Survey of the Invasive Mite *Phyllocoptes Fructiphilus* Rose Rosette Virus (RRV) and of its Predatory Mites in Northern Florida

PI: Xavier Martini, xmartini@ufl.edu
Co-PI: Austin N. Fife

ABSTRACT

We surveyed roses in landscape settings across North Florida to map the distribution of the eriophyid mite *Phyllocoptes fructiphilius*, the vector of rose rosette disease. The good news is that the current infestation seems contained, as out of 355 roses samples, only those in Leon county had *P. fructiphilius*. However, the population in Leon county increased by 10-fold in 4 months indicating that the mite has the potential to establish in North Florida. In addition, we found a number of different predatory (phytoseiid) mites which will be identified and considered for their potential as biocontrol of *P. fructiphilus*.

OBJECTIVES AND METHODS

We conducted a survey across northern Florida to scout for *Phyllocoptes fructiphilus*, the vector of rose rosette virus and predatory mites on roses.

Objectives of the survey were fourfold:

1. Detect *P. fructiphilus* and/or Rose Rosette Disease if present in northern Florida
2. Identify native predatory mites with bio-control potential
3. Identify other possible vectors of Rose Rosette Virus
4. Detect other mite species of concern on Florida roses

A survey of roses in the landscape was conducted following a transect of northern Florida from west to east, Pensacola to Jacksonville. Cities with populations over 1,000 were visited along this route and cuttings were taken from various roses in each city. Rose cultivar/species, sun exposure and GPS coordinates were recorded to map out sites which had predatory mites, eriophyid mites, or possibly symptoms of Rose Rosette Disease. Rose tissue samples were taken from the periphery of various roses in the landscape; sampling was focused on the flowering tips of roses and included a mixture of flowers, fruits, buds, and short lengths of rose cane. Samples were trimmed with bypass pruners which were routinely sanitized with 70% ethanol between cuts. Samples were stored in 500 mL Nalgene™ Wide-Mouth Polypropylene Copolymer bottles (ThermoFisher Scientific, Waltham, MA, USA) with ~10 mL of 95% ethanol. The rose samples then were gently shaken to coat the rose tissues sampled with ethanol. Doing so made sure that the sampled mites were killed and acted to preserve both mites and rose tissues until samples could be processed further and checked for mites.

Samples were processed using a washing method derived from Monfreda et al. (2007) used to detect eriphyoid mites such as *P. fructiphilius*: The sampling bottles with ethanol and rose tissues were vigorously shaken to dislodge any mites, then the ethanol in the container was poured over a stack of sieves with decreasing screen sizes: 180 µm, 53 µm, and 25 µm. The bottle and rose pieces were then further rinsed with 95% ethanol over the sieve stack to dislodge any remaining mites.

The 53 µm and 25 µm sieves were processed separately; the 53 µm sieve retained larger mites while the 25 µm sieve retained smaller mites, including *P. fructiphilus*. The sieves were then back-washed from the underside of their screen with a 95% ethanol-filled wash bottle, starting from the highest point of a sieve and working to the bottom to flush any trapped debris and mites into a 50 mL centrifuge tube for storage and future observations.

The ethanol solutions of mites and plant debris were allowed to settle until excess ethanol could be siphoned off, allowing us to then pour this concentrated plant-mite mixture into a thin, small petri dishes to be observed under a dissecting microscope. Mites found among the plant debris were counted, then siphoned off with a glass pipette and subsequently stored in micro-centrifuge containers with 95% ethanol as a preservative. 5-10 specimens from each sample were made into prepared microscope slides: Mites were cleared and mounted using the methods of Faraji and Bakker (2008): mites were simultaneously cleared and stained with Faraji and Bakker's modified clearing solution and heated on a hot plate until the specimens were clear. Subsequently these mites were moved with an eyelash tool into an iodine-modified Hoyer's slide mounting media (Hempstead Halide®, Inc., Galveston, Texas, USA), underneath a 12 mm glass cover-slip. The prepared slide was then dried at 90 °C before sealing the slide by painting a ring of alkyd insulating enamel (Red Glyptal® 1201, Chelsea, MA, USA) over the edges of the cover-slip to seal the slide, to protect it from damage by air incursion and moisture. These slides could then be observed under a compound microscope with phase-contrast objectives to identify the mite families and species if necessary.

After mite quantities and species were recorded, a representative sample of eriophyoids putatively identified as *P. fructiphilus* had their identity verified with the acarologist, Dr. Sam Bolton of the Florida Department of Agriculture and Consumer Services, Division of Plant Industry (FDACS-DPI) to ensure accuracy.

Roses which appeared to show symptoms of Rose Rosette Disease, or which had populations of *P. fructiphilus* present were tested by the Plant Disease Diagnostic Clinic at the NFREC. Plant tissues were tested for Rose rosette virus by Dr. Fanny Iriarte using the currently accepted molecular methods described in Babu et al. (2016), Babu, Washburn, Ertek, et al. (2017), and/or Babu, Washburn, Miller, et al. (2017).

Impact of the Covid-19 epidemic on the research

Our plan was to survey extensively North Florida from February to June 2020. However due to the Covid-19 outbreak and the lockdown of the university our sampling effort has been restrained. In addition, research activities were forbidden for almost 2 months, which slowed us down in the identification of the predatory mites.

RESULTS

We have been able to collect over 218 additional samples for our dataset, for a total of 355 rose samples overall from 29+ cities. We have not encountered P. fructiphilus nor any other eriophyoid mites on roses beyond the infestation in Leon County to date. In Leon county population of P. fructiphilus increased by more than 10-fold in 4 months indicating that the mite has the potential to establish in North Florida (**Figure 1**).

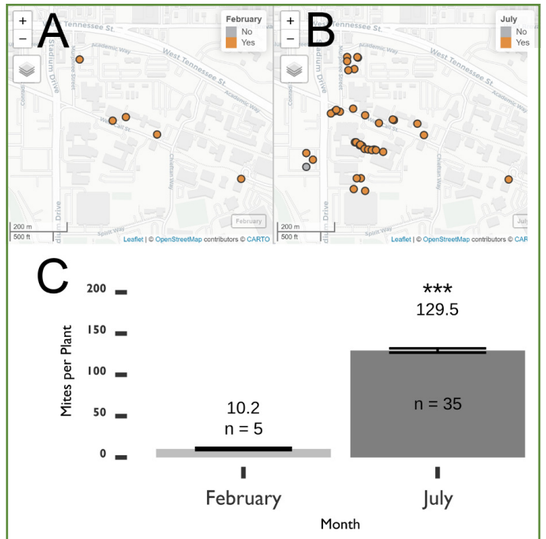

Figure 1. Presence of Phyllocoptes fructiphilus in Leon County, Florida, USA, in (A) Feb 2019 and (B) Jul 2019. Orange dots indicate sites sampled that had P. fructiphilus. Gray dots indicate surveyed areas where no P. fructiphilus were found. (C) Average number of P. fructiphilus per rose sample. Samples were taken from sites in Leon County, Florida, on 14 Feb and 16 Jul 2019. Asterisks represent significant differences as calculated by pairwise t-tests of the 5 sites tested for P. fructiphilus during both mo. P-value < 0.001 (From Fife et al. 2020).

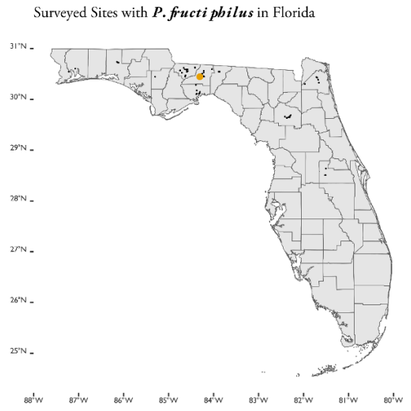

Figure 2. Black dots indicate individual sites which have been surveyed for Phyllocoptes fructiphilus. Orange dots indicate a number of sites with populations of P. fructiphilus detected in Leon county, Florida. No symptoms of Rose Rosette Disease have been seen on these plants to date.

During our survey, we have not encountered any species of concern or any other eriophyoid mites which show potential to transmit rose rosette virus other than *P. fructiphilus*. Our mite collecting and washing methods appear to be effective: we have encountered plant-feeding mites of various species and sizes, including common plant-associated mites in the following families: Tetranychidae, Tarsonemidae, and Tenuipalpidae. We have also collected a few predatory mites in the family phytoseiidae which have been mounted and now await identification. We also have identified to order a few Oribatids and other mites which are commonly associated with plants, but are not considered predators nor herbivores. Please see **Figure 2** to see the county where *P. fructiphilus* have been encountered.

CONCLUSIONS

Our surveys were proposed to identify areas with greater disease risk for *P. fructiphilus* and/or Rose Rosette Virus and to understand the movements of mite populations within Florida. These data are contrary to previous reports of a southern boundary for populations of *P. fructiphilus* (Solo et al. 2020). The presence of *P. fructiphilus* in Florida represents a risk for the introduction of RRD, but so far we have found no evidence that this population of *P. fructiphilus* has spread beyond Leon County. We also have not seen any evidence of RRD in Florida during our surveys. Together, these data suggest that neither *P. fructiphilus* nor RDD are widespread in Florida, which provides us with an opportunity to control localized outbreaks.

RECOMMENDATIONS AND REFERENCES

With the present threat of *P. fructiphilus* in Florida, we recommend developing control methods to prevent further spread of the mite. By controlling the mite vector, it may be possible to reduce or even prevent the incidence of RRD in the following years. Furthermore, we recommend collaborating with FDACS and the public to detect any new instances of RRD in the state.

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