

MITE-VIRUS-PLANT COMPLEXES OF IMPORTANCE FOR FLORIDA
AGRICULTURE: EARLY DETECTION, CHEMICAL ECOLOGY AND BIOCONTROL
OF *PHYLLOCOPTES FRUCTIPHILUS* AND *BREVIPALPUS CALIFORNICUS*

By
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‘For Liz, Violet, Juniper and Fifes to come’

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Major: Entomology and Nematology

'Rose Rosette Virus (genus Emaraviridae) is the most devastating disease of roses.

Rose Rosette Virus (RRV) creates witches brooms, rosetting, deforms flowers, increases prickle density, elongates shoots, reddens of plant tissues, causes dieback and ultimately plant death. RRV is spread by a microscopic eriophyid mite known as *Phyllocoptes fructiphilus* Keifer (Trombidiformes: Eriophyidae). Few management options are available:

Current mite control is achieved by removing infected roses and frequent pesticide applications. Growers are interested in alternative and less expensive management options to combat *P. fructiphilus* and RRV. Predatory mites have potential to fulfill this need: mites from the family Phytoseiidae are being investigated as biocontrol agents for the management of *P. fructiphilus*. Preliminary data suggest that the phytoseiid mite *Amblyseius swirskii* Athias-Henriot (Mesostigmata: Phytoseiidae) orients itself towards volatiles of RRV-infected roses. This attraction may have synergistic effects for *P. fructiphilus* control. *A. swirskii* and three other commercially-available phytoseiid mites will be tested in olfactometer choice tests to identify specific volatile compounds which may be causing this behavior. Findings will help to develop chemical lures and promote depredation on *P. fructiphilus* in Rose Rosette-infected roses. This research will contribute a biocontrol option for the management of *P. fructiphilus* in southern Georgia

and northern Florida. 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: Nolinoidaea) 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 (Nolinoidaea), 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): *Brevipalpus californicus* (Banks) *sensu lato*, *B. obovatus* Donnadeieu, and *B. confusus* Baker. In conclusion, OFV orchid strains are present in northern Florida, representing a risk for susceptible plants 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 **pains of** mite identification. 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 injury** (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 Tetranychoidea 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 (Brattsten and Ahmad 1986). Herbivorous mite damage is dependent on the specific mite-plant interactions for a given plant spp. 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. 2018b), 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 discolored (Oldfield 1996a). Feeding on young tissues often forms distortions and delays plant growth, and some mites—many eriophyoidea and two spp. 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 Co-evolved plant specialists: the eriophyoidea

The eriophyoidea are the second most economically-important group of herbivorous mites, right behind Tetranychidae. Where eriophyoids lose out in damage output, they pull ahead in diversity: 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 (Manson and Oldfield 1996). Many eriophyoid mites have summer forms (protogynes) which readily proliferate and winter forms (deutogynes), which are able to survive harsher conditions until they can disperse when environmental

conditions are moderate (Kassar and Amrine Jr 1990). Eriophyids are not able to disperse very far by walking (Calvet et al. 2020), but are known to disperse aerially (Kuczyński et al. 2020) and passively (Galvão et al. 2012), rarely by phoresy on other animals (Li et al. 2018). 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 eriophyids is superficial and causes minimal damage to their host plant (Krantz and Lindquist 1979, Oldfield and Proeseler 1996). To date, eriophyids have only been associated with plant viruses (Lillo et al. 2018), which may be explained by the small size of eriophyoid 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 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 Virus, the causal agent of Rose Rosette Disease

Phyllocoptes fructiphilus Keifer, (Acari: Trombidiformes: Prostigmata: Eriophyoidea: Eriophyidae) is an eriophyoid mite from the Prostigmata group. Like many other eriophyoids, 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), but it doesn't create noticeable damage by feeding. Instead, the increased interest in *P. fructiphilus* stems from its relationship with a virus known as Rose Rosette Virus (RRV) Emaraviridae (Allington et al. 1968, Tzanetakis et al. 2006, Laney et al. 2011). *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, creating millions of dollars of losses for growers (Babu et al. 2014) and threatening the ornamental rose industry Rwahnih et al. (2019). 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 pestilent roses (Tipping and Sindermann 2000). The relationship between, *R. multiflora*, mite and virus also has a positive influence on *P. fructiphilus* fecundity: 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 found a new colony without being fertilized *a priori* to dispersion (Helle and Wysoki 1996). Together, these factors likely contribute to *P. fructiphilus*'s dispersal ability. RRD and *P. fructiphilus* can spread through the landscape in various ways: RRV can be spread 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), and can be spread by humans moving infested plants (Navia et al. 2009). 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 RRD in 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

Integrated Pest Management (IPM) is a philosophy of 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



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.

specific pest density has been exceeded, and interventions are required to prevent the crop from reaching the EIL (Stern et al. 1959). A useful framework for controlling plant pathogens has been developed in the concept of the disease triangle. For a long time, plant pathologists have recognized the importance of pathogen, environment and host on disease proliferation (Gäumann 1950), and all of these factors must be present in sufficient quantity and quality for disease to occur (McNew 1960, N 1960, Agrios 2004). Extensions of the basic disease triangle have been considered, including adding extra dimensions to account for more variables (Francl 2001), or applying it to different systems (Scholthof 2006), but the basic concept is that removing of one of these main factors of disease gives a point of attack for control efforts: Management may focus on removing a suitable host by crop rotation, or by using cultivars resistant/tolerant to pests and/or pathogens. Pathogens can be excluded from the crop via sanitation measures such as insect screens, tissue culture, seed certification programs, cleaning harvesting equipment, or by growing

crops in a greenhouse. Environment can also be manipulated by growing crops in areas where the pest/pathogen isn't present or can't survive under normal conditions. The IPM paradigm encourages the combination of as many of these methods as possible for improved pest control. Although there is some overlap in the technologies and terminologies used, pest management traditionally divides pest interventions into four main 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, harm beneficial insects/pollinators, leave residues on crops meant for consumption, and can harm the environment through runoff/drift, polluting surface and groundwater (Driesche et al. 2007, Marquina et al. 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 (roguing) by hand, creating physical barriers such as raised beds, insect screens, organic or plastic/reflective mulch, solarizing/heating the soil, sticky traps/barriers, ~~and~~ 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).

1.2.1 Current Management of RRD is not effective

Nursery managers have been recommended to manage RRD by removing sick plants and spraying acaracides (Hong et al. 2012, Olson et al. 2017, “Control - rose rosette” 2018). 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, including *Phyllocoptuta oleivora* (Ashmead) and *Acalitus vaccinii* (Keifer) becoming 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*. Pesticide applications are further complicated by the biology of the mite: *P. 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 all help shield the mites from conventional pesticide treatments. Furthermore, a single mite is potentially enough to transmit the virus (Di

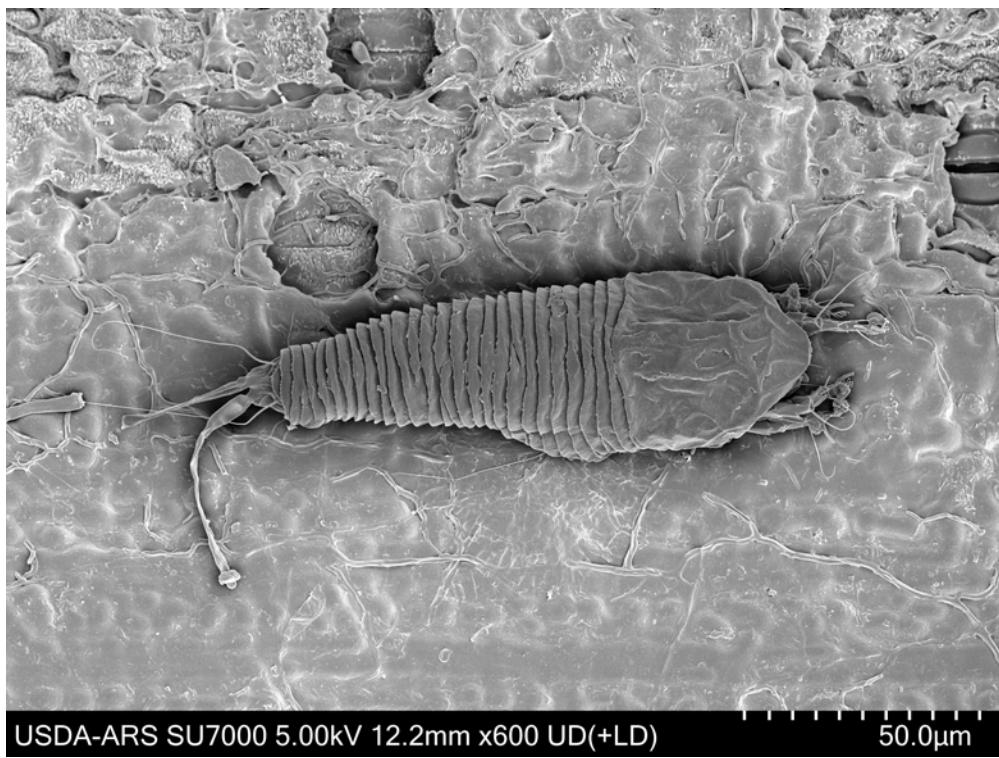


Figure 1-2. Cryo-SEM image of Eriophyid mite infected with unidentified fungus, collected from *Liriope muscari*. Photo Credit: Dr. Gary R. Bauchan, USDA-ARS, 2020.

Bello et al. 2017), which can infect a rose in less than an hour, yet plants can remain sick and symptomless for months to years (Amrine Jr 1996, Di Bello et al. 2017). This slow onset of disease symptoms creates an additional challenge for management (Di Bello et al. 2017), because by the time the disease is noticed, the mites may have already spread to the whole garden. Disease detection is also difficult: Symptoms can appear similar to natural plant growth or herbicide damage, making it hard to diagnose in the field (Hong et al. 2012). 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 to test for 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. Host plant resistance is not a viable option for controlling RRD: 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), 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 Phytoseiids mites: good options for biological control of mites?

Many mites species have potential as a biological control (Gerson et al. 2003, Carrillo et al. 2015). Their efficiency as predators of pest species has been recognized for many years: One of the earliest recorded attempts at biological control was of a mite *Tyroglyphus phylloxerae* (Riley & Planchon), which was imported to France from the USA in an attempt to control the grape phylloxera, *Daktulosphaira vitifoliae* (Fitch 1855) (Riley 1874, Dent 2000, Kirchmair et al. 2009). Although this early attempt was unsuccessful, several mite species have been successful in controlling pest species (Bellows Jr et al. 1996, Driesche et al. 2010). The most well-studied family of predatory mites used for biological control are the Phytoseiidae (Gerson et al. 2003, Farragut et al. 2010, Carrillo et al. 2015). The lifestyles of Phytoseiid mites are generally split into four categories based on feeding guilds as described in McMurtry and Croft (1997): Type I mites belong to the genus *Phytoseiulus*, the only group considered to be specialists on spider mites from the genus *Tetranychus*. These mites reproduce faster than other phytoseiid groups, but only thrive on spider mite prey. They also respond to kairomones emitted by *Tetranychus* feeding (McMurtry and Croft 1997, Farragut et al. 2010). Type II phytoseiids are also heavily associated with *Tetranychus*, including representatives from the genera *Neoseiulus*, *Galendromus*, and *Typhlodromus*, but Type II mites also can feed on other mite groups, such as eriophyid, tydeid, and tarsonemid mites. They also can feed on pollen or plant exudates if necessary (McMurtry and Croft 1997, Farragut et al. 2010). Both Type I and Type II mites often live in the web colonies of their Tetranychid hosts, and have longer

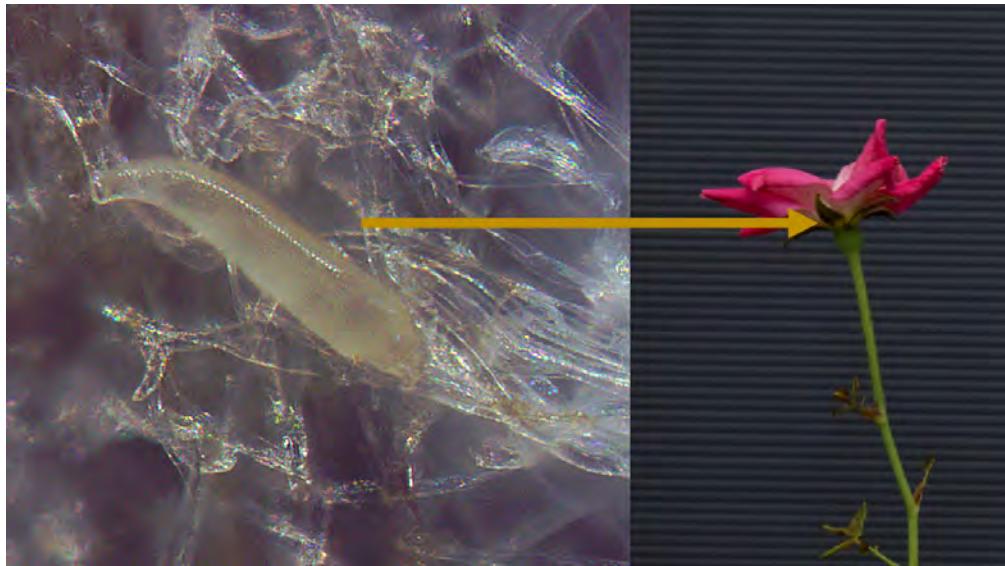


Figure 1-3. Illustration of the typical location of *Phyllocoptes fructiphilus* on roses. *P. fructiphilus* are difficult to manage with pesticides due to the protection offered by the sepals.

dorsal setae to avoid entanglement (McMurtry and Croft 1997, Farragut et al. 2010). Type III phytoseiids are considered to be generalists, and can grow and reproduce on a variety of different mite groups, including Eriophyoid, Tetranychoid, Tarsonemid and Acarid mites. They also feed on plant pollen, nectar, and insects such as whiteflies and thrips (McMurtry and Croft 1997, Farragut et al. 2010). The breadth of their feeding guild has encouraged their use in biological control programs (Farragut et al. 2010). Type III mites are more likely to feed on other mites of the same species, and require more prey for development than the specialist phytoseiid groups (McMurtry and Croft 1997, Farragut et al. 2010). Type III live on plants rather than in spider mite colonies, and accordingly have shorter dorsal setae. Type III mites will still feed opportunistically on *Panonychus*, a group of Tetranychid mites which produce less dense webbing (McMurtry and Croft 1997, Farragut et al. 2010). Lastly, Type IV mites belong to the genus *Euseius*, which are polyphagous mites which primarily feed on pollen, although they will feed on other mites and small insects as well. They have short dorsal setae (McMurtry and Croft 1997, Farragut et al. 2010). Phytoseiid mites of all four types have been integrated successfully

into various pest management programs. Many phytoseiids have been tested with various combinations of other biocontrol agents, such as predatory bugs and *Beauveria bassiana* (Chow et al. 2010, Midthassel et al. 2016, Bouagga et al. 2018, Freitas et al. 2021), as well as certain pesticides (Trumble and Morse 1993, Nicetic et al. 2001, Fernández et al. 2017). Phytoseiid reproduction has been studied to develop methods for mass-rearing and releasing 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, Carrillo et al. 2011a, Carrillo and Peña 2011, Sarwar 2017, Knapp et al. 2018, Argolo et al. 2020). One of the more popular species of commercially-available predatory mite is *Amblyseius swirskii* Athias-Henriot (Calvo et al. 2014). *A. swirskii* is a Type III mite species (Farragut et al. 2010), which has been used successfully in agriculture for pest control of crop pests such as whiteflies (Bolckmans et al. 2005), spider mites (McMurtry et al. 1970), rust mites (Park et al. 2010, Onzo et al. 2012), broad mites (Tarsonemidae) (Lopez et al. 2016), and thrips (Wimmer et al. 2008). *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). *A. swirskii* can be reared on artificial diets (Nguyen et al. 2013), natural and supplemental pollen (Loughner et al. 2011, Park et al. 2011, Delisle et al. 2015) and/or other arthropods present in the environment even when the pest of concern is absent (Janssen and Sabelis 2015, Kumar et al. 2015). This allows *A. swirskii* to be released periodically as a preventative measure instead of reacting to an outbreak (Kutuk and Yigit 2011). Volunteer and banker plants near cropping systems can also provide shelter for phytoseiids to live in (Smith and Papacek 1991, Coli et al. 1994, Xiao et al. 2012, Nunes et al. 2020). Type III 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). For example, Type III phytoseiids prefer to live on ‘hairy’ plants with dense trichomes, and will leave glabrous leaf surfaces (Loughner et al. 2010a, 2010b), often laying their eggs in the densest patches of plant hair, or the thick tufts 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), possibly to avoid predation (Faraji et al. 2002). Predator-plant mutualisms also extend into the realm of chemical communications: many types of phytoseiids learn to associate their prey with Volatile Organic Compounds (VOCs) released when plants are injured by pests or infected with pathogens (Sabelis et al. 1999, Maeda and Takabayashi 2001, Boom et al. 2002, Boer and Dicke 2004a, 2004b, 2005). VOCs can repel (Moraes et al. 2001) attract (Nomikou et al. 2005, Gadino et al. 2012), encourage predation (Kessler and Baldwin 2001, Halitschke et al. 2007), or poison arthropods (Vancanneyt et al. 2001). Plant responses to herbivory differ between plant and predator species (Maeda and Liu 2006, Qualley and Dudareva 2008), underlining the importance of studying the specific interactions for each crop (Boom et al. 2004).

1.3 Induced plant defenses for biological control

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



Figure 1-4. *Phytoseiulus persimilis* are type I phytoseiid mites: specialists of spider mites from the genus *Tetranychus* [@Farragut2010; @McMurtry1997].

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).

1.3.1 Can systemic acquired resistance be used to reduce mite herbivory?

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 long term resistance to future attack, 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 Ca^{2+} influx at the site of injury (Vincent et al. 2017). Another 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 (Conrath et al. 2006, Vlot et al. 2009, Zhang et al. 2010). SAR-induction increases levels of β -1,3-glucanase and chitinases (Bronner et al. 1991a, Ward et al. 1991), which prevent fungal disease development (Goy et al. 1992, Xue et al. 1998, Narusaka et al. 1999, Suo and Leung 2001). These proteins have potential for the biological control of pest mites: Bronner et al. (1991b); Bronner et al. (1991a) observed that feeding by the gall mite *Aceria cladophthirus* (Nalepa) triggered the hypersensitive response on *Solanum dulcamara*, producing chitinase and β -1,3-glucanase activity, which suggested that these may have a role in plant defenses against herbivores. Subsequent experiments by Westphal et al. (1991) also found that *S. dulcamara*'s SAR response to *A. cladophthirus* produced long lasting protection from subsequent colonization by more *A. cladophthirus* or another eriophyid, *Thamnacus solani* Boczek and Michalska, a rust mite. Unfortunately, later research by Westphal et al. (1992) demonstrated that these induced responses were not enough not protect the plant from *Tetranychus urticae* Koch, but instead increased their fecundity. This type of interplay between induced defenses is common, and varies by plant species (Boom et al. 2004). The induction of SA and JA are known to exhibit negative cross-talk in some plant systems (Baldwin et al. 1997, Belliure et al. 2010, Thaler et al.

2012): 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. (2018a) found that SA and JA induction was sufficient to control erineum forming *Colomerus vitis* (Pagenstecher). 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 induces 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* spp. with CiLV-C induces SA and suppresses JA/ET pathways through crosstalk (Arena et al. 2016). A follow-up 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 (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).

1.4 A second mite-plant-pathogen system: *Brevipalpus californicus* and Orchid fleck virus

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, 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, 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

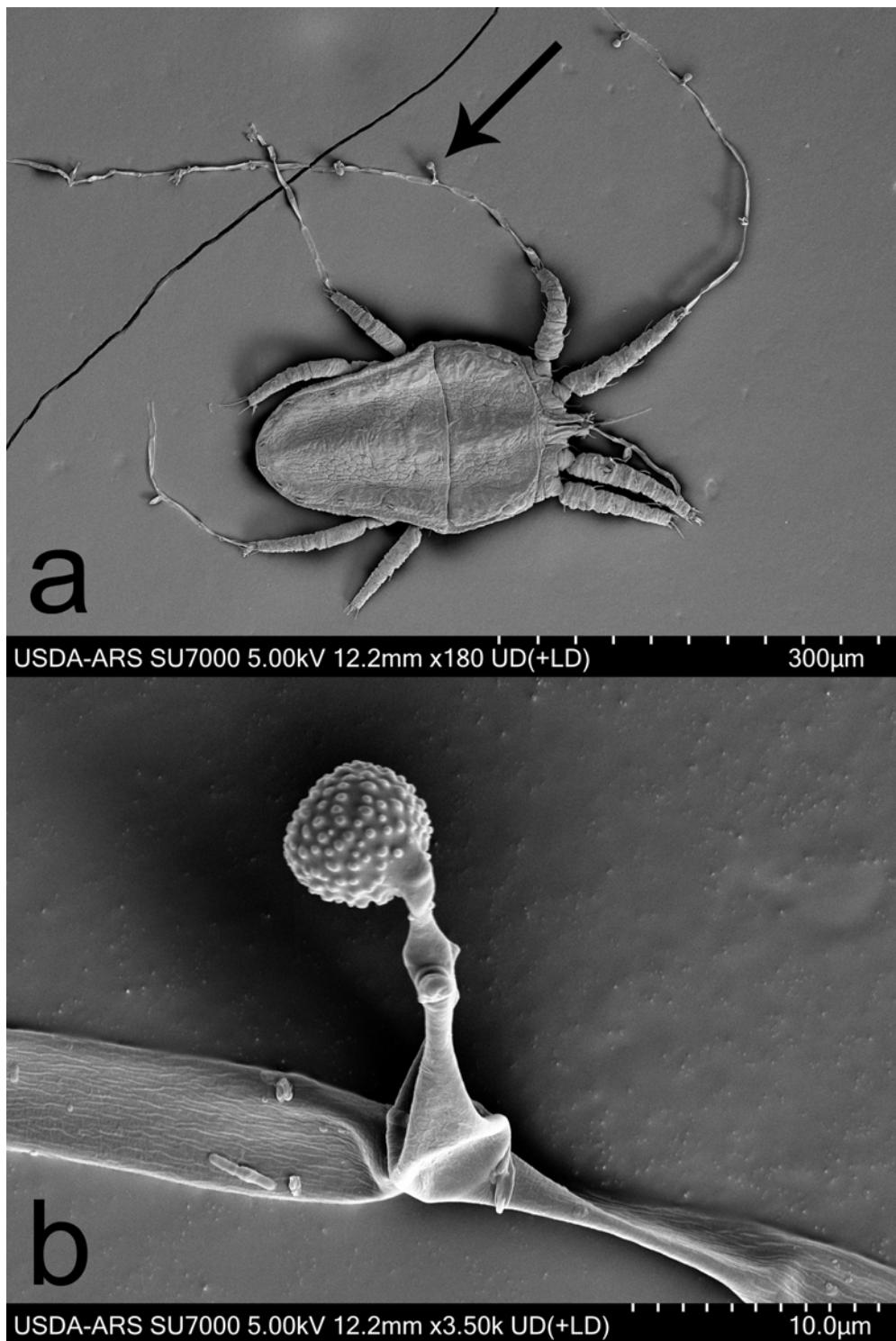


Figure 1-5. a) Cryo-SEM image of Tenuipalpid mite infected with unidentified fungus, collected from *Liriope muscari* b) detail of sporangia. Photo Credit: Dr. Gary R. Bauchan, USDA-ARS, 2020

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 1-6. *Oncidium* orchid infected with Orchid flea 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 rouging 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 et al. (2020), Solo (2018), see [2-1](#)). They encountered *P. fructiphilus* in Thomas County and Lowndes County, GA ([2-1](#)), less than 20 miles from the northern border of Florida. RRD has been detected in previously in southern Florida (Babu et al. 2014), but no mites were detected at that time, and no RRD has been documented in those areas since.

2.2 Surveying for *P. fructiphilus*, RRD and predatory mites in northern Florida

A key part of *P. fructiphilus* control is vector and disease monitoring. Populations of *P. fructiphilus* are easily overlooked, the mites are microscopic and cryptic in habits,

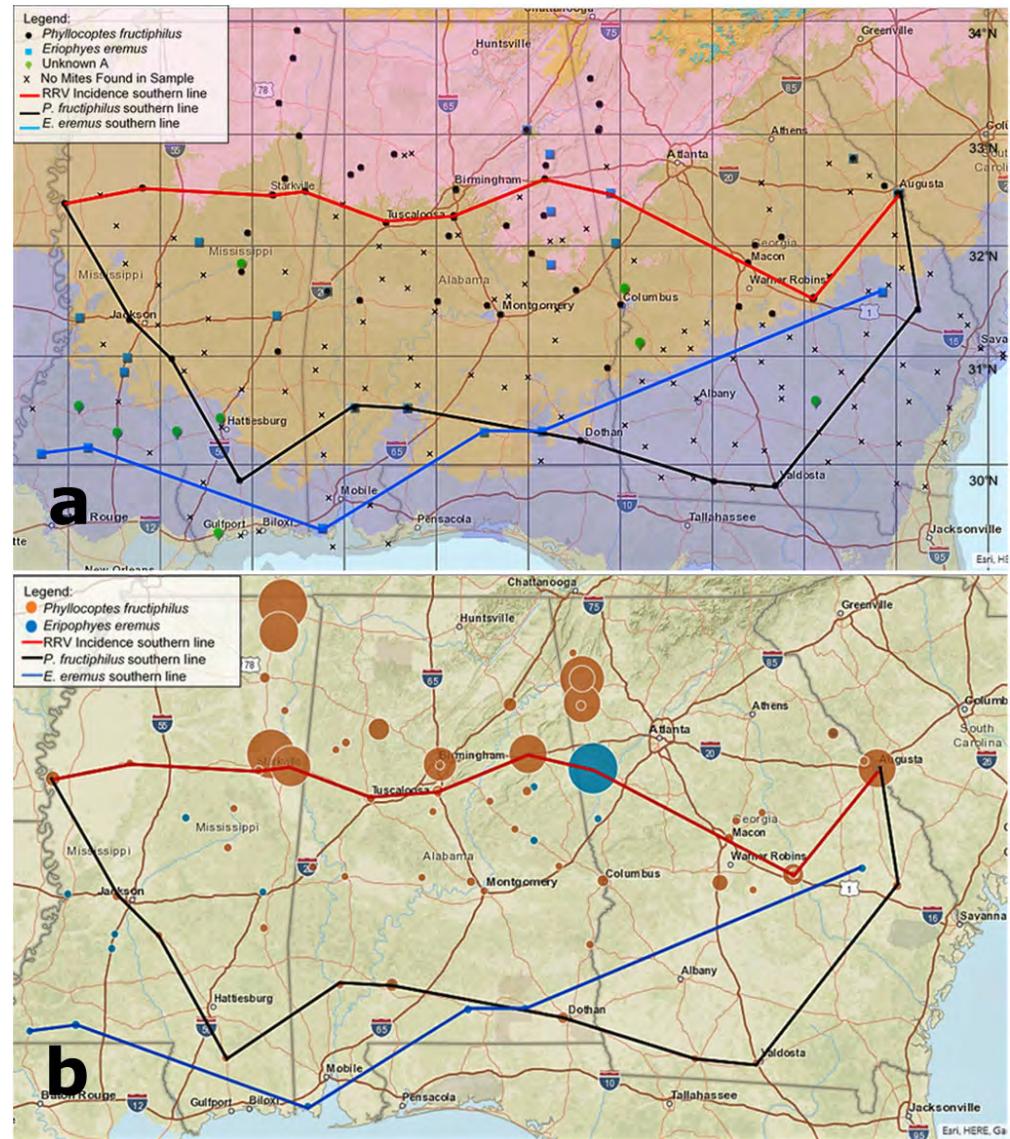


Figure 2-1. From Solo et al. 2020: a) 'Map of the southern incidence line of Rose rosette virus (RRV) and eriophyid mites in Alabama, Georgia, and Mississippi in 2017. Plant hardiness Zone 7b is in pink, Zone 8a is brown, Zone 8b is blue, and Zone 9a is in gray. Note that there are five locations in which two mite species were found on the same rose sample' b) '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

primarily located under the sepals of rose flowers, near glandular trichomes on the tips of rose canes (Jesse et al. 2006, Bauchan et al. 2019). Furthermore, previous surveys of the southeastern United States did not extend into Florida (Solo et al. (2020), see [2-1](#)). It is possible that *P. fructiphilus* and/or RRV are present in northern Florida or other parts of the state. 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 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 of *P. fructiphilus* and/or RRD.

2.2.1 Phenology of natural populations of *P. fructiphilus* in northern Florida

As a result of these surveys, populations of eriophyoid mites suspected to be *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 phenology of *P. fructiphilus* in that area from 2020-2021.

2.3 Materials & Methods

2.3.1 Mite Survey

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™ 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).

2.3.2 Phenology

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 plots of ~3 m², with approximately 12 roses per plot. Samples were processed as previously described in [2.3.1](#). After washing, plant tissues were placed

into in 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. About ~5% of early samples collected were missing dry weights, missing data was imputed using Multivariate Imputation by Chained Equations via the mice package (Buuren and Groothuis-Oudshoorn 2011) using R version 4.1.1 (R Core Team 2021).

2.4 Results

2.4.1 Survey

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.

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
Macclemmy	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

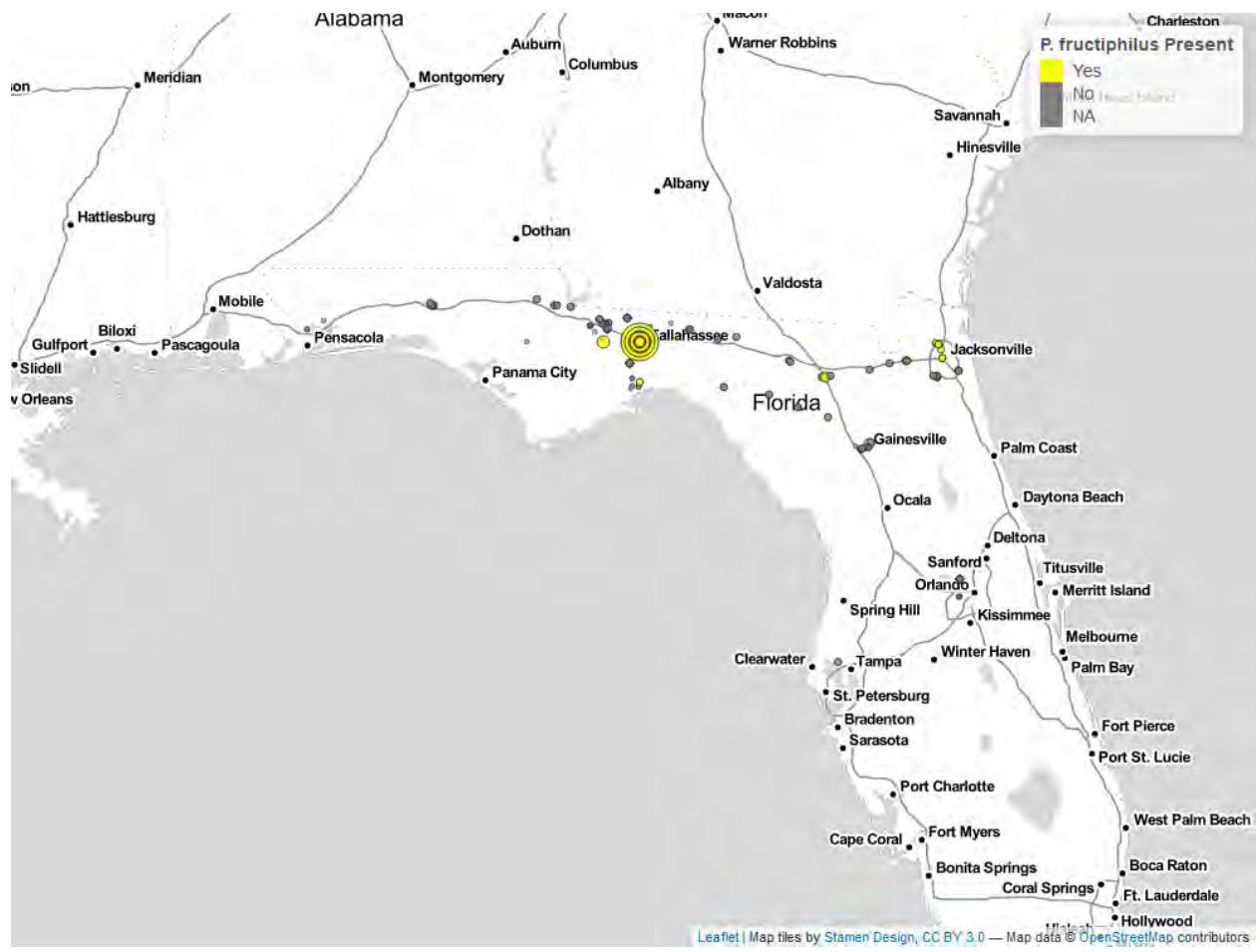


Figure 2-2. *P. fructiphilus* mites recovered during surveys of roses in Florida, 2017-2021.

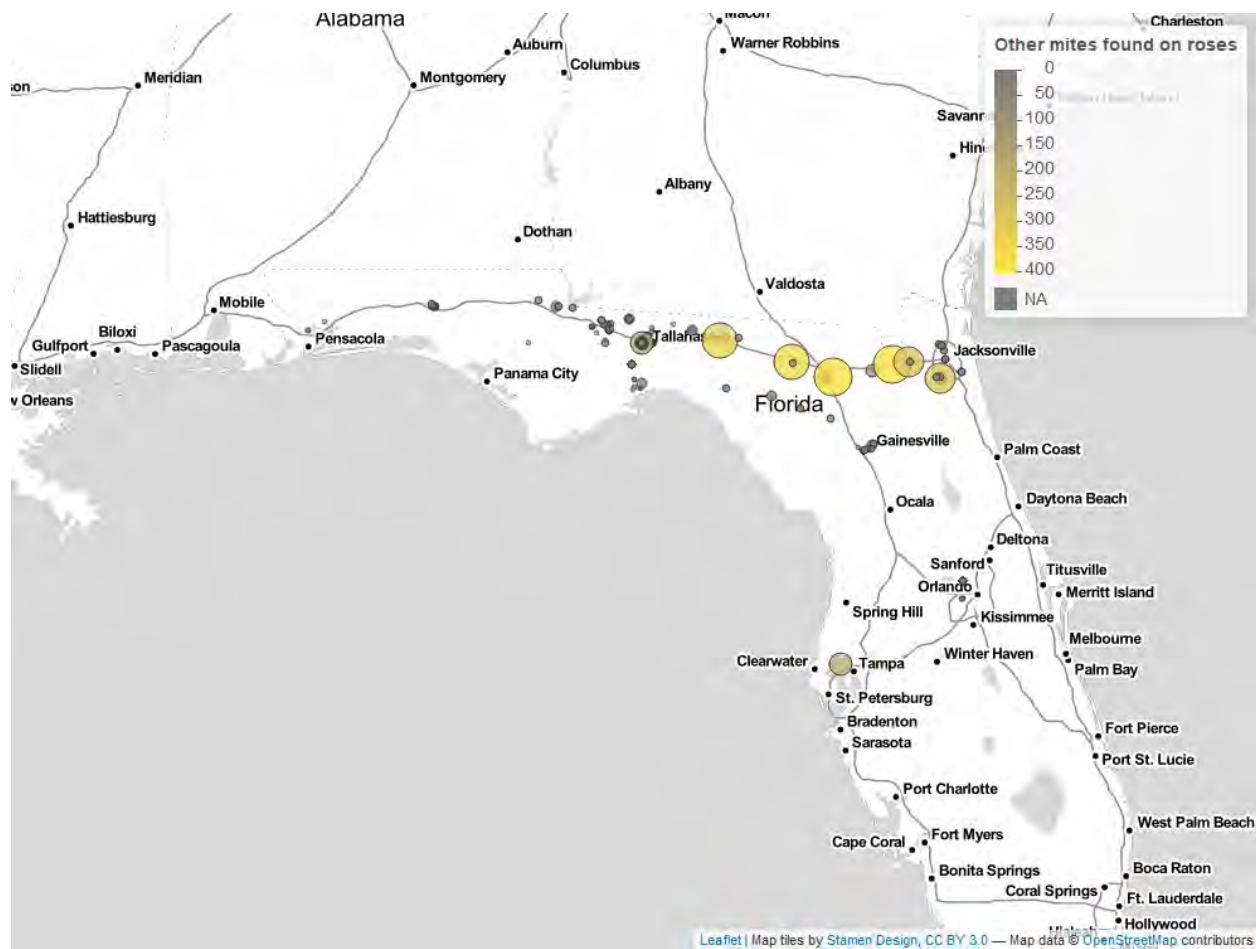


Figure 2-3. Other mites recovered during surveys of roses in Florida, 2017-2021.

Eriophyoid mites found on roses in northern Florida

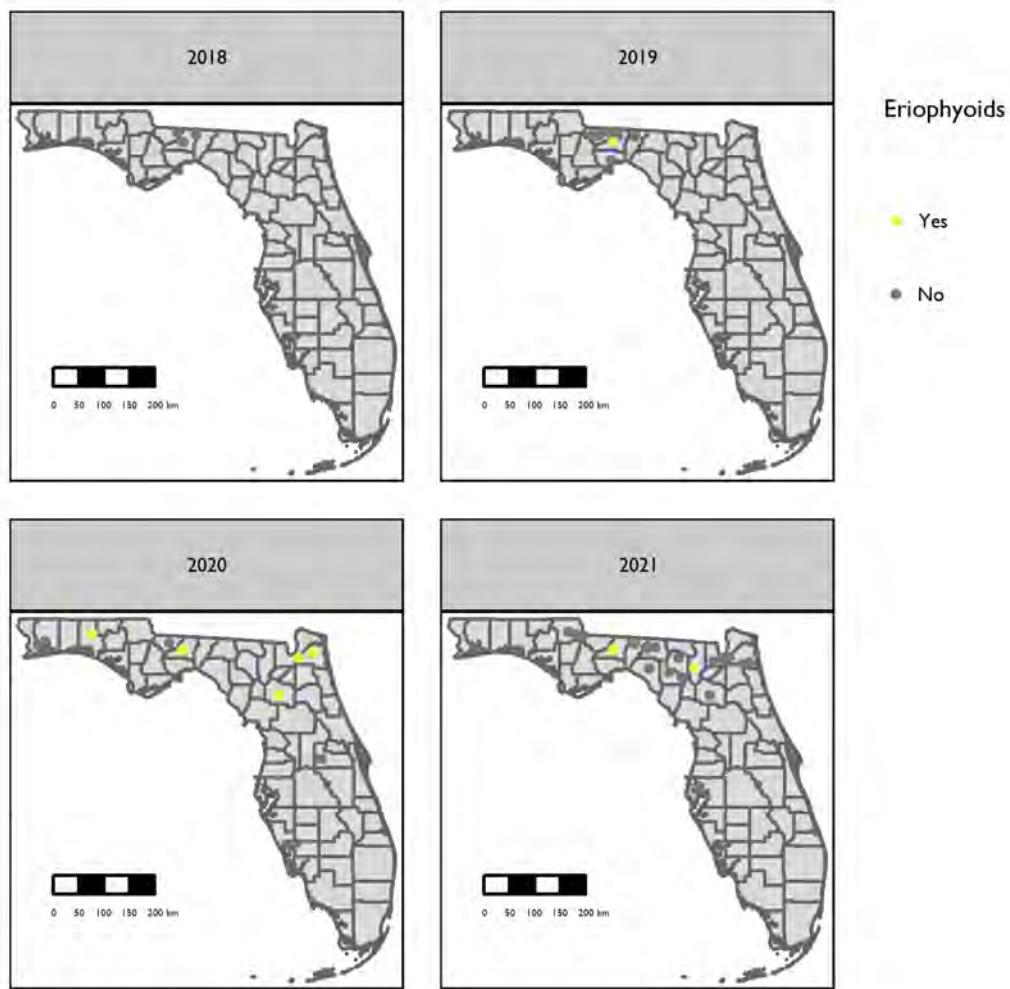


Figure 2-4. Location of populations of eriophyoid mites found on roses in northern Florida 2018-2021.

Other mites found on roses in northern Florida

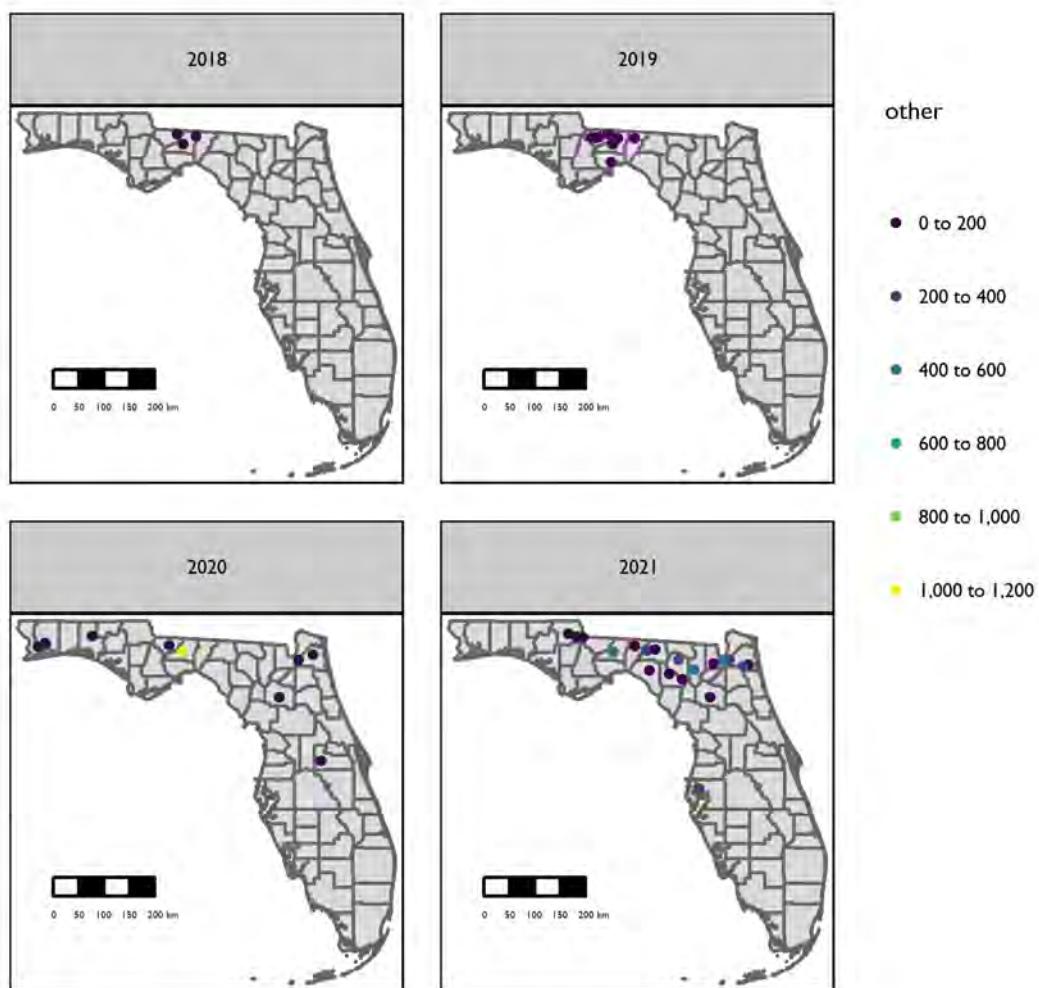


Figure 2-5. Locations of other mites recovered during surveys of roses in Florida, 2018-2021.

Mean Number of *P. fructiphilus* collected per gram of rose dry weight

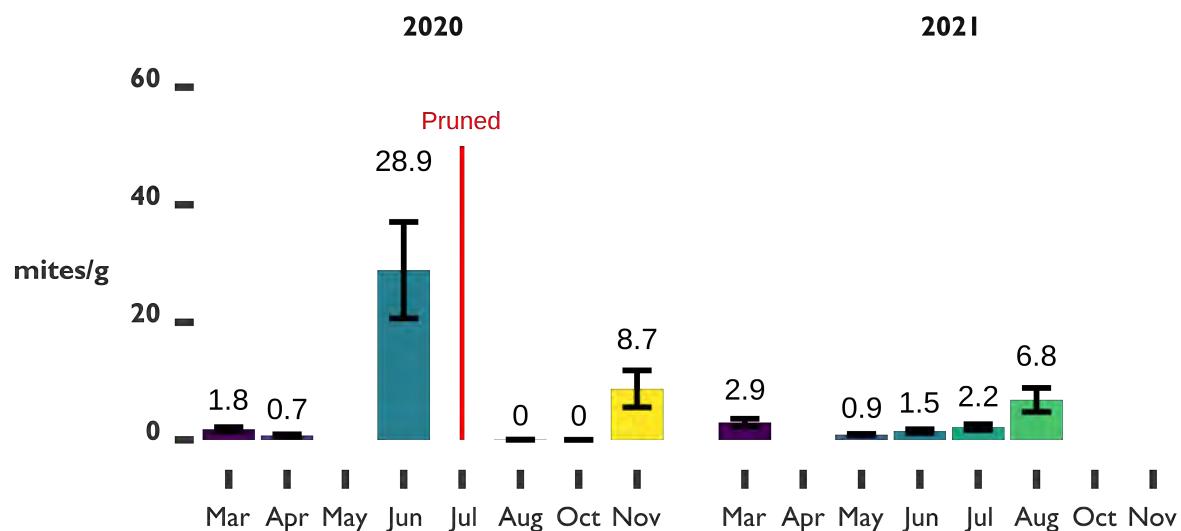


Figure 2-6. Phenology of *P. fructiphilus* mite populations on roses in Leon County, Florida 2020-2021. Roses were pruned back heavily on July 9, 2020.

2.4.2 Phenology

Populations of *P. fructiphilus* showed seasonal fluctuations in mite numbers, with highest populations achieved during June 2020. Rose pruning in July reduced the numbers of *P. fructiphilus* collected for 3 months, populations began to recover that November. 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.

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). 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 site with *P. fructiphilus*. 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, and unfortunately our observations are not sufficient to describe a mechanism of invasion. Eriophyoid mites are known to disperse in a variety of ways: they may be windblown, transported with infected plants, move on contaminated equipment or clothes, or rarely, through phoresy (Sabelis and Bruin 1996). 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, Katsianis 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-eriphoid mites have been collected as well, but it is beyond our ability to identify many of them. Phytoseiid and other predatory mites require expert identification by mite taxonomists, and many species have been misidentified by amateurs either through carelessness or ignorance (Demand et al. 2021). The large reductions of *P. fructiphilus* seen post pruning suggests its potential as a method of cultural mite control. It may be possible to combine pruning with acaricide treatments for improved control of *P. fructiphilus*, but this hypothesis requires further study. Tracking populations for longer periods of time with additional climatic data could be used to determine the best times to prune to reduce mite numbers. 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.



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

Austin Fife¹, Samuel Bolton², Jessica L. Griesheimer¹, Mathews Paret¹, and Xavier Martini^{1,*}

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 ± 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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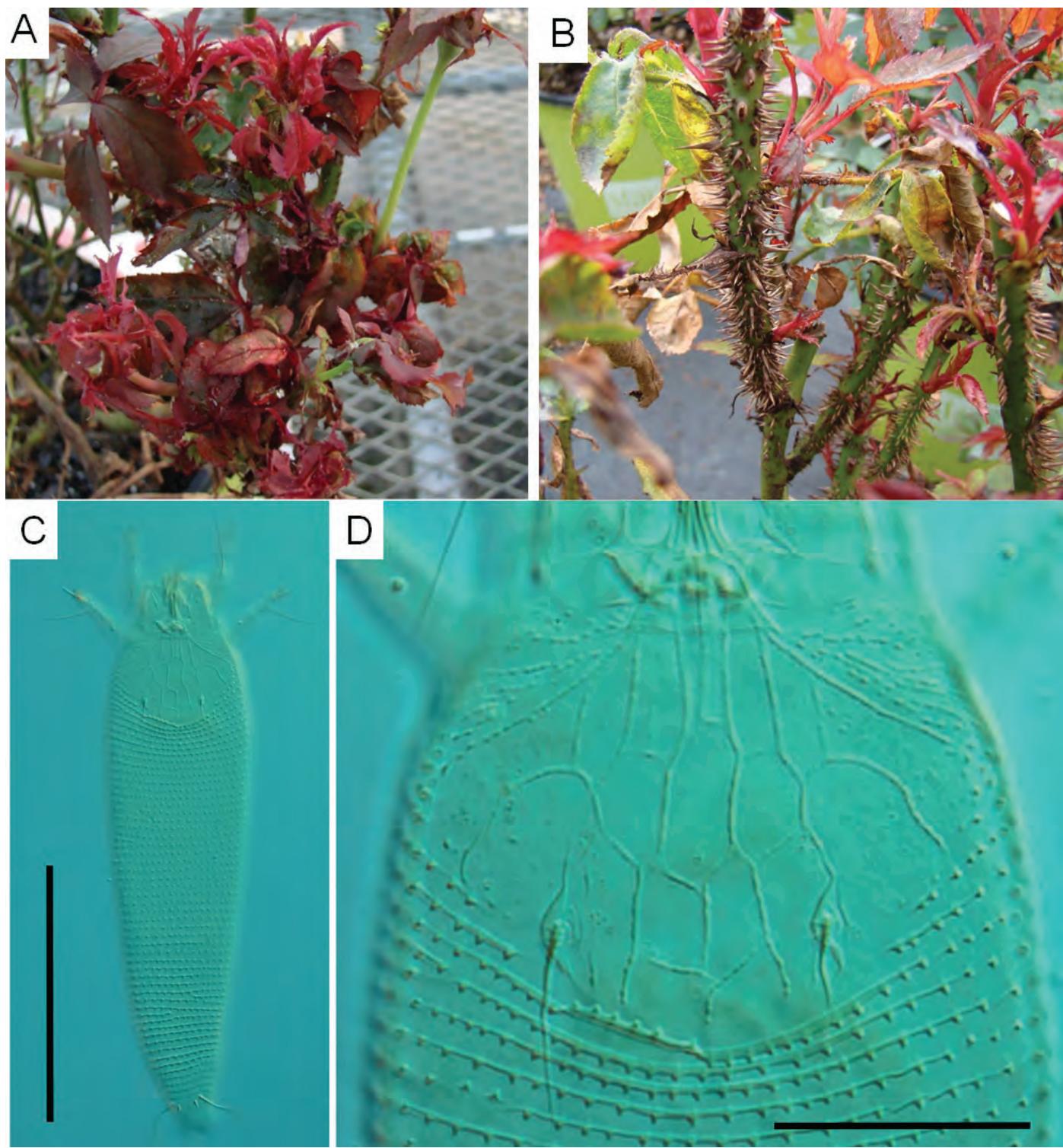


Fig. 1. (A) Symptoms of rose rosette disease: witches' broom, and (B) excessive thorn proliferation; (C) *Phyllocoptes fructiphilus* Keifer (female) from Leon County, Florida, USA: body (scale bar = 100 μm); (D) enlargement of *P. fructiphilus* prodorsal shield to show detail (scale bar = 20 μm).

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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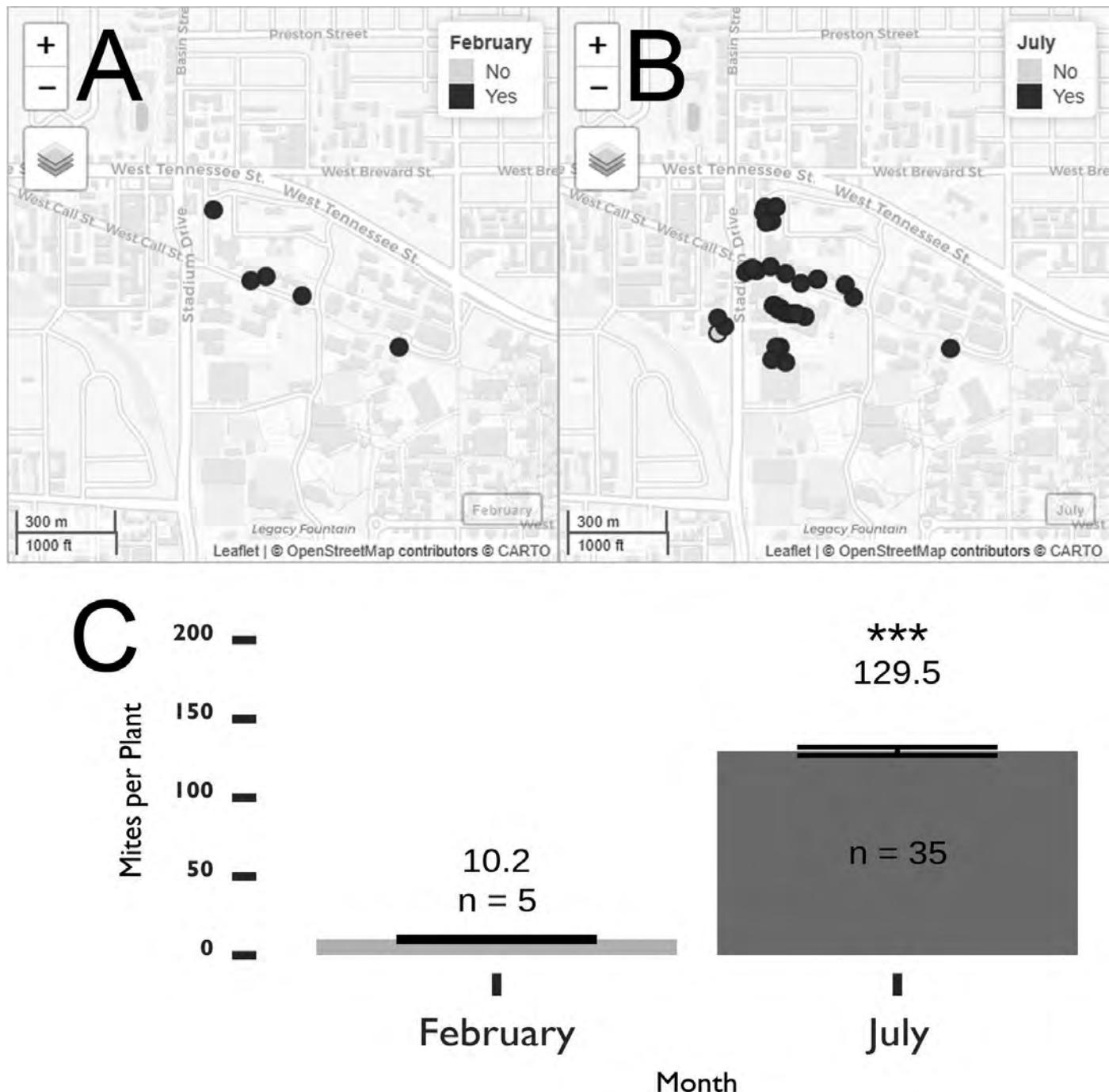


Fig. 2. Presence of *Phyllocoptes fructiphilus* in Leon County, Florida, USA, in (A) Feb 2019 and (B) Jul 2019. Orange dots indicate sites sampled that had *P. fructiphilus*. Gray dots indicate surveyed areas where no *P. fructiphilus* were found. (C) Average number of *P. fructiphilus* per rose sample. 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.

ease (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

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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CHAPTER 3

CHANGES IN HEADSPACE VOLATILES FOR RRD-INFECTED ROSES

3.1 Introduction

3.1.1 Rose Rosette Disease, Predatory mites and Plant Defense: Why are *Amblyseius swirskii* attracted to 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 alternative and less expensive management options to combat *P. fructiphilus* and RRD. Predatory mites may have potential to fulfill this need (Gerson et al. 2003, Farragut et al. 2010, Gerson 2014, Carrillo et al. 2015): Many predatory mites species have been successfully integrated into pest management programs along with other biocontrol agents (Chow et al. 2010, Midthassel et al. 2016, Bouagga et al. 2018, Freitas et al. 2021), and certain pesticides (Trumble and Morse 1993, Nicetic et al. 2001, Fernández et al. 2017). Predatory mites from the family phytoseiidae live in close association with their host plants and are effective predators of many pest species (Gerson et al. 2003, Farragut et al. 2010, Carrillo et al. 2015). One of the most popular species of commercially-available phytoseiid mites is *Amblyseius swirskii* Athias-Henriot (Mesostigmata: Phytoseiidae)(Calvo et al. 2014).

A. swirskii are considered to be a generalist species which feed on a variety of different arthropod pests (McMurtry and Croft 1997), including eriophyoid mites (Park et al. 2010, 2011). *A. swirskii* are able to be purchased in slow release sachets and safely shipped to growers that need them (Buitenhuis et al. 2014, Calvo et al. 2014, Lopez and Smith 2016). They are commonly used in biological control programs (Calvo et al. 2014), partially because of their ability to survive on alternative food sources when pests are not present (Nguyen et al. 2013). They are also able to be released before pests arrive (Kutuk and Yigit 2011), surviving on banker and native plants in the environment until pest populations increase (Xiao et al. 2012). This intimate relation with plants extends into the realm of chemical communications: Phytoseiid like *A. swirskii* have no eyes, instead relying on plant Volatile Organic Compounds (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, 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). Methyl Salicylate (MeSA) is a VOC commonly produced under many types of pathogen attack (Park et al. 2007, Arimura et al. 2011). This plant volatile is derived from Salicylic Acid (SA), an important plant hormone involved in activating the hypersensitive response to pathogens (Gaffney et al. 1993, Goodman and Novacky 1994, Park et al. 2007, Vlot et al. 2009). Volatilized MeSA can initiate the signalling pathway which primes a plant's immune system against attacks by pathogens (Shulaev et al. 1997, Conrath et al. 2006, Tieman et al. 2010, Gozzo and Faoro 2013). Activation of these plant defenses creates long term resistance against future pathogen attack throughout the plant, through a mechanism known as systemic acquired resistance (SAR) (Boller and Felix 2009, Vlot et al. 2009, Zhang et al. 2010). These same pathways can be induced in a plant by chemical analogues to SA, such as the benzothiadiazoles, including acibenzolar-S-methyl (ASM) (Narusaka et al. 1999, Ziadi et

al. 2001, Tripathi et al. 2010, Darolt et al. 2020). 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. 1991b, 1991a, Westphal et al. 1991). Infested plants produced β -1,3-glucanase and chitinases, which were hypothesized to contribute to defenses against these eriophyoid mites (Bronner et al. 1991a, Ward et al. 1991). Similar increases in β -1,3-glucanase and chitinase activity was seen in roses treated with ASM, restricting the growth of a fungal pathogen (Suo and Leung 2001). This suggests that SAR-induction in roses may have biological activity against eriophyoid mites, including *P. fructiphilus*. 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 to create a successful pest management program. Our study was designed to investigate some of the interactions between plant volatiles and their effects on predatory mites: Preliminary data suggest that *A. swirskii* are attracted towards volatiles of RRD-infected plants. Similarly, MeSA has been reported to attract phytoseiid mites towards their prey to varying degrees of success (Boer and Dicke 2004a, 2004b, James and Price 2004, Gadino et al. 2011, 2012). We hypothesized that infection with RRD was triggering the release of MeSA, creating *A. swirskii*'s attraction to the infected plants. 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. The results will help inform future assays involving predatory mites and their prey-seeking behaviors in relation to rose RRV-infection status and the use of SAR-inducers for biological control of *P. fructiphilus*.

3.2 Materials & Methods

3.2.1 Collection of headspace volatiles from roses

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

3.2.1.1 Volatile Collection Trap Method

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. 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~~ from Pink Double Knock Out® roses. Bags were allowed to inflate in order to detect excessive leaks, if no large leaks were detected, the corner of the bag would be cut and a VCT would be inserted. Traps were 3.5" with 28 ± 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 µLs 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. 4 extractions were completed from graft-inoculated RRD-positive roses (Doudrick et al. 1987), grown in a quarantine greenhouse under similar parameters. Headspace samples of the RRD-infected roses were taken prior to the quarantine measures requested by federal agencies. Samples were taken at ~20 °C and RH 50-66%.

3.2.1.2 Solid Phase Micro Extraction Method

The SPME field 90 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 RRD-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 RRD-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 (Clear₂O® 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 RRD-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%.

3.2.1.3 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.

3.2.2 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 volatile sources and the arms of the Y-tube. The olfactometer was held vertically with a lab stand, and illuminated with a 60 watt household 5000K white LED



Figure 3-1. Volatile collection system 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 μ 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.

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



Figure 3-2. Volatile collection system 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 μ L of Nonyl Acetate is added as an internal standard, and then the sample is processed using a coupled GC-MS for chemical identification.

to have made ‘no choice’ and were removed. Assays with roses involved bagging the roses as previously described, but with outlet air lines 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 RRD-infected roses. Synthetic volatiles were selected from the top ten chemicals seen in the contributions table from the PCA analysis ([3-3](#), [3-1](#)). Assays of synthetic volatiles were conducted by a selected VOC with dicholoromethane. 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 at were tested, at a concentration of 1 $\mu\text{g}/\mu\text{L}$. 100 μL of dicholoromethane was used as a control.

Responses of *A. swirkii* were compared with χ^2 tests using R version 4.1.1 (R Core Team 2021).

3.3 Results

3.3.1 Volatile differences between infected, healthy and induced roses

The top six contributing VOCs for VCT were γ -Murrolene, β -Ocimene, (E,E)- α -Farnesene, β -Caryophyllene, Methyl Salicylate and β -Pinene ([3-1](#)). The top six contributing chemicals for SPME were γ -Murrolene, β -Caryophyllene 1R- α -Pinene, Mesitylene, β -Pinene and m-Xylene ([3-3](#)). Roses infected with RRD were generally similar to uninfected roses for both VCT and SPME methods, ([3-4](#), [3-9](#), [3-6](#), [3-11](#)), but ASM-treated roses had less chemical variance than either group ([3-9](#), [3-11](#)). For VCT samples, γ -Murrolene explained most of the variance in uninfected roses, while SPME samples showed the opposite: γ -Murrolene and β -Caryophyllene were primarily found in infected roses.

3.3.2 *A. swirkii* attraction to VOCs

A. swirkii 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. ([3-18](#)). Choices for DL-Limonene

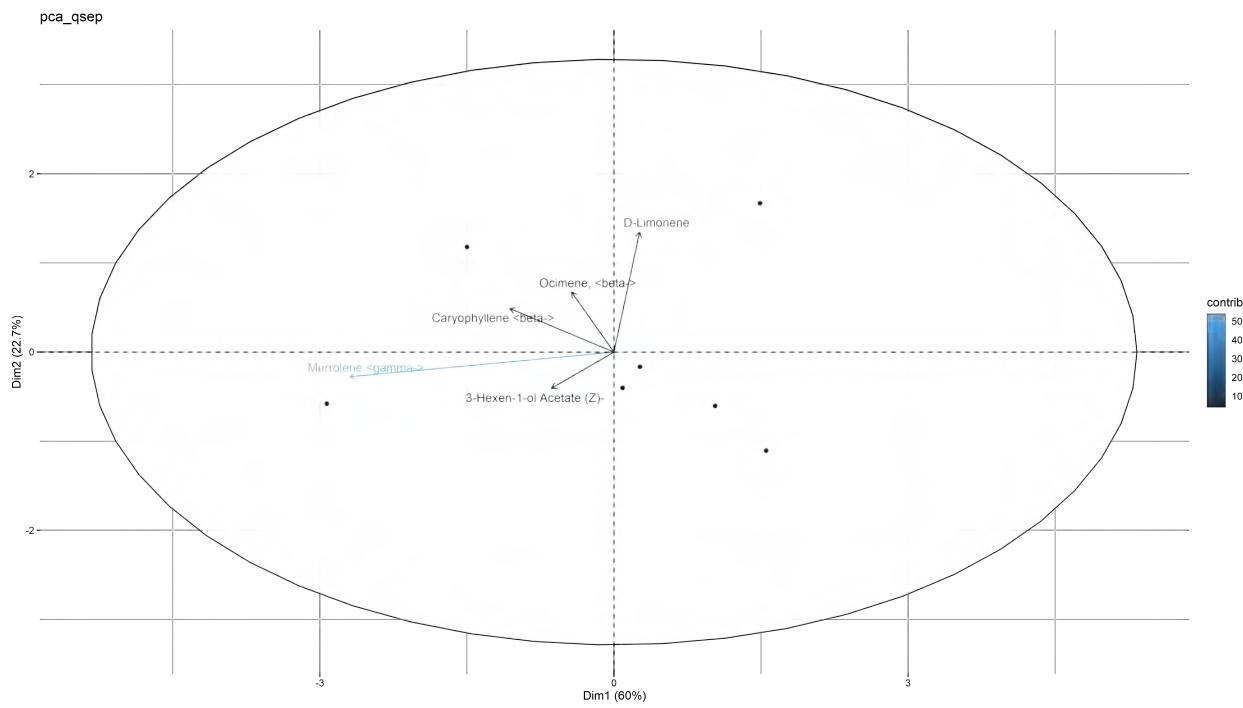


Figure 3-3. PCA biplot of volatiles collected with VCT method.

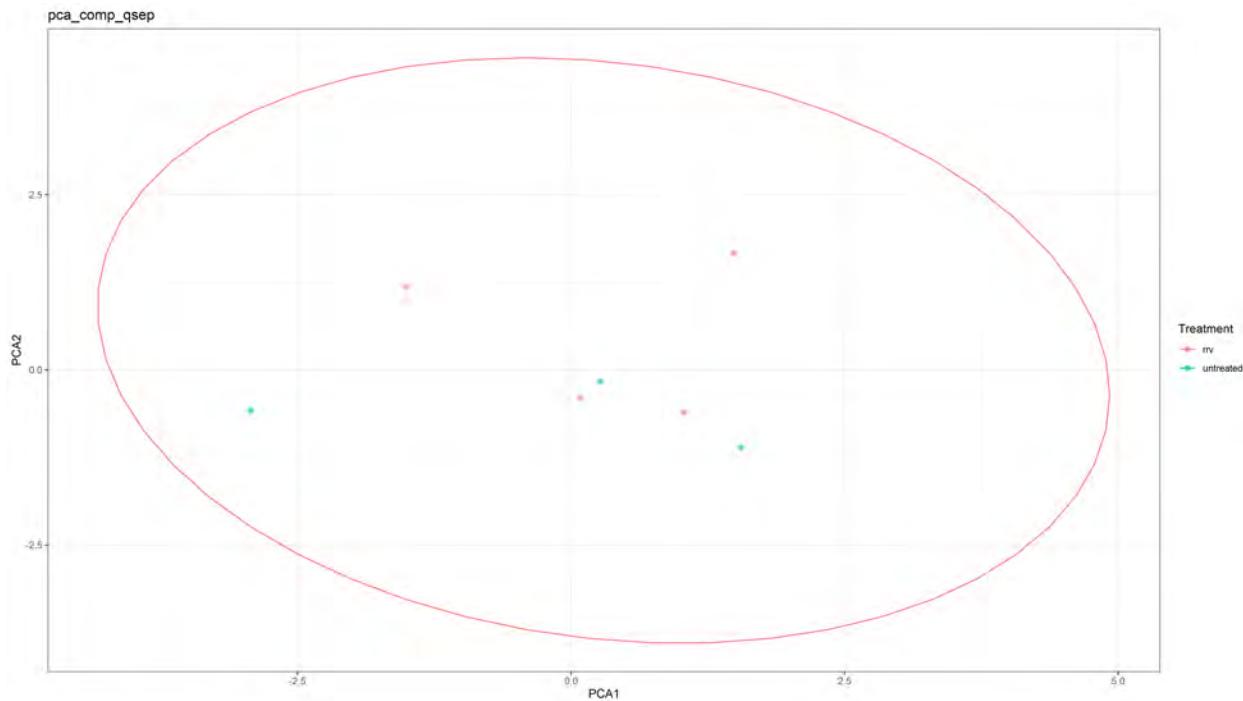


Figure 3-4. Comparison of VCT Principal Components. Ellipses represent 95% confidence intervals.

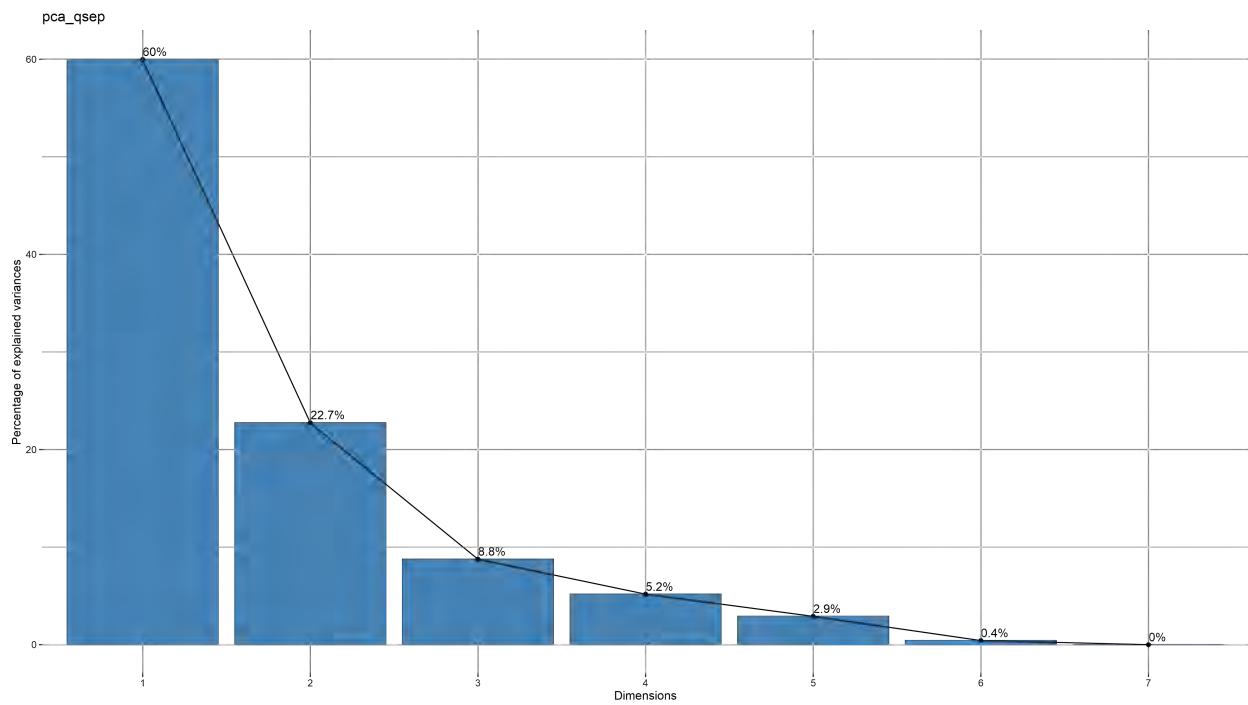


Figure 3-5. Scree plot of VCT Principal Components

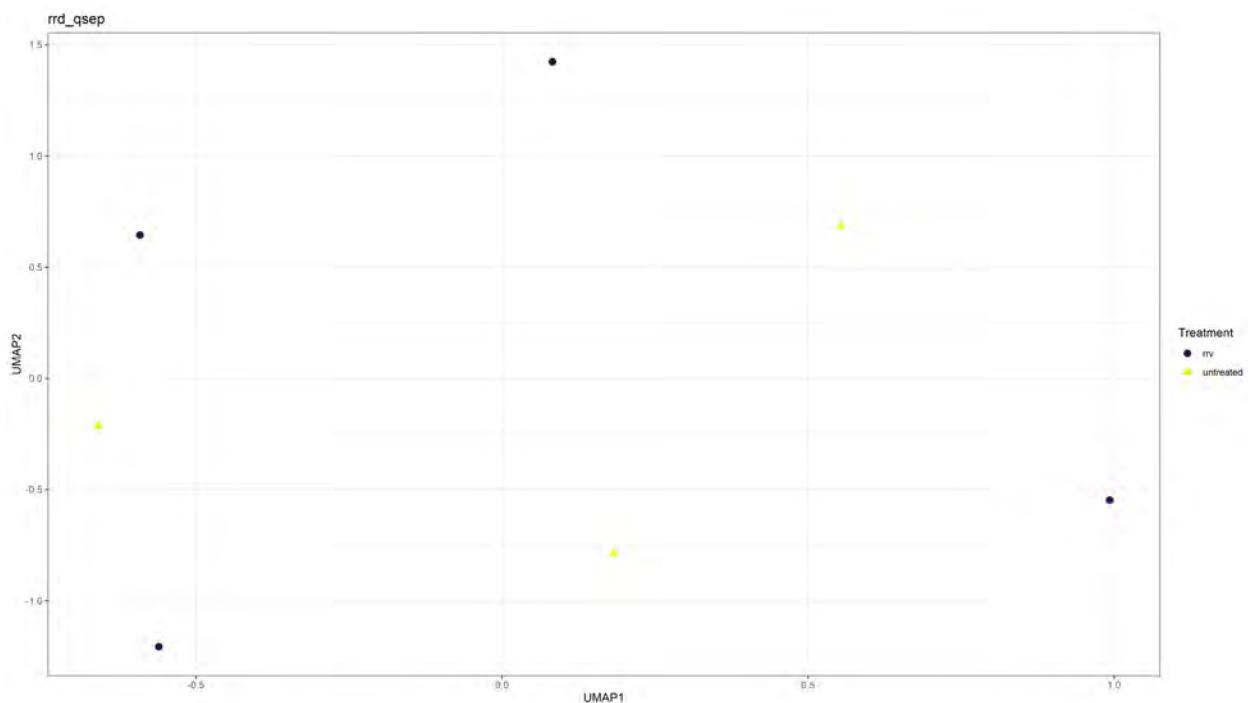


Figure 3-6. Uniform Manifold Approximation and Projection (UMAP) of VCT samples.

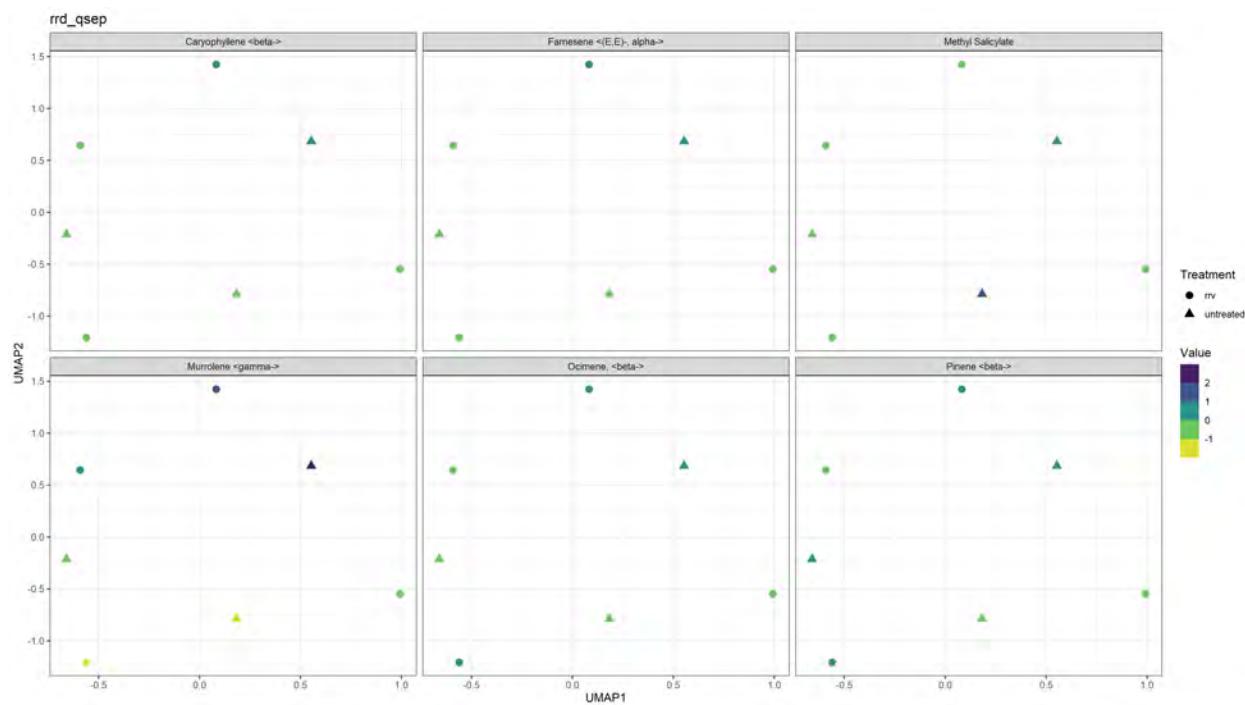


Figure 3-7. Uniform Manifold Approximation and Projection (UMAP) of VCT method's six largest contributions to volatile compositions.

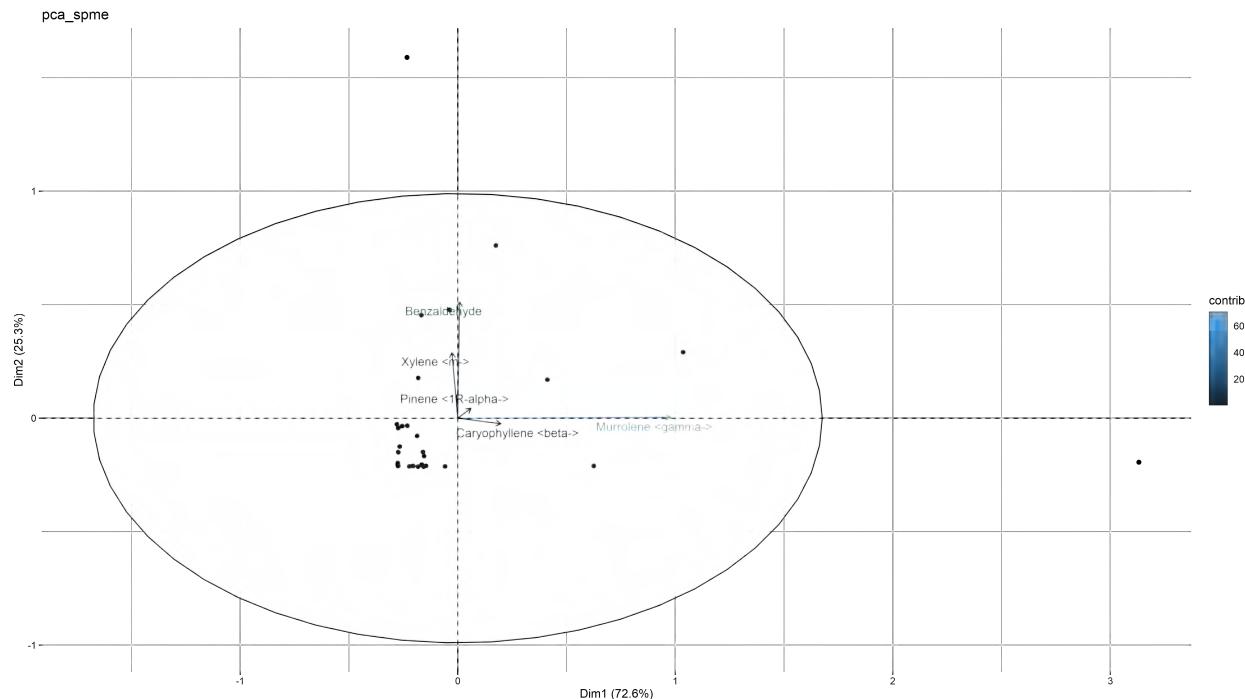


Figure 3-8. PCA biplot of volatiles collected with SPME method.

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

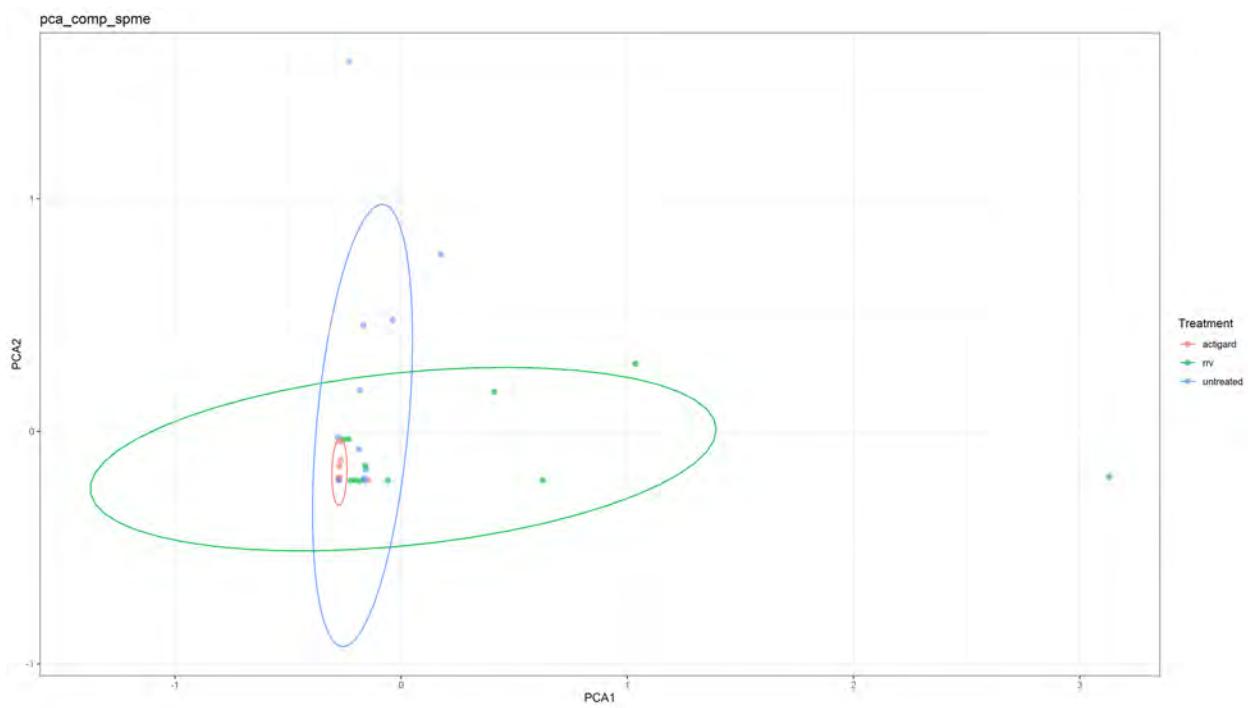


Figure 3-9. Comparison of SPME Principal Components. Ellipses represent 95% confidence intervals.

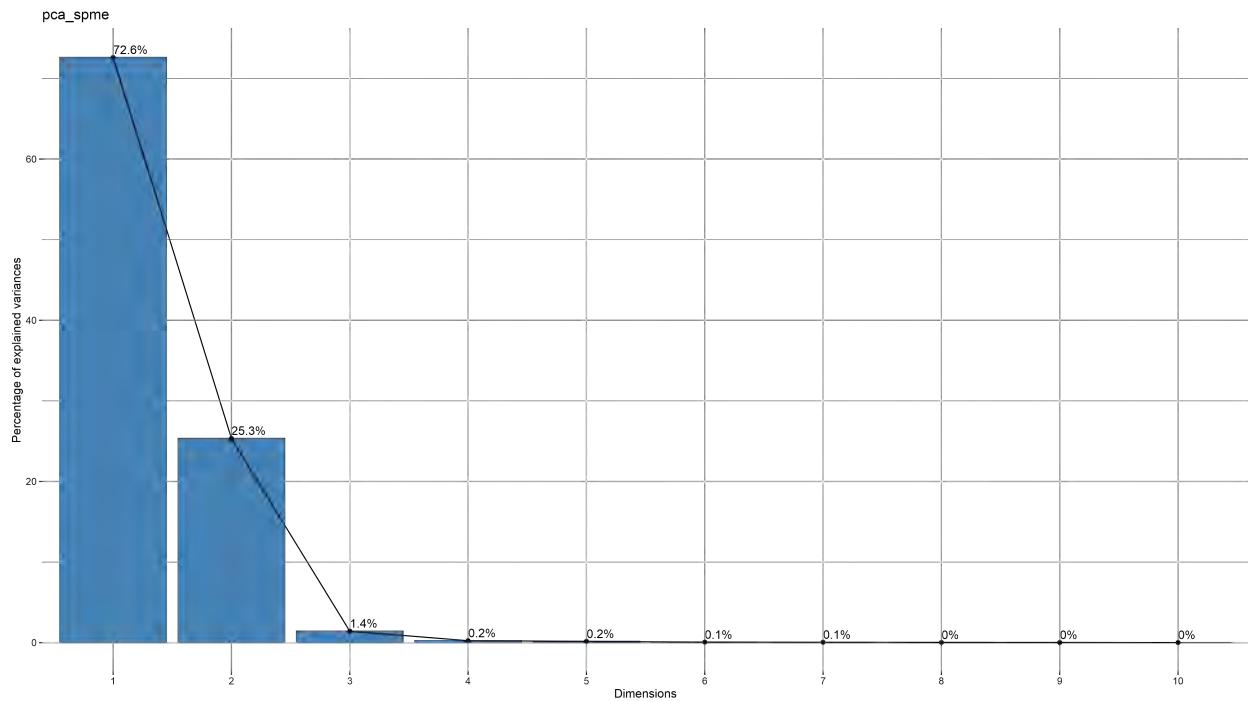


Figure 3-10. Scree plot of SPME Principal Components

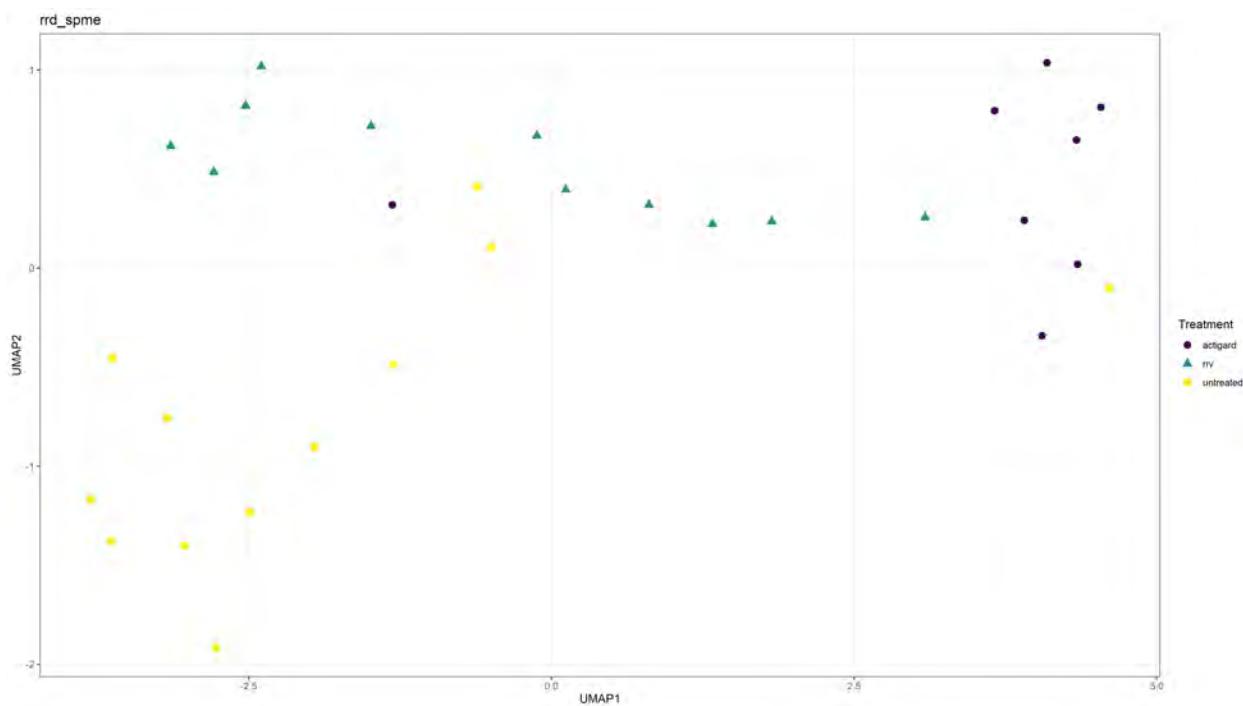


Figure 3-11. Uniform Manifold Approximation and Projection (UMAP) of SPME method volatiles.

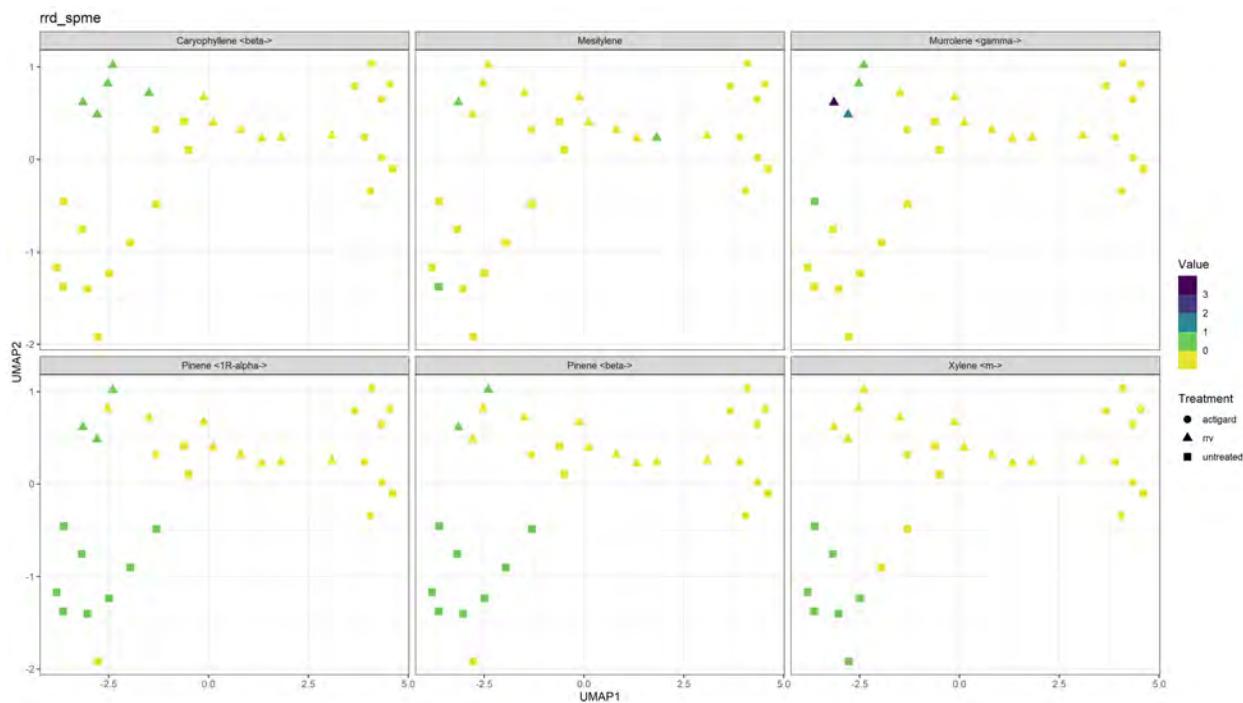


Figure 3-12. Uniform Manifold Approximation and Projection (UMAP) of SPME method's six largest contributions to volatile compositions.

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

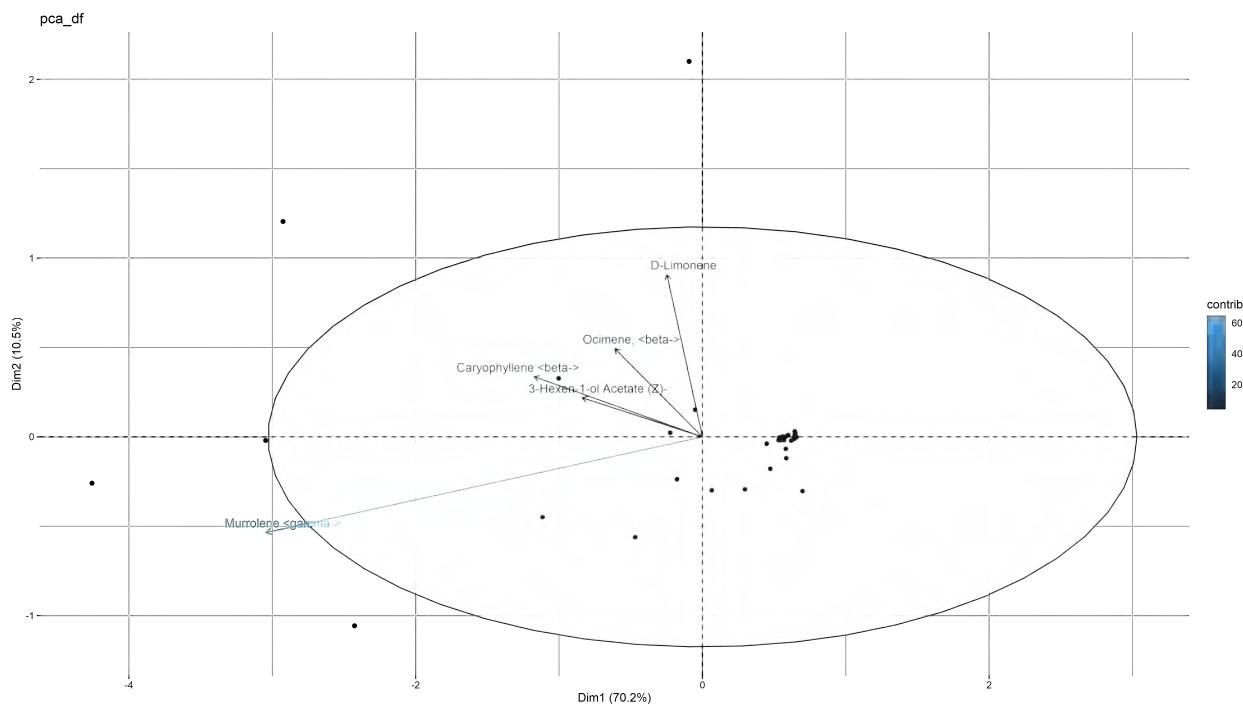


Figure 3-13. PCA biplot of volatiles collected with VCT+SPME methods.

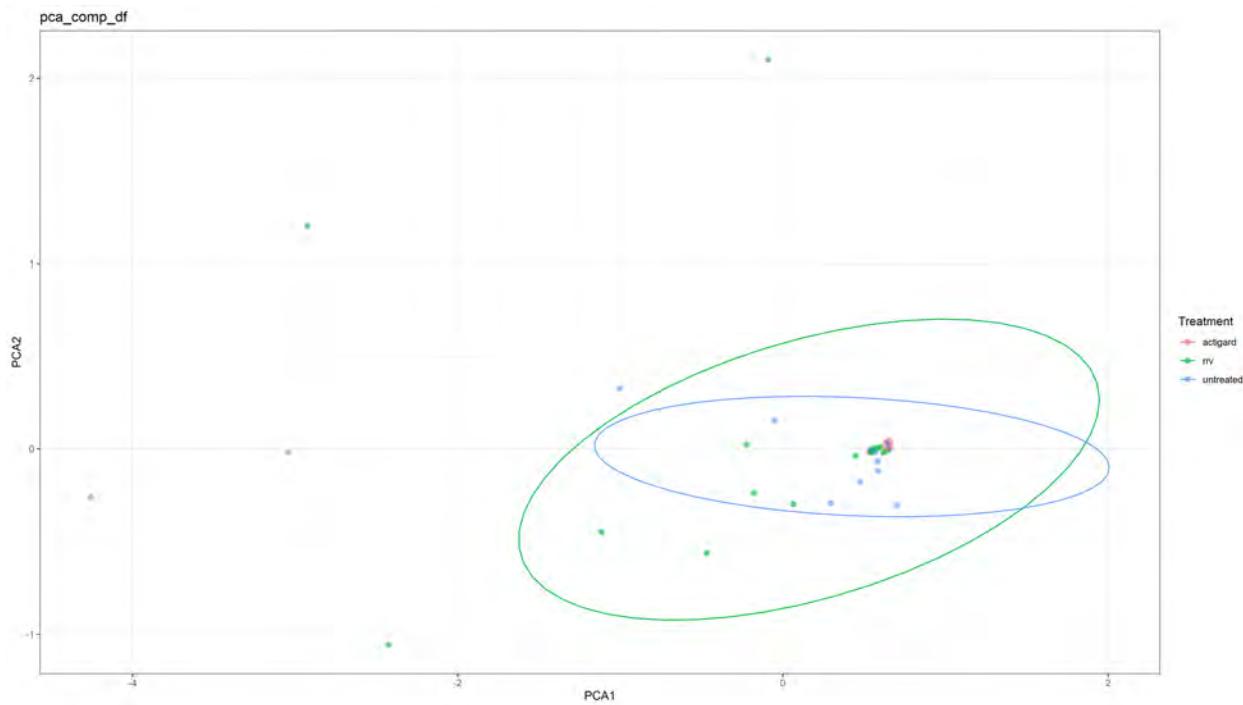


Figure 3-14. Comparison of VCT+SPME Principal Components. Ellipses represent 95% confidence intervals.

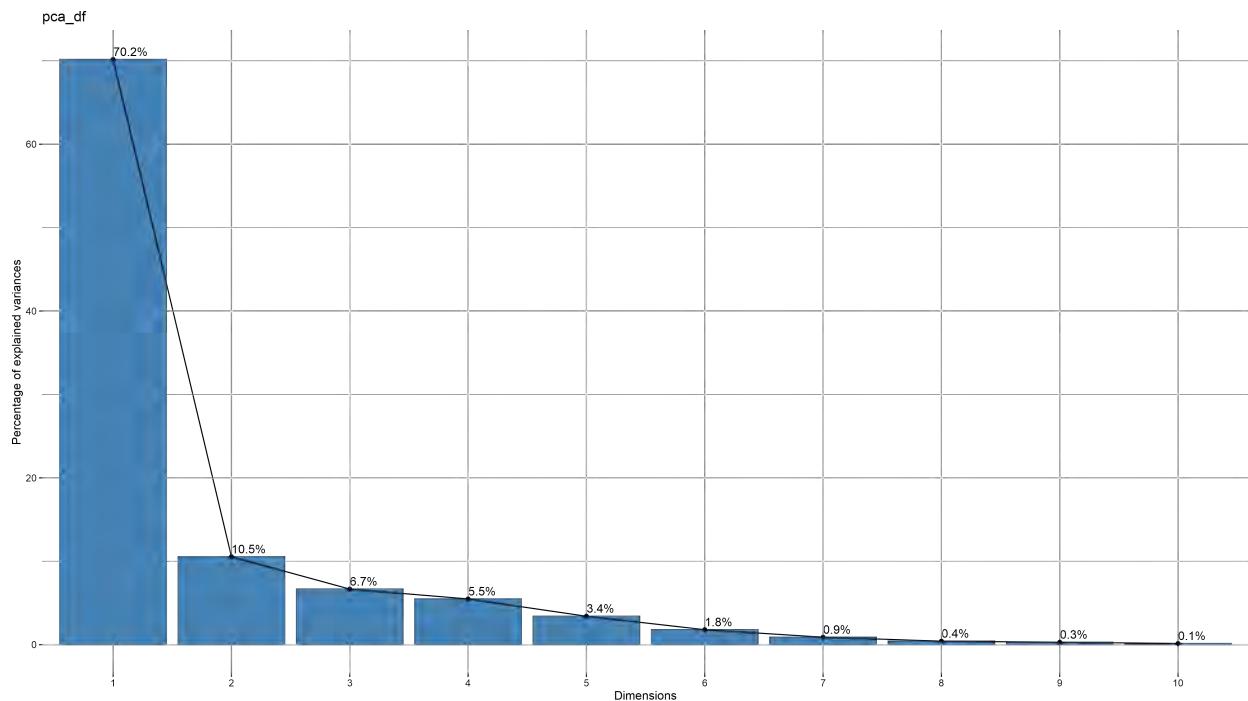


Figure 3-15. Scree plot of VCT+SPME Principal Components.

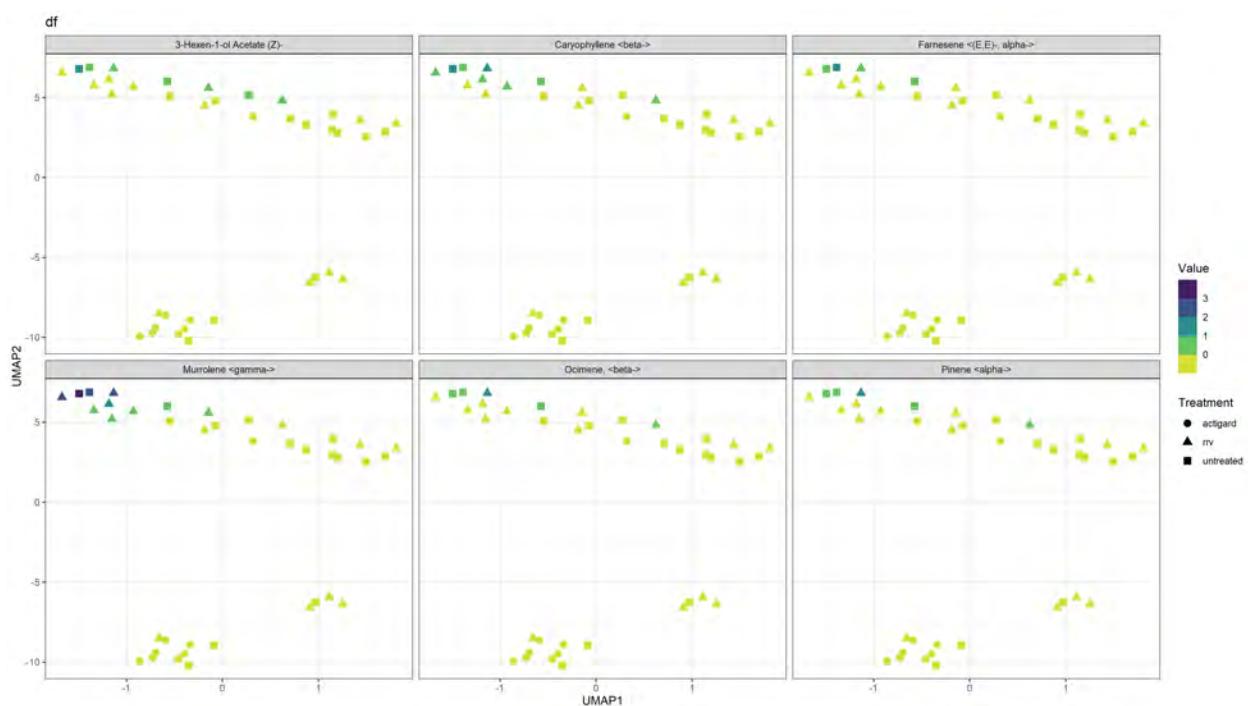


Figure 3-16. Uniform Manifold Approximation and Projection (UMAP) of all VCT+SPME volatiles.

Table 3-5. Contribution table for PCA of headspace VOCs collected with VCT+SPME methods from Pink Double Knock Out® roses.

Chemical	PCA1	PCA2	PCA3
Murrolene <gamma->	72.0312150	14.8458386	2.2559910
Caryophyllene <beta->	10.7352857	5.8896031	0.8573628
3-Hexen-1-ol Acetate (Z)-	5.4873502	2.4907075	15.1134127
Ocimene, <beta->	2.9063582	12.6409633	0.4596114
Pinene <alpha->	2.2118248	10.8381397	0.3605728
Farnesene <(E,E)-, alpha->	2.0526949	0.8704001	0.2158671
Pinene <beta->	1.3622616	3.8858693	0.3120422
Methyl Salicylate	1.0705440	0.1803856	44.7435245
D-Limonene	0.4783855	42.7586741	6.7797188
Germacrene D	0.3878739	0.0496878	0.0054492
Nona-1,3,7-triene <4-8-dimethyl-, (E)->	0.2421617	0.0465500	0.1880732
3-Hexen-1-ol, (Z)-	0.2309602	0.0253246	0.8083669
Benzaldehyde	0.2290871	2.8464099	21.0212998
Copaene <beta->	0.0890619	0.0051737	0.0022856
Xylene <m->	0.0773006	0.4316211	5.6984868
Cadinene <delta->	0.0722424	0.0269341	0.0053198
Calamenene	0.0527682	0.5593544	0.1657161
Humulene	0.0452563	0.0316314	0.0031415
Aromadendrene	0.0383456	0.0126573	0.0399404
Ocimene, <trans-beta->	0.0312297	0.0108197	0.0054715
2-Hexenal	0.0304577	0.0046560	0.0224511
Caryophyllene <9-epi-(E)->	0.0298231	0.0631881	0.0059091
2,4,6-Octatriene, 2,6-dimethyl-, (E,Z)-	0.0233474	0.0270255	0.0009908
unknown	0.0232887	0.0000441	0.0406786
Camphor	0.0107637	0.0000204	0.0188011
Nerolidol	0.0082779	0.0004926	0.0092053
Farnesene <(E)-, beta->	0.0079276	0.0000150	0.0138472
5-Hepten-2-one, <6-methyl->	0.0076194	0.4623979	0.0993761
Trivertal	0.0060998	0.3219597	0.0388516
Copaene <alpha->	0.0038213	0.0005826	0.0031106
Mesitylene	0.0036797	0.0431734	0.0303253
Elemene <beta->	0.0028054	0.0000053	0.0049003
Benzaldehyde <para-ethyl->	0.0021270	0.0000040	0.0037366
Myrcene <beta->	0.0017155	0.1178053	0.0151776
2-Thujene	0.0016199	0.0705489	0.0080563
Murrolene <alpha->	0.0013953	0.0209536	0.0153921
2-Hexen-1-ol, acetate, (E)-	0.0009637	0.0778123	0.0032888
Pinene <1R-alpha->	0.0005408	0.3041847	0.5552174
Decanal	0.0004416	0.0358075	0.0548420
Sulindac sulfide	0.0003181	0.0001405	0.0004729

Table 3-6. Correlation table for PCA of headspace VOCs collected with VCT+SPME methods from Pink Double Knock Out® roses.

Chemical	PCA1	PCA2	PCA3	
Benzaldehyde	0.0569214	-0.0777897	-0.1680448	
Xylene <m->	0.0330649	-0.0302918	-0.0874933	
Sulindac sulfide	0.0021211	-0.0005466	-0.0007970	
p-Cymene	0.0016826	-0.0003702	-0.0005673	
Squalene	0.0016661	-0.0003497	0.0000317	
Nonanal	0.0013973	0.0000050	0.0002608	
Pentadecane	0.0003956	-0.0001613	-0.0002113	
Dodecane	0.0001816	-0.0000086	0.0000845	
Hexadecane, 1-bromo-	0.0000403	0.0000000	0.0000200	
Bergamotene <alpha-, cis->	0.0000165	-0.0000132	-0.0000235	
Terpineol <alpha->	0.0000036	0.0000000	0.0000002	
Limonene oxide, trans-	0.0000022	-0.0000041	-0.0000010	
Undecane	0.0000016	-0.0000065	-0.0000002	
Hexanal <n->	0.0000000	0.0000000	0.0000000	
Naphthalene, 1,6-dimethyl-4-(1-methylethyl)-)	0.0000000	0.0000000	0.0000000	
Toulene	0.0000000	0.0000000	0.0000000	
Phellandrene <beta->	-0.0000091	-0.0001475	-0.0001614	
Zonarene	-0.0000499	-0.0001263	-0.0000693	
Caryophyllene oxide	-0.0000610	-0.0002356	-0.0000683	
Cubebene <beta->	-0.0001444	-0.0003589	-0.0001396	
Acetic acid, hexyl ester	-0.0001900	-0.0000032	0.0000774	
Cadina-1,4-diene <trans->	-0.0004390	-0.0000074	0.0001788	
Bourbonene <beta->	-0.0005735	-0.0017143	-0.0012675	
Carene <delta-3>	-0.0007037	-0.0013095	-0.0005611	
Pentane, 3-ethyl-2,2-dimethyl-	-0.0013798	0.0001407	0.0039958	
Decanal	-0.0024992	-0.0087249	-0.0085833	
Pinene <1R-alpha->	-0.0027658	-0.0254298	-0.0273103	
2-Hexen-1-ol, acetate, (E)-	-0.0036919	0.0128617	-0.0021019	
Murrolene <alpha->	-0.0044423	-0.0066743	-0.0045472	
2-Thujene	-0.0047865	0.0122467	-0.0032897	
Myrcene <beta->	-0.0049257	0.0158254	-0.0045154	
Benzaldehyde <para-ethyl->	-0.0054848	-0.0000920	0.0022404	
Elemene <beta->	-0.0062990	-0.0001063	0.0025657	
Mesitylene	-0.0072141	-0.0095804	-0.0063826	
Copaene <alpha->	-0.0073516	-0.0011129	0.0020442	
Trivertal	-0.0092882	0.0261622	-0.0072244	
5-Hepten-2-one, <6-methyl->	-0.0103809	0.0313532	-0.0115541	
Farnesene <(E)-, beta->	84	-0.0105888	-0.0001787	0.0043130
Nerolidol	-0.0108202	-0.0010233	0.0035165	
Camphor	-0.0123383	-0.0002083	0.0050256	

vs. control and Filtered Air vs. Healthy Roses were not significantly different, but *A. swirskii* mites were significantly more attracted to RRV-infected roses.

3.4 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 ([3-17](#)). 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 prey items (Janssen et al. 1990), so it is possible that infected roses release volatiles that *A. swirskii* would associate with prey. 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 ([3-9](#), [3-16](#)). Another possibility is that the time of sampling affected the outcome. ASM-treated plants were sampled at the end of a 12-week application period, by which it may no longer be producing MeSA at high levels. 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

A. swirskii attraction to RRV-infected roses



Figure 3-17. *Amblyseius swirskii* attraction to healthy and Rose Rosette Virus-infected Pink Double Knock Out® roses. Asterisks represent significant differences as calculated by χ^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: $\chi^2 = 0.47$, $df = 1$, $\alpha = 0.05$, $p - value = 0.4913$.

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 defense related terpenes ([citation?](#)). γ -Murrolene and β -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. In conclusion, we have found preliminary data about

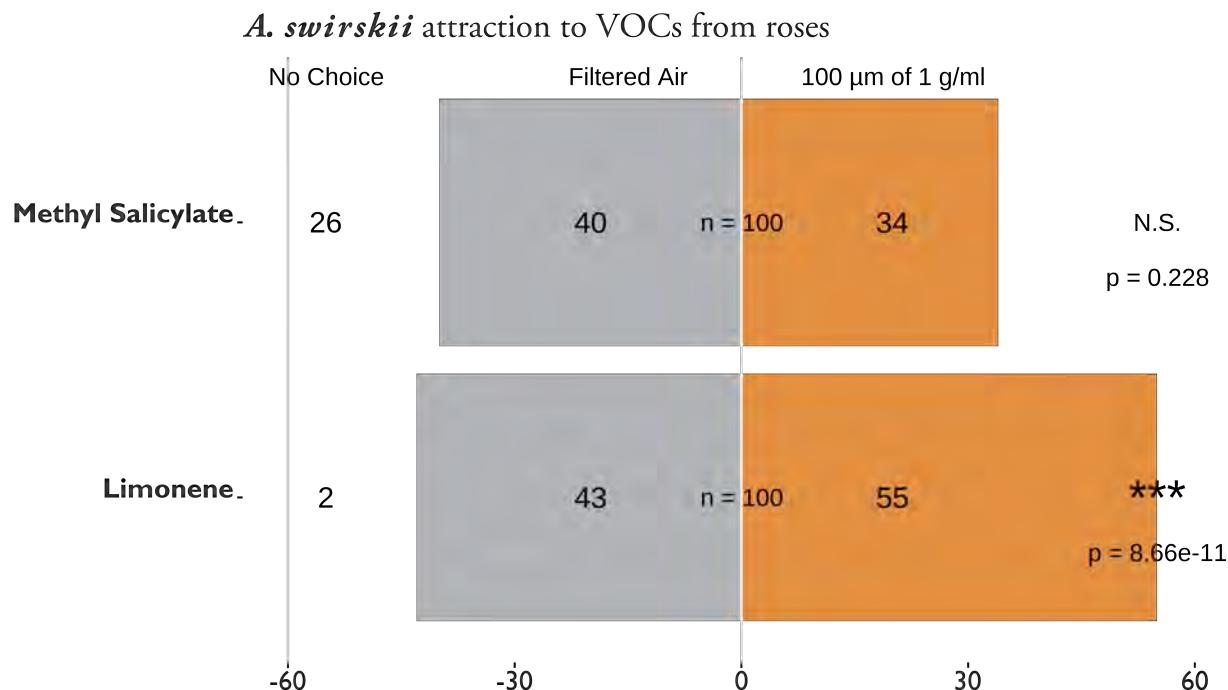


Figure 3-18. *Amblyseius swirskii* attraction to Methyl Salicylate (MeSA) and D-L Limonene vs Filtered Air at concentrations of 1 g/ μL . 100 μL s of chemical was applied to 3 cm of dental wick inside of erlenmeyer flasks inline with the filtered air from the olfactometer. Asterisks represent significant differences as calculated by χ^2 contingency table tests for given probabilities. N.S. = not significant. MeSA vs Air: $\chi^2 = 0.48649$, $df = 1$, $\alpha = 0.05$, $p - \text{value} = 0.4855$. D-L Limonene vs Air: $\chi^2 = 0.94737$, $df = 1$, $\alpha = 0.05$, $p - \text{value} = 0.3304$.

volatile changes in infected roses and SAR-induced roses, and their putative relationships with *A. swirskii*.

CHAPTER 4

INTEGRATED PEST MANAGEMENT OF *PHYLLOCOPTES FRUCTIPHILUS*

4.1 Introduction: *Phyllocoptes fructiphilus* - an increasingly large problem

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 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 *P. 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). A major obstacle to controlling RRD is the small size and habits of *P. fructiphilus*. Eriophyoid mites are hard to control via conventional methods (i.e. pesticides) due to their small size and their cryptic habits: *P. fructiphilus* hide in tight spaces such as under rose sepals and petioles, and under glandular plant hairs (trichomes) (Jesse et al. 2006, Otero-Colina et al. 2018, Bauchan et al. 2019). This means that management which relies on contact with the pest

is unlikely to reach these mites under normal circumstances. It is possible that predatory mites can circumvent this problem and combat *P. fructiphilus* *in situ*. Predatory mites in the family Phytoseiidae can manage a variety of agricultural pests (Gerson et al. 2003, Carrillo et al. 2015), and some species are small enough that they may be able to find and feed on *P. fructiphilus* in their hiding places (Carillo, personal communication). As part of the efforts to develop novel mite management methods, 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. Attraction to these odorants is usually suggestive of predatory mite feeding preferences, and is a fast way to judge their potential for use in biological control (Janssen et al. 1990). 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 RRD (see [3.3.2](#)). This was noteworthy because *A. swirskii* are generalist predators which feed on other common agricultural pests such as whiteflies (Bolckmans et al. 2005), spider mites (McMurtry et al. 1970), and thrips (Wimmer et al. 2008). *A. swirskii* can also persist on pollen (Loughner et al. 2011, Delisle et al. 2015) and other arthropods even when the pest of concern is absent (Janssen and Sabelis 2015). This allows *A. swirskii* to be released as a preventative measure instead of reacting to an outbreak (Kutuk and Yigit 2011). Furthermore, phytoseiid mites integrate well into pest management programs and are compatible with certain pesticides (Trumble and Morse 1993, Nicetic et al. 2001, Fernández et al. 2017) and other bio-control agents (Midthassel et al. 2016). Although *A. swirksii* mites are likely too large to infiltrate into the tight spaces needed to feed on *P. fructiphilus*, they remain a good model organism for testing combinations of different pest

management treatments. These results compelled 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) ([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 RRD influences the prey-seeking behaviors of *A. swirksi*, which has implications for how effective predatory mite biocontrol will 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 Salicylic Acid 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). ASM application also has shown chitinase activity in roses (Suo and Leung 2001). Mites have an exoskeleton comprised of chitin (Nuzzaci and Alberti 1996), and some studies have shown that the hypersensitive response and SAR interfere with the ability of eriophyoid mites to feed or grow on induced plants (Bronner et al. 1991a, 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). We conducted a number of field studies from 2018-2021 in order to test the integration of predatory mites with SAR.

4.2 Materials & Methods

4.2.1 SAR-induction with ASM to reduce populations of *P. fructiphilus*

We tested the efficacy of SAR-induction against mites in field trials conducted at Griffin and Athens, GA, sites with low and high *P. fructiphilus* pest pressure, respectively.

Roses This will be a 12-week experiment conducted from August to October simultaneously in Griffin, GA and Athens, GA. Each site will be given 48 Pink Double Knock Out® Roses (Star Roses and Plants, West Grove, PA, USA) which will be planted in 1 gallon buckets filled with potting soil and mixed with granular slow-release fertilizer. Plants will be placed on black plastic mulch and be watered weekly with overhead impact sprinklers.

Mites *Phyllocoptes fructiphilus* are present in the landscape of Georgia. Tissue from RRD-infected roses will be placed onto roses during the first week and 5th week

Spray rates We will be applying the Acibenzolar-S-methyl (ASM) based Actigard50WG® (Syngenta AG, Basel, Switzerland), at two different rates: 50 mg/L (Half

rate) and 100 mg/L (High rate) to observe the effects of inducing Systemic Acquired Resistance (SAR) on *Phyllocoptes fructiphilus* Kiefer. We will have two field sites in Georgia with local populations of *P. fructiphilus*: Griffin GA and Athens, GA. The tests in Griffin will have two controls for chemical applications in this experiment: The first will be the miticide Kontos (Bayer CropScience LP, Cary, NC, USA), at the label rate as a positive control and the second will be water as a negative control. The tests in Athens will have a control of untreated roses as well as water as a negative control.

Data Collection Rose/rosebud cuttings ~10 cm will be taken from each plant before the first treatment to determine the initial populations of *P. fructiphilus* on the roses. We will then take a subset of samples from each rose treatment weekly, rotating samples until each rose plant has been sampled three times. We will also collect samples from all roses at the end of the trial. This experiment will be repeated for two seasons. Rose samples will be placed in 50 mL centrifuge tubes and refrigerated or frozen until floral samples can be processed. Samples will be processed using the washing methods of Monfreda et al. (2007), eriophyoid mites will be counted and identified as previously described.

Plot Design - 2018 Plot Design - 2019

4.2.2 Integrating Pest Management Methods to control *Phyllocoptes fructiphilus*

We tested ASM alongside a different a SAR-inducer (SP2700, Trade name ‘Ninja,’ SePro) and combined one of the SAR-inducers with predatory mites to observe any synergistic effects. We used an acaricide as a negative control and water as a positive control. (see [4-7](#)).

Spray applications done weekly for 12 weeks, on the same day each week, weather permitting. The site had divided into two blocks, with ten plots of roses in each block. Each plot was 3 m² with approximately 12 roses, the six roses at the center of the plot were treated, while the adjacent roses on either side was left as a buffer between plots to avoid treatment drift.

Infected Rose Patch

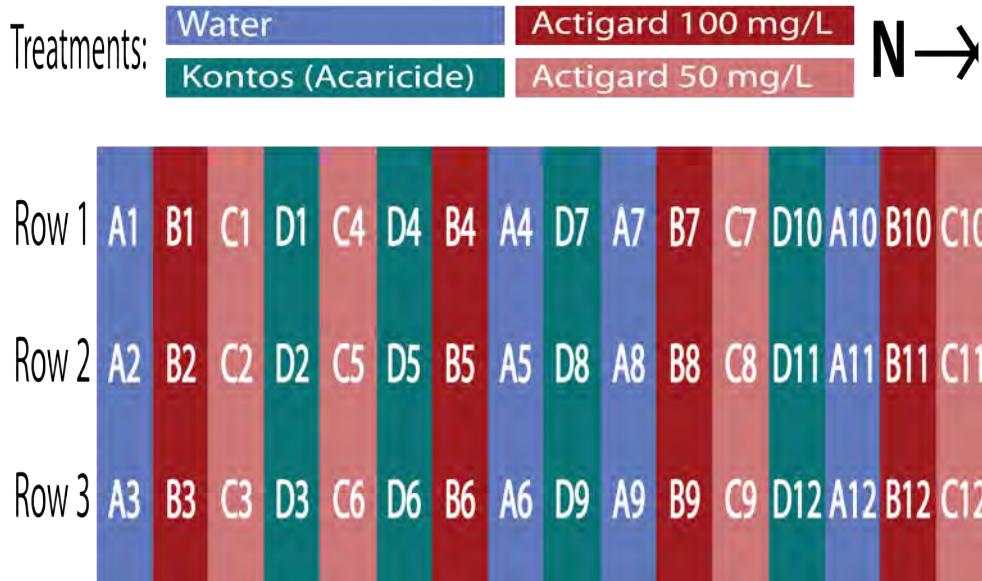


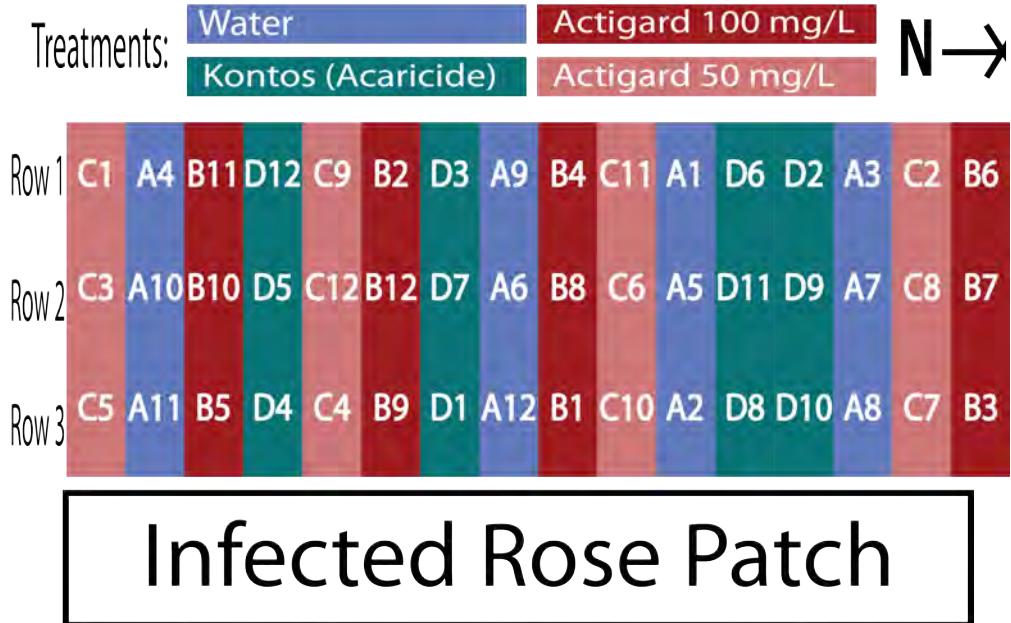
Figure 4-1. Field design for testing the potential of Acibenzolar-S-Methyl to reduce populations of *P. fructiphilus* by inducing Systemic Acquired Resistance in Pink Double Knock Out® roses. Trials were conducted for three months from September to December 2019 in Griffin, GA. Four treatments were applied 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.

Five treatments were applied: tap water as a positive control, Kontos (Spirotetramat) Bayer - High rate as a negative control, Actigard 50 WG (acibenzolar-S-methyl) Syngenta - 100 mg/L, *Amblyseius swirskii* mini sachets with hooks (Ambly-S, Arbico Organics, Oro Valley, AZ, USA) - two sachets per rose in treated plot, and a combined treatment of Actigard - 100 mg/L + *A. swirskii* - two sachets per rose in treated plot.

Samples were collected and processed using the same methods as previously described in 2.3.1.

- 95% Ethanol
- 500 mL Nalgene Bottles (labeled with date, plot # and treatment)
- Pruners/Secateurs

Infected Rose Patch



Infected Rose Patch

Figure 4-2. Field design for testing the potential of Acibenzolar-S-Methyl to reduce populations of *P. fructiphilus* by inducing Systemic Acquired Resistance 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.

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. These sachets contain live colonies of *A. swirskii* and a mite which they consume for food. There is a small hole at the bottom of the sachet where the mites are able to leave and climb on the roses searching for food, accordingly, the sachet was hung from rose canes in the center of each rose. The *A. swirskii* plots were also treated with water weekly in order to keep conditions similar to other treatments.

Flower cuttings were taken weekly from each of the six roses in the center of each plot.

Three flowers (buds if no flowers present) from each of the six flagged roses, for a total of 18 flowers/buds per bottle for each plots. **Make sure to collect 18 flowers/buds from any rose in the untreated plots 11-14 for the phenology study as well.**

Place the flowers/buds into the ethanol-filled bottles provided, make sure the lid is tight, then shake the bottle vigorously for a few seconds to coat the rose tissue with ethanol and help dislodge any mites.

- Use the sieves to separate mites from the plant tissues. (see *name of protocol sheet* for more information).
- Dry plant tissues in the appropriately labeled, high-tech *paper bag* and put into the oven until dry (~48 hrs at 50 °C), then weigh the rose tissue and record the dry weight (remember to tare the scale with a paper bag for slightly improved accuracy).

We propose testing two different SAR-inducers as well as predatory mites for their ability to reduce populations of *P. fructiphilus*. We also intend to combine the effects of predatory mites with a SAR-inducer to determine if these treatments are compatible. All testing will be done in areas with high pest pressure in Georgia. Our hypothesis is that there will be fewer *P. fructiphilus* on plants treated with the SAR-inducers when compared to the water treated control group, and even fewer mites found on plants treated with the combination of a SAR-inducer and predatory mites.

4.2.3 Materials & Methods

Our studies are designed to investigate if predatory phytoseiid mites such as *A. swirskii* can be combined with roses' natural systemic activated resistance (SAR) to manage populations of the plant-parasitic mite, *P. fructiphilus*, the vector of Rose Rosette Virus (RRV). Our findings will be used to develop Integrated Pest Management (IPM) programs for *P. fructiphilus* management.

Roses This will be a 12-week experiment conducted from August to October simultaneously in Griffin, GA and Athens, GA. The Athens site will be given 96 Pink Double Knock Out® Roses (Star Roses and Plants, West Grove, PA, USA), while Griffin will use

54 roses due to the smaller plot area available. Bare root roses will be planted 2 months before the trials begin to allow new flush to form. Rose planting media and environmental conditions will be the same as previously described.

Mite Infestation *Phyllocoptes fructiphilus* are present in the landscape of Georgia. Rose cuttings ~10 cm will be taken from roses showing symptoms of Rose Rosette Disease in the landscape and placed in each rose pot on the 1st, 5th and 9th week of the experiment.

Predatory mites

Amblyseius swirskii mites will be applied on the 1st, 5th and 9th week of the experiment. These mites are deployed from polyethylene fiber sachets containing live colonies of *A. swirskii* and a mite which they consume for food. There is a small hole at the bottom of these sachets which allows the mites to be slowly released into the environment.

Field Treatments

1. Water - Control
2. Actigard - 100 mg/L
3. Ninja - label rate
4. Kontos - label rate
5. *A. swirskii* (one sachet per rose treated)
6. *A. swirskii* + Ninja (one sachet per rose treated, label rate)

Data Collection

Georgia collaborators will be collecting flower samples 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, Georgia collaborators will collect flower samples starting from the top rows of each block every week, until each row has been sampled three times (see [4-3](#) and [4-4](#)). Georgia collaborators rate disease severity for each rose every week before they spray, rating roses according to the Horsfall-Barratt Scale (Horsfall 1945). Roses

displaying symptoms of RRD will have tissues sent to the Plant Disease Diagnostic Clinic at the North Florida Research and Extension Center(PDC) for virus confirmation.

Sample Processing

- A flower cutting of about ~12 cm will be taken and placed the flower petal side down into 50 ml centrifuge tubes filled with 15 ml of 95% ethanol so the entire flower is submerged over the sepals. Once the lid is secure, the tube will be shaken vigorously for a few seconds to help dislodge any mites. Samples will be processed using the washing methods of Monfreda et al. (2007), eriophyoid mites will be counted and identified as previously described.

Plot Design - Athens

The site at Athens, GA has space for five blocks: A, B, C, D and E. Each block is a 3 × 6 plot with 18 plants, with three plants in each treatment. The experiments will be run for 12 weeks. We will be sampling flower cuttings from two rows each week, starting with the top rows (1-15 and 16-30 for week one) of each block and rotating to the next row each week (31-45 and 46-60 on week 2) continuing until all rows have been sampled three times. In order to avoid confusion, each rose pot will be labeled with a stake that has the plant number and treatment abbreviation: (W, A, K, M, N, +) written on it. Applications will be done on the same day each week, weather permitting, preferably at the beginning of the week. **Plot Design - Griffin**

The site at Griffin, GA has space for three blocks: X, Y, and Z. Each block is a 3 × 6 plot with 18 plants, with three plants in each treatment. This experiment was run for 12 weeks as well. We will be sampling flower cuttings from two rows each week, starting with the top rows (1-9 and 10-18 for week one) of each block and rotating to the next row each week (19-27 and 28-36 on week 2) continuing until all rows have been sampled three times. Labels and applications were conducted in the same manner as previously described.

Water			Actigard			Kontos		
Mites			Ninja			Ninja + Mites		
Week 1	1	2	3	4	5	6	7	8
	16	17	18	19	20	21	22	23
Week 2	31	32	33	34	35	36	37	38
	46	47	48	49	50	51	52	53
	61	62	63	64	65	66	67	68
	76	77	78	79	80	81	82	83
	10	11	12	13	14	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 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.

4.3 Results

Combining predatory mites with a SAR-inducer was as effective as the miticide alone, and controlled herbivorous mite populations more than either SAR-induction or predatory mites alone (see 4-7).

4.4 Discussion

Our results suggest that SAR-induced plant defenses have the potential to manage populations of *P. fructiphilus* and other herbivorous mites, especially when integrating SAR-induction with predatory mites.

Water	Actigard			Kontos		
Mites	Ninja			Ninja + Mites		
Week 1	1	2	3	4	5	6
	10	11	12	13	14	15
Week 2	19	20	21	22	23	24
	28	29	30	31	32	33
	37	38	39	40	41	42
	46	47	48	49	50	51
						N →

Figure 4-4. Field design for Integrated Pest Management 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.

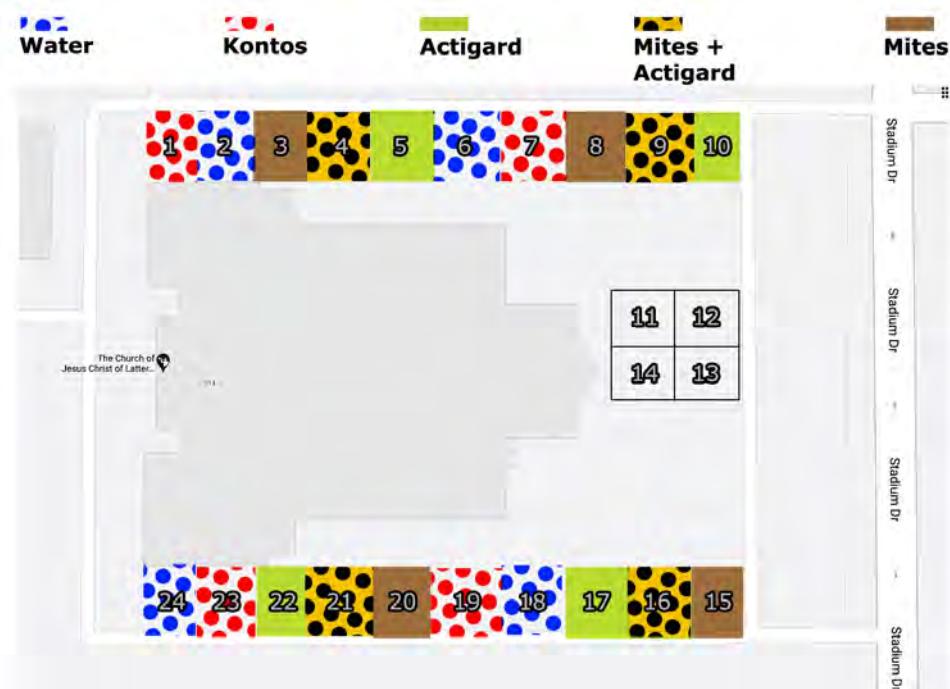


Figure 4-5. Field design for Integrated Pest Management trials on Pink Double Knock Out® roses to control *P. fructiphilus* in Tallahassee, FL with five treatments: Water, Actigard50WG, Kontos, *Amblyseius swirskii* predatory mite sachets, and *A. swirskii* + Actigard combined treatments. All products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

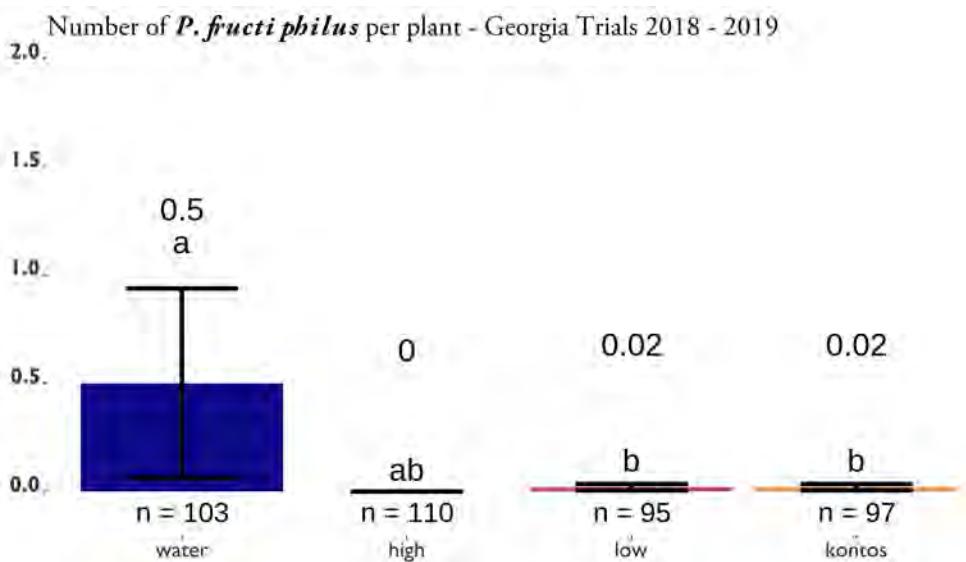


Figure 4-6. SAR-induction trials on Pink Double Knock Out® roses to control *Phyllocoptes fructiphilus* in Athens and Griffin, GA. Statistical significance was determined using Tukey contrasts for multiple Comparisons of means. Groups which share letters are not statistically different from one another. $\alpha = 0.05$.
 water = Water Control, High = 100 mg/L Actigard® 50WG (Syngenta, Greensboro, NC, USA) acibenzolar-S-methyl (ASM), low = 50 mg/L Actigard® 50WG (Syngenta, Greensboro, NC, USA) acibenzolar-S-methyl (ASM), kontos = Kontos® Miticide Insecticide - Spirotetramat (Bayer Corporation, Whippny, New Jersey, USA), untreated = No treatment. All products were applied at their label rates for 12 weeks. Flower cuttings were taken weekly to record the numbers of herbivorous mites.

Number of herbivorous mites per plant - IPM Trials 2019

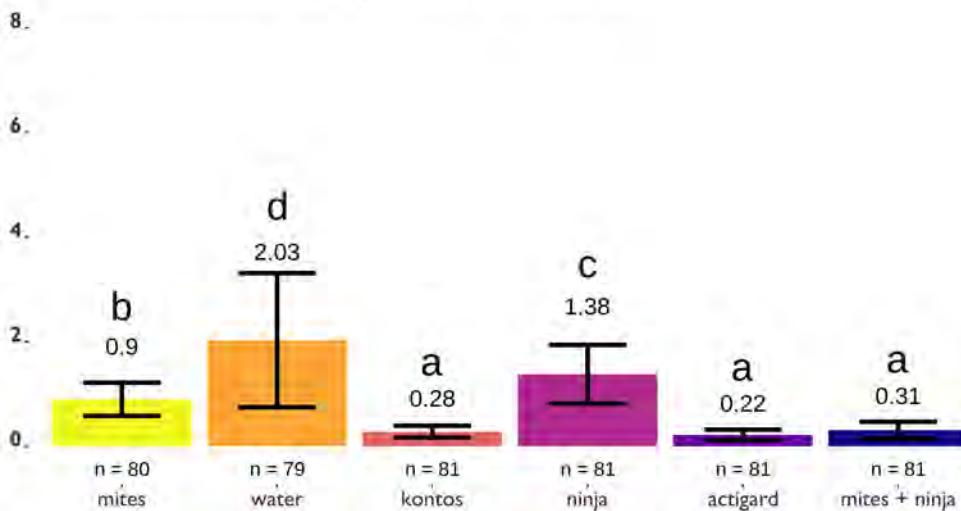


Figure 4-7. Integrated Pest Management trials on Pink Double Knock Out® roses to control *Phyllocoptes fructiphilus* in Athens and Griffin, GA with five treatments. Statistical significance was determined using Tukey contrasts for multiple Comparisons of means. Groups which share letters are not statistically different from one another. $\alpha = 0.05$. water = Water Control, actigard = Actigard® 50WG (Syngenta, Greensboro, NC, USA) acibenzolar-S-methyl (ASM), kontos = 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), ninja = SP2700 (Trade name: Ninja™, SePro, Carmel, IN, USA), mites + ninja = *A. swirskii* + Ninja combined treatments. All products were applied at their label rates for 12 weeks. Flower cuttings were taken weekly to record the numbers of herbivorous mites.

Mean of eriophyoid mites per gram dry weight - Tallahassee IPM Trials 2020-2021

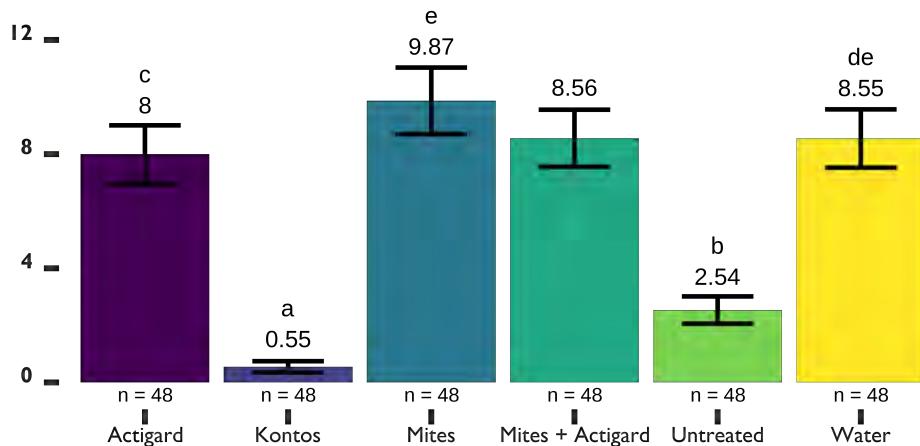


Figure 4-8. Integrated Pest Management trials on Pink Double Knock Out® roses to control *Phyllocoptes fructiphilus* in Tallahassee, FL with five treatments. Statistical significance was determined using Tukey contrasts for multiple Comparisons of means. Groups which share letters are not statistically different from one another. $\alpha = 0.05$. Water = Water Control, Actigard = Actigard® 50WG (Syngenta, Greensboro, NC, USA) acibenzolar-S-methyl (ASM), Kontos = 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), Mites + Actigard = *A. swirskii* + Actigard combined treatments. Untreated = No treatment. 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.

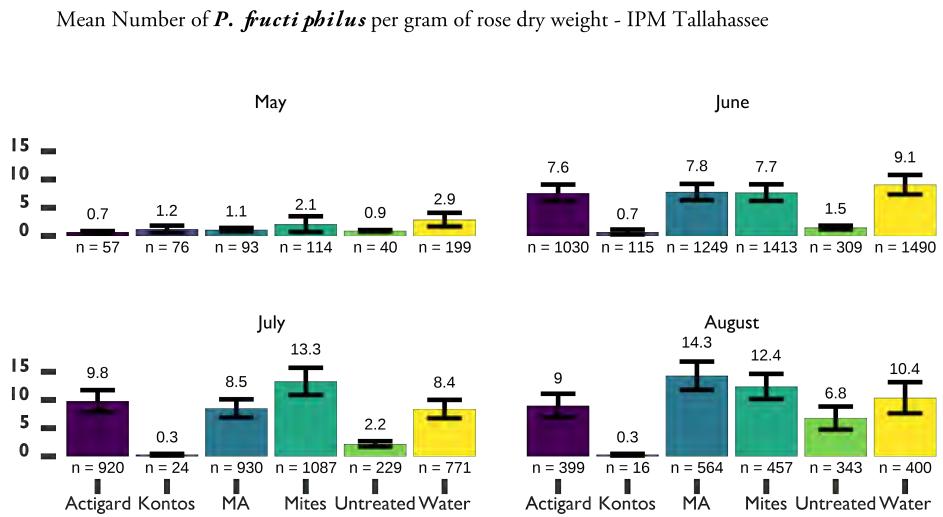


Figure 4-9. Integrated Pest Management trials on Pink Double Knock Out® roses to control *Phyllocoptes fructiphilus* in Tallahassee, FL with five treatments. Water = Water Control, Actigard = Actigard® 50WG (Syngenta, Greensboro, NC, USA) acibenzolar-S-methyl (ASM), Kontos = Kontos® Miticide Insecticide - Spirotetramat (Bayer Corporation, Whipppany, 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. Untreated = No treatment. 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.

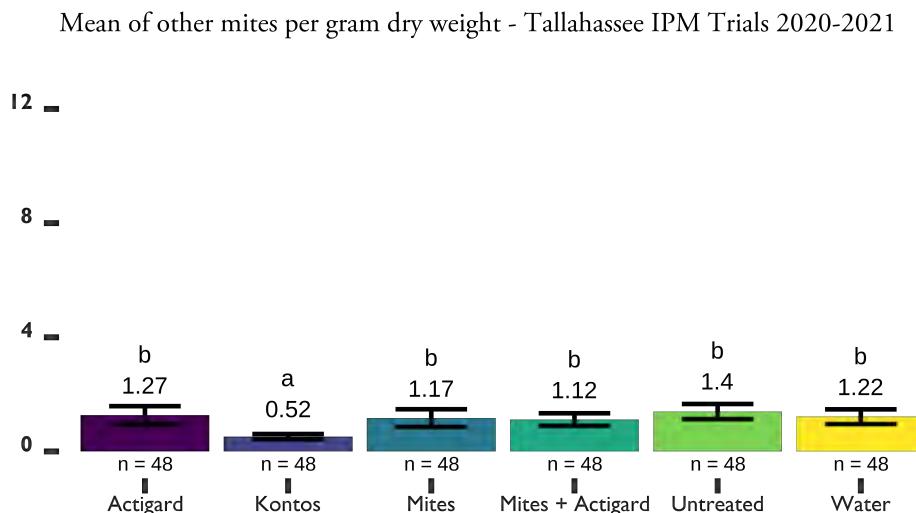


Figure 4-10. Integrated Pest Management trials on Pink Double Knock Out® roses to control *Phyllocoptes fructiphilus* in Tallahassee, FL with five treatments. Statistical significance was determined using Tukey contrasts for multiple Comparisons of means. Groups which share letters are not statistically different from one another. $\alpha = 0.05$. Water = Water Control, Actigard = Actigard® 50WG (Syngenta, Greensboro, NC, USA) acibenzolar-S-methyl (ASM), Kontos = Kontos® Miticide Insecticide - Spirotetramat (Bayer Corporation, Whipppany, New Jersey, USA), Mites = *Amblyseius swirskii* predatory mite mini sachets on hooks (Ambly-S, Arbico Organics, Oro Valley, AZ, USA), Mites + Actigard = *A. swirskii* + Actigard combined treatments. Untreated = No treatment. 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.

CHAPTER 5

BREVIPALPUS-TRANSMITTED ORCHID FLECK VIRUS 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 Lilyturf *Liriope* spp., cv. ‘Gigantea’ in a landscape of Leon County, Florida ([5-1](#)). *Liriope* 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). *Liriope* 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* spp. 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 orchid fleck virus, 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 coinfecte 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 Mites

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



Figure 5-1. Variety of symptoms seen on *Liriope* spp. infected with orchid flea 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*

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. (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



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

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 (Andrade et al. 2010, 2019). 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). In addition, 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, predatory mites (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]. 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 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.

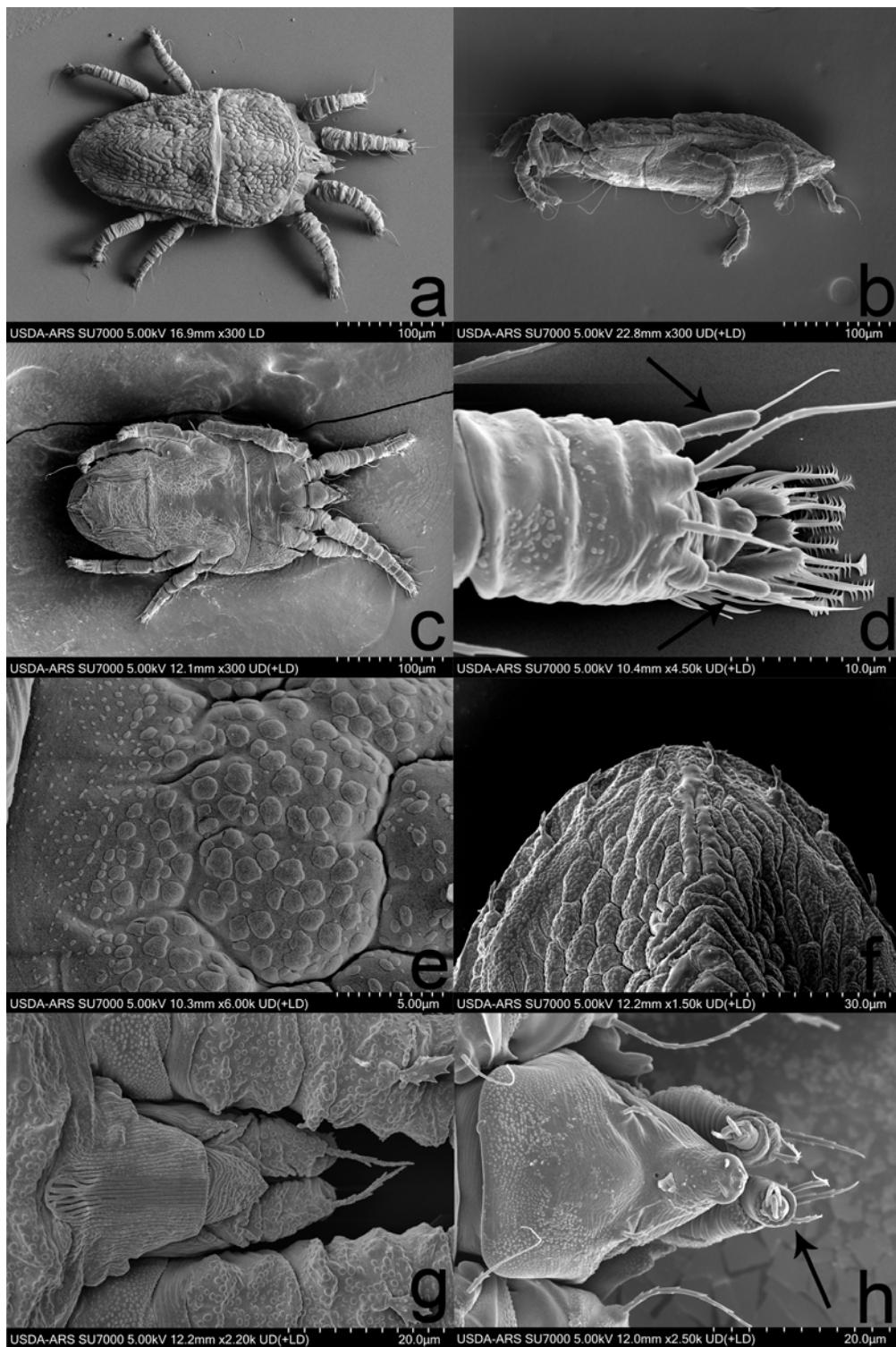


Figure 5-3. Cryo-SEM images of *Brevipalpus californicus* *sensu lato* displaying various characters used for identification (Baker and Tuttle 1987, Beard et al. 2015) (a) Dorsum (b) Lateral view (c) Venter (d) Close up of distal end of leg 2, with arrows indicating paired solenidia, characteristic of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

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BIOGRAPHICAL SKETCH

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