Evaluation of Eriophyid Mite Collection Methods and Environmental Parameters that Affect Populations of *Phyllocoptes fructiphilus*

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Tara Michelle Barnette

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Abstract

Rose rosette disease (RRD) is an infectious and lethal disease of roses, caused by *Rose rosette virus* (RRV). The eriophyid mite *Phyllocoptes fructiphilus* transmits the virus through feeding on healthy tissue after feeding on infected tissue. Infected plants must be destroyed, as there is currently no cure. An outbreak of RRV can cost a grower millions in lost product. Rose rosette disease has been reported in 39 states within the U.S., and is a major threat to the country's billion dollar rose industry. Prevention is key to an effective RRV management program. Tracking the emergence, rise, and decline of *P. fructiphilus* populations will give a more accurate timeframe for miticide applications to be most effective. Four counties within Tennessee were surveyed for populations of *P. fructiphilus* by collecting symptomatic rose tissue, which harbors the mite. *P. fructiphilus* travels from infected to healthy plants by "ballooning" on air currents. Mites were present July through November; densities were significantly greater on Knock Out roses than on multiflora roses. Three eriophyid mite collection methods were evaluated to sample aerial dispersing mites. Pan and bottle traps were effective in collecting mites; samples on sticky traps were not identifiable. Color of water pan traps did not significantly impact mite densities. Environmental factors can influence eriophyid mite behaviors such as ballooning and can thus affect population densities. Mite populations were surveyed through tissue sampling roses. Temperature, humidity, and precipitation, negatively impacted mite densities, while densities were positively influenced by solar radiation. These results will better inform management of RRD and RRV on roses.

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Chapter 1

Literature Review

Introduction

Roses (*Rosa* spp.) are among the most popular flowers in the world and a perennial favorite among gardeners, landscapers, and florists. According to fossil records, roses have existed on earth for at least 40 million years (Guoliang 2003). Humans have cultivated roses for at least 5,000 years (Gudin 2003). Though valued for its timeless beauty and heavenly scent, humans have found a myriad of uses for the plant beyond its aesthetic value through medicinal applications, perfume, religious ceremonies, livestock hedges and culinary consumption. American's infatuation with the rose led to the rose being proclaimed as the national flower in 1986 (National Archives and Records Administration 1986).

Not only are roses important symbolically to the U.S., but the rose industry also is important economically. The rose industry as a whole is estimated to contribute $1 billion to the country's economy (Babu et al. 2018). Ornamental roses are grown in greenhouses, poly houses, container yards and in the field. Roses are harvested and sold as bareroot roses or container roses. End users for roses include homeowners, landscapers and public and private gardens. In a U.S. landscape, roses can number from one to thousands and be grown in landscape beds or large containers. According to the USDA's 2019 Census of Horticulture Specialties, the wholesale and retail market values cut roses, shrub roses and potted roses at more than $200 million (USDA NASS 2020). Using the USDA's estimated value of landscape roses per state, the leading

rose-producing states are Florida at $33 million, California at $21 million, Arizona and Oregon at $12 million, and Texas at $10 million (USDA NASS 2020).

The U.S. rose industry is threatened by rose rosette disease (RRD) which can be lethal in as little as 1-3 years (Amrine 2002, Epstein and Hill 1995). The disease is caused by *Rose rosette virus* (RRV), an emaravirus (Laney et al. 2011), which is primarily vectored by the eriophyid mite *Phyllocoptes fructiphilus* Keifer (Allington et al. 1968). The disease was first reported in Manitoba, Canada in 1940 and in Wyoming and California in 1941. Since the first reports, it has been found in more than 30 states (Conners 1941, Thomas and Scott 1953, Shires and Ong 2018). In 2017, Weeks Roses (a large commercial rose business) discovered an outbreak of RRD in their fields in Wasco, CA. The outbreak cost them millions of dollars in control costs, including destroying hundreds of thousands of roses, aerial applications of miticides, lost reputation and loss of sales (no roses were sold in the western half of the country in 2018 and sales were significantly reduced in the eastern U.S.).

Within the last decade, public parks and gardens have increasingly had to destroy rose plantings due to RRD outbreaks. In 2013, the Tulsa Municipal Rose Garden in Oklahoma destroyed and replaced 1,500 of their rose bushes after finding RRD (Stanley 2013). Texas has been struggling with outbreaks of the disease and many public gardens have abandoned the use of roses. In 2015, an outbreak at the Fort Worth Botanic Garden culminated in the removal of 2,000 rose bushes (Copeland 2018). Over 5,000 roses in medians and parks in Southlake, TX were also removed and replaced due to RRD in 2015, costing the city an estimated $500,000 (Bahari 2015). In 2015, in Allen, TX, an alliance of rose growers, city officials and Texas A&M University

personnel was formed to eradicate diseased roses from the city (Cook 2015). The city passed an ordinance that required the public to remove their diseased roses. Hang tags, printed in English and Spanish (Figure 1), were hung on residence doors throughout the city to encourage residents to remove and bag diseased roses, and then to call a hotline telephone number to have a special garbage truck retrieve the bagged roses. The hang tags educated the public on RRD and what to do if they found diseased roses on residential property. Symptomatic shrubs were found in 2018 at the Carleen Bright Arboretum in Tyler, TX, the city colloquially referred to as “the rose capital of America” (Copeland 2018). The eastern U.S. also has been impacted by RRD. For example, the Malcolm W. Gross Memorial Rose Garden in Allentown, PA lost 700 plants to an outbreak of RRD in 2019 (Merlin 2019).

The Host – *Rosa* spp.

The rose genus (*Rosa*) is in the Rosaceae family, which includes roses and many other economically and agriculturally important crops, such as apple, pear, cherry, peach, strawberry, and almond. The number of *Rosa* species is estimated to be between 140 and 360. The discrepancy in the numbers is due to some taxonomists clumping similar species into one species. Currently, more than 20,000 cultivars of *Rosa* species and interspecific crosses exist worldwide (Leus et al. 2018). Roses are divided into three types: old garden or antique roses, wild roses, and modern garden roses. These classifications are subdivided into other groups based on horticultural characteristics and include climbing, hybrid tea, floribunda, grandiflora, floribunda miniature, musk and shrub roses (Dirr and Dirr 2009).

Mulitflora rose, *Rosa multiflora* Thunberg, is a wild type rose species, native to Japan, Korea and Eastern China (Amrine 2002). Initially, multiflora rose was imported from East Asia for use as rootstock in the 1860s (Dirr and Dirr 2009). In the 1930s, millions of *R. multiflora* were planted throughout the eastern U.S. for erosion control due to the plant's robust root system (Amrine 2002). The plant's ability to form a sprawling thicket made multiflora rose an ideal shelter for wildlife and it was recommended by the U.S. government for use as a "living fence" for livestock (Hindal 1988). One multiflora rose plant can produce up to 500,000 seeds, making it an abundant source of food for wildlife (Amrine 2002). In some states, multiflora rose was planted in highway medians to reduce headlight glare. A dark pink variant of multiflora rose, Seven Sisters, was introduced to the U.S. in 1817 (Warner 2017). The rose has also escaped cultivation and is often seen growing along roadsides, eroded banks and old homesites.

By the 1960s, the same characteristics that popularized multiflora rose also made it a nuisance. Its hardiness and prolific reproduction allowed multiflora rose to freely establish itself in areas where it was undesired. Since it escaped cultivation, the rose has reduced usable farm acreage, taken over abandoned, unmanaged land and crowded out native plants in natural areas. Since it has escaped cultivation and reproduces so prolifically, multiflora rose is now classified as an exotic, invasive species (Dirr and Dirr 2009, Kurtz and Hansen 2013). Once *R. multiflora* was declared as a noxious weed, RRD was studied as a possible biological control method due to its limited host range (only *Rosa* species) and lethal nature (Amrine and Hindal 1988, Epstein and Hill 1995, Hindal and Wong 1988). The use of RRD to control multiflora rose was never fully accepted because of the potential risk of the pathogen spreading to

ornamental roses (Rohozinski et al. 2001) and its use as a biological control strategy was abandoned. Despite this precaution, RRD has become the preeminent menace to ornamental roses.

The modern rose cultivars of today are the descendants of 8 to 15 *Rosa* species with attributes humans found desirable that were selectively cultivated and crossbred for thousands of years (Leus et al. 2018). Today's cultivated roses most likely originated from China, West Asia and North Africa; however, modern rose cultivars with genes of *Rosa* species native to the U.S. include *R. acicularis* Lindl, *R. arkansana* Porter*, R. blanda* Aiton*, R. californica* Chamisso & Schlechtendal*, R. foliolosa* Nuttall & Gray*, R. gymnocarpa* Nuttall*, R. minutifolia* Engelmann*, R. palustris* Marshall*, R. setigera* Michaux*, R. stellata* Wooton*, R. virginiana* Mill*, and R. woodsia* Lindl (Dirr and Dirr 2009). Modern rose breeding can be challenging due to the polyploidy of rose genetics and complications with pollination, seed production, and/or germination (Gudin 1999). Roses have chromosomes in sets of seven; modern cultivars of roses are diploid, triploid or tetraploid whereas wild rose species vary from diploid up to decaploid (Byrne et al. 2018).

No commercially available rose cultivars are resistant to RRD. A few wild type rose species, such as *R. palustris* and *R. setigera,* have shown promising levels of resistance (Martin 2014). However, the characteristics that native roses possess (such as single flowers with 2.5 – 7.6 diameter, consisting of five pink petals that bloom once per season) are not the qualities that are desirable in today's rose market. Rose breeding programs have been developing resistant plants by crossbreeding popular commercial cultivars with disease-resistant species. Researchers have looked for genes

that contribute to RRD resistance, to modify the germplasm of *R.* species already possessing the desired characteristics (Bryne et al. 2018). After years of screening dozens of species and thousands of cultivars for RRD resistance the loci for resistance has been identified (Lau et al. 2022). While this discovery is a major breakthrough, it could still take 11-20 years to get a RRD/RRV-resistant rose in the commercial market.

The Disease – Rose Rosette Disease

Roses are susceptible to several plant diseases, including RRD. In the 1940s this disease was first recorded in Manitoba, Canada after being observed on a rose plant that displayed witches' broom-like growth (a prolific cluster of lateral shoots with stunted apical growth) (Conners 1941). In the U.S. a disease was observed on roses in Wyoming and California with symptoms of witches' broom, distorted leaves, dwarfed stems and chlorotic leaves (Thomas and Scott 1953). An outbreak of a disease causing the same symptoms was reported in Nebraska with additional symptoms of thickened stems, short internode growth and persistent red coloration of leaves and the eventual death of the plant (Allington et al. 1968). Today, RRD is found in 39 states in the U.S. (Figure 2).

The symptoms of RRD can be categorized into three stages. The first stage describes early symptoms that include excessive thorning, thickened stems, and strappy leaves (Figure 3), leaf distortion, leaf mosaic and mottled leaf coloration (Figure 4) (Epstein and Hill 1995). The second stage symptoms involve intense reddening, reduced bud or flower production, stunted growth, shortened petioles, and rosette or witches' broom formation (Figure 5) (Epstein and Hill 1995). The third stage symptoms

include more witches' brooming, reduced leaves, stunted vertical growth, proliferation of lateral shoots, and reddened tissue (Epstein and Hill 1995). However, symptoms of RRD can vary among ornamental rose cultivars, and symptoms produced on multiflora rose can vary from those produced on ornamental roses (Figure 6) (Epstein and Hill 1999). Symptoms on ornamental roses include excessive thorning, distorted leaves, witches' brooming, and hyper pigmentation (Amrine 1996). Multiflora rose symptoms include reddening of leaves and stems (Figure 5), mosaic patterns on leaves and witches' brooms (Amrine and Hindal 1988). Some symptoms of RRD can be mistaken for those related to other issues, such as the red pigment seen in new growth which will change to green over time. Additionally, herbicide damage can induce stunted growth, distorted or thin leaves but not excessive thorn production. Symptoms of RRD usually occur within 30-146 days after infection takes place (Allington et al. 1968). While symptoms of this disease can vary based on the cultivar and age of the plant, the disease usually kills plants within 2-5 years, once symptoms start to show (Epstein and Hill 1995).

The Pathogen - Rose Rosette Virus

The causal agent of rose rosette disease (RRD) is *Rose rosette virus* (RRV), a seven segmented single stranded negative sense RNA virus, having a spherical shaped virion 120-150 nm in diameter, enclosed in a double membrane and a member of the genus *Emaravirus* (Gergerich and Kim 1983, Laney et al. 2011, Di Bello et al. 2015). RRV has low genetic variability between isolates which may be due to its limited host range. Low genetic variability and limited host range is not uncommon with *Emaravirus*

species (Laney et al. 2011, Katsiani et al. 2020). Emaraviruses are members of the Fimoviridae virus family (Order Bunyavirales). Other members in the genus *Emaravirus* are *European Mountain Ash Ringspot-associated Emaravirus*, *Fig Mosaic Emaravirus*, *Raspberry Leaf Blotch*, *Pigeon Pea Sterility Mosaic Emaravirus* 1 & 2, *Redbud Yellow Ringspot-associated Emaravirus*, *Actinidia Chlorotic Ringspot-associated Emaravirus* and *High Plains Wheat Mosaic Emaravirus*. Emaraviruses are enveloped virion spheres, 80-100 nm in diameter, and have genomes consisting of four to eight negative-sense single strand RNAs (Elbeanio et al. 2018). Eriophyid mites vector many Emaraviruses (Mielke-Ehret and Muhlbach 2012).

Plant viruses often cause leaf yellowing, mosaics, crinkling and stunted growth, as these symptoms were seen in the first reports of RRD, it was theorized early that the causation of RRD was a viral pathogen (Thomas and Scott 1953). After a severe outbreak in Nebraska, a grafting study determined the cause to be associated with the mite *P. fructiphilus*, either as a viral vector or through feeding-induced damage (Allington et al. 1968). In 1980, the RRD was theorized to be caused by a phytoplasma, but this theory was discarded after antibiotic-treated plants symptomatic with RRD failed to recover (Slykhuis 1980, Epstein and Hill 1995). Researchers in 1983 observed symptomatic plant tissue to contain spherical “virus-like particles” which reinforced the theory that RRD was caused by a virus (Gergerich and Kim 1983). A transmission study in 1988 confirmed *P. fructiphilus* to be a vector of the disease and by 1990 researchers were able to isolate dsRNA from roses displaying RRD symptoms (Amrine et al. 1988, Di et al. 1990).

The virus genome was partially sequenced using dsRNA isolated from symptomatic *R. multiflora*, using genomes from other Emaraviruses as a guide, European Mountain Ash Ringspot-Associated Emaravirus (EMARaV) and Fig Mosaic Emaravirus (FMV), four single strand negative sense segments of RNA were identified (Laney et al. 2011). Using the RNA segments found, a RT-PCR assay was created, this detection method found the virus to be present in 84 of 84 RRD-symptomatic plants, confirming the pathogen that causes RRD is a viral pathogen called *Rose rosette virus* and classified it into the Emaravirus genus (Laney et al. 2011). Di Bello et al. (2015) isolated RNA segments 5-7 from an ornamental rose cultivar in 2015. A single primer for RRV detection (RRV2F/2R) was developed for use with TaqMan-quantitative RT-PCR (RT-qPCR), SYBR Green RT-qPCR, or Endpoint RT-PCR assays of RRV (Dobhal et al. 2016). In 2017, a highly specific RRV assay was created that used isothermal reverse transcription-recombinase polymerase amplification (RT-RPA) using primer sets RPA-267, RPA-131, and RPA-321 (Babu et al. 2017b). Shires (2020) discovered that primers RRV2 and RRV (db/271) can be used to detect the presence of RRV in asymptomatic roses (Shires 2020).

The primary method of RRV transmission is by the eriophyid mite *P. fructiphilus* and only one infectious mite is needed to transmit the virus to a healthy plant (Epstein et al. 1997, Di Bello et al. 2015). The secondary method of RRV transmission is through grafting infected tissue on to healthy rose plants (Allington et al. 1968, Amrine 2002). RRV can also be transmitted through infected seeds (at very low rates) and may be transmitted through roots, but sap transmission is not likely (Shires 2020).

The Vectors – *Phyllocoptes fructiphilus* and *Phyllocoptes arcani*

In a 1968 study, rose plants were successfully infected with RRV by grafting but also by the eriophyid mite *P. fructiphilus* (Allington et al. 1968). *P. fructiphilus* was the only known vector of RRV until an August 2023 study which proved *Phyllocoptes arcani* Druciarek, first described in 2021, can successfully vector the virus as well (Druciarek et al. 2021, 2023). *P. fructiphilus* was first described by Keifer (1940) after it was found on a rose in California in the U.S. and is thought to be native to North America. *P. fructiphilus* has a two-segmented, cone-shaped body consisting of the gnathosoma (mouthparts) and a tapered abdomen (opisthosoma); an adult mite of this species is approximately 140-170 um long, 43 um wide (Keifer 1940). Like all eriophyid mites, *P. fructiphilus* has no eyes or wings; they do have two pairs of legs located anteriorly. The prodorsal shield, which has a pattern of 10-12 irregular rectangular shaped raised cells, partially covers the gnathosoma (Keifer 1940). Adult females have a genital coverflap with 6 to 7 longitudinal ridges located ventrally (Keifer 1940). Juveniles do not have genital coverflaps (Otero-Colina et al. 2018).

The lifecycle of *P. fructiphilus* consists of four stages: egg, protonymph, deutonymph, and adult (Kassar and Amrine 1990, Amrine 1996). Females lay eggs which are small, round, gelatinous, and translucent or white (Amrine 1996, Manson and Oldfield 1996) (Figure 7). An egg will hatch after approximately four days and a protonymph will emerge (Kassar and Amrine 1990). Protonymphs lack external genitalia, their bodies are transparent but shifts to a milky white over time; this stage lasts for 3 days until the juvenile mite enters a pharate resting state where the body will become swollen and shiny (Kassar and Amrine 1990). After two to three days a white

deutonymph emerges, its size and shape are similar to that of an adult but this form still lacks external genitalia (Kassar and Amrine 1990, Manson and Oldfield 1996). Over three days deutonymphs change from white to yellowish white until they enter their final pharate from which the adult will exit after 2-3 days (Kassar and Amrine 1990). The adult mite lifestage can last for 14 days and the overall average lifespan of *P. fructiphilus* is 30 days (Kassar and Amrine 1990, Amrine 1996). Adult mites can begin feeding soon after leaving their last pharate form (Kassar and Amrine 1990).

Once adult females exit their pharate form they can start laying eggs within 12-24 hours (Kassar and Amrine 1990). *P. fructiphilus* reproduces by indirect insemination in which an adult male will deposit a spermatophore (a sperm sac on a stalk) onto the substrate surface (Figure 7); if an adult female encounters a spermatophore, she will hover over it, taking the spermatophore into her sperm receptacle, the spermatheca where the female can fertilize the egg (Oldfield and Michalska 1996). Fertilized eggs will develop into females, whereas unfertilized eggs develop into males (Oldfield and Michalska 1996). Adult females can lay one egg each day (Amrine 1996). *P. fructiphilus* females have two forms, protogynes are the typical, primary form and deutogynes are the mated overwintering form (Manson and Oldfield 1996). After overwintering, deutogynes emerge and feed on tender new growth (Amrine 2002). As a refuge mite, *P. fructiphilus* lives within remote niches of a rose plant, often found in the hard to access regions of the plant, such as sepals, petiole connections, and bud scales (Otero-Colina et al. 2018).

Roses are the only known host of *P. fructiphilus*. This mite feeds on the rose plant sap; this species of eriophyid mite is found more frequently in the sepals and

petioles of a symptomatic rose plant, which may be due to the plant tissue in these areas having more tender tissue while offering the mite protection from predators (Monterrosa et al. 2022). After *P. fructiphilus* feeds on the sap of a RRV-infected rose plant, it may take up to five days for the mite to become viruliferous; a viruliferous mite can then infect a healthy rose plant in less than 1 hour (Di Bello et al. 2018).

While few studies have been conducted on general populations of *P. fructiphilus*, populations are thought to be relatively low in early spring, with peak population density occurring in the summer until the population declines in late fall (Amrine et al. 1990). In Nebraska, populations of *P. fructiphilus* were low until mid-June to early July, peaking in September, though large numbers of mites were still found on roses in December (Allington et al. 1968). In a field of multiflora roses in Iowa, populations of *P. fructiphilus* peaked in August or September (Epstein et al. 1997). Studies have also shown that *P. fructiphilus* populations are more abundantly found on the tissue of RRV-infected plants in quantities 14 to 40 times greater, than on noninfected plants (Amrine 1996, Epstein and Hill 1999, Solo et al. 2019a). In a preliminary study conducted in 2020 in Knoxville, TN, populations of *P. fructiphilus* remained static till mid-June and then leveled off in August as previously reported in other publications (Barnette, unpubl. data) (Figure 8).

Eriophyid mites are slow, traveling short distances by crawling. They can move from one plant to another by crawling, if the plants overlap or touch. To travel long distances eriophyid mites ride on air currents, called “ballooning,” which is their main mode of transport (Oldfield and Michalska 1996). To balloon the mite excretes a sticky substance from their posterior end on to the surface of a leaf, allowing the mite to raise up its anterior end and waive its legs to catch an air current (Michalska et al. 2010).

Mites can travel 100 m or more on air currents but have no way to control where they land (Epstein et al. 1997). Ballooning is performed almost entirely by adult eriophyid mites (Nault and Styler 1969).

Eriophyid mite species *Phyllocoptes adalius* Keifer, *Phyllocoptes chorites* Keifer*, Phyllocoptes fructiphilus* Keifer*, Eriophyes eremus* Druciarek & Lewandowski and *Callyntrotus schlechtendali* Nalepa have been found on rose plants with RRD symptoms, however, only *P. fructiphilus* and *P. arcani* are known to vector RRV (Otero-Colina et al. 2018, Druciarek et al. 2023) (Figure 9). To identify these eriophyid mites, their prodorsal shield patterns and genital coverflaps are examined microscopically (Manson and Oldfield 1996). The prodorsal shield of *E. eremus* has no frontal lobe, the dashed median ridge is between two admedian unbroken lines extending the length of the shield and there are no cross dorsal ridges, only faint longitudinal ridges or dashes (Otero-Colina et al. 2018). Adult female *E. eremus* possess coverflaps bearing two parallel ridges on each side, the base of the coverflap is covered in protuberances (Otero-Colina et al. 2018). The prodorsal shield of *C. schlechtendali* has a protruding frontal lobe covering the gnathosoma; the shield does not have a median ridge, or any cross ridges (Otero-Colina et al. 2018). The dorsal side of *C. schlechtendali* has longitudinal wax-like ridges extending the length of the mite's body (Otero-Colina et al. 2018). Adult *C. schlechtendali* females possess coverflaps that bear 12-13 longitudinal ridges with 3 to 5 transverse ridges on the base of the coverflap (Otero-Colina et al. 2018). Mites of *C. schlentendali* can easily be identified from the other two using a stereoscope (70X) due to their waxy white color and broad bodies. Separation of *E. eremus* and *P. fructiphilus* may be more difficult at that magnification since body shapes

and color are similar and distinctions of prodorsal shields and of coverflaps may be indistinctive at that magnification.

Eighteen *Rosa* species were evaluated for resistance to *P. fructiphilus* (increase in mite populations over time) in 2016-2017 (Solo et al. 2019b). In the first year, *R. carolina* and *R. hugonis* harbored significantly lower populations than the susceptible control (*R. multiflora*). However, in 2017, mite populations were low on all *Rosa* species and differences in mite populations were indiscernible.

Management and Control

Hundreds of rose species and cultivars have been screened for natural resistance to RRV using PCR testing to detect the presence of the virus. Zary (1994) concluded that resistance would “turn the battle against RRD” but would take decades and be expensive due to the lengthy process of breeding a plant with polyploid genetics. Technological advances, such as digital genotyping, may speed the development of RRD-resistant roses (Byrne et al. 2015). A recent study found 14 rose species to be tolerant of RRV; these 14 species test positive for RRV but stay symptom-free for at least two years (Shires 2020). The Texas A&M Rose Breeding and Genetics program has assessed data collected from germplasm populations at the University of Tennessee AgResearch Plateau Research and Education Center. This genetic factor appears to inhibit the ability of the virus to replicate in the host. The preliminary analysis indicates that roses with this locus may be resistant to the virus (Lau et al. 2022). Quantitative trait loci for reduced susceptibility to RRD has been found within diploid

rose groups in linkage groups 1, 3, 5, 6 and in 1, 5, 6, 7 in tetraploid groups; additionally genes on linkage group 5 may contain disease-resistant genes (Hochhaus et al. 2023).

Rose species, infected with RRV, but with few or no symptoms of RRD, have been identified by researchers at the University of Tennessee, and these roses have been designated as RRD-tolerant roses (Windham 2021). These roses are *Rosa arkansana* Porter*, Rosa bracteate* Wendl*, Rosa carolina* Linnaeus*, Rosa folialosa* Nuttall*, Rosa rugosa* Bailey Thunberg, *Rosa virginiana* Miller, *Rosa wichurana* Franchet & Rochebrune *Rosa woodsii* Lindl. However, these roses may cause serious problems by serving as an undetectable (or difficult to detect) source for virus inoculum for other roses in a rose garden. Other strategies aside from resistance need to be investigated and implemented to control RRD so both the rose industry and the rose-loving public can survive until RRV-resistant roses are available.

Best management practices relating to the control of RRD should encourage the immediate removal of the entire rose plant at detection of symptoms. Roses should be inspected often, every one to two weeks, for symptoms of RRD in order to prevent further spread of the disease. As eriophyid mites travel on air currents, leaf blowers should not be used in the garden. All multiflora roses within 100 m of ornamental roses should be removed and destroyed to remove possible viral reservoirs.

Chemical control is effective in RRD prevention. Field studies found that populations of *P. fructiphilus* on roses were significantly reduced with biweekly applications of a miticide containing the active ingredients bifenthrin, fenpyroximate, spiromesifen, or spirotetramat; however, bifenthrin is the only miticide of this group available for consumers without commercial applicator licenses (Windham 2021). As

best management practices for pesticide usage involve using the correct pesticide for the target pest in the least amount needed while still effectively controlling the pest to prevent pesticide resistance, further research is needed to identify the maximum interval between miticide applications while still being effective. To be effective, miticides must be applied before populations of *P. fructiphilus* escalate, but more data are needed to determine when populations rise and decline, which may differ depending on the region, climate or cultivar the mite is inhabiting (Windham 2021).

Barriers of nonhost plants can be an effective means of reducing the spread of RRD in the garden. In a study that planted blocks of *Miscanthus sinensis* Andersson behind roses the incidence of RRD was reduced by 77% compared to rose plantings without the *M. sinensis* barrier (Windham 2021). Because establishing a 1.8 to 2.1 meter dense wall of *M. sinensis* may not be possible or desirable for many rosarians, other nonhost plants should be investigated for use as potential barriers to reduce the entry of eriophyid mite populations and to slow the spread of RRD if introduced.

In situations where the immediate removal of RRD-symptomatic roses is strongly advised, it may not always be possible. In these situations, the symptomatic tissue should be pruned and destroyed (Windham et al. 2016). Populations of eriophyid mites on symptomatic rose tissue were found to be 40 times greater than mite populations found on healthy tissue (Solo et al. 2019a). Pruning cannot cure a plant once it is infected with RRV, however, it can reduce eriophyid mite populations which may reduce or slow the spread of RRD until a time when the total plant can be removed.

Chapter 2

Emergence and Seasonality of *Phyllocoptes fructiphilus* in Eastern and Middle Tennessee

Introduction

The health and survival of roses (*Rosa* spp.), as well as their continued use in landscapes and gardens, is threatened by rose rosette disease (RRD). The *Rose rosette virus* (RRV) that causes this disease, which can kill roses in as few as 2-3 years, is vectored by the eriophyid mite *Phyllocoptes fructiphilus* Keifer (Epstein 1995). Generally, *P. fructiphilus* overwinters as deutonymphs, the third instar stage of a mite, with adults emerging in the spring. Adult females will breed, laying one egg per day on average over their 30-day lifespan. During their lifecycle, some adult *P. fructiphilus* will move to new host plants on air currents; this movement may be due to competition for resources. This reproduction and movement will continue until the population declines; usually as temperatures become colder.

Because *P. fructiphilus* is dependent on favorable environmental conditions for survival, detailed information on fluctuations in populations densities (such as increases and decreases) and ballooning is essential. Population densities generally increase as the temperatures increase. The early suppression of populations may prevent the build up of populations and the subsequent dispersal of adults to healthy plants. Emergence, seasonality and decline of populations of *P. fructiphilus* vary over the U.S. A better understanding of these population fluctuations would enhance control recommendations that are more location-specific. For example, Tennessee is a large state that encompasses four climate distinct divisions (Division 1 - East Tennessee, Division 2 -

Cumberland Plateau, Division 3 - Middle Tennessee, Division 4 – West Tennessee) (Voss et al. 2014). These four climate regions represent uniquely different topography, temperatures, and other environmental features. It is important to understand when emergence of *P. fructiphilus* occurs each year, and how populations fluctuate within the state.

The objective of this study was to determine, using generational population curves, when emergence of *P. fructiphilus* occurs and how populations fluctuate by monitoring populations of *P. fructiphilus* in three of the four climate divisions in Tennessee (Division 1 – East Tennessee, Division 2 – Cumberland Plateau, and Division 3 – Middle Tennessee). Suppression of early generations of *P. fructiphilus* before population densities reach damaging levels is crucial. The information gained in this study will enable rose producers to better manage the mite that vectors RRV. It will also aid in more precise miticide application intervals, allowing for less usage of miticides, resulting in a reduction in the development of pesticide resistance by the mite.

Materials and Methods

In this study, RRV-infected wild and cultivated roses were monitored monthly from June to November at 18 locations in Eastern and Middle Tennessee (Table 1). On each monthly sampling date, tissue samples were collected; mites were extracted from these samples and counted to obtain population data.

The wild and cultivated roses used for tissue collection in this study were *Rosa multiflora* (multiflora rose) and *Rosa* ‘Radrazz’ (Knock Out rose), respectively. Both of these rose types are *Rosa* species that are known hosts of *P. fructiphilus*. The tissue

collected in this study consisted of the preferred tissue of *P. fructiphilus* mites: tender new growth, leaf petioles, sepals, bud scales, and flower buds. For the first two months of the study in 2021 at the two Knoxville Knock Out rose locations, 20g of tissue was obtained from each location in total, consisting of 10g of bud tissue (closed to partially open buds) and 10g of petiole tissue. The purpose of this collection was to confirm or rebuke findings in 2020 in Knoxville which showed a greater number of mites were found in closed and partially open buds than were found in petiole and leaf tissue. The tissue obtained from Knock Out roses was combined at each location to form a composite sample.

Tissue samples from Knock Out roses were collected from two locations in East (Knoxville [Knox Co.]) and three locations in Middle (Crossville [Cumberland Co.] and Nashville [Davidson Co.]) Tennessee (Table 1). The two locations used to obtain Knock Out rose tissue in Knox County were located at Dunkin Donuts, 720 Merchant Drive, Knoxville, TN (GPS 36°00'21.8"N 83°58'43.2"W), and Kroger Fuel Center, 4918 Kingston Pike, Knoxville, TN (35°56'22.5"N 83°59'08.2"W). From each location in Knoxville, tissue (10 g) was collected from eight Knock Out rose shrubs. Tissue (10 g) was collected from a composite of eight Knock Out roses located at Burke's Outlet, 86 The Crossings, Crossville, TN (35°58'50.4"N 85°02'17.1"W). Additionally, 10g of tissue was collected from a composite of eight roses at Comfort Suites, 258 E 1st St, Crossville, TN (35°57'42.2"N 84°59'01.2"W). Samples of tissue from Knock Out roses were collected by cooperators at one location in Nashville, TN (Davidson County) (36°7'2"N 86°46'26"W) and shipped via FedEx to Knoxville, TN, where they were processed.

Tissue samples from multiflora rose were collected from two locations in East (LaFollette [Campbell Co.]) and two locations in Middle (Crossville [Cumberland Co.]) Tennessee (Table 1.). Two multiflora rose plants were used for tissue collection in Campbell County in LaFollette, TN (36°20'07.2"N 84°04'01.9"W). The multiflora rose tissue from the two locations in Cumberland County was collected from *R. multiflora* growing wild at the UT AgResearch Plateau Research and Education Center, Crossville, TN; 10g of tissue was collected from one plant located at GPS coordinates 36°00'48.4"N 85°07'40.1"W and 10g of tissue from one plant located at GPS 36°00'46.2"N 85°07'57.9"W.

After collecting the samples, the mites were extracted from the rose tissue by placing 10g of tissue into a 400 ml beaker. The tissue was covered with 250 ml of 70% ethanol and then stirred for 5 minutes to dislodge mites. A series of mesh sieves were placed on top of an empty beaker, arranged top to bottom from sieves no. 80 (180 mm), no. 270 (53 mm), and no. 500 (25 mm). The stirred tissue and ethanol in the beaker was poured into the top sieve (no. 80) and this tissue was then rinsed with 70% ethanol from a squeeze bottle (Figure 10). The top sieve (no.80) was removed, and the no. 270 sieve was then rinsed with ethanol. The no. 500 sieve was removed and held over a counting dish, the sieve surface was rinsed into the counting dish. The counting dish was placed under a dissecting microscope and allowed to sit for 5 minutes to allow the contents to settle so the mites could be counted. The number of mites found per gram of tissue was recorded. Slides were made for mite identification. Using a glass pipette the mites were removed from the ethanol in the counting dish and placed on a blank microscope slide to allow the excess ethanol to evaporate. Using a hair loop or minuten

pin tool, the mites were transferred onto a slide prepared with modified Berlese medium and then covered with a coverslip. The slides were cured on a hot plate at 85^{o} C for 1 hour. Slides were examined with a stereoscope at 40x magnification to identify the mite species using dorsal shield and coverflap markings.

Data Analysis

All data were analyzed using ANOVA GLIMMIX. LSMeans was used to determine differences among the means. For significance, p values were set at 0.05.

Results

The total number of mites collected in 2021 (20.98 +/- 17.54) and 2022 (14.95 +/) were not significantly different. Combined for 2021 and 2022, densities of *P. fructiphilus* were significantly greater on Knock Out roses than on multiflora roses (26.79 and 6.82 mites per sample, respectively) (Figure 11). In 2021 and 2022, combined for all counties, densities of mites were not significantly different among months (Figure 12). Densities of mites were significantly greater in Knox County (33.27) than in the other counties (5.28 in Campbell, 14.10 in Cumberland and 25.67 in Davidson) (Figure 13).

Multiflora Rose

Campbell County

Combined for 2021 and 2022, in Campbell County, mites were not recovered from *R. multiflora* samples collected in June, but they were present on samples collected in July, August, September, and October (Table 2). In July, density of *P. fructiphilus* was 0.22 mites/g of tissue from multiflora rose. Populations peaked in

August at 0.45 mites/g, declined in September at 0.28 mites/g, before decreasing in October to 0.23 mites/g.

Cumberland County

In 2021 and 2022, in Cumberland County, *P. fructiphilus* was recovered from *R. multiflora* samples collected in June, July, August, September, and October (Table 2). In June, the density of *P. fructiphilus* was 0.47 mites/g of tissue. By August and September, the densities of *P. fructiphilus* peaked at 0.51 and 0.52 mites/g of tissue, respectively. Population densities of *P. fructiphilus* decreased slightly in October when the mite population decreased to 0.40 mites/g of tissue.

Knock Out Roses

Cumberland County

In 2021 and 2022, *P. fructiphilus* on Knock Out roses in Cumberland County was present in June at 0.93 mites/g of tissue (Table 3). The *P. fructiphilus* population remained steady from June to October (1.00 mites/g of tissue in July, 0.94/g in August, and 0.92 mites/g in September and 0.80 in October). At one location in Knox County in 2020, populations of *P. fructiphilus* were 3-7X more abundant than they were at any of the locations in 2021 or 2022, although they followed the same pattern as the previous year (unpublished data).

Davidson County

In Davidson County, samples were not collected from Knock Out roses in June 2021 or 2022. Density of *P. fructiphilus* was 1.0 mites/g on each sampling date when

combined for both years. It is important to note that samples were not collected in some months in one of the years (Table 3).

Knox County

In 2021 and 2022, populations of *P. fructiphilus* on Knock Out roses in Knox County were present from June to November (Table 3). Populations of *P. fructiphilus* remained steady, around 1 mite/g of tissue, throughout the sampling period.

Discussion

This study monitored the eriophyid mite populations on cultivated and wild roses in Knox, Campbell, Cumberland, and Davidson Counties in Tennessee. On Knock Out roses in Davidson County, *P. fructiphilus* was present in August 2021 and July 2022. The population was most abundant in September during both 2021 and 2022. In 2021 the population declined in November; in 2022, the population declined in October. In Cumberland County, on Knock Out roses, *P. fructiphilus* was present in June, the population was most abundant in September during both 2021 and 2022. The population declined in October in both 2021 and 2022. In 2021 and 2022 in Knox County, *P. fructiphilus* populations were present in June. The population was most abundant in September 2021 while in 2022 it was most abundant in August. The population declined in October of both 2021 and 2022.

On multiflora rose in Cumberland County, *P. fructiphilus* populations were present in July in 2021 and 2022. The population was most abundant in August in 2021, but 2022 was most abundant in September. The population declined in October of both years. In Campbell County on multiflora rose, *P. fructiphilus* populations were present in

July 2021 and 2022. The population was most abundant in September 2021 and October 2022. The population declined in October 2021. There was no data for November 2022 to obtain when the population declined in that year.

On multiflora rose, in both 2021 and 2022, densities of *P. fructiphilus* were much lower than on Knock Out roses in both Campbell and Cumberland Counties, with the largest density collected at 16.8 mites/g. With mite populations that low it may be a appropriate to remove multiflora rose bushes entirely, to prevent them from being a host plant for *P. fructiphilus*, than to spray miticides.

Results will influence current management practices relating to *P. fructiphilus*. As RRD has become more prevalent in the world, a better understanding of the annual cycle of *P. fructiphilus*, as well as its emergence and seasonality will help growers and producers strategize management tactics. This information can be utilized for miticide spray practices. For example, rose growers In Knox County should begin applications of recommended miticides in July when the mite is present but before its populations begin to increase and surge. More research is needed in Davidson County in order to determine when applications of miticides should be applied in Davidson County. In Cumberland County, miticide applications should begin in September on Knock Out roses.

Chapter 3

Evaluation of Methods to Collect Eriophyid Mites

Introduction

The eriophyid mite, *Phyllocoptes fructiphilus* Keifer, vectors *Rose rosette virus* (RRV) which causes rose rosette disease (RRD). This disease can kill roses, such as the popular *Rosa* spp. found in landscapes and gardens, in as few as 2-3 years. Eriophyid mites are slow and can travel short distances on the plant by crawling. Mites can also transport themselves from one plant to another by crawling, if the plants overlap or touch. To travel longer distances, eriophyid mites ride on air currents, called "ballooning," which is their main mode of transport (Oldfield 1996). The ballooning process begins when mites excrete a sticky substance from their posterior end on to the surface of a leaf. This sticky substance enables the mite to raise up its anterior end and wave its legs to catch an air current (Michalska 2010). Mites can travel 100 m or more on air currents but have no way to control where they land (Epstein 1997). Ballooning is performed almost entirely by adult eriophyid mites (Nault & Styler 1969).

Knowing when *P. fructiphilus* populations balloon to a new host will improve understanding of the mite's behavior and this knowledge will aid growers in devising better management strategies for RRD. Common methods used to study the aerial dispersal of eriophyid mites include sticky traps and water traps. The Woody Ornamental Laboratory at the University of Tennessee Knoxville currently uses a modified sticky trap to sample for ballooning mites. The drawback to using a sticky trap is that the adhesive substance can distort and modify the appearance of the mites,

making species identification difficult. Attempting to remove the mites from the adhesive can destroy the mites making identification impossible (Monfreda 2009). It is important to have a simple, easy way to collect mites that leave them in a recognizable condition once they are collected. This study compared two nondestructive collection methods to a destructive sticky trap for sampling mites that disperse aerially.

Materials and Methods

This study was conducted for 18 weeks in 2021 at the University of Tennessee AgResearch Plateau Research and Education Center in Crossville, TN. One type of destructive collecting method (spinning sticky trap) and two types of nondestructive collecting methods (spinning bottle trap and water pan trap) and were evaluated. These collection methods were placed among wild and cultivated roses that were known to be positive for RRV. Each collection method was replicated five times.

Collection Method 1. Spinning Bottle Trap

The first eriophyid mite, nondestructive sampling method evaluated was the battery-operated spinning bottle trap that was suspended from a shepherd's hook (Figure 14). Each trap consisted of a wooden dowel, 15 cm long and 1.3 cm wide, a battery-operated rotating spinner, two clear plastic bottles with screw top caps, metal hooks, wire, and D-batteries. The plastic bottles used were 18 cm in length, 5 cm width, with a 7 cm x 5 cm "window" removed from one side which would be the entrance to the collection chamber. A hole was drilled through the center of each bottle cap where a cup hook with screw was attached and secured with glue. The wooden dowels had grooves cut into the upper surface, 2.5 cm from each end, where the hook that was

attached to the bottle was placed to allow the bottle to be suspended. The dowels had a small hole drilled through the center and were placed on the attachment point at the base of the rotating spinner and secured with wire. The bottles were oriented vertically on the dowels with the “window” facing the windward direction (southwest) to catch ballooning mites. The spinning bottle trap was suspended from metal shepherds hooks 60 cm from the ground surface. Each bottle was filled with 50 mL of water with 1 drop of dish detergent to act as a surfactant.

Samples were collected once each week by pouring the solution from the bottle through stacked mesh sieves (180 mm, 53 mm, and 25 mm, respectively). The 25 mm sieve was rinsed through a funnel into a 50 mL centrifuge tube. Samples in the centrifuge tube were taken to the laboratory where the contents were rinsed with an ethanol-filled bottle into a counting dish. The solution was observed under a stereoscope, and any mites present were counted. Mites were removed from the solution then placed on a microscope slide to be viewed under a compound microscope for species identification. This process was repeated for 18 weeks.

Collection Method 2. Water Pan Trap

The second eriophyid mite, nondestructive collection method evaluated was a modified water pan trap (Figure 15). In a similar study, water pan traps were found to be more efficient than sticky pan traps for aerial dispersing mite studies (Zhao and Amrine 1997a). In this study, the pan trap consisted of a gray, metal cake pan of 22.9 cm diameter and 3.8 cm height which was 3/4 filled with water and a drop of dish detergent. The pan filled with water was placed on top of a wooden support in the ground. The detergent was added to act as a surfactant to promote the mites breaking through the

water surface. The base support for the pan trap was constructed out of an 18 cm x 30 x 2.54 x 1.27 cm square wood plank attached perpendicular to a 5 cm wooden stake, which was driven into the ground so that the pan rested 60 cm from the ground surface. To discourage wind or other weather events from dislodging the pan, wire brads were hammered into the wooden board around the perimeter of the pan. Eye hooks were screwed into the upper surface of the wood planks at each corner and a bungee cord was stretched over the top of the pan to strap the pan to the board, providing additional stability against blowing over.

After consulting with Dr. Ronald Ochoa (USDA ARS, Beltsville, MD) regarding the collection methods in this study, an additional 10 water pan traps were added to the study in September 2021; five of these were spray painted yellow and five were spray painted red. The yellow and red pan traps were implemented during week eleven between September 27 to October 1. The additional water pans were added to observe the possible light sensing capabilities of eriophyid mites. Since insects are most attracted to ultraviolet light, yellow was used for its ability to reflect UV light. Eriophyid mites are thought to have no eyes, though this topic has not been widely studied. If the mite has any ocular light sensing capability, perhaps they may be attempting to direct themselves toward a particular color.

Samples from the pans were collected once each week by pouring the soap and water solution through mesh sieves stacked top to bottom (180 mm, 53 mm, and 25 mm, respectively). The sieves were rinsed with a water-filled spray bottle to separate the mites from other insects and debris. The bottom sieve was rinsed through a funnel into a 50 mL centrifuge tube, which was labeled with the date, trap type, and location of

the trap. In the lab, the centrifuge tube was rinsed into a counting dish using an ethanol-filled spray bottle. The mites were counted under a stereoscope, mounted onto slides using modified Berlese medium. The mite species were identified under a compound microscope. This process was repeated for 18 weeks.

Collection Method 3. Spinning Sticky Traps

The third eriophyid mite collection method evaluated was a spinning sticky trap (this trap is the destructive collecting method and was included for comparison to the other two trap types) (Figure 16). This trap consisted of a 3D printed rod, 25 cm long, fashioned to hold a vertically aligned microscope slide on each end; this rod was attached to the base of a battery-operated rotating spinner. The spinning sticky trap was hung from a metal shepherd's hook 60 cm above the ground. The spinning traps were placed close enough to the roses to intercede ballooning mites, but the traps did not touch any foliage to prevent mites from crawling on to the sticky traps. The spinning sticky slide traps were placed in the field for five days; they were then collected and transported to the laboratory, where the slides were viewed under a microscope and the mites were counted and the number of mites recorded. If present, the mites were identified as eriophyid mites but this method does not allow species identification. This process was repeated every week for 18 weeks.

As a control, to assess at what level eriophyid mites were present in the rose field, symptomatic tissue from nearby rose plants was gathered. The tissue was collected from plants that were located within the same row and block that each trap was located, instead of collecting tissue from the plant that was nearest each trap. This selection was done to minimize interference with ballooning mites, which the trap would

potentially collect. Sampling tissue from nearby plants, instead of the plants closest to the traps, will confirm mite presence in the field while minimizing interference of the mite populations closest to the traps.

Data Analysis

All data were analyzed using ANOVA GLIMMIX. LSMeans was used to determine differences among the means. To assess significance, p values were set at 0.05.

Results

Due to inclement weather, traps were not placed in the field for 11 of the 18 weeks of the study in 2021, resulting in no data collections during the following weeks: June 28-July 2, July 5-9, July 12-16, August 16-20, August 30-September 3, September 6-10, September 13-17, September 20-24, October 4-8, and October 25 to 29 (Table 4). The greatest number of mites collected from all pans, bottles, and slides combined were found during week 17 (October 18-22). In 2021, no mites were collected in any pan, bottle or slide trap during week 5 (July 26-July 30).

During 2021, 34 mites were collected using bottle traps, 23 mites were collected using spinning sticky slide traps, and 36 mites were collected using gray pan traps over the course of the study. Pan traps and bottle traps were more effective methods of eriophyid mite collection than spinning sticky traps (Figure 17 and 18). Significantly greater numbers of mites were collected in water pan traps than in bottle traps or sticky traps (Table 5). Significantly greater numbers of mites were collected in bottle traps than in sticky traps.

Over the four-week period in 2021 to assess mite collections among the different pan colors, 21 mites were collected from gray pans, 20 mites were collected from red pans, and 11 mites were collected from yellow pan traps. No significant differences among pan colors was documented (Figure 19 and 20). Densities of all eriophyid mites combined, as well as *P. fructiphilus*, were not impacted by trap color (Tables 6 and 7).

In 2022, no data were collected during the following weeks: July 25-29, August 1-5, October 10-14, October 17-21, and October 24-28 (Table 8). The spinning sticky traps collected a total of 125 eriophyid mites. The most mites (n=25) were collected during the week of September 28-October 2, and the least number (n=0) of mites were found during the weeks of August 8-12 and August 15-22.

The spinning bottle traps collected a total of 48 mites. The most mites (n=11) were collected using this method during the week of September 28-October 2. No (n=0) mites were collected during the weeks of July 4-8, August 29-September 2, and September 3-7.

In 2022, a total of 354 mites were collected using the gray, red, and yellow water pan traps. The yellow pan traps collected the greatest total number (n=142) of mites, while the gray pan traps and red pan traps collected 112 and 100 total mites, respectively. Regarding the yellow pan traps, the greatest number (n=49) of mites were collected during September 12-16, while no (n=0) mites were collected from yellow pan traps during the week of June 27-July 1.Regarding the gray pan traps, the greatest number (n=39) of mites were collected during the week of September 12-16, and the least number (n=2) of mites were collected from gray pan traps during the weeks of June 27-July 1 and August 8-12. Regarding red pan traps, the greatest number (n=18)

of mites were collected from red pan traps during the weeks of September 12-16 and September 28-October 2, and the least number (n=2) of mites were collected during August 8-12.

After comparing the number of mites collected in the bottle, pan, and slide traps, pan traps and bottle traps were the most effective method of eriophyid mite collection (Figure 17 and 18). This study found that there was no significant difference between the color of the pan traps among the yellow, gray, and red pans (Figure 19 and 20).

Discussion

The vector of RRD is the size of a pin head, making monitoring extremely difficult. The purpose of this study was to evaluate two of the current eriophyid mite collection methods, the spinning sticky trap and pan trap. Additionally, this study evaluated a new trap type, the spinning bottle trap, for its potential in eriophyid mite collection. The pan trap and the spinning bottle trap was significantly more effective at eriophyid mite collection than the spinning sticky trap. The spinning bottle trap was not significantly more effective at collecting eriophyid mites than the water pan traps. However, the water pan traps have a few advantages over the spinning bottle trap. For example, the water pan traps do not rely on a battery, which was replaced frequently during the study (once per week). Additionally, the water pan trap was easier to install than the bottle traps which required cutting into each plastic bottle and drilling into the plastic lids. The water pan trap required fewer parts which were easily found at a local big box store (wood plank, wood stake, metal pan, water, dish detergent, and nails) versus the bottle trap which required a spinning motor, battery, wooden dowels, hooks,

plastic bottles, water, dish detergent, and shepherd's hook, some of which had to be ordered online. Mite densities were not significantly different among trap color. Further research is needed to determine any ocular capabilities of eriophyid mites.

Summary

This study evaluated one destructive and two nondestructive eriophyid mite collection methods: a spinning sticky slide trap, a spinning bottle trap, and a water pan trap. Gray, red, and yellow metal pans were used in the water pan traps. The water pan traps and the spinning bottle traps outperformed the spinning sticky traps. The water pan traps and spinning bottle traps were significantly more effective at collecting eriophyid mites during the two year study, when compared to sticky traps. No significant difference was found between the number of eriophyid mites collected using the water pan trap or the spinning bottle trap. However, for ease of use, the water pan trap was the more efficient trap of the two nondestructive eriophyid mite collection methods. No significant difference in the color of the metal pans used was documented.

Rose growers would greatly benefit from an early detection method for the presence of *P. fructiphilus*. Water pan traps would be simple, cheap, and effective way for rose growers to monitor for *P. fructiphilus*. Water pan traps are easy to install, requires little monitoring, requires no specialized skill, utilizes basic equipment, and is an effective way to collect eriophyid mites. Early monitoring for *P. fructiphilus* would allow rose growers to be proactive in monitoring for *P. fructiphilus* instead of waiting until rose shrub display symptoms of RRD, by which point the shrub cannot be saved.

Chapter 4

Environmental Effects on Eriophyid Mites

Introduction

Environmental factors (such as temperature and wind) can influence the behavior of many arthropod species. These factors can directly or indirectly impact the movement, behavior, and population densities of mites, especially eriophyid mites that depend upon ballooning to move from plant to plant. One of these types of eriophyid mites dependent upon ballooning is *Phyllocoptes fructiphilus* Keifer, the primary vector of *Rose rosette virus* (RRV) that causes rose rosette disease (RRD). This eriophyid mite becomes active once the temperature increases in the spring, and then the females will feed on rose tissue and lay eggs. Under greenhouse conditions at 23° C, on average, a *P. fructiphilus* egg will hatch after 4 days and the lifespan of an adult mite is ~14 days; the average female can live up to 30 days (Kassar and Amrine 1990). A study in Georgia that used temperature-controlled growth chambers documented greater population densities of *P. fructiphilus* at 32° C than at 15° C or 21° C (Joseph 2022).

Aerial dispersal is the most frequently used method of transport by eriophyid mites. In West Virginia, more eriophyid mites were collected on windy days than on less windy days (Zhao and Amrine 1997b). In a study of the eriophyid mite *Floracarus pepprepae* Knihinicki & Boczek, a gall mite, population density was positively associated with wind speed (David 2019). Eriophyid mites were more likely to balloon when wind speeds were between 11 to 19 km/hr (Davis 1964). Ballooning is less likely to occur in periods with low wind speeds, low temperatures, and during dark periods (Sabelis & Bruin 1996). In a study observing grass mites in Ohio, the greatest level of

eriophyid mite aerial dispersal activity occurred during periods when temperatures were greater than 18° C and wind speed was greater than 24 km/hr (Nault & Styer 1969).

In addition to temperature, humidity factors also can impact eriophyid mite behavior and survival. Generally, eriophyid mites disperse under conditions of high humidity and high temperatures (Michalska 2010). In the case of the pigeonpea mite, *Aceria cajani* Channabasavanna, the eriophyid mite that vectors Pigeon pea sterility mosaic virus, mites were less abundant in periods with greater amounts of rainfall; however precipitation had less influence on population density than did temperature and humidity (Kaushik et al. 2013). In periods of relative humidity between 61% and 64%, mite populations were greatest, but in periods of higher humidity (68% and 79%), the mites did not thrive. In *P. fructiphilus* specifically, low humidity may inhibit growth, but this species may not need extreme levels of high humidity as refuge mites are less at risk for desiccation (Hoy 2013, Sabelis & Bruin 1996). One study observed that population densities of *P. fructiphilus* were greater at 60% relative humidity than at 20% or 95% relative humidity within a controlled growth chamber (Monterrosa et al. 2021). Within controlled growth chambers, the eriophyid mite *Aceria tulipae* Keifer dispersed more frequently in periods of light than dark (Nault & Styer 1969). Populations of the pear rust mite, *Epitrimerus pyri* Nalepa, were more abundant in periods of light than dark in both controlled and field conditions (Bergh 1992).

As temperatures in field conditions are less precise than studies conducted in controlled growth chambers, this field study examined the temperature range that coincides with declines in populations of *P. fructiphilus*, but more importantly also the temperature range in which populations of *P. fructiphilus* boom. This study also

observed the effects of precipitation, temperature, wind speed, humidity, and solar radiation on populations of eriophyid mites (*P. fructiphilus, Eriophyes eremus* Druciarek & Lewandowski and *Callyntrotus schlechtendali* Nalepa) in Tennessee.

Materials and Methods

This study was conducted from June 28 to October 29, 2021 and June 27 to October 28, 2022 (18-week period each year) in the rose rosette trial field at the University of Tennessee AgResearch Plateau Research and Education Center, Crossville, TN. The elevation at this location is 551.7 m. The weather station, located 85 m from the rosette trial field, monitored temperature, precipitation, wind speed, solar radiation, and humidity. These environmental parameters were monitored to observe how the combination of these environmental factors affect populations of *P. fructiphilus*. Roses were visually assessed for RRD symptoms and tested for RRV using TaqMan RT-qPCR. The roses were positive for RRV. Mite populations were surveyed using the trap types described in Chapter 3. Tissue samples were collected from the roses, once each month, to ensure the presence of eriophyid mites. The type of rose tissue collected was that which displayed symptoms of RRD (see Chapter 1 for description of symptoms), primarily the sepals, buds, stipules, and petioles that *P. fructiphilus* most often inhabits (Otero-Colina et al. 2018).

Samples were taken to the laboratory, where the mites were extracted from the rose tissue by placing 10g of tissue into a 400 ml beaker; 250ml of 70% ethanol was then added and stirred for 5 minutes. Mesh sieves were stacked in order as top no. 80 (180 mm), middle no. 270 (53 mm), and bottom no. 500 (25 mm). The ethanol and

tissue was poured into the stacked sieves and rinsed with 70% ethanol. The top sieve was removed and the middle sieve was rinsed with 70% ethanol and then removed. The contents of the bottom sieve was rinsed into a counting dish and then placed under a dissecting microscope. After 5 minutes, any mites present were counted. For identification, mites were siphoned from the ethanol onto a glass microscope slide using a glass pipette to allow the excess ethanol to evaporate; filter paper was also used to wick away excess ethanol from the slide as needed. A drop of modified Berlese medium was placed on a separate glass slide into which mites were positioned, either ventral or dorsally, using a pin tool. After a coverslip was applied, the slides were cured at 85° C for 1 hour on a hotplate. The slides were viewed under a stereoscope to identify the mite species using dorsal shield and coverflap markings.

Temperature, precipitation, wind speed, solar radiation, and relative humidity data collected at the weather station were downloaded from the National Centers for Environmental Information. These environmental data were compared with the mite data obtained from weekly eriophyid mite trap collection described in Chapter 3 to determine their influence on populations of eriophyid mites.

Five spinning sticky slide traps mounted on a metal shepherd's hook were placed in the rose rosette trial field located 85 m from the weather station to collect ballooning eriophyid mites. The spinning sticky slide trap was loaded with two Tanglefoot-coated glass slides, one on each end of the spinning rod. These glass slides were placed in the rose bed for five days once each week, throughout the 18-week study. After five days the glass slides were taken to the laboratory and observed under a stereoscope. The

number of eriophyid mites present on the slides were counted; however, these mites were not identified to species.

Five spinning bottle traps were placed in the rose rosette trial field. The bottle traps were hung from shepherd's hooks placed near RRD-symptomatic roses. The bottle traps were filled with soapy water and left in the field for five days, then the water was poured through mesh sieves stacked in order as top no. 80, middle no. 270, and bottom no. 500. The contents of the bottom sieve was rinsed with 70% ethanol into a collection tube and then taken to the laboratory. The contents of the tubes were poured into counting dishes and then placed under a dissecting microscope. The mites present were counted. If mites were present, up to 20 mites were mounted onto a slide in Berlese medium and a coverslip placed on top. The slides were cured at 85° C for 1 hour on a hotplate. The slide was placed under a stereoscope for species identification.

Fifteen water pan traps were placed in the rose rosette trial field, near roses that were symptomatic for RRD (these are the same water pan traps used in Chapter 3). The traps were filled with soapy water then left in the field for five days. The water from the pans was poured through stacked sieves (no. 80, no. 270, and no. 500). The contents of the bottom sieve were rinsed with 70% ethanol into a collection tube and then taken to the laboratory. The contents of the tube were poured into a counting dish, and any mites present were counted; if present up to 20 mites were mounted in Berlese medium then identified under a microscope.

Data Analysis

All data were analyzed using ANOVA GLIMMIX. LSMeans was used to determine differences among the means. For significance, p values were set at 0.05.

Results

All average environmental data and average mite densities collected during 2021 and 2022 are presented in Table 9. In 2021, the mite population spiked during September 27-October 01. The average temperature during this week was 18.1° C with no recorded precipitation. The wind speed was 4.4 10 meters per second (10 m/s). The solar radiation was 17.7 MJ/M2. The average relative humidity was 89.4%. In 2022, the fewest mites were collected the August 8-12. During this week, temperatures averaged 22.0° C, precipitation 3.9 mm, wind speed 5.3 10 m/s, solar radiation 17.6 MJ/M2, and relative humidity 89.9%. The largest number of mite collected was during the week of September 12-16; the temperature was 16.5° C, zero precipitation, 4.9 10 m/s wind speed, 19.6 MJ/M2 solar radiation, and 82.8% relative humidity. The following results are for all combined data collected in 2021 and 2022.

The average number of mites/trap was significantly ($p<0.002$) impacted by average weekly temperature, and this effect was a negative relationship (Figure 21). As the temperature increased, the average number of mites decreased. Previous studies show a positive correlation between temperature and mite populations (Joseph 2022). However, this difference between studies may be attributed to the methodology of each study, as the present study was conducted under ambient, field conditions, while the Joseph (2022) study was conducted in a temperature-controlled growth chamber.

The number of mites/trap was significantly ($p<0.001$) impacted by average weekly precipitation, and this effect was a negative relationship (Figure 22). As the amount of precipitation increased, the number of mites collected decreased. More mites were collected during periods with less precipitation. These data agrees with previous findings in literature (Muraleedharan & Chandrasekharan 1981).

Wind had no statistically significant ($p>0.05$) effect on the number of mites collected. Previous studies found mite population density positively associated with wind speed. Further study may be needed to determine why the findings in this study deviated from previous findings, and if perhaps the other four environmental factors had an interactive influence on the possible impact of wind speed.

Solar radiation had a significant ($p<0.0001$) effect on the number of mites collected ($p<0.0001$), and this effect was a positive relationship. As average solar radiation decreased, the number of mites also decreased. For example, solar radiation steadily decreased from July to October, and mite populations also decreased steadily from August to October. These data agree with previous findings in literature (Sabelis & Bruin 1996).

The average relative humidity had a significant ($p<0.0001$) effect on the average number of mites/trap, and this effect was a negative relationship (Figure 23). Relative humidity varied from 78.9% to 94.8% during 2021 and from 77.1% to 94.1% in 2022. Previous studies, using a growth chamber, found *P. fructiphilus* populations were greatest at a relative humidity at 60% than at low (20%) or high (95%) relative humidities. Further research that includes observations during months with lower relative humidity is needed in Tennessee.

Eriophyid mites were present the week of July 20-24 2021 and June 27-July 1 2022. In 2021, the fewest mites (n=0) were collected during July 26-30. During this period, the average temperature was 23.8° C which aligns with a previous study which documented less population densities at temperatures of 15° or 21° C than at or 32° C (Joseph 2022). The precipitation was 0.7 mm. The wind speed was 5.4 10 m/s but wind speed did not significantly impact mite populations during this study. Solar radiation during this period was an average of 23.6 MJ/M2, which was the highest level of solar radiation during the two-year study. This period had an average relative humidity of 91.2% which agrees with a study that showed that populations of *P. fructiphilus* thrived at 60% humidity as opposed to 95% or 20% (Monterrosa et al. 2021).

Discussion

This study documented a significant influence of temperature on eriophyid mite populations. A negative correlation was found between the number of mites collected and temperature; these findings did not agree with previous literature, which documented a positive correlation (Joseph 2022). As mentioned previously, this difference between studies may be attributed to the methodology of each study, as the present study was conducted under ambient, field conditions, while the Joseph (2022) study was conducted in a temperature-controlled growth chamber. Further research is under field conditions is needed to determine if this phenomenon holds true when data are collected throughout an entire year, including winter months.

Fewer mites were collected during periods of precipitation, which previous studies have documented. The speed of wind had no significant effect on the mites during this study.

Solar radiation was found to have a positive effect on eriophyid mite populations, agreeing with previously published literature. Relative humidity was found to have an inverse relationship with the population of eriophyid mites. The results of this study will help growers determine and refine integrated pest management programs for *P. fructiphilus* on roses.

Summary

This 18-week study collected data from a weather station located at the University of Tennessee AgResearch Plateau Research and Education Center in Crossville, TN. Mites were collected weekly using eriophyid mite collection traps. These mite collections were compared with data collected from the weather station and then downloaded from the National Centers for Environmental Information.

Temperature, precipitation, and relative humidity had an inverse relationship to the number of mites collected. Wind had no statistically significant effect on the number of mites collected. Solar radiation had a positive correlation on the number of mites collected.

Rose growers may experience higher *P. fructiphilus* populations during periods of higher temperatures, low precipitation, higher solar radiation, and medium levels of relative humidity. Knowing the environmental factors that influence eriophyid mite populations and how these factors influence population growth or decline can help rose growers take proactive measures against *P. fructiphilus*.

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Appendix

Table 1. Locations sampled and number of plants sampled.

Rosa spp. Sampled	Year	County	No. of Locations	No. of Plants
Rosa multiflora (multiflora rose)	2021	Campbell	2	2
	2022	Campbell	2	2
	2021	Cumberland	2	4
	2022	Cumberland	2	4
Rosa sp. (Knock Out rose)	2021	Cumberland	2	8
	2022	Cumberland	2	8
	2021	Davidson	1	8
	2022	Davidson	1	8
	2021	Knox	2	8

Table 2. Densities of *Phyllocoptes fructiphilus* on multiflora rose in Campbell and Cumberland Counties, Tennessee, 2021 and 2022.

County	Month	2021	2022	2021 and 2022 combined
Campbell County	June	No Data	0.00* ± 0.00	0.00* ± 0.00
	July	0.02 ± 0.02	0.43 ± 0.61	0.22 ± 0.42
	August	0.08 ± 0.11	0.83 ± 0.04	0.45 ± 0.44
	September	0.13 ± 0.43	0.43 ± 0.61	0.28 ± 0.41
	October	0.24 ± 0.34	0.22 ± 0.05	0.23 ± 0.20
	November	No Data	No Data	No Data
Cumberland County	June	0.50 ± 0.71	0.43 ± 0.54	0.47 ± 0.52
	July	0.50 ± 0.71	0.15 ± 0.21	0.33 ± 0.47
	August	0.50 ± 0.71	0.53 ± 0.60	0.51 ± 0.54
	September	0.44 ± 0.62	0.61 ± 0.39	0.52 ± 0.43
	October	0.30 ± 0.12	0.49 ± 0.36	0.40 ± 0.25
	November	No Data	No Data	No Data

* Number (± SD) of mites/g of rose tissue.

Table 3. Densities of *Phyllocoptes fructiphilus* on Knock Out roses in Cumberland, Davidson, and Knox Counties, Tennessee, 2021 and 2022.

County	Month	2021	2022	2021 and 2022 combined
Cumberland County	June	1.00 ± 0.00	0.85 ± 0.21	0.93 ± 0.15
	July	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
	August	0.95 ± 0.07	0.93 ± 0.11	0.94 ± 0.08
	September	0.96 ± 0.05	0.87 ± 0.18	0.92 ± 0.12
	October	0.76 ± 0.34	0.84 ± 0.06	0.80 ± 0.21
	November	No Data	No Data	No Data
Davidson County	June	No Data	No Data	No Data
	July	No Data	1.00 ± 0.00	1.00 ± 0.00
	August	1.00 ± 0.00	No Data	1.00 ± 0.00
	September	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
	October	No Data	1.00 ± 0.00	1.00 ± 0.00
	November	1.00 ± 0.00	No Data	1.00 ± 0.00
Knox County	June	0.99 ± 0.01	1.00 ± 0.00	0.99 ± 0.01
	July	0.96 ± 0.01	1.00 ± 0.00	0.98 ± 0.02
	August	0.99 ± 0.01	1.00 ± 0.00	0.99 ± 0.01
	September	1.00 ± 0.00	0.97 ± 0.04	0.99 ± 0.03
	October	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
	November	1.00 ± 0.00	No Data	1.00 ± 0.00

* Number (± SD) of mites/g of rose tissue.
[t] Samples in Davidson and Knox Counties were collected only one year in some months.

Table 4. Influence of trap type on number of eriophyid mites collected using three trap types on roses, Crossville, TN, 2021.

Week	Collection Period	Spinning Sticky Trap	Spinning Bottle Trap	Water Trap Gray	Water Trap Red	Water Trap Yellow
1	06/28/21-0702/21	N/A	N/A	N/A	N/A	N/A
2	07/5/21-07/9/21	N/A	N/A	N/A	N/A	N/A
3	07/12/21-07/16/21	N/A	N/A	N/A	N/A	N/A
4	07/20/21-07/24/21	1	3	2	N/A	N/A
5	07/26/21-07/30/21	0	0	0	N/A	N/A
6	08/02/21-08/06/21	7	0	1	N/A	N/A
7	08/09/21-08/13/21	9	1	0	N/A	N/A
8	08/16/21-08/20/21	N/A	N/A	N/A	N/A	N/A
9	08/23/21-08/26/21	3	4	2	N/A	N/A
10	08/30/21-09/03/21	N/A	N/A	N/A	N/A	N/A
11	09/06/21-09/10/21	N/A	N/A	N/A	N/A	N/A
12	09/13/21-09/17/21	N/A	N/A	N/A	N/A	N/A
13	09/20/21-09/24/21	N/A	N/A	N/A	N/A	N/A
14	09/27/21-10/01/21	1	15	3	2	6
15	10/04/21-10/08/21	N/A	N/A	N/A	N/A	N/A
16	10/11/21-10/15/21	1	1	2	3	0
17	10/18/21-10/11/21	1	4	9	0	8
18	10/25/21-10/29/21	N/A	N/A	N/A	N/A	N/A

* Number of mites collected weekly per trap type.

Table 5. Comparison of average total number of eriophyid mites collected using three trap types on roses, Crossville, 2021 and 2022.

Trap Type	Average Total No. Mites/Trap
Spinning Bottle Trap	0.60 ± 1.31 a*
Water Pan Trap	1.28 ± 2.14 b
Spinning Sticky trap	0.09 ± 0.46 c

*Data analyzed using GLIMMIX LSMeans ($p < 0.05$); means followed by the same letter are not significantly different.

Table 6. Comparison of average total number of eriophyid mites collected using three colors of pan traps on roses, Crossville, 2021 and 2022.

Pan Trap Color	Average Total No. Mites/Trap
Gray	1.04 ± 1.81 a*
Red	1.26 ± 1.61 a
Yellow	1.63 ± 2.89 a

*Data analyzed using GLIMMIX LSMeans ($p < 0.05$); means followed by the same letter are not significantly different.

Table 7. Comparison of average number of *P. fructiphilus* collected using three colors of pan traps on roses, Crossville, 2021 and 2022.

Pan Trap Color	Average No. Mites/Trap
Gray	0.60 ± 1.29 a*
Red	0.79 ± 1.31 a
Yellow	1.09 ± 2.52 a

*Data analyzed using GLIMMIX LSMeans ($p < 0.05$); means followed by the same letter are not significantly different.

Table 8. Influence of trap type on number of eriophyid mites collected using three trap types on roses, Crossville, TN, 2022.

Week	Collection Period	Spinning Sticky Trap	Spinning Bottle Trap	Water Trap Gray	Water Trap Red	Water Trap Yellow
1	06/27/22-07/01/22	13	9	2	3	0
2	07/04/22-07/08/22	11	0	4	4	5
3	07/11/22-07/15/22	3	4	5	14	3
4	07/18/22-07/22/22	7	3	4	4	3
5	07/25/22-07/29/22	N/A	N/A	N/A	N/A	N/A
6	08/01/22-08/05/22	N/A	N/A	N/A	N/A	N/A
7	08/08/22-08/12/22	0	1	2	2	2
8	08/15/22-08/19/22	0	1	5	4	2
9	08/22/22-08/26/22	4	3	4	8	8
10	08/29/22-09/02/22	12	0	15	11	9
11	09/03/22-09/07/22	11	0	5	5	7
12	09/12/22-09/16/22	8	6	39	18	49
13	09/19/22-09/23/22	10	4	14	4	15
14	09/28/22-10/02/22	25	11	4	18	25
15	10/03/22-10/07/22	21	6	9	5	14
16	10/10/22-10/14/22	N/A	N/A	N/A	N/A	N/A
17	10/17/22-10/21/22	N/A	N/A	N/A	N/A	N/A
18	10/24/22-10/28/22	N/A	N/A	N/A	N/A	N/A

* Number of mites collected weekly per trap type.

Table 9. Comparison of environmental factors and eriophyid mite densities in roses, Crossville, TN 2021 and 2022.

Year	Month	Week	Collection Date	Temperature C	Precipitation MM	Wind Max 10S	Solar Radiation MJ/M2	Average Relative Humidity %	Total Mites Collected
2021	June	1	07/02/21	N/A	N/A	N/A	N/A	N/A	0
2021	July	2	07/09/21	N/A	N/A	N/A	N/A	N/A	0
2021	July	3	07/16/21	N/A	N/A	N/A	N/A	N/A	0
2021	July	4	07/24/21	21.96	0.0	4.14	23.0	88.96	6
2021	July	5	07/30/21	23.84	0.7	5.375	23.6	91.19	0
2021	August	6	08/06/21	20.0	0.0	4.8	21.5	78.94	8
2021	August	7	08/13/21	23.8	0.2	5.5	19.9	83.42	10
2021	August	8	08/20/21	21.6	5.1	4.8	11.1	93.70	N/A
2021	August	9	08/26/21	23.1	0.7	4.0	19.8	87.22	9
2021	September	10	09/03/21	19.9	10.7	5.6	13.7	90.18	N/A
2021	September	11	09/10/21	18.6	0.0	6.0	20.8	80.99	N/A
2021	September	12	09/17/21	20.5	0.6	5.4	12.9	88.38	N/A
2021	September	13	09/24/21	15.5	5.6	6.2	12.1	89.79	N/A
2021	October	14	10/01/21	18.1	0.0	4.4	17.7	89.45	27
2021	October	15	10/08/21	17.9	21.3	5.5	8.1	94.75	N/A
2021	October	16	10/15/21	19.3	1.4	5.2	10.3	90.55	7
2021	October	17	10/22/21	11.8	1.3	5.3	11.9	86.03	22
2021	October	18	10/29/21	10.3	2.5	7.3	7.0	88.27	N/A
2022	June	1	07/01/22	22.3	0.0	4.9	22.8	77.39	27
2022	July	2	07/08/22	24.4	6.4	7.6	21.7	85.34	24
2022	July	3	07/15/22	22.2	1.3	5.5	23.3	80.74	29
2022	July	4	07/22/22	23.8	14.5	6.5	18.8	89.80	21
2022	July	5	07/29/22	24.1	22.0	6.9	14.1	86.66	N/A
2022	August	6	08/05/22	23.0	1.6	6.0	17.5	87.59	N/A
2022	August	7	08/12/22	22.0	3.9	5.3	17.6	89.97	7
2022	August	8	08/19/22	20.2	0.0	4.8	17.5	86.15	12
2022	August	9	08/26/22	21.0	1.4	4.0	17.4	89.03	27
2022	August	10	09/02/22	21.3	0.0	5.5	19.8	81.62	47
2022	September	11	09/07/22	21.1	20.2	4.9	10.3	94.09	28
2022	September	12	09/16/22	16.5	0.0	4.9	19.6	82.83	120
2022	September	13	09/23/22	19.1	0.0	4.5	17.0	78.80	47
2022	September	14	10/03/22	12.0	0.0	7.7	18.2	77.22	83
2022	October	15	10/07/22	12.4	0.0	5.5	16.7	77.05	55
2022	October	16	10/14/22	N/A	N/A	N/A	N/A	N/A	N/A
2022	October	17	10/21/22	N/A	N/A	N/A	N/A	N/A	N/A
2022	October	18	10/28/22	N/A	N/A	N/A	N/A	N/A	N/A

*Total mites collected from eriophyid mite traps (see Chapter 3).



Figure 1. Comparison of environmental factors and eriophyid mite densities in roses, Crossville, TN 2021 and 2022.

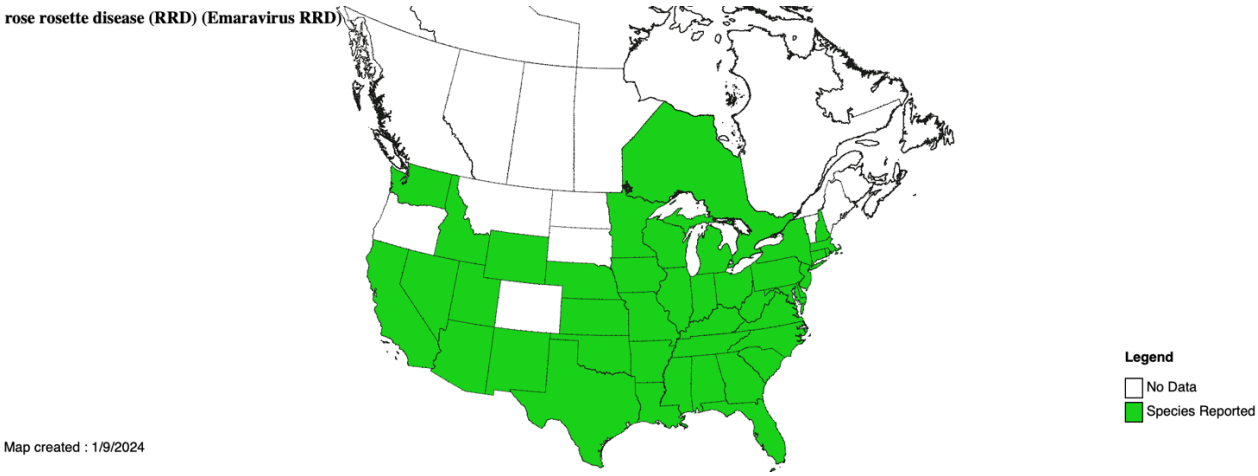


Figure 2. Rose rosette disease (RRD) distribution map as of January 9, 2024 (EDDMapS 2024).



Figure 3. Symptom of Rose rosette disease (RRD) classified as excessive thorning. *Note.* RRD symptoms seen in photo: excessive thorn production, thickened stems, excessive red pigmentation, and strappy leaves. Photo credit: Alan Windham, 2017, UT Soil, Plant and Pest Center. https://www.facebook.com/SoilPlantPestCenter/photos/a.255177287859526/1556533437723898



Figure 4. Symptom of Rose rosette disease (RRD) classified as leaf mosaic/mottling. *Note*. Photo shows leaf distortion and mosaic/mottling pattern. Photo credit: Jennifer Olson, 2015, Oklahoma State University, Bugwood.org. https://www.ipmimages.org/browse/detail.cfm?imgnum=5529048



Figure 5. Symptom of Rose rosette disease (RRD) classified as witches' broom.

Note. Ornamental rose displaying normal growth (bottom) and RRD symptoms (top): witches' broom, hyper pigmentation, and leaf distortion. Photo credit: Alan Windham, 2014, UT Soil, Plant and Pest Center. https://www.facebook.com/SoilPlantPestCenter/photos/pcb.750662748310975/750660921644491/



Figure 6. Rose rosette disease (RRD) symptoms on *Rosa multiflora*.

Note. Multiflora rose displaying RRD symptoms of distorted leaves and red pigmentation. Photo credit: James W. Amrine Jr., 2002, West Virginia University, Bugwood.org. https://www.ipmimages.org/browse/detail.cfm?imgnum=0002054

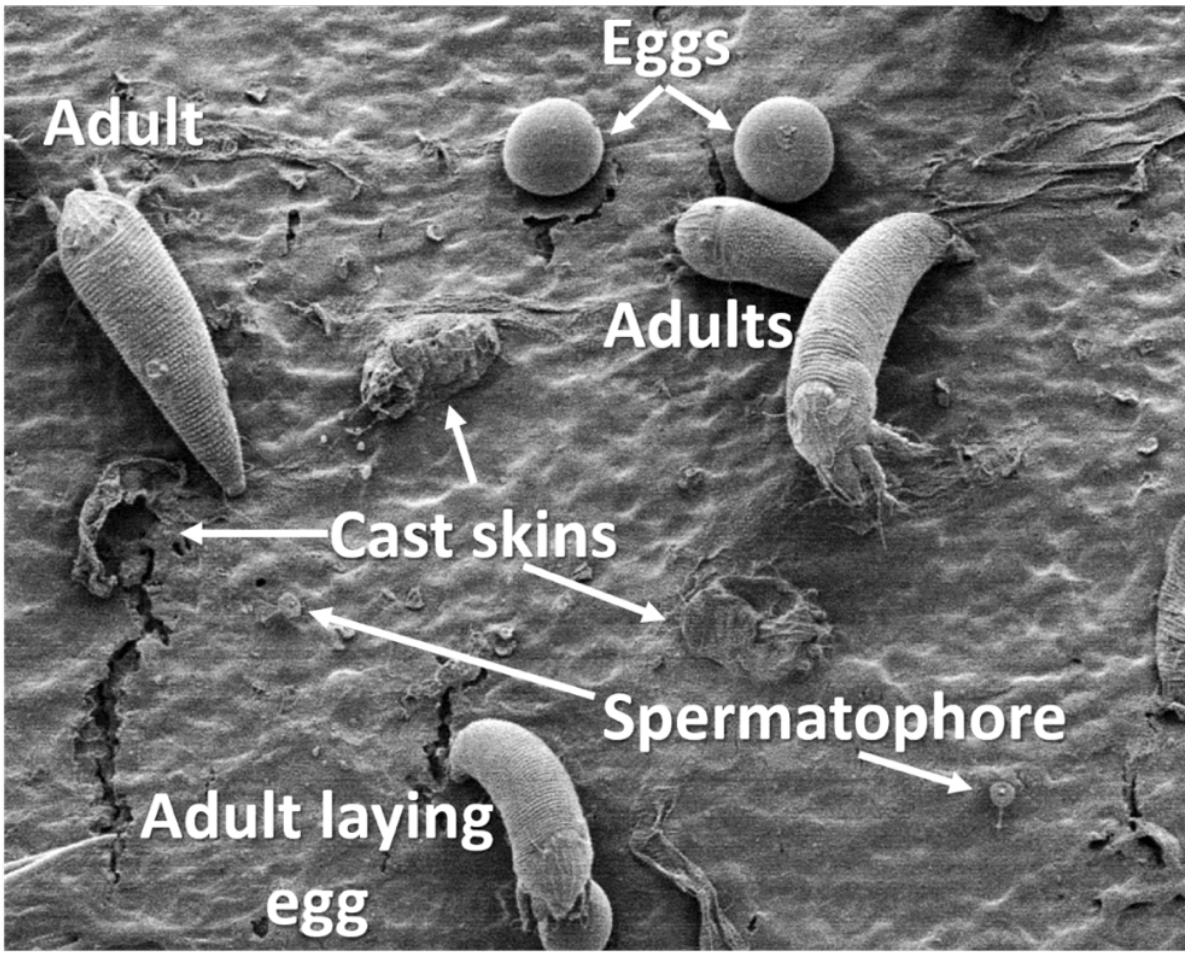


Figure 7. *Phyllocoptes fructiphilus* adults, eggs, spermatophores, and cast skins. *Note*. Some of the pictured adults may be deutonymphs but this cannot be confirmed due to mite orientation. Photo credit: G. Bauchan and R. Ochoa, USDA ARS, Beltsville, MD.

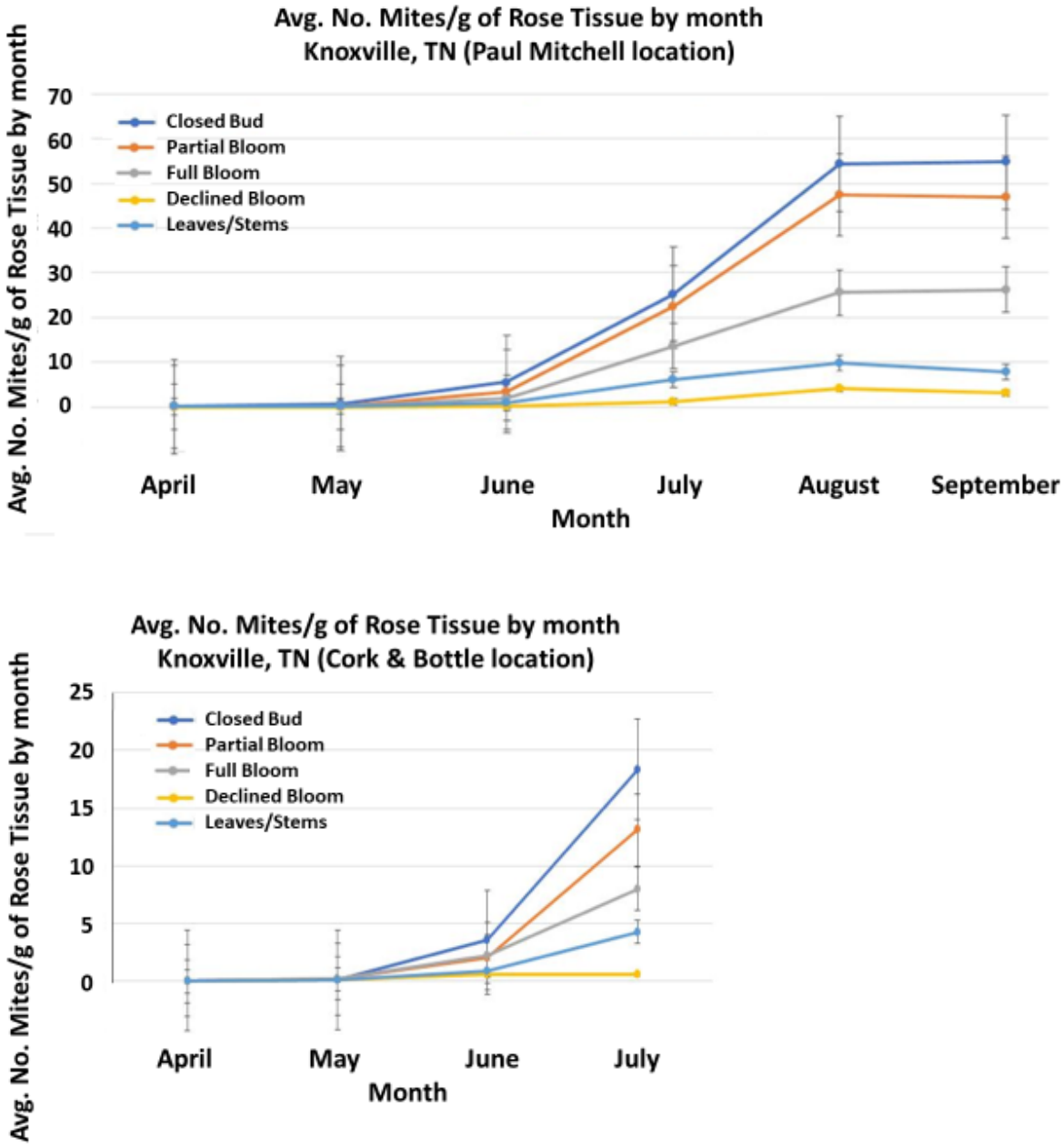


Figure 8. Population levels of *Phyllocoptes fructiphilus* on Knock Out roses 2020. *Note*. Population levels of *P. fructiphilus* at two locations in Knoxville, TN for mites collected on different types (as well as ages) of plant parts.

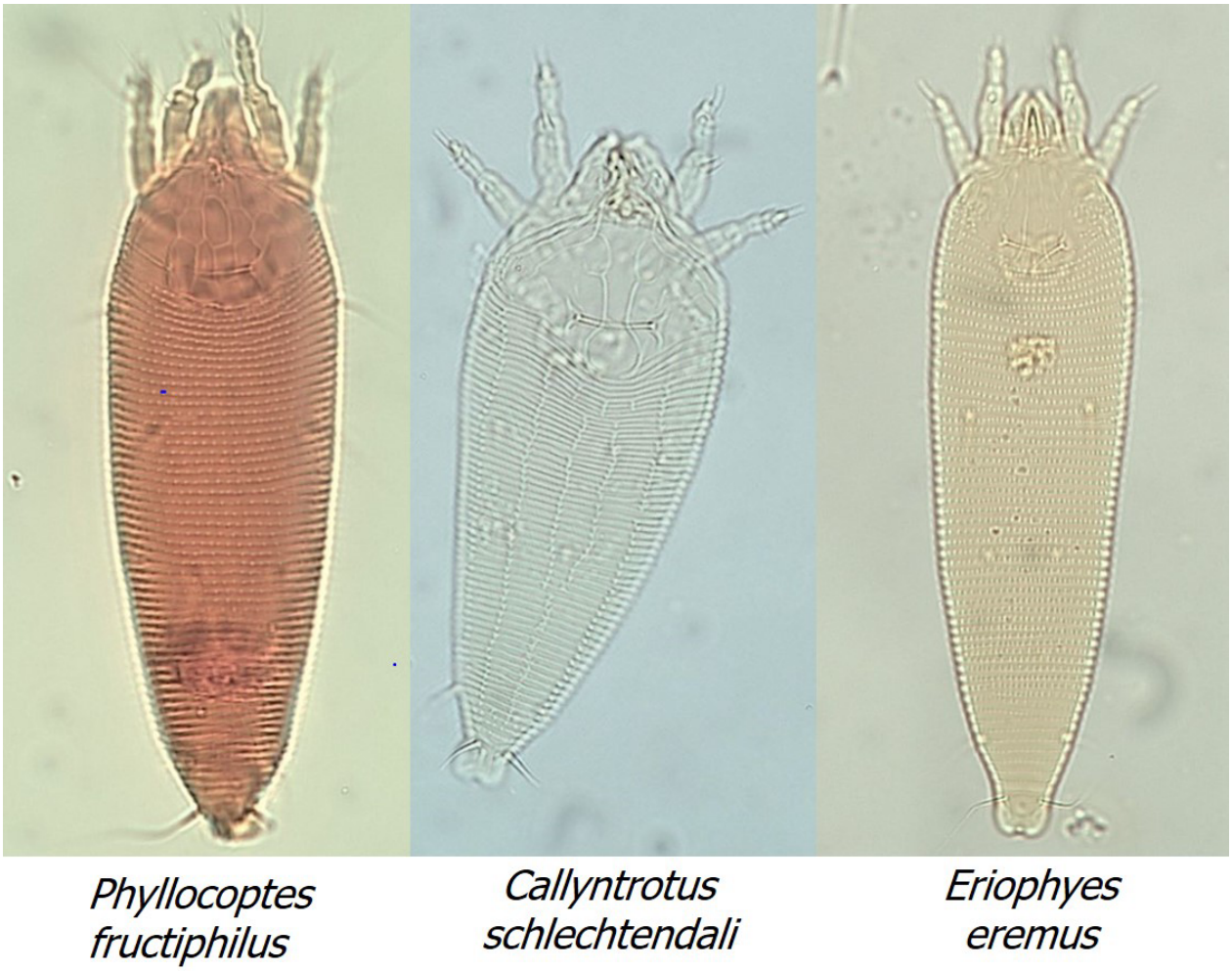


Figure 9. Comparison of *Phyllocoptes fructiphilus*, *Callyntrotus schlechtendali*, and *Eriophyes eremus*.
Note: Photo credit: Sara Collins and Katherine Solo, University of Tennessee.



Figure 10. Mite extraction using ethanol.

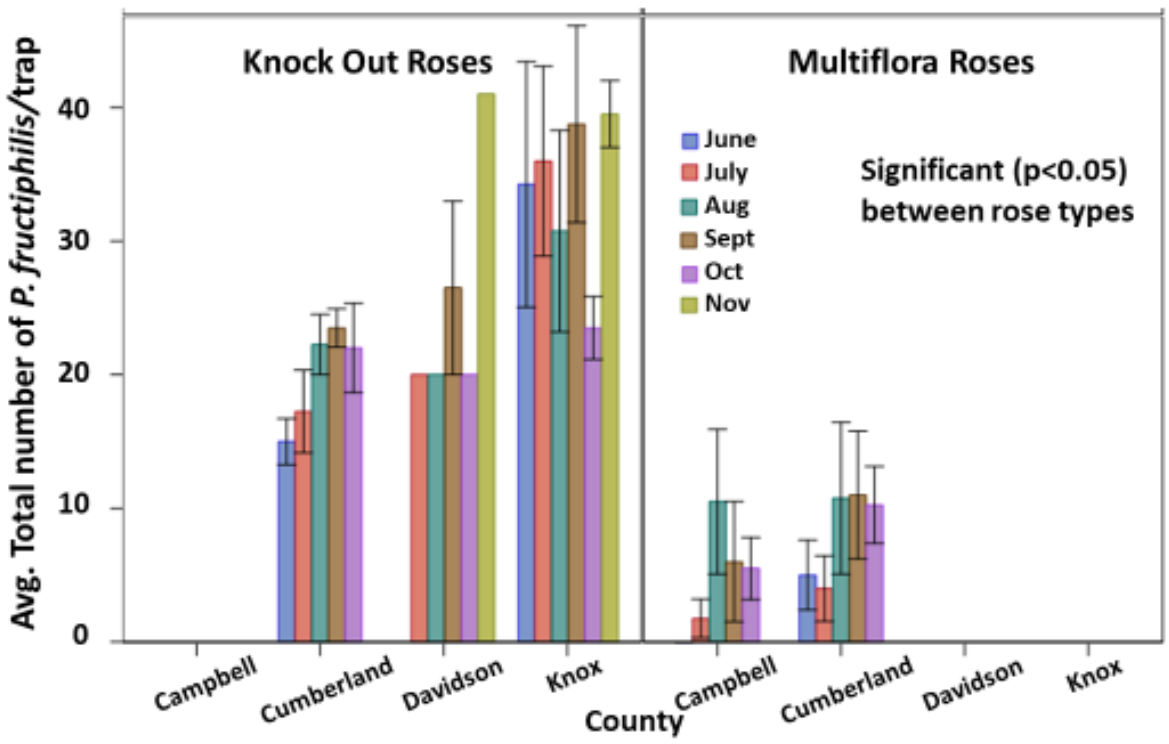


Figure 11. Total number of *P. fructiphilus* on Knock Out roses and *R. multiflora* combined for 2021 and 2022 in Tennessee.

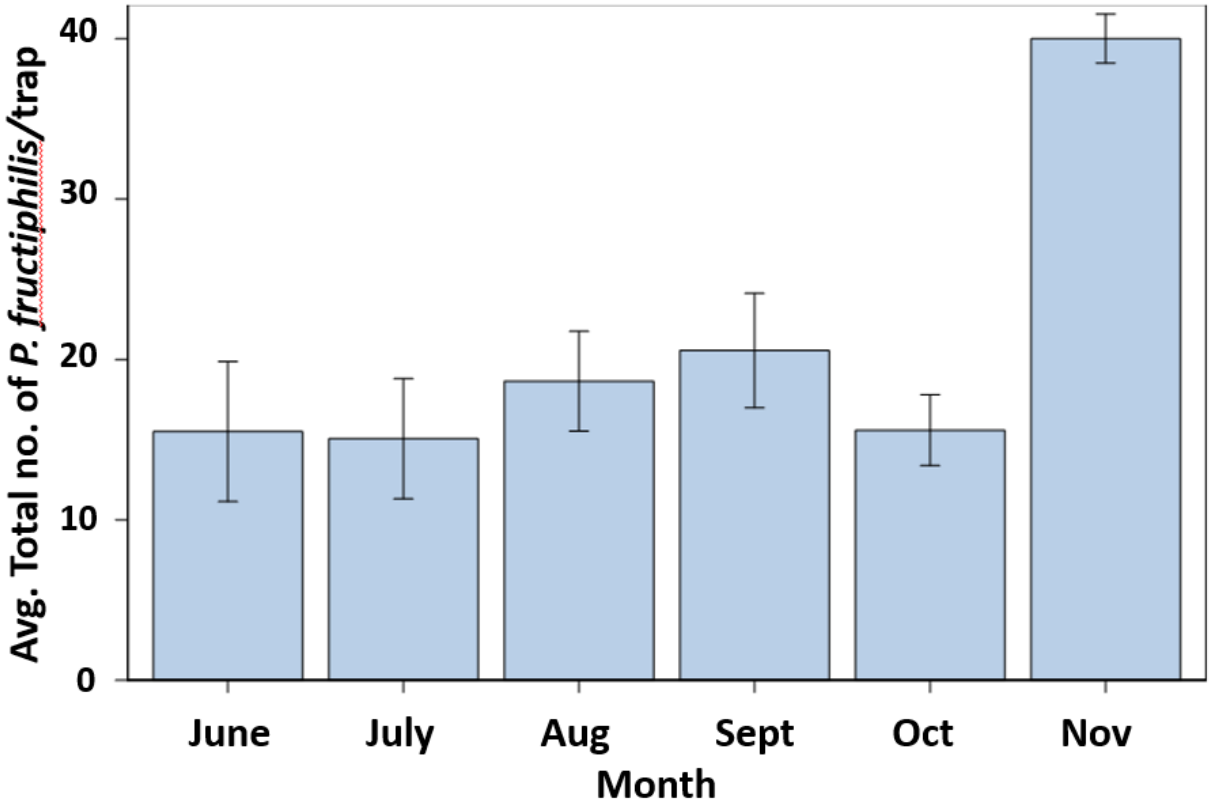


Figure 12. Seasonal incidence of populations of *P. fructiphilus* on roses combined for 2021 and 2022 in Tennessee.

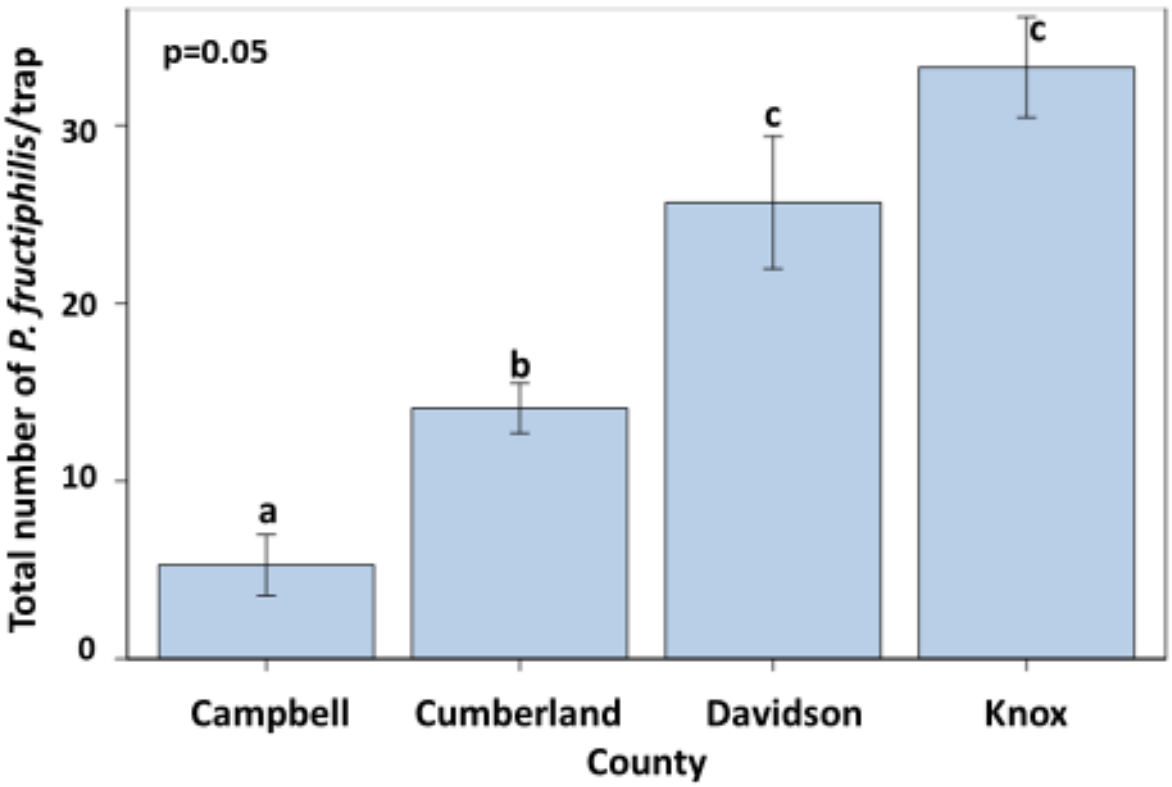


Figure 13. Densities of *P. fructiphilus* on roses in four counties in Tennessee, combined for 2021 and 2022.



Figure 14. Spinning bottle trap used to assess ballooning eriophyid mites on roses, Crossville, TN.

Note. Designed by Sara Collins, University of Tennessee.



Figure 15. Water pan trap used to assess ballooning eriophyid mites on roses, Crossville, TN.



Figure 16. Spinning sticky trap used to assess ballooning eriophyid mites in roses, Crossville, TN.

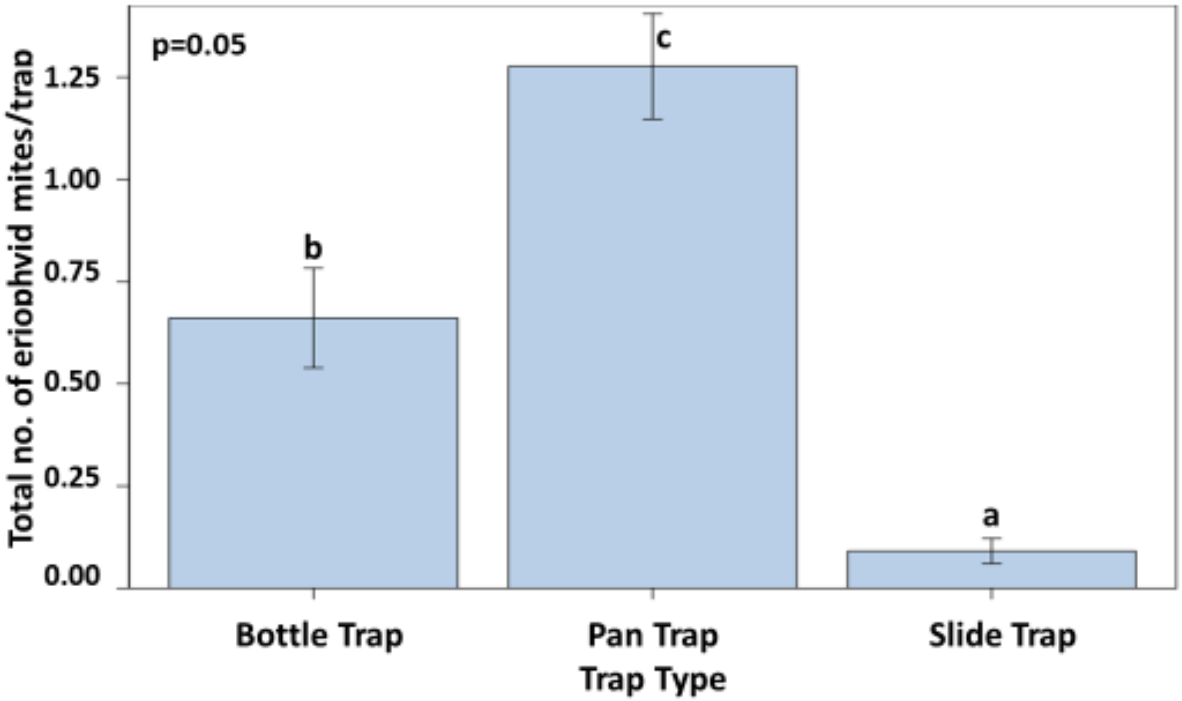


Figure 17. Comparison of total number of eriophyid mites collected in three trap types placed in roses, Crossville, TN, combined for 2021 and 2022.

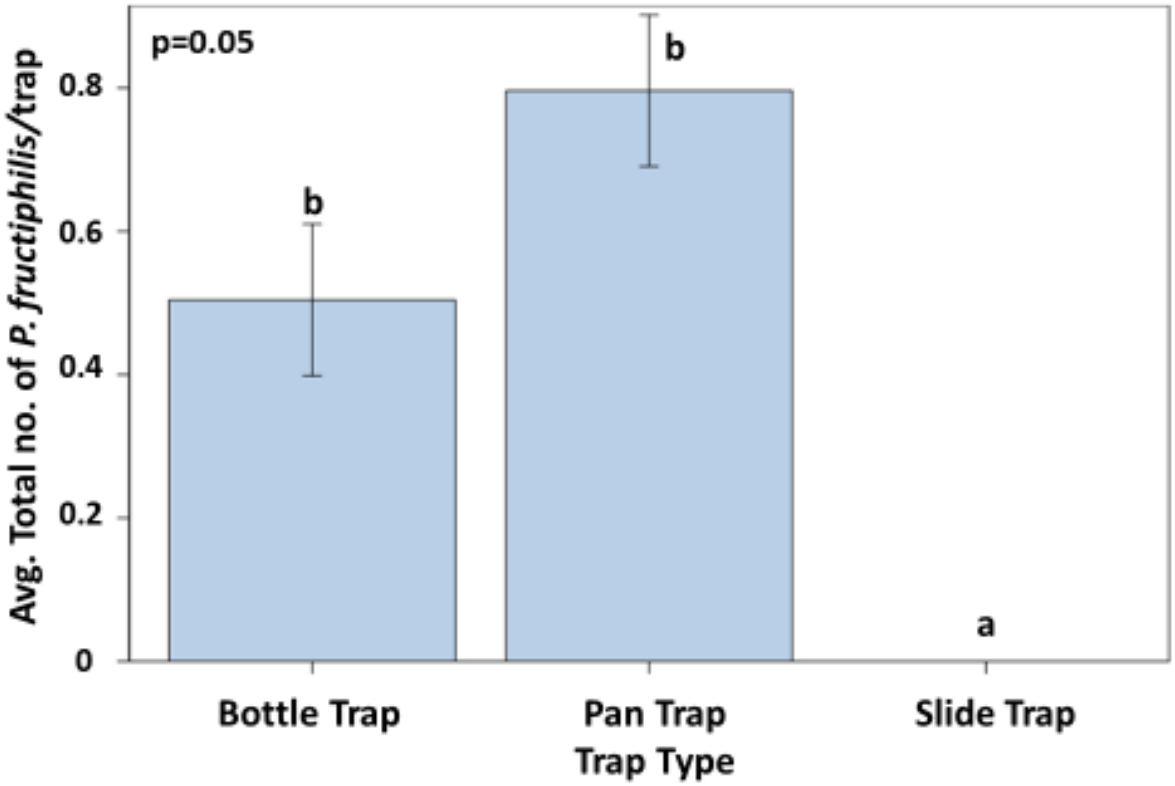


Figure 18. Comparison of total number of *Phyllocoptes fructiphilus* collected in three trap types placed in roses, Crossville, TN, combined for 2021 and 2022.
Note. Mites collected on sticky traps could not be identified to species.

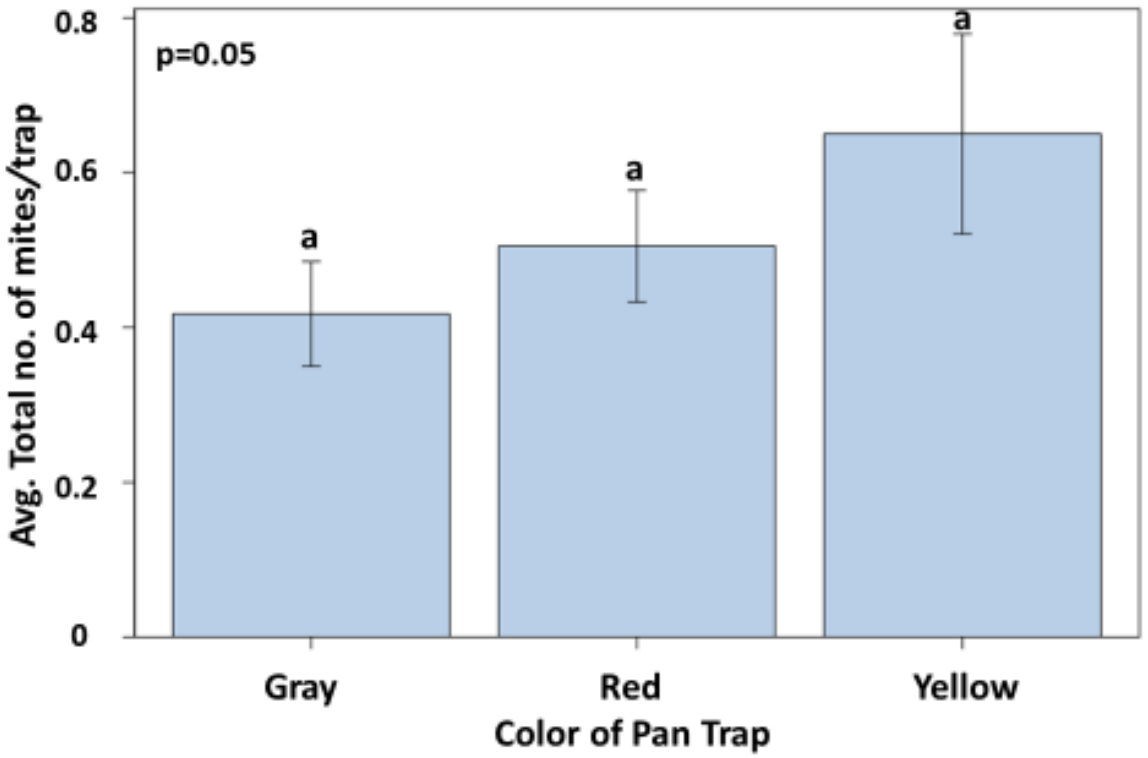


Figure 19. Influence of trap color of water pan traps on total number of eriophyid mites collected in roses, Crossville, TN, combined for 2021 and 2022.

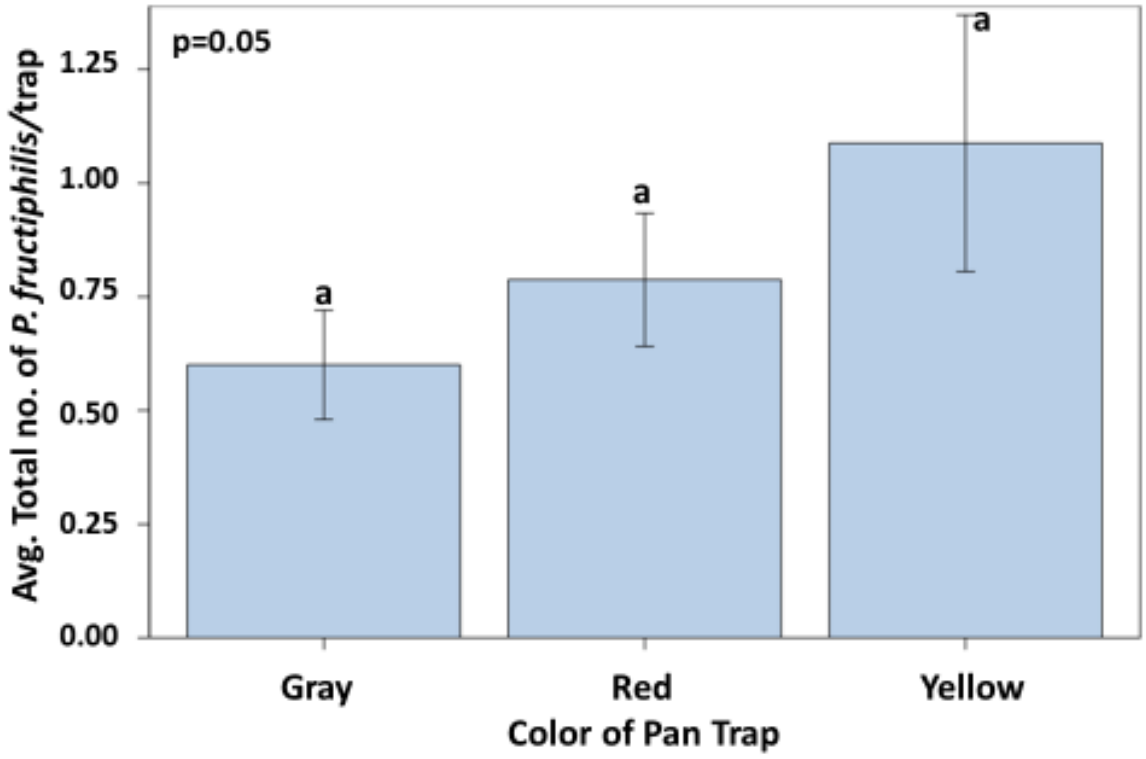


Figure 20. Influence of trap color of water pan traps on total number of *Phyllocoptes fructiphilus* collected in roses, Crossville, TN, combined for 2021 and 2022.

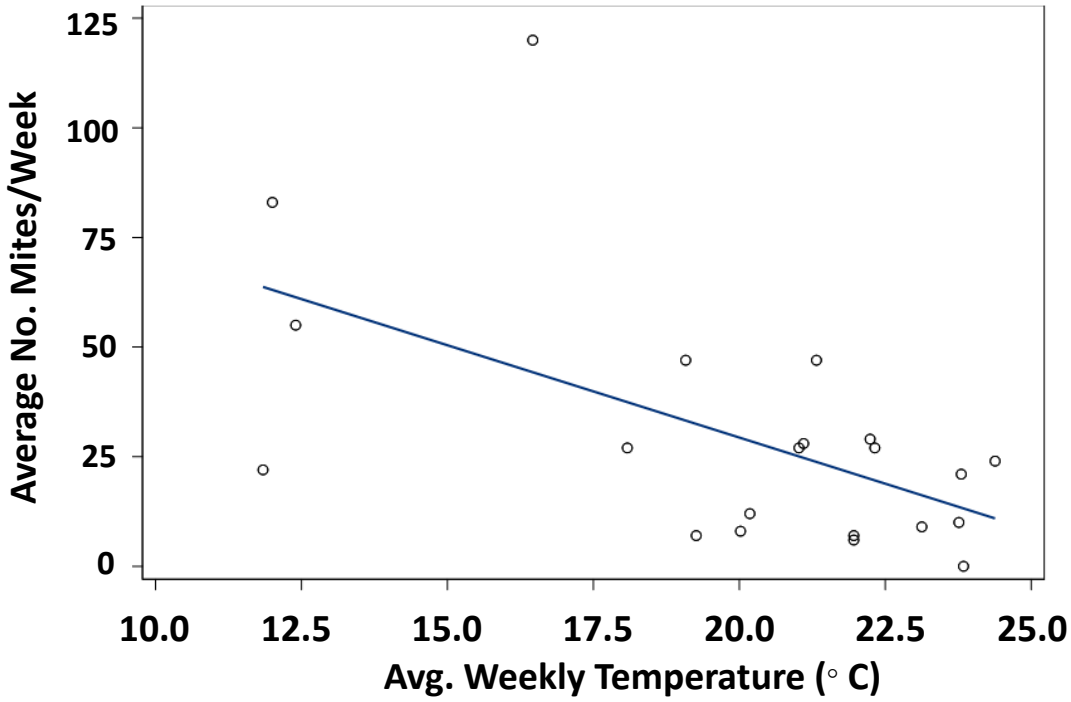


Figure 21. Influence of average weekly temperature on the average number of mites/week.

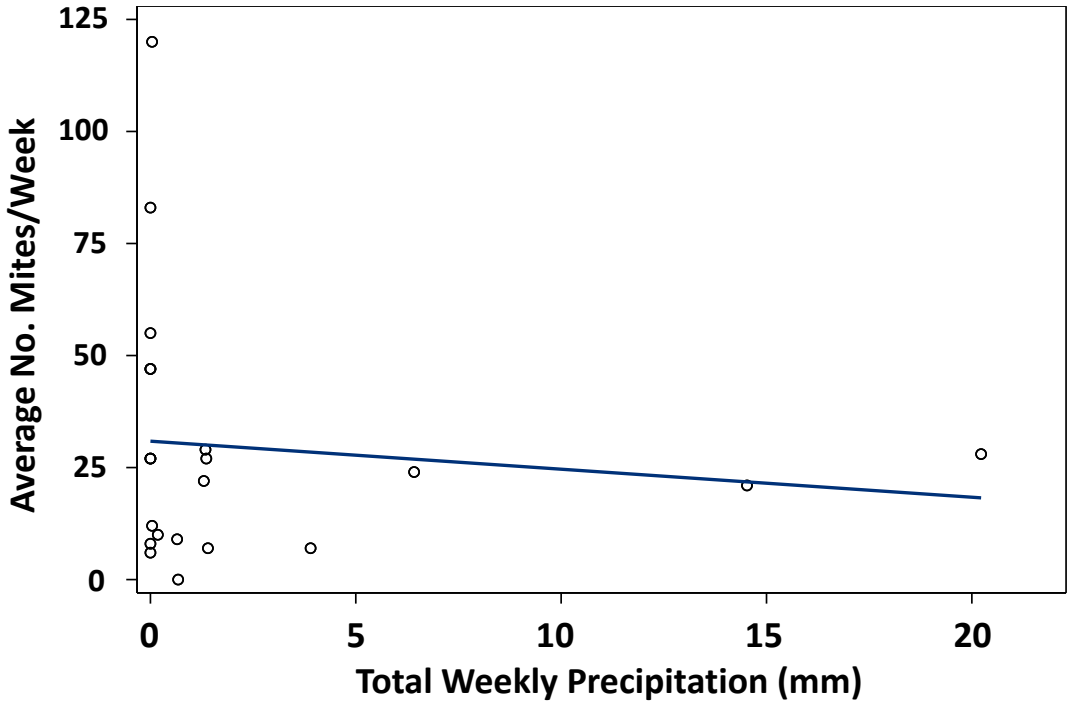


Figure 22. Influence of total weekly precipitation (mm) on the average number of mites/week.

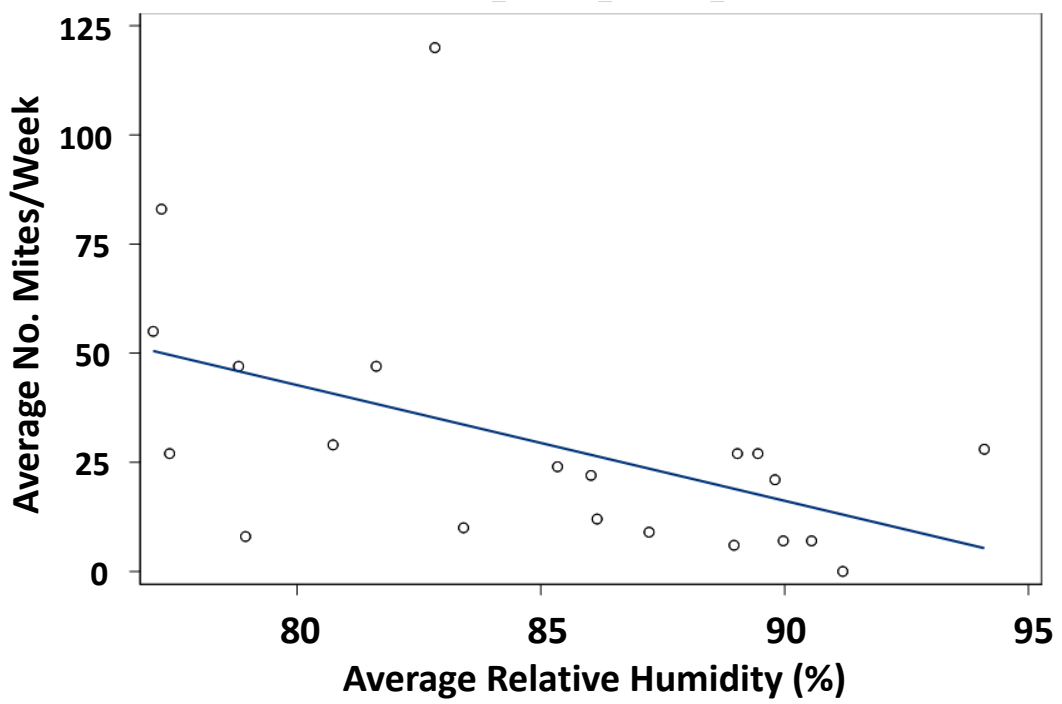


Figure 23. Influence of average relative humidity (%) on the average number of mites/week.

Vita

Tara Barnette grew up in Wise, Virginia, and she spent her summers in Baltimore, Maryland. After high school, she worked in customer service. She relocated to Knoxville, Tennessee, where she attended Pellissippi State Community College and received an Associate of Science degree. She transferred to the University of Tennessee, Knoxville, where she earned a Bachelor of Science degree in Plant Science in 2020. She is currently pursuing a Master of Science degree in Entomology and Plant Pathology with a concentration in Plant Pathology.