

# Plant cell piercing by a predatory mite: evidence and implications

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**Abstract** Omnivorous arthropods can play an important role as beneficial natural enemies because they can sustain their populations on plants when prey is scarce, thereby providing prophylactic protection against an array of herbivores. Although some omnivorous mite species of the family Phytoseiidae consume plant cell-sap, the feeding mechanism and its influence on the plant are not known. Using scanning electron microscopy we demonstrated that the omnivorous predatory mite *Euseius scutalis* penetrates epidermal cells of pepper foliage and wax membranes. Penetration holes were teardrop shape to oval, of 2–5 µm diameter. The similarities between penetration holes in pollen grains and in epidermal cells implied that the same penetration mechanism is used for pollen feeding and plant cell-sap uptake. Variation in shape and size of penetration holes in leaves and a wax membrane were attributed to different mite life stages, depth of penetration or the number of chelicerae puncturing (one or both). Punctured stomata, epidermal and vein cells appeared flat and lacking turgor. When the mite penetrated and damaged a single cell, neighboring cells were most often intact. In a growth chamber experiment very large numbers of *E. scutalis* negatively affected the growth of young pepper plants. Consequently caution should be taken when applying cell-piercing predators to young plants. Further studies are needed to take advantage of the potential sustainability of plant cell-sap feeding predators.

**Keywords** *Amblyseius swirskii* · *Euseius scutalis* · Phytoseiidae · Plant cell-sap feeding · Scanning electron microscopy

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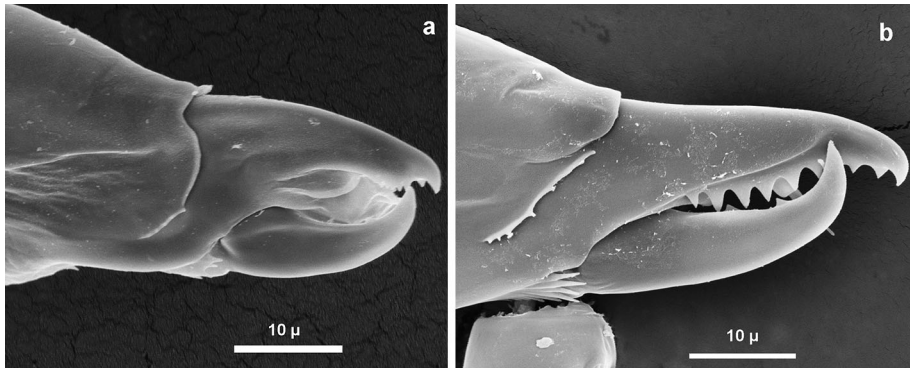
## Introduction

True omnivores can feed on both prey and plants (Coll and Guershon 2002). Plants provide alternative food such as pollen and nectar in order to attract true omnivores for their protection (Wackers et al. 2005). This is because omnivorous arthropods can sustain themselves longer on these plants and can therefore prevent herbivore outbreaks (Sabelis et al. 2005; Van Rijn et al. 2002). Some acarine species of the family Phytoseiidae are well known natural enemies used for the biological control of agricultural pests (Gerson et al. 2003). Though many phytoseiid genera are technically defined as true omnivores, as they feed on plant foods such as pollen and prey, they are usually referred to as predators (McMurtry and Croft 1997; McMurtry et al. 2013), and will thus be referred to herein. Consequently, their chelicerae are adapted to a heterogeneous diet enabling the predator to grasp and penetrate pollen or prey (Flechtmann and McMurtry 1992a, b). In addition to pollen, several studies have suggested that they also feed from the plant (Chant 1959; Kreiter et al. 2002; Magalhaes and Bakker 2002; Nomikou et al. 2003a; Porres et al. 1975). Plant feeding by other biological control agents such as the omnivorous predatory bugs (Heteroptera: Miridae) may damage the host plant (Castañe et al. 2011). To the best of our knowledge, plant injury by cell-sap feeding phytoseiids was recorded only once (Sengonca et al. 2004), who found scars on apple leaves and fruits caused by *Typhlodromus pyri* Scheuten, but effects on plant growth were not assessed. Cellsap feeding was suggested to benefit the phytoseiids (e.g. Grafton-Cardwell and Ouyang 1996; Kreiter et al. 2002; Nomikou et al. 2003a) and the host plant by extending predator survival when prey and pollen are scarce (Magalhaes and Bakker 2002; Sengonca et al. 2004).

Recently we provided filmed evidence of *Euseius scutalis* Athias-Henriot and *Iphiseius degenerans* (Berlese) (Phytoseiidae) probing and penetrating leaf tissue (Adar et al. 2012) (see supplemental material/online resource files 1–9, <http://link.springer.com/article/10.1007/s10493-012-9589-y>). Using a scanning electron microscope (SEM), we identified morphological differences between the chelicerae of plant cell-sap and non cell-sap feeding phytoseiids (Fig. 1a,b; Adar et al. 2012). The movable digit of the non cell-sap feeders was longer than the fixed digit, whereas in cell-sap feeders the movable digit was shorter and less curved. Accordingly, we proposed that the longer fixed digit presses the surface while the short slightly curved dagger-like movable digit penetrates the epidermal cell. However, the magnification and resolution in this study were not sufficient to determine which plant tissue is penetrated or injured.

Potentially, cell-sap feeding phytoseiids could cause damage to the host plant compared to other omnivorous predators that feed on nutritious exudates secreted by leaf epidermis cells such as pearl bodies (Ozawa and Yano 2009). Cell-sap feeding mites will be more prone to systemic pesticides (Magalhaes and Bakker 2002; Nomikou et al. 2005). Furthermore, mites feeding on epidermal cells may need to deal with plant defensive metabolites, and can be more influenced by leaf texture such as wax layer thickness, and therefore be more plant-specific. This may explain why *Euseius hibisci* (Chant) commonly found on avocado but not on citrus (Congdon and McMurtry 1985) was able to uptake radioactive labeled plant cell-sap from avocado but not from lemon (Porres et al. 1975).

In the present study we set out to determine whether phytoseiids penetrate epidermal cells during cell-sap feeding. If so, could this impact plant growth negatively? We first describe penetration holes made by *E. scutalis*, a pollen feeding phytoseiid with cell piercing abilities (McMurtry et al. 2013), using SEM. To elucidate the penetrating mechanism, we studied SEM images of leaves and wax membranes with high densities of



**Fig. 1** Scanning electron microscope images of chelicerae, paraxial profile taken at  $\times 2,700$ , of **a** *Euseius scutalis* and **b** *Amblyseius swirskii*. SEM preparations followed the methodology presented in Adar et al. (2012). White scale bars 10  $\mu$ m

*E. scutalis*. These were compared to similar leaf surfaces with large numbers of *Amblyseius swirskii* Athias-Henriot, a generalist predator (McMurtry et al. 2013) who also feeds on pollen but lacks cell piercing abilities (Adar et al. 2012; Nomikou et al. 2003a). Finally, we evaluated the effect of epidermal cell piercing on the growth of pepper plants.

## Materials and methods

### Mite cultures

*Euseius scutalis* collected from avocado leaves were reared on potted plants of *Solanum nigrum* (black nightshade) in a growth chamber maintained at  $24 \pm 4$  °C,  $60 \pm 13$  % RH, 16L:8D at the Neve Ya'ar Research Center, Israel. *Quercus ithaburensis* (Tabor oak) pollen stored at  $-20$  °C was applied to leaves of potted plants twice a week as the sole food source. *Amblyseius swirskii*, originally collected from citrus foliage and subsequently reared on *Carpoglyphus lactis* L. (Acari: Carpglyphidae), were received from Bio-Bee Biological Systems, Sdeh Eliyahu, Israel.

### SEM observations of cheliceral penetrations in leaf and wax membrane

Microscopic observations were performed by cryogenic scanning electron microscopy (Cryo-SEM, model specified below). Two types of samples were prepared:

#### *Mites on leaves*

Prior to cryo-SEM imaging phytoseiids were reared at room temperature on potted pepper *Capsicum annuum* (cultivar Red-rock 7180, Hishtil, Israel) or nightshade plants for at least 2 weeks. Pollen of *Q. ithaburensis* was provisioned twice a week, by placing a pinch on the leaf with a spatula. Highly populated leaves were removed from the plant and were taken either to sample preparation without preliminary treatment or sprayed prior to preparation with ethyl chloride cooling spray (I.G.S, Germany) to arrest any activity of the predators

before handling the leaves. Small rectangular (approximately 15 × 10 mm) leaf specimens were then cut adjacent to the main vein, bottom side up (where most predators were found).

### *Mites on wax paper*

Wax membrane samples for cryo-SEM imaging were prepared by placing approximately 100 motiles of either *E. scutalis* or *A. swirskii* on a 7 cm diameter circle of silk paper coated with beeswax (Adar et al. 2012). Phytoseiids were collected into micropipette tips that were later cut and placed on the waxed paper for their release. The paper was placed over a water soaked sponge, serving as a barrier to prevent mite escape and as a water source. The phytoseiids were fed with *Q. ithaburensis* pollen placed in a small heap on the wax surface twice a week, and synthetic cotton wool was provided for oviposition (Argov et al. 2002). After 2 weeks, 0.5 cm diameter circles of phytoseiid aggregations were cut out of the wax sheet for imaging without further preparation.

### *Imaging*

Samples of both leaf and wax were immediately frozen in one of two ways: (a) Glued to a stub which was screwed to a Bal-Tec specimen holder for SEM stubs, using a conducting carbon cement (Leit-C; Plano, Wetzlar, Germany), and then swiftly submerged under either liquid nitrogen (LN<sub>2</sub>) or liquid ethane (−196, −183 °C, respectively). (b) Glued using Tissue-Tek medium (OCT Compound; Ted Pella, Redding, CA, USA) to a 15 × 30 mm copper foil, flat, or 60° tilted. The foil with the samples was cooled to −196 °C before the insertion to LN<sub>2</sub> by attaching it with a screw to the sample holder for SEM stubs (Bal-Tec, Balzers, Lichtenstein) that was half immersed in LN<sub>2</sub>. After 30 s, the screw was fastened and the foil with the attached sample was covered with LN<sub>2</sub> according to Ochoa et al. (2011).

The rapid cooled samples were transferred with the VCT100 shuttle (Bal-Tec) to the LN<sub>2</sub> cooled Bal-Tec cryo-stage in the Zeiss Ultra Plus HRSEM (Carl Zeiss SMT, Oberkochen, Germany), kept at −145 °C. In some samples the surface was contaminated with condensed water vapor that was removed by thermally etching inside the HRSEM by raising the temperature of the cryo-stage to −100 °C for 3 min. Following etching, the temperature was lowered below −130 °C. Because of the use of low acceleration voltage (1 kV), no specimen coating was needed. Imaging was done at a working distance (WD) of 3–5 mm using the two secondary electron detectors: either the Everhart–Thornley detector or the high-resolution in-the-column detector (SE2 and In-Lens detector following Zeiss nomenclature). A mixture of both signals was often used to allow high resolution and good perception of the specimen topography. The respective preparation methods used are indicated in each figure legend. Measurements of holes attributed to cheliceral penetration were performed on images of leaves (n = 25) and wax membranes (n = 17).

### Effect of epidermal cell piercing by *Euseius scutalis* on pepper plant growth

We evaluated the effect of epidermal cell penetration by *E. scutalis* on 2 months old potted pepper plants *C. annuum* (cultivar Red-rock 7180). Pots (250 cc) with pepper plants were placed in a growth chamber in October 2012 and maintained at 27 ± 2 °C, 51 ± 9 % RH, 16L:8D. Flower buds were removed regularly prior to flowering to prevent access to pollen

and nectar. To prevent movement of predators among plants, each pot was placed in a plastic container (270 cc, Meitav, Plastic and Packaging Products, Holon, Israel) that retained the excess irrigation water, and was set on its lid, the latter placed upside down, with the lid groove filled with Parafin oil (light USP, Romical, Beer-Sheva, Israel), serving as moat.

The plants were randomly assigned to two treatments: a) an initial release of 50 motiles of *E. scutalis* and a pollen coated twine (21 plants); b) no predators and an uncoated twine (15 plants). As a food source for the predators we used pollen coated twines (Adar et al. 2014a). A 15 cm long segment of twine was tied in a ring and coated with pollen of *Q. ithaburensis* by shaking it in a closed container 1/5 filled with pollen. The excess pollen was then removed by holding individual rings with a tweezers and shaking them above the container. The twine was placed over the petiole of the youngest leaf that was longer than 3 cm. Additional twine rings were applied using the same procedure after 2 and 5 weeks from setup. After 6 weeks, at the end of the experiment, images of all plants from above were taken and their leaf-cover areas (LCA) were calculated using an image processing algorithm (Lati et al. 2011) executed in MATLAB (version 7.12.0.635 R2011a, Math-Works, Natick, MA). Plant height was measured and number of leaves (longer than 1 cm) counted. Twine rings were removed and rinsed in ethanol 70 % for mite extraction prior to plant weighing. Plants (without the roots) were then weighed (fresh weight), rinsed in ethanol 70 % and subsequently dried for 4 days at 40 °C and weighed again (dry weight). To estimate *E. scutalis* population we counted the number of motiles in the ethanol rinses of both plant and twine. To test the hypothesis that large populations of *E. scutalis* negatively impact the growth of pepper plants, we compared the height, number of leaves, fresh weight, dry weight and leaf cover area between plants with and without high populations of *E. scutalis*. Data were analyzed by one-tailed *t* test after a Shapiro–Wilk *W* test for normality (JMP 7.0 SAS).

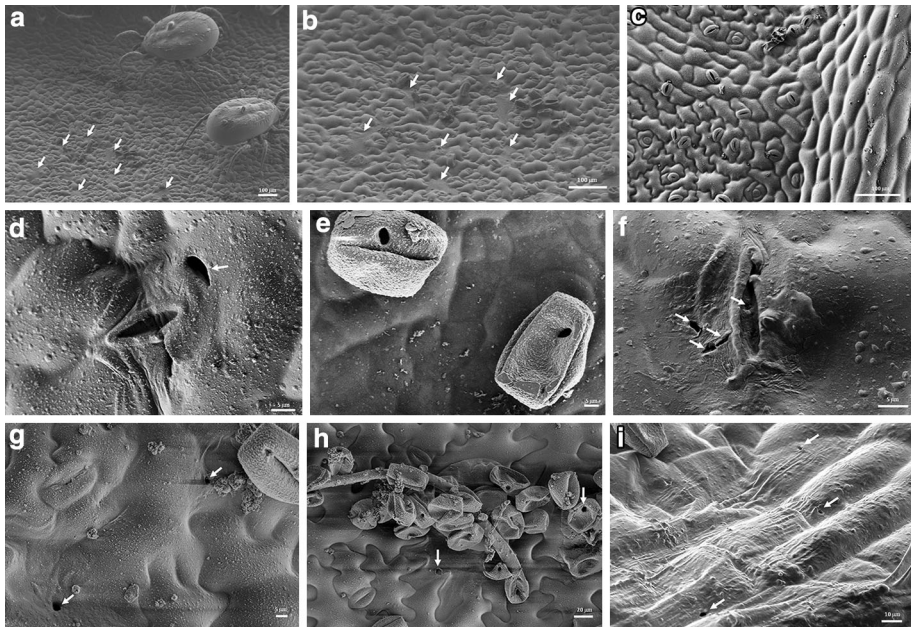
While feeding on pollen, *E. scutalis* spreads pollen grains, creating a thin yellow layer on the leaves that may potentially affect photosynthesis and negatively impact plant growth. Therefore we evaluated the effect of two pollen densities on pepper growth. As described above, two month old potted pepper plants were placed in a growth chamber (January 2013) and maintained at  $27 \pm 1$  °C,  $39 \pm 6$  % RH, 16L:8D for 6 weeks. The plants were randomly assigned to one of three treatments (10 replicates each): heavy pollen cover, light pollen cover and no pollen cover. To achieve heavy and light pollen cover, *Typha latifolia* (bulrush) pollen stored at  $-20$  °C, was applied evenly using a 53 micron sieve, covering all leaves once a week, and once every 3 weeks, respectively. The heavily covered treatment leaves were thus covered throughout the experiment, whereas plants of the lightly covered treatment had several uncoated young leaves throughout most of the experiment. The latter treatment is typical of plants populated with a high density of phytoseiids where the mature leaves are covered with a pollen layer, whereas the heavy treatment is an exaggerated density (E. Adar, personal observations). During the experiment one of the heavily covered plants died and was removed from the analysis. At the end of the experiment, LCA, height, number of leaves, fresh weight and dry weight were recorded as described above, except that the pollen was delicately wiped from the leaves with cotton swabs prior to weighing and drying and that ethanol plant washes for mite extraction were not conducted. The data were compared with a one-way ANOVA after a Shapiro–Wilk *W* test for normality (JMP 7.0 SAS).

## Results

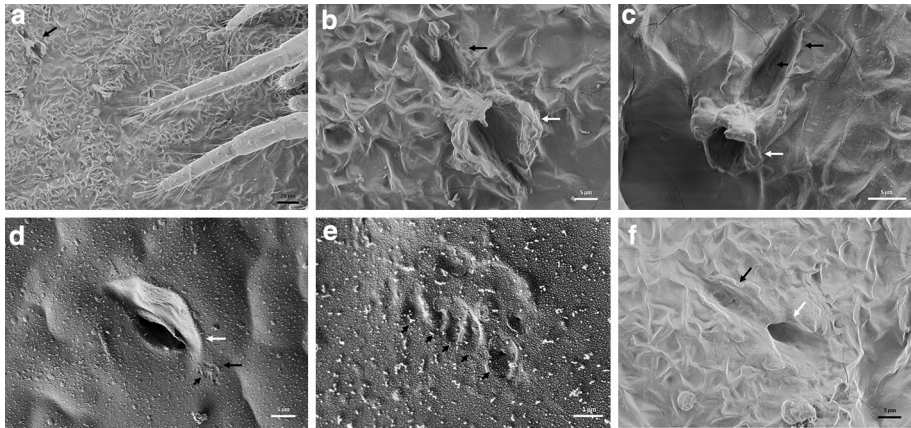
### SEM observations on cheliceral penetration in leaf and wax membrane

Flattened epidermal cells and stomata, appearing deflated (Fig. 2a, b—closeup with arrows) were found scattered on the underside of pepper and nightshade leaves harboring *E. scutalis*. In contrast, in uninhabited leaf surfaces (Fig. 2c), all epidermal, stomatal and vein cells seemed normal with an inflated appearance.

Penetration holes varied in location, size and shape. In the vicinity of stomatal cells (Fig. 2d) and pollen (Fig. 2e) we mostly found teardrop-shaped holes, 4–6  $\mu\text{m}$  in length and 1–2  $\mu\text{m}$  wide. Occasionally there were smaller holes or more than one hole near stomata (Fig. 2f). Many of the holes detected in epidermal cells were smaller than those near stomata (Fig. 2g), being more rounded or slightly elliptical ( $\sim 1\text{--}3\ \mu\text{m}$  diameter), but in some cases larger holes were observed too ( $\sim 5\ \mu\text{m}$  diameter). One such round puncture type with a notch, depicting a ‘key hole’, was observed in an epidermal cell and in nearby



**Fig. 2** Scanning electron microscope images of penetration holes inflicted by *Euseius scutalis* in pepper leaves and pollen grains. **a** *Euseius scutalis* on a pepper leaf with scattered flattened epidermal cells (arrows). **b** Enlargement of same pepper leaf with scattered flattened epidermal cells. **c** Pepper leaf without *E. scutalis* presented as a non-damaged reference. **d** Large (5–6  $\mu\text{m}$  length) teardrop shaped penetration hole (arrow) near a flattened stoma aperture. **e** Large teardrop shaped penetration holes in pollen grains that were found on a pepper leaf populated with *E. scutalis*. **f** Small (2–4  $\mu\text{m}$  length) penetration holes near flattened stoma (arrows). **g** Small holes in an epidermal cell (arrows). **h** Large (5  $\mu\text{m}$  diameter) round notched penetration hole in an epidermal cell (arrow), and a same shaped penetration in a nearby pollen grain (arrow). **i** Penetration holes in vein cells (arrows). Specimens were either without preliminary preparations (**a–h**) or were sprayed with ethyl chloride cooling spray (**i**). Specimens were then either (**a**) glued to a stub and swiftly submerged under either liquid nitrogen  $\text{LN}_2$  (**c**, **d**), or liquid ethane (**e**, **f**), or (**b**) glued to a copper foil, flat (**g**, **h**) or  $60^\circ$  tilted (**a**, **i**), that was later cooled by attaching it to a stub submerged in  $\text{LN}_2$  for 30 s, then covered with  $\text{LN}_2$  itself. Scales are noted above bars



**Fig. 3** Scanning electron microscope images of cheliceral imprints in wax and on pepper leaves. **a** *Euseius scutalis* on a wax surface with a penetration hole imprint (black arrow). **b** Enlargement of image (a), a symmetrical heart shaped deep penetration hole (white arrow) with adjacent shallow grooves (black arrow), apparently created by two chelicerae. **c** Imprint of penetration hole with teeth marks at the distal tip of the shallow groove imprinted by the fixed digits in the wax surface (short black arrow). **d** Deep penetration hole in the leaf (white arrow) in what appears to be a torn stoma aperture and adjacent two rows of teeth (black arrows). **e** Enlargement of the two rows of teeth (black short arrows) imprint in the leaf. **f** Smaller shallower wax imprint of a single chelicerae penetration hole by the movable digit (white arrow) and the fixed digit (black arrow). Specimens were either **a** glued to a stub and swiftly submerged under either liquid nitrogen LN<sub>2</sub> (**d**, **e**), or glued to a flat copper foil (**a**, **b**, **c**, **f**) that was later cooled by attaching it to a stub submerged in LN<sub>2</sub> for 30 s, then covered with LN<sub>2</sub> itself. To remove condensed water vapor contamination the specimen was thermally etched by raising the temperature of the cryo-stage to  $-100\text{ }^{\circ}\text{C}$  for 3 min then lowering it below  $-130\text{ }^{\circ}\text{C}$  (**a**, **b**, **c**, **f**). Scales are noted above bars

perforated oak pollen grains (Fig. 2h). Penetration holes were also observed in vein cells (Fig. 2i) but their size and shape could not be measured accurately as images of vein cells were taken with a  $60^{\circ}$  tilt.

On the wax membrane, we found imprints of chelicerae (Fig. 3a). The most common imprints were symmetrical paired holes, evidently made by both chelicerae (Fig. 3b). A heart-shaped hole ( $\sim 5\text{--}6\text{ }\mu\text{m}$  wide) with wax displaced from within to the side and above, was aligned in a straight line with a shallow depression with no wax displacement; in some cases two rows of small pits were visible toward the shallower depression distal tip ( $\sim 3\text{ }\mu\text{m}$  length, 5 teeth pits, Fig. 3c). Similar shallow imprint pits were found on the pepper leaves with *E. scutalis* (Fig. 3d,e). On the wax surface we also observed narrow single imprints ( $\sim 3\text{ }\mu\text{m}$  wide, Fig. 3f) that included a deep hole and a shallow groove just as in the paired symmetrical penetrations, but these seemed to be made by only one chelicera.

#### *Control: leaf and wax surfaces with Amblyseius swirskii*

As expected, no penetration holes were found on leaf or wax surfaces that harbored large populations of *A. swirskii*, a non-cell piercing phytoseiid, for at least 2 weeks. Holes on waxed paper did not differ from random holes on uninhabited wax, evidently made during preparation of the waxed silk paper. Penetration holes found in oak pollen grains were shaped rounder and were almost twice as big as the holes made by *E. scutalis* ( $\sim 9\text{--}11\text{ }\mu\text{m}$  diameter compared with  $\sim 5\text{--}6\text{ }\mu\text{m}$  of the latter) (Fig. 4).

**Fig. 4** Large penetration hole in pollen grain (9  $\mu\text{m}$  diameter), created by *Amblyseius swirskii*. Specimen was glued to a flat copper foil that was later cooled by attaching it to a stub submerged in  $\text{LN}_2$  for 30 s, then covered with  $\text{LN}_2$  itself. Scale is noted above *bar*



### The effect of *Euseius scutalis* on pepper plants

Dense *E. scutalis* populations ( $1,305 \pm 81/\text{plant}$ ) developed on pepper plants with pollen supply. Despite our efforts to keep the control plants mite-free they did contain a few *E. scutalis* ( $13.3 \pm 3.6/\text{plant}$ ). The height ( $t_{1,34} = 1.78$ ,  $p < 0.05$ ), fresh weight ( $t_{1,34} = 2.67$ ,  $p < 0.01$ ), dry weight ( $t_{1,34} = 2.43$ ,  $p < 0.05$ ) and leaf cover area ( $t_{1,34} = 3.10$ ,  $p < 0.01$ ) of the control pepper plants were significantly higher than of plants with *E. scutalis* (Fig. 5). There were no significant differences in the number of leaves between treatments.

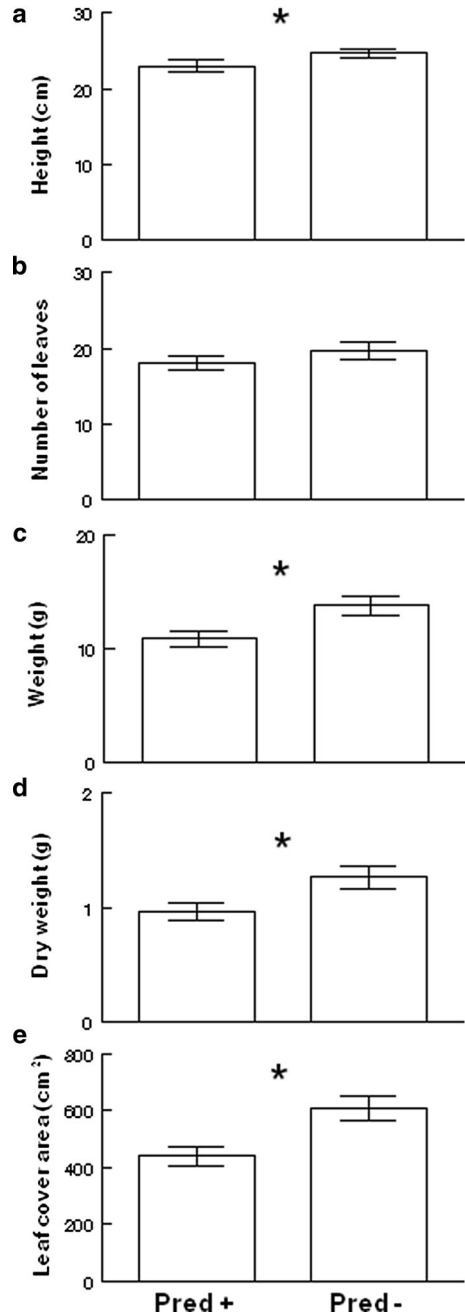
Pollen cover alone did not affect plant growth. The height ( $22.6 \pm 0.3$  cm (mean  $\pm$  SE);  $F_{2,26} = 0.57$ ,  $p > 0.5$ ), fresh weight ( $15.1 \pm 0.5$  g;  $F_{2,26} = 0.42$ ,  $p > 0.5$ ), dry weight ( $1.46 \pm 0.04$  g;  $F_{2,26} = 0.3$ ,  $p > 0.5$ ), number of leaves per plant ( $30.6 \pm 0.6$ ;  $F_{2,26} = 1.25$ ,  $p > 0.1$ ) and leaf cover area ( $617.4 \pm 20.8$   $\text{cm}^2$ ;  $F_{2,26} = 0.38$ ,  $p > 0.5$ ) were similar in plants that were heavily covered with pollen, lightly with pollen and plants that were not covered with pollen at all. All these values resemble the growth parameters of the unpopulated plants of the experiment above.

### Discussion

We have demonstrated that the phytoseiid *E. scutalis* penetrates epidermal cells when feeding on plant cell-sap, whereas *A. swirskii* does not. The similar size and shape of penetration holes in pollen grains and leaves suggests a similar mode of penetration. The shape of the hole supports the penetration mechanism of prey as described by Flechtmann and McMurtry (1992b), and the hypothesis proposed for plant feeding suggested by Adar et al. (2012). Both studies hypothesized that the two movable digits are inserted into the prey/plant, while the fixed digits are pressed against the surface. The length of the larger penetration holes indeed matches the median height of the movable digit (half way between base and tip, Fig. 1a) of an adult *E. scutalis*, and the width of the hole matches the width of two movable digits pressed together. The images (Fig. 3b,c,f) of the wax samples confirm that the large penetration holes were created by the movable digits as the wax displacement, set to the sides and above the hole, matches the direction of cheliceral penetration. The paired grooves, adjacent to the hole, fit the external cheliceral ‘hold’ of the fixed digits as they are shallow and elongated without apparent wax displacement but with the occasional apparent imprint of what seems to be two teeth rows towards the distal tip of the digit, both in the wax and on the leaf. Shape and size of leaf penetration holes



**Fig. 5** Mean ( $\pm$  SE) plant growth parameters of pepper plants after 6 weeks of either hosting a large population of *Euseius scutalis* (Pred+, N = 21) or no predators (Pred–, N = 15). Plant growth parameters are: height (a), number of leaves (b), weight (c), dry-weight (d) and leaf cover area (e). Asterisks indicate significant differences ( $p < 0.05$ )



varied from large teardrop-like, apparently made by both movable digits pressed together, to smaller and more oval or elliptic. Penetration by various life stages and depth of penetration differentiation could partially account for this variation that might also be caused by the penetration of a single digit. The clear imprints of single chelicerae

penetrations that were smaller and more elliptic than teardrop- or heart-shaped holes found in the wax support this hypothesis. It is possible that the small holes result from the mite's probing and crimping prior to feeding (Adar et al. 2012).

In the present study injured epidermal cells and stomata appeared flattened and drained, probably because of the loss of turgor following phytoseiid cell-sap feeding. It is important to note that our images of microscopic injuries differ substantially from those reported by Sengonca et al. (2004), where the phytoseiid *T. pyri* was implicated in causing visible injuries (to the naked eye) to apple leaves and fruits. Additional SEM studies at the cellular level are needed to determine whether the nature of cell injury caused by *T. pyri* in apple corresponds or differs from that reported in the present study.

Interestingly, the single cell damage inflicted by *E. scutalis* resemble those of the phytophagous mite *Halotydeus destructor* (Tucker) (Acari: Penthalidae) (Ridsdill-Smith 1997). The movable digits of that pest are slightly curved and dagger-shaped and similar in size to those of *E. scutalis*, as was the proposed cell piercing mechanism (Adar et al. 2012; Ridsdill-Smith 1997). Despite the exceedingly high densities of *E. scutalis*, the cells surrounding the single pierced cells were mostly intact and unharmed, in contrast to the very large patches of punctured epidermal cells inflicted by *H. destructor*. Thus it is the difference in the extent of damage, not the mode, that differentiates the omnivorous phytoseiid predator that complements its diet by plant feeding from the phytophagous mite that is dependent on the plant as its sole food source. Once saliva is secreted into a plant, a more collateral damage is expected, including damage to the adjacent cells (Tomczyk and Kropczynska 1985) and pathogens transmission (Mitchell 2004; Powell 2005). That being said, there is no evidence in the SEM images presented that damage extends beyond the penetrated cell and as far as we know there is no indication in the literature on plant disease transmission by phytoseiids.

Some of the zoophytophagous mirids (Hemiptera) are natural enemies that feed on plants and under certain conditions may become pests themselves. Castañe et al. (2011) concluded that levels of damage inflicted by mirids is strongly correlated with the plant tissue from which these omnivores feed. In the present study high density (approx. 1,000/plant) of *E. scutalis* negatively affected pepper plant weight, height and leaf cover area. As pollen densities on leaves had no effect on plant performance, we attribute these negative effects on pepper growth to leaf damage caused by cheliceral penetration of *E. scutalis*. Indeed a thorough examination of the meristematic tissue should be conducted in plants that are populated with *E. scutalis* to determine whether any damage is inflicted to these sensitive organs, and at which predator density. The mirids seem to switch between prey and plant feeding (Gabarra et al. 1988; Sanchez 2009), which under certain conditions may lead to damaging their host plant when prey is scarce (Castañe et al. 2011; Sampson and Jacobson 1999; Sanchez 2009; Sanchez and Lacasa 2008). In contrast, phytoseiids pierce cells and feed on plant cell-sap complementary to their main diet, being prey and/or pollen, without the ability to complete their immature development on cell-sap alone (Nomikou et al. 2003b), excluding the one report on *T. pyri* (Sengonca et al. 2004). Thus while phytoseiids and mirids are omnivores, phytoseiids are not expected to become pests when prey and pollen are scarce. Additionally, in our experimental setup, plant growth was apparently restricted by small pot containers of 250 cc (done purposely to prevent replicates from touching). This led to the development of very high populations of *E. scutalis* on particularly small plants (~1,000 predators on a plant with 20 leaves). However, in commercial agro-ecosystems, large populations of cell piercing predators are not needed to achieve pest control nor are easy to attain even after augmentative releases and provisioning of alternate food. For example, only five *E. scutalis* per pepper leaf yielded

effective broad mite, *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae) control without apparent damage to the crop (Adar et al. 2014b). Low populations (~100 per plant) of *I. degenerans*, another phytoseiid with cell piercing abilities, provided control of thrips on cucumber plants when regularly supplied with pollen (Van Rijn et al. 1999). Hence, the agricultural implications of plant growth damage inflicted by high populations of cell piercing phytoseiid predators are limited.

Plant cell-sap-feeding may be of importance in biological control or IPM programs as these predators could be more susceptible to systemic pesticides than non-sap feeding predators (Magalhaes and Bakker 2002; Nomikou et al. 2003a). Furthermore, cell piercing predators are more exposed to secondary compounds in cell-sap, and may therefore be more host-plant specific (Porres et al. 1975). On the other hand, the sustainability of plant cell-sap feeding phytoseiids may be better than non cell-sap feeders when prey is scarce however, this advantage may be host plant and predator species specific and it cannot be generalized as was concluded for Heteroptera by Naranjo and Gibson (1996). Whether these acarine predators obtain water, nutrients or both from the plant is still an open question. Recently it was demonstrated that *Amblydromalus limonicus* Garman & McGregor, assumed to be a plant cell-sap feeder (based on gut color observations), completed its larval development (moulting to the protonymphal stage) on leaf discs but not on water. Implying that at least for this species cell-sap contains nutrients that support larval development and survival (Vangansbeke et al. 2014).

In conclusion, using SEM imaging we demonstrated that plant cell-sap uptake by *E. scutalis* is performed by penetrating the epidermis, leaving discrete holes in its surface that are surrounded by intact cells. Because high *E. scutalis* densities may damage young plants, we suggest that caution should be taken when applying plant sap feeding phytoseiids prophylactically on young plants with limited foliage. While we did not assess yield, we do not anticipate that these predators will have any negative impact in agricultural systems, as their numbers per leaf are typically low in these crops. To take advantage of the sustainability of plant cell-sap feeders, further studies are needed to determine the host plant compatibility of these predators.

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