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# Mine-damaged leaves by *Phyllocnistis citrella* Stainton provide refuge for phytoseiids on grapefruit in Florida and Texas*

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## Abstract

Damages caused by pests to leaves can indirectly affect populations of other associated arthropods. The relative abundance of mites was compared across young healthy leaves, mature healthy leaves and mature leaves damaged by the citrus leafminer, *Phyllocnistis citrella* Stainton, on grapefruit in Florida and Texas. The spider mite *Eotetranychus sexmaculatus* (Riley) (Tetranychidae) was significantly more abundant on mined leaves in Florida, whereas in Texas tetranychids were found sporadically. Predaceous phytoseiid mites (Phytoseiidae) were significantly more abundant on mature mined leaves than on mature leaves without mines. *Iphiseiodes quadripilis* (Banks) (n= 139), *Typhlodromalus peregrinus* (Muma) (n= 122) and *Euseius mesembrinus* (Dean) (n= 18) were the most abundant phytoseiids in Florida; *E. mesembrinus* was the dominant species in Texas [>90% of identified specimens (n=13)]. Although relatively high numbers of predaceous stigmaeid mites (Stigmaeidae) were found in some occasions in Florida, they had a patchy distribution, resulting in no significant differences between mined and unmined leaf types in most sampling dates. They were not found in Texas.

**Key words:** Citrus, leafminer, mite-refuge, *Phyllocnistis citrella,* phytoseiid.

## Introduction

Alterations of the leaf surface due to feeding and/or damage by pests may produce changes in the distribution of small arthropod populations, including mites. The citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), is one of the pests that change the surface of attacked leaves. In the United States, it was first reported in Dade County, Florida in 1993, spreading quickly throughout the rest of this state (Heppner, 1993) and to neighboring states, including Alabama, Louisiana and Texas (Legaspi *et al*., 1999). *Phyllocnistis citrella* was initially considered a serious pest in Florida but native natural enemies (Amalin *et al*., 2001), introduced parasitoids (Hoy & Nguyen, 1997) and new IPM programs had an impact on its population densities. Today, *P. citrella* is considered a secondary pest problem (Browning *et al*., 2001), although infested plants become vulnerable to infection by Asiatic citrus canker, caused by the bacterium *Xanthomonas axonopodis* pv. *citri* (Hasse). Adult *P. citrella* females deposit single eggs on young citrus foliage (flush), preferring the midrib on the abaxial surface and occasionally green twigs and young fruits. Immediately after hatching, a larva bores through the leaf epidermis and forms a mine. The mine is serpentine in form and each succeeding larval instar widens the tunnel. Mines caused by *P. citrella* larvae prevent young leaves from expanding uniformly, causing the folding and/or twisting of leaves as they get older. The third instar causes most of the damage, with the prepupa preparing a pupal chamber by folding the edges of the leaf down or upwards and binding them together with silk (Hoy & Nguyen, 1997).

Folded leaves are sheltered spaces that provide refugia to other small arthropods. These spaces may provide more favorable microenvironment conditions than leaves without *P. citrella* damage.

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In fact, they may function in a similar way to domatia, structures present in leaves of many tropical and subtropical plants that provide refuge for microarthropods (O'Dowd & Willson, 1989). Domatia occur in many different forms, and are located at the junction of the primary and secondary veins; they are sometimes lined with leaf hairs (O'Dowd & Willson, 1997; Norton *et al*., 2001; Kreiter *et al*., 2002). The main objective in this study was to compare the relative abundance of predatory and phytophagous mite populations on undamaged young and mature leaves and on mature leaves damaged by *P. citrella*.

## Materials and Methods

### Florida study

In Florida, weekly samples were taken from eleven-year-old red 'Ruby' grapefruit trees (n =10) at the Citrus Research and Education Center in Lake Alfred, from 2 February to 13 April 2001. All ten sampled trees were in a row and were selected at random. Samples were taken between 10:00 and 11:00 AM and consisted of terminals with at least five leaves. In the first seven sampling dates, terminal shoots evaluated were of two types: with mature leaves undamaged by *P. citrella* and with mature leaves damaged by *P. citrella*. In the last four sampling dates, young terminals were also sampled. Only five leaves of each terminal were examined. The first two types of terminals had leaves that flushed the previous year, whereas the third type started to flush around mid-March and were sampled only from 23 March through 13 April. This grove had not received any pesticide sprays since September of the previous year. The terminals were taken to the laboratory, where predatory and phytophagous mite eggs and motile stages were counted using a stereomicroscope. Data were transformed using $\sqrt{X + 0.5}$, where *X*= number of eggs or motile mites per 5-leaves. One-way ANOVA was used to analyze the data; LSD tests (Multiple Range Test for separation of means) were used to separate means of the last four sampling dates, when three shoot types were evaluated. Statistical analyses were done using Statistica® (Stat Soft, Inc. 2000). Mean ± SEM values shown here are untransformed data.

### Texas study

In Texas, five undamaged leaves and five leafminer-damaged leaves were sampled weekly from six randomly selected trees in a 4-year-old commercial organic 'Rio Red' grapefruit orchard located in Weslaco, from 6 April to 2 June 2010. Leaves were processed as previously mentioned. Comparison of predaceous phytoseiid mite (Phytoseiidae) numbers between undamaged leaves and leaves damaged by *P. citrella* was conducted for each date with z-tests, using Statistica® (Stat Soft, Inc. 2000).

## Results

### Florida study

In Florida, the following phytophagous mites were found: Texas citrus mite, *Eutetranychus banksi* (McGregor) (Tetranychidae), six-spotted mite, *Eotetranychus sexmaculatus* (Riley) (Tetranychidae), citrus rust mite, *Phyllocoptruta oleivora* (Ashmead) (Eriophyidae), pink citrus rust mite, *Aculops pelekassi* (Keifer) (Eriophyidae) and a few *Brevipalpus* sp. (Tenuipalpidae). Among the predators, several phytoseiid species (see species identified below) and the stigmaeid *Agistemus* sp. were found. In addition, Tydeidae were also present, but their populations were low with no significant differences found between the three types of terminals ($p > 0.05$).

Fig. 1 (A) and (B) present the means (± SEM) for *E. sexmaculatus* eggs and motiles. Significantly higher numbers of eggs were found on mined leaves on 23 February, 2, 8, 23 and 31 March, 7 and 13 April (Fig. 1A), whereas significantly higher numbers of motiles were found on mined leaves on 23 February; 2, 8, 23 and 31 March; and 7 and 13 April (Fig. 1B).

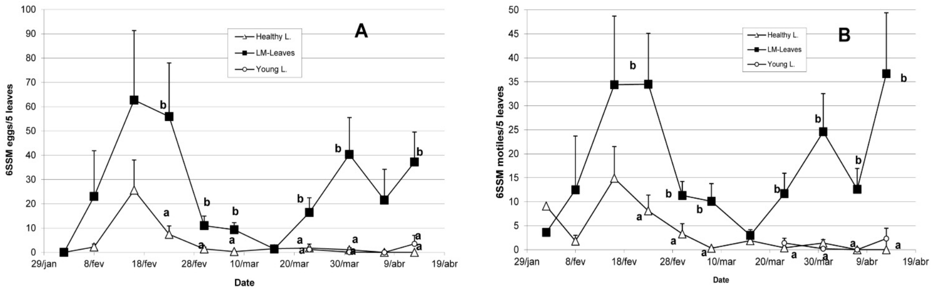


**FIGURE 1.** Mean numbers (± SEM) of six-spotted spider mite, *Eotetranychus sexmaculatus* (6SSM), eggs (A) and motiles (B) on healthy mature (Healthy L.), leafminer-damaged mature (LM-Leaves) and young leaves (Young L.) in Florida. At each sampling date, values bearing the same letter were not significantly different in an ANOVA or LSD test ($p > 0.05$).

No significant differences ($p > 0.05$) were found for eriophyids (citrus and pink rust mites) on any of the dates sampled. Peak eriophyid population occurred in mid February (ca. 100 eriophyids/5 leaves on healthy mature leaves and 75 eriophyids/5 leaves on mined leaves). This population decreased to its lowest levels on 9 April (<5 eriophyids/5 leaves on all three leaf types). The fluctuation of the populations of other phytophagous mites was not evaluated, because they were found in low numbers.

Significantly higher numbers of phytoseiids were found on mined leaves on all sampling dates, except 8 and 16 February and 13 April (Fig. 2A). No significant differences were observed between phytoseiid densities on young undamaged leaves and on mature undamaged leaves on any sampling date. Phytoseiid numbers increased from 8 February until the end of March, reaching averages of 8.9 ± 2.0 (mean ± SEM) motiles/5 mined leaves and 1.9 ± 0.6 motiles/5 healthy leaves on March 23.

Fig. 2B presents the means for motile stigmaeids. Although relatively high numbers were found in some occasions on mined leaves, significant differences were only observed on 23 February, due to the patchy distribution of these mites (Fig. 2B).

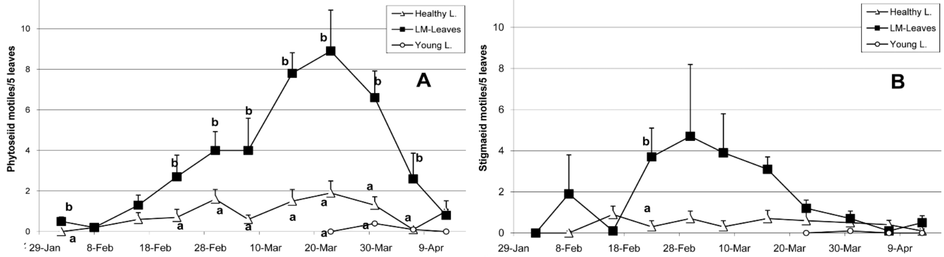


**FIGURE 2.** Mean numbers (± SEM) of predatory mites: Phytoseiidae (A) and Stigmaeidae (B) motiles on healthy mature (Healthy L.), leafminer-damaged mature (LM-Leaves) and young leaves (Young L.) in Florida. At each sampling date, values bearing the same letter were not significantly different in an ANOVA or LSD test ($p > 0.05$).

Most of the phytoseiids collected on 16, 23 and 31 March were slide mounted for identification. They were identified as *Iphiseiodes quadripilis* (Banks) (n= 139), *Typhlodromalus peregrinus* (Muma) (n= 122), *Euseius mesembrinus* (Dean) (n= 18), as well as *Phytoscutus sexpilis* and *Galendromus helveolus* (n= one each). On each of the three sampling dates, the prevalent species were by far *I. quadripilis* and *T. peregrinus* (Fig. 3).

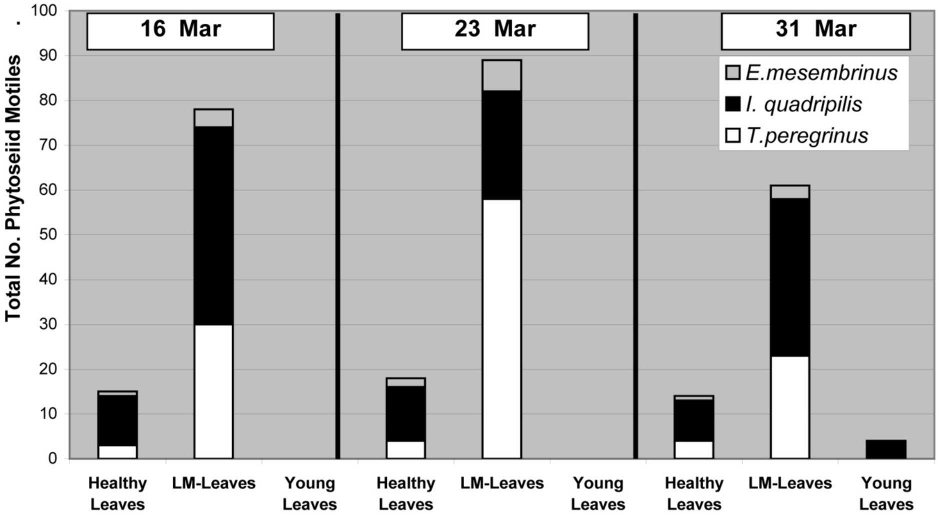


**FIGURE 3.** Relative abundance of the phytoseiids *Euseius mesembrinus, Iphiseiodes quadripilis* and *Typhlodromalus peregrinus* on healthy mature (Healthy L.), leafminer-damaged mature (LM-Leaves) and young leaves (Young L.) on the three sampling dates with the highest numbers of phytoseiids recorded in Florida.`

## Texas study

Presence of phytophagous mites was insignificant although some *E. banksi* and *Panonychus citri* (McGregor) were observed. With regard to the phytoseiids, in Texas we found a trend similar to that determined for Florida, that is, more phytoseiids were found on *P. citrella* mined leaves compared with mature undamaged leaves; differences were statistically significant on most dates, except 12 May and 2 June (Table 1). No stigmaeids were found.

**TABLE 1.** Mean numbers of phytoseiid mites per leaf (± SEM) on mature leaves damaged by *Phyllocnistis citrella* and on mature undamaged leaves of 'Rio Red' grapefruit in Weslaco, Texas, 2010.

| Date | Mined Leaves | Healthy leaves | Z-value | Significance |
|---|---|---|---|---|
| 06-Apr | 1.1 ± 0.3 | 0.1 ± 0.1 | $5.8 \times 10^{-6}$ | ** |
| 14-Apr | 0.8 ± 0.2 | 0.3 ± 0.1 | 0.030 | * |
| 21-Apr | 2.0 ± 0.4 | 0.3 ± 0.2 | $7.9 \times 10^{-16}$ | ** |
| 28-Apr | 1.2 ± 0.3 | 0.2 ± 0.1 | $2.9 \times 10^{-6}$ | ** |
| 06-May | 0.7 ± 0.1 | 0.2 ± 0.1 | 0.013 | * |
| 12-May | 0.7 ± 0.2 | 0.4 ± 0.2 | 0.24 | ns |
| 19-May | 1.7 ± 0.3 | 0.1 ± 0.1 | $6.4 \times 10^{-12}$ | ** |
| 26-May | 0.7 ± 0.2 | 0.1 ± 0.1 | 0.008 | ** |
| 02-Jun | 1.1 ± 0.3 | 0.8 ± 0.2 | 0.24 | ns |

## Discussion

The preference of the spider mite *E. sexmaculatus* for mined leaves could be due to one or more of the following: it being seasonally more abundant following cold winter months; its preference for older, harder leaves (Childers, 1994); or that the irregularity of the mined leaves facilitates the webbing process.

Mined leaves have been shown to provide protective sheltered spaces not only for predators, but also for phytophagous mites. Villanueva & Harmsen (1996) observed similar effects with mines produced by the spotted tentiform leafminer *Phyllonorycter blancardella* Fabricius (Lepidoptera: Gracillariidae) on apple in Ontario. In apple, the stigmaeid *Zetzellia mali* (Ewing) and the phytoseiids *Typhlodromus caudiglans* Schuster and *Neoseiulus fallacis* (Garman) were found in old mines feeding on *Panonychus ulmi* (Koch) and on mites of the family Tarsonemidae (Villanueva & Harmsen, 1996, 1998). Tarsonemids laid higher numbers of eggs around the frass and exuviae of *P. blancardella* inside the mines. This behavior may be important for phytoseiids especially in the winter, when prey is scarce. Frass and exuviae are also found in mines produced by *P. citrella*.

In relation to predators found in this study, unpublished laboratory observations by the first author showed that *I. quadripilis* and *E. mesembrinus* feed on phylloplane fungi that grow more abundantly on mined than on healthy leaves. Zemec & Prenerova (1997) observed that *Typhlodromus pyri* Scheuten fed on the conidia of the powdery mildew *Erysiphe orontii* Castellani from tobacco and *Oidium fragariae* Harz from strawberry in the laboratory. *Typhlodromus pyri* reproduced and completed its life cycle on *E. orontii*.

*Iphiseiodes quadripilis* and *T. peregrinus* combined corresponded to more than 90% of the total number of phytoseiids found on the leaves in Florida. This supports observations of Villanueva & Childers (2005) and C. C. Childers (unpublished) showing *T. peregrinus* and *I. quadripilis* as the second and third most abundant phytoseiids in seven central Florida orchards. *Euseius mesembrinus* was the most abundant phytoseiid in Childer's survey (unpublished) but this species was not abundant in the current study. The presence of these fungi can also affect phytophagous species as it was shown by Belczewski & Harmsen (1997), they found that sprays of the phylloplane fungus *Alternaria alternata* Keissler on apple leaves enhanced population growth of *Tetranychus urticae* Koch compared to that of *P. ulmi*. This change could, in turn, affect the relative dominance of predatory mites.

*Iphiseiodes quadripilis* and *T. peregrinus* are generalist predators (Muma, 1961) and the former shows preference for grapefruit over orange as a substrate (Villanueva & Childers, 2006). Stigmaeids were found throughout the duration of this study in Florida, where their patchy distribution and variable numbers were similar to what was reported by Muma & Selhime (1971) for the stigmaeid *Agistemus floridanus* Gonzalez in Florida.

This paper describes the refuge provided by mined leaves and its effect on phytoseiid populations on citrus in Florida and Texas. The mine provides phytoseiids with shelter and food. Phytoseiids utilize mines as refugia, spaces where they can increase more than on un-mined leaves. Although the citrus leafminer is a pest, changes in leaf morphology produced by its feeding are used positively by phytoseiids and thus could enhance pest mite control. In addition to the phylloplane fungi that can develop on mined leaves, McMurtry & Croft (1997) indicated that leaf morphology can strongly influence the abundance of generalist phytoseiids by providing sheltered habitats that may be more important than food availability. This result should be taken into consideration when designing sampling plants for those predatory mites. However, it may also be possible that only some predatory species, such *I. quadripilis* and *T. peregrinus*, exploit this type of habitat, while other phytoseiid species avoid it. This study only focused on acarine populations but insects such thrips, curculionids, hemipterans including psyllids, and spiders were observed more frequently on mined leaves than on undamaged leaves. Complementary research is necessary to clarify how mining by *C. citrella* and other insect species affects beneficial arthropod numbers on grapefruit and other plant species.

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