A Survey of *Rose rosette virus* and Eriophyid Mites Associated with Roses in the Southeastern United States

Katherine M. Solo and Sara B. Collins

Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN 37996

Madalyn K. Shires

Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77845

Ron Ochoa

U.S. Department of Agriculture, Agricultural Research Service, Systematic Entomology Laboratory, Beltsville, MD 20705

Gary R. Bauchan

U.S. Department of Agriculture, Agricultural Research Service, Electron and Confocal Microscopy Unit, Beltsville, MD 20705

Liesel G. Schneider

Department of Animal Science, University of Tennessee, Knoxville, TN 37996

Alan Henn

Department of Biochemistry, Molecular Biology, Entomology, and Plant Pathology, Mississippi State University, Starkville, MS 39762

James C. Jacobi

Alabama Cooperative Extension System, Auburn University, Birmingham, AL 35223

Jean L. Williams-Woodward

Department of Plant Pathology, University of Georgia, Athens, GA 30602

M.R. Hajimorad

Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN 37996

Frank A. Hale

Department of Entomology and Plant Pathology, University of Tennessee, Nashville, TN 37211

John B. Wilkerson

Department of Biosystems Engineering and Soil Science, University of Tennessee, Knoxville, TN 37996

Alan S. Windham

Department of Entomology and Plant Pathology, University of Tennessee, Nashville, TN 37211

Kevin L. Ong

Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77845

Mathews L. Paret

Plant Pathology Department and North Florida Research and Education Center, University of Florida, Quincy, FL 32351

Xavier Martini

Entomology and Nematology Department, North Florida Research and Education Center, University of Florida, Quincy, FL 32351

David H. Byrne

Department of Horticultural Sciences, Texas A&M University, College Station, TX 77845

Mark T. Windham

Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN 37996

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Abstract. The eriophyid mite, *Phyllocoptes fructiphilus*, vectors the causal agent, *Rose rosette virus* (RRV), that results in rose rosette disease. Parts of the southeastern United States have remained free of the disease, except for infected plant material introductions that were eradicated. A survey of sampling points through Alabama, Georgia, and Mississippi (n = 204) revealed the southeastern border of RRV. The presence of RRV in symptomatic plant tissue samples (n = 39) was confirmed by TaqMan-quantitative reverse transcription polymerase chain reaction (RT-qPCR). Samples were also collected at every plot for detection of eriophyid mites, specifically for *P.*

***fructiphilus*.** Three different species of eriophyid mites were found to be generally distributed throughout Alabama, Georgia, and Mississippi. Most of these sites ($n = 60$) contained *P. fructiphilus*, found further south than previously thought, but in low populations (<10 mites/gram of tissue) south of the RRV line of incidence. Latitude was found to be significantly correlated with the probability of detecting RRV-positive plants, but plant hardiness zones were not. Plot factors such as plant size, wind barriers, and sun exposure were found to have no effect on *P. fructiphilus* or the presence of RRV. The reason for the absence of RRV and low populations of *P. fructiphilus* in this southeast region of the United States are unclear.

Rose rosette disease (RRD) has become established across much of the United States. The causal agent is *Rose rosette virus* (RRV) (genus *Emaravirus*) (Di Bello et al., 2015; Laney et al., 2011), with the main mode of natural transmission being the eriophyid mite, *Phyllocoptes fructiphilus* Keifer (Allington et al., 1968; Amrine et al., 1988; Baughan et al., 2019; Gergerich and Kim, 1983), although the virus can be spread through grafting (Amrine et al., 1988; Gergerich and Kim, 1983) and movement of infected plant material. Eriophyid mites can use wind currents to disperse long distances to new locations (Galvao et al., 2012; Lindquist and Oldfield, 1996; Zhao and Amrine, 1997). The relationship between virus and vector and mode of virus transmission are unknown. However, more than just passive transport is suspected. Five other eriophyid mites are known to infest roses in the United States but are not known to transmit RRV: *P. adalius*, *P. linegranulatus*, *P. chorites*, *Call-yntrotus schlechtdali* (Baker et al., 1996; Keifer, 1939a, 1939b, 1940, 1972; Otero-

Colina et al., 2018; Styer, 1974), and *Eriophyes eremus* (Otero-Colina et al., 2018).

RRV is lethal to roses; and, like most other plant virus infection, there is no cure once roses are infected. Cultivated roses and many *Rosa* species are susceptible, including the noxious weed *R. multiflora* Thunb. (multiflora rose). Beds of ornamental roses and wild multiflora roses can serve as reservoirs of both the mite and the virus. Although control of multiflora rose is recommended, it is often difficult due to the numerous seeds produced annually, remote growing location, and regeneration of shoots from roots left in soil (Denight et al., 2008). Beds of landscape roses not frequently inspected for RRD symptomatic plants may serve as reservoirs for mites and virus.

RRD was first documented in Manitoba, Canada in 1940 (Connors, 1941). Shortly after, there were similar reports from Wyoming (in 1941) and California (in 1942) (Thomas and Scott, 1953). RRD has since spread to the south and east of the United States, and it is currently documented from 33 states, including: Alabama, Arkansas, California, Colorado, Connecticut, Delaware, Georgia, Illinois, Indiana, Iowa, Kansas, Kentucky, Louisiana, Maryland, Massachusetts, Michigan, Mississippi, Missouri, Nebraska, New Jersey, New York, North Carolina, Ohio, Oklahoma, Pennsylvania, South Carolina, Tennessee, Texas, Utah, Virginia, West Virginia, Wisconsin, and Wyoming (Amrine, 2002; Baughan et al., 2019; Denight et al., 2008; Dobhal et al., 2016; Shires and Ong, 2018). Despite the wide incidence of RRD, the boundaries of the distribution are unknown, notably in the southeastern United States. With reports of RRD in the northern parts of Alabama, Georgia, and Mississippi (Shires and Ong, 2018) the southward extent of disease incidence in those states was unknown. There have been anecdotal reports of RRD infections as far south as Interstate I-20, a highway extending from Augusta through Atlanta, GA; Birmingham, AL; and Jackson, MS; but the absence of reports south (excepting reports from Florida discussed in the following) was intriguing.

Distribution of the vector, *P. fructiphilus*, has also not been clearly determined, though previously reported in North America (Hoy, 2013) and as “widely distributed” on both wild and ornamental roses (Amrine, 2002). Because the natural spread of RRV relies on *P. fructiphilus*, the distribution of RRV could correlate with the distribution of these mites. However, it is possible that the mite may be present in the absence of the disease, including on rose species not prone to infection (e.g., *R. palustris*).

The mite has so far not been reported from the southern halves of the U.S. Gulf States (Alabama, Georgia, and Mississippi) (Hoy, 2013). In Nov. 2013, a case of RRV-infected ‘Knock Out’ roses was discovered in Florida and confirmed using reverse transcription polymerase chain reaction (RT-PCR); but no eriophyid mites were recovered from the infected roses (Babu et al., 2014). This discovery suggests that infected roses were shipped into Florida. Following quarantine and destruction of the symptomatic roses that tested positive for RRV, no spread of the disease has been observed (Babu et al., 2014). Subsequently, three more cases were confirmed in Florida from 2014 to 2018 in symptomatic plants shipped from other states, but no eriophyid mites were found on the plants.

A better understanding of the distribution of the mite could identify the risk of spread of RRV, given that the mite is the primary vector, along with transport of infected plants to areas where the disease is unknown to occur. Therefore, more information on the incidence of *P. fructiphilus* can help in predicting potential for locations where natural spread of RRV can occur. Nursery growers, public and private gardens, and those overseeing natural areas with invasive and native wild roses in the Deep South (the southeastern region of the United States including Alabama, Georgia, and Mississippi) could benefit from more information when assessing the potential for impact the disease may have. This study surveyed this region by sampling for both RRV-infection and populations of eriophyid mites on cultivated roses. The objectives of this study were to determine if *P. fructiphilus* and RRD were present in this region in managed areas; if certain factors such as wind barriers, sun exposure levels, or plant size were associated with the presence of eriophyid mites in these sites; and if plant hardiness zones or latitude could be used to predict the prevalence of the *P. fructiphilus* or RRD in the region.

Materials and Methods

Collection of samples. Samples were collected from June through November in 2017 from public and commercial cultivated rose landscaping plots in multiple cities within Alabama, Georgia, and Mississippi, with preference given to cities with populations greater than 1000 due to the likelihood of finding roses. There were 72 samples collected in Alabama, 83 in Georgia, and 49 in Mississippi (Fig. 1). At each sample location, information was recorded, including factors such as GPS coordinates, plant hardiness zone (USDA-ARS, 2012), sunlight exposure,

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K.M.S. is the corresponding author. E-mail: kbaker46@vols.utk.edu.

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presence of barriers (a physical obstruction to guard from wind), RRD symptoms, rose variety, plant size, as well as the number roses surrounding sampled plants. At every location, a sample of rose tissue was collected for future testing to determine if eriophyid mites were present: a 10-cm terminal section—including stem and leaves—was placed in a 50-mL tube with a screw cap containing 70% ethanol to preserve any collected eriophyid mites. Samples were placed in a cooler and stored at 5 °C until processed. At sites where roses exhibited typical RRD symptoms, additional terminal samples were collected ($n = 39$) and placed in a cooler to determine if RRV was present. Symptoms suggestive of RRD included excessive thorniness, witch's-broom rosettes, an abundance of lateral shoots, shoots with abnormally persistent red coloration, mottled or deformed leaves and flowers, and lateral shoots with a wider girth than the parent cane (Amrine, 1996a; Di Bello et al., 2015; Hoy, 2013; Laney et al., 2011). Upon return to the laboratory, RRD samples were stored at -20 °C until processed.

Mite extraction. Each rose sample collected for eriophyid mite detection ($n = 204$) had the ethanol decanted and the tissue cut into ≈ 1 -cm pieces. Mites were extracted using a modified version of the Monfreda et al. (2007) procedure. The tissue was placed in an 80-mesh sieve with 270-mesh and 500-mesh sieves placed below in a series; mesh openings were 180, 53, and 25 μ m, respectively (Hogentogler, Columbia, MD). Ethanol was poured through the ascending sieve series. The tissue was rinsed with $\approx 2,000$ mL of 70% ethanol. The 500-mesh screen was rinsed with 70% ethanol into a petri dish and examined under a stereomicroscope for the presence of eriophyid mites. Mites were isolated using a glass pipette and kept in a 1.5-mL Eppendorf tube with 70% ethanol. Isolated mites were stored at 5 °C until mounted on a microscope slide for identification.

Mounting mites. Mites were mounted in a modified Berlese mounting medium according to the procedure described by Amrine and Manson (1996). Mites were transferred from the tubes of ethanol using a glass pipette. Excess ethanol was absorbed by small pieces of filter paper. A minuten pin probe was used to transfer mites into a small drop of mounting medium. One slide was made per sampling location with mites recovered from the ethanol, with a maximum of 25 mites per slide. Using the minuten pin probe, the mites were oriented dorsally and ventrally for identification. This orientation allows for both the ventral coverflap and dorsal prodorsal shield to be viewed, both distinguishing morphological characteristics of eriophyid mites. A 12-mm coverslip was added, and the slide was cleared on a hot plate for 3 h at 50 °C. The slides were labeled and placed in an oven at 40 °C for 3–4 weeks to continue clearing and curing the medium. The slides were then sealed with clear nail polish and stored at room temperature.

Mite identification. Slides were viewed under a variety of microscopes, including an

Olympus BX-53 phase contrast microscope equipped with a 17-megapixel DP73 camera (Olympus, Tokyo, Japan) and an Olympus BX-63 differential interface contrast microscope equipped with a 14-megapixel Q-camera (Olympus). Ornamentation of the ventral genital coverflaps and prodorsal shields were the morphological characteristics used to identify the mite species. Additionally, several mites samples were viewed directly with a tabletop scanning electron microscope at the USDA-ARS in Beltsville, MD. Mites from the alcohol samples were placed on 25-mm SEM specimen stubs (Ted Pella, Redding, PA) with ultra-smooth, round (25 mm diameter) carbon adhesive tabs (Electron Microscopy Sciences, Inc., Hatfield, PA) affixed to the surface of the stub. The mites were simply removed from alcohol, briefly air-dried, and then mounted on the SEM stubs. Images of the mites were obtained using a Hitachi TM 3030 + Table Top Scanning Electron Microscope (Hitachi High Technologies America, Inc., Dallas, TX) with a Deben TM-3000 Coolstage (Deben UK Ltd., Suffolk, U.K.) set at -25 °C. Images were viewed using the secondary electron detector set at charge reduced mode (variable pressure mode) with 15 kV accelerating voltage, a working distance of 8–12 mm, and a magnification between 1000x and 2500x.

RNA purification. Total RNA, including the RRV RNA, was extracted from rose samples exhibiting RRD symptoms ($n = 39$) using the modified direct antigen capture method (Shires et al., 2018). Using a mesh ELISA bag (Agdia, Elkhart, IN), a pestle, 7 mL 1% PBST (phosphate-buffered saline with Tween 20), and 2 g of leaves from each sample, cell contents were released from the sample. One mL of the sap and buffer solution was transferred to an Eppendorf tube and incubated in the refrigerator at 5 °C for 2 min. The contents of the Eppendorf tubes were then poured out and discarded, and the tubes were rinsed six times with 1 mL of PBST, making certain no liquid was left in the tube after the last rinse. Tubes were placed on a 95 °C heating block for 1 min and then were transferred back to -20 °C for about 5 min. The RNA elution master mix was prepared by combining 2 μ L of RNasin (Promega, Madison, WI) with 30 μ L of sterile water. Then, 32 μ L of master mix was added to each sample to elute RNA from the Eppendorf tube surfaces. Samples were vortexed, centrifuged at 2680 g for 10 s, and stored at -20 °C until TaqMan RT-qPCR was performed.

TaqMan RT-qPCR. TaqMan-quantitative reverse transcription polymerase chain reaction (RT-qPCR) assays were performed using a *Rose rosette virus* forward primer (RRV2F), a reverse primer (RRV2R), and a TaqMan RRV2 Probe (Dobhal et al., 2016), using an Applied Biosystems QuantStudio 6 Flex Fast Real-time PCR System (Thermo-Fisher, Carlsbad, CA). The reaction mix consisted of 10.1 μ L RNase free water; 5 μ L Fast Virus 1 Step Master Mix (containing

polymerase, enzyme, dNTPs, recombinant ribonuclease inhibitor, dye, and buffer) (Thermo-Fisher); 2 μ L (10 μ M) Primer mix F/R RRV; 0.5 μ L (10 μ M) TaqMan RRV Probe; 0.4 μ L Rox Reference Dye (50X) (Thermo-Fisher); and 2 μ L of RNA template for a final reaction volume of 20 μ L. The controls included two positives and three negatives, of which one was RNase-free water. The positive RRV RNA controls came from roses naturally infected with RRV from Durant, OK. One positive control was extracted and stored at -20 °C for 1 month, and another was extracted at the time of this experiment. The negative controls were two asymptomatic roses from the University of Tennessee Plateau AgResearch and Education Center in Crossville, TN. The TaqMan RT-qPCR amplification protocol consisted of an initial stage 50 °C for 5 min, a holding stage of 95 °C for 20 s, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min, according to the manufacturer's suggestions. Data acquisition and analysis were performed using the Applied Biosystem's QuantStudio 6 software.

Statistical analysis. Three separate generalized linear mixed models with binary distributions and logit link were developed to test factors associated with the probabilities of observing symptoms, finding *P. fructiphilus*, and *E. eremus* mites within sampled plants. Models were developed using the GLIMMIX procedure in SAS 9.4 (Littell et al., 2006). Factors included in model building were latitude, plant hardness zone, cultivar, plant size, light availability, and wind barrier locations. Plants that were symptomatic were further analyzed for RRV by TaqMan RT-qPCR. Multilevel logistic regression was performed to test factors associated with the probability of detecting RRV from tested plants.

Manual forward model selection was used to determine the best fitting explanatory variables for all analyses. Odds ratio estimates, 95% confidence limits, and model-adjusted probabilities were obtained. Statistical significance was determined at $\alpha = 0.05$.

Results

Eriophyid mites were recovered from about half of the sample locations (Fig. 2). Of the 106 samples that contained eriophyid mites, 91 samples of mites could be morphologically identified. Three different species of eriophyid mites were found in different sampling locations in at least two states, including *P. fructiphilus* (Figs. 3 and 4), *E. eremus*, and an unidentified mite denoted as 'Unknown A' based on descriptions by Amrine (1996b) and Druciarek and Lewandowski (2016).

Eriophyid mites were found on samples taken from throughout Alabama, Georgia, and Mississippi. *P. fructiphilus*, formerly not thought to be present in the southern half of the Deep South, was found in 60 locations and as far south as the cities of Wiggins,

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

MS; Dothan, AL; and Valdosta, GA, in many cases not in association with RRV symptoms. *E. eremus* was found in 24 locations, across all three states. “Unknown A” was found in nine locations in Georgia and Mississippi (Fig. 1). There were five locations where two mite species were found on the same sample. Four of these locations had both *P. fructiphilus* and *E. eremus*, while one location had *P. fructiphilus* and “Unknown A.”

When roses exhibited RRD symptoms, a sample of rose plant tissue was taken for RRV detection ($n = 39$). Of the total 39 samples that were tested for RRV, 29 tested positive by Taqman RT-qPCR, or 74.4% (Table 1). The positive samples were mapped to show the southern edge of the distribution of RRV in the southeastern United States (Fig. 1).

The latitude of the sampled plants was significantly correlated with the probability of detecting RRV ($P = 0.01$) in plants with symptoms; however, plant hardiness zone was not ($P = 0.83$). For each degree increase in latitude, the odds for RRV-positive results increased by 8.2 times over the chance of detection in samples from one degree less in latitude (OR: 8.2, 95% CI: 1.58–42.6).

The number of eriophyid mites in a sample ($P = 0.001$) and the plant hardiness zone ($P = 0.006$) were both significantly correlated with the probability of observing symptoms in sampled plants. As the number of mites increased by 10, the symptoms of RRD were 1.25 times more likely to be observed (OR: 1.25, 95% CI: 1.1–1.4) (Fig. 2).

Table 1. Characteristics of rose rosette samples from Alabama, Georgia, and Mississippi.

Rose cultivar	Location	Observable symptoms	CT value	RRV presence
Knock Out	Anniston, AL	Yes	27.593	Positive
Unknown	Birmingham, AL	Yes	25.751	Positive
Knock Out	Gadsden, AL	Yes	24.846	Positive
Unknown	Livingston, AL	Possible	No CT	Negative
Knock Out	Northport, AL	Yes	24.875	Positive
Knock Out	Oxford, AL	Yes	23.240	Positive
Unknown	Pelham, AL	Possible	28.646	Positive
Knock Out	Townley, AL	Yes	20.213	Positive
Drift	Augusta, GA	Possible	27.794	Positive
Drift	Augusta, GA	Yes	25.646	Positive
Knock Out	Augusta, GA	Yes	26.266	Positive
Knock Out	Bremen, GA	Yes	31.197	Positive
Knock Out	Brunswick, GA	Possible	No CT	Negative
Knock Out	Cedartown, GA	Yes	27.431	Positive
Knock Out	Cedartown, GA	Yes	27.099	Positive
Knock Out	Columbus, GA	Possible	No CT	Negative
Knock Out	Dublin, GA	Yes	23.786	Positive
Knock Out	Dublin, GA	Yes	24.509	Positive
Knock Out	Evans, GA	Possible	No CT	Negative
Knock Out	LaGrange, GA	Possible	No CT	Negative
Knock Out	Lincolnton, GA	Yes	23.948	Positive
Knock Out	Macon, GA	Possible	No CT	Negative
Knock Out	Madison, GA	Yes	26.701	Positive
Knock Out	Madison, GA	Yes	29.535	Positive
Knock Out	Pine Mountain, GA	Possible	No CT	Negative
Knock Out	Rome, GA	Yes	27.307	Positive
Knock Out	Rome, GA	Yes	25.944	Positive
Knock Out	Roopville, GA	Yes	24.066	Positive
Knock Out	Summerville, GA	Possible	No CT	Negative
Knock Out	Tybee, GA	Possible	No CT	Negative
Knock Out	Boonville, MS	Yes	23.982	Positive
Knock Out	Columbus, MS	Yes	25.044	Positive
Knock Out	Corinth, MS	Yes	23.421	Positive
Knock Out	Greenville, MS	Yes	23.063	Positive
Knock Out	Greenwood, MS	Yes	23.701	Positive
Knock Out	Indianola, MS	Possible	No CT	Negative
Knock Out	Starkville, MS	Yes	30.210	Positive
Unknown	Tupelo, MS	Yes	23.910	Positive
Knock Out	West Point, MS	Yes	23.949	Positive

Fig. 2. Map of the southern incidence line of *Rose rosette virus* (RRV), southern distribution of *Phyllocoptes fructiphilus* and *Eriophyes eremus*, and the population densities of eriophyid mites found on rose samples in Alabama, Georgia, and Mississippi in 2017. The larger the circle, the more mites found in the sample.

USDA plant hardiness Zone 9a (USDA-ARS, 2012) had few observations, therefore Zones 8b and 9a were combined for analysis (referred to as “Zone 8b”). Of the samples collected, 13.2% were in Zone 7b, 49.5% were in Zone 8a, and 36.8% were in Zone 8b. RRD symptoms were 18.4 times more likely to be found in Zone 7b compared with Zone 8b (OR = 18.4; 95% CI: 3.2–107.7) and 8 times more likely in Zone 8a compared with Zone 8b (OR = 7.96; 95% CI: 1.6–38.8).

Plant hardiness zones ($P = 0.006$) and presence of RRD symptoms ($P = 0.0002$) were correlated with the probability of finding *P. fructiphilus*. While we saw no difference between Zones 7b and 8a in the odds of finding *P. fructiphilus* ($P = 0.69$), *P. fructiphilus* was significantly more likely to be found in Zone 7b compared with Zone 8b (OR = 3.591; 95% CI: 1.01–12.81). Likewise, *P. fructiphilus* was significantly more likely to be found in Zone 8a vs. Zone 8b (OR = 4.43; 95% CI: 1.78–11.02). Where RRD symptoms were observed, the presence of *P. fructiphilus* was significantly more likely (OR = 10.64; 95% CI: 3.4–33.3) compared with plants where no RRD symptoms were observed.

Plot factors were also analyzed for the presence of *E. eremus*. Both cultivar ($P = 0.02$) and light conditions ($P = 0.05$) were correlated with the presence of *E. eremus*. *E. eremus* was significantly more likely to be found on ‘Drift’ vs. ‘Knock Out’ roses (OR =

Fig. 3. Table Top—SEM images of *Phyllocoptes fructiphilus* adult, vector of *Rose rosette virus*: (A) dorsal view, (B) ventral view, (C) prodorsal shield, and (D) ventral cover flap.

5.45; 95% CI: 1.7–17.5). There appeared to be an elevated correlation of finding *E. eremus* in samples from full sun conditions compared with partial sun (OR = 0.383; 95% CI: 0.15–1.0).

Discussion

There are increasing reports of both RRV from around the United States and the eriophyid mites that are found on roses, but there

Fig. 4. Differential Interference Contrast Microscopy images of *Phyllocoptes fructiphilus*, vector of *Rose rosette virus*: (A) dorsal view and (B) ventral view.

are no prior studies focusing on these two factors to better understand their distribution and association, especially in the southeastern United States, as done in this study.

Previously, a geographical limit for incidence for RRV was unknown. As shown in Fig. 1, it appears the southeastern distribution and southern limit has now been defined for symptomatic RRV infections. The sampling techniques used in this study allowed a snapshot of RRV incidences occurring in the same year to be recorded instead of just integrating and cataloging all historic cases of RRD. In this way, our line of incidence might be considered as the disease's current front. This line of reference will be useful in future studies to track and compare the spread of RRD. It was decided to use visible RRD symptoms as a prerequisite for sample collection due to the cost of testing each sample for RRV RNA with TaqMan RT-qPCR. However, because of the collection of samples in this study, asymptomatic infections of RRV have been discovered. Although we are confident in our current geographical southern boundary for incidence of symptomatic RRV infection, it is possible the virus may reside in asymptomatic roses further south of the line.

A similar survey in 2017 in northern and central Florida (Martini, data not shown) found RRD only on a few plants recently introduced into the state, but the disease did not become established. Following the same sampling method as in our current research, no eriophyid mite vectors were found at the Florida sites, or in the Florida landscape. However, the lack of vector presence and the failure of the disease to become established does not absolve rose growers located below the RRV and *P. fructiphilus* lines of vigilance, because both disease and vector can be transported wherever roses are shipped.

In cooler zones (Zones 7b and 8a in this study), there were much higher chances of observing symptoms of RRD in roses. Although, plant hardiness zones were not asso-

ciated with the positive RRV detections. This could be because not all samples were tested for RRV presence, but only those samples from symptomatic plants. Symptomatic roses growing at higher latitudes had significantly higher chances of testing positive for RRV compared with symptomatic plants at lower latitudes. Although we suggest that any rose grower in this region remain vigilant for symptoms of RRD, our results indicate that latitude could be used as a tool to predict if RRD might be present in roses or not.

Factor such as wind barriers, sun exposure levels, and plant size were taken at each sampling locations. It is unknown if any of these factors affect the presence of eriophyid mites or RRD in roses. Eriophyid mites spread naturally by floating on wind currents. We wanted to specifically look at the size of the plant and if there were any wind barriers nearby such as walls, trees, or other large plants that could prevent mites from accessing the plant by wind. However, plant size and wind barriers were not found to be significantly associated with any outcome of interest tested. Likewise, sun exposure (full sun, part sun, and shade) conditions were also examined and had no significant association with *P. fructiphilus* or the presence of RRD. *E. eremus* was found to be significantly associated with full sun environments and 'Drift' roses, factors that may influence future research with this species.

The presence of eriophyid mites in most samples, both symptomatic and asymptomatic for RRV, was not surprising. However, finding *P. fructiphilus* in most of the samples was unexpected in the southern halves of the states where RRD was not observed. *E. eremus* was described in 2016 (Druciarek and Lewandowski, 2016) and has only recently been found in five states, including Georgia (Otero-Colina et al., 2018). This study also found *E. eremus* in Alabama and Mississippi, resulting in a first report for this mite in those states. Most interestingly, *E. eremus* was found at a high population level (258 mites/g of tissue) on a rose plant that was severely symptomatic for RRD and TaqMan RT-qPCR positive. No other mite species were present on this sample, not even *P. fructiphilus*. There has not been any damage observed to host plants due to *E. eremus* feeding (Otero-Colina et al., 2018) that may imitate certain RRD symptoms. Because this mite was newly found in North America, the only continent in which RRV is known to naturally occur, further examination is required to discover whether *E. eremus* can play a role in RRV transmission. Better characterization of the relationship of rose eriophyid mites with other species occupying the same rose plant, and on both wild and cultivated species, is also critically needed information. Five of the 204 samples in this study had two species of eriophyid mites. Both *E. eremus* and *P. fructiphilus* are refuge-seeking mites and may compete for the same feeding locations and niches for protection from predators and the environment. We are also not certain whether there is

a strong host or cultivar preference, particularly in *E. eremus*.

The unidentified mite, "Unknown A," was found in nine different samples in both Georgia and Mississippi. One sample from a location in Mississippi contained both "Unknown A" and *P. fructiphilus*. We were unable to place "Unknown A" to genus using a key by Lindquist and Amrine (1996). Additional live specimens are needed to collect morphological, behavioral, and genetic information before this mite can be identified.

Figure 1 shows the distribution of the three mites found in this study. Our work shows the distribution of *P. fructiphilus* much farther south than previously thought (Hoy, 2013) throughout all three states, albeit in small populations. In fact, the largest populations of eriophyid mites, including *P. fructiphilus* were found either at (or north) of the RRV line of incidence, with no populations greater than 10 mites per gram south of the RRV line (Fig. 2). It is also known that *P. fructiphilus* tends to be more abundant on roses symptomatic with RRD than asymptomatic roses (Amrine, 1996a; Epstein and Hill, 1999; Jesse et al., 2006). Future research will need to examine what factors might limit the mites' presence south of this RRV incidence line, such as humidity, temperature, wind patterns, plant physiology, lack of RRD symptomatic roses, pathogenic fungi, or predatory mites. In this study, mite identification was prioritized over testing them for presence of RRV; however in future studies, this could be an interesting factor, especially in mite species other than *P. fructiphilus*, to better understand the relationship between virus and host, as well as learn whether there may be other vectors of RRV.

This is the first comprehensive examination of rose eriophyid mites in the Deep South. This data will be instrumental in forming future research directions regarding eriophyid mites and RRD. Future surveys can be used in combination with this study to assess the spread of this disease and its vector(s).

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