

Eriophyoid Mites Found on Healthy and Rose Rosette Diseased Roses in the United States¹

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Abstract

Phyllocoptes fructiphilus (Keifer), known as the rose bud mite, is an eriophyoid mite that has been shown to be the vector of Rose rosette virus (RRV), an *Emaravirus*, and the causal agent of rose rosette disease (RRD). Studies were conducted of mites found on roses, using various microscopy techniques including wide field, phase contrast and differential interference contrast light microscopy, and table top and low temperature scanning electron microscopy. Surveys of roses from several states within the US indicate the presence of three species of eriophyid mites: *Phyllocoptes fructiphilus*, *Eriophyes eremus* (Druciarek & Lewandowski), and *Callyntrotus schlechtendali* (Nalepa). *Phyllocoptes fructiphilus* was found primarily under the petioles (stipules), inside the flower sepals appressed to the ovary/seeds, and on the surface of the leaves. It was collected on plants with or without symptoms of RRD and often hides amongst the dense simple and bulbous, glandular hairs or under the stipules/petioles. *Eriophyes eremus* was found under the stipules of roses and is now recorded for the first time in the Americas. *Callyntrotus schlechtendali* was found on the open surface of the older leaves. The latter two species were not observed to be associated with obvious plant injury. In addition, predatory mites were found associated with these mites which may be useful as biological control agents of the eriophyid mites.

Index words: insect vectors, disease spread, predatory mites, viruses.

Species used: Rose bud mite [*Phyllocoptes fructiphilus* (Keifer)]; Roses (*Rosa* spp.).

Significance to the Horticulture Industry

Eriophyoid mites are extremely small mites among which *Phyllocoptes fructiphilus* has been shown to be the vector of Rose Rosette Disease. Various microscopy techniques were used in this study to produce high resolution images of outstanding quality, for the correct identification of the mites and their locations on the rose plants. Surveys of roses from several states within the US indicate the presence of three species of eriophyid mites, *Phyllocoptes fructiphilus*, *Eriophyes eremus*, and *Callyntrotus schlechtendali*. Several predatory mites of the families Phytoseiidae, Cheyletidae and Bdellidae, as well as Tydeidae and Iolinidae, which are predacious of mites as well as being fungi feeders, were observed on some of the rose samples. The correct identification of these three species of eriophyoid roses is the first step in studying their biology and distribution and may aid in preventing the introduction of these exotic and potentially harmful species into other areas. In addition, this information will be useful to rose producers, breeders, growers, plant protection officers, entomologists, biologists and horticultural scientists who are interested in solving the rose rosette disease problem.

Introduction

The rose bud mite, *Phyllocoptes fructiphilus* (Keifer) (Acari: Trombidiformes: Eriophyoidea), is an eriophyoid

mite first collected from seeds and fruits and around the petiole bases of *Rosa californica* (Cham & Schltdl) in Clarksburg, California in 1940 (Keifer 1940). In 1941, a disease affecting an unidentified rose variety was detected in Canada (Connors 1941); its nature remained unclear for many years although it was thought to be a virus (Di et al. 1990) vectored by *P. fructiphilus* (Allington et al. 1968). Laney et al. (2011) found an *Emaravirus* which was present only in rose tissues showing symptoms of the rose rosette disease (RRD), characterized its genome, and named it the Rose Rosette Virus (RRV). Later, Di Bello et al. (2015) conclusively proved that RRV is the only etiological agent of RRD, and that it is vectored by *P. fructiphilus*. More recently, Di Bello et al. (2018) also demonstrated that *P. fructiphilus* was able to establish, lay eggs and develop nymphs and adults on 20 tested rose genotypes.

Currently, *P. fructiphilus* is suspected to be widely distributed in the U.S. on wild and commercial roses (Amrine 2002). RRV and its mite vector were shown to be a method to control *Rosa multiflora* (Thunb), a plant categorized as being an invasive weed (Amrine 2002). However, since commercial roses are also affected by both the virus and the mite, the use of these organisms as biological control agents of *R. multiflora* is now highly discouraged (Hoy 2013).

Eriophyid mites are tiny, 140-175 µm in length and 40-50 µm in width, soft-bodied, transparent, worm-like and with only two pairs of legs. Species identification of eriophyoid mites is usually based on their unique morphology and requires microscopic observation at high magnification, although ecological differences could offer important clues. For instance, some mites hide inside the buds or plant malformations that they induce, whereas others wander on the open leaf surface (Sabelis and Bruin 1996).

There are 20 species of eriophyoid mites that belong to 10 different genera known worldwide on roses (de Lillo

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Table 1. List of rose varieties and species studied, with (+) symptoms of rose rosette disease, undefined (?) or apparently healthy (-). The microhabitat of *Phyllocoptes fructiphilus* was recorded, when this mite was found. None* designates that *P. fructiphilus* was not found but does not mean the variety is resistant.

Collection site, date and collector's name	Rose Species/Variety	Rose rosette disease (+/-)	Microhabitat
Oklahoma State University, Stillwater, OK. October 2015, Jennifer Olson	'Belinda's Dream'	+	Under leaf bud stipule
	'Blushing Knockout'	+	Under leaf bud stipule
	'Father Hugo'	+	Under flower sepals
	'Grenada'	+	Open leaves & under flower sepals
	'Gypsy'	+	Open leaves & under flower sepals
	Hearth of Gold	+	None*
	'Lady Elsie May'	+	Under flower sepals
	'Little Mischief'	+	Under flower sepals
	'Mystic Fairy'	+	Under leaf bud stipule
	'Red Knockout'	+	Under leaf bud stipule
	'Sophy's Rose – David Austin'	+	None*
	'The Fairy'	+	Under flower sepals
	'Bright Melodies'	-	None*
	'Burgundy Iceberg'	-	None*
	'Chrysler Imperial'	-	None*
	'Electron'	-	None*
	'Home Run'	-	Open leaves & under flower sepals
	'Love and Peace'	-	None*
	'Oklahoma'	-	None*
	'Papa Hemeray'	-	None*
	'Pink Knockout'	-	None*
Texas A&M University, College Station, TX. May 2016, David Byrne	'Yellow Submarine'	-	Under flower sepals
	'Lafter'	?	None*
	'My Girl'	?	None*
	'Swamp'	?	None*
	'Tea Rose'	?	None*
University of Delaware Newark, DE. February 2016, Tom Evans	'Sir Thomas Lipton'	?	None*
	'Frau Dagmar'	?	None*
	'Purple Pavement'	?	None*
	<i>Rosa rugosa</i>	?	None*
	'Elle'	?	None*
Oklahoma State University, Stillwater, OK. April, 2016, Jen Olsen	'Glowing Peace'	?	None*
	'Grenada'	?	Under flower sepals
	'Gypsy'	?	None*
	'Home Run'	?	None*
	'Lyda'	?	None*
	'Palace Eve'	?	Under flower sepals
	'Pretty Lady'	?	Open leaves & under leaf bud stipule
	'Renaissance Clair'	?	Under leaf bud stipule
	'Renaissance Naiomi'	?	Under flower sepals
	'Renaissance Natalie'	?	Under flower sepals
	'Scarborough Faire'	-	Under flower sepals
	'Town and Country Cottage Rift'	?	Under flower sepals
	'Town and Country Luray'	?	Under flower sepals
Beltsville Agricultural Research Center, Beltsville, MD. February-April, 2016, Ron Ochoa	'Wettersonic'	?	None*
	Red Knockout	-	None*
	<i>Rosa multiflora</i>	-	None*
National Arboretum, Washington, DC. April, 2016 Gabriel Otero-Colina	<i>Rosa banksii</i>	-	None*
	<i>Rosa fakundii</i>	-	None*
	<i>Rosa roxburghii</i>	-	None*

(Dip. Scienze del Suolo, Università di Bari Aldo Moro, Bari, Italy) and Amrine personal databases; Druciarek and Lewandowski 2016). As part of the US. National Project on RRD led by D.H. Byrne at Texas A&M University, we surveyed rose samples from several U.S. states using modern microscopy techniques including wide field, phase contrast, differential interference contrast microscopy, table top scanning electron microscopy and low temperature scanning electron microscopy. The goal of this survey was to determine which species of mites feed on roses,

identify where they are found on roses, and to discuss their importance as pests.

Methods and Materials

Rose samples at various stages of development were obtained between October 2015 and May 2016 from several states including: Delaware, Georgia, Indiana, Maryland, North Carolina, Oklahoma, Pennsylvania, South Carolina, Texas, Washington, DC and West Virginia (see Table 1 for collection data of Rose varieties and species

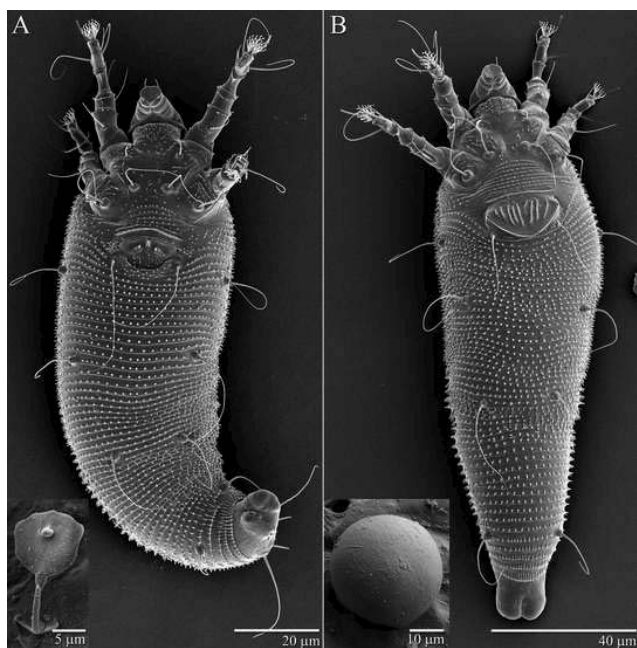


Fig. 1. Low Temperature – Scanning Electron Microscopy (LT-SEM) images of *Phyllocoptes fructiphilus* adult used to describe sexual dimorphism and spermatophores. A) male with detail of spermatophore. B) female with detail of egg.

surveyed for *Phyllocoptes fructiphilus*). Plant samples consisting of 5 plants per variety were separated into stems, leaves, and flowers and examined using a variety of light and electron microscopy techniques. A minimum of 5 stems per plant were examined using a variety of light and electron microscopy techniques. Special care was taken to look for mites in protected niches on the plants, such as under the expanded petioles (stipules), the calyx and corolla, where many eriophyoids find shelter (Sabelis and Bruin 1996).

Light microscope. Initially, observations and images were obtained using a wide field Zeiss AxioZoom microscope (Carl Zeiss Microscopy Thornwood, NY). Samples were examined using a 1x 0.25NA or 2.3X 0.25NA PlanNeoFluor objectives with LED lighting and an AxioCam HRC color camera was used to capture the images. Representative mites were mounted on slides with Hoyer's fluid (Krantz and Walter 2009), with observations conducted with a Zeiss Axioplan 2 microscope (Carl Zeiss Microscopy Thornwood, NY) using phase contrast and differential interference contrast microscope with 40x PlanNeoFluor 1.3NA, 63x Apochromat 1.4NA, and 100x Apochromat 1.3NA objectives.

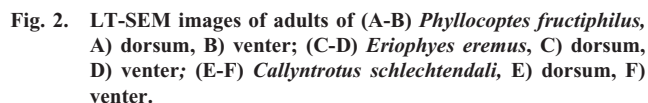
Table top scanning electron microscope (ttSEM). Plant parts observed to have mites on them and individual mites were removed from live plants and placed on 25 mm scanning electron microscope (SEM) specimen stubs (Ted Pella, Redding, PA) with ultra-smooth, round (25 cm diameter) carbon adhesive tabs (Electron Microscopy Sciences, Inc., Hatfield, PA, USA) for observation in the Hitachi TM3030+ (Hitachi High Technologies Dallas, TX) table top scanning electron microscope (ttSEM), coupled to

a Deben TM-3000 Coolstage (Deben UK Ltd. Suffolk, UK) set at -25 °C. The Coolstage reduces the amount of evaporation that takes place inside the microscope and provides a longer period of time for observations prior to sample shriveling. Images were viewed using the charge reduced mode (variable pressure mode) with 15kV accelerating voltage, a working distance of between 8 and 12 mm, magnification of between 40 and 1000x, and with backscatter or secondary electron detectors. This method preserves the true nature of the mites as opposed to pre-treating the sample with a fixative, or critical point drying it and then coating it with a metal conductor, which may create artefacts.

Low temperature scanning electron microscope (LT-SEM). Plant parts and mites were studied using the LT-SEM techniques outlined by Bolton et al. (2014), which are described briefly here. The specimens were placed on 15 by 30 mm copper plates using ultra smooth, round (12 mm diameter) carbon adhesive tabs (Electron Microscopy Sciences, Inc. Hatfield, PA, USA), then frozen conductively in a Styrofoam box, by placing the sample plates on the surface of a pre-cooled (-196°C) brass bar whose lower half was submerged in liquid nitrogen. After 20–30 seconds, the holders containing the frozen samples were transferred to the Quorum PP2000 cryo-prep-chamber (Quorum Technologies, East Sussex, UK) attached to an S-4700 field emission scanning electron microscope (Hitachi High Technologies America, Inc. Dallas, TX, USA). The specimens were etched inside the cryotransfer system to remove any surface contamination (condensed water vapor) by raising the temperature of the stage to -90°C for 10–15 min. Following etching, the temperature inside the chamber was lowered below -130°C, and the specimens were coated with a 10nm layer of platinum using a magnetron sputter head equipped with a platinum target. The specimens were transferred to a pre-cooled (-130°C) cryostage in the SEM for observation. An accelerating voltage of 5 kV was used to view the specimens. Images were captured using a 4pi Analysis System (4pi Durham, NC).

Results and Discussion

Three species of eriophyid mites were identified on roses in this study, *Phyllocoptes fructiphilus*, *Eriophyes eremus*, and *Callyntrotus schlechtendali*, based on descriptions and illustrations by Amrine (1996), Baker et al. (1996), and Druciarek and Lewandowski (2016). All eriophyoid, males can be identified by having genital coverflaps on the venter (underside of the body) medially, just posterior to the second pair of legs with two paired pointed projections (sensory pegs) on the coverflap and opening towards its gnathosoma (mouthparts) (Fig. 1A). Males deposit free-standing spermatophores (Fig. 1A insert) on the surface of the plant. The spermatophores are 7–8 µm in width and 10–12 µm in height with the base of the stalk firmly attached to the surface. The females can be identified by having genital coverflaps with distinctive patterns on their ventral side (underside of body) which open posteriorly (Fig. 1B).



Phyllocoptes fructiphilus (Eriophyidae) (Figs. 1 A, B; 2 A, B; 3 A, D; 4; 5).

The female genital coverflap found on its venter has five to nine longitudinal ridges, slightly directed medially. Above the genital coverflap there is a single ridge followed by eight short dotted ridges progressing towards the anterior part of the mite (Fig. 2B and 3D).

Remarks. *Phyllocoptes fructiphilus* was the eriophyoid mite most frequently encountered in the collection sites (Table 1). All plants showing symptoms of RRD were infested by *P. fructiphilus*, but several symptom-less roses were also infested by this species. A majority (55%) of the varieties and species observed did not have *P. fructiphilus*; however, if no mites were found in the roses it does not mean the variety or species is resistant to the mite. Rather, the roses did not have the mites, thus additional observations need to be conducted. The mites were typically located under the petioles and stipules of the vegetative buds and under the sepals of flower buds, or in open flowers, especially at the base of the sepals. During the early stages of flower development, the sepals and petals are too tightly appressed for even mites to readily gain access; however, during the later stages of floral bud development, when spaces have developed between sepals and petals, the mites are able to gain access to the inside of the buds, and probably remain there throughout the floral development. Populations of mites were observed even within winter-collected flower buds or rosehips (fruit). This species appears to have an affinity for feeding at the base of the sepals under the dense covering of trichomes (hairs), especially close to the base of bulbous, glandular hairs in both floral and vegetative buds. The epidermis in this area is very thin and thus the mites have easy access to the vascular system to nourishment (Fig. 5). The trichomes appear to offer *P. fructiphilus* potential protection from the much larger predatory mites and other insect predators also observed on roses. This is of interest because glandular hairs are typically regarded as physical barriers or deterrents against other plant microbiota feeders (Hashidoko et al. 2001). However, *P. fructiphilus* takes advantage of the glandular hairs by feeding and laying eggs at their bases. Specimens of *P. fructiphilus* may get trapped in the sticky matrix at the tip of the gland cells of the hairs, but they are strong enough to wiggle free of them (J.W. Amrine, personal observations).

Eriophyes eremus (Eriophyidae) (Figs. 2 C, D; 3 B, E; 6).

Diagnostic characters. This species can be distinguished from the other mites found on roses by the prodorsal shield having a dashed median ridge with a pair of admedian lines on either side extending across the entire prodorsal shield (Fig. 2C and 3B). On either side of the admedian lines there is a series of short ridges. The prodorsal shield lacks the frontal lobe (Figure 3B arrow) and closed cells (Fig. 3B). Note, the prodorsal shield of immature deutonymphs of *P. fructiphilus* can have a very similar shape as that of the adults, however, they lack the genital coverflap present on the venter of the adults. The female genital coverflap consists of two concentric ridges on both sides of its shield and a series of raised nodules on the basal coverflap. Above

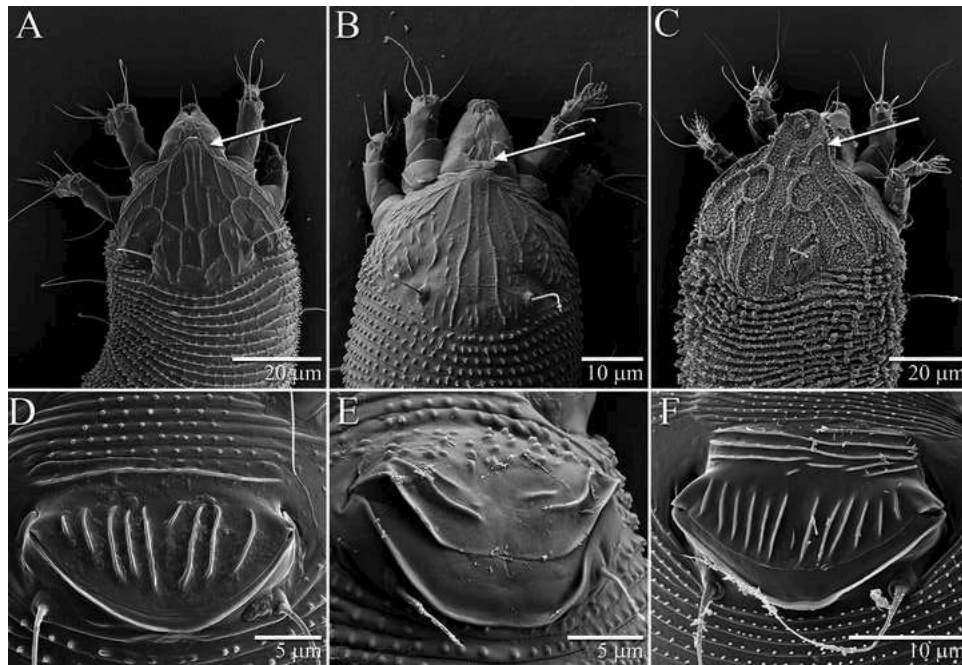


Fig. 3. LT-SEM images of (A-C) prodorsal shield: A) *Phyllocoptes fructiphilus*, B) *Eriophyes eremus*, and C) *Callyntrotus schlechtendali*; and (D-F) of ventral genital coverflap D) *Phyllocoptes fructiphilus* E) *Eriophyes eremus*, and F) *Callyntrotus schlechtendali*. Arrow indicates location of frontal lobe of the prodorsum.

the genital coverflap are four rows of coxisternal annuli bearing microtubercles (Fig. 2D and 3E).

Rose varieties examined of Eriophyes eremus. A commercial rose variety ‘Red Knockout’ was examined by Gabriel Otero-Colina, between February to April 2016 at the Beltsville Agricultural Research Center (BARC), USDAARS, Beltsville, Maryland (Table 1). Mites were discovered in the folded leaf stipules. Additional observations of this mites species were found on *Rosa multiflora* in Georgia, North Carolina, South Carolina and West Virginia

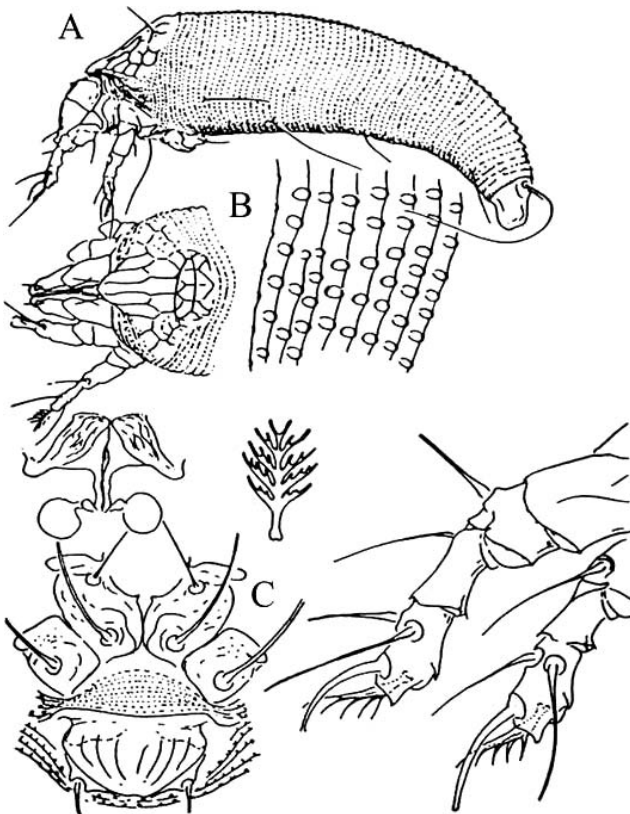


Fig. 4. Line drawings of *Phyllocoptes fructiphilus* (A-C). A) Lateral view, B) Dorsal view of prodorsal shield, and C) Venter female coverflap. (after Keifer, 1939).

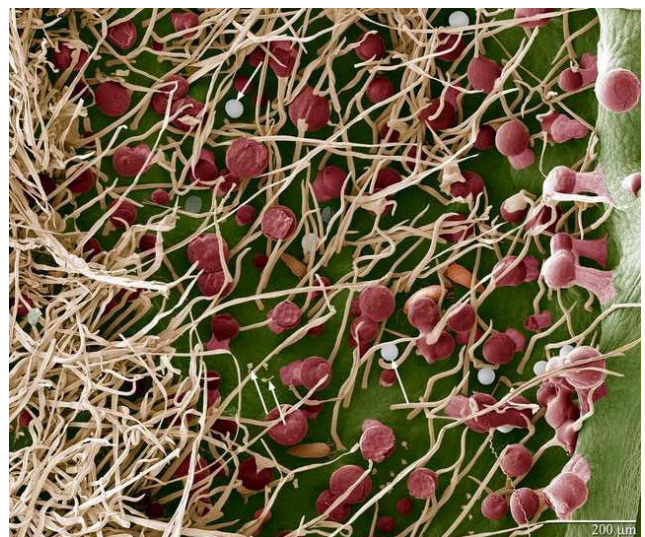


Fig. 5. Colorized LT-SEM image of the base of a flower buds sepal with *Phyllocoptes fructiphilus* feeding at the base of bulbous, glandular hairs and simple hairs. Note presence of both eggs (arrow) and spermatophores (double arrows).

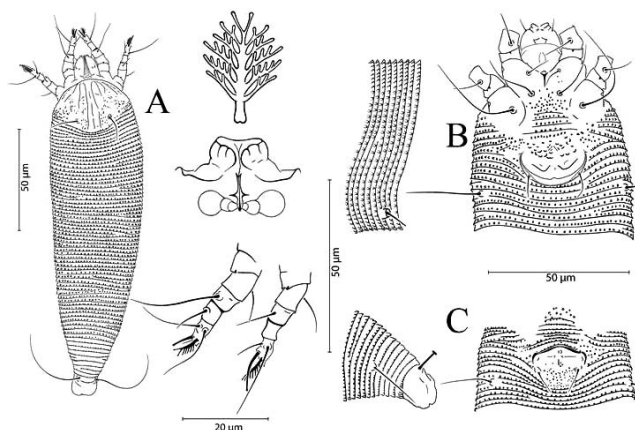


Fig. 6. Line drawings of *Eriophyes eremus* (A-C). A) Dorsum showing prodorsal shield, B) Venter female coverflap, and C) Venter male coverflap. (after Druciarek and Lewandowski, 201)

from 1990 to the present (J.W. Amrine collection, West Virginia).

Remarks. This species was described by Druciarek and Lewandowski (2016) from specimens found inside the flower buds and in petiole bases on an unknown cultivar of *Rosa x hybrida* in Israel. No apparent damage has been observed associated with its presence. This species has been collected by J.W. Amrine in several sites since the 1990's; it has a wide distribution in the US, at least in several Eastern states (J.W. Amrine collection, West Virginia). In Georgia and the Carolinas, mites were found under the stipules that were closely appressed to the stem; in some cases, the mites were found developing in small pits in the stem. The finding of this species in our study represent the first record of this species in the United States and Western Hemisphere.

***Callyntrotus schlehtendali* (Eriophyidae) (Figs. 2 E, F; 4 E, F; 7).**

Diagnostic characters. This species is easily distinguished from the other mites by the characteristics of its dorsum which has a very wax-like white appearance with several straight-line ridges of wax running the length of the mite's body (Fig. 2E). The prodorsal shield has two large distinctive eye-like circular rings at the anterior center of the shield and the central posterior portion of the shield is rounded, protruding into the opisthosoma (posterior) of the mite (Figures 2E and 3C). The prodorsal shield has a large frontal lobe (Fig. 2E and 3C arrow) which completely covers the underlying gnathosoma. The female genital coverflap on the ventral side is wide and shallow and has 12 to 13 longitudinal ridges. The basal coverflap has three to five transverse ridges, some not entire length of shield. Above the coverflap are 10-12 coxigenital annuli bearing small microtubercles (Fig. 2F and 3F).

Rose varieties examined. A commercial rose variety 'Red Knockout' was examined by Gabriel Otero-Colina, Andrew Ulsamer and Jenny Bo between February to April 2016 at the Beltsville Agricultural Research Center

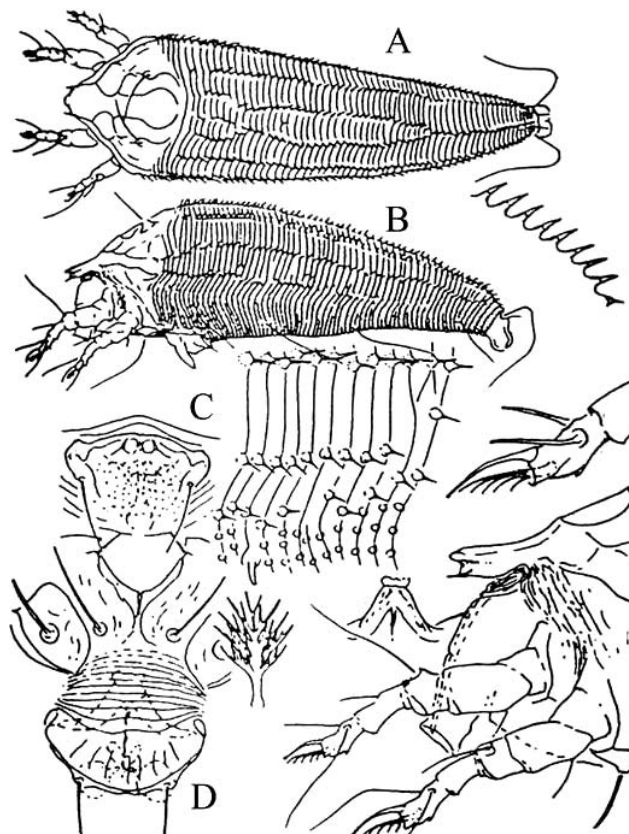


Fig. 7. Line drawings of *Callyntrotus schlehtendali* (A-C). A) Dorsum showing prodorsal shield, B) Lateral view, C) Venter male coverflap, and D) Venter female coverflap. (after Keifer, 1940).

(BARC), USDA-ARS, Beltsville, Maryland. In addition, specimens were examined from collections of this species made in West Virginia and Indiana from 1991 to present (J.W. Amrine collection, West Virginia).

Remarks. Unlike the other eriophyoids found on roses, *C. schlehtendali* was found on the open surfaces of leaves (mainly the abaxial surface) and petioles. No damage was observed that was attributable to this mite (Data not shown).

Other mites. Several predatory mites of the families Phytoseiidae, Cheyletidae and Bdellidae, as well as Tydeidae and Iolinidae mites which are predacious as well as being fungivores (fungus feeders), were observed on some of the rose samples. Some phytophagous mites (plant feeding) in the family Tetranychidae were encountered in some samples as well. Many of these predatory mites were found roaming around the bases of the leaf and flower buds, crevasses of the petiole, and on the leaf surfaces. None of these mites are small enough to find their way into the confined spaces within the buds.

Phyllocoptes fructiphilus is believed to be widely distributed in the U.S. Since it is considered the only vector of RRV (Amrine *et al.* 1994), the distribution of the virus and vector should coincide. Surprisingly, this is not necessarily true as in Florida, Babu *et al.* (2014) detected RRV, but not the mite. A possible explanation may be that

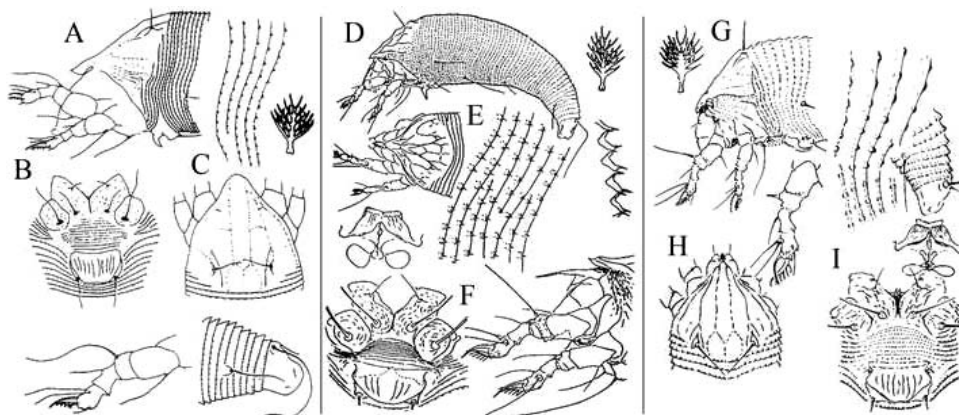


Fig. 8. Line drawings of Eriophyoid mites found on roses in the US but not observed in this study (A-I). *Phyllocoptes linegranulatus* (A-C): A) Lateral view, B) Venter of female coverflap, and C) Prodorsal shield; *P. adalium* (D-F): D) Lateral view, E) Prodorsal shield, and F) Venter female coverflap; *P. chorites* (G-I): G) Lateral view, H) Prodorsal shield, and I) Venter female coverflap. (after Keifer, 1940)

the plants were already infected by the virus when they were introduced into that area and the mites had been controlled chemically or naturally, or had moved on following feeding. We confirmed the presence *P. fructiphilus*, independent of the RRV; however, in all cases where plants were RRD-symptomatic, *P. fructiphilus* was also present, a fact consistent with the role of this mite as a RRV vector. RRD-infected roses have been purchased in supermarkets and other stores (J.W. Amrine, personal communication). A survey of multiflora roses in Indiana from 1989 to 1994 found that about 10% of the roses from Indiana were symptomatic, lost the symptoms and became symptomatic again before dying. It is possible to buy asymptomatic plant which are in fact infected roses with the Rose Rosette Virus (J.W. Amrine unpublished data).

It is noteworthy that *P. fructiphilus* were frequently found in flowers of *R. multiflora* but never in mature fruit. In contrast, Keifer (1940) collected this mite for the first time inside fruits (rose hips) of *Rosa californica* Cham. & Schltdl. (probably in fruits that had fissures or openings). However, in most studies this mite has been found under stipules or vegetative bud scales (Amrine 2002, Amrine et al. 1988). The finding of this mite in flowers is relevant because flowers are intensely traded, so the risk of dissemination of both the mite and RRV is high.

Out of the 20 species of eriophyoid mites living on roses, only the following three species, *E. eremus*, *C. schlehtendali*, and *Phyllocoptes adalium* (Keifer), have a distribution that includes two or more continents, while 15 species are known only in their type location (Druciarek and Lewandowski 2016). So far, seven species are known to occur in the USA: *C. schlehtendali*, *Phyllocoptes linegranulatus* (Styer 1974), *E. eremus*, *P. adalium*, *Phyllocoptes chorites* (Keifer) (Keifer 1972), *P. fructiphilus*, and an undescribed species of the genus *Diptacus* that needs to be formally described once more material is available for its description. *Phyllocoptes adalium*, *P. chorites* and *P. linegranulatus*, (Fig. 8) although previously reported in the US by Keifer (1939), Keifer (1972), and Styer (1974), respectively, were not found during our surveys.

Owing to the importance of the trade of live rose plants, it is expected that the distribution of some of these species will or has already expanded. This may be

especially true due to the introduction and popularity of landscape roses, which have been planted by landscapers in large groups in addition to their low maintenance appeal by homeowners. Once these mites are transported to new areas, the local environmental conditions such as temperature, rain, and winds can contribute to their development and further spread with their associated disease, particularly by the wind. Economic injury of eriophyid mites to roses has been attributed to *Acerimima bajgahi* Kamali, Doryanizadeh & Akrami in Iran (Kamali et al. 2015), *Paraphytoptus rosae* (Domes) in Germany (Domes 2000), *Phyllocoptes resovius* (Druciarek & Lewandowski) in Poland and *P. fructiphilus* in the US (Hoy 2013, Windham et al. 2014). In addition, *P. adalium*, although not so important in the US and unable to vector RRV (Amrine et al. 1994), is reported to reach high populations and cause severe injury to roses in Poland (Druciarek et al. 2016). These data highlight the importance of the correct identification of eriophyoid mites infesting roses as an aid to prevent the introduction of exotic, potentially harmful species.

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