



Herbivore Exploits Chink in Armor of Host

R. Ochoa, J. J. Beard, G. R. Bauchan,
E. C. Kane, A. P. G. Dowling, and E. F. Erbe

Abstract: Knowledge of plant-feeding mite diversity, their interactions with host plants, and their ecological impact are not clear or have been too narrowly studied in the past. The mite superfamily Tetranychoidae has been identified around the world feeding on almost every plant known. Spider mites (Tetranychidae) are the most common mite recognized by farmers, researchers, and any person with an ornamental plant at home. On the other hand, flat mites (Tenuipalpidae) are also common on plants, but are often overlooked due to their small size and camouflaging colors. Mites in the flat mite genus *Raoiella* Hirst are obligate plant parasites that feed via stylet-like mouthparts adapted to pierce plant tissues. A species of particular interest in this genus, the red palm mite, *R. indica* Hirst, is currently spreading aggressively throughout the Americas on species of palm (Arecaceae). How they feed on the plant and how they are able to multiply into huge populations on one host plant have only recently been studied. Here, using low-temperature scanning electron microscopy, *R. indica* and several other species in this genus were captured and observed for the first time feeding via the stomatal aperture of host plants in several different families. These findings on their different host plants challenged our common belief that all plant-feeding mites feed by piercing the epidermis. Stomatal feeding could negatively affect the plant's ability to regulate gas and water exchange. Further examination of feeding methods for other herbivorous mites is necessary.

Phytophagous mites in the genus *Raoiella* Hirst (Tenuipalpidae) are of major concern to quarantine worldwide due to the rapid spread of one species, the red palm mite, *R. indica* Hirst. The red palm mite is currently devastating palm crops across the world and spreading aggressively throughout the Americas (Flechtmann and Etienne 2004, Kane and Ochoa 2006, Dowling et al. 2008), and is the only *Raoiella* species known to feed on monocot hosts (mainly palms, Arecaceae). Our research indicates that most species of *Raoiella* actually feed on dicots; in particular, species in the family Myrtaceae.

Raoiella, like all other species in the Tetranychoidae (spider mites and their relatives), are obligate plant feeders. The moveable digits of tetranychoid chelicerae are highly modified to form a pair of elongate stylets, which are protracted during feeding and lock together in a unique "tongue and groove" arrangement to form a single hollow tube specialized for piercing plant tissues (Summers et al. 1973). Mites in this group are assumed to feed on the internal mesophyll tissues by first piercing the epidermis of leaves (Summers and Stocking 1972, Hislop and Jeppson 1976). However, because the epidermis and underlying hypodermis of a plant play a critical role in its survival and defense by enabling the plant to protect itself against excessive water loss and the entry of harmful substances and pathogens (Reina-Pinto and Yephremov 2009), it is often thick, fibrous, or covered in dense wax layers. The leaves of the Myrtaceae and Arecaceae are no exception, with protective outer layers that must be penetrated by the mites in order to feed.

In many Myrtaceae, the cuticle, or outer covering of the epidermal

cells, is raised into mounds or ridges of various shapes, producing a particular pattern (Fig. 1). The cuticle in *Eucalyptus*, for example, can be thick enough to support the development of permanent folds, mounds, or ridges, which often form over the subsidiary cells associated with a stomatal opening and may be as thick as 30 µm (Car and Car 1987). The cuticle of a eucalypt leaf also extends over the surface of the guard cells of the stomata and the mesophyll cells that line the walls of the stomatal chamber (Car and Car 1978), though it is often much thinner at the line of closure of the guard cells (Car and Car 1979).

Conversely, the epidermis and hypodermis of palms, which together form the surface layer, are tightly bonded and appear to function as a unit that acts like a rind over the leaf (Horn et al. 2009). As an indication of how strong the bond between these two layers is, the epidermis of palm leaves cannot be removed by peeling, but must be scraped away (Tomlinson 1990). Although normal epidermal cells in stomata-bearing regions vary a great deal in wall thickness, the epidermal cell wall exposed on the leaf surface is usually thick and heavily cutinised (Tomlinson 1990). Likewise, the hypodermal cells below the epidermis are usually larger and distinctly thicker-walled than the epidermal cells (Tomlinson 1990).

Despite these obvious mechanical defences, *Raoiella* is able to feed on these hosts and reach populations in the tens of millions per tree (Kane et al. 2005, Pons and Bliss 2007). To find out how this is possible, we examined red palm mites feeding on a preferred host, coconut palm, *Cocos nucifera* L. (Arecaceae), using low-temperature scanning electron microscopy (LTSEM) (Achor et al. 2001). All active

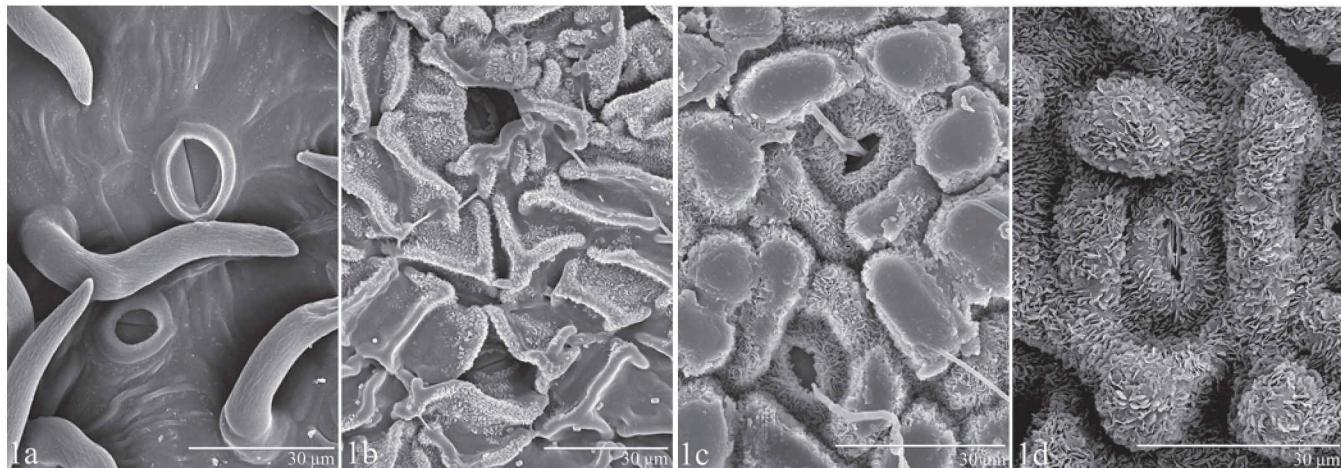


Fig. 1. Detail of stomatal complex and epidermis of host plants in the Family Myrtaceae (a) *Lophostemon confertus* (R.Br.); (b) *Angophora subvelutina* F. Muell.; (c) *Eucalyptus marginata* Sm.; (d) *Corymbia calophylla* (Lindl.).

stages of *R. indica* were observed with their stylets inserted between the guard cells of a stomatal complex (Fig. 2a) (Pons and Bliss 2007), not into the epidermal cells as had previously been assumed. This observation triggered a detailed study of red palm mites and other *Raoiella* species to determine the consistency of this remarkable feeding behaviour.

Materials and Methods

Low-temperature SEM observations were performed using an S-4700 field emission scanning electron microscope (Hitachi High Technologies America, Inc., Pleasanton, CA) equipped with a Polaron Polar Prep 2000 (Energy Beam Sciences, East Grandby, CT) cryotransfer system. To prepare specimens, samples of mites on leaves were excised and placed on flat specimen holders consisting of 16 x 30 mm copper plates that contained a thin layer of Tissue Tek (OCT Compound, Ted Pella, Inc., Redding, CA), which acted as the cryo-adhesive upon freezing. The samples were frozen conductively, in a Styrofoam box, by placing the plates on the surface of a pre-cooled (-196°C) brass bar whose lower half was submerged in liquid nitrogen (LN₂). After 20–30 s, the holders containing the frozen samples were transferred to a LN₂ Dewar for future use or cryotransferred under vacuum to the cold stage in the pre-chamber of the cryotransfer system. The specimens were etched inside the cryotransfer system to remove any surface contamination (condensed water vapour) 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 very fine layer of platinum using a magnetron sputter head equipped with a platinum target. The specimens were transferred to a pre-cooled (-140°C) cryostage in the SEM for observation. An accelerating voltage of 10 kV was used to view the specimens. Scan rate of the electron beam was 80 s and images were produced at a 2560 x 1920 pixel capture resolution with a resulting 4.8 Mb image. Images were sized and placed together to produce figures using Adobe® Photoshop 7.0.

Passing the mechanical host plant defences

Using LTSEM, red palm mite was observed feeding via stomatal apertures on several species of palm in addition to coconut, including date palm, *Phoenix dactylifera* L., and parlor or bamboo palm, *Chamaedorea* sp. Red palm mite was also observed feeding in the same manner on other monocot hosts in different families,

including banana (*Musa* spp., Musaceae) (Fig. 2b) and *Heliconia* sp. (Heliconiaceae).

An undescribed species of *Raoiella* was observed feeding via the stomatal opening of two plants in the family Myrtaceae, brush box, *Lophostemon confertus* (R.Br.) (Fig. 3a), and broad-leaved apple, *Angophora subvelutina* F. Muell. (Fig. 3b). An additional nine undescribed species of *Raoiella* from Australia were also observed feeding via stomata on various plant species and genera within the family Myrtaceae, including *Eucalyptus* (Fig. 4a, b) and *Corymbia* (formerly *Eucalyptus*) (see Fig. 1d).

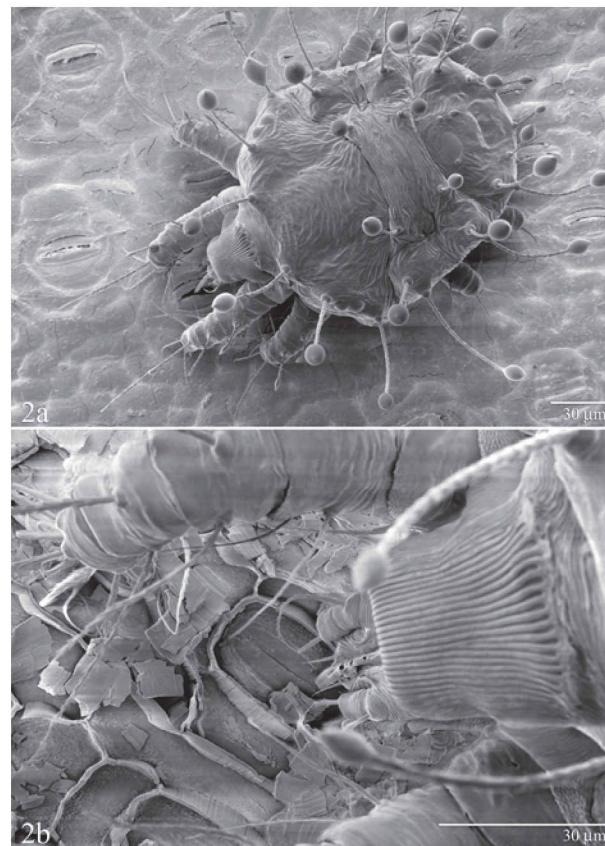


Fig. 2. Adult female *Raoiella indica* Hirst (Tenuipalpidae) feeding via a stomatal aperture (a) of coconut *Cocos nucifera* L. (Arecaceae); (b) of banana *Musa* spp. (Musaceae), in detail.

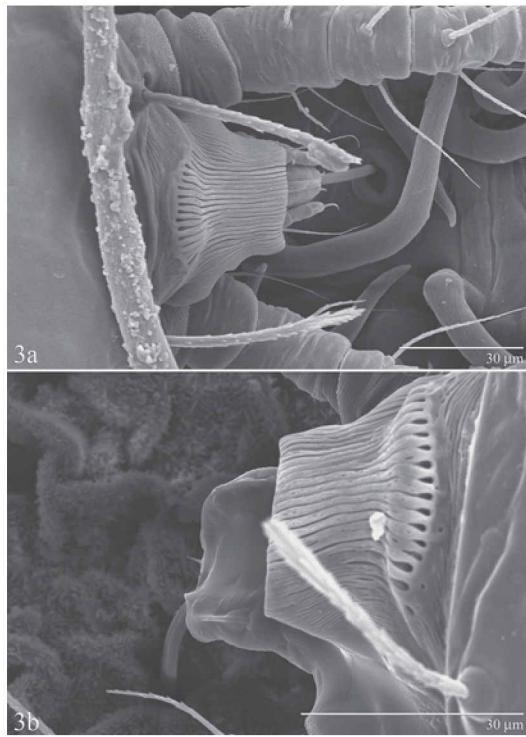


Fig. 3. Detail of an adult female of an undescribed species of *Raoiella* feeding via a stomatal aperture (a) of *L. confertus*; (b) of *A. subvelutina*.

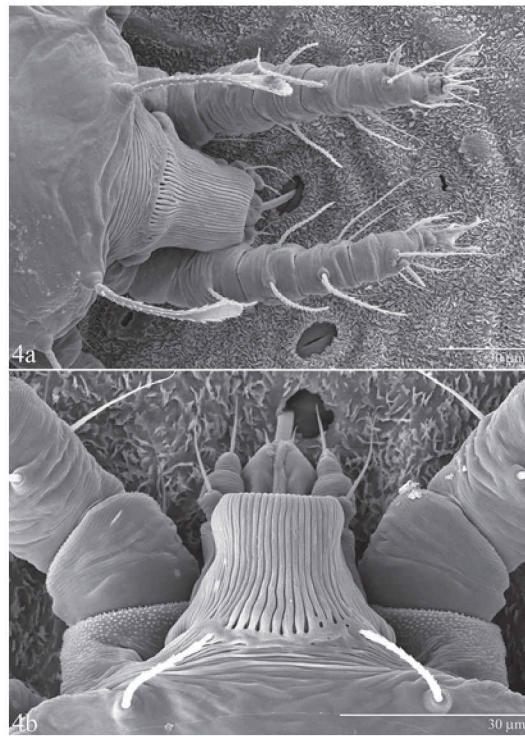


Fig. 4. Detail of adult females of two undescribed species of *Raoiella* feeding via a stomatal aperture (a) of *E. wandoo* Blakely; (b) of *Eucalyptus* sp.

These mites actively bypass the mechanical defences by exploiting a series of structural weakness in the architecture of their hosts:

1. the thick-walled hypodermal cells in palms are always interrupted below each guard cell to form a substomatal chamber;
2. the walls of palm guard cells that face the inside of the stomatal chamber are usually thinner than the walls exposed on the leaf surface;
3. except for one genus with lignified cells (*Johannesteijsmannia*), the substomatal cells are always thin-walled and sometimes contain chloroplasts.

Meanwhile, the cuticle of many eucalypts is much thinner along the line of closure of the guard cells (Carr and Carr 1979), at the same location that *Raoiella* mites insert their stylets. The conclusion is that stomata are a major chink in the plants' armor and represent an obvious point of attack for these mites.

Other plant-feeding arthropods

Although *Raoiella* are the first mites to be observed feeding via the stomata, they are not the only arthropod herbivores to do so. Several lace bugs (Heteroptera: Tingidae) and at least one aphid (Hemiptera: Aphididae) have been observed feeding via the stomata. The green spruce aphid, *Elatobium abietinum* (Walker) (Parry 1971), and the lace bugs *Stephanitis pyrioides* Scott (Butntin et al. 1996, Ishihara and Kawai 1981) and *S. typica* (Distant) (Mathen et al. 1988) are known to always feed via the stomata of their host plants, spruce (*Picea* spp., Pinaceae), azalea (*Rhododendron* spp., Ericaceae), and coconut, respectively. In addition, the lace bugs *Urentius aegyptiacus* Bergevin and *S. rhododendri* Horvath occasionally utilize stomatal openings, though they usually feed by directly penetrating the leaf epidermis (Johnson 1937, Pollard 1959). To feed, adult and nymphal *S. pyrioides* insert their stylets (penetration depth > 500 µm) through the stomata (Mathen 1988), which are located on the lower leaf surface only, penetrate the spongy mesophyll parenchyma intercellularly, and

selectively pierce and remove the contents of palisade cells near the upper surface of the leaves, creating the characteristic white stippling associated with lace bug damage (Buntin et al. 1996). The stylets of *Raoiella* mites (approx. 170 µm) are not as long as those of tingids; however, though the mites are most likely not feeding on tissues near the opposite surface of leaves, their feeding does cause significant colour changes to both surfaces of the host leaves, from green to purple in the Myrtaceae and green to yellow in Arecaceae.

Implications for the host plant and other pathogens

The specialized feeding found in *Raoiella* has potentially far-reaching implications in terms of damage to the host plant. For example, feeding by lace bugs increases stomatal closure, which in turn results in the plant suffering reduced rates of photosynthesis and respiration (Buntin et al. 1996). The primary role of stomata is the regulation of gas and water exchange between the inside and outside of the leaves through the use of the guard cells that control the opening and closing of the stomatal aperture. Even though the stomata occupy only a small percent of the total leaf surface area (when completely open, between 0.5 and 5%), the enormity of their role should not be underestimated. Not only does almost all water transpired and CO₂ absorbed by a plant pass through the stomata (Lawson 2008), they also play a key role in intra-plant communication (Brownlee 2001, Lake et al. 2001). Short-term gas exchange is regulated within a plant by directly controlling the stomatal aperture. However, the overall gas exchange of a plant is regulated through the total number of stomata present on all leaf surfaces. In the long term, plants are able to regulate the density of stomata on a leaf surface through a communication system between mature leaves, which monitor CO₂ and light levels, and the developing leaves (Brownlee 2001, Lake et al. 2001). Thus, mature leaves act as monitors of environmental conditions for the developing leaves so that they can regulate their stomatal density in anticipation of the conditions they are likely to be exposed to when developed (Lake et al. 2001). Large populations of *Raoiella* feeding on mature leaves could potentially alter the signal

being delivered and have an effect on the density of stomata that will ultimately occur on developing leaves.

It is still uncertain if *Raoiella* actively kill the guard cells during feeding. However, when guard cells no longer function and stomata remain permanently open to the external atmosphere, not only does a plant lose its ability to regulate gas exchange and water loss, but its internal tissue will be directly exposed to attack by fungi and pathogens (e.g. Fig. 1c) (Plotnikova et al. 2000, Reina-Pinto and Yephremov 2009). For example, red palm mite feeds readily on banana (Pena et al. 2006, Welbourn 2006, Cocco and Hoy 2009), and due to the massive population sizes developed, this mite could be a major factor in the spread of ascomycete fungi such as *Mycosphaerella fijiensis* Morelet and *M. musicola* Mulder, which cause the devastating banana diseases Black Sigatoka and Yellow Sigatoka, respectively.



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- Ronald Ochoa** is a research entomologist with the USDA-ARS, Beltsville Agricultural Research Center, Systematic Entomology Laboratory in Beltsville, MD. His research focus is on plant—feeding mite systematics and their association with host plants. Ron.ochoa@ars.usda.gov. **Jenny Beard** is an acarologist currently with the Department of Entomology, University of Maryland, College Park, MD. She studies systematics and biology of phytophagous mites in the superfamily Tetranychoidea and their associated acarine predators, particularly the family Phytoseiidae. **Gary Bauchan** is the Director of the Electron and Confocal Microscopy Unit at the USDA-ARS, Beltsville Agricultural Research Center, in Beltsville, MD. He is an expert in low temperature SEM and confocal microscopy. Gary.bauchan@ars.usda.gov. **Ethan Kane** is a program manager with USDA-APHIS-PPQ, Riverdale, MD and formerly an entomologist with the USDA-ARS, Systematic Entomology Laboratory in Beltsville, MD. His research interests include the functional morphology and systematics of plant-feeding mites. **Ashley Dowling** is an Assistant Professor in the Department of Entomology at the University of Arkansas, Fayetteville. He studies systematics and historical ecology of small arthropods, primarily mites. **Eric Erbe** is a retired electron microscopist with the USDA-ARS, Electron Microscopy Unit, Beltsville Agricultural Research Center in Beltsville, MD. He pioneered the use of LT-SEM for the study of soft body arthropods with special interest on mites.