to the east toward a large field that was maintained as grass through mowing. A stand of heritage fruit trees including apple, plum, and *Prunus avium* (L.) L. (sweet cherry) extended from 100–150m upslope of the study area. To the north of the study area were additional research plantings of wine grapes. Commercial vineyards of small or medium size were to the east, south, and west.

CJV (elev. 445m) was surveyed multiple times in 2016–2018 for incidence of treehopper populations and associated feeding damage. A resident population of *T. albidosparsus* was first observed on young grapevines at CJV in 1986 shortly after vineyard establishment (R. J. Hilton, *personal communication*).
CJV was a small vineyard (3.25 ha) containing two adjacent blocks of wine grapes planted in a north-south orientation and isolated from other vineyards by more than 2000 meters. Banning loam constituted the soil type of the western block of self-rooted *V. vinifera* 'Pinot gris' that was planted in 1990. The eastern block was planted in 1985 to *V. vinifera* 'Pinot noir' clones Wädenswil, Dijon clone 115, and Early Muscat on Takilma very cobbly loam soil. At CJV, surrounding habitat was dominated by *Arbutus menziesii* Pursh (madrone), *Arctostaphylos* Adans. spp. (manzanita), and hawthorn located primarily on adjacent parcels that were not otherwise associated with the vineyard. A pond (0.1 ha) was centrally located between the vineyard blocks.

EPV was planted in 2012 and 2013 to diverse wine grape cultivars including *V. vinifera* 'Pinot noir' clone 115 and 'Merlot' clone 181 on 3309 rootstock in the upper block (5–20% slopes), and *V. vinifera* 'Syrah' clone 470, 'Chardonnay' clone 15, 'Viognier' clone 1 and 'Sauvignon Blanc' clone 376 on 101-14 rootstock in the lower block (1-5% slope). The vineyard was 9.5 ha in area and was surveyed for treehoppers from 9–13 July 2018 (elev. 450–490m). For both blocks, within-row vine spacing was 1.22m, and spacing between rows was 2.13m. Carney clay soils underlaid the vineyard.

As determined in a previous study, significant spread of GRBV occurred at JV (elev. 470–480m) from 2014 to 2016 (Dalton et al. 2019). Surveys for potential GRBV vector insects using sweep netting and beat sheeting methodologies took place at JV from 2014–2018. The 10-ha block was planted on prime farmland to various clones of self-rooted 'Pinot noir' from 2009 to 2012. Four soil types of roughly equal proportions underlaid the block, including Coleman loam, Gregory silty clay loam, Medford silty clay loam, and Ruch silt loam. A narrow ribbon of Manita loam was at the southern edge. Vines had been planted in a north-south orientation at 1.8m in-row spacing and 2.25m between-row spacing on a slight north slope. Vines were removed following the 2016 growing season due to grower concern that the widespread presence of GRBV could act as a reservoir of infection for the rest of the vineyard. Irrigation ditches were to the north and east, an alfalfa field to the west, and deciduous oak forest to the south of the plot.

Appendix Table A.1 Historical specimens of *Spissistilus festinus* in the Oregon State Arthropod Collection.

Specimen#	Location	County	Date	Collector	Latitude	Longitude
00000001540	Unity	Baker	10-Jun-1955	Joe Schuh	44.438° N	118.192° W
00000001541	Corvallis	Benton	25-Apr-1937	C. G. Thompson	44.570° N	123.275° W
00000001542	Corvallis	Benton	25-May-1937	C. G. Thompson	44.570° N	123.275° W
00000001543	Corvallis	Benton	02-07 May-1937	C. G. Thompson	44.570° N	123.275° W
00000001544	Corvallis	Benton	10-May-1930	Itol Wilcox	44.570° N	123.275° W
00000001545	Valley of the Rogue Park	Douglas	20-May-1972	Musgrave	42.409° N	123.134° W
00000001546	Applegate	Jackson	11-May-1969	Paul Oman	42.257° N	123.169° W
00000001547	Griffin Creek	Jackson	03-May-1956	Schuh & Vertrees	42.278° N	122.936° W
00000001548	Sams Valley	Jackson	01-May-1970	Oman	42.492° N	122.975° W
00000001549	Warm Springs	Jefferson	25-Apr-1977	Oman	44.792° N	121.326° W
00000001550	Grants Pass	Josephine	08-Aug-1941	S. C. Jones	42.439° N	123.328° W
00000001551	Dodson	Multnomah	27-Jul-1923		45.605° N	122.038° W
00000001552	Forest Grove	Washington	19-May-1938	S. E. Crumb Jr.	45.519° N	123.130° W
00000001553	Forest Grove	Washington	07-Apr-1938	S. E. Crumb Jr.	45.519° N	123.130° W
00000001554	Forest Grove	Washington	06-Aug-1918	J. M. Langston	45.519° N	123.130° W
00000001555	McMinnville	Yamhill	15-Apr-1980	K. Fender	45.204° N	123.183° W
00000001556	McMinnville	Yamhill	1935	K. M. and D. M. Fender	45.204° N	123.183° W

Appendix Table A.2 List of treehopper voucher specimens collected in Benton, Josephine, and Yamhill Counties, Oregon, USA and identified to species by Dr. Dennis Kopp in 2019. Specimens were designated with unique Oregon State Arthropod Collection (OSAC) identification number and deposited into OSAC on 3 December 2019.

Appendix Table A.2

OSAC Identifier	Species	Gender	Date	Plant Host	Collector	County (USA: Oregon)	Latitude	Longitude
1229081*	*Stictocephala basalis*	female	9–13 July	*Daucus carota* (nymph)	D. Dalton	Benton	44.573° N	123.330° W
1229082	*Stictocephala basalis*	female	9–13 July	*Daucus carota* (nymph)	D. Dalton	Benton	44.573° N	123.330° W
1229083	*Stictocephala basalis*	male	9–13 July	*Daucus carota* (nymph)	D. Dalton	Benton	44.573° N	123.330° W
1229084	*Stictocephala basalis*	female	9–13 July	*Daucus carota* (nymph)	D. Dalton	Benton	44.573° N	123.330° W
1229085	*Stictocephala basalis*	female	9–13 July	*Daucus carota* (nymph)	D. Dalton	Benton	44.573° N	123.330° W
1229086*	*Stictocephala basalis*	male	9–13 July	*Daucus carota* (nymph)	D. Dalton	Benton	44.573° N	123.330° W
1229087	*Stictocephala basalis*	male	9–13 July	*Daucus carota* (nymph)	D. Dalton	Benton	44.573° N	123.330° W
1229088	*Stictocephala basalis*	female	9–13 July	*Daucus carota* (nymph)	D. Dalton	Benton	44.573° N	123.330° W
1229089	*Stictocephala basalis*	male	9–13 July	*Daucus carota* (nymph)	D. Dalton	Benton	44.573° N	123.330° W
1229090	*Stictocephala basalis*	male	9–13 July	*Daucus carota* (nymph)	D. Dalton	Benton	44.573° N	123.330° W
1229091	*Tortistilus albidosparsus*	female	July–Sept	unknown	D. Dalton	Yamhill	45.300° N	123.108° W
1229092	*Tortistilus albidosparsus*	male	July–Sept	unknown	D. Dalton	Yamhill	45.300° N	123.108° W
1229093	*Tortistilus albidosparsus*	female	July–Sept	unknown	D. Dalton	Yamhill	45.300° N	123.108° W
1229094	*Tortistilus albidosparsus*	female	July–Sept	unknown	D. Dalton	Yamhill	45.300° N	123.108° W
1229095	Uncertain ID	male	July–Sept	unknown	D. Dalton	Yamhill	45.300° N	123.108° W
1229096	Uncertain ID	female	July–Sept	unknown	D. Dalton	Yamhill	45.300° N	123.108° W
1229097*	*Tortistilus albidosparsus*	female	July–Sept	unknown	D. Dalton	Yamhill	45.300° N	123.108° W
1229098*	*Tortistilus albidosparsus*	male	July–Sept	unknown	D. Dalton	Yamhill	45.300° N	123.108° W
1229099	*Stictocephala basalis*	male	10-Jul	*Daucus carota* (nymph)	D. Dalton	Yamhill	45.300° N	123.108° W
1229100	*Stictocephala basalis*	male	10-Jul	*Daucus carota* (nymph)	D. Dalton	Yamhill	45.300° N	123.108° W
1229101	*Tortistilus albidosparsus*	female	16-Jul	*Vitis vinifera*	D. Dalton	Josephine	42.105° N	123.583° W
1229102	*Tortistilus albidosparsus*	male	16-Jul	*Vitis vinifera*	D. Dalton	Josephine	42.105° N	123.583° W
1229103	*Tortistilus albidosparsus*	female	16-Jul	*Vitis vinifera*	D. Dalton	Josephine	42.105° N	123.583° W
1229104*	*Tortistilus albidosparsus*	female	16-Jul	*Vitis vinifera*	D. Dalton	Josephine	42.105° N	123.583° W
1229105	*Tortistilus albidosparsus*	female	16-Jul	*Vitis vinifera*	D. Dalton	Josephine	42.105° N	123.583° W
1229106	*Tortistilus albidosparsus*	female	16-Jul	*Vitis vinifera*	D. Dalton	Josephine	42.105° N	123.583° W
1229107	*Tortistilus albidosparsus*	male	16-Jul	*Vitis vinifera*	D. Dalton	Josephine	42.105° N	123.583° W
1229108*	*Tortistilus albidosparsus*	male	16-Jul	*Vitis vinifera*	D. Dalton	Josephine	42.105° N	123.583° W
1229111*	*Stictocephala basalis*	female	June–Sept	unknown	D. Dalton	Yamhill	45.325° N	123.158° W
1229112	*Stictocephala basalis*	female	June–Sept	unknown	D. Dalton	Yamhill	45.325° N	123.158° W
1229113	*Stictocephala basalis*	male	June–Sept	unknown	D. Dalton	Yamhill	45.325° N	123.158° W
1229114	*Stictocephala basalis*	female	June–Sept	unknown	D. Dalton	Yamhill	45.325° N	123.158° W
1229115	*Stictocephala basalis*	male	June–Sept	unknown	D. Dalton	Yamhill	45.325° N	123.158° W
1229116	*Stictocephala basalis*	male	June–Sept	unknown	D. Dalton	Yamhill	45.325° N	123.158° W
1229117*	*Stictocephala basalis*	male	June–Sept	unknown	D. Dalton	Yamhill	45.325° N	123.158° W
1229118	*Stictocephala basalis*	female	June–Sept	unknown	D. Dalton	Yamhill	45.325° N	123.158° W
1229119	*Stictocephala basalis*	male	June–Sept	unknown	D. Dalton	Yamhill	45.325° N	123.158° W
1229120	*Stictocephala basalis*	female	June–Sept	unknown	D. Dalton	Yamhill	45.325° N	123.158° W
1229121	*Stictocephala basalis*	female	12-Jul	under *Crataegus* spp. (nymph)	D. Dalton	Yamhill	45.297° N	123.092° W
1229122	*Stictocephala basalis*	male	12-Jul	under *Crataegus* spp. (nymph)	D. Dalton	Yamhill	45.297° N	123.092° W

* specimens deposited at the Smithsonian National Museum of Natural History, Washington, D.C.

Appendix Table B.1 List of voucher specimens. All were collected on *Medicago sativa*, in Jackson County, Oregon (42.389° N 122.940° W) by M. Stowasser and determined by D. Kopp in 2018.

OSAC Identifier	Species	Sex	Date
0001229067	*Spissistilus festinus*	female	21 July 2018
0001229068	*Spissistilus festinus*	female	21 July 2018
0001229069	*Spissistilus festinus*	male	21 July 2018
0001229070	*Stictocephala bisonia*	male	27 July 2018
0001229071	*Stictocephala bisonia*	male	27 July 2018
0001229072	*Stictocephala bisonia*	female	27 July 2018
0001229073	*Stictocephala bisonia*	female	27 July 2018
0001229074	*Stictocephala bisonia*	female	27 July 2018
0001229075	*Stictocephala bisonia*	female	27 July 2018
0001229076	*Stictocephala bisonia*	female	27 July 2018
0001229077	*Stictocephala bisonia*	male	27 July 2018
0001229078	*Stictocephala bisonia*	male	27 July 2018
0001229079	*Tortistilus albidosparsus*	male	27 July 2018
0001229080	*Tortistilus albidosparsus*	female	27 July 2018

**Appendix Table C.1** Julian date calendar for leap year.

day	January	February	March	April	May	June	July	August	September	October	November	December
1	1	32	61	92	122	153	183	214	245	275	306	336
2	2	33	62	93	123	154	184	215	246	276	307	337
3	3	34	63	94	124	155	185	216	247	277	308	338
4	4	35	64	95	125	156	186	217	248	278	309	339
5	5	36	65	96	126	157	187	218	249	279	310	340
6	6	37	66	97	127	158	188	219	250	280	311	341
7	7	38	67	98	128	159	189	220	251	281	312	342
8	8	39	68	99	129	160	190	221	252	282	313	343
9	9	40	69	100	130	161	191	222	253	283	314	344
10	10	41	70	101	131	162	192	223	254	284	315	345
11	11	42	71	102	132	163	193	224	255	285	316	346
12	12	43	72	103	133	164	194	225	256	286	317	347
13	13	44	73	104	134	165	195	226	257	287	318	348
14	14	45	74	105	135	166	196	227	258	288	319	349
15	15	46	75	106	136	167	197	228	259	289	320	350
16	16	47	76	107	137	168	198	229	260	290	321	351
17	17	48	77	108	138	169	199	230	261	291	322	352
18	18	49	78	109	139	170	200	231	262	292	323	353
19	19	50	79	110	140	171	201	232	263	293	324	354
20	20	51	80	111	141	172	202	233	264	294	325	355
21	21	52	81	112	142	173	203	234	265	295	326	356
22	22	53	82	113	143	174	204	235	266	296	327	357
23	23	54	83	114	144	175	205	236	267	297	328	358
24	24	55	84	115	145	176	206	237	268	298	329	359
25	25	56	85	116	146	177	207	238	269	299	330	360
26	26	57	86	117	147	178	208	239	270	300	331	361
27	27	58	87	118	148	179	209	240	271	301	332	362
28	28	59	88	119	149	180	210	241	272	302	333	363
29	29	60	89	120	150	181	211	242	273	303	334	364
30	30		90	121	151	182	212	243	274	304	335	365
31	31		91		152		213	244		305		366

Appendix Table C.2 Julian date calendar for non-leap year.

day	January	February	March	April	May	June	July	August	September	October	November	December
1	1	32	60	91	121	152	182	213	244	274	305	335
2	2	33	61	92	122	153	183	214	245	275	306	336
3	3	34	62	93	123	154	184	215	246	276	307	337
4	4	35	63	94	124	155	185	216	247	277	308	338
5	5	36	64	95	125	156	186	217	248	278	309	339
6	6	37	65	96	126	157	187	218	249	279	310	340
7	7	38	66	97	127	158	188	219	250	280	311	341
8	8	39	67	98	128	159	189	220	251	281	312	342
9	9	40	68	99	129	160	190	221	252	282	313	343
10	10	41	69	100	130	161	191	222	253	283	314	344
11	11	42	70	101	131	162	192	223	254	284	315	345
12	12	43	71	102	132	163	193	224	255	285	316	346
13	13	44	72	103	133	164	194	225	256	286	317	347
14	14	45	73	104	134	165	195	226	257	287	318	348
15	15	46	74	105	135	166	196	227	258	288	319	349
16	16	47	75	106	136	167	197	228	259	289	320	350
17	17	48	76	107	137	168	198	229	260	290	321	351
18	18	49	77	108	138	169	199	230	261	291	322	352
19	19	50	78	109	139	170	200	231	262	292	323	353
20	20	51	79	110	140	171	201	232	263	293	324	354
21	21	52	80	111	141	172	202	233	264	294	325	355
22	22	53	81	112	142	173	203	234	265	295	326	356
23	23	54	82	113	143	174	204	235	266	296	327	357
24	24	55	83	114	144	175	205	236	267	297	328	358
25	25	56	84	115	145	176	206	237	268	298	329	359
26	26	57	85	116	146	177	207	238	269	299	330	360
27	27	58	86	117	147	178	208	239	270	300	331	361
28	28	59	87	118	148	179	209	240	271	301	332	362
29	29		88	119	149	180	210	241	272	302	333	363
30	30		89	120	150	181	211	242	273	303	334	364
31	31		90		151		212	243		304		365

Appendix Figure A.1 Oregon State Arthropod Collection (OSAC) voucher specimens of *Stictocephala basalis* female, OSAC_0001229084 (a); male, OSAC_0001229119, abdomen removed (b). Scalebar=1 mm.
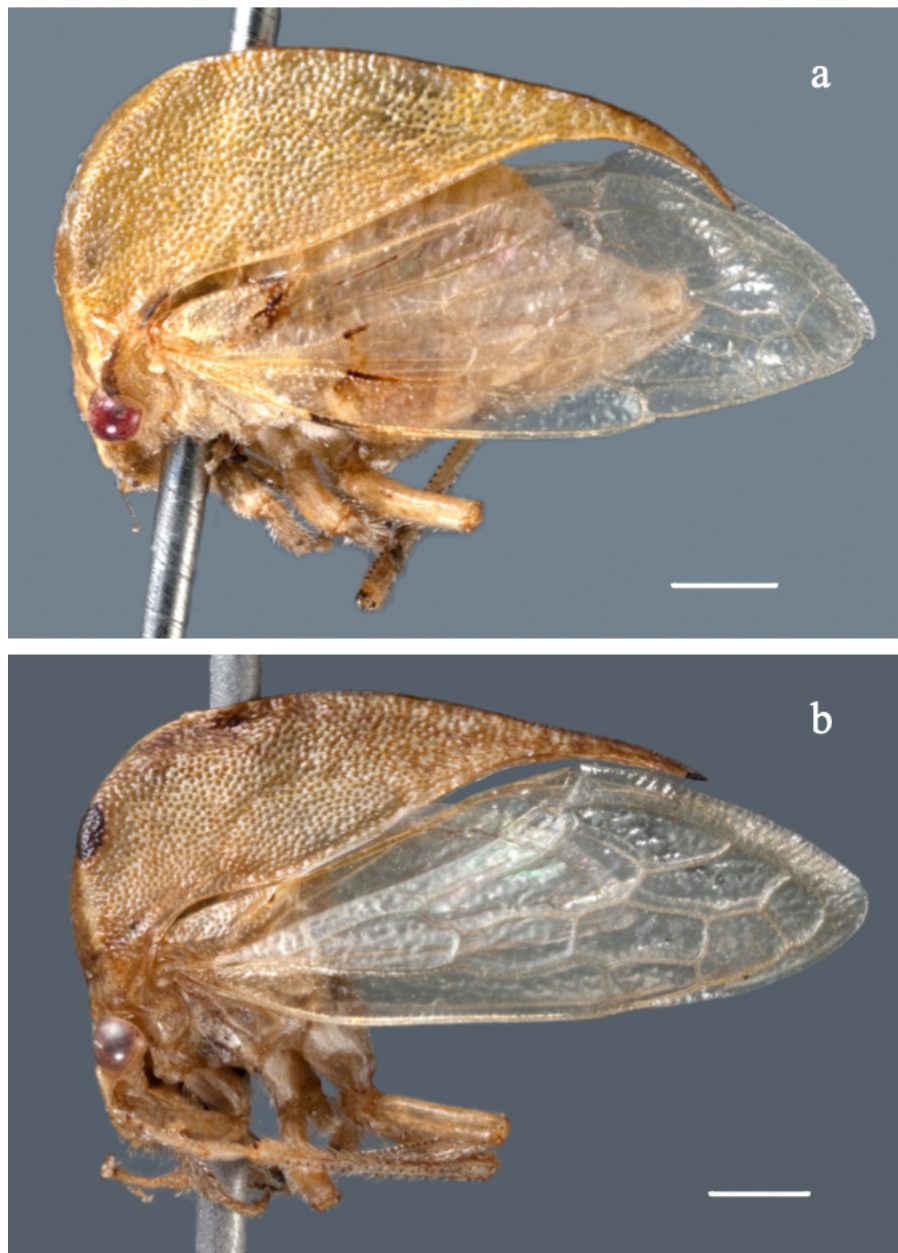
Appendix Figure A.2 Oregon State Arthropod Collection (OSAC) voucher specimens of *Tortistilus albidosparsus* female, OSAC_0001229096 (a); male, OSAC_0001229095, abdomen removed (b). Scalebar=1 mm.

Appendix Figure B.1 Oregon State Arthropod Collection (OSAC) voucher specimens of *Spissistilus festinus* female, OSAC_0001229067 (a); male, OSAC_0001229069, abdomen removed (b). Scalebar=1 mm.

Appendix Figure B.2 Oregon State Arthropod Collection (OSAC) voucher specimens of *Stictocephala bisonia* female, OSAC_0001229075 (a); male, OSAC_0001229071, abdomen removed (b). Scalebar=1 mm.

Appendix Figure B.3 Oregon State Arthropod Collection (OSAC) voucher specimens of *Tortistilus albidosparsus* female, OSAC_0001229080 (a); male, OSAC_0001229079, abdomen removed (b). Scalebar=1 mm.
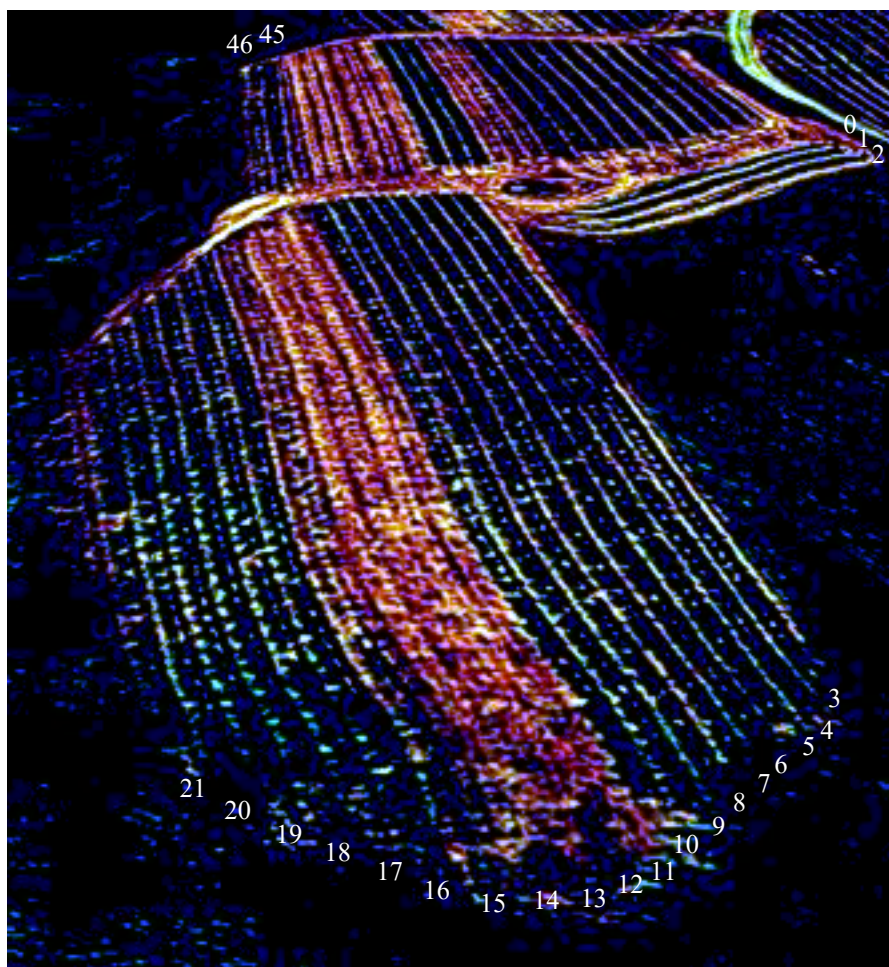
Appendix Figure C.1 Three-dimensional layout of vineyard blocks at YV vineyard site (view of south aspect facing north). Orange areas indicate access roads or areas of blocks that had been removed prior to the study. Thick line at upper right-hand corner indicates gravel road. Top left: west block rows 45–46; top right: Southwest Block rows 0–2; middle: southwest block rows 3–21.