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**First report of *Phyllocoptes fructiphilus* Keifer, the vector of the *Rose Rosette Virus*, in Florida**

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*Phyllocoptes fructiphilus* Keifer (1940) is a microscopic plant-feeding mite (Acari: Eriophyidae). Eriophyid mites are very host specific (Oldfield 1996; Skoracka *et al.* 2009) and *P. fructiphilus* only feeds on plants in the genus *Rosa* (Amrine 1996). *Phyllocoptes fructiphilus* is the vector of *Rose rosette virus* (RRV). RRV infection is commonly associated with the following symptoms: witches’ broom, rosetting, deformed flowers, increased thorn density, elongated shoots, reddened leaves and stems, and increased die-back which ultimately kills the rose host (Amrine 1996). This disease is known as Rose Rosette Disease (RRD) and is the most serious illness of roses, affecting the U.S. commercial rose industry which worth millions of dollars. Rose Rosette Disease and *P. fructiphilus* have invaded the southeastern United States as they followed the range expansion of the non-native noxious weed *Rosa multiflora* (Thunb.) towards the east coast (Amrine 2002; Otero-Colina *et al*. 2018).

RRD was detected in Florida in 2014 on 15 plants. The plants were destroyed and the mites (*P. fructiphilus*) were not detected on the roses at that time (Babu *et al.* 2014). In 2018 we began a series of surveys along the borders of northern Florida and southern Georgia to determine if this mite was present and acting as a vector for the disease.

Survey efforts initially focused on counties around Leon County. Rose tissue samples were taken from the periphery of various roses: samples included a mixture of flowers, fruits, buds and short lengths of rose cane, trimmed with bypass pruners and stored in quart sized plastic bags. Pruners were sanitized with 70% ethanol between cuts. Rose species and coordinates were recorded to map out sites that had predatory mites, eriophyid mites, or possibly RRD.

Samples were processed using a washing method derived from Monfreda *et al.* (2007): 10 cm cut roses were soaked in a 500 mL beaker with a solution of 1:1 bleach:water with a few drops of dishwasher detergent. The solution was stirred vigorously with a glass rod to dislodge any mites, then poured over a stack of sieves with decreasing screen sizes: 180 *μm*, 53 *μm* and 25 *μm*. The beaker and rose pieces were further rinsed with tap water over the sieve stack to dislodge any remaining mites. The 25 *μm* sieve screen traps mites that are less than the average size of *P. fructiphilus*. This sieve was then backwashed from the underside of the screen with a water-filled wash bottle, starting from the highest point of the sieve and working to the bottom of the sieve to flush the trapped debris into a 50 ml centrifuge tube for storage and future observation. Samples were observed under a dissecting microscope. Mites found among the plant debris were siphoned off with a glass pipette and subsequently stored in micro-centrifuge containers with 95% ethanol as a preservative. Some specimens were mounted directly into Hoyer’s slide mounting media (Hempstead Halide, Inc. Galveston, TX), dried at 90°C, then ringed with nail polish.

On February 14, 2019, we found a total of 42 eriophyid mites from six samples obtained while surveying roses in Tallahassee, Leon County, Florida (see Fig. 1A). The mites were sent to the Florida Department of Agriculture and Consumer Services - Division of Plant Industry (FDACS-DPI) and were all identified as *P. fructiphilus* based, among other characters, on the distinctive pattern of ridges on the prodorsal shield (Bauchan *et al.* 2019) (Fig. 2). The roses did not show signs or symptoms of RRD. These roses were tested for RRV with RT-qPCR and Reverse Transcription Recombinase Polymerase Amplification (RT-RPA) (Babu *et al.* 2016, 2017). However, none of the plants infested with *P. fructiphilus* were positive for RRV.

On July 16th we conducted an additional survey of 33 roses near the initial site of discovery, including the rose sites where *P. fructiphilus* was originally detected (Fig. 1B). Each sample contained more than 50 eriophyid mites, with some samples containing over 300 mites. We compared the samples collected during February and July with a paired t-test and we found a significant increase in the *P. fructiphilus* population between the two sampling dates (seeFig. 1C; p-value = 0.001, α = 0.05, df = 4). Mites that were slide mounted were subsequently confirmed as *P. fructiphilus*. Additional rose samples were tested for RRV by RT-qPCR, but no virus was detected.

This is the first record for *P. fructiphilus* in Florida. Based on our data, RRV is currently not established in Florida. None of the mite-infested roses showed symptoms of RRD and none were positive for RRV. However, the presence of *P. fructiphilus*, along with past detections of RRV in Florida warrants increased monitoring for the mite and virus in Florida. There is a critical need to develop methods to manage *P. fructiphilus* and RRV, or the US rose industry stands to lose millions in the coming years.

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**Summary**

The invasive mite *Phyllocoptes fructiphilus* Keifer (Acari: Trombidiformes, Eriophyidae) feeds on plants in the genus *Rosa* and is known as the primary vector of *Rose rosette virus*, the causal agent of Rose Rosette Disease (RRD) (Bunyavirales: Emaravirus). The mite *P. fructiphilus* is reported for the first time in the state of Florida, USA. No roses showed signs or symptoms of viral infection, and no virus was detected using molecular methods. *P. fructiphilus* represents a potential threat to the Florida rose industry if RRD becomes established.

Key Words: Rose Rosette Disease; *Rose rosette virus*; Emaravirus

**Resumen**

El ácaro invasivo *Phyllocoptes fructiphilus* Keifer (Acari: Trombidiformes, Eriophyidae) se alimenta sobre plantas del género *Rosa* y es reconocido principalmente como vector del Virus del Rosetón Rosal, el agente causal de la Enfermedad Rosetón Rosal (ERR) (Bunyavirales: Emaraviridae). El ácaro *P. fructiphilus* se reporta por primera vez para el estado de laFlorida, USA. Ninguna rosa mostró señales o síntomas de una infección viral, y ningun virus fue detectado con el uso de métodos moleculares. *P. fructiphilus* representa una amenaza potencial para la industria de la rosa en la Florida si la ERR se llega a establecer.

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**Figure Caption**

**Fig. 1:** Presence of *Phyllocoptes fructiphilus* in Leon County, Florida in (A) February 2019 and (B) July 2019. Orange dots indicate sites sampled which had *P. fructiphilus*. Gray areas 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 February 14 and July 16th, 2019. Asterisks represent significant differences as calculated by pairwise t-tests of the 5 sites tested for *P. fructiphilus* during both months. P-value < 0.001.

**Fig. 2:** *Phyllocoptes fructiphilus* Keifer (Female) from Leon County, Florida: (A) Body (scalebar = 100 µm); (B) Prodorsal shield (scalebar = 20 µm).

A close up of a map

Description automatically generated

**Figure 1**

A picture containing animal

Description generated with very high confidence

**Figure 2**