

DETECTION OF TWO MITE-PLANT-VIRUS PATHOSYSTEMS IN FLORIDA,  
INCLUDING CHEMICAL ECOLOGY OF *Amblyseius swirskii* and INDUCTION OF  
SYSTEMIC ACQUIRED RESISTANCE ON ROSES

By

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For Liz, Violet, Juniper and Fifes to come

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The invasive *Phyllocoptes fructiphilus* (Trombidiformes: Eriophyidae) is the vector of *Rose rosette emaravirus* (RRV), the causal agent of Rose Rosette Disease (RRD). Growers are interested in more management options to combat this mite-plant-virus pathosystem. Surveys were conducted from 2019-2021 in northern Florida, searching for RRD, *P. fructiphilus*, predatory mites, and other mites on roses in the landscape. As a result, *P. fructiphilus* were discovered for the first time in Florida. Various other unidentified mites were collected, but no RRD was found. Some growers of ornamental roses are interested in use of the predatory mite, *Amblyseius swirskii* Athias-Henriot (Parasitiformes: Phytoseiidae) for biological control of various pests in roses. Olfactometer tests revealed *A. swirskii* attraction to RRV-infested roses. To investigate this attraction, volatile organic chemicals (VOCs) were collected with two different methodologies from the headspace of roses treated in the field and in the lab. Volatiles were collected from roses treated with acibenzolar-S-methyl (ASM, a systemic acquired resistance (SAR) inducer), healthy and RRV-infected roses. The volatiles  $\beta$ -Caryophyllene and  $\gamma$ -Murrolene were found in greater amounts in RRV-infected roses using the solid phase microextraction field method. These chemicals may explain *A. swirskii* attraction to RRV-infected roses. We conducted field trials of ASM, spirotetramat and *A. swirskii* releases on roses in Griffin and Athens, GA, as well as Tallahassee, FL. We found that spirotetramat significantly reduced populations of *P. fructiphilus*. Pruning may also reduce populations of *P. fructiphilus* in non-viruliferous roses. We describe the first detection of *Orchid fleck dichorhavirus*, orchid infecting subgroup (OFV-Orc) infecting three unreported ornamental hosts in Leon and Alachua Counties, FL. Plants from the Orchidaceae, Asparagaceae (Nolinoidae), and Rutaceae can become infected with OFV-Orc. Infection causes citrus leprosis-like symptoms in the Rutaceae. The vector, *Brevipalpus californicus* (Banks) *sensu lato* (Trombidiformes: Tenuipalpidae) were collected and identified from OFV-Orc-infected

plants. Coinfections of two different OFV-Orc strains were detected in both counties. In conclusion, Florida has many plants in the landscape threatened by these mite-plant-virus pathosystems, each of which represent a risk for economic losses for the ornamental plant industry in the southeastern US.

## CHAPTER 1

### LITERATURE REVIEW

#### 1.1 A Small Introduction to Some Herbivorous Acari

Mites and ticks belong to a subclass of small arachnids known as the Acari, an incredibly diverse group of arthropods. Despite their ubiquity, mites and invertebrates in general remain understudied relative to other animal fauna (Grodsky et al. 2015, Rosenthal et al. 2017, Titley et al. 2017). Hoy (2011) speculates that our understanding of mite diversity and abundance may be around 50-100 years behind the taxonomy of the Insecta: The small size and cryptic habits of mites make them easy to overlook, and difficult to observe. In addition, their disputed placement in the Arachnida (Giribet 2018) presents a real challenge for taxonomists (Giangrande 2003). Lastly, many mite species have been misclassified, and cryptic species make taxonomic certainty elusive (Bickford et al. 2007). In spite of these impediments, technological improvements, such as Low-Temperature Scanning Electron Microscopy (Achor et al. 2001, Wergin et al. 2006), Confocal Laser Scanning Microscopy (Chetverikov 2012, Chetverikov et al. 2012), X-ray computed tomography (Dunlop et al. 2011, Facchini et al. 2019), and advances in molecular biology—including high-throughput sequencing—(Dasch et al. 2019), have helped to alleviate the difficulty and ambiguity of mite identification via solely morphological characters. The combination of these techniques allows for greater taxonomic certainty (Chetverikov et al. 2012). The most well known species of mites have gained scientific recognition primarily due to their pest status (Savory 1964, Jeppson et al. 1975, Hoy 2011): many acari are parasites of plants and animals, causing disease and economic losses (Jeppson et al. 1975, Hoy 2011, Walter and Proctor 2013). Even so, the majority of mite species are of no economic importance, and are harmless, or even beneficial: many show promise as biological control agents for weeds and arthropod pests (Gerson et al. 2003, Carrillo et al. 2015). Although the majority of arachnids are predatory, mites are unique in that there are species which feed on plants (Savory 1964). Phytophagy arose at least seven times in the Trombidiformes: Parasitengonae, Tetranychoidea, Raphignathoidea, Heterostigmata, Eupodoidea, Tydeoidea and Eriophyoidea all have species which feed on plants (Lindquist

1999). Phytophagy is thought to be a facultative development for the majority of mite taxa outside of the Tetranychidae and Eriophyoidea; few species of Eupodoidea and Raphignathoidea are obligate herbivores—only *Halotydeus*, *Penthaleus* (Raphignathoidea: Penthaleidae) and *Eustigmaeus* (Raphignathoidea: Stigmeidae (Gerson 1971))—and the other mite groups have few morphological adaptations associated with plant feeding (Krantz and Lindquist 1979, Lindquist 1999). An important development in the evolution of acarine phytophagy is the reduction of the chelicerae into sharp stylets used for piercing plant tissues (Lillo et al. 2018). These styliform mouthparts are thought to reduce damage to the plant in order to avoid some of the toxic chemistry plants use to defend themselves from arthropod feeding (Mullin 1986). Herbivorous mite damage is dependent on the specific mite-plant interactions for a given plant species or cultivar (Petanović and Kielkiewicz 2010a). Mites generally feed on plant epidermal and mesophyll cells (McCoy and Albrigo 1996, Rancic et al. 2006). High mite populations can reduce the amount of chlorophyll available to the plant (Khederi et al. 2018c), primarily causing bronzing/russetting/silvering by direct feeding, but this damage often spreads to the surrounding tissues as the plant's immune system responds (Bensoussan et al. 2016). Mite salivary secretions can also cause a condition known as toxemia, which causes plant tissues to become chlorotic or discolor (Oldfield 1996a). Feeding on young tissues often forms distortions and delays plant growth, and some mites—many eriophyoidea and two species of Tenuipalpidae—form galls on their host plants (Jeppson et al. 1975, Westphal and Manson 1996, Oldfield 2005). The majority of herbivorous mites do not transmit pathogens (Oldfield and Proeseler 1996), but those which do act as vectors of plant viruses principally from two families from the Prostigmata: Eriophyidae and Tenuipalpidae (Slykhuis 1965). Members of these mite superfamilies are obligate herbivores considered to be some of the more ancient lineages of phytophagous mites (Lindquist 1999). There have been singular reports of spider mites—tetranychidae—associated with viruses (Slykhuis 1965, Robertson

and Carroll 1988), but other studies have failed to reproduce similar results (Granillo and Smith 1974). Plant mites are generally considered secondary pests, but often cause significant losses when conditions are optimal, due to the fast reproductive rate of many pest species (Gerson and Cohen 1989, Dutcher 2007).

### 1.1.1 Coevolved plant specialists: the eriophyoidea

The eriophyoidea are the second most economically-important group of herbivorous mites, right behind Tetranychidae. Although eriophyoids may be less damaging than the tetranychidae, they are one of the most specious groups of plant-feeding mites: It is estimated that the 2,838 species reported in the “Catalog of the Eriophyoidea of the World” represent only about 10% of the total number of species which exist (Amrine and Stasny 1994). Publications of new species descriptions of eriophyids averaged about 70 per year from 1996 to 2007, and have greatly increased since the publication of *Eriophyoid Mites – their Biology, Natural Enemies and Control* (Lindquist et al. 1996), a landmark publication for the field of eriophyoid studies. Eriophyids range in size from 80-500 µm long (Nuzzaci and Lillo 1996), and seem to have evolved specifically for plant feeding (Krantz and Lindquist 1979, Oldfield and Proeseler 1996, Lindquist 1999, Skoracka and Dabert 2010, Lillo et al. 2018): they have styliform chelicera covered with a protective sheath (Lindquist 1999, Bolton et al. 2018), elongate vermiform bodies, and adult mites have a reduced number of legs from the typical eight to four (Lindquist 1996). The stylets of eriophyids are short,  $\leq 20 \mu\text{m}$  (Oldfield and Proeseler 1996), which primarily limits their feeding to epidermal cells, with young/tender meristematic tissues being preferred (Petanović and Kielkiewicz 2010a, 2010b). Feeding is thought to rely on enzymes in their saliva to break down plant cells contents and feed on the resulting soup (Hoy 2011). Eriophyoid mites also have an abbreviated lifecycle, progressing from egg, to larva, followed by nymph, then adult (Kassar and Amrine Jr 1990, Manson and Oldfield 1996). Many eriophyoid mites have summer forms (protogynes) which readily proliferate and winter forms (deutogynes). Deutogynes are thought to be particularly adapted to withstand the

extreme conditions which may be encountered during long-distance dispersal (Zhao and Amrine 1997, Valenzano et al. 2019). Dispersal is thought to be a high-risk activity for eriophyids (Kiedrowicz et al. 2017), especially if mites land on a non-host plant, where they may not be able to feed or reproduce (Marini et al. 2021). Eriophyid mites are not able to disperse very far by walking (Calvet et al. 2020), but are thought to primarily disperse passively on the wind (Sabelis and Bruin 1996, Galvão et al. 2012, Kuczyński et al. 2020, Majer et al. 2021). Mites also can be transported on infected plants, move on contaminated equipment or clothes (Duffner et al. 2001), or rarely move by phoresy on other animals (Gibson and Painter 1957, Galvão et al. 2012, Li et al. 2018). Eriophyoids also exhibit unique behaviors though to be correlated with dispersal (Nault and Styler 1969): in response to increased windspeed or changes in other climatic conditions (Bergh 2001), mites will sometimes form chains (Laska et al. 2019), move to the tips of plants (Sabelis and Bruin 1996), or stand erect on their hind anal sucker (Nault and Styler 1969, Michalska et al. 2009, Kiedrowicz et al. 2017), possibly in order to ‘jump’ as described by Ozman and Goolsby (2005). Eriophyid lifestyles are generally sorted into leaf vagrants/rust mites and galling/bud forms, with rust mites being fusiform and living on the plant surfaces, while bud and gall mites tend to have more cryptic lifestyles, as suggested by their names (Hoy 2011). The majority of eriophyids studied to date are considered host specific, limited to feeding on a single genus, or one host plant. (Oldfield and Proeseler 1996, Lillo et al. 2018). The few mite species which appear to have a broader host range may be misidentified cryptic species (Navia et al. 2012, Skoracka et al. 2013), a hypothesis strengthened by their limited ability to disperse and close associations with their host plants (Magalhães et al. 2007). These lifestyles and feeding habits of the Eriophyoidea are thought to have shaped both their relationships with their host plants and their ability to transmit pathogens (Mauck et al. 2012, Biere and Bennett 2013): Eriophyid mites represent the majority of mites involved in virus transmission to plants Lillo et al. (2018). In the absence of an infectious agent, most damage from the feeding of vagrant forms of

eriohyids is superficial and causes minimal damage to their host plant (Krantz and Lindquist 1979, Oldfield and Proeseler 1996). To date, eriohyids have only been associated with plant viruses (Lillo et al. 2018), which may be explained by the small size of eriohyoid mouths and foreguts, which preclude the acquisition and circulation of large pathogens (Oldfield and Proeseler 1996). Although these developments can be considered an evolutionary advantage for the Eriophyoidea, it puts the lifestyles of these minute arachnids in direct conflict with the interests of modern agriculture, and motivates the need for management of mite populations.

### 1.1.2 *Phyllocoptes fructiphilus*: the vector of *Rose rosette emaravirus*, the causal agent of Rose Rosette Disease

*Phyllocoptes fructiphilus* Keifer, (Acari: Trombidiformes: Prostigmata: Eriophyoidea: Eriophyidae) is an eriohyoid mite from the Prostigmata group. Like many other eriohyoids, the relationships *P. fructiphilus* has with its host and virus are very specific, and create problems for growers (Krantz and Lindquist 1979, Oldfield and Proeseler 1996): *P. fructiphilus* only feeds on plants in the genus *Rosa* (roses). *P. fructiphilus* does not cause much noticeable feeding damage, but instead causes injury due to its relationship with a virus known as *Rose rosette emaravirus* (RRV) *Emaraviridae* (Allington et al. 1968, Tzanetakis et al. 2006, Laney et al. 2011, Pemberton et al. 2018). *P. fructiphilus* transmits RRV while feeding on the rose epidermis (Allington et al. 1968). A single mite is enough to transmit RRV, and can inoculate a rose in less than an hour (Di Bello et al. 2017). Infection creates the following symptoms: clusters of deformed flowers known as rosettes/witches' brooms, increased thorniness, elongated shoots, reddened leaves and stems, and increased cane die-back which ultimately kills the rose host (Epstein and Hill 1995). This disease is known as Rose Rosette Disease (RRD) and is the most serious disease of roses threatening the ornamental rose industry (Rwahnih et al. 2019). Roses represent a wholesale value of over \$151.5 million USD, and the southeastern region produces 39% of this revenue (Babu et al. 2014, White and Klingeman 2014). RRD was

first described in North America in 1941 from an outbreak in Manitoba, Canada (Conners 1941). *P. fructiphilus* later became recognized as the vector for RRV (Allington et al. 1968, Doudrick et al. 1986, Jesse et al. 2006) and RRV was eventually confirmed to be the casual agent for the RRD (Doudrick et al. 1987, Tzanetakis et al. 2006, Laney et al. 2011, Bello et al. 2015, Dobhal et al. 2016, Di Bello et al. 2017). The mite and virus were generally associated with invasive multiflora rose, *Rosa multiflora* (Thunb) (Amrine Jr 1996, 2002, Otero-Colina et al. 2018) and spread along with the rose throughout the central US (Crowe 1983), and the east (Hindal et al. 1988). Initially the relationship between *R. multiflora* and the pathogen was considered as a type of natural biological control (Epstein and Hill 1999), and some studies even considered artificially spreading viruliferous *P. fructiphilus* to eradicate these invasive roses (Tipping and Sindermann 2000). The relationship between, *R. multiflora*, mite and virus also has a positive influence on *P. fructiphilus* fecundity: Solo et al. (2019b) reported 46 times more *P. fructiphilus* per gram of rose tissue and Epstein and Hill (1999) reported a 17-fold increase in the mite population of diseased roses compared to uninfected plants. Like many other species of plant-feeding mites, *P. fructiphilus* reproduce via arrhenotokous parthenogenesis (Oliver 1971), meaning that unfertilized eggs become male, while fertilized eggs become female (Oldfield and Michalska 1996). *P. fructiphilus* grows from egg to adult in 11 days (Kassar and Amrine Jr 1990), which allows a single female to quickly initiate a new colony without being fertilized *a priori* to dispersion (Helle and Wysoki 1996). Together, these factors likely contribute to the dispersal ability of *P. fructiphilus*. Mite and virus spread through the landscape in various ways: RRV can be transmitted by grafting (Doudrick et al. 1987) and the mites can crawl from plant to plant or be blown by the wind over long distances (Zhao and Amrine 1997, Zhao and James 1997, Michalska et al. 2009). Unfortunately, *P. fructiphilus* and RRD have the ability to infest commercial rose cultivars as well (Epstein and Hill 1995, Byrne et al. 2018, Solo et al. 2019a), and can be spread by humans moving infested plants (Navia et al. 2009). Interceptions of RRD into Florida occurred in 2013,

when RRV-infected Knock Out® roses were received from out of state by a nursery in Gadsden county ([Babu et al. 2014](#)). No *P. fructiphilus* were detected at the time, and the roses were quarantined and destroyed ([Babu et al. 2014](#)). A survey for *P. fructiphilus* and RRD in the southeastern US ([Solo 2018, Solo et al. 2020](#)) found both mite and virus to be present in southern Georgia, and *P. fructiphilus* was recently detected in northern Florida ([Fife et al. 2020](#)). The presence of *P. fructiphilus* and the possibility of RRD invasion into the southeast emphasizes the need to monitor for and manage RRD to prevent its establishment in these rose growing regions.

## **1.2 Integrated Pest Management: Best Practices for Modern Agriculture**

Modern agriculture relies heavily on the concepts of Integrated Pest Management (IPM), a science-informed approach to pest control based on integrating as many different types of control to keep pest populations underneath their economic injury level (EIL) ([Stern et al. 1959, Flint and Bosch 1981](#)). The EIL is a breaking point, where the cost of controlling the damage from pests exceeds the costs of crop production ([Stern et al. 1959](#)). This EIL is informed by an economic threshold, a point where a specific pest density has been exceeded, and interventions are required to prevent the crop from reaching the EIL ([Stern et al. 1959](#)). The EIL is of a crop is more difficult to determine when a pathogen is involved, partially because many techniques to control pathogens are primarily preventative in nature ([Pedigo et al. 1986](#)). With this goal of prevention in mind, plant pathologists have created the concept of the disease triangle to emphasize the importance of convergence between pathogen, environment and host for disease proliferation to occur ([Gäumann 1950, McNew 1960, Dimond and Horsfall 1960, Agrios 2004](#)). Extensions of this basic disease triangle have been considered, including adding extra dimensions to account for more variables ([Franci 2001](#)), or applying it to different systems ([Scholthof 2006](#)). Even so, the basic tenants of the disease triangle prevail in that altering one of these main factors of disease (host, pathogen or environment) potentially can prevent proliferation of a disease ([Dumroese 2012](#)). Management further subdivides pest interventions into four main



Figure 1-1. Typical symptoms of Rose Rosette Disease (RRD), caused by *Rose rosette virus*: clusters of deformed flowers known as rosettes/witches' brooms, increased thorniness, elongated shoots, reddened leaves and stems. RRD ultimately kills the rose host.

categories: chemical, mechanical, cultural (environmental), and biological control methods (Bradley and Moore 2018). Chemical controls are an effective way to quickly control pest outbreaks, but pesticides have many drawbacks as well: they pose a risk to the applicator (Alavanja et al. 2004), harm beneficial insects/pollinators, leave residues on crops meant for consumption, and can harm the environment through runoff/drift (Fenner et al. 2013), and polluting surface and groundwater (Driesche et al. 2007, Marquina and Ferre 2010). Pesticides also can create secondary pest outbreaks (Gerson and Cohen 1989) and pest resurgence by killing natural predators in the environment (Driesche et al. 2007), while encouraging pesticide resistance in surviving pest populations (Dutcher 2007, Ciancio and Mukerji 2007). Even so, chemical controls remain useful when used judiciously in an IPM program (Dent 2000, Driesche et al. 2007). Mechanical control is the physical manipulation of plants to prevent pests and pathogens. Mechanical controls include pruning, raking, tilling, removing pests and infected plants (rouging) by hand, creating physical barriers such as raised beds, insect screens, organic or plastic/reflective mulch, solarizing/heating the soil, sticky traps/barriers, etc. (Ciancio and Mukerji 2007, Bradley and Moore 2018). Cultural control has some overlap with mechanical control methods, but cultural controls tend to refer to controlling the environment of your crops to exclude pests. Some examples include: selecting resistant cultivars, cultivating and planting in healthy soil, managing weeds, interplanting/trap cropping, aerating the soil, choosing appropriate planting dates, rotating crops, letting the field lie fallow, and etc. (Ciancio and Mukerji 2007, Bradley and Moore 2018). Biological control in the classical sense relies on reintroducing the various biological entities which keep pest populations in check in their natural/native environments (Heimpel and Mills 2017, Hajek and Eilenberg 2019). This concept is based off of the enemy release hypotheses from invasion ecology, which hypothesizes that introduced pest populations flourish because they have no natural enemies present to control their growth (Liu and Stiling 2006, Heger and Jeschke 2014). The corollary being that biological control agents such as parasitoids, predators, herbivores and pathogens can

injure pest populations sufficiently to provide control (Heimpel and Mills 2017, Hajek and Eilenberg 2019). One of the primary benefits of biological control is that these natural enemies can become established in the environment, creating long term control while adapting to fluctuations in pest populations over time (Hajek and Eilenberg 2019).

Biological control has found success in a variety of natural and agricultural environments, protecting crops against insect pests and combating invasive weed species (Driesche et al. 2010). In conclusion, implementation of these different control methods has potential to interfere with the proliferation of pests and pathogens in a variety of cropping systems.

### **1.2.1 Current management of Rose Rosette Disease is not effective**

There are few IPM methods which have been studied that are effective for managing RRD and/or *P. fructiphilus* (Windham et al. 2014). Currently, all roses are known to host *P. fructiphilus*, and few roses show signs of resistance to RRV (Di Bello et al. 2017, Byrne et al. 2018). Mechanical control via pruning and sanitation have not proven to be effective (Olson et al. 2017). The use of windbreaks has been suggested to reduce the number of mites landing on roses (Windham et al. 2014), but few effective control methods exist (Windham et al. 2014). Currently, rose growers, gardeners, and nursery managers have been recommended to rogue sick plants and spray acaracides to keep mite populations low (Hong et al. 2012, Olson et al. 2017, “Control - rose rosette” 2018). These methods are likely unsustainable in the long term, because although eriophyid mites are often controlled via chemical means (Messing and Croft 1996, Leeuwen et al. 2009), a handful of eriophyoid species have developed resistance to some acaricides. For example, some populations of *Phyllocoptrus oleivora* (Ashmead) and *Acalitus vaccinii* (Keifer) are reportedly resistant to dicofol (Omoto et al. 1994, 1995), while *Aculus cornutus* (Banks) and *Aculops lycopersici* (Tryon) have developed resistance to various organophosphates (Baker 1979, Abou-Awad and El-Banhawy 1985). In addition, to date there is limited information regarding the toxicity and effectiveness of acaricides used to combat *P. fructiphilus* (Windham et al. 2014). Pesticide applications are further complicated by the biology of the mite: *P.*

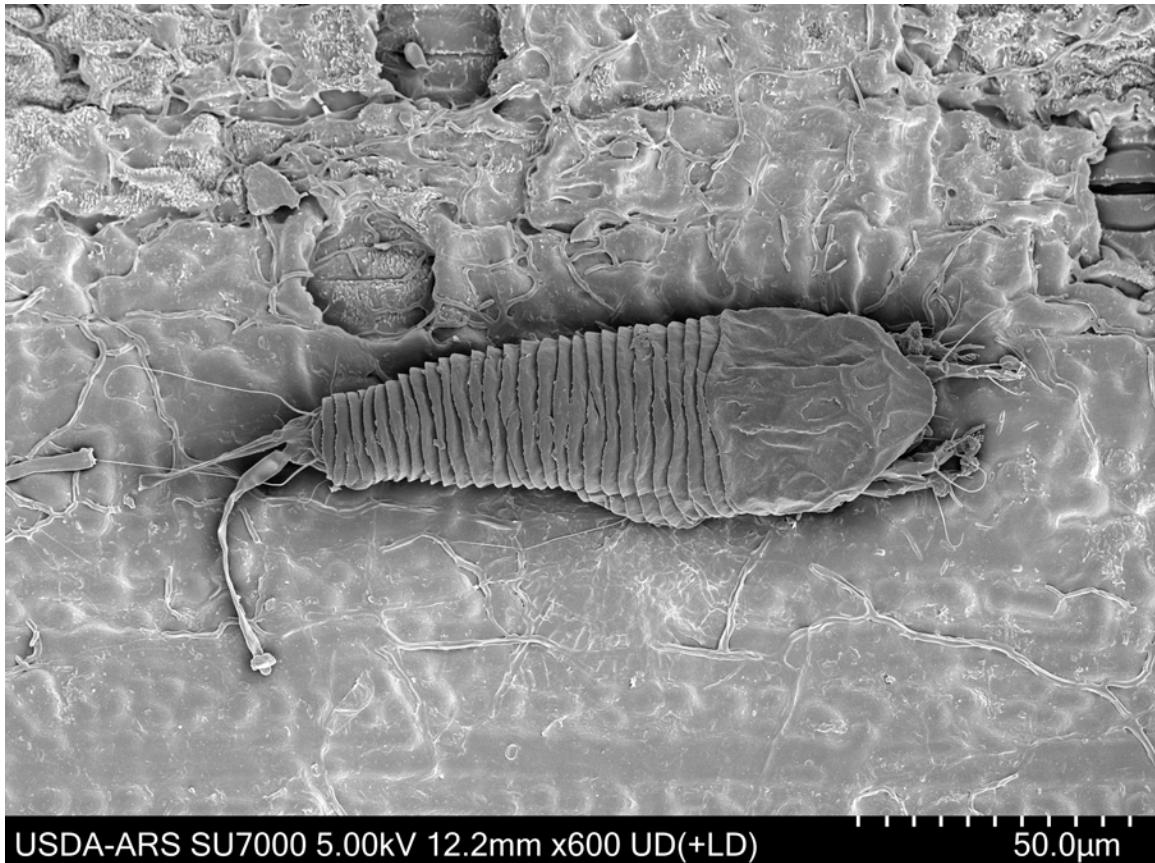


Figure 1-2. Cryo-SEM image of Eriophyid mite infected with unidentified fungus, collected from *Liriope muscari*., Photo Credit: Dr. Gary R. Bauchan, USDA-ARS, 2020.

*fructiphilus* are a refuge-seeking species of eriophyoids which prefer to feed on the small plant hairs (trichomes) on the sepals, underneath the petals (Amrine and Stasny 1994, Jesse et al. 2006, Lillo et al. 2018, Otero-Colina et al. 2018, Bauchan et al. 2019). The petals, sepals, and trichomes likely help shield the mites from conventional pesticide treatments (Xu et al. 2011), and some types of trichomes can protect the eriophyids from their natural enemies (Karioti et al. 2011). This issue of mite management is exacerbated by the fact that a single mite is potentially enough to transmit the virus (Di Bello et al. 2017), which they can do within an hour of feeding on a rose. After initial infection, roses may remain asymptomatic for months before the disease is noticed (Amrine Jr 1996, Di Bello et al. 2017). In addition, identification of RRD infections is error-prone when based on morphological characters, as early signs of RRD are easily confused with natural plant growth or herbicide damage (Ong et al. 2018), making it hard to diagnose in the field without further testing (Hong et al. 2012, Ong et al. 2018). Molecular methods for testing RRV are becoming readily available Di Bello et al. (2017), and newer technologies, such as Raman spectroscopy (Farber et al. 2019) are being developed which may be able to rapidly detect RRV, but it remains to be seen if these methods are capable of identifying asymptomatic infections or if these tests are suitable for disease monitoring on larger scales. The lack of management options for mites, as well as the increased cost of rose production due to RRV make it difficult for growers to compete with an increasingly competitive international market. Rose growers need better methods to combat *P. fructiphilus* and RRV.

### **1.2.2 Small phytoseiid mites could be an option for biocontrol if discovered**

Eriophyoid mites are hard to control via conventional methods (i.e. pesticides) due to their small size and their cryptic habits, so alternative methods of pest control are being investigated. There are currently no known natural enemies associated with *P. fructiphilus*, but surveys of eriophyoid-infested plants sometimes encounter predatory mites which may be suitable for biological control (Lawson-Balagbo et al. 2007b, Picoli et al. 2010, Azevedo

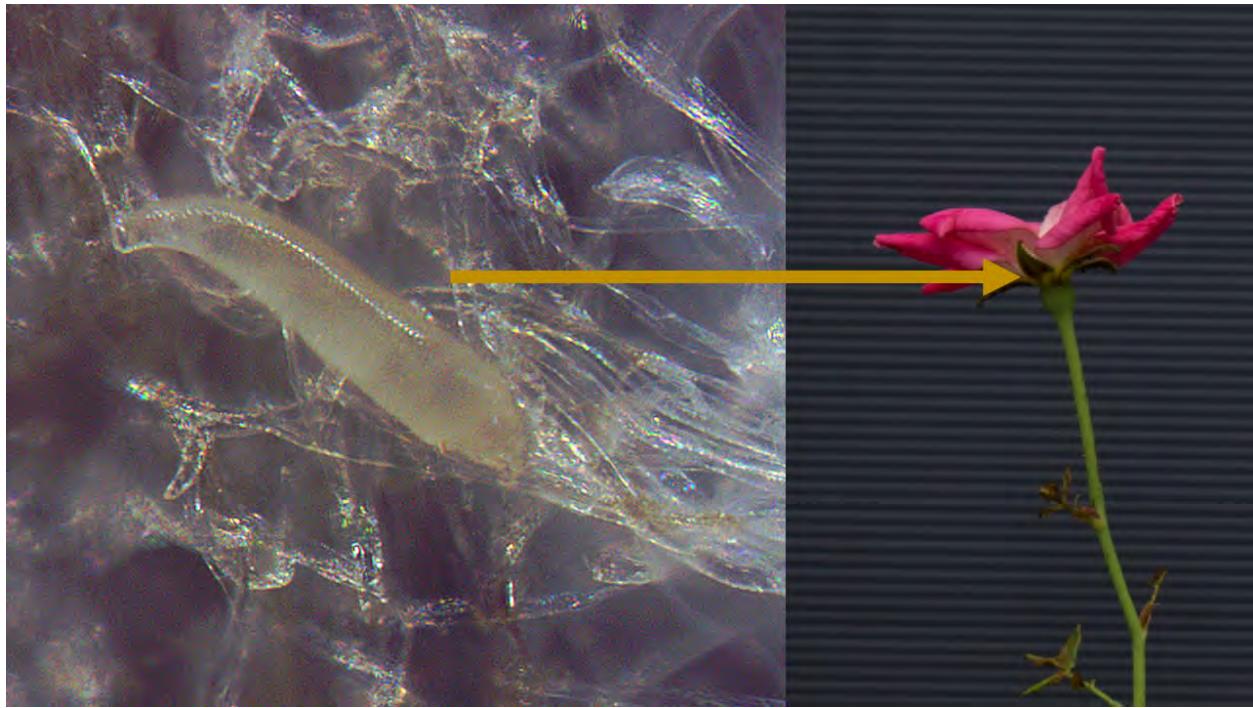


Figure 1-3. Illustration of the typical location of *Phyllocoptes fructiphilus* on roses. *P. fructiphilus* are difficult to manage with pesticides due to the protection offered by the sepals.

et al. 2016). The most well-studied family of predatory is the Phytoseiidae (Gerson et al. 2003, Farragut et al. 2010, Carrillo et al. 2015), which are sometimes released as biological control agents for thrips, whiteflies, spider mites (Tetranychidae), flat mites (Tenuipalpidae), scale insects and other pests (Gerson et al. 2003, Chen et al. 2006, Arthurs et al. 2009, Carrillo et al. 2011a, Carrillo and Peña 2011, Doğramacı et al. 2011, Sarwar 2017, Knapp et al. 2018, Argolo et al. 2020). Phytoseiid mites are generally associated with plants (Farragut et al. 2010), and do not survive well without them (Jung and Croft 2000). Plant structures affect many aspects of a phytoseiid's life (Cortesero et al. 2000, Schmidt 2013), influencing dispersal (Buitenhuis et al. 2013, Lopez et al. 2016), as well as performance as predators (Cédola et al. 2001, Seelmann et al. 2007, Buitenhuis et al. 2013). Some species such as *Neoseiulus paspalivorus* (De Leon) (Lawson-Balagbo et al. 2007a, Neglo et al. 2011, Lesna et al. 2014) and *Phytoseius intermedius* Evans & MacFarlane (Picoli et al. 2010, Azevedo et al. 2016) are small enough that they can feed

on refuge-seeking eriophyid mites hiding in difficult to reach areas, such as in erinea or under plant bracts/perianths (Lawson-Balagbo et al. 2007c, Azevedo et al. 2016, Silva 2016). Studies of predatory mites associated with roses can hopefully encounter a similar mite species able to find its way under the sepals, petioles, and glandular plant hairs (trichomes) to feed on hiding *P. fructiphilus* and provide some control (Howard and Rodriguez 1991, Jesse et al. 2006, Otero-Colina et al. 2018, Bauchan et al. 2019). However, prey-seeking on a large plant is a non-trivial task, accordingly, many species of mites learn to find their hosts or prey by relying on Volatile Organic Compounds (VOCs) to guide them released when plants are injured by pests or infected with pathogens, and use these VOCs to locate their prey (Sabelis et al. 1999, Maeda and Takabayashi 2001, Boom et al. 2002, Boer and Dicke 2004a, 2004b, 2005). The chemistry of VOCs varies depending on plant species, and can create many different effects on the arthropods which inhabit the affected plant: VOCs can repel (Moraes et al. 2001, Pichersky and Gershenson 2002, Agut et al. 2018) attract (Nomikou et al. 2005, Gadino et al. 2012, Avellaneda et al. 2019), encourage predation (Kessler and Baldwin 2001, Halitschke et al. 2007), or poison arthropods (Vancanneyt et al. 2001). By manipulating the VOCs which natural enemies or pests encounter on roses, we may be able to inform future research into push-pull strategies (Cook et al. 2007), improve retention of predators at release points, a common issue in mite biocontrol (Buitenhuis et al. 2010, 2013, 2015).

### **1.3 Induced Plant Defenses - Can Systemic Acquired Resistance Reduce Mite Herbivory?**

Plants are primarily sessile organisms which aren't able to run or hide, therefore undefended plants struggle to grow in the face of the constant threat of herbivory (Kessler et al. 2004). In order to combat being eaten, plants rely heavily on their ability to protect themselves *in-situ*, via a myriad of different physical and chemical defenses (Walling 2000). These defenses are categorized as either constitutive defenses or induced defenses (Farmer 2016). Constitutive defenses are always 'on', being produced by the plant constantly, such as tannins and latex, while inducible defenses rely on some sort of signal before the plant

will produce them. Physical defenses of herbivory includes spines, prickles, thorns, glandular trichomes, latex, sclereids, epicuticular wax, bark, thick cell walls, and compensatory growth to prevent tissue damage while increasing wear on herbivore mouthparts ([Farmer 2016](#)). In addition to these physical barriers to herbivory, plants are also efficient chemical factories which produce a bevy of secondary plant metabolites, including inhibitory proteins, enzymes, and toxins which reduce palatability of plant tissues, prevent uptake of essential amino acids, or kill the herbivore outright ([Farmer 2016](#)). It is hypothesized that inducible defenses must have evolved in response to threats that were sporadic in nature, but strong enough to necessitate a response ([Edelstein-Keshet and Rausher 1989](#), [Tollrain and Harvell 1999](#)). The idea is that inducible defenses allow plants a type of cost-saving for their limited resources (Optimal Defense Theory, [Steppuhn and Baldwin \(2008\)](#); [Adler and Karban \(1994\)](#)), or to avoid damaging themselves with the compounds used ([Steppuhn and Baldwin 2008](#)). Otherwise, the evolution of constitutive defenses would seem to be a better option for plant defense ([Karban and Myers 1989](#), [Järemo et al. 1999](#)). A corollary of the optimal defense theory is that inducible defenses should have cues that trigger dependably, accurately and be an effective deterrent once activated, so as to avoid opportunity costs ([Berenbaum and Zangerl 1999](#), [Järemo et al. 1999](#)). Induced chemical defenses are thought to have an added benefit of being faster and less costly for plants to produce than other types of defense, such as developing spines or thicker cell walls ([Berenbaum and Zangerl 1999](#), [Järemo et al. 1999](#)). Even so, chemical defenses still have drawbacks in allocation costs: plants investing energy into defenses are not using those resources for growth or reproduction ([Berenbaum and Zangerl 1999](#)). There are also probably genetic tradeoffs to keep inducible costs active rather than other essential plant functions, and there may be ecological compromises as well: adaptation to one form of defense may preclude the use of another ([Berenbaum and Zangerl 1999](#)). Another confounding factor with induced defenses occurs in the presence of specialist herbivores, many of which are especially adapted to overcoming a particular plant defense

(Ehrlich and Raven 1964, Schoonhoven et al. 2005, Farmer 2016). Järemo et al. (1999) considered the development of systemic responses to be more probable if plant defenses required larger doses for deterrence, and posited that localized responses to herbivory are benefit the plant when small amounts of initial damage are a reliable cues of larger damage to come. This framework readily considers the feeding activities of stylet feeders like mites, whose initial damages are minimal, but quickly accelerate due to mites' fast population growth rates. Accordingly, plants need to be responsive and accurate when identifying a threat before a defense can be mounted. Plants rely on pattern recognition receptors (PRRs) (Couto and Zipfel 2016), to detect pathogen-associated molecular patterns (PAMPS) (Boller and Felix 2009), and herbivore-associated molecular patterns (HAMPs) (Mithöfer and Boland 2008), molecules released from attacking pathogens and herbivores, respectively. These PRRs are part of innate plant immunity: PAMP-triggered immunity and effector-triggered immunity (ETI) (Chisholm et al. 2006, Jones and Dangl 2006). Plant cell-surface receptors detect common pathogen molecules, such as flagellar proteins, chitin and ergosterol. If activated PTI typically stops further invasion, by depositing callose at the site of infection, releasing reactive oxygen species (ROS), mitogen-activated protein kinases (MAPK, Howe and Jander (2008)), and inducing pathogen-responsive genes. If this first line of defense is surpassed, ETI has evolved to identify the proteins used to overcome PTI, by detecting pathogen effectors with the *R* proteins encoded by the corresponding *R*-genes in the plant (Boller and Felix 2009). One of the most well known effects of activating ETI is the hypersensitive response (HR), rapid localized cell death/necrosis at sites of infection. The HR also activates pathogenesis-related (PR) genes and upregulates intercellular Salicylic Acid (SA), which converts to the VOC, Methyl Salicylate (MeSA), a signal which propagates resistance throughout the plant. The increased expression of PR genes primes the plant for resistance to future attacks, via a process known as systemic acquired resistance (SAR) (Boller and Felix 2009, Vlot et al. 2009, Zhang et al. 2010). HAMPs work in a similar way to PAMPs, but instead of

detecting molecular patterns associated with pathogens, they detect molecules associated with herbivores, such as arthropod oral secretions, eggs, pheromones, or other chemicals conserved across a wide range of arthropods (Mithöfer and Boland 2008). Plants respond to triggered HAMPS in similar ways: they also trigger ROS, MAPKs, and  $\text{Ca}^{2+}$  influx at the site of injury (Vincent et al. 2017). A second important plant defense is the Jasmonic Acid (JA)-Ethylene (ET) signalling pathways. The JA/ET pathways are activated when JA upregulates in response to wounding and/or arthropod damage, and provides protection from herbivory as well as pathogen damage (Thaler et al. 2001, Farmer et al. 2003, Guo and Ecker 2004, Glazebrook 2005, Howe and Jander 2008). Plants can be primed directly through application of SA, MeSA or even synthetic chemical analogues, such as acibenzolar-S-methyl (ASM) to activate SAR (Anfoka 2000, Conrath et al. 2006, Vlot et al. 2009, Zhang et al. 2010, Jeschke 2015). Exogenous applications of ASM can protect plants in the same way as SA applications, by activating the SAR pathway, reducing disease incidence and severity of pathogenic fungi (Ziadi et al. 2001, Tripathi et al. 2010, Jeschke 2015), bacteria (Romero et al. 2001), phytoplasmas (Ratchaseema et al. 2021), and viruses (Anfoka 2000, Babu et al. 2021, Doungous et al. 2021). ASM application (SAR-induction) has also been noted for its ability to promote wound healing in potatoes (Jiang et al. 2019), and control whiteflies (Correa et al. 2005, Doungous et al. 2021) as well as aphids (Costa and Moraes 2006). Taken together, induced plant defenses offer an important potential role in the prevention of disease and control of insect pests.

### 1.3.1 Effects of Systemic Acquired Resistance on eriophyoid mites

Induced plant defenses have been studied somewhat for their effects on eriophyoid and other herbivorous mites: Bronner et al. (1991a), Bronner et al. (1991b), Westphal et al. (1991), and Westphal et al. (1992) observed that feeding by the gall mite *Aceria cladophthirus* (Nalepa) activated induced defenses, triggering the hypersensitive response in *Solanum dulcamara*. As part of SAR-induction these plants increased their levels of  $\beta$ -1,3-glucanase and chitinases (Bronner et al. 1991b, Ward et al. 1991, Bhardwaj et al.

2021), enzymes which together help to prevent fungal disease development (Mauch et al. 1984, 1988, Goy et al. 1992, Xue et al. 1998, Narusaka et al. 1999, Suo and Leung 2001, Anguelova-Merhar et al. 2008, Bhardwaj et al. 2021, Rajninec et al. 2021). Similar responses have been seen in grapes (*Vitis vinifera* L.) attacked by the erineum mite *Colomerus vitis* Pagenstecher (Khederi et al. 2018a), at least suggesting that the SAR-induction is not solely limited to the Solanaceae, and likely occurs in many eriophyid-plant systems. Induced plant defenses show promise for interfering with establishment of these mites: Experiments by Westphal et al. (1991) found that *S. dulcamara*'s SAR response to *A. cladophthirus* produced 40-day protection from subsequent colonization by more *A. cladophthirus* or another eriophyid, *Thamnacus solani* Boczek and Michalska, a rust mite. Westphal et al. (1992) continued to demonstrate that these induced responses were not enough not protect the plant from *Tetranychus urticae* Koch, but instead increased their fecundity, possibly due to negative cross-talk between SA and JA that is found in some plant systems (Baldwin et al. 1997, Belliure et al. 2010, Thaler et al. 2012). For example, Warabieda et al. (2020) observed that JA helped increase plant resistance to *T. urticae*, but applying JA and ASM together was less effective than applying JA alone, due to SA interference with JA pathways. Other studies have found opposite effects: Favaro et al. (2019) reported reduced numbers of *T. urticae* on strawberries post SA induction, and Khederi et al. (2018b) found that SA and JA induction was sufficient to control erineum forming *Colomerus vitis* (Pagenstecher). David et al. (2021) showed how damage from chewing herbivores and exogenous applications of JA reduced gall formation, but did not significantly reduce populations of the eriophyid mite, *Floracarus perrepae* Knihinicki and Boczek (David et al. 2021). This type of inconsistency is best explained by the results of Kant et al. (2007), which found examples of interspecific variation of *T. urticae*'s ability to induce—and resist—JA defenses. Furthermore, there have been a number of cases where mites have been known to avoid or prevent upregulation of plant defenses entirely: Both *T. urticae* and *Aculops lycopersici*

Massee, have been reported to suppress the JA pathways without relying on antagonistic cross-talk between the responses (Sarmento et al. 2011, Alba et al. 2014), instead by suppressing downstream accumulation of JA (Alba et al. 2014, Glas et al. 2014). Glas et al. (2014) observed that *A. lycopersici* would still induce SA defenses, while an inducer species (Kant et al. 2007) of *T. urticae* feeding on tomato (*Solanum lycopersicum*) induces both JA and SA pathways, but when both mites were introduced to the same plant, the JA response plummeted and SA doubled (Glas et al. 2014). This caused *A. lycopersici* populations to suffer while the *T. urticae* populations benefited from *A. lycopersici*'s reduction of JA (Glas et al. 2014). Vectors of plant pathogens create a similar struggle for their host plants by manipulating and suppressing the SA and JA/ET pathways for their mutual benefits (Agrawal and Karban 1999, Belliure et al. 2010). An example can be seen in the interactions of *B. yothersi*, the vector of *Citrus leprosis virus C* (CiLV-C): infection of *Arabidopsis thaliana* and *Citrus* species with CiLV-C induces SA and suppresses JA/ET pathways through crosstalk (Arena et al. 2016). Another study of *B. yothersi* feeding on *A. thaliana* was similar in result: mite feeding triggered both SA and JA/ET pathways, but *B. yothersi* reared on mutant *A. thaliana* with no SA response had lower fecundity (Arena et al. 2018), suggesting that *B. yothersi* rely on inducing SA to antagonize JA production. Inducing plant defenses can have negative consequences for the predators as well as the herbivores present in the system (Pappas et al. 2017): Ataide et al. (2016) observed that inducing a plant JA pathway reduced *T. urticae* and *T. evansi* mite performance, but also negatively affected ovophagy by *Phytoseiulus longipes*, which ate fewer eggs from mites living on induced plants (Ataide et al. 2016), slowing their reproductive rate. It is also possible that the differences feeding methods between mite species is creating different defense responses, as has been seen in other arthropod groups (Zarate et al. 2006, Zhang et al. 2009, Arimura et al. 2011). In conclusion, this type of interplay between induced defenses is common, sometimes host-specific, and can vary by plant species or cultivar

(Boom et al. 2004, Anguelova-Merhar et al. 2008, Petanović and Kielkiewicz 2010a). Plant defensive responses also change across life stages (Sobral et al. 2021), meaning that plant-pathogen-arthropod system should carefully examined mechanistically to better understand the multitrophic interactions at play (Turlings and Erb 2018).

### 1.3.2 Improving the staying power of biological control: Why are *Amblyseius swirskii* leaving rose patches?

One of the more popular species of commercially-available predatory mite is *Amblyseius swirskii* Athias-Henriot (Calvo et al. 2014). *A. swirskii* tolerate shipping well (Lopez and Smith 2016) and are often sold packaged in vermiculite or in sachets with wheat bran which allows the mites to slowly release into the environment (Buitenhuis et al. 2014, Calvo et al. 2014, Addesso et al. 2018). *A. swirskii* do not feed on leaves (Nomikou et al. 2003), and are considered a generalist predator capable of surviving on artificial diets (Nguyen et al. 2013), natural or supplemental pollen (Loughner et al. 2011, Park et al. 2011, Delisle et al. 2015, Ghasemzadeh et al. 2017), or a variety of other microarthropods present in the natural environment (Janssen and Sabelis 2015, Kumar et al. 2015).

Furthermore, *A. swirskii* have been tested for their compatibility with various combinations of other biocontrol agents, such as predatory mirids, minute pirate bugs, and *Beauveria bassiana* (Chow et al. 2010, Midthassel et al. 2016, Bouagga et al. 2018). Certain modern pesticides and reduced risk-fungicides had relatively low toxicity to *A. swirskii* while other agrichemicals increase mortality or reduce fecundity (Gradish et al. 2010, Masui et al. 2014, Fernández et al. 2017, Ersin et al. 2020, Havasi et al. 2021). Agrichemical products should be tested for their compatibility with biocontrol agents before they are utilized in a pest management program. *A. swirskii* have been used to control a variety of pests on greenhouse vegetable crops, including thrips (Calvo and Belda 2007, Messelink et al. 2008, Arthurs et al. 2009, Calvo et al. 2010), whiteflies (Nomikou et al. 2001, Nomikou et al. 2002, Calvo and Belda 2007, Messelink et al. 2008, Calvo et al. 2010), *Polyphagotarsonemus latus* (Banks) (Maanen et al. 2010, Onzo et al. 2012), and to a lesser

extent, *T. urticae* (Momen and Elsaway 1993, Messelink et al. 2009, Xu and Enkegaard 2010). It is difficult for *A. swirskii* to enter into the webbing of *T. urticae* (Messelink et al. 2009), and when given the choice between *Frankliniella occidentalis* (Pergande) and *T. urticae* as prey, about twice as many *F. occidentalis* were eaten (Xu and Enkegaard 2010). Although *A. swirskii* apparently do not prefer *T. urticae* they have been reported to feed on eriophyid mites: Momen and Abdel-Khalek (2008) found high fecundity and net reproductive rate for *A. swirskii* feeding on *Aculops lycopersici* (Massee), and Momen and Elsaway (1993) reported faster reproduction and development when *A. swirskii* fed on *Eriophyes dioscoridis* Soliman and Abou-Awad. The same study reported that *A. swirskii* preferred *A. lycopersici* over either *T. urticae* or castor bean pollen *Ricinus communis* (L.). Similarly, Park et al. (2010) showed increased oviposition and reproductive rates for *A. swirskii* feeding on *A. lycopersici*. They also saw more feeding on *A. lycopersici* than cattail (*Typha latifolia* [L.]) pollen, and very slight preference for *A. lycopersici* feeding over the common thrips species they had present in their study (*F. occidentalis* and *Thrips tabaci* Lindeman) (Park et al. 2011). Although the aforementioned thrips, whiteflies and mites are serious pests of ornamental greenhouse crops, predatory mites such as *A. swirskii* are seldom used (Buitenhuis et al. 2015). A number of studies have tested *A. swirskii* in roses for their suitability to control thrips, greenhouse whitefly and *T. urticae* (Chow et al. 2010, Hoogerbrugge et al. 2011, Hoogerbrugge et al. 2014, Alipour et al. 2016, 2019, Houten et al. 2016), but has been little adoption of biocontrol by growers in ornamental crops (Buitenhuis et al. 2015), or crops in general (Lenteren 2011). Even so, there is increasing interest by some growers to implement biological control into their greenhouse production systems (Buitenhuis et al. 2015). Unfortunately, in some releases of *A. swirskii* in roses, growers felt that the mites were not effective control of *Scirtothrips dorsalis* Hood (Schoeller et al. 2020), an unexpected outcome for a mite known to feed on many species of thrips, as previously mentioned. Schoeller et al. (2020) hypothesizes that this reduction in thrips control may be due to reduced trichomes on roses. This hypothesis agrees with the

reports of Loughner et al. (2010a) and Loughner et al. (2010b), who noted that phytoseiids tend to disperse away from plants with few trichomes. Even so, phytoseiids tend to oviposit on dense patches of trichomes along axillary veins known as ‘domatia’ on the underside of leaves (O’Dowd and Willson 1991, Walter 1992, 1996, Grostal and O’Dowd 1994, Agrawal and Karban 1997), a behavior which is thought to help to avoid predation (Faraji et al. 2002), so it is unclear if phytoseiids leaving roses is simply due to having few trichomes. Also, the idea that *A. swirskii* dislike glabrous surfaces contrasts with McMurtry et al. (2013)’s classification of *A. swirskii* as a type III-b phytoseiid mite: a generalist predator which lives on glabrous surfaces. This classification was recently challenged by Liu et al. (2017), who recovered only three feeding guilds of phytoseiids from detailed analysis of morphometric data. Liu et al. (2017)’s results were more similar to earlier classifications McMurtry and Croft (1997), which had separated the phytoseiids into three feeding groups: specialists of *Tetranychus* species (type I), generalist predators (type II), and *Euseius utilis*, a pollen feeding phytoseiid (type III) (McMurtry and Croft 1997, McMurtry et al. 2013, Liu et al. 2017). Even if *A. swirskii*’s preference for glabrous habitats is in question, there are other concerns with utilizing them for biological control of roses. Experiments with *T. urticae*-resistant roses revealed some issues when combining *A. swirskii* with another common predator of *T. urticae*, *Phytoseiulus persimilis* Athias-Henriot (Alipour et al. 2016, 2019). Alipour et al. (2016) saw that predators fed more on *T. urticae* on the resistant rose cultivar, but *P. persimilis* generally outperformed *A. swirskii*, with higher predation rates. This study seems to simply reaffirm prior knowledge about the feeding preferences of these mite species on *T. urticae*, but a follow-up study in 2019 found some antagonistic effects on the intrinsic rate of increase for both predators as well as *T. urticae* on the resistant roses (Alipour et al. 2019). These effects may be due to host plant resistance (antibiosis), or possibly induced plant defenses (Stout 2013) caused by *T. urticae*

feeding. These types of plant defense mechanisms have potential to help control mites such as *T. urticae* (Agut et al. 2018), but as demonstrated by the example of Alipour et al. (2019), the mechanisms of host plant resistance must be closely examined to avoid harming beneficial organisms in the same plant system (Pappas et al. 2017).

#### 1.4 A Second Plant-Mite-Pathosystem: *Brevipalpus californicus* and *Orchid fleck dichorhavirus*

The most important superfamily of herbivorous mites is the Tetranychoidea. The Tetranychoidea are comprised of 2,000 species divided into 5 families (Krantz 2009), two of which have economic significance, Tetranychidae—the spider mites—and Tenuipalpidae. Tenuipalpidae are known colloquially as the false spider mites, or flat mites due to their flattened character and superficial similarity to the Tetranychidae. In contrast to tetranychids, tenuipalpids do not spin webs and are considered to be a pest of reduced severity: Krantz (2009) places them as the ‘third most important family of phytophagous mites’. Flat mites are considered to be a tropical to subtropical group of mites (Gerson 2008), the majority of which are not of economic significance (Hoy 2011). Consequently, tenuipalpids have been studied much less than the Tetranychidae (Jeppson et al. 1975, Childers et al. 2003a, Gerson 2008), but flat mites from the genus *Brevipalpus* have been gaining importance in recent years as vectors of plant viruses (Chagas et al. 2003, Childers et al. 2003c, Childers and Derrick 2003, Kitajima et al. 2003, Rodrigues et al. 2003, Kitajima et al. 2008, Kitajima et al. 2010, Childers and Rodrigues 2011, Melzer et al. 2013, Rodrigues and Childers 2013, Ramos-González et al. 2017, Chabi-Jesus et al. 2018, Dietzgen et al. 2018a). Another major pest of modern concern is *Raoiella indica* (Hirst), a pest of palms (Arecaceae), ginger (Zingiberaceae), bananas (Musaceae), and bird of paradise plants (Strelitziaceae) (Jeppson et al. 1975, Etienne and Flechtmann 2006, Hoy 2011, Beard et al. 2012), which has been invading the Neotropics since their introduction to the Caribbean (Etienne and Flechtmann 2006, Rodrigues et al. 2007, Roda et al. 2008, Vásquez et al. 2008, Carrillo et al. 2011b, Dowling et al. 2011, Kane et al. 2012, Peña et al. 2012, Alcívar et al. 2020, Escobar-Garcia and Andrade 2020, Ramírez et al. 2020,

Rodrigues et al. 2020, Amaro et al. 2021). Flat mites are typically small (200 to 300 µm) red or green, and move slowly (Jeppson et al. 1975, Hoy 2011). Tenuipalpids feeding is typically restricted to a few hosts, and mites can usually be found on the underside of leaves, often along leaf veins or the midrib (Jeppson et al. 1975, Hoy 2011). Some species feed on grass, bark, flower heads, leaf sheaths, or form galls (Jeppson et al. 1975, Hoy 2011). Flat mites have egg, larva, protonymph, deutonymph and adult forms over an average of 3-4 weeks (Hoy 2011). A few species have only six legs as adults. Tenuipalpid mites can be difficult to classify correctly with light microscopy, due to distortions during mounting of characters used in species identification (Welbourn et al. 2003). Furthermore, flat mites are thelytokous parthenogenic: Males are seldom encountered, due to infections with the feminizing bacteria *Candidatus Cardinium*

(Cytophaga–Flavobacterium–Bacteroides phylum) Chigira and Miura (2005). This has caused some concern that some *Brevipalpus* species are actually isofemale lines specialized on their specific hosts (Groot et al. 2005), an idea which is further complicated by the occurrence of cryptic species in these groups (Navia et al. 2013, Skoracka et al. 2015). Few methods of pest management have been reported from past reviews of tenuipalpids (Jeppson et al. 1975, Gerson 2008, Hoy 2011): a number of different mite predators have been tested for their efficacy, as well as the pathogenic fungi *Hirsutella thompsonii* and *Metarhizium anisopliae* (Rossi-Zalaf and Alves 2006, Gerson 2008). Zheng et al. (2012) tested the ability of water and phytoseiids to reduce populations of *B. obovatus*. *Beauveria bassiana* has been tested to control *R. indica*, and is potentially compatible with the previously-tested phytoseiid species *Amblyseius largoensis* and *Typhlodromus ornatus* (Carrillo and Peña 2011, Freitas et al. 2021). Chemical applications are typically used to control tenuipalpids (Childers 1994), but some species have begun to develop chemical resistance to the more common applications (Campos and Omoto 2002, Rocha et al. 2021). One of the more cosmopolitan species of tenuipalpid is *Brevipalpus californicus* (Banks). *B. californicus* is a common pest with a large host range of agricultural and ornamental crops,

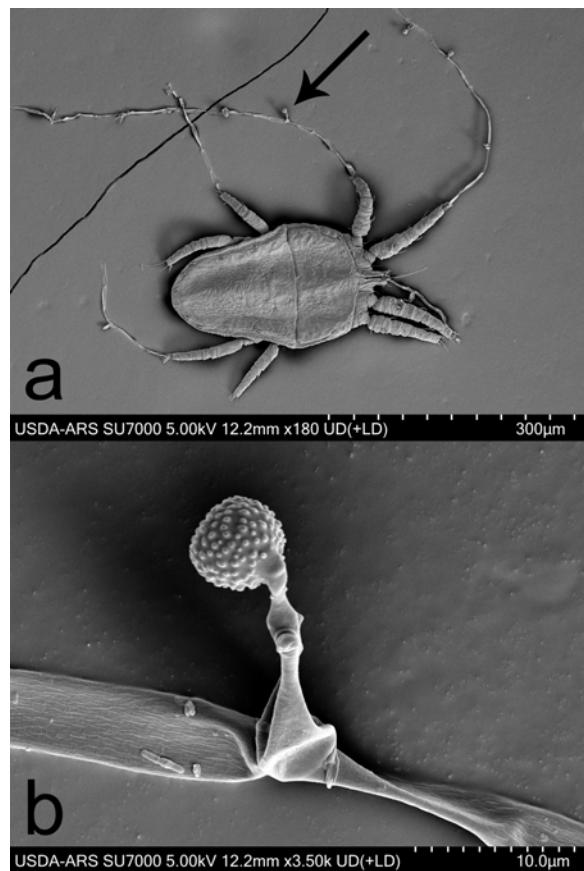


Figure 1-4. a) Cryo-SEM image of Tenuipalpid mite infected with unidentified fungus, collected from *Liriope muscari* b) detail of sporangia. Photo Credit: Dr. Gary R. Bauchan, USDA-ARS, 2020

including tea, orchids, citrus, cotton and tobacco (Jeppson et al. 1975, Hoy 2011). *B. californicus* acts as the primary vector for Orchid fleck dichorhavirus (OFV), the type member for the genus *Dichorhavirus*, family *Rhabdoviridae*; a bacilliform, nuclear rhabdoviruses composed of two segments of single-stranded, negative-sense RNA which infects plants (Dietzgen et al. 2014, Walker et al. 2018, Amarasinghe et al. 2019). Dichorhaviruses are only known to be transmitted by mites in the genus *Brevipalpus* (Dietzgen et al. 2014). Other members of this genus are: Citrus chlorotic spot virus, Citrus leprosis virus N, Clerodendrum chlorotic spot virus and Coffee ringspot virus (Dietzgen et al. 2018a). Many orchid genera are able to become infected with OFV (Kondo et al. 2006, 2006), as well as some Asparagaceae (Nolinoidaea) (Dietzgen et al. 2018a), and *Citrus* plants (Rutaceae), where it causes leprosis-like symptoms (Bastianel et al. 2010, Roy et al. 2013, García-Escamilla et al. 2018). Mechanical transmission of OFV is possible under lab conditions to various Chenopodiaceae, Aizoaceae, Fabaceae, and Solanaceae (Chang et al. 1976, Kondo et al. 2003, Peng et al. 2013). *B. californicus* has been collected from OFV-infected Nolinoidaea plants in Australia (Mei et al. 2016, Dietzgen et al. 2018b). *B. californicus* was historically associated with cases of citrus leprosis disease of Florida and Texas prior to 1925, when the disease mysteriously disappeared from the US sometime during the 1960s (Knorr et al. 1968, Childers et al. 2003b). Later studies of herbarium specimens from this time period revealed this disease to be caused by a Dichorhavirus, distantly related to modern OFV strains (Kitajima et al. 2011, Hartung et al. 2015). Maeda (1998) found evidence that *B. californicus* can transmit OFV in a persistent propagative manner, which means that the virus may replicate inside of its mite vector. OFV was first described infecting *Cymbidium* orchids in Japan (Doi et al. 1977). Many countries have reported OFV and OFV-like rhabdoviruses infecting orchids worldwide (Kondo et al. 2003), including Asia: [China (Peng et al. 2017), Korea (Peng et al. 2013)], Africa: [South Africa (Blanchfield et al. 2001, Cook et al. 2019), North America: [The US (Blanchfield et al. 2001), (Bratsch et al. 2015)]], South America: [Brazil Kitajima et al.

(2001), Colombia (Kubo et al. 2009b), Costa Rica (Freitas-Astúa et al. 2002), Paraguay (Ramos-González et al. 2015), Europe: (Begtrup 1972), Germany (Petzold 1971, Lesemann and Doraiswamy 1975) and Oceania: (Australia Lesemann and Begtrup 1971, Lesemann and Doraiswamy 1975, Gibbs 2000), Fiji (Pearson et al. 1993), Vanuatu (Pearson et al. 1993)]. The prevalence of OFV and its mite vector is thought to be associated with the importation of infected orchids (Dietzgen et al. 2018a). Orchids infected with OFV develop chlorotic/necrotic flecks on leaves and reduces plant vigor (Peng et al. 2013). Citrus infected with OFV develop chlorotic/necrotic bullseye lesions on leaves, fruits and bark (Roy et al. 2015, Ramos-González et al. 2017). Citrus-infecting strains of OFV have been encountered in Mexico (Roy et al. 2015) and recently in Hawaii (Ocenar 2020, Olmedo-Velarde et al. 2021), introduction of this virus is considered a threat to the billion dollar citriculture industries of the US.



Figure 1-5. *Oncidium* orchid infected with Orchid Fleck Virus

CHAPTER 2  
SURVEY AND PHENOLOGY OF NATURAL POPULATIONS OF THE INVASIVE  
MITE *PHYLLOCOPTES FRUCTIPHILUS* IN NORTHERN FLORIDA

**2.1 Introduction**

*Phyllocoptes fructiphilus* is a microscopic plant-feeding eriophyid mite. Eriophyoid mites are very host specific (Oldfield 1996b, Skoracka et al. 2009) and *P. fructiphilus* only feeds on plants in the genus *Rosa* (Amrine Jr 1996). *P. fructiphilus* is the vector of Rose Rosette Virus (RRV). RRV infection is commonly associated with the following symptoms: witches' brooms/rosetting, deformed flowers, increased prickle density, elongated shoots, reddened leaves and stems, and increased die-back which ultimately kills the rose host (Amrine Jr 1996). This disease is known as Rose Rosette Disease (RRD). and is the most serious disease of roses. Florida is the largest producer of roses with a total value exceeding \$30 million, and stands to lose millions of dollars if RRD and *P. fructiphilus* become established. There are few options available to control RRD, prevention of disease spread by quarantine and roguing infected roses is key to controlling the spread of this disease into Florida. Rose Rosette Disease and the mite have invaded the southeastern united states as they followed the range expansion of the non-native *Rosa multiflora* (Thunb) towards the coast (Amrine Jr 2002, Otero-Colina et al. 2018). In 2017, a group of researchers conducted a series of surveys for *P. fructiphilus* and RRD in the southeastern United States Solo (2018). RRD has been detected in previously in southern Florida (Babu et al. 2014), but no mites were detected at that time, possibly due to previous application of pesticides before shipping (as commented by Otero-Colina et al. (2018)), or perhaps by grafting of infected rootstock (Doudrick et al. 1987). No RRD or *P. fructiphilus* had been documented in those areas since, but populations of eriophyoid mites are easily overlooked: *P. fructiphilus* are microscopic and cryptic in habits, primarily located under the sepals of rose flowers, near glandular trichomes on the tips of rose canes, making their accidental collection unlikely (Jesse et al. 2006, Bauchan et al. 2019). Previous surveys detected larger populations of *P. fructiphilus* in northern states, with decreasing populations southwards Solo (2018), (see 2-1, 2-2). Previous surveys were also broader in scope,

covering either a broad region ([Solo et al. 2020](#)), or select sites known to harbor eriophyoids ([Otero-Colina et al. 2018](#)), meaning that the small populations or new introductions would be harder to detect. The data from these previous surveys gave rise to the concept of a ‘southern incidence line’ (*see Solo et al. (2020)*), and speculations regarding what may be constraining movement of *P. fructiphilus* and RRD further southwards. Following this line of reasoning, humidity was once hypothesized to be a limiting factor for growth and spread of *P. fructiphilus* populations, but Monterrosa et al. ([2021](#)) reported greater mite survival at moderate humidity (60% RH) and increased RRD severity in plants exposed to greater than 20% RH. Furthermore, previous surveys of the southeastern United States did not extend into Florida ([Solo \(2018\)](#)), (*see 2-1, 2-2*). Solo et al. ([2020](#)) found small population of *P. fructiphilus* in Thomas County and Lowndes County, GA, less than 20 miles from the northern border of Florida, as well as populations in Dothan, AL (*see 2-1, 2-2*), areas with climate trends similar to those of northern Florida ([Daly et al. 2012](#)). Therefore, we hypothesized that *P. fructiphilus* (and/or RRV) could be present in northern Florida or other parts of the state if introduced via the plant trade.

## **2.2 Surveying for *Phyllocoptes fructiphilus*, Rose Rosette Disease and Predatory Mites in Northern Florida**

In 2017, the entomology lab at the North Florida Research and Education Center (NFREC) in Quincy, FL, began a series of surveys of roses along the borders of northern Florida and southern Georgia. Our purpose was to estimate the distribution and populations levels of *P. fructiphilus*, as well as recording any RRD incidence in northern Florida. An additional goal of the rose surveys was to detect other predatory mites present on roses: there are many species of predatory phytoseiid mites present in Florida with potential to control agricultural pests such as *P. fructiphilus* ([Muma and Denmark 1970](#)). Encountering predatory mites native to the Florida landscape may help in the development

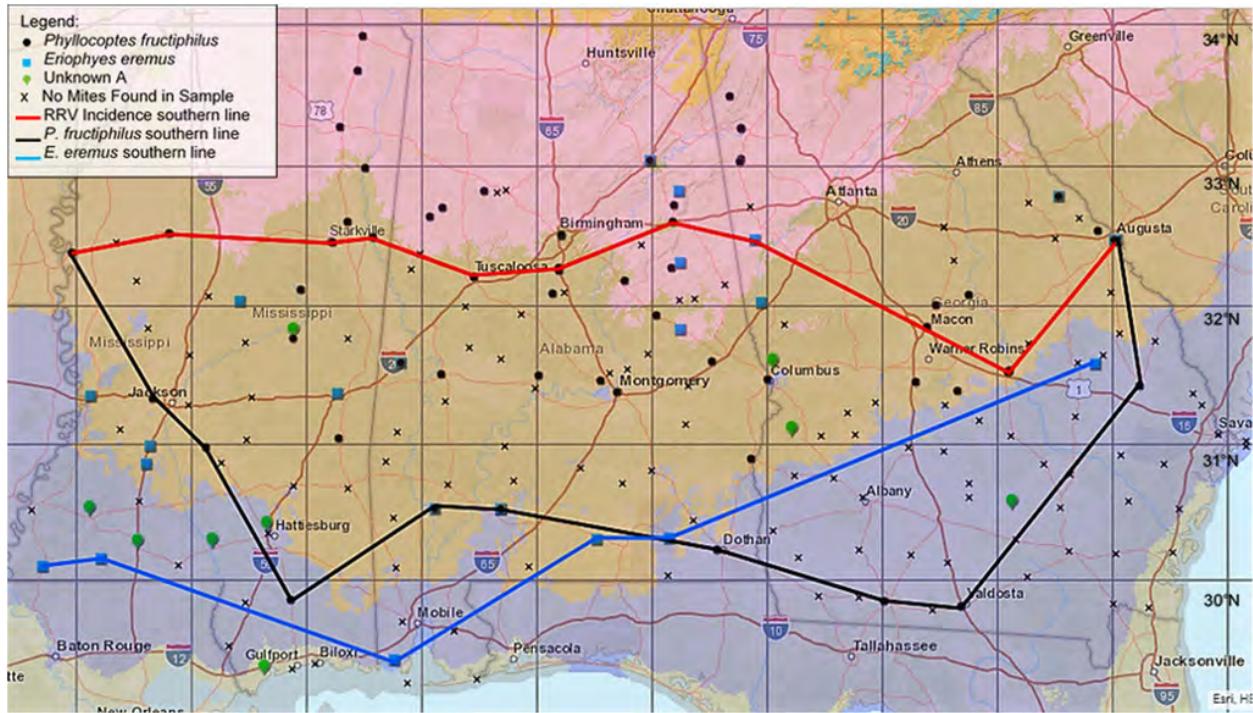


Figure 2-1. 'Map of the southern incidence line of *Rose rosette virus* (RRV) and eriophyid mites in Alabama, Georgia, and Mississippi in 2017. Plant hardiness Zone 7b is in pink, Zone 8a is brown, Zone 8b is blue, and Zone 9a is in gray. Note that there are five locations in which two mite species were found on the same rose sample.' Citation: HortScience horts 55, 8; 10.21273/HORTSCI14653-20, CC BY-NC-ND 4.0, Unmodified from the original version.

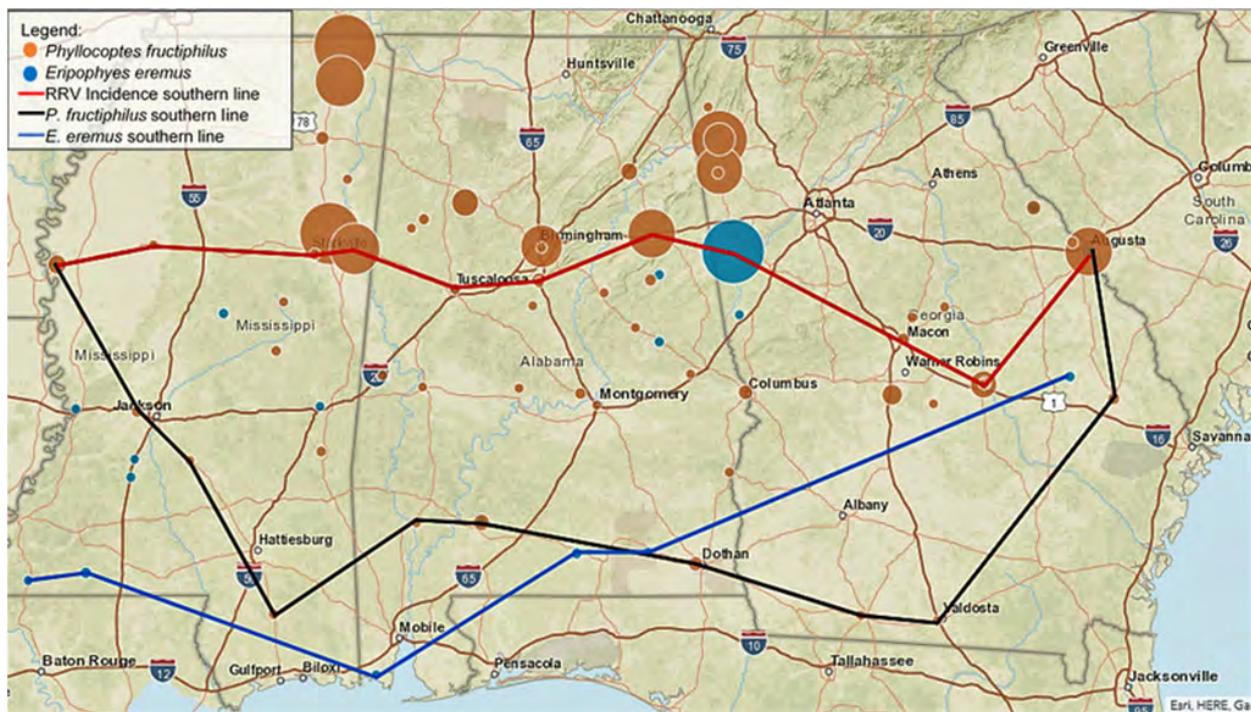


Figure 2-2. 'Map of the southern incidence line of Rose rosette virus (RRV), southern distribution of *Phyllocoptes fructiphilus* and *Eriophyes eremus*, and the population densities of eriophyid mites found on rose samples in Alabama, Georgia, and Mississippi in 2017. The larger the circle, the more mites found in the sample.' Citation: HortScience horts 55, 8; 10.21273/HORTSCI14653-20, CC BY-NC-ND 4.0. Unmodified from the original version.

of biological control methods for *P. fructiphilus*: native predatory mites sometimes have an advantage for bio-control because native mites have adapted to the environment where they will be released ([Gerson 2014](#)). Our results should help identify areas with greater risk for invasion, and help track the movement of *P. fructiphilus* and/or RRD into Florida.

### 2.3 Materials & Methods

A survey of roses in the landscape was conducted following a transect of northern Florida from west to east, Pensacola to Jacksonville. Cities with populations over 1,000 were visited along this route and cuttings were taken from various roses in each city ([see 2-1](#)). Rose cultivar/species, sun exposure and GPS coordinates were recorded to map out sites which had predatory mites, eriophyoid mites, or possibly symptoms of RRD. Rose tissue samples were taken from the periphery of various roses in the landscape; sampling was focused on the flowering tips of roses and included a mixture of flowers, fruits, buds, and short lengths of rose cane. Samples were trimmed with bypass pruners which were routinely sanitized with 70% ethanol between cuts. Samples were stored in 500 mL Nalgene<sup>TM</sup> Wide-Mouth Polypropylene Copolymer bottles (ThermoFisher Scientific, Waltham, MA, USA) with ~10 mL of 95% ethanol. The rose samples then were gently shaken to coat the rose tissues sampled with ethanol. Doing so made sure that the sampled mites were killed and acted to preserve both mites and rose tissues until samples could be processed further and checked for mites. Samples were processed using a washing method derived from Monfreda et al. ([2007](#)) used to detect eriophyoid mites such as *P. fructiphilus*: The sampling bottles with ethanol and rose tissues were vigorously shaken to dislodge any mites, then the ethanol in the container was poured over a stack of sieves with decreasing screen sizes: 180 µm, 53 µm, and 25 µm. The bottle and rose pieces were then further rinsed with 95% ethanol over the sieve stack to dislodge any remaining mites. The 53 µm and 25 µm sieves were processed separately; the 53 µm sieve retained larger mites while the 25 µm sieve retained smaller mites, including *P. fructiphilus*. The sieves were then backwashed from the underside of their screen with a 95% ethanol-filled wash bottle,

starting from the highest point of a sieve and working to the bottom to flush any trapped debris and mites into a 50 mL centrifuge tube for storage and future observations. The ethanol solutions of mites and plant debris were stained with a derivative of McBride's acid fuchsin stain to enhance contrast ([Backus et al. 1988](#)). Solutions were allowed to settle until excess ethanol could be siphoned off, making it possible to then pour this concentrated plant-mite mixture into a thin, small petri dishes or a glass plate for observation under a dissecting microscope. Mites found among the plant debris were counted, then siphoned off with a glass pipette and subsequently stored in micro-centrifuge containers with 95% ethanol as a preservative. 5-10 unstained specimens from each sample were made into prepared microscope slides: Mites were cleared and mounted using the methods of Faraji and Bakker ([2008](#)): mites were simultaneously cleared and stained with Faraji and Bakker's modified clearing solution and heated on a hot plate until the specimens were clear. Subsequently, these mites were moved with an eyelash tool into an iodine-modified Hoyer's slide mounting media (Hempstead Halide®, Inc., Galveston, Texas, USA), underneath a 12 mm glass coverslip. The prepared slide was then dried at 90°C before sealing the slide by painting a ring of alkyd insulating enamel (Red Glyptal® 1201, Chelsea, MA, USA) over the edges of the coverslip to seal the slide, to protect it from damage by air incursion and moisture. These slides could then be observed under a compound microscope with phase-contrast objectives to identify the mite families and species if necessary. After mite quantities and species were recorded, a representative sample of eriophyoids putatively identified as *P. fructiphilus* had their identity verified with the acarologist, Dr. Sam Bolton of the Florida Department of Agriculture and Consumer Services, Division of Plant Industry (FDACS-DPI) to ensure accuracy. Roses which appeared to show symptoms of RRD, or which had populations of *P. fructiphilus* present were tested by the Plant Disease Diagnostic Clinic at the NFREC. Plant tissues were tested for RRV by Dr. Fanny Iriarte using the currently accepted molecular methods described in Babu et al. ([2016](#)), Babu et al. ([2017a](#)), and/or Babu et al. ([2017b](#)).

## 2.4 Results

425 samples were taken from 33 sites from an east to west transect along the border northern Florida. Eriophyoid mites were recovered in rose samples from six cities. The first samples of *P. fructiphilus* were first encountered in Tallahassee during 2019; subsequent sampling efforts found in more eriophyoids in Jacksonville, Baldwin, Gainesville and Defuniak Springs in 2020, and Lake City in 2021. Other mites were collected from 68% of the cities visited. The largest populations of *P. fructiphilus* were seen in Tallahassee, with over 8,400 eriophyoid mites from 260 samples collected during 2019-2021 (*see 2-1*). No evidence of RRD was observed in northern Florida during the surveys, even in areas where abundant *P. fructiphilus* were found. Over 4,600 non-eriophyoid mites were collected from various cities during 2019-2021 *2-1*. Other non-eriophyoid mites collected primarily belong to the family Tetranychidae, but a small number of phytoseiidae and other predatory mites were recovered as well. These other mites currently await curation and expert identification.

## 2.5 Discussion

The presence of *P. fructiphilus* in northern Florida over multiple years and seasons provides evidence against a putative southern limit for the species ([Solo et al. 2020](#)). Unfortunately, our survey efforts were severely hampered by the COVID-19 pandemic, which limited opportunities to travel and collect mites. We expect that further investigations of roses in other Florida cities will reveal more *P. fructiphilus*-infested sites. The arrival of a competent vector is not a guarantee that its associated disease will follow suit, but it does provide a necessary component of the disease triangle: if the environmental conditions are suitable, and the rose host is sufficiently abundant, there is potential for disease to occur ([Franci 2001](#)). We did not see any signs of RRD in roses in northern Florida, but it is important to note that the delayed onset and difficulty of identifying symptoms makes it likely to miss detection until late stages of the disease. It is not known how *P. fructiphilus* have arrived in northern Florida, or how long they have been here, and unfortunately our

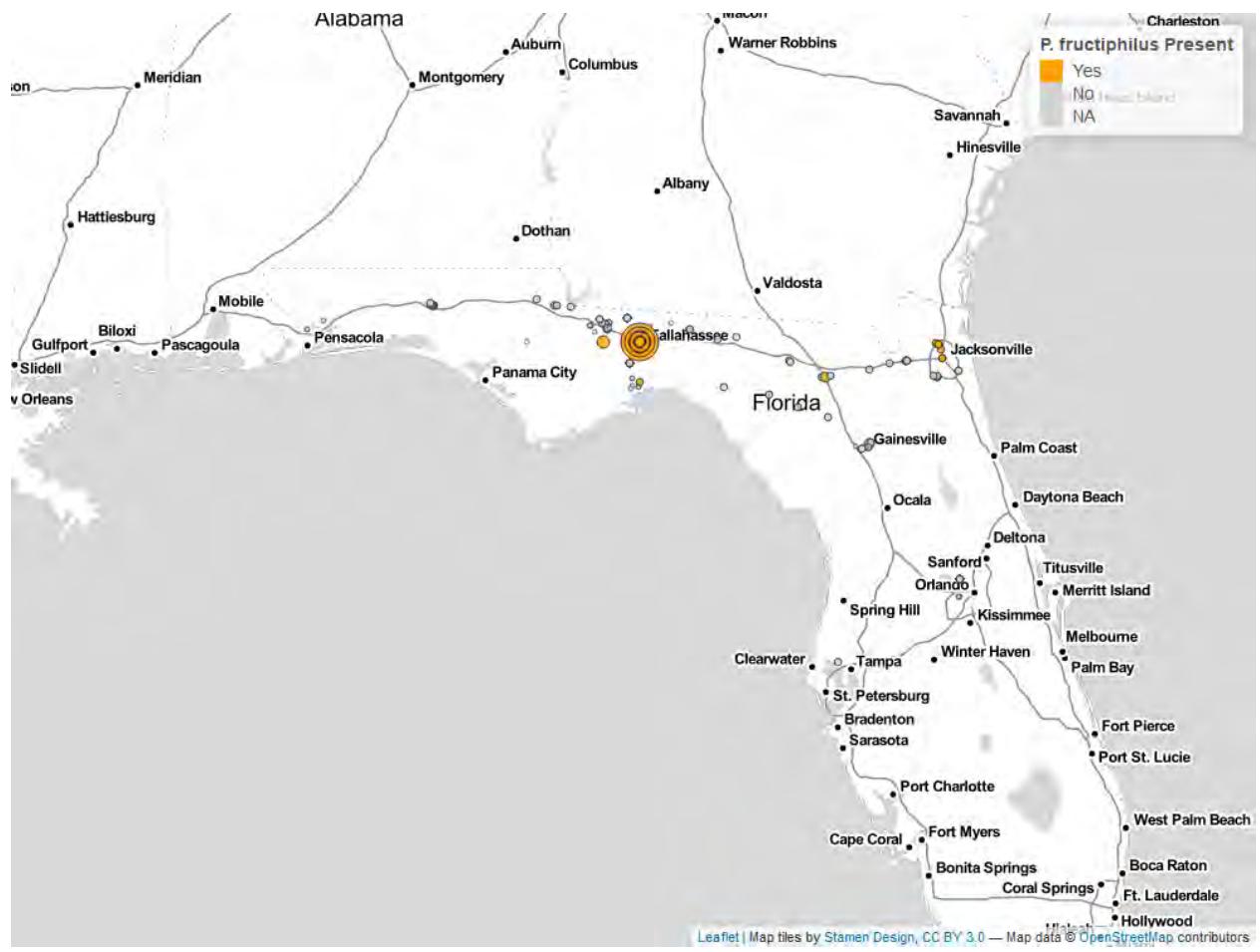


Figure 2-3. *P. fructiphilus* mites recovered during surveys of roses in Florida, 2017-2021.

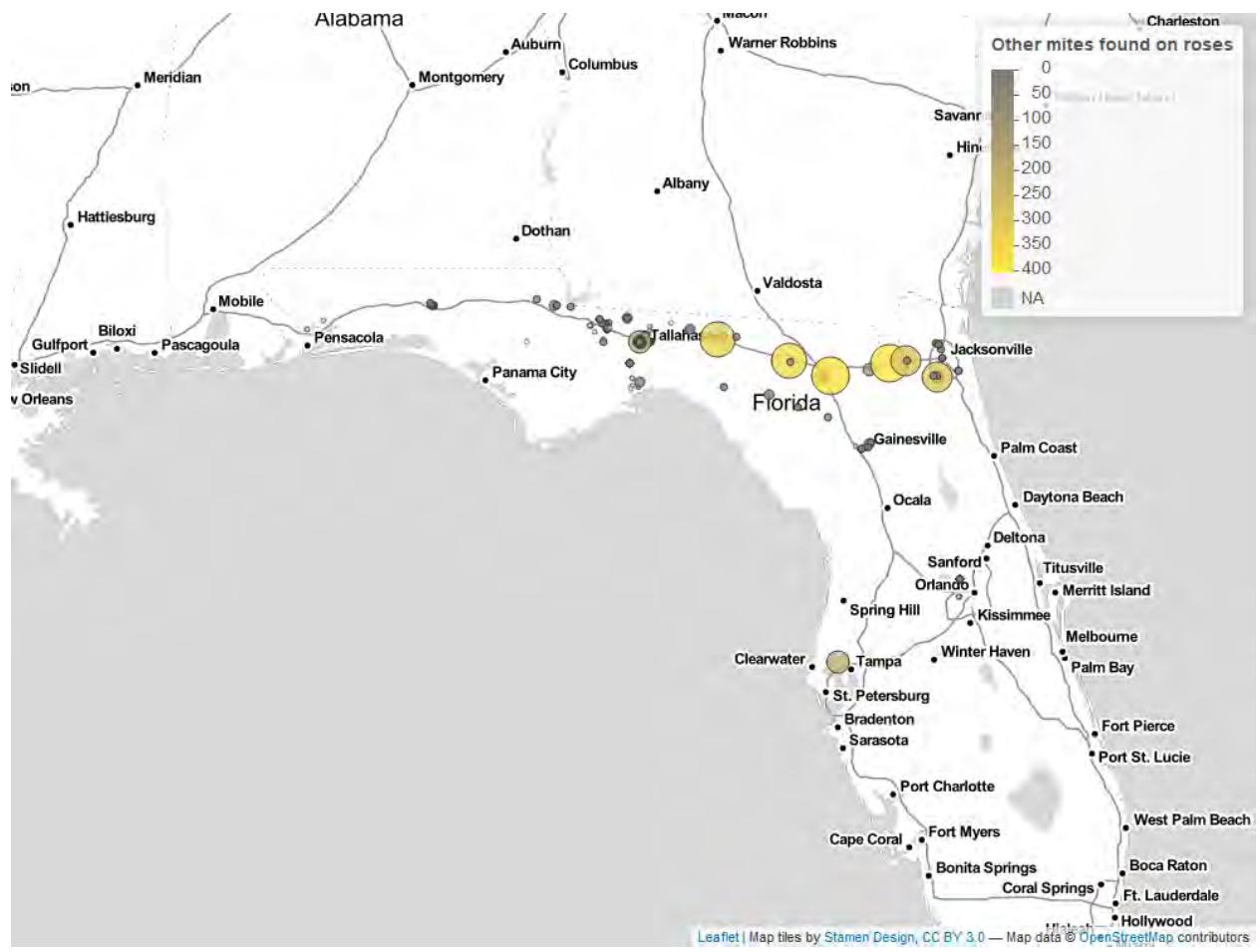


Figure 2-4. Other mites recovered during surveys of roses in Florida, 2017-2021.

### Eriophyoid mites found on roses in northern Florida

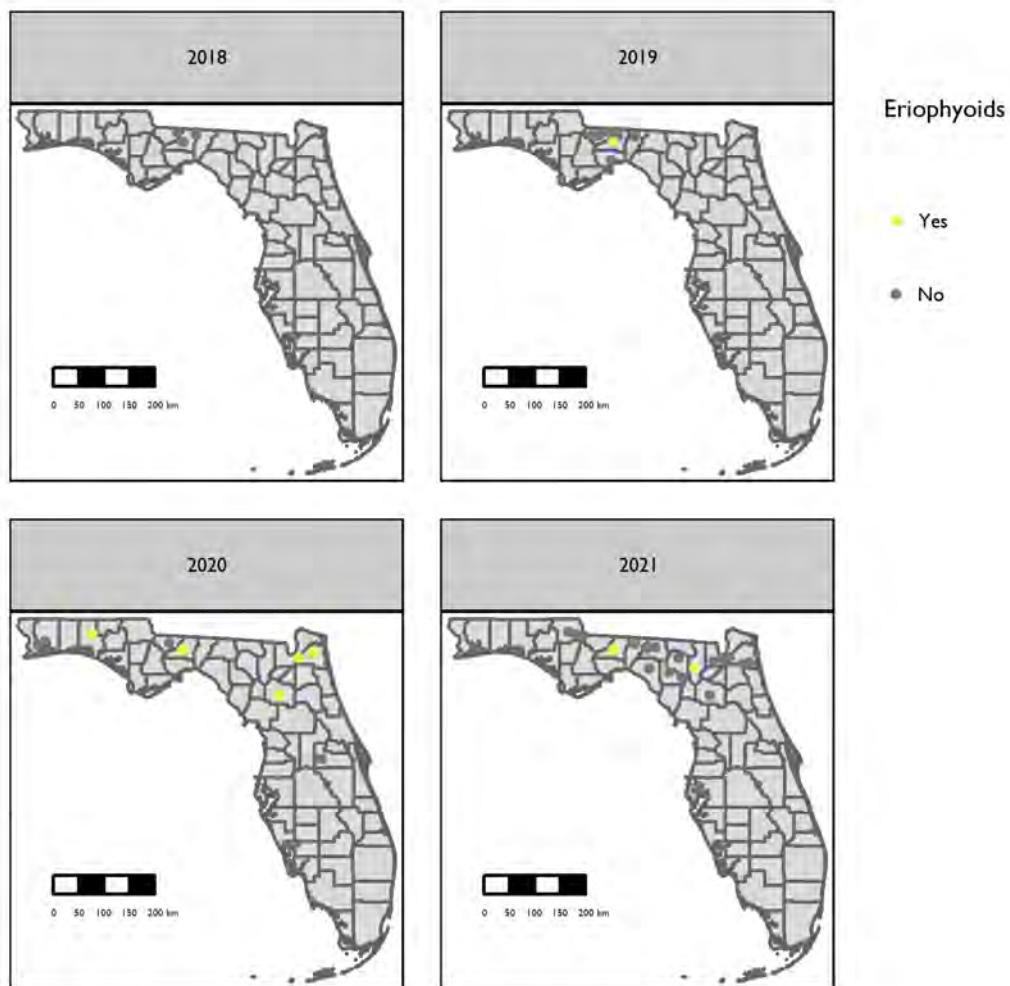


Figure 2-5. Location of populations of eriophyoid mites found on roses in northern Florida 2018-2021.

### Other mites found on roses in northern Florida

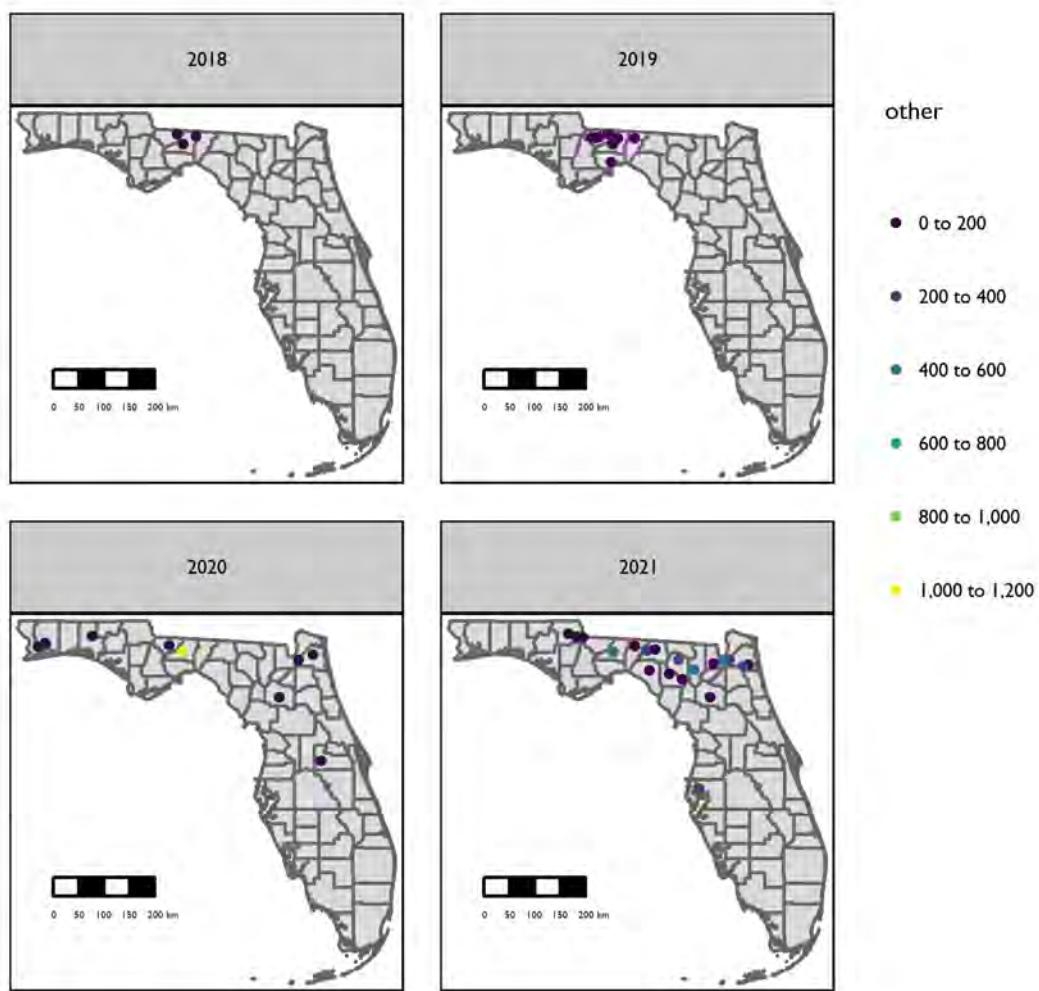


Figure 2-6. Locations of other mites recovered during surveys of roses in Florida, 2018-2021.

Table 2-1. Eriophyids and other mites recovered during surveys of roses in Florida, 2017-2021.

City	Year	Eriophyoids	Std. Error	Other Mites	Std Error	Samples per city	Eriophyoids per sample	Totals	Longitude	Latitude
Havana	2018	0	NA	0	NA	9	0.0	0	-84.41461	30.62618
Miccosukee	2018	0	NA	0	NA	1	0.0	0	-84.03900	30.59406
Tallahassee	2018	0	NA	0	NA	7	0.0	0	-84.29692	30.44227
Bradfordville	2019	0	NA	0	NA	2	0.0	0	-84.21892	30.54678
Crawfordville	2019	0	NA	0	NA	7	0.0	0	-84.35288	30.12877
Greensboro	2019	0	NA	0	NA	5	0.0	0	-84.74333	30.56824
Gretna	2019	0	0.00	5	1.77	4	0.0	5	-84.66470	30.55817
Havana	2019	0	0.00	49	6.08	5	0.0	49	-84.41501	30.62601
Midway	2019	0	0.00	0	0.00	2	0.0	0	-84.32201	30.44610
Monticello	2019	0	NA	0	NA	7	0.0	0	-83.87824	30.54454
Quincy	2019	0	0.00	9	1.13	7	0.0	9	-84.60175	30.56986
Tallahassee	2019	4704	9.86	47	0.29	79	59.5	4751	-84.30902	30.44618
Baldwin	2020	27	1.31	15	1.55	4	6.8	42	-81.97352	30.30268
Defuniak Springs	2020	20	2.06	29	0.70	6	3.3	49	-86.12718	30.72614
Ferry Pass	2020	0	NA	0	NA	2	0.0	0	-87.21893	30.54415
Gainesville	2020	25	3.23	55	4.60	20	1.2	80	-82.36118	29.64316
Jacksonville	2020	66	1.13	41	1.67	8	8.2	107	-81.68811	30.39848
Milton	2020	0	NA	0	NA	1	0.0	0	-87.07610	30.60700
Orlando	2020	0	NA	0	NA	4	0.0	0	-81.51605	28.50670
Quincy	2020	0	NA	0	NA	1	0.0	0	-84.57331	30.55638
Tallahassee	2020	3364	4.82	1150	0.79	157	21.4	4514	-84.30878	30.44297
Baldwin	2021	0	0.00	309	145.50	2	0.0	309	-81.97613	30.30260
Branford	2021	0	NA	12	NA	1	0.0	12	-82.92316	29.95685
Gainesville	2021	0	0.00	6	1.00	2	0.0	6	-82.36566	29.63691
Grand Ridge	2021	0	0.00	30	11.00	2	0.0	30	-85.04182	30.71944
Greenville	2021	0	NA	356	NA	1	0.0	356	-83.63092	30.46842
Greenwood	2021	0	NA	200	NA	1	0.0	200	-82.57149	28.00751
Jacksonville	2021	0	0.00	12	2.65	3	0.0	12	-81.59286	30.21992
Lake City	2021	49	9.80	472	77.72	5	9.8	521	-82.67590	30.12273
Live Oak	2021	0	0.00	376	182.00	2	0.0	376	-82.99862	30.30259
Macclenny	2021	0	NA	400	NA	1	0.0	400	-82.11797	30.28349
Madison	2021	0	NA	14	NA	1	0.0	14	-83.46405	30.48829
Marianna	2021	0	NA	11	NA	1	0.0	11	-85.20554	30.76829
Mayo	2021	0	NA	32	NA	1	0.0	32	-83.18075	30.05482
Monticello	2021	0	NA	20	NA	1	0.0	20	-83.87114	30.54514
Orange Park	2021	0	0.00	363	90.28	3	0.0	363	-81.70203	30.18646
Perry	2021	0	NA	3	NA	1	0.0	3	-83.57833	30.11150
Sanderson	2021	0	NA	78	NA	1	0.0	78	-82.29722	30.24098
Sneads	2021	0	NA	1	NA	1	0.0	1	-84.91279	30.71067
Tallahassee	2021	424	2.78	579	8.68	24	17.7	1003	-84.30731	30.44491
Grand Totals	2021	8679	NA	4674	NA	425	127.9	13353	-83.71379	30.25158

observations are not sufficient to describe a mechanism of invasion. Eriophyoid mites may be windblown, transported with infected plants, move on contaminated equipment or clothes, or rarely, are moved through phoresy ([Sabelis and Bruin 1996](#)). Previous Florida mite surveys were conducted in 2017 and 2018, along a north-south transect from Florida into Georgia, but *P. fructiphilus* were only detected in recent years. The 2017-2018 Florida surveys were focused on Cherokee roses *Rosa laevigata*, roses which are easiest to find during the spring and early summer, due to their ephemeral blooming cycle. Our recent surveys did not cover the same locations and focused on all species roses present in public or private plantings. In addition, we were unable to sample from Cherokee roses in Florida,

due to COVID-19 lockdowns travel restrictions during the blooming season of *R. laevigata*. The sites with the largest populations of *P. fructiphilus* in Florida are various plantings around of a college campus, with many landscaping projects, allowing for many potential introductions of *P. fructiphilus* on newly purchased plants. On the other hand, the current invasions of *P. fructiphilus* may have been introduced through their natural means of dispersal over time. The short distances between mite infested roses in Georgia, Alabama and Florida suggest the possibility of multiple routes of introduction, but the mechanisms of dispersal require further investigation for *P. fructiphilus*. In addition, the movements of plant pathogens such as RRD is thought to be partially driven by socioeconomic factors and the movement of plants by people ([Nelson and Bone 2015](#), [Katsiani et al. 2020](#)). Inspections and quarantines of mite-infested roses by wholesalers and larger growers is predicted to slow the spread of plant pathogen epidemics ([Nelson and Bone 2015](#)). A large number of other non-eriohyoid mites have been collected. Our findings were similar to the results of other surveys ([Jesse et al. 2006](#), [Otero-Colina et al. 2018](#), [Solo et al. 2020](#)): we primarily encountered plant-feeding mites, including mites from the families tetranychidae, tenuipalpidae, eriophyidae, and some predators, including chelytidae and phytoseiidae. We did encounter some tydeidae, but we are not certain of their feeding guild, as many tydeidae are fungivores rather than herbivores. Further examination of collected mites is expected to reveal a variety of species and overlooked individuals. Accurate identification will necessarily require collaboration with individuals better trained in mite taxonomy to avoid misidentifications ([Demand et al. 2021](#)). In conclusion, the presence of *P. fructiphilus* in Florida necessitates the development of mite control practices to prevent mite populations from surging, and to hopefully prevent the spread of RRD.

# CHAPTER 3

## CHANGES IN HEADSPACE VOLATILES FOR ROSES INFECTED WITH ROSE ROSETTE DISEASE

### 3.1 Introduction

#### 3.1.1 Plant defenses and volatiles: why are *Amblyseius swirskii* attracted infected roses?

Rose Rosette Virus (RRV) genus *Emaraviridae* is the the casual agent of Rose Rosette Disease (RRD), the most severe disease of roses ([Laney et al. 2011](#)). RRD is thought to have invaded the southeastern United States by the movement of its vector, the eriophyid mite, *Phyllocoptes fructiphilus* Kiefer (Trombidiformes: Eriophyidae), on multiflora rose (*Rosa multiflora* (Thunb)), as these organisms expanded their range from the US northwest, south and east towards the coast ([Amrine Jr 2002](#), [Otero-Colina et al. 2018](#), [Solo et al. 2020](#)). RRD is currently present throughout the US, and has been recently detected in Florida ([Fife et al. 2020](#)). Infection with RRD creates witches' brooms, rosetting, deforms flowers, increases prickle density, elongates shoots, reddens of plant tissues, causes die-back and ultimately plant death. Few management options are available to manage *P. fructiphilus*: Current mite control is achieved by removing infected roses and frequent pesticide applications ([Hong et al. 2012](#), [Olson et al. 2017](#), “Control - rose rosette” [2018](#)). Nursery managers are interested in additional management options to combat *P. fructiphilus* and RRD. Induced plant defenses offer an important potential role in the prevention of disease and control of insect pests ([Walling 2000](#), [Farmer 2016](#)). When plants detect elicitors from a pathogen or an herbivore ([Mithöfer and Boland 2008](#), [Boller and Felix 2009](#)), they respond by upregulating intercellular Salicylic Acid (SA), initiating a process known as systemic acquired resistance (SAR) ([Gaffney et al. 1993](#), [Goodman and Novacky 1994](#), [Park et al. 2007](#), [Vlot et al. 2009](#)). Induction of SAR helps to provide protection from herbivory as well as pathogen damage ([Cole 1999](#), [Anfoka 2000](#), [Babu et al. 2021](#), [Doungous et al. 2021](#)). Inducing SAR can have various positive effects on disease prevention: SAR induction is often used to combat fungal diseases ([Goy et al. 1992](#), [Xue et al. 1998](#), [Narusaka et al. 1999](#), [Suo and Leung 2001](#)), and some studies have found that SAR induction and the hypersensitive response can disrupt the establishment of eriophyoid

mites (Bronner et al. 1991a, 1991b, Westphal et al. 1991). Infested plants produce  $\beta$ -1,3-glucanase and chitinases, which were hypothesized to contribute to plant defenses against eriophyoid mites (Bronner et al. 1991b, Ward et al. 1991). Similar increases in  $\beta$ -1,3-glucanase and chitinase activity was seen in roses treated with acibenzolar-S-methyl (ASM), a benzothiadiazole known used to induce SAR (Cole 1999, Darolt et al. 2020, Doungous et al. 2021). Applications of ASM have also been reported to help restrict the growth of fungal pathogens in roses (Suo and Leung 2001), and purportedly control populations of whiteflies (Correa et al. 2005, Doungous et al. 2021) as well as aphids (Costa and Moraes 2006). We hypothesize that SAR-induction in roses may be able to reduce populations of *P. fructiphilus* as well. Unfortunately, inducing SAR can have negative effects on herbivores as well as predators (Kant et al. 2015, Ataide et al. 2016, Pappas et al. 2017), which necessitates careful study of all organisms involved in the system to create a successful pest management program. A handful of studies tested *Amblyseius swirskii* Athias-Henriot (Mesostigmata: Phytoseiidae), for their suitability to control pests on ornamental roses (Chow et al. 2010, Hoogerbrugge et al. 2011, Hoogerbrugge et al. 2014, Alipour et al. 2016, 2019). *A. swirskii* is one of the most popular species of commercially-available phytoseiid mites (Calvo et al. 2014), but has been seldom used in ornamental crops (Buitenhuis et al. 2015). *A. swirskii* are considered to be a generalist species which feed on a variety of different arthropod pests, including thrips, whiteflies, and occasionally other mites (McMurtry and Croft 1997, Bolckmans et al. 2005, Hoogerbrugge et al. 2014), and there is growing interest in using them on ornamental crops to control pests commonly encountered in roses (Chow et al. 2010, Buitenhuis et al. 2015, Schoeller et al. 2020). Phytoseiid like *A. swirskii* have no eyes, instead relying on plant VOCs to guide them to their prey (Gnanvossou et al. 2003, Boer and Dicke 2004a, Nomikou et al. 2005). Many plant VOCs are released when a plant is attacked by herbivores or pathogens (Shulaev et al. 1997, Sabelis et al. 1999, Ozawa et al. 2000, Halitschke et al. 2007), the composition of which varies, depending on the plant

species and type of damage/infection (Sabelis et al. 1999, Boom et al. 2004, Maeda and Liu 2006, Qualley and Dudareva 2008). Volatiles also deter herbivores or attract predators (Pichersky and Gershenson 2002), so it important to understand which chemicals may manipulate the behaviors of released biological agents in the same system. Studies by Farber et al. (2019) found differences between healthy and RRV-infected roses using Raman spectroscopy at a spectral band of  $1720 \text{ cm}^{-1}$ , a spectral region associated with carbonyl stretching vibrations. This indicates the presence of carbonyl compounds, a group functional group commonly seen in many organic compounds, including esters, aldehydes, carboxylic acids, and ketones. Farber et al. (2019) suggested that bands may be evidence for the presence of the carboxylic compounds Jasmonic Acid and Salicylic Acid, due to the association of these plant hormones with plant defenses against pathogens (Verma et al. 2016). When SAR is induced, SA becomes methylated and converts to Methyl Salicylate (MeSA), a volatile signal which helps to propagate resistance throughout the plant (Shulaev et al. 1997). This chemical is known to be attractive to phytoseiid mites towards their prey with varying degrees of success (Boer and Dicke 2004a, 2004b, James and Price 2004, Gadino et al. 2011, 2012), meaning that applications of ASM in the field may interfere with their efficacy as biological control agents of rose. Fortunately, the VOCs released from infected or induced plants can be analyzed using the methods of paired Gas Chromatography - Mass Spectrometry to determine the quantity and quality of plant headspace volatiles. Once the primary VOCs are identified, olfactometers assays can be used a preliminary method to screen the attractiveness of various VOCs to predatory mites (Dicke et al. 1988, Janssen et al. 1990, Nomikou et al. 2005, Halitschke et al. 2007). With that in mind, our studies were designed to investigate differences between RRV-infected and uninfected Pink Double Knock Out® roses and their volatiles, as well as the effects of SAR-induction on rose volatiles. We hypothesized that *A. swirskii* would be attracted to infected roses and SAR-induced plants due to increases in defensive volatiles consistent with SAR-induction, including MeSA. Our results should help provide preliminary

understanding of the effects of SAR-induction and RRV infection status on the VOCs released from roses, and determine any effects on *A. swirskii* behavior. An important limitation of the study was the inability to bring RRV-infected plants or *P. fructiphilus* into the lab for volatile extractions. After finding *P. fructiphilus* in northern Florida, our lab was asked to destroy graft-inoculated roses. Due to federal quarantines, the majority of volatiles samples of infected roses had to be taken in the field in Athens, GA, where the RRV pathogen is present in the landscape. Future studies will need to develop additional methods to overcome these restrictions, or should be done in areas where manipulations of *P. fructiphilus* and RRV are permitted in the lab for better environmental control of volatile collection.

### 3.2 Materials & Methods

#### 3.2.1 Which volatiles are most attractive?: two-arm olfactometer assays

*A. swirskii* mites were purchased online as mini sachets with hooks (Ambly-S, Arbico Organics, Oro Valley, AZ, USA). Sachets would be emptied into a plastic container before assays in order to facilitate finding mites. The mite colony was fed every 2 days with bee/pine pollen.

Filtered air was provided by a 4-Port Positive Pressure Flow Out/Dual Y-Tubes & Volatile Collection System (Sigma Scientific, Micanopy, FL USA) at 0.3 Ls/min for each arm of the Y-tube olfactometer. Air was humidified by passing air through a gas washing bubbler flask filled with water. The bubbler flasks were placed inline before any volatile sources and the arms of the Y-tube. Air was blown through a teflon tube into a nylon oven bag (GoBeGreen Turkey Oven Bags, GoBeGreen, Los Angeles, CA, USA.) that had been dried in an oven at 50 °C overnight. The bag would be sealed over the end of a flowering rose cane from Pink Double Knock Out® roses. Bags were allowed to inflate in order to detect excessive leaks, if no large leaks were detected, outlet air lines would be connected to the inlet of one arm of the Y-tube. In this manner, comparisons were made between an empty bag of air and a healthy rose, as well as uninfected roses to RRV-infected roses. The

olfactometer was held vertically with a lab stand, and illuminated with a 60 watt household 5000K white LED lightbulb. One arm of the Y-tube would be labelled as the experiment side, and the other arm would be the control chemical. Air lines would be switched after every 5 assays to avoid side bias in the Y-tube. After every 10 assays, the Y-tube was switched for a clean one. Individual *A. swirksii* mites were transferred to the mouth of the Y-tube olfactometer using a fine tipped paintbrush. Assays were recorded for 5 minutes. If a mite entered midway into the arm of an olfactometer, the time and label of the tube would be recorded, the mite removed, and the assay would end. Mites which failed to do so were considered to have made ‘no choice’ and were removed.

Additional olfactometer experiments were conducted after collecting and identifying headspace volatiles of interest from roses [3.2.2](#). Available synthetic volatiles were selected from the top ten chemicals seen in the contributions table from the PCA analysis of rose headspace volatiles ([3-3](#), [3-1](#)). Assays of synthetic volatiles were conducted diluting a selected VOC to dicholoromethane to a concentration of 1 µg/µL. The solution was then applied to a 3 cm dental wick. Dental wicks were placed in otherwise empty inline gas washing bubbler flasks, and left for 5 minutes before beginning an assay. The synthetic VOCs MeSA and DL-Limonene were tested. 100 µL of dicholoromethane was used as a control.

Responses of *A. swirkii* were compared with  $\chi^2$  tests using R version 4.1.1 ([R Core Team 2021](#)).

### **3.2.2 Collection of headspace volatiles from roses**

Two methods for collecting headspace volatiles were explored, Volatile Collection Traps (VCT) and Solid Phase Micro Extraction (SPME).

### **3.2.2.1 Volatile collection trap methodology**

The VCT method of collecting VOCs was based on a push-pull volatile collection method as illustrated in [3-1](#). Filtered air was provided by a 4-Port Positive Pressure Flow Out/Dual Y-Tubes & Volatile Collection System (Sigma Scientific, Micanopy, FL USA) at 0.2 Ls/min. Assays with roses involved bagging the roses as previously described, but the corner of the bag would be cut and a VCT would be inserted. Traps were 3.5" with  $28 \pm 5$  mg 80/100 mesh with HayeSep® Q adsorbent (Sigma Scientific, Micanopy, FL USA). Headspace volatiles were drawn through the filter by a vacuum line operating at 0.1 L/min. Bags were left at positive pressure during volatile collection for 24 hours. The VOCs adsorbed inside the VCT filter were then eluted with 150 µL of dichloromethane into a glass headspace vial, then 5 µL of 1 µL/g Nonyl Acetate were added to each solution as an internal standard. A baseline of 53 headspace VOC extractions were collected from 20 mite-free, one-year-old, uninfected Pink Double Knock Out® roses growing in 3 gallon buckets, 16:8 Light:Dark cycles. Extractions were conducted for ~12 hours. 4 extractions were completed from graft-inoculated RRV-positive roses ([Doudrick et al. 1987](#)), grown in a quarantine greenhouse under similar parameters. Headspace samples of the RRV-infected roses were taken prior to the quarantine measures requested by federal agencies. Samples were taken at ~20 °C and RH 50-66%.

### **3.2.2.2 Solid phase micro extraction (SPME) methodology**

The Solid Phase Micro Extraction (SPME) field method was created to overcome a specific study limitation: shortly after the detection of *P. fructiphilus* in Florida, NFREC researchers were restricted from working with RRV-infected plants by state agencies to prevent the introduction of the virus to the mites found in the region. In order to obtain VOCs from RRV-infected roses, extractions were taken *in situ* from roses in the landscape of Athens, GA [3-2](#). The SPME procedure was similar to the VCT method with a few exceptions: Oven bags were left over rose canes and flowers for an hour before extraction would begin. Filtered air was provided by a Kobalt 24v cordless high-volume inflator

(Lowe's, Mooresville, NC, USA) attached to a inline carbon block filter (Clear<sub>2</sub>O® RV and Marine Inline Water Filter - CRV2006, Miramar, FL, USA). Air flow was adjusted to 0.2 L/min with a variable flow meter (Sho-Rate™ Series Glass Tube Variable Area Flow Meter, model 1350CB1F, Brooks Instruments, Hatfield, PA, USA) and blown through a teflon tube into an oven bag surrounding the rose flowers and canes to agitate the headspace VOCs. An edge of the oven bag was removed and a disposable air filter/check valve inserted to prevent the oven bag from bursting due to over pressure. Once the bag was in place, an aluminum rod with burette holder/clamps was driven into the ground near the rose to be sampled, and the manual SPME injector (Supelco, Inc, Sigma-Aldrich, Bellefonte, PA, USA) clamped so that the needle of the fiber holder could pierce the inflated bag. Before VOC extraction, 1 µL of Nonyl Acetate was added to the bag as an internal standard. Extractions were done for 20 minutes. Injections were done using 24 Ga Stableflex SPME fibers with 50/30 µm DVB/CAR/PDMS coatings (Supelco, Inc, Sigma-Aldrich, Bellefonte, PA, USA). For SPME sampling, headspace VOCs were collected from 11 untreated roses from *P.fructiphilus*-infested roses in the landscape of Tallahassee, FL. 8 roses from the same location were treated with Actigard® 50WG (Syngenta, Greensboro, NC, USA), (ASM) 100 mg/L for 12 weeks before sampling, and 11 RRV-infected roses were sampled from three locations in Athens, GA on May 28th, 2021. Plants were of unknown age, growing in full sun, with symptoms of RRD. The daily temperature of the day of extractions ranged from 24-31 °C, RH 46-74%.

### **3.2.2.3 Analysis of headspace data**

Headspace extractions from VCT and SPME were injected into a paired Gas Chromatography-Mass Spectrometer (GC-MS) (ThermoFisher Trace 1310 Gas Chromatograph and ThermoFisher ISQ QD Single Quadrupole Mass Spectrometer, (ThermoFisher Scientific, Waltham, Massachusetts) and their spectra were analyzed with Chromeleon™ 7 Chromatography Data System software (ThermoFisher Scientific, Waltham, Massachusetts). GC-MS Injections were for 38 mins with 5 °C increase per

minute. Volatile collection equipment was cleaned according to manufacturer's instructions. Compounds were identified by comparisons of mass spectra to spectral databases for confirmation. The ratio of the area under the curve for each chemical peak was then divided by the area of the internal standard (Nonyl Acetate). These values were used for Principal Component Analysis (PCA) with the factoextra package ([Kassambara and Mundt 2020](#)), and Uniform Manifold Approximation and Projection (UMAP) ([Konopka 2020](#)) in R version 4.1.1 ([R Core Team 2021](#)), to visualize any clustering of data and to determine which VOCs are of interest.

### 3.3 Results

#### 3.3.1 *Amblyseius swirskii* were not attracted or repelled by the VOCs tested

*A. swirskii* were not significantly selective for MeSA at the concentrations we used, but mites were significantly more likely to make choices when testing with roses. DL-Limonene created more choices than other treatments. ([3-4](#)). Choices for DL-Limonene vs. control and Filtered Air vs. Healthy Roses were not significantly different, but *A. swirskii* mites were significantly more attracted to RRV-infected roses.

#### 3.3.2 Volatiles differ between RRV-infected, uninfected and induced roses

The top six contributing VOCs for VCT were  $\gamma$ -Murrolene,  $\beta$ -Ocimene, (E,E)- $\alpha$ -Farnesene,  $\beta$ -Caryophyllene, Methyl Salicylate and  $\beta$ -Pinene ([3-1](#)). The top six contributing chemicals for SPME were  $\gamma$ -Murrolene,  $\beta$ -Caryophyllene 1R- $\alpha$ -Pinene, Mesitylene,  $\beta$ -Pinene and m-Xylene ([3-3](#)). Roses infected with RRD were generally similar to uninfected roses for both VCT and SPME methods, ([3-7](#), [3-12](#), [3-9](#), [3-14](#)), but ASM-treated roses had less chemical variance than either group ([3-12](#), [3-14](#)). For VCT samples,  $\gamma$ -Murrolene explained most of the variance in uninfected roses, while SPME samples showed the opposite:  $\gamma$ -Murrolene and  $\beta$ -Caryophyllene were primarily found in infected roses.

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Figure 3-1. Volatile collection trap method for rose headspace sampling. An inert nylon bag is placed around the canes of interest, an air inlet is inserted and sealed at the base with a zip-tie to form a relatively air-tight seal around the base of the rose canes. Once the bag begins to inflate, a small hole is cut in the corner of the bag and a filter inserted and sealed with a zip-tie to form a second seal. The exterior end of the filter is attached to a vacuum airline set to allow for constant static pressure on the bag from inflation. The rose is then left for 24 hours, the filter is eluted with Dichloromethane into a gas chromatography vial, 1  $\mu$ L of Nonyl Acetate is added as an internal standard, and then the sample is processed using a coupled Gas Chromatography - Mass Spectrometer (GC-MS) for chemical identification.



Figure 3-2. Solid phase micro extraction (SPME) method for rose headspace sampling. An inert nylon bag is placed around the canes of interest, an air inlet is inserted and sealed at the base with a zip-tie to form a relatively air-tight seal around the base of the rose canes. Once the bag begins to inflate, a small hole is cut in the corner of the bag and a filter inserted and sealed with a zip-tie to form a second seal. 1  $\mu$ L of Nonyl Acetate is added as an internal standard. The needle of a SPME fiber holder is used to puncture the bag, and the fiber is exposed to the headspace for 20 minutes adsorb volatiles to the special coating of the SPME fiber. The fiber is then injected into a coupled GC-MS for chemical identification.

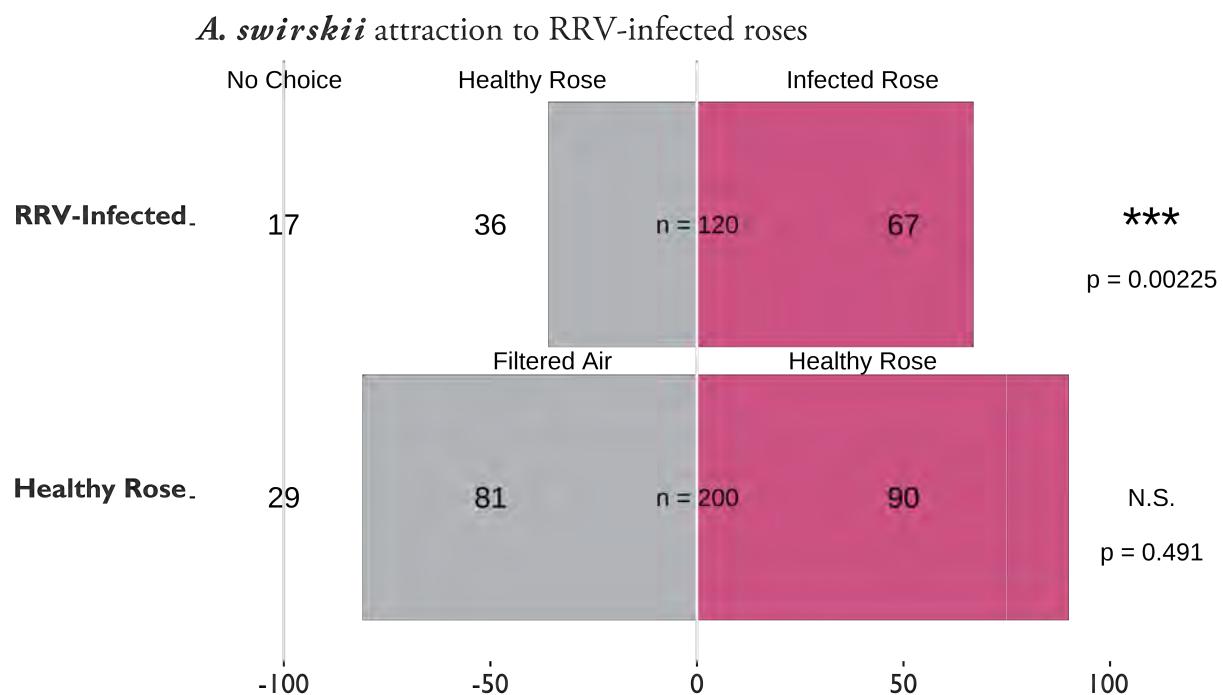


Figure 3-3. *Amblyseius swirskii* attraction to healthy and Rose Rosette Virus-infected Pink Double Knock Out® roses. Asterisks represent significant differences as calculated by  $\chi^2$  contingency table tests for given probabilities. N.S. = not significant. RRV-infected vs Healthy Rose:  $\chi^2 = 9.33$ ,  $df = 1$ ,  $\alpha = 0.05$ ,  $p - value = 0.002$ . Filtered Air vs Healthy Rose:  $\chi^2 = 0.47$ ,  $df = 1$ ,  $\alpha = 0.05$ ,  $p - value = 0.4913$ .

*A. swirskii* attraction to VOCs from roses

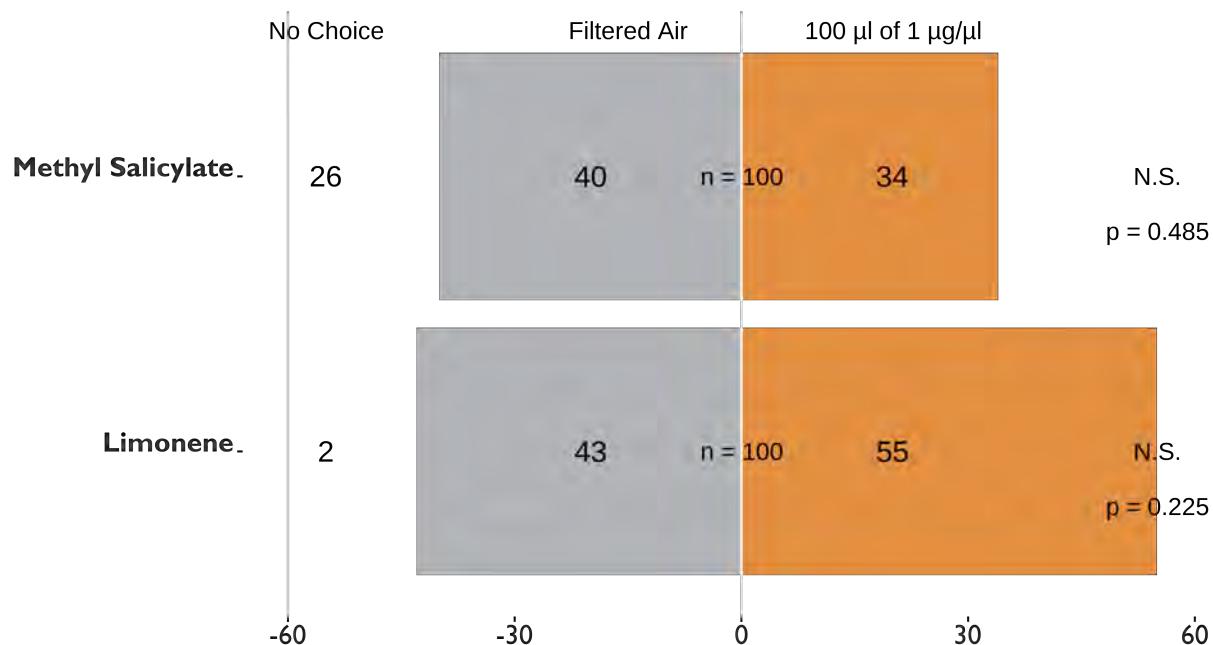


Figure 3-4. *Amblyseius swirskii* attraction to Methyl Salicylate (MeSA) and D-L Limonene vs filtered air at concentrations of 1 g/µL. 100 µLs of chemical was applied to 3 cm of dental wick inside of erlenmeyer flasks inline with the filtered air from the olfactometer. Asterisks represent significant differences as calculated by  $\chi^2$  contingency table tests for given probabilities. N.S. = not significant. MeSA vs Air:  $\chi^2 = 0.48649$ ,  $df = 1$ ,  $\alpha = 0.05$ ,  $p - value = 0.4855$ . D-L Limonene vs Air:  $\chi^2 = 0.94737$ ,  $df = 1$ ,  $\alpha = 0.05$ ,  $p - value = 0.3304$ .

*A. swirskii* attraction to VOCs from roses - time to choice

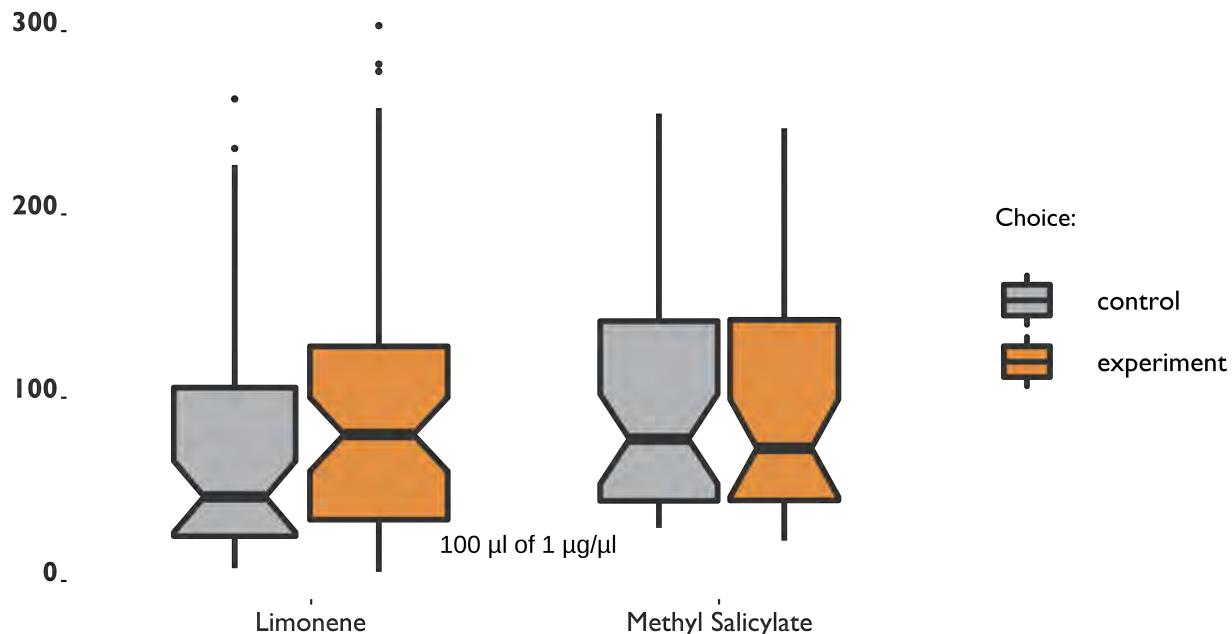


Figure 3-5. Time elapsed before choice of *A. swirskii* to MeSA and D-L Limonene vs filtered air at concentrations of 1 g/µL. 100 µLs of chemical was applied to 3 cm of dental wick inside of erlenmeyer flasks inline with the filtered air from the olfactometer.

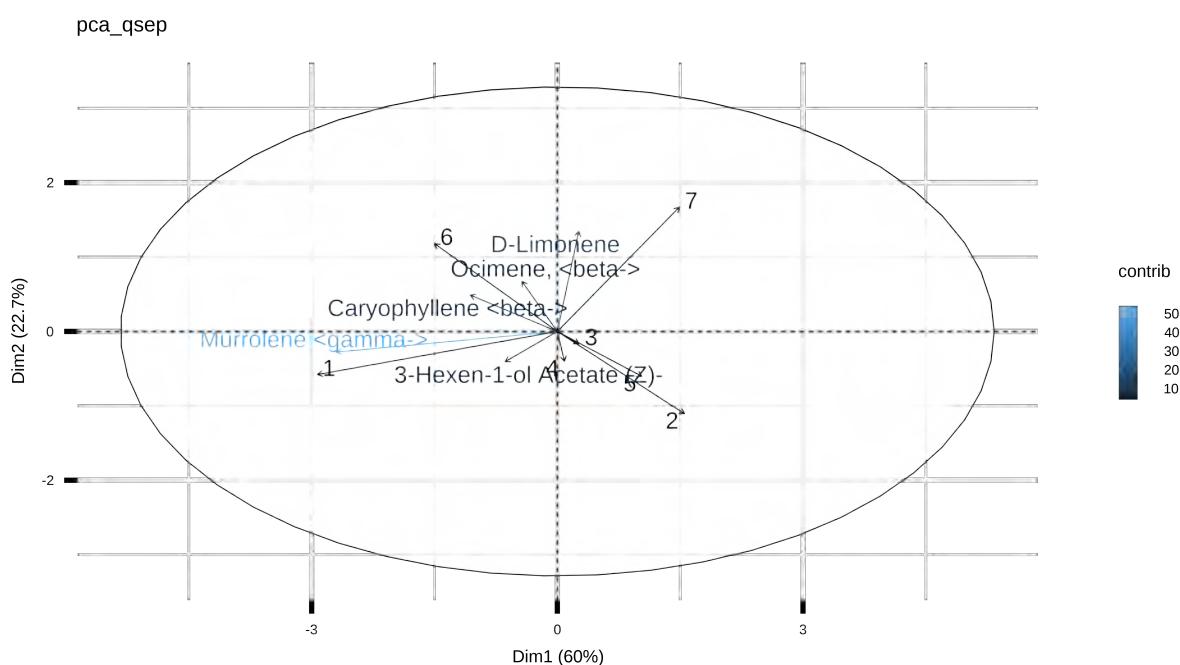


Figure 3-6. Principal component analysis (PCA) biplot of volatiles collected with VCT method.

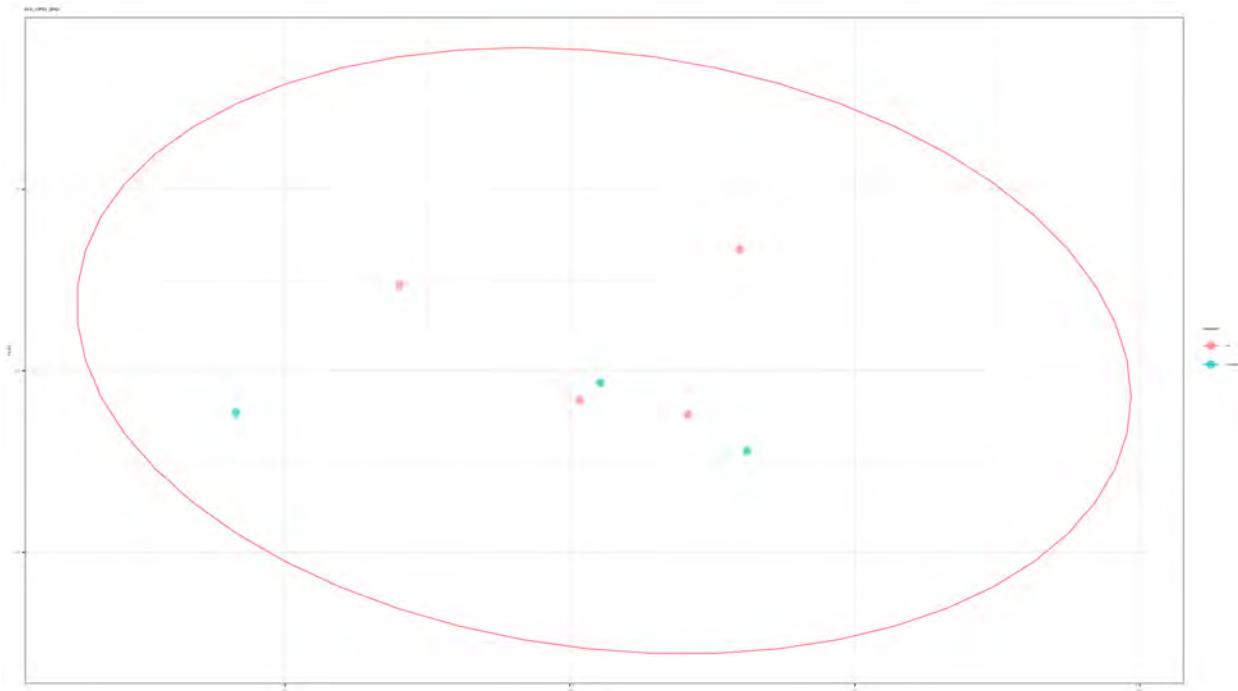


Figure 3-7. Comparison of VCT principal components. Ellipses represent 95% confidence intervals.

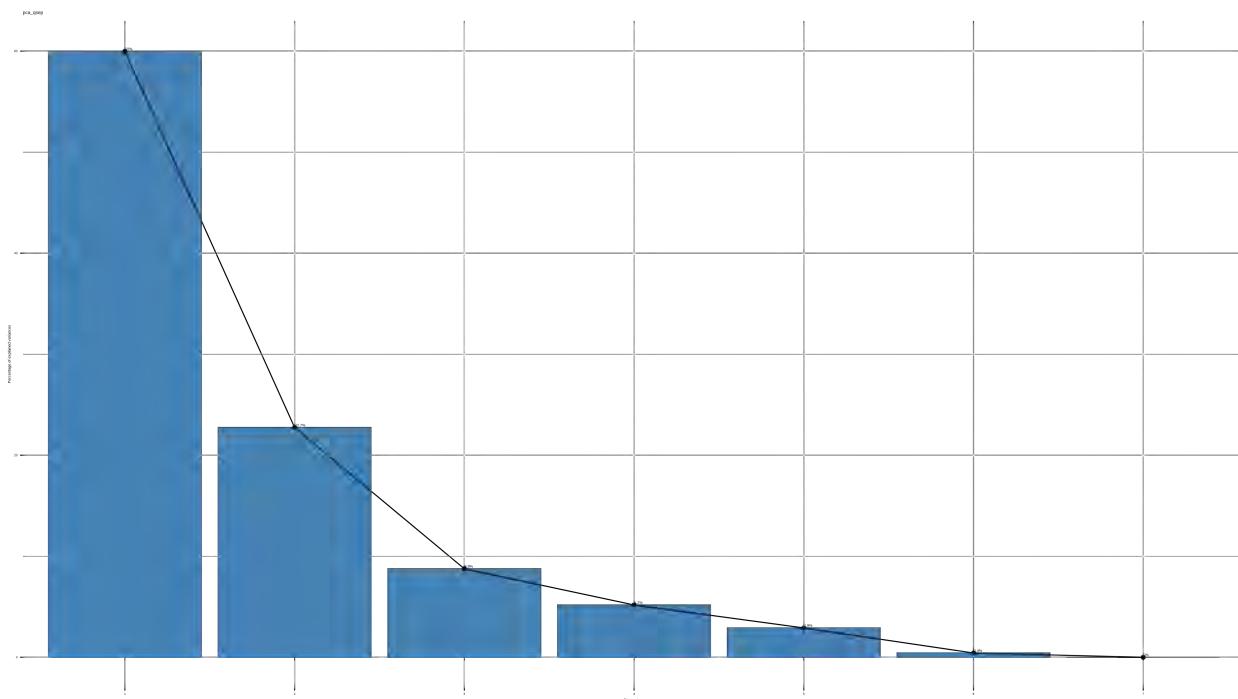


Figure 3-8. Scree plot of VCT principal components.

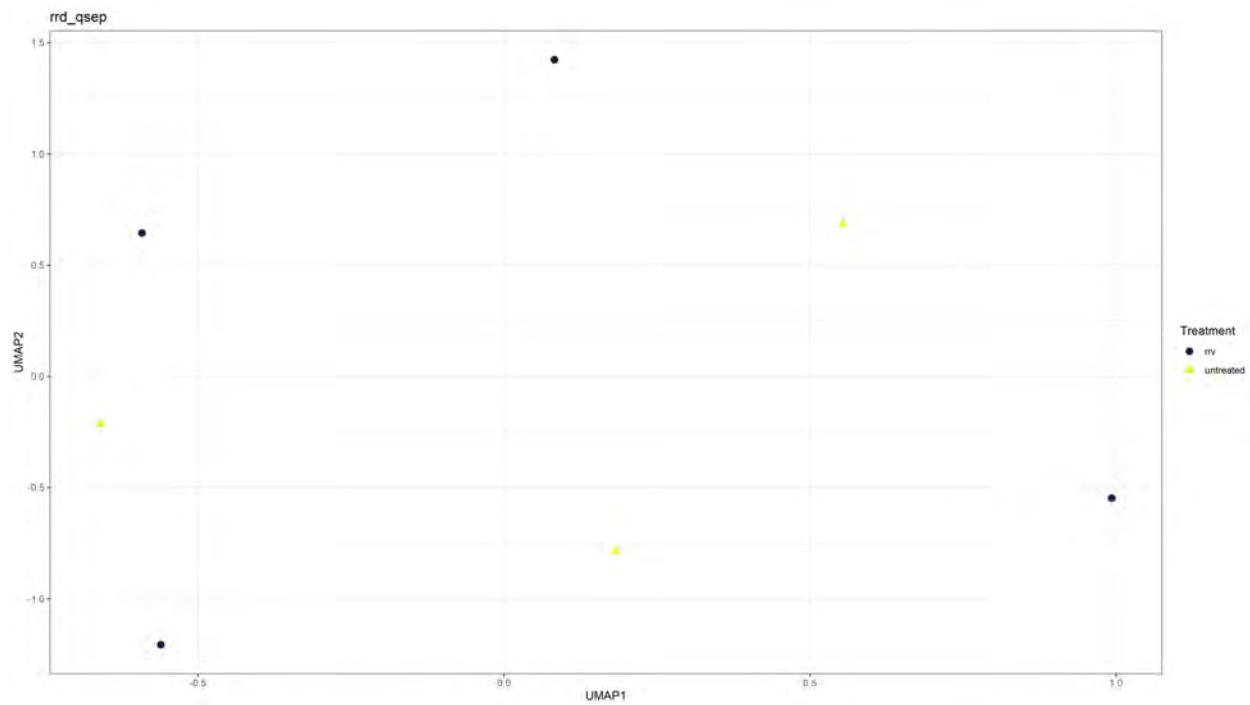


Figure 3-9. Uniform Manifold Approximation and Projection (UMAP) of VCT samples.

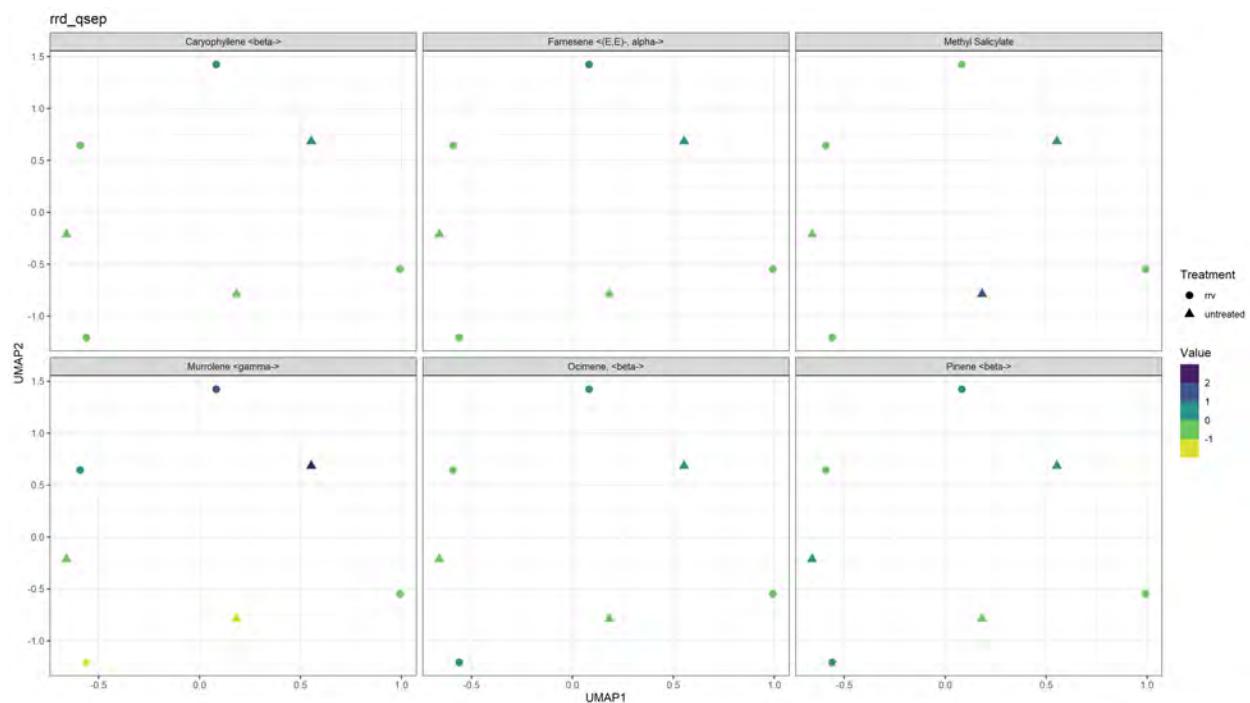


Figure 3-10. UMAP of VCT method's six largest contributions to volatile compositions.

Table 3-1. Contribution table for PCA of headspace VOCs collected with VCT methods from Pink Double Knock Out® roses.

Chemical	PCA1	PCA2	PCA3
Murrolene <gamma->	73.7200281	2.0505736	0.0590805
Caryophyllene <beta->	11.4442072	6.3932415	1.6136116
3-Hexen-1-ol Acetate (Z)-	4.1088721	4.3938742	0.0543187
Farnesene <(E,E)-, alpha->	2.1748819	0.6870580	1.2212043
Pinene <alpha->	1.9893868	7.6247823	1.5809223
Ocimene, <beta->	1.8922159	12.1385950	3.3711482
Pinene <beta->	1.2158051	3.0295147	1.3808570
Germacrene D	0.7637065	0.0169593	0.0005140
D-Limonene	0.6934082	48.5442320	4.7360377
Methyl Salicylate	0.6511928	12.4348187	84.5744765
3-Hexen-1-ol, (Z)-	0.4892346	0.9571750	0.1575944
Cadinene <delta->	0.1804902	0.1066432	0.2043152
Nona-1,3,7-triene <4-8-dimethyl-, (E)->	0.1485550	0.0001213	0.0034408
2-Hexenal	0.1396463	0.0379478	0.0191767
Copaene <beta->	0.1250792	0.0000533	0.0895675
Humulene	0.0717575	0.0196316	0.0003570
Aromadendrene	0.0486886	0.1419599	0.2551968
2,4,6-Octatriene, 2,6-dimethyl-, (E,Z)-	0.0352002	0.0348526	0.0458192
Calamenene	0.0292478	0.1013612	0.3343829
Caryophyllene <9-epi-(E)->	0.0238007	0.1026956	0.0680314
Ocimene, <trans-beta->	0.0200925	0.0135288	0.0222477
Nerolidol	0.0126271	0.0034313	0.0017340
2-Hexen-1-ol, acetate, (E)-	0.0093561	0.0819047	0.0016911
Pentane, 3-ethyl-2,2-dimethyl-	0.0051438	0.0121933	0.0941394
Myrcene <beta->	0.0029627	0.1348161	0.0120437
Trivertal	0.0027171	0.2966634	0.0007833
2-Thujene	0.0013261	0.0817092	0.0081560
5-Hepten-2-one, <6-methyl->	0.0003697	0.5596623	0.0891524

Table 3-2. Correlation table for PCA of headspace VOCs collected with VCT methods from Pink Double Knock Out® roses.

Chemical	PCA1	PCA2	PCA3
D-Limonene	0.1385033	0.7135872	0.1383390
Methyl Salicylate	0.1342210	-0.3611586	0.5845971
Calamenene	0.0284454	0.0326072	0.0367586
2-Hexen-1-ol, acetate, (E)-	0.0160884	0.0293111	0.0026141
Pentane, 3-ethyl-2,2-dimethyl-	0.0119291	-0.0113094	-0.0195040
Myrcene <beta->	0.0090533	0.0376053	0.0069762
Trivertal	0.0086701	0.0557841	0.0017791
2-Thujene	0.0060570	0.0292761	0.0057408
5-Hepten-2-one, <6-methyl->	0.0031980	0.0766198	0.0189803
Nerolidol	-0.0186904	-0.0059994	0.0026470
Ocimene, <trans-beta->	-0.0235766	0.0119126	0.0094815
Caryophyllene <9-epi-(E)->	-0.0256602	0.0328212	0.0165803
2,4,6-Octatriene, 2,6-dimethyl-, (E,Z)-	-0.0312060	0.0191204	0.0136069
Aromadendrene	-0.0367011	-0.0385888	-0.0321125
Humulene	-0.0445553	0.0143501	-0.0012011
Copaene <beta->	-0.0588245	0.0007475	-0.0190245
2-Hexenal	-0.0621556	-0.0199513	0.0088029
Nona-1,3,7-triene <4-8-dimethyl-, (E)->	-0.0641076	-0.0011280	-0.0037288
Cadinene <delta->	-0.0706630	-0.0334461	-0.0287334
3-Hexen-1-ol, (Z)-	-0.1163387	-0.1002014	-0.0252352
Germacrene D	-0.1453546	0.0133377	-0.0014412
Pinene <beta->	-0.1833992	0.1782645	0.0746984
Ocimene, <beta->	-0.2287973	0.3568309	0.1167148
Pinene <alpha->	-0.2345985	0.2828082	0.0799268
Farnesene <(E,E)-, alpha->	-0.2452920	0.0848936	0.0702475
3-Hexen-1-ol Acetate (Z)-	-0.3371529	-0.2146852	0.0148153
Caryophyllene <beta->	-0.5626760	0.2589637	0.0807489
Murrolene <gamma->	-1.4280990	-0.1466614	-0.0154511

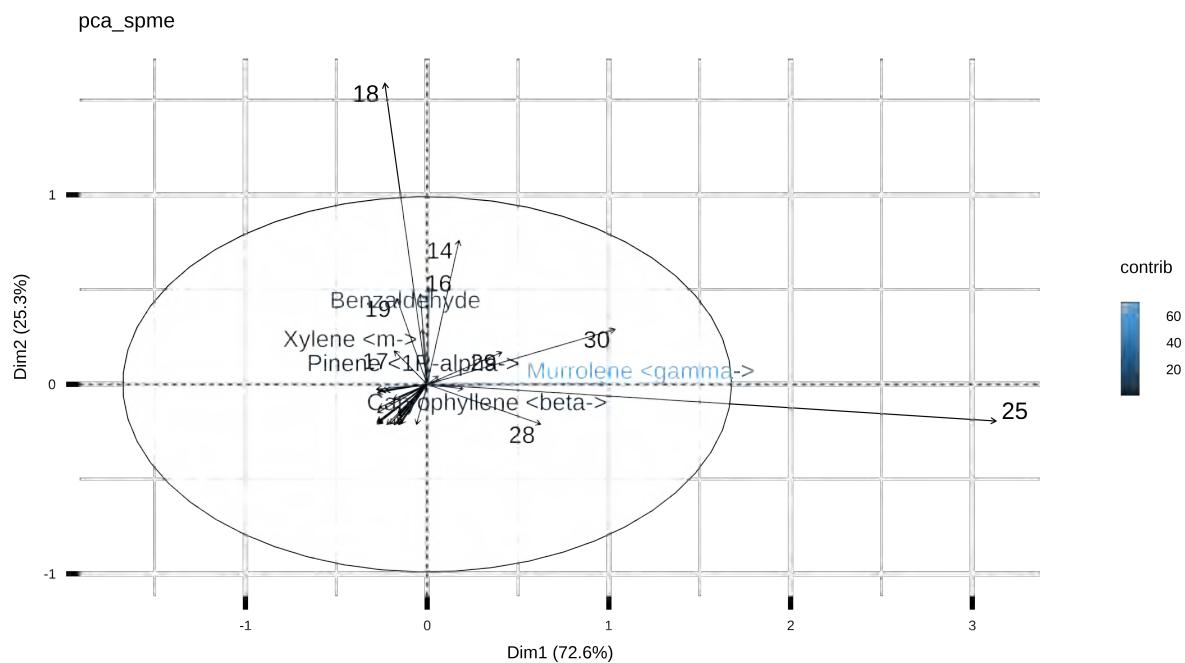


Figure 3-11. PCA biplot of volatiles collected with SPME method.

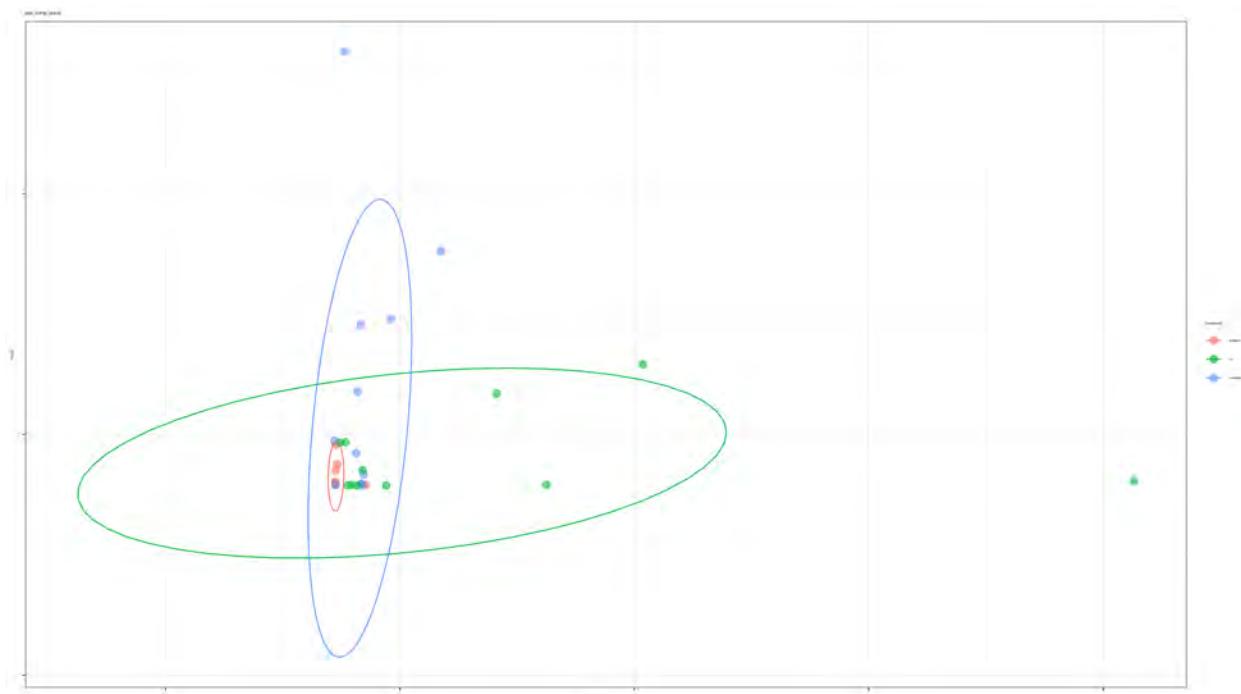


Figure 3-12. Comparison of SPME principal components. Ellipses represent 95% confidence intervals.

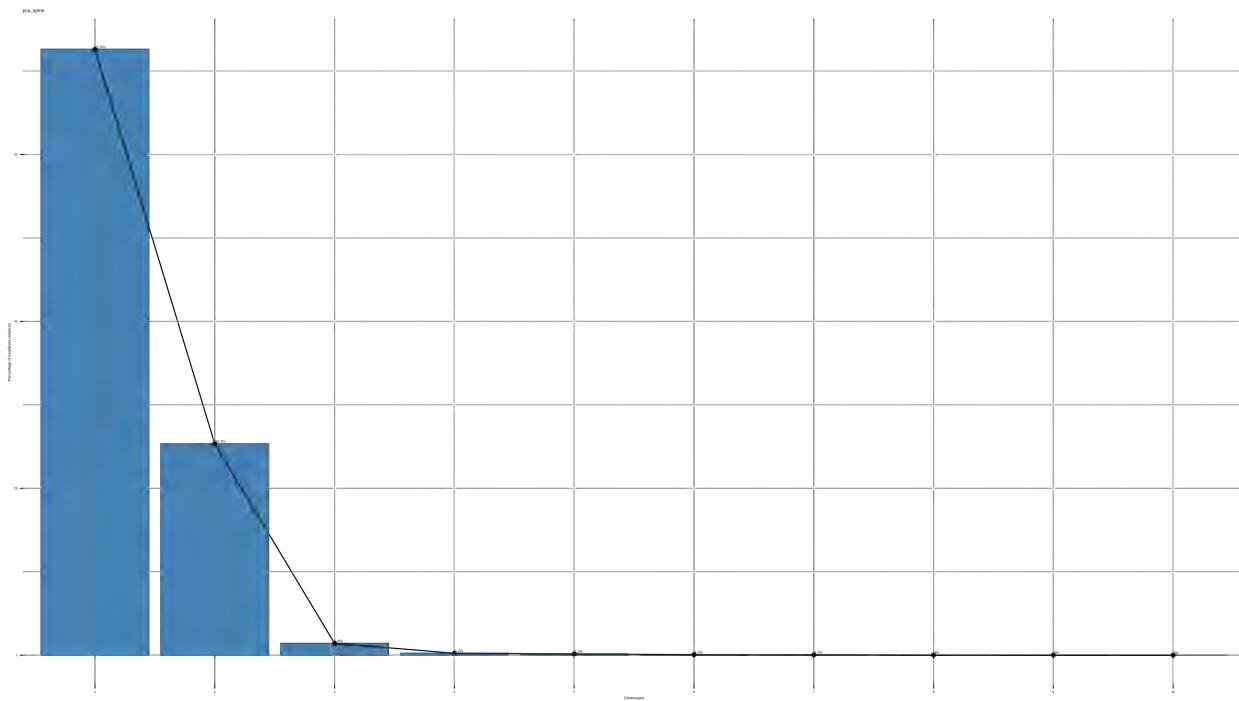


Figure 3-13. Scree plot of SPME principal components

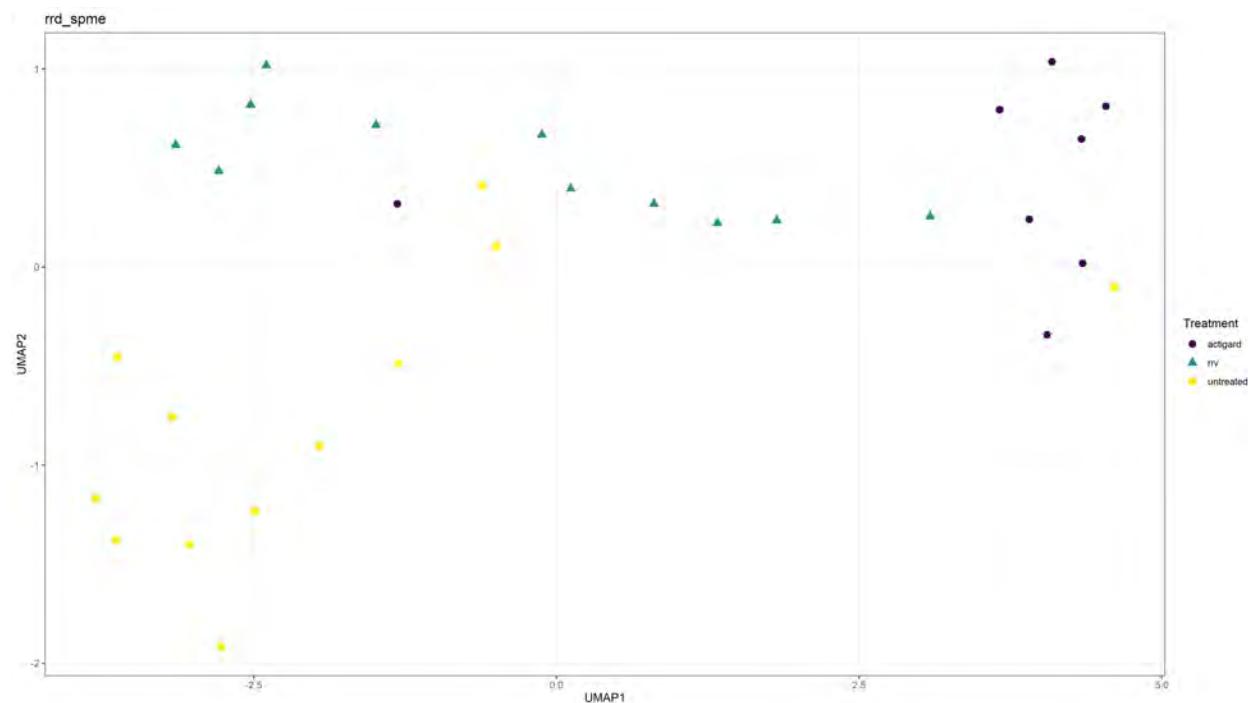


Figure 3-14. UMAP of SPME method volatiles.

Table 3-3. Contribution table for PCA of headspace VOCs collected with SPME methods from Pink Double Knock Out® roses.

Chemical	PCA1	PCA2	PCA3
Murrolene <gamma->	95.2415076	0.0053449	0.3441858
Caryophyllene <beta->	3.9342124	0.1693947	10.4160243
Pinene <1R-alpha->	0.3768098	0.5546385	2.2986334
Mesitylene	0.1335730	0.0002688	0.8001631
Pinene <beta->	0.1116905	0.1813244	2.4829953
Xylene <m->	0.0713400	23.9618474	62.3968052
Decanal	0.0581282	0.0397120	0.0663818
Murrolene <alpha->	0.0574696	0.0000490	0.0049504
Benzaldehyde	0.0078500	75.0832447	20.3483361
Carene <delta-3>	0.0024585	0.0008599	0.0266662
Bourbonene <beta->	0.0022327	0.0003698	0.2020302
Copaene <alpha->	0.0007166	0.0003087	0.0899490
Sulindac sulfide	0.0005104	0.0008275	0.0002247
Nonanal	0.0004008	0.0004141	0.1045067
p-Cymene	0.0003913	0.0004774	0.0565775
Pinene <alpha->	0.0001973	0.0000038	0.0013929
Squalene	0.0001843	0.0006171	0.3553188
Cubebene <beta->	0.0001687	0.0000857	0.0017078
Caryophyllene oxide	0.0000720	0.0000732	0.0017161
Farnesene <(E,E)-, alpha->	0.0000449	0.0000114	0.0003219
Zonarene	0.0000171	0.0000013	0.0000096
Phellandrene <beta->	0.0000120	0.0000197	0.0001646
Pentadecane	0.0000089	0.0000522	0.0007362
Dodecane	0.0000034	0.0000497	0.0001903
Hexadecane, 1-bromo-	0.0000002	0.0000026	0.0000055
Undecane	0.0000000	0.0000001	0.0000016
Benzaldehyde <para-ethyl->	0.0000000	0.0000001	0.0000001
Limonene oxide, trans-	0.0000000	0.0000000	0.0000030
Terpineol <alpha->	0.0000000	0.0000000	0.0000013
Bergamotene <alpha-, cis->	0.0000000	0.0000011	0.0000006

Table 3-4. Correlation table for PCA of headspace VOCs collected with SPME methods from Pink Double Knock Out® roses.

Chemical	PCA1	PCA2	PCA3
Murrolene <gamma->	0.6348800	0.0028099	0.0053536
Caryophyllene <beta->	0.1290350	-0.0158185	-0.0294511
Pinene <1R-alpha->	0.0399337	0.0286233	-0.0138352
Mesitylene	0.0237759	-0.0006301	-0.0081628
Pinene <beta->	0.0217414	0.0163660	-0.0143793
Decanal	0.0156845	0.0076591	-0.0023511
Murrolene <alpha->	0.0155954	0.0002690	-0.0006421
Benzaldehyde	0.0057639	0.3330322	0.0411637
Carene <delta-3>	0.0032256	-0.0011270	-0.0014902
Bourbonene <beta->	0.0030739	0.0007391	0.0041016
Copaene <alpha->	0.0017414	0.0006753	0.0027368
Pinene <alpha->	0.0009137	0.0000754	-0.0003406
Cubebene <beta->	0.0008449	-0.0003558	-0.0003771
Caryophyllene oxide	0.0005519	-0.0003289	-0.0003780
Farnesene <(E,E)-, alpha->	0.0004358	-0.0001300	-0.0001637
Zonarene	0.0002692	-0.0000444	-0.0000283
Phellandrene <beta->	0.0002257	0.0001706	-0.0001171
Undecane	0.0000126	-0.0000138	-0.0000117
Limonene oxide, trans-	0.0000065	-0.0000065	-0.0000158
Bergamotene <alpha-, cis->	-0.0000028	0.0000410	0.0000070
Terpineol <alpha->	-0.0000035	-0.0000009	-0.0000102
Benzaldehyde <para-ethyl->	-0.0000065	-0.0000093	-0.0000023
Hexadecane, 1-bromo-	-0.0000287	-0.0000616	-0.0000215
Dodecane	-0.0001204	-0.0002710	-0.0001259
Pentadecane	-0.0001940	0.0002776	0.0002476
Squalene	-0.0008831	-0.0009547	-0.0054395
p-Cymene	-0.0012868	0.0008397	0.0021706
Nonanal	-0.0013024	-0.0007821	-0.0029500
Sulindac sulfide	-0.0014697	0.0011056	-0.0001368
Xylene <m->	-0.0173758	0.1881373	-0.0720828

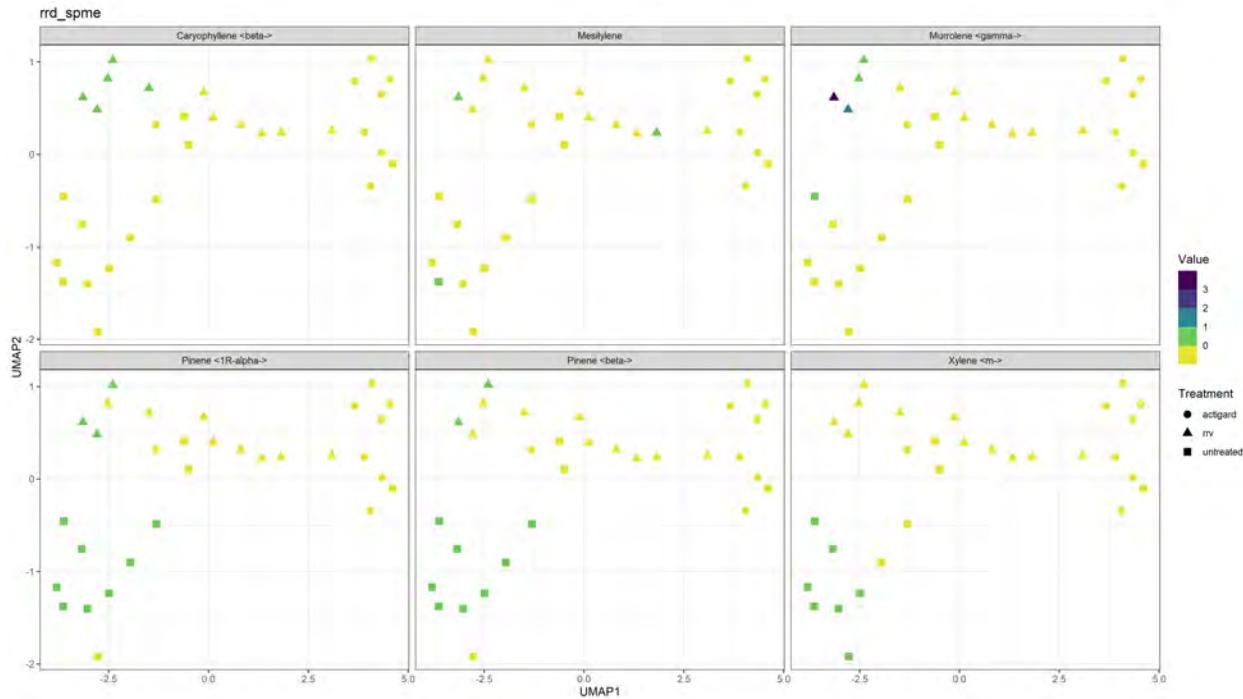


Figure 3-15. UMAP of SPME method's six largest contributions to volatile compositions.

### 3.4 Discussion

Plants play a large role in the lives of phytoseiid mites ([Cortesero et al. 2000, Schmidt 2013](#)): for example the trichome density of plants affects their dispersal ([Loughner et al. 2010a, 2010b, Buitenhuis et al. 2013, Lopez et al. 2016](#)), oviposition, ([O'Dowd and Willson 1991, Walter 1992, 1996, Grostal and O'Dowd 1994, Agrawal and Karban 1997](#)), performance as predators ([Cédola et al. 2001, Seelmann et al. 2007, Buitenhuis et al. 2013](#)) and their ability to avoid predation ([Faraji et al. 2002](#)). Therefore, it is not unusual to see a preference towards plant VOCs, but it is interesting to note their attraction to an infected rose ([3-3](#)). Future tests should try varying the concentrations of these chemicals, and testing the other chemicals detected from the volatile extractions. Olfactometers are considered a useful way to determine predatory mite attraction to different plant volatiles ([Janssen et al. 1990](#)). It is interesting to note that VCT methods found that levels of MeSA were lower for infected plants; SPME methods did not recover MeSA. It may be that SPME fibers were not exposed to VOCs long enough on infected roses to reach

equilibrium, or MeSA is very low in severely infected plants. It is also peculiar to see that MeSA levels were not recovered from ASM-treated plants ([3-3](#), [3-4](#)). ASM induces SAR, again possibly that SPME fibers may not be adsorbing MeSA in our assays for some reason. Furthermore, ASM-treated plants had lower volatile activity overall, compared to other roses, which may explain the clustering we see in our figures ([3-12](#), [3-7](#)). Unfortunately, these data are not truly comparable due to the inherent differences in the collecting methods used. Another possibility is that the time of sampling affected the outcome, as SPME is affected by temperature and plant volatiles are regulated by both time of day and UV exposure ([Griebel and Zeier 2008](#), [Escobar-Bravo et al. 2019](#)). Double Knock Out® roses are known to have less fragrance than other rose cultivars, suggesting lower levels of volatile emissions from petals ([Bergougnoux et al. 2007](#)), which may have affected the quantities of the VOCs collected. Another issue comes from the field aspects of the study, with multiple herbivores present on the roses in the landscape which could activate different plant defenses. For example, *T. urticae* are known to activate both SA and JA signalling pathways during feeding ([Ozawa et al. 2000](#)), which may be interfering with volatiles which would normally be introduced by an infestation of just *P. fructiphilus*. Herbivore induced plant volatiles often last only for a few days after induction ([Choh and Takabayashi 2007](#)), but ASM-treated plants were sampled at the end of a 12-week application period, so these plants may have downregulated their production of MeSA by the time of sampling. This is an unfortunate side effect of sampling in the field at a remote site rather than in the lab. The SPME method is limited to short extractions, partially due to overheating of the inflator pumps. It is harder to have high confidence about results without VCT validation, due to the many variables and possible confounding effects such as plant age, measurement error, sample contamination, temperature, etc.. Sampling recently SAR-induced plants with VCT methods would give a good point of reference to resolve this discrepancy. If MeSA levels are truly low on infected roses, it may suggest that MeSA was not the primary chemical involved with attracting *A. swirskii* to roses. The

most common VOCs extracted from roses were terpenes.  $\gamma$ -Murrolene and  $\beta$ -Caryophyllene were the largest contributors to the VOC composition from the roses collected, so it would be worth testing these chemicals with *A. swirskii* to see if these chemicals play a role in *A. swirskii* attraction to infected roses. Mites were not more attracted to Limonene than filtered air in our preliminary trials, but there were differences in time to choice ([3-5](#)) and a significant difference between making a choice vs. no choice ([3-4](#)). On the contrary to the Limonene results, *A. swirskii* appeared to have random behaviors between choices and no-choice, and did not vary in their time to choice ([3-4](#), [3-5](#)). We predict that reducing the concentrations of these synthetic VOCs may produce different responses, more experiments are warranted. In conclusion, we have found preliminary data about volatile changes in infected roses and SAR-induced roses, and their putative relationships with *A. swirskii*.

CHAPTER 4  
MANAGEMENT OF *PHYLLOCOPTES FRUCTIPIHIULUS* WITH SYSTEMIC  
ACQUIRED RESISTANCE

#### 4.1 Introduction: *Phyllocoptes fructiphilus* - Small Mites Creating Big Problems

*Phyllocoptes fructiphilus* Keifer (Trombidiformes: Eriophyidae) is a microscopic plant-feeding arachnid known as an eriophyid mite. *P. fructiphilus* is host specific, only feeding on plants in the genus *Rosa*, and normally cause little damage by itself. Unfortunately, *P. fructiphilus* has become infamous due to Rose Rosette Virus (RRV), a pathogen which the mite transmits while feeding. RRV infection creates the following symptoms: witches' brooms/rosetting, deformed flowers, increased prickle density, elongated shoots, reddened leaves and stems, and increased die-back which ultimately kills the rose host ([Allington et al. 1968](#), [Tzanetakis et al. 2006](#), [Laney et al. 2011](#)). This disease is known as Rose Rosette Disease (RRD) and is widely considered the most serious disease of roses in the US. RRD and the mite have invaded the southeastern US as they followed the range expansion of the non-native *Rosa multiflora* (Thunb) towards the coast ([Amrine Jr 1996, 2002](#), [Otero-Colina et al. 2018](#)). RRD afflicts a passionate group from different sectors of the US rose industry, including homeowners, commercial landscapers, nurseries, conservationists, and rosarians, all of whom stand to lose millions of dollars and many established roses plantings in the coming years [Rwahnih et al. \(2019\)](#). Florida, as the nation's largest producer of roses, has a special interest developing methods to better control *P. fructiphilus* and RRD. There is a critical need to improve management of *P. fructiphilus* and RRD. Unfortunately, few commercially available roses have resistance to RRD ([Di Bello et al. 2017](#), [Byrne et al. 2018](#)). Presently, growers are recommended to manage the *P. fructiphilus* by removing plants and spraying pesticides ([Hong et al. 2012](#), [Olson et al. 2017](#), “Control - rose rosette” [2018](#)). However, pesticides have come under increased public scrutiny due to concerns about health, the environment, pest resistance, and harm to pollinators [Vanbergen and Insect Pollinators Initiative \(2013\)](#). Increased pesticide applications also decrease grower profits and reduce competitiveness with foreign markets. The lack of alternative or complementary management options exacerbates this

issue: rose growers need more options for *P. fructiphilus* control, especially methods which can be integrated into existing management programs. In 2013, nursery workers in Quincy, Gadsden County, Florida, USA, detected unusual red growths, deformed stems and extra thorniness on 15 knockout roses which had been imported from out of state. Eight symptomatic plants were tested by our Plant Diagnostic Clinic at the University of Florida's North Florida Research and Education Center in Quincy, FL, and found to be positive for RRD, but the *P. fructiphilus* mites were not detected at that time ([Babu et al. 2014](#)). On February 14, 2019, populations of *P. fructiphilus* were encountered on roses in Tallahassee, Leon County, Florida ([Fife et al. 2020](#)). The existence of *P. fructiphilus* in northern Florida increases the the possibility of introducing RRD from areas where the disease had become established, including the neighboring states of Georgia and Alabama ([Solo 2018, Solo et al. 2020](#)).

#### **4.1.1 Integrating pest management: what are the effects of systemic acquired resistance on *Amblyseius swirskii* and *Phyllocoptes fructiphilus*?**

Integrated Pest Management (IPM) is the combination of science-informed best practices designed to keep the cost of pest control below the value of the crop damages which would occur without intervention (i.e. economic injury level) ([Stern et al. 1959, Flint and Bosch 1981, USDA-ARS 2018](#)). In practice, IPM is informed by an understanding of the pest's biology and the judicious use of chemical controls, natural predators (biological controls), plant breeding, plant immune systems and physical (cultural) controls as needed to control pests as efficiently as possible ([Bradley and Moore 2018](#)). Our lab has been investigating the potential of inducing systemic acquired resistance (SAR) to provide protect from mite herbivory as well as pathogen damage ([Cole 1999, Anfoka 2000, Babu et al. 2021, Doungous et al. 2021](#)). Induction of SAR may be able to disrupt the establishment of eriophyoid mites ([Bronner et al. 1991a, 1991b, Westphal et al. 1991](#)). Infested and SAR-induced plants produce β-1,3-glucanase and chitinases, which were hypothesized to contribute to plant defenses against eriophyoid mites ([Bronner et al. 1991b, Ward et al.](#)

1991). Similar increases in  $\beta$ -1,3-glucanase and chitinase activity was seen in roses treated with acibenzolar-S-methyl (ASM), a benzothiadiazole known used to induce SAR (Cole 1999, Darolt et al. 2020, Doungous et al. 2021). Applications of ASM have also been reported to help restrict the growth of fungal pathogens in roses (Suo and Leung 2001). We hypothesize that SAR-induction in roses may be able to reduce populations of *P. fructiphilus* as well. Unfortunately, inducing SAR can have negative effects biological control agents as well as pest species (Kant et al. 2015, Ataide et al. 2016, Pappas et al. 2017), emphasizing the importance of studying the interactions between pests, predators and agrichemical applications to create a successful pest management program. The Swirskii mite, *Amblyseius swirskii* Athias-Henriot (Mesostigmata: Phytoseiidae), is one of the most popular species of commercially-available phytoseiid mites (Calvo et al. 2014). *A. swirskii* are typically released in greenhouse vegetable crops (Messelink et al. 2008, Arthurs et al. 2009, Gradish et al. 2010, Doğramacı et al. 2011), but there is growing interest to use them as biocontrol for ornamental crops (Buitenhuis et al. 2015, Ghasemzadeh et al. 2017). *A. swirskii* are considered to be a generalist species which feed on a variety of different arthropod pests, including some of the thrips, whiteflies, and mites which affect roses (Chow et al. 2010, Hoogerbrugge et al. 2011, Hoogerbrugge et al. 2014, Buitenhuis et al. 2015, Alipour et al. 2016, 2019, Schoeller et al. 2020). Phytoseiid like *A. swirskii* have no eyes, instead relying on plant VOCs to guide them to their prey (Gnanvossou et al. 2003, Boer and Dicke 2004a, Nomikou et al. 2005). Many plant VOCs are released when a plant is attacked by herbivores or pathogens (Shulaev et al. 1997, Sabelis et al. 1999, Ozawa et al. 2000, Halitschke et al. 2007), the composition of which varies, depending on the plant species and type of damage/infection (Sabelis et al. 1999, Boom et al. 2004, Maeda and Liu 2006, Qualley and Dudareva 2008). Volatiles also deter herbivores or attract predators (Pichersky and Gershenson 2002), so it important to understand which chemicals may manipulate the behaviors of released biological agents in the same system. Studies by Farber et al. (2019) found differences between healthy and RRV-infected roses

using Raman spectroscopy at a spectral band of  $1720\text{ cm}^{-1}$ , a spectral region associated with carbonyl stretching vibrations. This indicates the presence of carbonyl compounds, a group functional group commonly seen in many organic compounds, including esters, aldehydes, carboxylic acids, and ketones. Farber et al. (2019) suggested that bands may be evidence for the presence of the carboxylic compounds Jasmonic Acid and Salicyclic Acid, due to the association of these plant hormones with plant defenses against pathogens (Verma et al. 2016). When SAR is induced, SA becomes methylated and converts to Methyl Salicylate (MeSA), a volatile signal which helps to propagate resistance throughout the plant (Shulaev et al. 1997). Fortunately, the VOCs released from infected or induced plants can be analyzed using the methods of paired Gas Chromatography - Mass Spectrometry to determine the quantity and quality of plant headspace volatiles. Once the primary VOCs are identified, olfactometers assays can be used a preliminary method to screen the attractiveness of various VOCs to predatory mites (Dicke et al. 1988, Janssen et al. 1990, Nomikou et al. 2005, Halitschke et al. 2007). With that in mind, our studies were designed to investigate differences between RRV-infected and uninfected Pink Double Knock Out® roses and their volatiles, as well as the effects of SAR-induction on rose volatiles.

Our lab has leveraged its experience with chemical ecology to conduct a series of preliminary two-choice maze (Y-tube olfactometer) trials with various species of commercially-available predatory mites. In these trials, mites can be exposed to chemical odorants which are correlated with the pest arthropod, such as samples droppings or sheds of the pest, a substrate which the pest has walked on, eggs of the pest, or plants which have been attacked by the pest (Janssen et al. 1990). Florida is one of the main producers of woody ornamentals in the US, While testing the compatibility of our rose system with various commercially-available predatory mites, we observed that *Amblyseius swirskii* Athias-Henriot (Mesostigmata: Phytoseiidae) mites were attracted towards roses which were infected with RRV (see Chapter 3 - Results). These results encouraged us to further

investigate the differences between the chemical odorants (headspace volatiles) released from RRV-infected and uninfected roses using coupled Gas Chromatography-Mass Spectroscopy analysis (GC-MS). The GC-MS data suggested that RRV-infected roses had low levels of an important plant hormone known as Methyl Salicylate (MeSA) ([1.3](#)). MeSA typically increases during an immune response, such as when a plant is attacked by herbivores or pathogens ([Shulaev et al. 1997](#), [Park et al. 2007](#), [Tieman et al. 2010](#)). We expected high levels of MeSA in these infected roses, because they were in the middle of experiencing a pathogen attack, but contrary to this expectation, we found low levels of MeSA emitted from the RRV-infected roses. MeSA is also known to be an attractive odor to some many predatory mites, which use MeSA to locate their prey [Boer and Dicke \(2004a\)](#), and are often attracted to the Volatile Organic Compounds (VOCs) released when plants are injured by pests or infected with pathogens ([Boer and Dicke 2004b](#)). Our results suggest that either *A. swirski* are attracted to very low levels of MeSA, or perhaps this attraction is caused by other plant chemical cues besides MeSA. Either way, identification of these VOCs gives us insight into how ASM applications influence the dispersal behaviors of *A. swirksii*, which has implications for how effective predatory mite biocontrol might be. The most interesting part about low levels of MeSA is the role which this phytohormone typically plays in pathogen resistance [Park et al. \(2007\)](#). MeSA is derived from Salicylic Acid (SA) ([Tieman et al. 2010](#)), a chemical involved when inducing a plant's immune response, known as Systemic Acquired Resistance (SAR) ([Gaffney et al. 1993](#), [Gozzo and Faoro 2013](#)). SAR protects plants from fungi, bacteria and viral pathogens when induced, and affects all tissues in the plant [Ryals et al. \(1994\)](#). Low levels of MeSA suggest that RRV interferes with the rose's ability to defend itself against the pathogen. A possible way to avoid this negative feedback loop is to use SA to induce SAR *before* RRV infection, a procedure which would increase the rose's resistance to pathogens before exposure [Kalaivani et al. \(2016\)](#). In light of this knowledge, we collaborated with the University of Georgia in to test how SAR-induction might protect roses from *P. fructiphilus* and/or

RRD. Acibenzolar-S-methyl (ASM) is a benzothiadiazole, a SAR-inducing chemical which works like SA to induce plant defenses against viruses and bacteria Ziadi et al. (2001). ASM is currently used by growers to protect plants from fungal infection (Ziadi et al. 2001, Tripathi et al. 2010, Jeschke 2015). ASM application also has shown chitinase activity in roses (Suo and Leung 2001), and reduces the severity of RRD infection (Babu et al. 2021). Some studies have also shown that the hypersensitive response and SAR interfere with the ability of eriophyoid mites to feed or grow on induced plants (Bronner et al. 1991b, Westphal et al. 1991, 1992). A remaining concern is the effect that SAR-induction may have on predatory mite releases: although predatory mites do not feed directly on plants, they may still be harmed via direct and indirect effects of SAR-induction Pappas et al. (2017), or change their distributions due to the attractive and/or repellent effects of VOCs released from SAR-induction (Boer and Dicke 2004a, 2004b, James and Price 2004, Gadino et al. 2011, 2012). We hypothesized that the systemic acaricide and ASM would reduce populations of *P. fructiphilus* and *T. urticae* more than the control, and that combined treatments of ASM with *A. swirskii* would reduce populations of *T. urticae* more than ASM alone. Furthermore, we hypothesized little harm to *A. swirskii* from either ASM or the acaricide, as *A. swirskii* are reportedly unharmed by spirotetramat-based products (Fernández et al. 2017). In order to test these hypotheses, we conducted a number of field studies from 2018-2021 to test the integration of predatory mites with SAR for control of herbivorous mites.

#### **4.1.2 Phenology of populations of *Phyllocoptes fructiphilus* in northern Florida**

*P. fructiphilus* were encountered on roses in Tallahassee, Leon County, Florida, on February 14, 2019 ([Fife et al. 2020](#)). Accordingly, the NFREC reported the find to the Florida Department of Agriculture and Consumer Services, who were able to confirm the mite species as *P. fructiphilus*. Researchers at the NFREC monitored the local populations of *P. fructiphilus* in that area from 2020-2021, before conducting the IPM field trial in Tallahassee.

## **4.2 Materials & Methods**

### **4.2.1 Inducing systemic acquired resistance with acibenzolar-S-methyl to reduce populations of *Phyllocoptes fructiphilus***

We tested the efficacy of SAR-induction against mites in field trials during 12 weeks, from August to October at Griffin and Athens, GA. Griffin and Athens sites had low and high *P. fructiphilus* pest pressure, respectively.

***Phyllocoptes fructiphilus* Infestations** Potted plants were inoculated with putatively *P. fructiphilus*-infested canes from nearby RRV-infected roses in the landscape. Rose canes from infected roses were placed onto potted roses during the first and fifth weeks of the experiment.

**Roses** Field sites had 48 Pink Double Knock Out® Roses (Star Roses and Plants, West Grove, PA, USA) planted in 1 gallon buckets filled with potting soil and mixed with a granular slow-release fertilizer. Roses were placed on black plastic mulch with approximately 2 ft of space between pots. Plants were watered daily with overhead impact sprinklers. Plants in Griffin were placed alongside a plot of RRV-infected roses during 2018, and a second plot of RRV-infected roses was planted on the opposite side during 2019 (see [4-1](#) and [4-2](#)). Plots were designed with 12 roses total for each treatment, with 16 pots

in 3 rows. 4 columns of 3 roses per block were assigned to the same treatment, and the same plants were rotated to new blocks of the same treatment during the second year (see [4-1](#) and [4-2](#)). Plants were monitored in a greenhouse to observe potential disease progress during the winter of 2018.

**Treatments** Actigard50WG® (Syngenta, Greensboro, NC, USA), (acibenzolar-S-methyl, ASM) was applied at two different rates: 50 mg/L (Low rate) and 100 mg/L (High rate) to observe the effects of inducing Systemic Acquired Resistance (SAR) on *P. fructiphilus*. A spirotetramat based miticide was applied at the label rate (Kontos® Miticide Insecticide, Bayer Corporation, Whippany, New Jersey, USA), and tap water was used as a control. Trials in Athens had the same treatments.

#### **Plot Design ASM Trials, Griffin - 2018 Plot Design ASM Trials, Griffin - 2019**

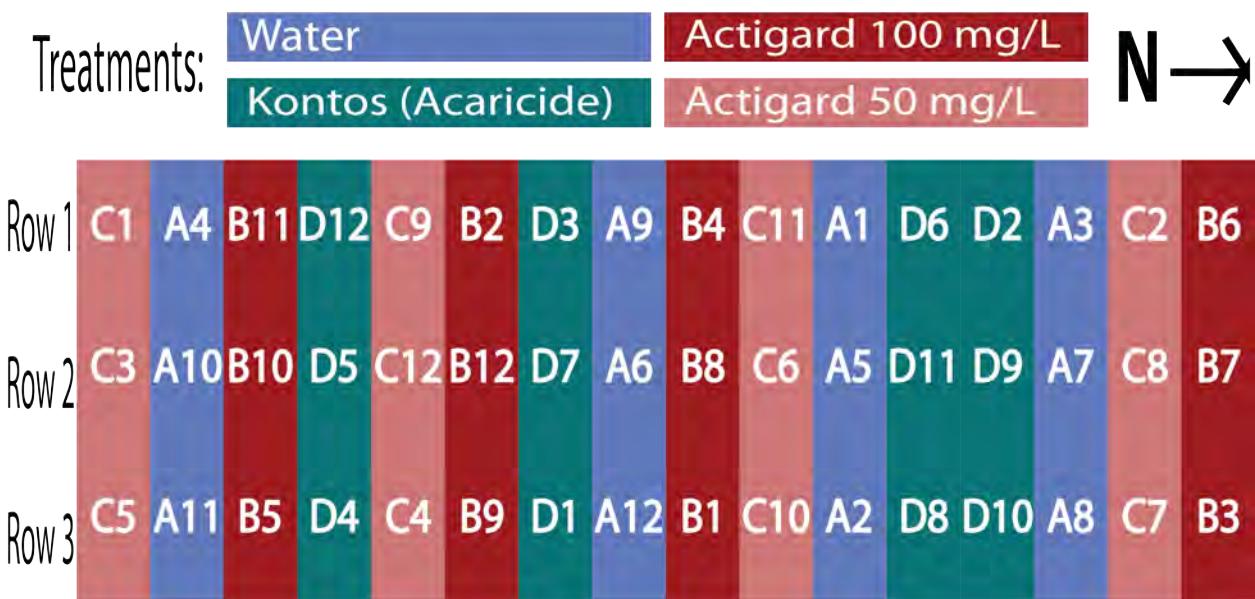
**Data Collection for ASM Trials** Rose/rosebud cuttings of about ~10 cm of cane were taken from each rose before the first treatments to determine the initial populations of *P. fructiphilus* on the roses. A stratified subset (one row across all blocks) of samples was taken from each rose treatment weekly, rotating samples until each rose plant has been sampled three times. Samples were collected from all roses at the end of the trial. The field trial was repeated for two seasons during the growing seasons of 2018 and 2019. Rose samples were placed in 50 mL centrifuge tubes and refrigerated or frozen until floral samples could be processed with 90% ethanol according to the methods of Monfreda et al. ([2007](#)). Eriophyoid mites and other mites collected were counted. Eriophyoid mites were identified after clearing and mounting in Hoyer's iodine-modified medium as described by Faraji and Bakker ([2008](#)).

# Infected Rose Patch

Treatments:	Water	Actigard 100 mg/L	N →													
	Kontos (Acaricide)	Actigard 50 mg/L														
Row 1	A1	B1	C1	D1	C4	D4	B4	A4	D7	A7	B7	C7	D10	A10	B10	C10
Row 2	A2	B2	C2	D2	C5	D5	B5	A5	D8	A8	B8	C8	D11	A11	B11	C11
Row 3	A3	B3	C3	D3	C6	D6	B6	A6	D9	A9	B9	C9	D12	A12	B12	C12

Figure 4-1. Field design for testing the potential of acibenzolar-S-methyl (ASM) to reduce populations of *P. fructiphilus* by inducing Systemic Acquired Resistance (SAR) in Pink Double Knock Out® roses. Trials were conducted for three months from September to December 2019 in Griffin, GA. Four treatments were applied weekly for 12 weeks: Blue = Water Red = Actigard50WG® 100 mg/L (High rate), Pink = Actigard50WG® mg/L (Half rate) Turquoise = Kontos® (Label rate). Flower cuttings were taken weekly to record *P. fructiphilus* numbers.

# Infected Rose Patch



# Infected Rose Patch

Figure 4-2. Field design for testing the potential of ASM to reduce populations of *P. fructiphilus* by inducing SAR in Pink Double Knock Out® roses. Trials were conducted for three months from September to December 2019 in Griffin, GA. Four treatments were applied weekly for 12 weeks: Blue = Water Red = Actigard50WG® 100 mg/L (High rate), Pink = Actigard50WG® 100 mg/L (Half rate) Turquoise = Kontos® (Label rate). Flower cuttings were taken weekly to record *P. fructiphilus* numbers.

#### **4.2.2 Integrating management options to control *Phyllocoptes fructiphilus***

During 2019 we conducted a second set of field trials in Griffin and Athens in addition to the ASM trials. We tested ASM alongside a different a SAR-inducer (SP2700, Trade name ‘Ninja’, SePro) and combined this SAR-inducer with predatory mites releases to observe if these two treatments would have greater reductions of *T. urticae* than either treatment on its own. We again tested a spirotetramat-based miticide and used water as a positive control (see [4-3](#) and [4-4](#)).

**Mite Infestation** *P. fructiphilus* are present in the landscape of Georgia. Cuttings of ~10 cm or rose cane were taken from roses showing symptoms of RRD in the landscape and placed in each rose pot on the 1st, 5th and 9th weeks of the experiment.

**Predatory mites** *Amblyseius swirskii* predatory mites were applied/released on the 1st, 5th and 9th week of the experiments. Mites were deployed from polyethylene fiber sachets (Ambly-S, Arbico Organics, Oro Valley, AZ, USA), containing live colonies of *A. swirskii* and a mite which they consume for food ([Midthassel et al. 2014](#)). There is a small hole at the bottom of these sachets which allowed the mites to be slowly released into the environment. The sachets were hung from rose canes in the center of each rose canopy to encourage successful mite release ([Buitenhuis et al. 2014](#), [Addesso et al. 2018](#)).

**Roses** The experiments were run for 12 weeks from August to October simultaneously in Griffin, GA and Athens, GA. Bare root roses were planted 2 months before the trials began to allow new flush to form. Rose planting media and environmental conditions were the same as previously described. The Athens site received and planted 96 Pink Double Knock Out® Roses (Star Roses and Plants, West Grove, PA, USA), while the Griffin site received 54 roses due to the smaller plot area available. The site at Athens had space for five blocks, while the site at Griffin, GA had space for three blocks. Each block was a  $3 \times 6$  plot with 18 plants, with three roses for each treatment. Flower cuttings were sampled from two rows each week, starting with the top rows of each block and rotating to the next row each week, continuing until all rows were sampled three times.

**Treatments** Actigard50WG® (Syngenta, Greensboro, NC, USA), (acibenzolar-S-methyl, ASM) was applied at 100 mg/L, SP2700 (Trade name: Ninja™, SePro, Carmel, IN, USA) was applied at its labeled rate, A spirotetramat based miticide was applied at the label rate (Kontos® Miticide Insecticide, Bayer Corporation, Whippany, New Jersey, USA), and tap water was used as a control. *A. swirskii* mites were deployed with one sachet per rose treated, and *A. swirskii* + Ninja treatments were applied with one sachet per rose treated, and SP2700 applied at its labelled rate. Treatments were done on the same day each week, weather permitting. Trials in Athens had the same treatments.

**Plot Design - Athens IPM 2019 Plot Design - Griffin IPM 2019 Data Collection for Georgia IPM Trials** Flower samples were collected from all roses once before beginning the treatments on week 1 and once at the end of the experiment on week 12. For weeks 2 through 11, flower samples were collected weekly. starting from the top rows of each block, until each row was sampled three times (see [4-3](#) and [4-4](#)). Disease severity was recorded weekly according to the Horsfall-Barratt Scale ([Horsfall 1945](#)). Roses displaying symptoms of RRD had tissues sent to the Plant Disease Diagnostic Clinic at the North Florida Research and Extension Center(PDC) for virus confirmation. Flower cuttings of about ~12 cm were taken and placed in 50 ml centrifuge tubes. Flowers were placed flower side down and submerged with 15 ml of 95% ethanol. Once the lid was secured, the tube was shaken vigorously for a few seconds to help dislodge any mites. Samples were processed using the washing methods of Monfreda et al. ([2007](#)), eriophyoid mites were counted and identified as previously described.

#### **4.2.3 Phenology field study**

In order to be sure that a further series of IPM trials would be successful, we monitored a population of *P. fructiphilus* in Tallahassee which had mites but no RRD. Rose cuttings were collected periodically from four plots in the landscape of a church in Leon County, FL from 2020-2021: The site had two plantings of 2-3 years old Double Pink Knockout roses, with open sun exposure, ~0.3 m spacing, and natural watering. Blocks were divided into 24

Water			Actigard			Kontos		
Mites			Ninja			Ninja + Mites		
1	2	3	4	5	6	7	8	9
16	17	18	19	20	21	22	23	24
31	32	33	34	35	36	37	38	39
46	47	48	49	50	51	52	53	54
61	62	63	64	65	66	67	68	69
76	77	78	79	80	81	82	83	84
10			13			15		
25	26	27	28	29	30			
40	41	42	43	44	45			
55	56	57	58	59	60			
70	71	72	73	74	75			
85	86	87	88	89	90			

Figure 4-3. Field design for Integrated Pest Management (IPM) trials on Pink Double Knock Out® roses to control *P. fructiphilus* in Athens, GA with five treatments. W = Water A = Actigard50WG, K = Kontos®, M = *A. swirkii* predatory mite sachets, N = SP2700 (Trade name: Ninja, SePro), + = *A. swirkii* + Ninja combined treatments. All products were applied at their label rates for 12 weeks. Flower cuttings were taken weekly to record *P. fructiphilus* numbers.

Water			Actigard			Kontos		
Mites			Ninja			Ninja + Mites		
1	2	3	4	5	6	7	8	9
10	11	12	13	14	15	16	17	18
19	20	21	22	23	24	25	26	27
28	29	30	31	32	33	34	35	36
37	38	39	40	41	42	43	44	45
46	47	48	49	50	51	52	53	54

Figure 4-4. Field design for IPM trials on Pink Double Knock Out® roses to control *P. fructiphilus* in Griffin, GA with five treatments. W = Water A = Actigard50WG, K = Kontos®, M = *A. swirkii* predatory mite sachets, N = SP2700 (Trade name: Ninja, SePro), + = *A. swirkii* + Ninja combined treatments. All products were applied at their label rates for 12 weeks. Flower cuttings were taken weekly to record *P. fructiphilus* numbers.

plots of ~3 m<sup>2</sup>, with approximately 12 roses per plot. Samples were processed as previously described in 2.3. After washing, plant tissues were placed into paper bags and put into a drying oven for ~48 hrs at 50 °C, after which dry weight was recorded. Mites were counted and recorded to track changes in the mite populations. Roses were pruned once on the beginning of July 2020 by a professional landscaping crew.

#### 4.2.4 IPM field trials, Tallahassee 2021

A second trial of IPM was conducted in Tallahassee, FL. This site did not have RRD present, but had verified populations of *P. fructiphilus* present in the landscape (see APPENDIX). Spray applications were done weekly for 12 weeks from May to August 2021. Treatments were made on the same day each week, weather permitting.

**Roses** The site was divided into two blocks, with ten plots of roses in each block. Each plot was 3 m<sup>2</sup> with approximately 12 roses, the six roses at the center of the plot were treated, while the adjacent roses on either side were left as a buffer between plots to avoid treatment drift.

**Treatments** Five treatments were applied: tap water as a control, Actigard50WG® (acibenzolar-S-methyl, Syngenta, Greensboro, NC, USA) - 100 mg/L, *Amblyseius swirskii* mini sachets with hooks (Ambly-S, Arbico Organics, Oro Valley, AZ, USA) - two sachets per rose in treated plots, and a combined treatment of Actigard50WG® - 100 mg/L + *A. swirskii* - two sachets per rose in treated plots, and a spirotetramat miticide (Kontos® Miticide Insecticide, Bayer Corporation, Whippany, New Jersey, USA), (see @(*talla-ipm-2021*)).

**Mite Infestation** *P. fructiphilus* are present in the landscape of Tallahassee. Populations were present on the roses, as verified by the phenology experiments.

**Predatory mites** The two sachets of *A. swirskii* mites were applied to each of the six treated roses on the 1st, 5th and 9th week of the experiment, following the application instructions from the supplying company (Ambly-S, Arbico Organics, Oro Valley, AZ, USA). The sachets were hung from rose canes in the center of each rose. The *A. swirskii* plots were also treated with water weekly when the other plots were sprayed in order to keep conditions similar to other treatments.

**Data Collection for Tallahassee IPM Trials** Samples were collected and processed using similar methods as previously described in [2.3](#): Flower cuttings were taken weekly from each of the six roses in the center of each plot. Three flowers (or buds if no flowers were present) were taken from each of the six central roses in each plot, for a total of 18 flowers/buds per bottle for each sampling bottle. The flowers/buds were placed into the ethanol-filled bottles and shaken vigorously for a few seconds to coat the rose tissue with ethanol and help dislodge any mites. Plant samples were processed according to the methods described in Monfreda et al. ([2007](#)). Plant tissues were retained after mites were washed off, and dried in kraft paper bags and put into the oven until dry (~48 hrs at 50 °C), after which the dry weight of the rose tissues was recorded.

#### 4.2.5 Analysis of field trial data

All data from all trials was processed and analyzed using R version 4.1.1 ([R Core Team 2021](#)). The data were all count data, so a Zero-Inflated Poisson Model ([Zeileis et al. 2008](#)) was required for the ASM data ([Hothorn et al. 2008](#)), while Generalized Linear Mixed Models ([Bates et al. 2015](#)) were used for all of the IPM and phenology trials. Estimated marginal means (least squares means) ([Lenth 2021](#)) and Tukey Contrasts Multiple Comparisons of Means were used to determine significant effects. In addition, about ~5% of early samples collected from the Tallahassee field trials were missing dry weights. Missing weight data was estimated and imputed using Multivariate Imputation by Chained Equations via the mice package ([Buuren and Groothuis-Oudshoorn 2011](#)).

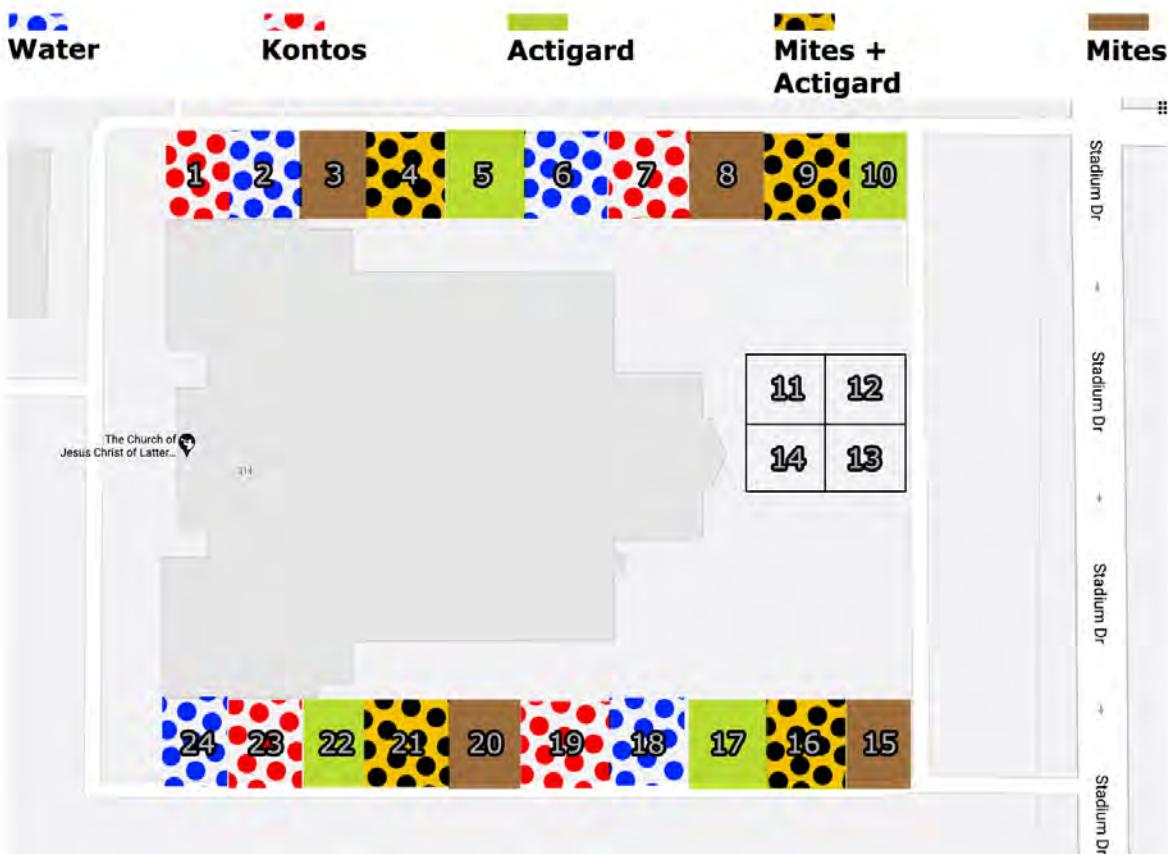


Figure 4-5. Field design for IPM trials on Pink Double Knock Out® roses to control *P. fructiphilus* in Tallahassee, FL with five treatments: Water, Actigard50WG®, Kontos®, *Amblyseius swirskii* predatory mite sachets, and *A. swirskii* + Actigard combined treatments. All products were applied at their label rates for 12 weeks. Flower cuttings were taken weekly to record *P. fructiphilus* numbers.

## 4.3 Results

### 4.3.1 ASM trials

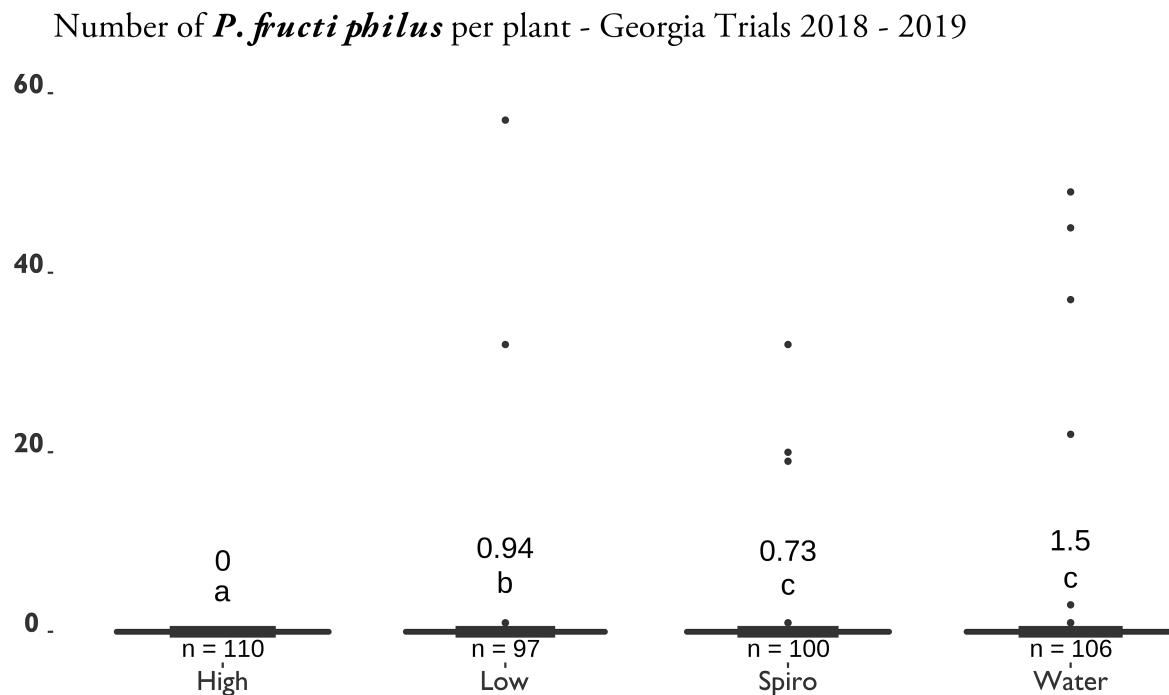


Figure 4-6. SAR-induction trials on Pink Double Knock Out® roses to control *Phyllocoptes fructiphilus* in Athens and Griffin, GA. Statistical significance was determined using Tukey contrasts for multiple Comparisons of means. Groups which share letters are not statistically different from one another.  $\alpha = 0.05$ . water = Water Control, High = 100 mg/L Actigard50WG® (Syngenta, Greensboro, NC, USA) acibenzolar-S-methyl (ASM), low = 50 mg/L Actigard50WG® (Syngenta, Greensboro, NC, USA) acibenzolar-S-methyl (ASM), Spiro = Kontos® Miticide Insecticide - Spirotetramat (Bayer Corporation, Whippany, New Jersey, USA), All products were applied for 12 weeks. Flower cuttings were taken weekly to record the numbers of herbivorous mites.

Table 4-1. Progression of RRD on SAR-induced Pink Double Knock Out® roses in Athens, GA 2018.

Treatment	Block	2018-08-31	2018-09-07	2018-09-14	2018-09-21	2018-09-28	2018-10-05	2018-10-12	2018-10-19	2018-10-26	2018-11-02	2018-12-19	AUDPC	Final disease severity (%)	Sample #	Plant	RRD	P.fructiphilus	Other Mites	Field	
Water	1	0	0	0	0.0	0.0	0.0	1.5	1.5	4.5	1.5	17.5	124.25	17.5	1	W1	S	2	0	Athens	
Water	2	0	0	0	0.0	0.0	0.0	0.0	1.5	4.5	1.5	0.0	52.50	0.0	2	W2	A?	0	0	Athens	
Water	3	0	0	0	0.0	0.0	0.0	0.0	1.5	4.5	1.5	1.5	57.75	1.5	3	W3	A	0	0	Athens	
Water	4	0	0	0	0.0	0.0	0.0	0.0	1.5	1.5	0.0	1.5	0.0	31.50	0.0	4	W4	A	0	0	Athens
Water	5	0	0	0	0.0	0.0	0.0	0.0	1.5	1.5	0.0	0.0	9.0	52.50	9.0	5	W5	S	0	0	Athens
Water	6	0	0	0	0.0	0.0	0.0	0.0	1.5	1.5	1.5	9.0	73.50	9.0	6	W6	S	1	1	Athens	
Water	7	0	0	0	0.0	0.0	0.0	0.0	0.0	1.5	1.5	1.5	26.25	1.5	7	W7	A	0	0	Athens	
Water	8	0	0	0	0.0	0.0	0.0	0.0	0.0	1.5	1.5	4.5	36.75	4.5	8	W8	A?	0	0	Athens	
Water	9	0	0	0	0.0	0.0	0.0	0.0	1.5	1.5	1.5	0.0	42.00	0.0	9	W9	A	0	0	Athens	
Water	10	0	0	0	0.0	0.0	0.0	0.0	0.0	1.5	1.5	0.0	31.50	0.0	10	W10	A?	0	0	Athens	
Water	11	0	0	0	0.0	0.0	0.0	1.5	0.0	0.0	1.5	1.5	36.75	1.5	11	W11	A?	0	0	Athens	
Water	12	0	0	0	0.0	0.0	0.0	0.0	1.5	1.5	1.5	17.5	103.25	17.5	12	W12	S	0	1	Athens	
Low	1	0	0	0	0.0	0.0	0.0	0.0	0.0	1.5	4.5	1.5	0.0	52.50	0.0	13	L1	A	0	0	Athens
Low	2	0	0	0	0.0	0.0	0.0	0.0	0.0	1.5	1.5	4.5	36.75	4.5	14	L2	A	0	0	Athens	
Low	3	0	0	0	0.0	0.0	0.0	0.0	4.5	1.5	4.5	0.0	78.75	1.5	15	L3	S	0	0	Athens	
Low	4	0	0	0	0.0	0.0	0.0	1.5	1.5	1.5	0.0	0.0	36.75	1.5	16	L4	A	0	0	Athens	
Low	5	0	0	0	0.0	0.0	0.0	1.5	0.0	0.0	1.5	17.5	82.25	17.5	17	L5	S	0	0	Athens	
Low	6	0	0	0	0.0	0.0	0.0	0.0	0.0	0.0	1.5	9.0	42.00	9.0	18	L6	A	0	0	Athens	
Low	7	0	0	0	1.5	1.5	1.5	0.0	0.0	1.5	1.5	17.5	113.75	17.5	19	L7	S	0	0	Athens	
Low	8	0	0	0	0.0	1.5	1.5	4.5	1.5	1.5	1.5	4.5	99.75	4.5	20	L8	A	0	0	Athens	
Low	9	0	0	0	0.0	0.0	0.0	1.5	0.0	0.0	0.0	17.5	71.75	17.5	21	L9	S	0	0	Athens	
Low	10	0	0	0	0.0	0.0	0.0	1.5	1.5	0.0	1.5	4.5	47.25	4.5	22	L10	A	0	0	Athens	
Low	11	0	0	0	0.0	0.0	0.0	0.0	1.5	4.5	1.5	0.0	52.50	0.0	23	L11	A	0	0	Athens	
Low	12	0	0	0	0.0	0.0	0.0	1.5	0.0	0.0	1.5	1.5	36.75	1.5	24	L12	A	0	0	Athens	
High	1	0	0	0	0.0	0.0	0.0	1.5	0.0	4.5	4.5	1.5	78.75	1.5	25	H1	S?	0	0	Athens	
High	2	0	0	0	0.0	0.0	0.0	1.5	0.0	0.0	0.0	17.5	71.75	17.5	26	H2	S	0	0	Athens	
High	3	0	0	0	0.0	0.0	0.0	1.5	1.5	1.5	1.5	17.5	103.25	17.5	27	H3	S	0	0	Athens	
High	4	0	0	0	0.0	0.0	0.0	0.0	0.0	1.5	1.5	1.5	26.25	1.5	28	H4	A	0	0	Athens	
High	5	0	0	0	0.0	0.0	0.0	0.0	1.5	1.5	1.5	4.5	47.25	4.5	29	H5	S	0	0	Athens	
High	6	0	0	0	0.0	0.0	0.0	0.0	1.5	1.5	1.5	0.0	31.50	0.0	30	H6	A	0	0	Athens	
High	7	0	0	0	0.0	0.0	0.0	0.0	0.0	1.5	4.5	26.25	4.5	31	H7	S	0	0	Athens		
High	8	0	0	0	1.5	1.5	1.5	1.5	1.5	4.5	4.5	4.5	62.5	62.5	32	H8	S	1	0	Athens	
High	9	0	0	0	0.0	0.0	0.0	1.5	1.5	1.5	1.5	17.5	103.25	17.5	33	H9	S	0	0	Athens	
High	10	0	0	0	0.0	0.0	0.0	1.5	1.5	1.5	1.5	0.0	42.00	0.0	34	H10	A	0	0	Athens	
High	11	0	0	0	0.0	0.0	0.0	0.0	0.0	1.5	1.5	1.5	5.25	1.5	35	H11	A	0	0	Athens	
High	12	0	0	0	0.0	0.0	1.5	0.0	0.0	0.0	0.0	10.50	0.0	36	H12	A	0	0	Athens		
NoTrt	1	0	0	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	37	C1	A	0	0	Athens	
NoTrt	2	0	0	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.5	5.25	1.5	38	C2	A	0	0	Athens	
NoTrt	3	0	0	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	39	C3	A	0	0	Athens	
NoTrt	4	0	0	0	0.0	0.0	0.0	0.0	0.0	0.0	1.5	5.25	1.5	40	C4	A	0	0	Athens		
NoTrt	5	0	0	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	41	C5	A	0	0	Athens	
NoTrt	6	0	0	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	42	C6	A	0	0	Athens	
NoTrt	7	0	0	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	43	C7	A	0	0	Athens	
NoTrt	8	0	0	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	44	C8	A	0	0	Athens	
NoTrt	9	0	0	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	45	C9	A	0	0	Athens	
NoTrt	10	0	0	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.5	5.25	1.5	46	C10	A	0	0	Athens	
NoTrt	11	0	0	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	47	C11	A	0	0	Athens	
NoTrt	12	0	0	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.0	48	C12	A	0	0	Athens	

#### 4.3.2 Phenology

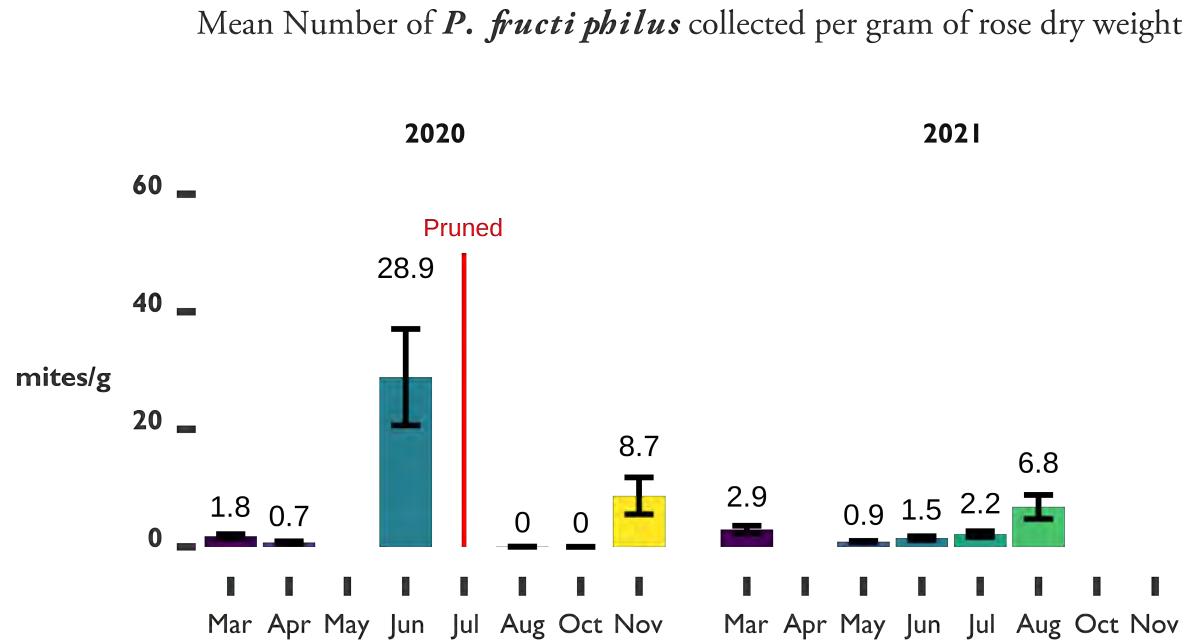


Figure 4-7. Phenology of *P. fructiphilus* mite populations on roses in Leon County, Florida 2020-2021. Roses were pruned back heavily on July 9, 2020.

#### 4.3.3 IPM trials

### 4.4 Discussion

We had low recovery of *P. fructiphilus* for the ASM and IPM trials in Georgia. This lead us to the phenology study, to ensure that there had been adequate establishment of *P. fructiphilus* to create measurable effects. The phenology study was successful in measuring population fluctuations of eriophyid mites, and helped guarantee the presence of *P. fructiphilus* before beginning the IPM trials in Tallahassee. Our results from the the IPM trials in Tallahassee suggest that spirotetramat were able to significantly reduce populations of *P. fructiphilus*, an effect seen in all of the field trials conducted. Numbers of *P. fructiphilus* on ASM-treated plants were were not significantly different than the control (see 4-8). *A. swirskii* were recovered in smaller numbers than the other species of mites (see 4-12). Although we did not create barriers to prevent dispersal of *A. swirskii*, these mites were most frequently recovered from the same plots where they were released.

### Mean Number of *P. fructiphilus* Per Gram of Rose Dry Weight - IPM Trials

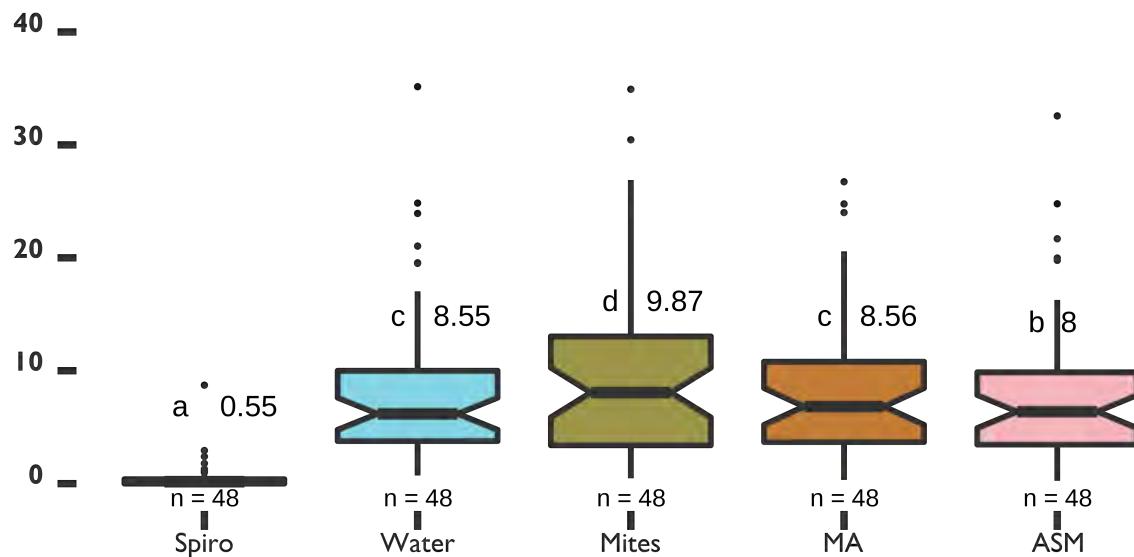


Figure 4-8. IPM trials on Pink Double Knock Out® roses to control *P. fructiphilus* in Tallahassee, FL with five treatments. Statistical significance was determined using Tukey contrasts for multiple Comparisons of means. Groups which share letters are not statistically different from one another.  $\alpha = 0.05$ . Water = Water Control, ASM = Actigard50WG® (Syngenta, Greensboro, NC, USA) acibenzolar-S-methyl (ASM), Spiro = Kontos® Miticide Insecticide - Spirotetramat (Bayer Corporation, Whippany, New Jersey, USA), Mites = *Amblyseius swirskii* predatory mite mini sachets on hooks (Ambly-S, Arbico Organics, Oro Valley, AZ, USA), MA = *A. swirskii* + Actigard combined treatments. All products were applied at their label rates for 12 weeks. Flower cuttings were taken weekly to record the numbers of *P. fructiphilus* and other herbivorous mites.

Mean Number of *P. fructiphilus* Per Gram of Rose Dry Weight - IPM Trials

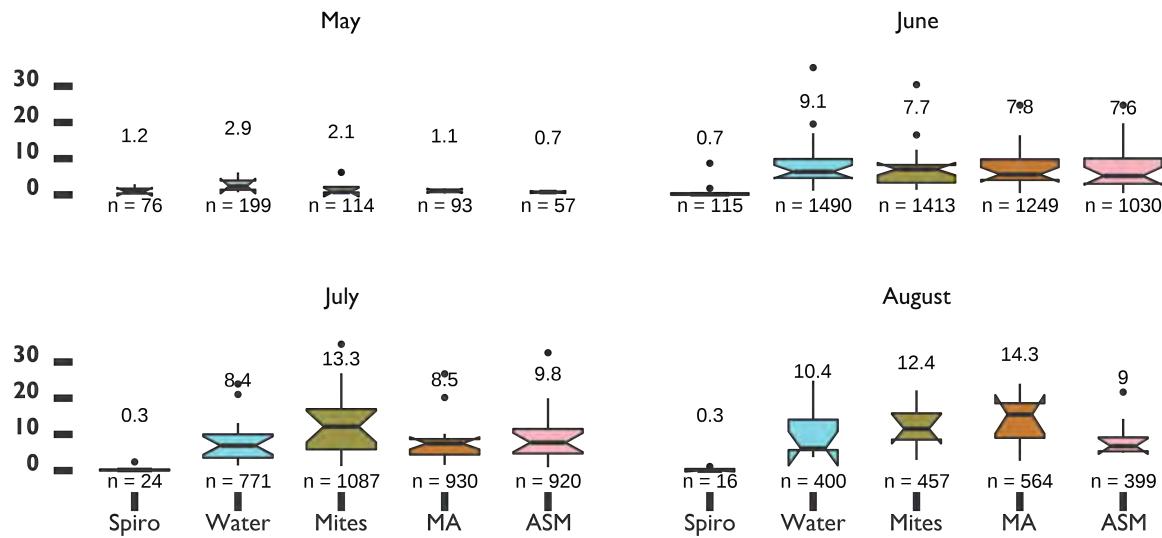


Figure 4-9. IPM trials on Pink Double Knock Out® roses to control *P. fructiphilus* in Tallahassee, FL with five treatments

Mean of Tetranychoid Mites Per Gram Dry Weight - IPM Trials

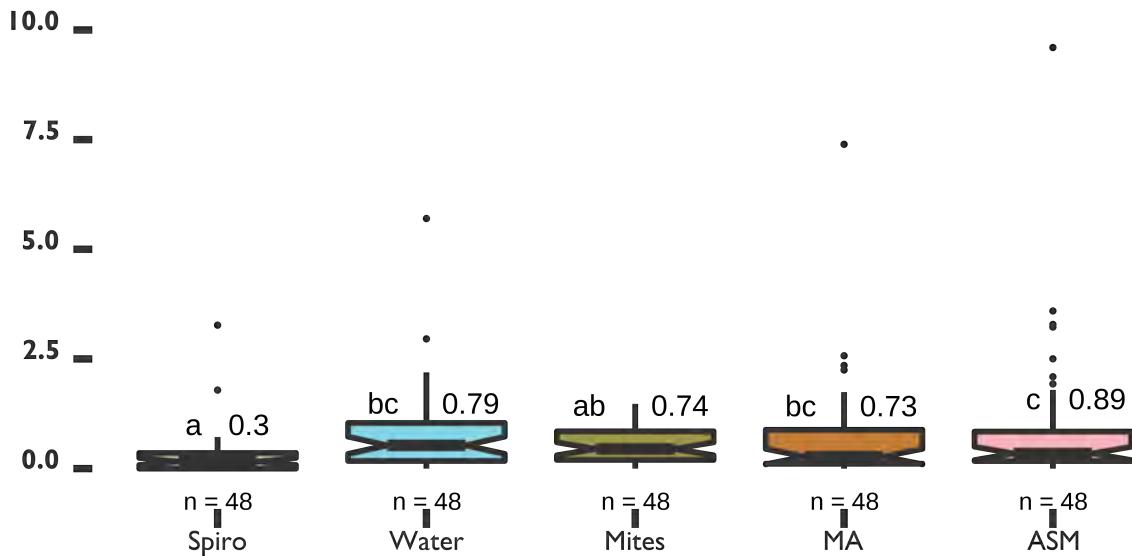


Figure 4-10. IPM trials on Pink Double Knock Out® roses to control *P. fructiphilus* in Tallahassee, FL with five treatments

Mean Number of Tetranychoid Mites Per Gram of Rose Dry Weight - IPM Trials

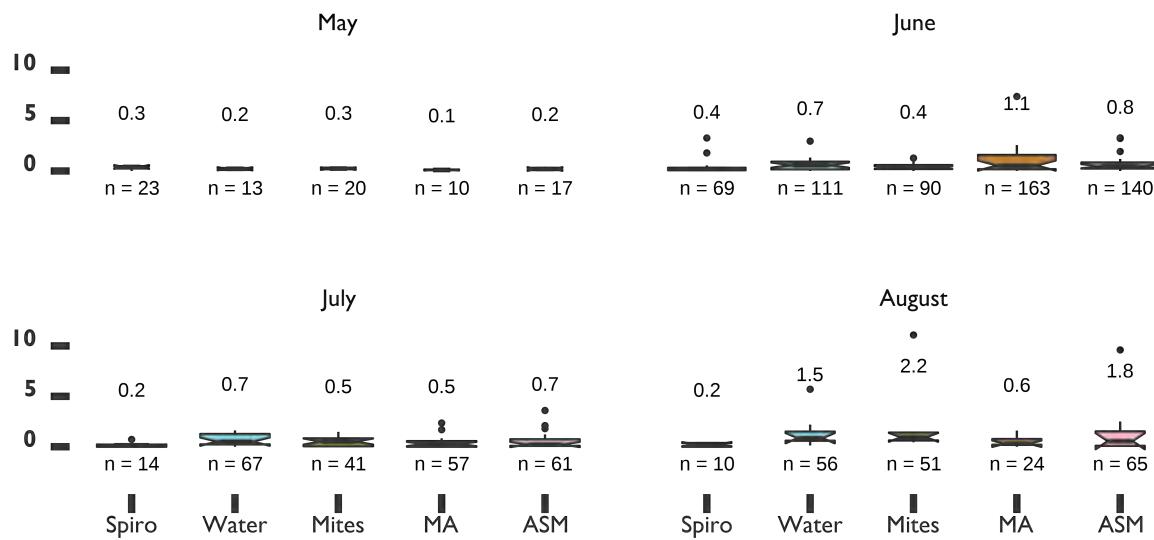


Figure 4-11. IPM trials on Pink Double Knock Out® roses to control *P. fructiphilus* in Tallahassee, FL with five treatments

Phytoseiid Mites Recovered Per Gram of Rose Dry Weight - IPM Trials

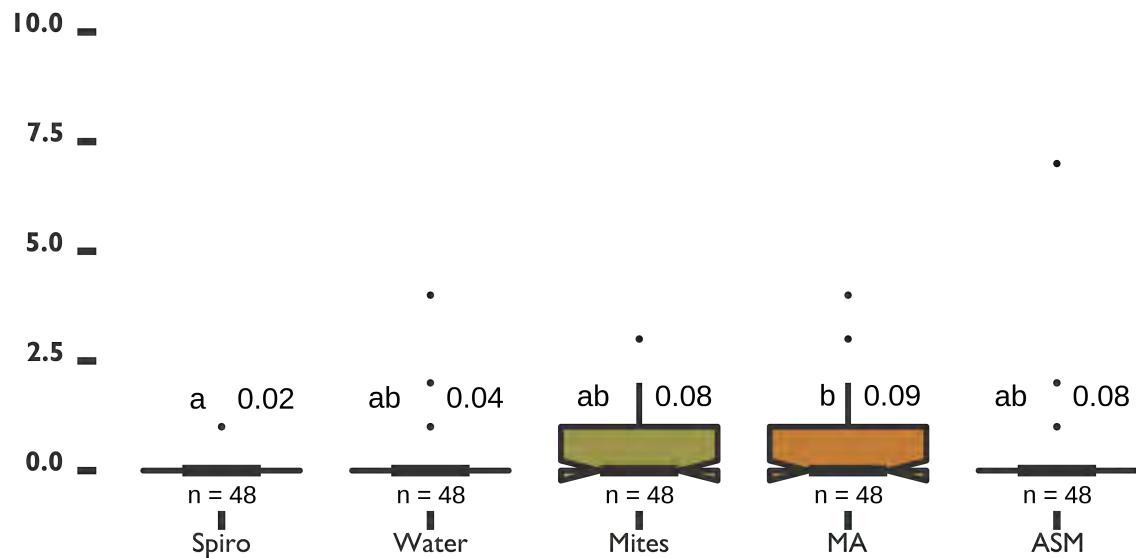


Figure 4-12. IPM trials on Pink Double Knock Out® roses to control *P. fructiphilus* in Tallahassee, FL with five treatments

### Number of Phytoseiid Mites Recovered Per Gram of Rose Dry Weight - IPM Trials

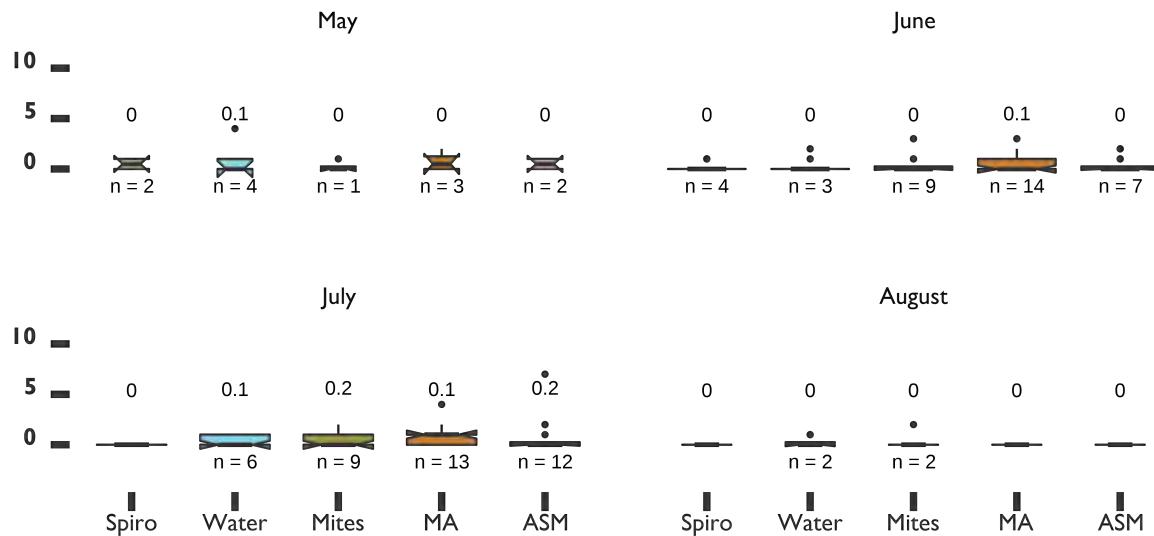


Figure 4-13. IPM trials on Pink Double Knock Out® roses to control *P. fructiphilus* in Tallahassee, FL with five treatments

It is possible that we encountered few *A. swirskii* because they dispersed to the point of leaving the rose plots ([Addesso et al. 2018](#)). This would make sense in the light of other studies: Buitenhuis et al. ([2013](#)) noted the ability of *A. swirskii* to move quickly on roses due to fewer trichomes, and recommended adjusting their release rates accordingly to maintain high populations on plants. *A. swirskii* are not expected to disperse very far off of plants, but canopy connectivity between plants has been noted to increase their dispersal ([Buitenhuis et al. 2010](#)). Ornamentals such as roses tend to be grown in separately spaced pots. Although the roses at our site were planted in the ground, the plots we tested did not have overlapping canopies, which may have reduced dispersal of *A. swirskii* into other nearby roses. We anticipated seeing lower numbers of *T. urticae* in plots where *A. swirskii* had been released, but we did not see any correlations between releases of *A. swirskii* on *T. urticae*. Other studies have found *A. swirskii* predation to be negatively correlated with trichome density ([Buitenhuis et al. 2013](#)), suggesting that *A. swirskii* are unlikely to feed on *P. fructiphilus* hidden in the tightly appressed surfaces

between rose sepals and petals. These areas also contain dense glandular trichomes, which tend to accumulate high quantities of VOCs (Tissier et al. 2017), defensive compounds (Gerhold et al. 1984, Ambrósio et al. 2008, Karabourniotis et al. 2019), or possibly entrap predators (Nihoul 1994), benefits which erinea-producing eriophyids readily exploit to protect themselves (Karioti et al. 2011, Karabourniotis et al. 2019). We did not see any correlations between *A. swirskii* and *P. fructiphilus*, as anticipated. Spirotetramat is known to less toxic to *A. swirskii* (Fernández et al. 2017), but no correlations emerged for *A. swirskii* for either spirotetramat or ASM treatments. Some evidence has been given that slow release mite sachets which are sheltered and provided with felt patches (Shimoda et al. 2017), and/or moisture in the form of water-absorbant sodium polyacrylate gels had better releases of predators (Shimoda et al. 2018). It is also possible that there was an excess of pollen or other acceptable food sources which *A. swirskii* preferred over the *T. urticae* found in roses (Leman and Messelink 2014), or competition with other predators encountered on the roses (Döker et al. 2021). A cursory examination of non-mite arthropods in rose samples revealed a number of unidentified species of thrips. *A. swirskii* are known to feed on thrips, so there may be correlations between the predatory mites and the number of thrips in each treatment, but this requires a reexamination of the bycatch data. Another factor in our studies is the absence of RRV in our plots, which might otherwise mediate multitrophic interactions between the *P. fructiphilus*, *T. urticae* and *A. swirskii* indirectly through induction of different plant defenses (Belluire et al. 2010). Some species of tetranychoids, including *T. urticae*, are known to be able to suppress some plant defenses directly (Sarmento et al. 2011, Alba et al. 2014, Arena et al. 2018, Blaazer et al. 2018). This interference with plant defenses can interfere with the entire herbivore community (Kant et al. 2015), including the eriophyoids feeding on the same plant (Thaler et al. 2001, Glas et al. 2014), which should be examined for *P. fructiphilus* control in future studies. Populations of *P. fructiphilus* showed seasonal fluctuations in mite numbers, with highest populations achieved during June 2020 and July 2021. Rose pruning

in July 2020 reduced the numbers of *P. fructiphilus* collected for 3 months, populations began to recover that November. However, the number of *P. fructiphilus* collected was not significantly different between the years 2020 and 2021 for the phenology trials. Mite numbers remained relatively lower during 2021 compared to the previous year. None of the mite-infested roses have shown symptoms of RRD to date. Tracking populations for longer periods of time with additional climatic data could be used to determine the best times to prune and apply chemistry to reduce mite numbers. The large reductions of *P. fructiphilus* seen post pruning suggests its potential as a method of cultural mite control. The combination of pruning with acaricide treatments has been previously tested to control *P. fructiphilus*, with limited success ([Windham et al. 2014](#)), but these studies were conducted in areas of high pest pressure, with the presence of RRD. It is unlikely that acaricides or pruning are sufficient to prevent RRD infection: pruning does not remove the virus ([Di Bello et al. 2017](#)), and the inoculation access period for transmission of RRD is surprisingly short, with mites able to transmit the virus within an hour of plant feeding ([Di Bello et al. 2017](#)). Even so, the efficacy of combining pruning and acaricides has not been tested in areas with recent introductions of the mite without the virus. Combined management treatments may prove more effective in maintaining low populations of non-viruliferous *P. fruciphilus*, and should be investigated for preventing the spread of the mite, rather than preventing the disease proper.

CHAPTER 5  
*BREVIPALPUS*-TRANSMITTED *ORCHID FLECK DICHORHAVIRUS* INFECTING  
THREE NEW ORNAMENTAL HOSTS IN FLORIDA

Orchid fleck virus (OFV), is the type member for the genus *Dichorhavirus*, family *Rhabdoviridae*. The virus is a bacilliform, nuclear rhabdovirus composed of two segments of single-stranded, negative-sense RNA which infects plants (Dietzgen et al. 2014, Walker et al. 2018, Amarasinghe et al. 2019). Only Flat mites (Trombidiformes: Tenuipalpidae) from the genus *Brevipalpus* are known to transmit dichorhaviruses (Maeda 1998). Plants infected with OFV exhibit chlorotic and necrotic flecks on their leaves (Kubo et al. 2009b, Kubo et al. 2009a, Dietzgen et al. 2018b). The virus was first described as infecting *Cymbidium* orchids in Japan (Doi et al. 1977). There have been reports of OFV and OFV-like rhabdoviruses infecting orchids in Asia, Africa, North America, South America, Europe, and Oceania. The prevalence of OFV and its mite vector is thought to be associated with the movement of infected orchids (Dietzgen et al. 2018a). More than fifty species of Orchidaceae (Kitajima et al. 2010, Peng et al. 2013) can naturally become infected with OFV, as well as some Asparagaceae (Nolinoidaea) (Mei et al. 2016, Dietzgen et al. 2018b), and Rutaceae, where infection causes citrus leprosis-like symptoms (Roy et al. 2015, 2020, Cook et al. 2019, Olmedo-Velarde et al. 2021). Mechanical transmission of OFV is possible under laboratory conditions to the plant families Chenopodiaceae, Aizoaceae, Fabaceae, and Solanaceae (Chang et al. 1976, Kondo et al. 2003, Peng et al. 2013).

### 5.1 Virus Detection

During June 2020, chlorotic flecks and ringspot patterns of unknown etiology were observed on Giant Liliy turf *Liriopsp* spp., cv. ‘Gigantea’ in a landscape of Leon County, Florida (5-1). *Liriopsp* belong to a group of plants in the family Asparagaceae, subfamily Nolinoidaea, comprised of grass-like monocotyledonous liliod plants native to southeastern Asia (Chase et al. 2009, Meng et al. 2021). *Liriopsp* and the closely related *Ophiopogon* (Asparagaceae: Nolinoidaea) are considered the most important ground cover plant in the southeastern United States (Mcharo et al. 2003). Viral infections of suspected leaf samples were initially tested at the Plant Disease Diagnostic Clinic at the North Florida Research

and Education Center (NFREC) in Quincy, FL. All the samples were tested with one step conventional RT-PCR, and were found negative for begomovirus, carlavirus, potyvirus, tospovirus, cucumber mosaic virus and tobacco mosaic virus. As initial diagnostics were inconclusive, samples were taken of putatively infected plants with ringspot symptoms during July and August of 2020. Leaves were taken from *Liriope* and *Ophiopogon* spp., as well as the *Aspidistra elatior* Blume (Asparagaceae: Nolinoidaea), nearby, which appeared sickly and chlorotic (5-2). Plant materials were sent to the Florida Department of Agriculture and Consumer Services (FDACS) for identification. The FDACS determined that the pathogen was OFV using previously published primers and methods to conduct RT-PCR and Sanger sequencing (Kubo et al. 2009b, Kubo et al. 2009a, Ramos-González et al. 2015). The identity of the virus was verified as OFV Orchid strain 1, (OFV-Orc1), following the methods described in Kondo et al. (2017). Nucleotide sequencing shared 98% nucleotide identity with the OFV-isolates So (Accession No. AB244418) and Br (Accession No. MK522807), which belong to orchid subgroup I (Kondo et al. 2006, 2017). These samples from FDACS were subsequently retested by the USDA-APHIS-PPQ S&T Beltsville laboratory, in conjunction with tests of fresh samples from both Alachua and Leon counties. The USDA used RT-PCR, RT-qPCR, and High Throughput Sequencing (HTS) to reconfirm the presence of OFV. Conventional RT-PCR with Generic R2-Dicho-GF and R2-Dicho-GR primers amplified ~800 nt amplicons of the L-gene (RNA2) (Roy et al. 2020), to detect both OFV-Orc1 and OFV-Orc2 in *O. intermedius* and *A. elatior* from Leon County (Kondo et al. 2017) (GenBank Accession Numbers: MZ852004, MZ852005 MZ852006, and MZ852007). 99% nucleotide sequence identity is shared between OFV-Orc1 and OFV-Orc2 for the RNA2 genome, whereas 90% sequence identity was found between these two reassortment strains. The presence of OFV-Orc1 and OFV-Orc2 in Leon and Alachua counties was reaffirmed with HTS data (5-1): Analysis of HTS data from Leon County found that the symptomatic *L. muscari* were coinfecte with both OFV-Orc1 and OFV-Orc2, while the symptomatic *A. elatior* were solely infected with

OFV-Orc1. Sequence data of symptomatic *L. muscari* from Alachua County revealed infections with OFV-Orc2 (GenBank Accession MZ852006). After the initial identification by FDACS of OFV-Orc, mite samples were collected from symptomatic Asparagaceae in Leon County. Most mites collected were Tenuipalpid mites (flat mites or false spider mites), a pest of ornamental plants, some of which are known to act as vectors for plant viruses ([Childers et al. 2003b](#), [Childers and Rodrigues 2011](#)).

Table 5-1. List of Asparagaceae (Nolinoidae) species infected with OFV, collected from the landscape of northern Florida.

Scientific Name	Common Names	County	Strains Detected
<i>Liriope muscari</i> cv. ‘Gigantea’* (Decaisne) Bailey	Lilyturf, Orchardgrass, Monkeygrass	Leon & Alachua	OFV-Orc1 & OFV-Orc2
<i>Ophiopogon intermedius</i> † Don	Aztec Grass, Argenteomarginatus	Leon	OFV-Orc1 & OFV-Orc2
<i>Aspidistra elatior</i> Blume	Cast Iron Plant, Bar-room Plant	Leon	OFV-Orc1 & OFV-Orc2

*Note:*

Both OFV-Orc1 and OFV-Orc2 were detected in each species tested, many plants were coinfected with both strains’.

\* *L. muscari* cv. ‘Gigantea’ has been traditionally classified as *L. gigantea* Hume by Broussard 2007 and Fantz et al. 2015, although this distinction has been challenged by Wang et al. 2014 and Masiero et al. 2020.

† *O. intermedius* is sometimes misclassified as *L. muscari* ‘Variegated Evergreen Giant’ or ‘Grandiflora White’ (Fantz 2008b, 2009).

## 5.2 A Comment on the Status of *Brevipalpus* in Florida

Mite taxonomy is complicated by cryptic species complexes which occur in many plant-feeding groups of the Acari ([Umina and Hoffmann 1999](#), [Skoracka and Dabert 2010](#), [Arthur et al. 2011](#), [Skoracka et al. 2013](#)), including tenuipalpid mites from the genus *Brevipalpus* ([Navia et al. 2013](#)). The commonly used phase-contrast microscopy is insufficient to detect some diagnostic characters for separation of cryptic species, instead best practices recommend the combination of Differential Interference Contrast (DIC) Microscopy and Scanning Electron Microscopy along with molecular methods to separate cryptic species ([Beard et al. 2015](#)). The flat mites collected were initially suspected to belong to *B. californicus* after inspection with phase contrast microscopy. Subsequent observation via DIC microscopy at FDACS agreed with this tentative identification. Unfortunately, the *B. californicus* s.l. species group, *sensu* Baker and Tuttle ([1987](#)) is suspected to contain cryptic species ([Childers and Rodrigues 2011](#), [Rodrigues and Childers 2013](#)). New mite samples were collected from symptomatic liriopogons and *A. elatior* in Leon County and sent to USDA-ARS’s Electron and Confocal Microscopy Unit for



Figure 5-1. Variety of symptoms seen on *Liriope* spp. infected with Orchid Fleck Virus (OFV): (a) symptoms on *Liriope muscari* cv. 'Gigantea' (b-c) Details of symptoms on *L. muscari* cv. 'Gigantea' (d) rust colored spots on *Ophiopogon intermedius*



Figure 5-2. Symptoms seen on *Aspidistra elatior* infected with OFV: (a) Detail of leaf chlorosis (b) Chlorosis appears similar to sun damage (c-d) Chlorotic flecks may indicate early symptoms of OFV

analysis. Three mite species were recovered and examined under cryo-scanning electron microscopy (Cryo-SEM): *B. californicus* s.l. (5-3), *B. obovatus* Donnadiue and *B. confusus* Baker. The recent report of OFV in the US is thought to be Ko et al. (1985) which describes nuclear inclusions caused by an undescribed bacilliform rhabdovirus in *Brassia* orchids. The significance of this report is their description of the spoke-wheel configurations of the viral particles (Ko et al. 1985), a sign typically associated with OFV infection (Chang et al. 1976). Unfortunately, this article made no mention of mites or further investigations of the virus. The first report of OFV in the continental US was Bratsch et al. (2015), who confirmed the presence of OFV in *Phalaenopsis* hybrids using Transmission Electron Microscopy of ultrathin sections of plant tissue as well as molecular sequence analysis. They also discuss the association of OFV with *Brevipalpus* mites, but the authors did not make a conclusive species identification beyond suggesting that the mite vector belonged to the *B. californicus* group, referring to Kondo et al. (2003)'s publication (Bratsch et al. 2015). Later reports of OFV described OFV infecting a previously undescribed Nolinoidaea hosts in Australia (Mei et al. 2016, Dietzgen et al. 2018b), including *Liriope spicata* (Thunb.) Lour, a different species of liriopogon than those identified from the Florida sites. We are not aware of any reports of OFV infecting liriopogons, *A. elatior* nor other Nolinoidaea in the US. Although Peng et al. (2013) had mentioned an association between *B. californicus* and *A. elatior*, they never reported symptoms of OFV-Orc in this plant. We believe that our findings indicate the first report of OFV-Orc infecting ornamental Nolinoidaea in Florida, and possibly the US. This publication also marks the first reports of *A. elatior* and *Ophiopogon* spp. as natural hosts of OFV-Orc. There are two orchid strains of OFV (OFV-Orc1 and OFV-Orc2), and two citrus strains (OFV-Cit1 and OFV-Cit2) (Beltran-Beltran et al. 2020, Roy et al. 2020). The OFV strains detected in Florida are identical in genome sequence to the orchid strains of OFV infecting citrus in Hawaii, Mexico, Colombia, and South Africa (Beltran-Beltran et al. 2020, Roy et al. 2020). Both OFV-Orc1 and OFV-Orc2 infect citrus (Roy et al. 2020),

but none of the citrus strains have been reported from any orchid species. The *Brevipalpus* mites collected from liriopogons and *A. elatior* in Leon County were abundant on OFV-infected plants very near to citrus trees, some plants even surrounding the trunk. *B. californicus* s. l. has been reported as a pest of citrus ([Childers et al. 2003b](#)) and are often collected from citrus fruits ([Baker 1949](#), [Baker and Tuttle 1987](#), [Vacante 2010, 2016](#)). The proximity of these mite vectors to citrus raises the question: why these trees are not currently infected with OFV-Orc? It is important to note the uncertainty surrounding the vector for OFV-Orc. There are three mite species which have been recovered from OFV-Orc infected plants: *B. obovatus*, and *B. confusus* and *B. californicus* s.l., but only *B. californicus* has been described as a vector of OFV. Even so, the *B. californicus* which we find on liriopogons and *A. elatior* may not be the same cryptic species as those found on citrus. Transmission of OFV from populations of *B. californicus* liriopogon/*A. elatior* to citrus may be limited by host preferences, vectorial capacity, viral propagation/circulation in the vector, viral acquisition times, or feeding times required for transmission to citrus. Even so, these types of questions require future study to determine the potential of nolinoidaea to citrus transmission. Best practices for integrated pest management have not been created for controlling *Brevipalpus* mites on these ornamentals, but methods designed to control *Brevipalpus* in other systems may be applicable. The most common method used to control *Brevipalpus* are synthetic acaricides such as spirodiclofen and cyflumetofen ([Andrade et al. 2010, 2019](#), [Leeuwen et al. 2015](#), [Vechia et al. 2018](#)). Unfortunately, some acaricides and their residues can harm beneficial predatory mites as well ([Fernández et al. 2017](#)), even at low doses ([Havasi et al. 2021](#)), and mixing different chemistries can be detrimental for mite control ([Vechia et al. 2018](#)). Furthermore, pesticide resistance has been reported in various *Brevipalpus* populations ([Alves et al. 2000](#), [Omoto et al. 2000](#), [Campos and Omoto 2002](#), [Rocha et al. 2021](#)), due to exposure to pesticides used to control other arthropod pests ([Vechia et al. 2021](#)). In addition, phytoseiid predatory mites such as *Amblyseius largoensis* (Muma) and *Galendromus helveolus* (Chant) ([Chen et al. 2006](#),

Argolo et al. 2020), entomopathogenic fungi (Magalhães et al. 2005, Rossi-Zalaf et al. 2008, Peña et al. 2015, Revynthi et al. 2019) have shown promise for controlling other *Brevipalpus* mites. Moreover, it is often possible to integrate different control techniques for improved management, such as combining predatory mites with compatible acaricides and entomopathogenic fungi (Reddy 2001, Midthassel et al. 2016, Andrade et al. 2019). In conclusion, detecting OFV in Florida represents a concern for horticulturists who grow orchids, *Liriope*, *Ophiopogon*, or other susceptible Asparagaceae species which are commonly used in landscaping. Florida is also home to a plethora of native and naturalized orchid species, many of which are threatened, including cultivated *Vanilla* in southern Florida (Chambers et al. 2019) and the famous Ghost Orchid, [*Dendrophylax lindenii* (Lindl.) Benth. ex Rolfe]. Furthermore, Citrus leprosis was present in Florida during the 1860's and almost eradicated by the mid-1960s (Knorr 1968, Knorr et al. 1968, Childers et al. 2003b). An examination of herbarium specimens of Florida citrus found that one strain of this historical virus, Citrus leprosis dichorhavirus-N0, is distantly related to the modern isolates of OFV (Kitajima et al. 2011, Hartung et al. 2015, Roy et al. 2020). The recent detection of OFV-Orc1 in South Africa (Cook et al. 2019) in *C. sinensis* (Navel and Valencia orange) and OFV-Orc2 in Hawaii (Olmedo-Velarde et al. 2021) in *C. reticulata* (mandarin) and *C. jambhiri* (rough lemon) associated with leprosis-like symptoms highlights the potential threat of different isolates of OFV on citrus, which will be a definite concern to the US multi-billion-dollar citrus industry already impacted by the Huanglongbing disease. *B. californicus*, *B. yothersi*, and *B. obovatus* are all present in Florida (Childers et al. 2003b, Akyazi et al. 2017), and are difficult to identify by non-experts, or without advanced methodologies. DNA barcoding (Armstrong and Ball 2005) or a similarly simple and accurate method for identification of these mite complexes is vital to identify mite populations which need to be monitored or controlled. By doing so, we can determine the risk OFV-Orc represents for the native plants, agriculture and the ornamental/landscaping industries of Florida and the surrounding regions.

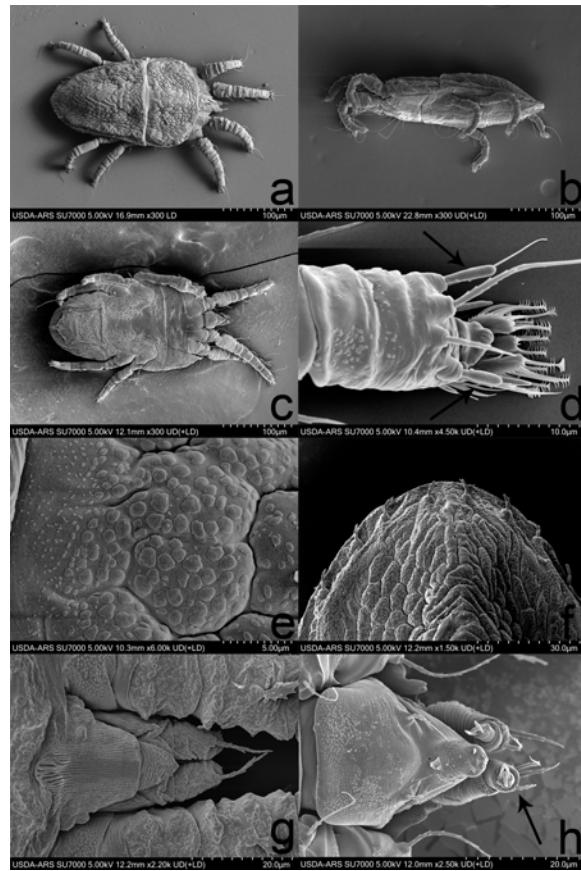


Figure 5-3. Cryo-SEM images of *Brevipalpus californicus* *sensu lato* displaying various characters used for identification (Baker and Tuttle 1987, Beard et al. 2015) (a) Dorsum (b) Lateral view (c) Venter (d) Close up of distal end of leg 2, with arrows indicating paired solenidia, characteristic of the genus *Brevipalpus* (e) Enlargement of the microplates of the mite cerotegument (f) Dorsal view of the distal portion of mite opisthosoma (g) Dorsal view of the mite rostrum (h) Ventral view of mite rostrum, observe 3 distal setae.

CHAPTER 6  
CONCLUSIONS: INVASIVE MITES ARE AN INCREASINGLY LARGE PROBLEM  
FOR FLORIDA

## 6.1 Main Findings

The principal findings of our experiments are:

1. *Phyllocoptes fructiphilus* is present in multiple cities in northern Florida
2. The predatory mite *Amblyseius swirskii* is attracted to roses infected with Rose Rosette Disease (RRD)
3. Infected roses have higher levels of defense-related terpenes, including  $\gamma$ -Murolene,  $\beta$ -Caryophyllene, and D-Limonene, some of which may be attractive to *A. swirskii*
4. ASM-treated plants had higher similarity in their Volatile Organic Compound (VOC) profiles to one another than to the VOCs released from healthy or RRV-infected plants
5. Systemic Acquired Resistance (SAR) via acibenzolar-S-methyl (ASM) does not appear to significantly reduce populations of *P. fructiphilus*
6. Preliminary evidence that *P. fructiphilus* populations may be reduced by spirotetramat applications, or possibly heavy pruning when RRD is not present
7. Orchid Fleck Virus (OFV) is present in common ornamental groundcover plants in Florida.
8. *Brevipalpus californicus sensu lato*, a known vector of OFV, were found on OFV-infected plants

## 6.2 Invasive Species Continue to be a Problem

The aim of our research was to address the emerging problems caused by the recent invasions of mites and the plant viruses they vector to northern Florida.

Florida is a hotspot for invasive species in the mainland US, due to a combination of its unique geography, climate, rapid development and a multitude of invasion pathways (Simberloff et al. 1997, Williams et al. 2007, Card et al. 2018). Invasive species are often harmful to ecosystem services and modify ecosystems to the detriment of native species (Gordon 1998). Efforts to manage invasive species costs the state of Florida ~\$45M each

year (Hiatt et al. 2019). Many Florida programs have been developed to raise public awareness about the threats of invasive species, as well as encouraging the reporting of invasive species by the public (Wallace et al. 2016). *Phyllocoptes fructiphilus*, *Rose rosette emaravirus*, and *Orchid fleck dichorhavirus* all represent yet another chapter in the story of Florida invasion ecology. These organisms have a high possibility of becoming established invasive species in Florida if preventative actions are not taken.

Invasive species are thought to mainly disperse through short distances via local spread around established focal populations (diffusive dispersal), while long-distance jumps are more often due to human movements (Ibrahim et al. 1996, Higgins and Richardson 1999, Novak and Mack 2001, Suarez et al. 2001, Garnas et al. 2016).

A further examination of population dynamics, genetic variation and/or spatial structure of mite populations should provide evidence for which pathways of introduction are most prevalent (Navia et al. 2005, Williams et al. 2007, Boubou et al. 2010, 2012).

The ease of arthropod movement is thought to have been generally underestimated in the past (Garnas et al. 2016).

In the last three years our lab has discovered recent introductions of two invasive of mite-virus-pathosystems previously unreported in Florida. The new invasive mite (*P. fructiphilus*) and the new mite-vectored virus (OFV) were both encountered on ornamental plants commonly planted in Florida landscapes.

Eriophyoid mites are known to disperse aerially over a hundred meters away from infested areas (Bergh and Mccoy 1997),

Eriophyids have also been found to move on contaminated clothing and equipment (Duffner et al. 2001).

Similarly, tenuipalpid mites are not known to disperse very far by walking, and few (< 1%) mites were seen to disperse even when wind speeds reached 30 and 40 km/h<sub>-1</sub> in lab experiments (Alves et al. 2005), suggesting that their potential for natural dispersal is low.

On the other hand, the current invasions of *P. fructiphilus* may have been introduced through their natural means of dispersal over time. The short distances between mite infested roses in Georgia, Alabama and Florida suggest the possibility of multiple routes of introduction, but the mechanisms of dispersal require further investigation for *P. fructiphilus*.

In addition, the movements of plant pathogens such as RRD is thought to be partially driven by socioeconomic factors and the movement of plants by people (Nelson and Bone 2015, Katsiani et al. 2020). Inspections and quarantines of mite-infested roses by wholesalers and larger growers is predicted to slow the spread of plant pathogen epidemics (Nelson and Bone 2015).

This is likely an unfortunate byproduct of the global plant trade and the inadvertent introduction of invasive species (Westphal et al. 2007, Chapman et al. 2017). Another risk factor for the spread of RRV is the movement of plants and their pathogens via the movement of infected materials and seed through trade networks (Jeger et al. 2007, Westphal et al. 2007, Hulme et al. 2008, Moslonka-Lefebvre et al. 2011, Liebhold et al. 2012, Pautasso and Jeger 2014, Schoebel et al. 2014a). Studies and simulations of seed networks have revealed that larger seed companies and operations have greater potential to spread plant pathogens than informal seed systems run by smaller entities (Biemond et al. 2013, Coomes et al. 2015). This is partially due to their greater ability to spread infected plant materials at a larger scale than smaller trade systems (Biemond et al. 2013).

Similarly, Nelson and Bone (2015) simulated the effects of quarantines on preventing the spread of a pathogen through an idealized plant trade network. In their simulations, they found that increasing the number of growers and wholesalers generally an increased number of infections, but that more inspections decreased the chances of infection. They concluded that were most likely to be prevented via quarantine of growers and wholesalers of plants (Nelson and Bone 2015).

### 6.3 Future Areas of Study

APPENDIX A  
FIRST REPORT OF *PHYLLOCOPTES FRUCTIPHILUS* KIEFER (ERIOPHYIDAE),  
THE VECTOR OF THE ROSE ROSETTE VIRUS, IN FLORIDA, USA



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## **First Report of *Phyllocoptes fructiphilus* Keifer (Eriophyidae), the Vector of the Rose Rosette Virus, in Florida, USA**

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# First report of *Phyllocoptes fructiphilus* Keifer (Eriophyidae), the vector of the rose rosette virus, in Florida, USA

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*Phyllocoptes fructiphilus* Keifer (Acari: Eriophyidae) is a microscopic eriophyid mite. Eriophyid mites are very host specific (Oldfield 1996; Skoracka et al. 2009) and *P. fructiphilus* feeds only on plants in the genus *Rosa* (Amrine 1996). *Phyllocoptes fructiphilus* is associated with the rose rosette emaravirus (rose rosette virus) and acts as the only known vector of rose rosette virus. Infection is associated commonly with the following symptoms: witches' broom, rosetting, deformed flowers, increased thorn density, elongated shoots, reddened leaves and stems, and increased die-back that ultimately kills the rose host (Amrine 1996) (Fig. 1A, B). This disease is known as rose rosette disease and is the most serious illness of roses, affecting the US commercial rose industry which is worth millions of dollars. Rose rosette disease and *P. fructiphilus* have invaded the southeastern US as they followed the range expansion of the non-native *Rosa multiflora* (Thunb.) (Rosaceae) towards the east coast (Amrine 2002; Otero-Colina et al. 2018).

In 2013, a nursery in Quincy, Gadsden County, Florida, USA, detected witches' brooms and other rose rosette disease symptoms on 15 knockout roses that had been imported from out of state. Eight symptomatic plants were tested and found to be positive for rose rosette disease, but *P. fructiphilus* was not detected on the roses at that time (Babu et al. 2014). In 2018, we began a series of surveys along the borders of northern Florida and southern Georgia to determine if this mite was present and acting as a vector for the disease.

Survey efforts initially focused on counties around Leon County, Florida. Rose tissue samples were taken from the periphery of various roses in the landscape; sampling was focused on the flowering tips of roses and included a mixture of flowers, fruits, buds, and short lengths of rose cane. The average sample contained  $26.8 \pm 1.5$  g of undried plant tissue. Samples were trimmed with bypass pruners, dried plant tissue with 70% ethanol between cuts and stored in quart sized plastic bags (Ziploc®, S.C. Johnson & Son, Racine, Wisconsin, USA). Rose cultivars, species, and coordinates were recorded to map out sites that had predatory mites, eriophyid mites, or possible rose rosette disease.

Samples were processed using a washing method derived from Monfreda et al. (2007); cut roses were soaked in a 500 mL beaker with a solution of 1:1 bleach:water with a few drops of concentrated liquid dish washing detergent. The solution was stirred vigorously with a glass rod to dislodge any mites, then poured over a stack of sieves with decreasing screen sizes: 180 µm, 53 µm, and 25 µm. The bea-

ker and rose pieces were further rinsed with tap water over the sieve stack to dislodge any remaining mites. The 53 µm and 25 µm sieves were processed separately; the 53 µm sieve retained larger mites while the 25 µm sieve retained smaller mites, including *P. fructiphilus*. The sieves were then backwashed from the underside of their screen with a water-filled wash bottle, starting from the highest point of a sieve and working to the bottom to flush any trapped debris and mites into a 50 mL centrifuge tube for storage and future observation. Samples were observed under a dissecting microscope. Mites found among the plant debris were siphoned off with a glass pipette and subsequently stored in micro-centrifuge containers with 95% ethanol as a preservative. Some specimens were mounted directly into Hoyer's slide mounting media (Hempstead Halide, Inc., Galveston, Texas, USA), dried at 90 °C, then a ring of nail polish was painted over the edges of the coverslip to seal the slide.

On 14 Feb 2019, we found a total of 42 eriophyid mites from 6 samples obtained from Pink Double Knock Out® roses while surveying roses in the landscape in Tallahassee, Leon County, Florida, USA (Fig. 2A). The mites were sent to the Florida Department of Agriculture and Consumer Services, Division of Plant Industry and were identified as *P. fructiphilus* based, among other characters, on the distinctive pattern of ridges on the prodorsal shield (Bauchan et al. 2019) (Fig. 1C, D). Whereas 2 other eriophyid mites *Eriophyes eremus* Druciarek & Lewandowski and *Phyllocoptes adalius* Keifer (both Acari: Eriophyoidea) are found in roses in the central and eastern US, neither of them were found in the samples analyzed. The roses did not show signs or symptoms of rose rosette disease.

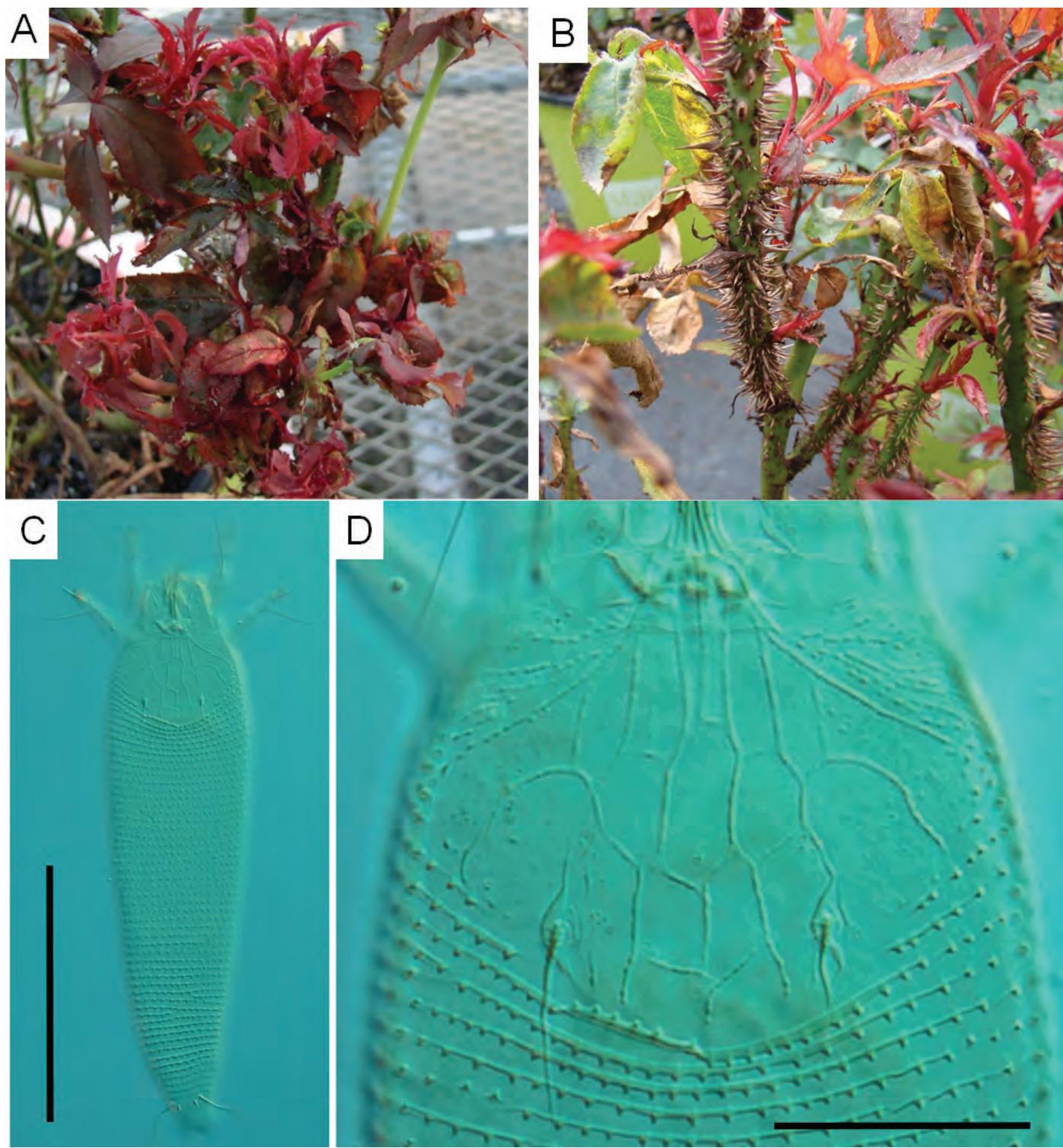
On 16 Jul 2019, we conducted an additional survey of 33 sites with Pink Double Knock Out® roses near the initial site of discovery, including the rose sites where *P. fructiphilus* was detected originally (Fig. 2B). Each sample contained more than 50 eriophyid mites, with some samples containing over 300 mites. We compared the samples collected during Feb and Jul in the same locations with a paired t-test and found a significant increase in the *P. fructiphilus* population between the 2 sampling dates (see Fig. 1C;  $P = 0.001$ ;  $\alpha = 0.05$ ;  $df = 4$ ). Mites that were slide mounted were confirmed subsequently as *P. fructiphilus*.

This is the first record for *P. fructiphilus* in Florida. None of the mite-infested roses showed symptoms of rose rosette disease and none tested positive for rose rosette virus based on detection tools devel-

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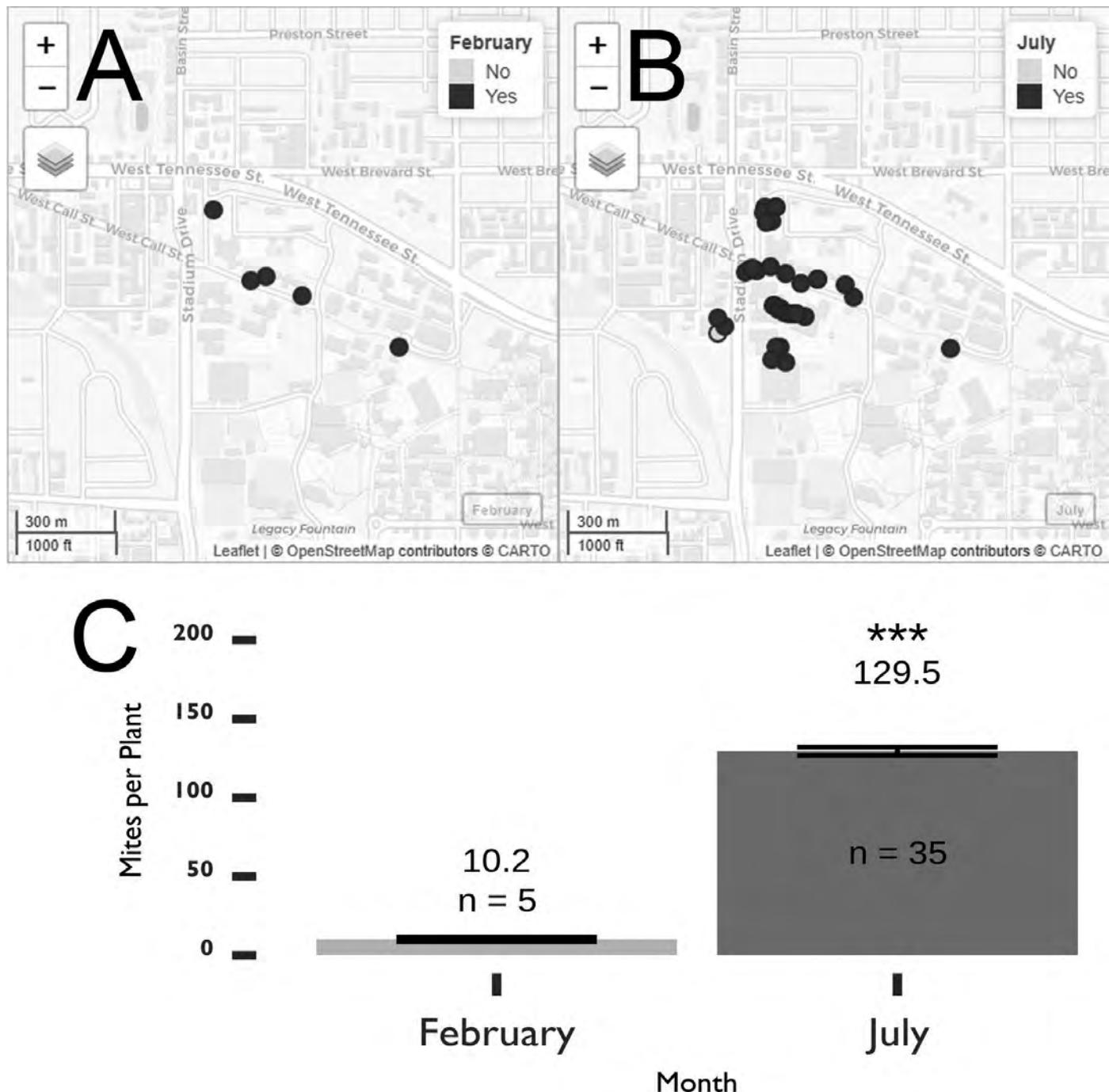
\*Corresponding author; E-mail: xmartini@ufl.edu



**Fig. 1.** (A) Symptoms of rose rosette disease: witches' broom, and (B) excessive thorn proliferation; (C) *Phyllocoptes fructiphilus* Keifer (female) from Leon County, Florida, USA: body (scale bar = 100  $\mu\text{m}$ ); (D) enlargement of *P. fructiphilus* prodorsal shield to show detail (scale bar = 20  $\mu\text{m}$ ).

oped to date. However, the presence of *P. fructiphilus*, along with past detections of rose rosette virus in Florida warrants increased monitoring for the mite and virus in Florida. There is a critical need to develop methods to manage *P. fructiphilus* and rose rosette virus, or homeowners, commercial landscapers, and the US rose industry stands to lose millions of dollars and established plantings in the coming yr.

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**Fig. 2.** Presence of *Phyllocoptes fructiphilus* in Leon County, Florida, USA, in (A) Feb 2019 and (B) Jul 2019. Orange dots indicate sites sampled that had *P. fructiphilus*. Gray dots indicate surveyed areas where no *P. fructiphilus* were found. (C) Average number of *P. fructiphilus* per rose sample. Samples were taken from sites in Leon County, Florida, on 14 Feb and 16 Jul 2019. Asterisks represent significant differences as calculated by pairwise t-tests of the 5 sites tested for *P. fructiphilus* during both mo.  $P$ -value < 0.001.

## Summary

The invasive mite *Phyllocoptes fructiphilus* Keifer (Acar: Eriophyidae) feeds on plants in the genus *Rosa*. *Phyllocoptes fructiphilus* is associated with the rose rosette emaravirus (rose rosette virus) and acts as the only known vector of rose rosette virus, the causal agent of rose rosette disease (Emaravirus).

The mite *P. fructiphilus* is reported for the first time in the state of Florida, USA. No roses showed signs or symptoms of viral infection, and current molecular methods were unable to detect the virus. *Phyllocoptes fructiphilus* represents a potential threat to the Florida rose industry if rose rosette disease becomes established.

Key Words: rose rosette disease; rose rosette virus

## Sumario

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El ácaro invasivo *Phyllocoptes fructiphilus* Keifer (Acari: Eriophyidae) se alimenta sobre plantas del género *Rosa*. *Phyllocoptes fructiphilus* se asocia con rose rosette emaravirus (virus del arrosetamiento de la rosa), es reconocido principalmente como vector de la virus del arrosetamiento de la rosa, el agente causal de la enfermedad del arrosetamiento de la rosa (Emaraviridae). El ácaro *P. fructiphilus* se reporta por primera vez para el estado de la Florida, USA. Ninguna rosa mostró señales o síntomas de una infección viral, y ningún virus fue detectado con el uso de métodos moleculares de hoy en día. *Phyllocoptes fructiphilus* representa una amenaza potencial para la industria de la rosa en la Florida si Emaraviridae se llega a establecer.

Palabras Clave: virus del arrosetamiento de la rosa; enfermedad del arrosetamiento de la rosa; emaravirus

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APPENDIX B  
*BREVIPALPUS*-TRANSMITTED ORCHID FLECK VIRUS INFECTING THREE NEW  
ORNAMENTAL HOSTS IN FLORIDA

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Issues



## ***Brevipalpus*-transmitted Orchid Fleck Virus Infecting Three New Ornamental Hosts in Florida**

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### **Abstract**

We describe the first detection of orchid fleck virus (OFV) infecting three unreported hosts: *Liriope muscari*, cv. 'Gigantea' (Decaisne) Bailey, *Ophiopogon intermedius* Don and *Aspidistra elatior* Blume (Asparagaceae: Nolinoidae) in Leon and Alachua Counties, FL. The orchid-infecting subgroup (Orc) of OFV infects over 50 plant species belonging to the plant families Orchidaceae, Asparagaceae (Nolinoidae), and causes citrus leprosis disease in *Citrus* (Rutaceae). The only known vectors of OFV-Orc are the flat mites, *Brevipalpus californicus* (Banks) *sensu lato* (Trombidiformes: Tenuipalpidae). Florida has various plants in the landscape which *Brevipalpus* spp. feed on, which are susceptible to infection by OFV-Orc. Chlorotic ringspots and flecking were seen affecting Liriopogons (*Liriope* and *Ophiopogon* spp.) in Leon County, FL. Nearby *A. elatior* also appeared chlorotic. Local diagnostics returned negative for common plant pathogens, therefore new samples were sent to the Florida Department of Agriculture and Consumer Services (FDACS) and USDA-ARS for identification. Two orchid-infecting strains of OFV were detected via combinations of conventional RT-PCR, RT-qPCR, Sanger sequencing, and high-throughput sequencing (HTS). Amplicons shared 98% nucleotide identity with OFV-Orc1 and OFV-Orc2 RNA2 genome sequences available in NCBI GenBank. Coinfections were detected in each county, but single strains of OFV-Orc were detected in *L. muscari* (Alachua, OFV-Orc2) and *A. elatior* (Leon, OFV-Orc1). Three potential mite vectors were identified via cryo-scanning electron microscopy (Cryo-SEM): *B. californicus* *sensu lato*, *B. obovatus* Donnadeau, and *B. confusus* Baker. In conclusion, OFV orchid strains are present in northern Florida, representing a risk for susceptible plants in the southeastern United States.

### **Resumen**

Se describe la primera detección del virus de orchid fleck virus (OFV), infectando a tres huéspedes no reportados: *Liriope muscari*, cv. 'Gigantea' (Decaisne) Bailey, *Ophiopogon intermedius* Don and *Aspidistra elatior* Blume (Asparagaceae: Nolinoidae) para los condados de Leon y Alachua, FL. Los subgrupos de OFV que infectan a las orquídeas (Orc) pueden infectar más de 50 especies de plantas pertenecientes a las familias Orchidaceae, Asparagaceae (Nolinoidae), e infecta *Citrus* (Rutaceae) como la enfermedad de la leprosis de los cítricos. Los únicos vectores de OFV-Orc son los ácaros planos *Brevipalpus californicus* (Banks) *sensu lato* (Trombidiformes: Tenuipalpidae). La Florida tiene varias plantas en el campo ornamental, por lo cuáles las especies de *Brevipalpus* se puedan alimentarse, y esas plantas son susceptibles a las infecciones de OFV-Orc. Se observaron manchas anulares cloróticas y salpicaduras en las hojas de las Serpentinas (*Liriope* y *Ophiopogon* spp.) y también se vieron hojas cloróticas en el *A. elatior* adyacente, situado en el condado de Leon, FL. Los diagnósticos del laboratorio local fueron negativos para los patógenos

comunes, por lo tanto, se enviaron nuevos ejemplares al Florida Department of Agriculture and Consumer Services (FDACS) y el USDA-ARS para su identificación. Se detectaron dos cepas del OFV mediante la combinación de RT-PCR convencional, RT-qPCR, secuenciación de Sanger y secuenciación de alto rendimiento. Las amplificaciones compartían una identidad de nucleótidos del 98% con las secuencias del genoma de ARN2 de OFV-Orc1 y OFV-Orc2 disponibles en el NCBI GenBank. Se detectaron coinfecciones del virus en cada condado, pero se detectaron cepas únicas de OFV-Orc en *L. muscari* (Alachua, OFV-Orc2) y *A. elatior* (Leon, OFV-Orc1). Se identificaron tres ácaros mediante microscopía electrónica de barrido criogénico (Cryo-SEM) con potencial de ser vectores: *B. californicus sensu lato*, *B. obovatus* Donnadieu y *B. confusus* Baker. En conclusión, OFV está presente en el norte de Florida, lo que representa un riesgo para las plantas susceptibles en el sureste de Estados Unidos.

**Key words:** false spider mite, flat mite, liriopogons, Rutaceae, Citrus leprosis

Orchid fleck virus (OFV) is the type member for the genus *Dichorhaviruses*, family *Rhabdoviridae*. The virus is a bacilliform, nuclear rhabdovirus composed of two segments of single-stranded, negative-sense RNA which infects plants (Dietzgen et al. 2014, Walker et al. 2018, Amarasinghe et al. 2019). Only Flat mites (Trombidiformes: Tenuipalpidae) from the genus *Brevipalpus* are known to transmit dichorhaviruses (Maeda 1998). Plants infected with OFV exhibit chlorotic and necrotic flecks on their leaves (Kubo et al. 2009b, Kubo et al. 2009a, Dietzgen et al. 2018a). The virus was first described as infecting *Cymbidium* orchids in Japan (Doi et al. 1977). There have been reports of OFV and OFV-like rhabdoviruses infecting orchids in Asia, Africa, North America, South America, Europe, and Oceania. The prevalence of OFV and its mite vector is thought to be associated with the movement of infected orchids (Dietzgen et al. 2018a). More than fifty species of Orchidaceae (Kitajima et al. 2010, Peng et al. 2013) can naturally become infected with OFV, as well as some Asparagaceae (Nolinoidae) (Mei et al. 2016, Dietzgen et al. 2018b), and Rutaceae, where infection causes citrus leprosis-like symptoms (Roy et al. 2015, 2020; Cook et al. 2019; Olmedo-Velarde et al. 2021). Mechanical transmission of OFV is possible under laboratory conditions to the plant families Chenopodiaceae, Aizoaceae, Fabaceae, and Solanaceae (Chang et al. 1976, Kondo et al. 2003, Peng et al. 2013).

## Virus Detection

During June 2020, chlorotic flecks and ringspot patterns of unknown etiology were observed on Giant Lilyturf *Liriope* spp., cv. 'Gigantea' in a landscape of Leon County, Florida (Fig. 1). *Liriope*

belong to a group of plants in the family Asparagaceae, subfamily Nolinoidae, comprised of grass-like monocotyledonous lilioid plants native to southeastern Asia (Chase et al. 2009, Meng et al. 2021). *Liriope* and the closely related *Ophiopogon* (Asparagaceae: Nolinoidae) are considered the most important ground cover plant in the southeastern United States (Mcharo et al. 2003, Fantz 2008). Viral infections of suspected leaf samples were initially tested at the Plant Disease Diagnostic Clinic at the North Florida Research and Education Center (NFREC) in Quincy, FL. All the samples were tested with one step conventional RT-PCR, and were found negative for begomovirus, carlavirus, potyvirus, tospovirus, cucumber mosaic virus, and tobacco mosaic virus. As initial diagnostics were inconclusive, samples were taken of putatively infected plants with ringspot symptoms during July and August of 2020. Leaves were taken from *Liriope* spp. and *Ophiopogon* spp., as well as the *Aspidistra elatior* Blume (Asparagaceae: Nolinoidae), nearby, which appeared sickly and chlorotic (Fig. 2). Plant materials were sent to the Florida Department of Agriculture and Consumer Services (FDACS) for identification. The FDACS determined that the pathogen was OFV using previously published primers and methods to conduct RT-PCR and Sanger sequencing (Kubo et al. 2009b, Kubo et al. 2009a, Ramos-González et al. 2015). The identity of the virus was verified as OFV Orchid strain 1 (OFV-Orc1), following the methods described in Kondo et al. (2017). Nucleotide sequencing shared 98% nucleotide identity with the OFV-isolates So (Accession No. AB244418) and Br (Accession No. MK522807), which belong to orchid subgroup I (Kondo et al. 2006, 2017). These samples from FDACS were subsequently retested by the



**Fig. 1.** Variety of symptoms seen on *Liriope* spp. infected with orchid fleck virus (OFV): (a) symptoms on *Liriope muscari* cv. 'Gigantea' (b–c) Details of symptoms on *L. muscari* cv. 'Gigantea' (d) rust colored spots on *Ophiopogon intermedius*.

USDA-APHIS-PPQ S&T Beltsville laboratory, in conjunction with tests of fresh samples from both Alachua and Leon counties. The USDA used RT-PCR, RT-qPCR, and high-throughput sequencing (HTS) to reconfirm the presence of OFV. Conventional RT-PCR with Generic R2-Dicho-GF and R2-Dicho-GR primers amplified ~800 nt amplicons of the L-gene (RNA2) (Roy et al. 2020), to detect both OFV-Orc1 and OFV-Orc2 in *O. intermedius* and *A. elatior* from Leon County (Kondo et al. 2017) (GenBank Accession Numbers: MZ852004, MZ852005 MZ852006, and MZ852007). Ninety-nine percent nucleotide sequence identity is shared between OFV-Orc1 and OFV-Orc2 for the RNA2 genome, whereas 90% sequence identity was found between these two reassortment strains. The presence of OFV-Orc1 and OFV-Orc2 in Leon and Alachua counties was reaffirmed with HTS data (Table 1): Analysis of HTS data from Leon County found that the symptomatic *L. muscari* were coinfecte

both OFV-Orc1 and OFV-Orc2, while the symptomatic *A. elatior* were solely infected with OFV-Orc1. Sequence data of symptomatic *L. muscari* from Alachua County revealed infections with OFV-Orc2 (GenBank Accession MZ852006). After the initial identification by FDACS of OFV-Orc, mite samples were collected from symptomatic Asparagaceae in Leon County. Most mites collected were Tenuipalpid mites (flat mites or false spider mites), a pest of ornamental plants, some of which are known to act as vectors for plant viruses (Childers et al. 2003, Childers and Rodrigues 2011).

## A Comment on the Status of *Brevipalpus* in Florida

Mite taxonomy is complicated by cryptic species complexes which occur in many plant-feeding groups of the Acari (Umina and Hoffmann 1999, Skoracka and Dabert 2010, Arthur et al. 2011, Skoracka et al. 2013), including tenuipalpid mites from the genus *Brevipalpus* (Navia et al. 2013). The commonly used phase-contrast microscopy is insufficient to detect some diagnostic characters for separation of cryptic species, instead best practices recommend the combination of differential interference contrast (DIC) microscopy and scanning electron microscopy along with molecular methods to separate cryptic species (Beard et al. 2015). The flat mites collected were initially suspected to belong to *B. californicus* after inspection with phase contrast microscopy. Subsequent observation via DIC microscopy at FDACS agreed with this tentative identification. Unfortunately, the *B. californicus* s.l. species group, sensu Baker and Tuttle (1987) is suspected to contain cryptic species (Childers and Rodrigues 2011, Rodrigues and Childers 2013). New mite samples were collected from symptomatic liriopogons and *A. elatior* in Leon County and sent to USDA-ARS's Electron and Confocal Microscopy Unit for analysis. Three mite species were recovered and examined under cryo-scanning electron microscopy (Cryo-SEM): *B. californicus* s.l. (Fig. 3), *B. obovatus* Donnadeau and *B. confusus* Baker. The recent report of OFV in the United States is thought to be Ko et al. (1985) which describes nuclear inclusions caused by an undescribed bacilliform rhabdovirus in *Brassia* orchids. The significance of this report is their description of the spoke-wheel configurations of the viral particles (Ko et al. 1985), a sign typically associated with OFV infection (Chang et al. 1976). Unfortunately, this article made no mention of mites or further investigations of the virus. The first report of OFV in the continental United States was Bratsch et al. (2015), who confirmed the presence of OFV in *Phalaenopsis* hybrids using transmission electron microscopy of ultrathin sections of plant tissue as well as molecular sequence analysis. They also discuss the association of OFV with *Brevipalpus* mites, but the authors did not make a conclusive species identification beyond suggesting that the mite vector belonged to the *B. californicus* group, referring to Kondo et al. (2003). Later reports of OFV described OFV infecting a previously undescribed Nolinoidaea hosts in Australia (Mei et al. 2016, Dietzen

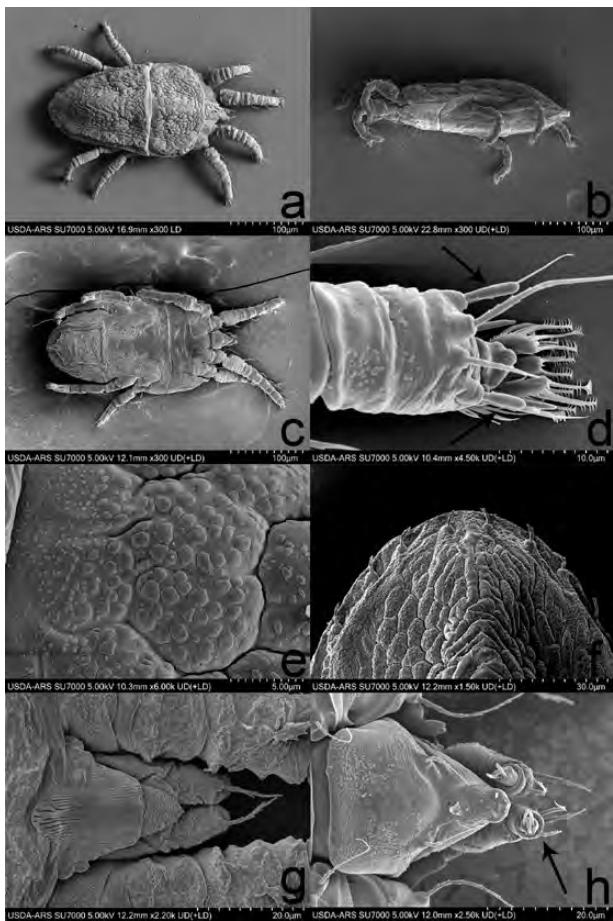


**Fig. 2.** Symptoms seen on *Aspidistra elatior* infected with OFV: (a) Detail of leaf chlorosis (b) Chlorosis appears similar to sun damage (c-d) Chlorotic flecks may indicate early symptoms of OFV.

**Table 1.** List of Asparagaceae (Nolinoidaea) species infected with orchid flea virus, collected from the landscape of northern Florida

Scientific name	Common names	County	Strains detected
<i>Liriope muscari</i> cv. 'Gigantea' <sup>a</sup> (Decaisne) Bailey	Lilyturf, Orchardgrass, Monkeygrass	Alachua & Leon	OFV-Orc1 & OFV-Orc2
<i>Ophiopogon intermedius</i> <sup>b</sup> Don	Aztec Grass, 'Argenteomarginatus'	Leon	OFV-Orc1 & OFV-Orc2
<i>Aspidistra elatior</i> Blume	Cast Iron Plant, Bar-room Plant	Leon	OFV-Orc1 & OFV-Orc2

<sup>a</sup>*Liriope muscari* cv. 'Gigantea' has been traditionally classified as *L. gigantea* Hume by Broussard (2007) and Fantz et al. (2015), although this distinction has been challenged by Wang et al. (2014) and Masiero et al. (2020). <sup>b</sup>*O. intermedius* is sometimes misclassified as *Liriope muscari* 'Variegated Evergreen Giant' Fantz (2009) or 'Grandiflora White' (Fantz 2009). Both OFV-Orc1 and OFV-Orc2 were detected in each species tested, many plants were coinfecte



**Fig. 3.** Cryo-SEM images of *Brevipalpus californicus* sensu lato displaying various characters used for identification (Baker and Tuttle 1987, Beard et al. 2015) (a) Dorsum (b) Lateral view (c) Venter (d) Close up of distal end of leg 2, with arrows indicating paired solenidia, characteristic of the genus *Brevipalpus* (e) Enlargement of the microplates of the mite cerotegument (f) Dorsal view of the distal portion of mite abdomen (g) Dorsal view of the mite rostrum (h) Ventral view of mite rostrum, observe three distal setae.

et al. 2018b), including *Liriope spicata* (Thunb.) Lour, a different species of liriopogon than those identified from the Florida sites. We are not aware of any reports of OFV infecting liriopogons, *A. elatior* nor other Nolinoidaea in the US. Although Peng et al. (2013) had mentioned an association between *B. californicus* and *A. elatior*, they never reported symptoms of OFV-Orc in this plant. We believe that our findings indicate the first report of OFV-Orc infecting ornamental Nolinoidaea in Florida, and possibly the United States. This publication also marks the first reports of *A. elatior* and *Ophiopogon* spp. as natural hosts of OFV-Orc. There are two orchid strains of OFV (OFV-Orc1 and OFV-Orc2), and two citrus strains (OFV-Cit1 and OFV-Cit2) (Beltran-Beltran et al. 2020, Roy et al. 2020). The OFV strains detected in Florida are identical in genome sequence to the orchid strains of OFV infecting citrus in Hawaii, Mexico, Colombia, and South Africa (Beltran-Beltran et al. 2020, Roy et al. 2020). Both OFV-Orc1 and OFV-Orc2 infect citrus (Roy et al. 2020), but none of the citrus strains have been reported from any orchid species. It is important to note the uncertainty surrounding the vector for OFV-Orc. There are three mite species which have been recovered from OFV-Orc infected plants: *B. obovatus*, and *B. confusus* and *B. californicus* s.l., but only *B. californicus* has been described as a vector of OFV. Even so, these types of questions require future study

to determine the potential of nolinoidaea to citrus transmission. Best practices for integrated pest management have not been created for controlling *Brevipalpus* mites on these ornamentals, but methods designed to control *Brevipalpus* in other systems may be applicable. The most common method used to control *Brevipalpus* are synthetic acaricides, such as spirodiclofen and cyflumetofen (Andrade et al. 2010, 2019, Leeuwen et al. 2015, Vechia et al. 2018). Unfortunately, some acaricides and their residues can harm beneficial predatory mites as well (Fernández et al. 2017), even at low doses (Havasi et al. 2021), and mixing different chemistries can be detrimental for mite control (Vechia et al. 2018). Furthermore, pesticide resistance has been reported in various *Brevipalpus* populations (Alves et al. 2000, Omoto et al. 2000, Campos and Omoto 2002, Rocha et al. 2021), due to exposure to pesticides used to control other arthropod pests (Vechia et al. 2021). In addition, phytoseiid predatory mites such as *Amblyseius largoensis* Muma and *Galendromus helveolus* Chant (Mesostigmata: Phytoseiidae) (Chen et al. 2006, Argolo et al. 2020), entomopathogenic fungi (Magalhães et al. 2005, Rossi-Zalaf et al. 2008, Peña et al. 2015, Revynthi et al. 2019) have shown promise for controlling other *Brevipalpus* mites. Moreover, it is often possible to integrate different control techniques for improved management, such as combining predatory mites with compatible acaricides and entomopathogenic fungi (Reddy 2001, Midthassel et al. 2016, Andrade et al. 2019). In conclusion, detecting OFV in Florida represents a concern for horticulturists who grow orchids, *Liriope*, *Ophiopogon*, or other susceptible Asparagaceae species which are commonly used in landscaping. Florida is also home to a plethora of native and naturalized orchid species, many of which are threatened, including cultivated *Vanilla* in southern Florida (Chambers et al. 2019) and the famous Ghost Orchid, [*Dendrophylax lindenii* (Lindl.) Benth. ex Rolfe]. Furthermore, Citrus Leprosis was present in Florida during the 1860s and almost eradicated by the mid-1960s (Knorr et al. 1968, Childers et al. 2003). An examination of herbarium specimens of Florida citrus found that one strain of this historical virus, Citrus leprosis dichorhavirus-N0, is distantly related to the modern isolates of OFV (Kitajima et al. 2011, Hartung et al. 2015, Roy et al. 2020). The recent detection of OFV-Orc1 in South Africa (Cook et al. 2019) in *C. sinensis* (Navel and Valencia orange) and OFV-Orc2 in Hawaii (Olmedo-Velarde et al. 2021) in *C. reticulata* (mandarin) and *C. jambhiri* (rough lemon) associated with leprosis-like symptoms highlights the potential threat of different isolates of OFV on citrus. *B. californicus*, *B. yothersi*, and *B. obovatus* are all present in Florida (Childers et al. 2003, Akyazi et al. 2017), and are difficult to identify by non-experts, or without advanced methodologies. DNA barcoding (Armstrong and Ball 2005) or a similarly simple and accurate method for identification of these mite complexes is vital to identify mite populations which need to be monitored or controlled. By doing so, we can determine the risk OFV-Orc represents for the native plants, agriculture, and the ornamental/landscaping industries of Florida and the surrounding regions.

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## BIOGRAPHICAL SKETCH

Austin N Fife received his B.S. degree in biology - zoology emphasis at Brigham Young University - Idaho at Rexburg. During his undergraduate degree, he conducted research on the distribution of the endemic St. Anthony Dunes Tiger Beetle *Cicendela arenicola*. He went on to receive a M.S. in entomology at the University of Idaho while working at the Kimberly Research and Extension Center on potato psyllids *Bactericera cockerelli* and host plant resistance. He received his PhD in entomology and nematology at the University of Florida in 2021 while working at the North Florida Research and Education Center in Quincy, FL. There, his research focused on *Phyllocoptes fructiphilus* and *Rose rosette emaravirus*, the causal agent of Rose Rosette Disease. His research covered a variety of topics, including acarology, chemical ecology, induced plant defenses, biological control, and plant-pathogen-arthropod interactions. As a former Eagle Scout from Idaho, he loves to spend time outdoors with his wife, Liz and his two (soon to be three) lovely daughters, Violet, Juniper and {insert plant name here}.