Interaction between cucumber plants and the broad mite, *Polyphagotarsonemus latus*: from damage to defense gene expression

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Abstract

The broad mite (BM), *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae), is a minute polyphagous mite that has severe effects on the host plant. Mechanisms of BM injury and subsequent plant responses are unclear. In this study we characterized the morphological and molecular reactions of cucumber plants (*Cucumis sativus* L., Cucurbitaceae) to BM infestation. Infested plants showed growth inhibition and a decrease in leaf number and leaf area. There was also an increase in the firmness of the infested leaves, as measured by a texture analyzer. Broad mite feeds on the epidermis, but structural and ultrastructural studies revealed aberrations in the whole leaf tissue. Severe infestation led to a complete loss of epidermis and an increase in mesophyll cell size and number. In transmission electron microscope (TEM) images, the entire epidermal tissue appeared to have collapsed, and the mesophyll cell walls appeared thick and distorted. In the infested leaves, a Northern blot analysis revealed the induction of genes related to the jasmonic acid (JA)/ethylene and salicylic acid (SA) pathways, such as lipoxygenase (*LOX*) and β-1,3 glucanase (*BGL2*), and an induction of oxidative stress-responsive transcripts, such as peroxidase (*PRX*). Transcript levels of ACC oxidase (*ACO*) that participate in ethylene biosynthesis, remained relatively constant. This work reveals that BM feeding causes dramatic morphological, structural, and ultrastructural changes, along with an induction of genes involved in defense pathways. Further studies are needed to evaluate whether the observed changes in leaf structure and ultrastructure affect the mite, and how the induction of the defense pathways affects the susceptibility of plants to BM infestations.

Introduction

Small-sized herbivorous mites are difficult to detect, but their damage often produces dramatic effects on plant morphology and physiology. The broad mite, *Polyphagotarsonemous latus* (Banks) (Acari: Tarsonemidae), is extremely polyphagous, and is found on more than 60 plant families (Gerson, 1992). This minute organism (0.2 mm long) causes severe damage to many greenhouse crops, including plants of the Solanaceae family, such as pepper (Cross, 1979; Cho et al., 1996a; De Coss-Romero & Peña, 1998) and the Cucurbitaceae, such as cucumber (Bassett, 1981; Cross, 1979). The mites are usually found on the upper part of the plant, feeding on the apical shoots and the abaxial side of young leaves. Broad mites (BM) are believed to be cell feeders, having styliform simple chelicerae that are only slightly reversible (Jeppson et al., 1975), with an approximate extended length of 43 microns (Gui et al., 2001). BM feeding causes a variety of symptoms in different hosts and plant organs. In general, plant growth is inhibited (Peña & Bullock, 1994; Cho et al., 1996a,b). Usually, the young apical leaves are heavily damaged, seem distorted, more rigid, and their edges curl downwards (Bassett, 1981; Cross & Bassett, 1982; Gerson, 1992; Cho et al., 1996b). The fruits, if any appear, may be cracked and sometimes reticulated (Bassett, 1981; Cross & Bassett, 1982; Gerson, 1992; Cho et al., 1996b).

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While the typical symptoms of BM on cucumber, such as chlorotic distorted shoots, brittle and down curled leaves, and distorted fruits have been qualitatively described (Bassett, 1981), the impact of the pest on plant morphology has not been assessed thoroughly. Studies by Nemesthoty et al. (1982) of BM infestation on *Hedera helix* (L.) and *Fatshendera lizei* leaves revealed a variable histological injury which was dependent on leaf age. The area injured by the mites became callous and sank into the leaf surface, while in the surrounding tissues cell proliferation and the formation of crystals were observed. Tissue differentiation failed, and the mesophyll remained homogeneous. In the older leaves, the tissue appears disorganized. These symptoms could result from local damage to the meristematic tissue in the apical shoot, but could also be explained as a systemic plant response to mite feeding. Whether changes in leaf structure are part of a general BM infestation syndrome in plant tissues is unknown. Moreover, the leaf ultrastructure following infestation of BM has not been studied.

On the other hand, as they are constantly exposed to herbivore damage, plants are known for their ability to defend themselves against pathogens and herbivores (Karban & Baldwin, 1997). Not much is known about plant defense responses to small arthropods that pierce single plant cells and feed on intracellular fluids, such as broad mites. The interaction of the gall mite, *Aceria cladophthirus* (Nalepa), with its host plant *Solanum dulcamara* (L.), following stylet penetration, resulted in a local hypersensitive response (HR), accompanied by the accumulation of pathogenesis-related (PR) proteins in resistant varieties, while the susceptible ones reacted with pronounced cytological and ultrastructural changes that culminated in gall formation (Bronner et al., 1989, 1991; Westphal & Manson, 1996). Another defense strategy was observed in response to plant infestation by the spider mite, *Tetranychus urticae* (Koch). In the case of the spider mite, the jasmonic acid (JA)/ethylene and salicylic acid (SA) mediated defense pathways were apparently induced (Walling, 2000). This included the induction of genes encoding lipoxygenase (LOX; Arimura et al., 2000a), phenylalanine ammonia lyase (PAL; Arimura et al., 2000a), and PR proteins such as β-1,3 glucanase (BGL2; basic type of PR2) and chitinase (basic PR3). In a spider mite infested cucumber, these defense pathways resulted in the production of cucurbitacin, which caused a reduction in mite population growth (Agrawal et al., 1999), and by the production of volatiles (Takabayashi et al., 1994; Agrawal et al., 2002). In the case of lima beans, induced volatiles also affected the neighboring plants, inducing them to express some defense genes in the leaves, e.g., LOX, PAL and peroxidase (Arimura et al., 2000a,b, 2001).

Little is known about plant defenses against BM. Constitutive defense to BM has been related to the sticky tips of foliar hairs in several *Solanum* species, creating a physical barrier that traps the mites when they attempt to move along petioles and stems to new leaves (Gibson & Valencia, 1978). The induction of any known plant-defense mechanism against BM has not yet been described.

In this study, we considered the interaction of BM with cucumber (*Cucumis sativus*). We characterized the damage and the plant response to infestation at morphological and anatomical levels. At the molecular level, we examined the transcription of a few selected defense genes following BM infestation.

Materials and methods

Broad mite culture

Broad mite culture was established on young potato foliage at the Plant Protection Institute of the Agricultural Research Organization (ARO), Bet-Dagan, Israel, as described in Palevsky et al. (2001), and maintained at 19–27 °C and 30–60% r.h.

Plant material

Cucumber plants (cv. 'M40', supplied by 'Zraim Gedera', Gedera, Israel) were grown in 1 l pots with standard fertilization in a growth room at ca. 24 °C. Plants at the 2–3 leaf stage (unless otherwise stated) were used for the experiments. Experimental plants were infested by placing infested potato foliage, with about 20 adult females, on a predetermined leaf, as estimated under a stereomicroscope (Olympus SZX12, at 40× magnification). Infested plants were isolated in cages covered with a fine (<2 mm) mesh.

Broad mite monitoring

The broad mite population on each cucumber plant at each time interval was assessed by washing an excised upper leaf (ca. 1.5 cm long) in 50 ml of 70% ethanol, and counting the washed mites under the stereomicroscope.

Morphological analysis

Total plant height and the number of expanded leaves were measured weekly. In order to estimate the damage to the 4th and 5th leaves, leaf area and rigidity were assessed. The latter was measured with a texture analyzer (TA-XT2, Micro Stable System, Surrey, UK) using a 2 mm diameter cylinder probe (SMS P/2). Resistance force data (in Newton, N) was processed by Texture Expert for Windows and expressed using the maximum resistance values (van Dijk et al., 2002).

Structural and ultrastructural studies

Strips of leaf tissue (ca. 1 × 3 mm), parallel to the major vein, were excised from selected leaves, fixed in 3.5% glutaraldehyde in sodium cacodylate buffer, pH = 7.0, at

4 °C for 2 h, washed in the buffer, and post-fixed in 1% OsO_4 in phosphate buffer, pH = 7.0, at 4 °C for 2 h. The fixed tissue was dehydrated in a graded series of ethanol, up to 100%, followed by acetone, and embedded in epoxy agar 100 resin (Agar Aids; Essex, UK).

Thin sections (2–3 μm) were prepared using a microtome (11800 Pyramitome, LKB-BROMMA, Stockholm, Sweden). Slides were stained with general tissue staining (Basic Fuchsin and Toluidine Blue) and observed under a light microscope (Axioskop, Zeiss-Germany).

Ultra-thin sections (600–800 Å) were prepared with an ultra-microtome (8800 Ultratome III, LKB-BROMMA, Stockholm, Sweden), and stained with uranyl acetate and lead citrate, and observed using a transmission electron microscope (Jeol 100-Cx) at 80 kV.

In order to evaluate the presence of lignin and/or suberin, strips of leaf tissue (ca. 2 × 3 cm) parallel to the major vein from selected leaves of infested and non-infested plants were excised and fixed in FAA (2% formaldehyde, 5% acetic acid, 60% ethanol) for 3 days. After fixation, the leaves were dehydrated with an alcohol series and embedded in Paraplast Plus (Sigma-Aldrich). The 20 μm thick sections were placed on glass slides (SuperFrost Plus, Menzel-Glaser, Germany) and allowed to dry for 2 h at 20 °C. The sections were stained with Safranin and Fast Green according to standard procedures (Ruzin, 1999).

Northern analysis

For the gene expression studies we used Northern analysis to assess transcript levels. Plants were infested with BM at the 6th leaf stage. Untreated and mechanically wounded plants served as control groups. Mechanical damage was inflicted by rubbing the two upper leaves with carborundum powder (0.1 mg/10 ml) using a rigid paint brush. This procedure was repeated every second day. The youngest upper leaves were sampled for RNA extraction after 24 h, 72 h, and 7 days. For each sample, leaves (6th or 7th) were pooled from three plants. Total RNA was extracted, using Tri-reagent buffer (Molecular Research Center Inc., OH). The Northern analysis followed a standard procedure (Ausubel et al., 1987). Gene probes were made by PCR amplification of plasmids harboring the following cloned gene fragments from cucumber (*Cucumis sativus*): β-glucanase 2 (*BGL2*; Ando et al., 2001), GenBank accession no. AB051372, nucleotides 1–360; lipoxygenase 1 (*LOX1*), accession BQ294486, nucleotides 1–743; lipoxygenase 2 (*LOX2*; Ando et al., 2001), AB051385, nucleotides 1–213; peroxidase (*PRX1*), accession no. M91372, nucleotides 708–958; ACC oxidase 1 (*ACO1*), AF033581, nucleotides 1–1185. The *BGL2* and *LOX2* clones were kindly provided by Drs S. Ando and S. Sakai, University of Tsukuba, Japan. These experiments were repeated three times.

Statistical analysis

To assess the effect of BM on the plant parameters that are known to distribute normally such as plant height, a students t-test was used. Data of leaf number was transformed by √x prior to t-test analysis. Parameters such as leaf area and rigidity were analyzed by the Mann–Whitney U-test. The analysis was done using STATVIEW 4.5 for the Apple Macintosh PC.

Results

Broad mite effects on growth and morphology

Two weeks after infestation, a severe reduction in plant height and leaf number was already visible (Figure 1A,B). However, the decrease in leaf number only became statistically significant after 3 weeks (Student's t-test; $P<0.05$), while significant inhibition in plant height was recorded after 4 weeks (Student's t-test; $P<0.001$). At the end of the experiment, the control plants were 90 cm high and had on average, 17 unfolded leaves, compared to the infested plants, which were severely stunted (26 cm), with only five well-developed leaves and many small, non-expanded ones.

The decrease in leaf area as the BM population increased is shown in Figure 2. The new leaves that developed under BM infestation grew less than controls (Mann–Whitney U-test: U = 5, P = 0.0011 and U = 7, P = 0.0184 for 4th and 5th leaf, respectively; Figure 2A,B). In addition, the infested

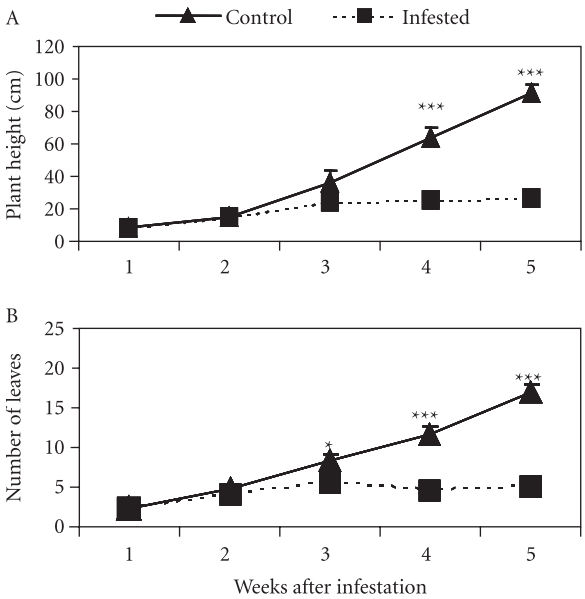

Figure 1 The effect of *Polyphagotarsonemus latus* infestation on (A) plant height, and (B) the number of unfolded leaves. Results are the mean ± SEM of 5 to 8 replicates. Asterisks denote significance in a t-test on raw data for height and the √x transformed data for the number of leaves: * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

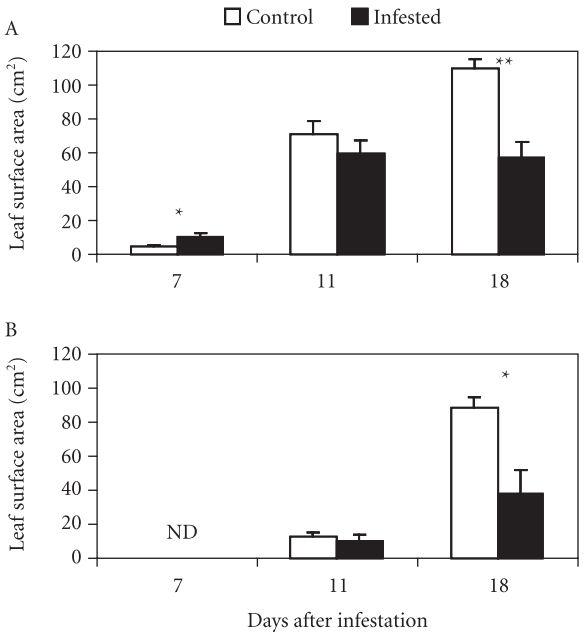

Figure 2 Change in cucumber leaf area following *Polyphagotarsonemus latus* infestation. (A) 4th leaf and (B) 5th leaf. The 5th leaf was still unfolded on day 7. The number of mites at the each time point was 45 ± 6, 280 ± 38, and 1158 ± 190 after 7, 11 and 18 days, respectively. Results are the mean ± SEM of 5 to 8 replicates. ND = No data. Asterisks denote significance in a t-test: * P<0.05; ** P<0.01.

leaves grew mechanically more resistant (Figure 3). Already 1 week after infestation, the 4th leaf was significantly more rigid than the control (Mann–Whitney U-test: U = 1.5, P = 0.0014), and this difference was maintained later (Figure 3A). A similar situation was observed with the 5th leaf (Figure 3B), when the rigidity of infested leaves was significantly higher than the controls at day 11 (Mann–Whitney U-test: U = 0, P = 0.0019) and increased over time (Mann–Whitney U-test: U = 0, P = 0.0003). Eighteen days post-infestation, a significant negative correlation was found between leaf rigidity and leaf area in the infested leaves (r = –0.81 and r = –0.83 in the 4th and 5th leaf, respectively). However, in both leaves and at all times tested, no consistent correlation was found between the level of mite infestation and the severity of the changes in leaf area and rigidity, with r-values ranging between –0.17 and 0.54.

Changes in leaf structure and ultrastructure

Time-dependent effects of BM infestation on leaf anatomy were revealed by light microscopy. Several replicates were analyzed, and representative pictures are shown (Figures 4 and 5). At day 7, young non-infested 4th and 5th leaves showed a distinct upper and lower epidermis, and their mesophyll layers were not fully differentiated (Figures 4A and 5A). In the non-infested leaves, the mesophyll layer differentiated with time into palisade and spongy mesophyll layers, which were especially prominent after 18 days (Figures 4C, 4E and 5C). The 4th leaf of the infested plant appeared morphologicaly similar to the parallel uninfested leaf (Figure 4B). However, after 11 days of infestation the 4th leaf exhibited prominent anatomical changes in comparison with non-infested ones (Figure 4D). The lower epidermis disappeared, the upper epidermis seemed collapsed, and it entirely disappeared after 18 days (Figures 4F and 5D). At the same time, the mesophyll cells of the infested leaf appeared larger and undifferentiated. Their intercellular spaces were small and the leaf was about twice as thick as the control. This phenomenon was even more prominent in the infested 5th leaf, in which both epidermal layers were much diminished (Figures 5B,D). Instead of the epidermal layers, a condensed layer of collapsed cells was obvious on both sides of the leaf. Safranin–Fast Green stained this area as dark red, suggesting that it contains high levels of phenolic substances, presumably lignin and/or suberin. In highly infested leaves, the same staining extended deeper into the intracellular spaces (data not shown).

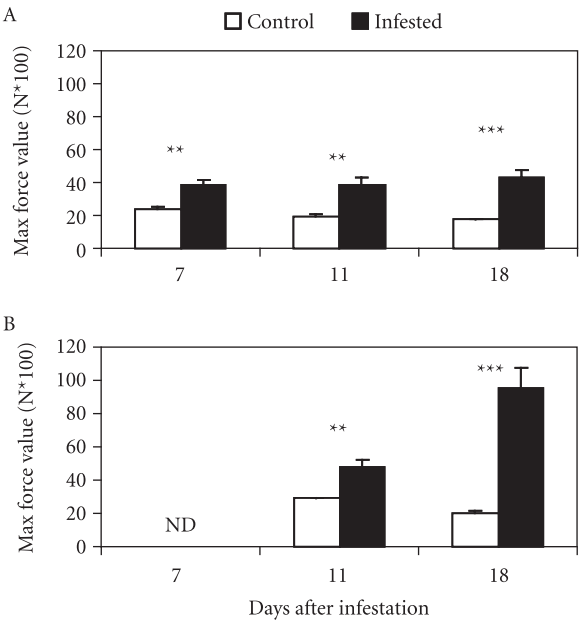

Figure 3 Change in cucumber leaf rigidity following *Polyphagotarsonemus latus* infestation. (A) 4th leaf and (B) 5th leaf. The 5th leaf was still unfolded on day 7. Number of mites at the each time point was 45 ± 6, 280 ± 38, and 1158 ± 190 after 7, 11 and 18 days, respectively. The results are the mean ± SEM of 5 to 8 replicates. ND = No data. Asterisks denote significance in a t-test: * P<0.05; ** P<0.01; ***P<0.001.

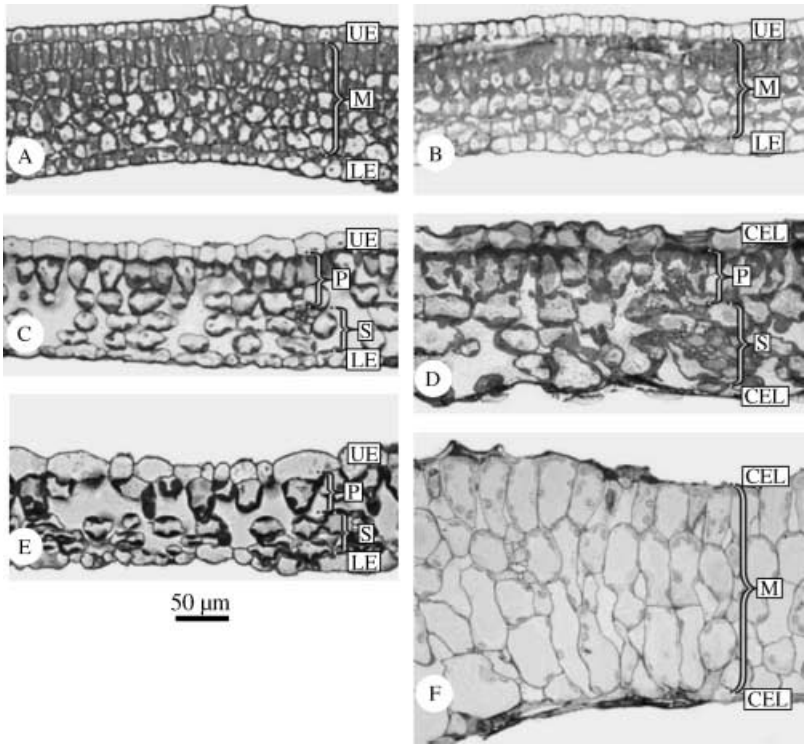

Figure 4 Light microscopy of 4th leaf sections from *Polyphagotarsonemus latus*-infested and control leaves. (A, C, E): control leaf 7, 11, and 18 days post-infestation, (B, D, F): infested leaf 7, 11, and 18 days post-infestation, respectively. CEL: Collapsed epidermal cells, LE: Lower epidermis, M: Undifferentiated mesophyll, P: Palisade mesophyll, S: Spongy mesophyll, UE: Upper epidermis.

The mesophyll tissue was thicker, less differentiated and lacking air spaces. The increase in thickness, about twofold, resulted from increases in both cell volume and cell number (five cell layers in the control leaf, compared to seven in the infested leaf, Figure 5C,D).

The ultrastructure of the 4th and the 5th leaves was examined under the electron microscope. Epidermal cells from healthy leaves (Figures 6A and 7A) had a thin cell wall (Figure 6C), covered at the external surface with a conspicuous cuticle and wax. In the infested 4th leaf at day 11, the external layer appeared to be composed of collapsed cells and non-defined components (Figure 6D). The cuticle was absent in several areas (Figure 6F). The epidermal cells of the 5th infested leaf were absent, and only cuticular residues

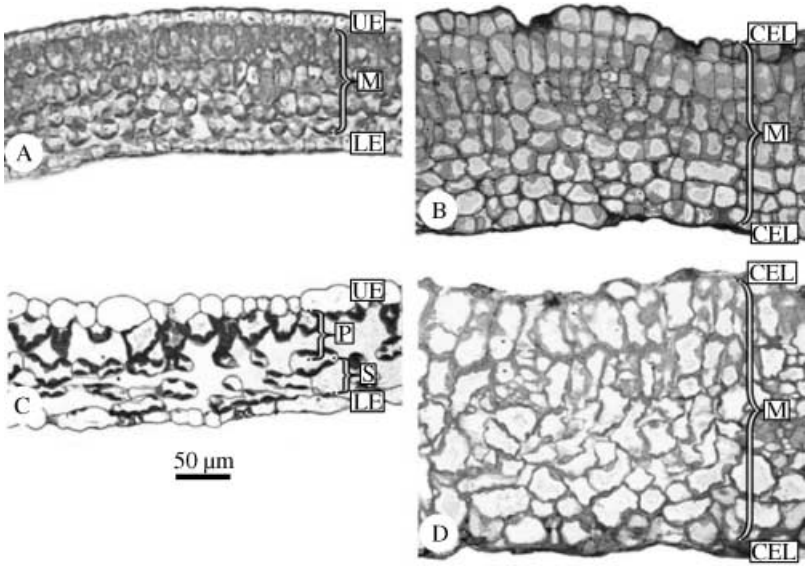

Figure 5 Light microscopy of the 5th leaf sections from *Polyphagotarsonemus latus*-infested and control leaves. (A, C): control leaf 11 and 18 days post-infestation and (B, D): infested leaf 11 and 18 days post-infestation, respectively. CEL: Collapsed epidermal cells, LE: Lower epidermis, M: Undifferentiated mesophyll, P: Palisade mesophyll, S: Spongy mesophyll, UE: Upper epidermis.

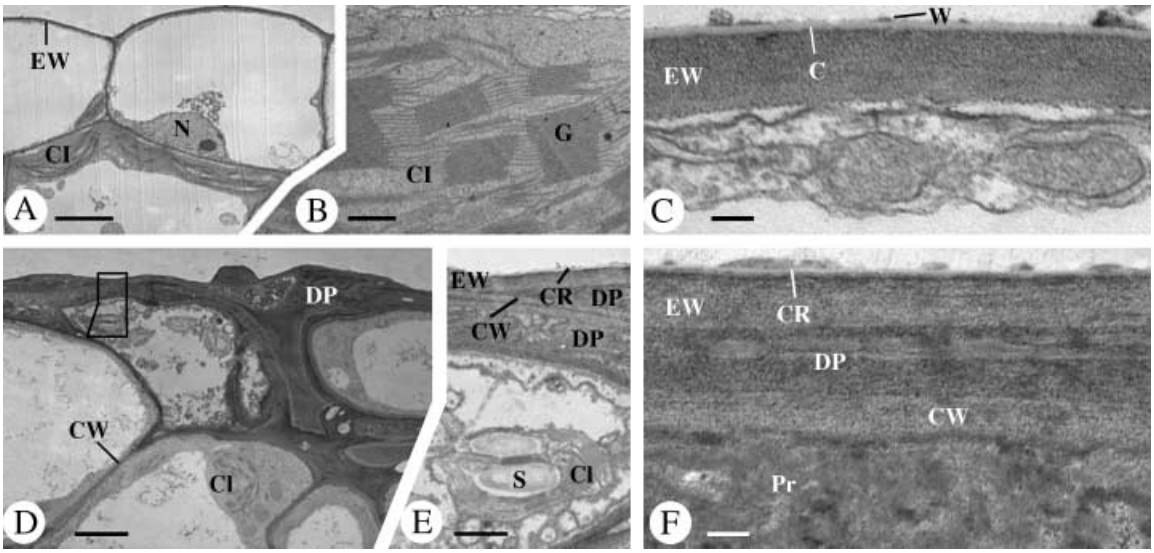

Figure 6 Leaf sections from *Polyphagotarsonemus latus*-infested and control leaves obtained by transmission electron microscopy, taken from the 4th leaf, 11 days post-infestation. (A) Cells of control leaf, bar = 3 μm; (B) chloroplast of control leaf, bar = 0.4 μm; (C) cell wall of control leaf, bar = 0.1 μm; (D) cells of infested leaf, bar = 4 μm; (E) enlarged section of (D), chloroplast of infested leaf, bar = 0.8 μm; (F) cell wall of infested leaf, bar = 0.1 μm. C: Cuticle, Cl: Chloroplast, CR: Cuticle residues, CW: Mesophyll cell wall, DP: Dense protoplast, EW: Epidermal cell wall, G: Grana, N: Nucleus, Pr: Protoplast, S: Starch body, W: Wax.

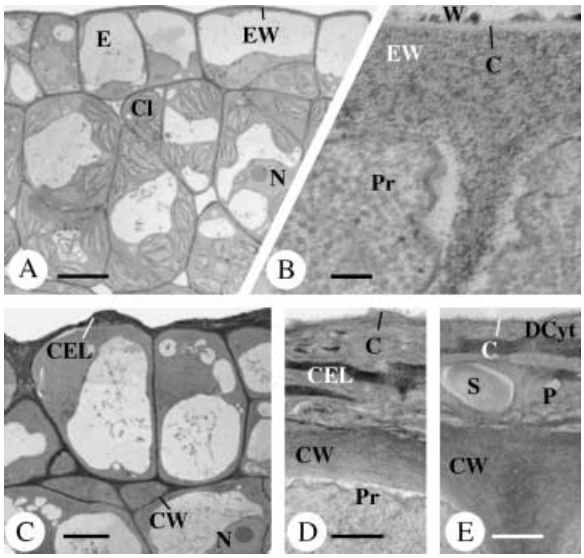

Figure 7 Leaf sections from *Polyphagotarsonemus latus*-infested and control leaves obtained by transmission electron microscopy, taken from the 5th leaf, 11 days post-infestation. (A) Cells of control leaf, bar = 3.5 μm; (B) Cell wall of control leaf, bar = 0.1 μm; (C) cells of infested leaf, bar = 4 μm; (D, E) cell walls of infested leaf, bars = 0.5 μm. C: Cuticle, CEL: Collapsed epidermal cells, Cl: Chloroplast, CW: Mesophyll cell wall, Dcyt: Dense cytoplasm, E: Epidermis, EW: Epidermal wall, N: Nucleus, Pr: Protoplast, S: Starch body, W: Wax.

were apparent (Figures 7D,E), and a thick external layer was present instead. Deformations of the cell walls were also observed in this outer layer (Figure 7D). Intercellular spaces were filled with electron dense substances that could be lignin or suberin, according to Safranin–Fast Green staining, as previously mentioned. The cytoplasm and vacuole appeared to contain electron-dense bodies. Starch grains were apparent among the chloroplast grana (Figure 6E); non-infested leaf chloroplasts had no starch grains (Figure 6B).

Changes in gene transcripts following broad mite infestation

We tested the expression of a number of defense genes that are known to respond to herbivores and other stresses in different plants. Figure 8 shows the prominent mite-specific induction of two lipoxygenase isozymes, of lipoxygenase 1 (*LOX1*), and lipoxygenase 2 (*LOX2*), β-glucanase 2 (*BGL2*) and peroxidase (*PRX*) transcripts after 24 h, often peaking in the 72-h samples and subsequently decreasing. The ACC oxidase 1 (*ACO1*) transcripts levels, however, remained constant. Mechanical wounding caused only a very slight induction of the *LOX1* and *PRX* transcripts.

Discussion

This study outlines the complexity of BM infestation syndrome, using *C. sativus* as a model for a susceptible host plant. We provide a first description of the plant's response to BM feeding at several levels: structural, ultrastructural,

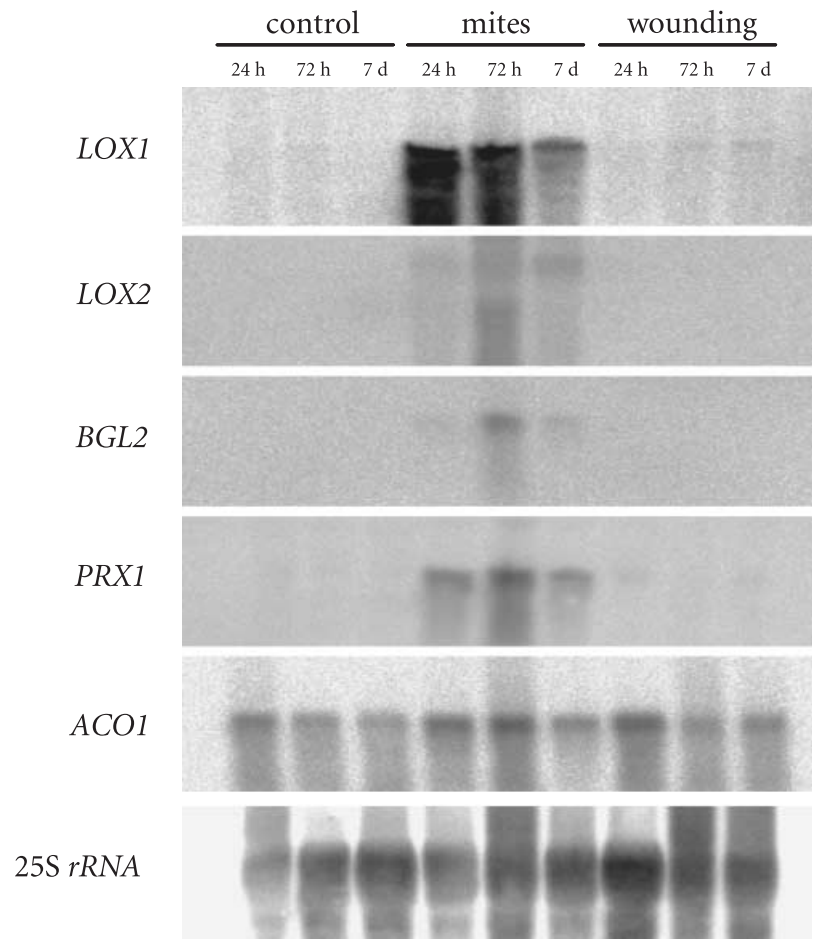

Figure 8 Transcript abundance of stress-related genes after *Polyphagotarsonemus latus* infestation. Total RNA (15 μg lane^{-1}) from BM-infested, mechanically wounded and untreated young apical leaves subjected to Northern analysis and hybridized to radiolabeled, cucumber gene probes. *LOX1*: lipoxygenase-1, *LOX2*: lipoxygenase-2, *BGL2*: β-1,3-glucanase-2, *PRX*: peroxidase, *ACO1*: ACC oxidase-1, *rRNA*: wheat 25S rRNA.

and molecular. BM inhibited the growth of cucumber plants, causing a substantial reduction (ca. 70%) in plant height and leaf number. This reduction, along with leaf darkening and curling downwards, was similar to the damage reported for cucumber and other plant species following broad mite attack (Hooper, 1957; Bassett, 1981; Gerson, 1992; Cho et al., 1996a,b). We also observed changes in leaf morphology, such as leaf growth inhibition, which resulted in smaller leaves. This was accompanied by an increased leaf rigidity, as was observed in the 4th leaf and, even more significantly, as BM infestation progressed, in the 5th leaf. We found no correlation between BM numbers on the plants and degree of damage as manifested by the decrease in leaf area and increase in leaf rigidity. This lack of correlation, and the fact that even a small initial number of mites (about 20 in our case) and even fewer mites in limes (Peña, 1990) and peppers (De Coss-Romero & Peña, 1998) is sufficient to invoke economic damage, raise the question of whether it is mostly direct damage (i.e., caused by mite feeding injury) or indirect [by chemical/hormonal changes induced by feeding (Gerson, 1992)].

BMs are believed to be cell feeders: their slightly reversible chelicerae enable them to feed only from the epidermal cell layer. However, the estimated length of the chelicerae (Gui et al., 2001) could imply that BM are able to reach into deeper layers within the leaf. In any case, the lower epidermis disappeared first, followed by the upper, supporting the idea that BM do feed mostly from the abaxial side of the leaf (Smith, 1935; Gerson, 1992). However, the striking sequential changes that were observed in the structure of all leaf layers seems to question the possibility that BM damage is only caused by a direct consumption of the cell content. A failure of tissue differentiation, and increased cell proliferation and cell volume in the infested plants, resulted in a twice as thick and very compact tissue, lacking air spaces. This explains well the increased rigidity of the infested leaves. The abnormal differentiation and increase in cell volume and proliferation are similar to those observed by Nemesthoty et al. (1982) in *Hedera helix* (L.) and *Fatshendera lizei* leaves attacked by BM. Profound ultrastructural changes in the BM-infested leaves were apparent using TEM. Intracellular starch accumulation was observed in

the chloroplasts of the BM infested leaves. This phenomenon has also been reported by Tanigoshi & Davis (1978) in apple leaves attacked by the spider mite *Tetranychus mcdanieli* (McGregor). Starch synthesis is related to photosynthetic rate (Carmi & Shomer, 1979), but has also been associated with the destruction and deformation of the grana and tylakoids in cases of herbivore attack and during leaf senescence (Crawford & Wilkens, 1996). On the other hand, starch accumulation may reflect abnormal sink strength or carbon metabolism induced by BM in the young leaves.

The cell wall aberrations in BM-infested leaves were further apparent using electron microscopy. It seems that the exaggerated cell growth in infested leaves could interfere with the cytoskeleton, creating a secondary, deformed, thick wall. The layering of components such as suberin or lignin in the middle lamella region, in place of collapsed epidermal cells, forms a barrier which inhibits further damage and/or pathogen ingression. The accumulation of phenolic compounds may indicate a defense response, and has previously been observed on cotton and cucumber hypocotyls after fungal infection (Sneh et al., 1989; Siegrist et al., 1994). Royalty & Perring (1988) reported the destruction of tomato epidermal cells by the rust mite *Aculops lycopersici* (Massee). In that case, a defense layer containing lignin appeared above the parenchyma, instead of the missing epidermal tissue, but unlike the response to BM, the underlying mesophyll layer was not affected. In any case, the detection of lignification implies an induction of putative defense mechanisms within the plant. In *Arabidopsis thaliana,* lignin synthesis is activated as part of the defense responses through jasmonate and ethylene (Caño-Delgado et al., 2003). On the other hand, in *Vigna unguiculata* (L.) SA was reported to enhance the activity of phenylalanine ammonia-lyase (PAL), an important enzyme in phenolic biosynthesis (Nandi et al., 2003).

A specific increase in transcription levels of *LOX1* and *LOX2*, known to participate in JA biosynthesis (He et al., 2002), provided evidence for the induction of the JA pathway in BM-infested leaves. *LOX* induction is up-regulated by both JA and ethylene (Walling, 2000), and is often associated with wounding by chewing insects (Stotz et al., 2000). It also occurred in beans that were attacked by spider mites (Arimura et al., 2000b). Mechanical wounding induced *LOX* expression in *Arabidopsis* (Reymond et al., 2000) and *Passiflora edulis f. flavicarpa* (Rangel et al., 2002), but not in cucumber leaves. This could suggest that the genes induced by BM in this study are not necessarily wound-inducible genes, and signals other than mechanical wounding by the mite mouth parts mediated the response. Alternatively, it could be that the wounding treatment that we devised was milder than the mechanical damage inflicted by BM. The other transcripts that were specifically expressed in BM infested leaves were *BGL2* and *PRX*. *BGL2* encodes a PR protein (*PR2*) and is thought to be one of the main markers of the salicylic acid (SA) pathway. Its expression is also induced during the systemic acquired resistance (SAR) response to aphids, whiteflies and spider mites (Walling, 2000; Glazebrook, 2001). *PRX* regulates the removal of reactive oxygen species and participates in the synthesis of lignin and other phenolic compounds (Arimura et al., 2000b; Hammond-Kosack & Jones, 2000). Spider mites induce the expression of this gene in lima beans (Arimura et al., 2000b), whereas Hiraga et al. (2000) observed *PRX* induction in mechanically wounded plants. The levels of *ACO1* transcripts were similar in all treatments. *ACO* is involved in ethylene biosynthesis, and *ACO* transcripts were induced by aphids (Moran et al., 2002) and spider mites (Arimura et al., 2000b); cucumber possesses multiple ACO genes and there could be other ACO family members that respond to BM feeding.

It is still unclear whether structural and biochemical changes occurring in the host affect the mite itself. Another question relates to the mechanism by which the BM's feeding caused profound changes in the entire leaf tissue. We hypothesize that the BM–host interaction involves plant manipulation, by the mite, with a possible systemic effect. Changes in growth and differentiation patterns could imply that BM induces a hormonal imbalance in the plant. Some of the observed symptoms, such as mesophyll hypertrophy, may be reminiscent of a 'primitive' galling response, providing a better food source for the BM. Gall mite signals can spread to neighboring cells and cause nucleolus swelling, compressed cytoplasm, a reduction in vacuole size and finally necrosis (Westphal & Manson, 1996). We can further suggest that under natural conditions, tissue manipulation by BM is confronted by the induction of plant defenses. The outcome of this confrontation is dependent on the balance between the two processes and will determine the damage to the infested plant and BM success. In susceptible crop varieties, such as *C. sativus* M40, the defense mechanisms are apparently inefficient in protecting the plant.

Taken together, BM infestation induces marked morphological, structural, and ultrastructural changes, as well as changes in gene expression. BM infestation activates the SA- and the JA-dependent pathways, and possibly an oxidative stress response. The simultaneous induction of several pathways was previously reported for various arthropod herbivores such as aphids (Moran & Thompson, 2001; Moran et al., 2002), and spider mites (Kant et al., 2004). Further studies involving more extensive transcript profiling of the response to BM will be required in order to determine the defense pathways that are induced by BM and to evaluate the impact of the latter on BM fitness.

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