

FINE STRUCTURAL ALTERATIONS OF BEAN PLANT LEAVES BY FEEDING INJURY OF *TETRANYCHUS URTICAE* KOCH (ACARI, TETRANYCHIDAE)

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INJURY
TO
PLANT
CELLS
BY
TETRANYCHIDS

ABSTRACT : Feeding injury of *Tetranychus urticae* Koch (Acari, Tetranychidae) on bean plant leaves was examined using light and electron microscopy.

Histological effects are directly related to the feeding intensity of the mites and are a function of time. The thickness of infested leaves is strongly reduced as a result of a reduction of both mesophyll layers. Cells which have been punctured by feeding mites collapse and exhibit no contents. Adjacent unpunctured cells show coagulated protoplasts and, as further damage, degenerative processes in the chloroplast structures. Thus, the feeding injury of *Tetranychus* is not limited to punctured cells, but cells of all mesophyll layers are affected. Possible mechanisms are discussed.

DAS
SCHADBILD
VON
TETRANYCHIDAE
AN
BOHNENBLÄTTERN

ZUSAMMENFASSUNG : Das Schadbild von *Tetranychus urticae* Koch (Acari, Tetranychidae) an Bohnenblättern wurde licht- und elektronenmikroskopisch untersucht.

Histologische Effekte sind direkt abhängig von der Fraßintensität der Milben und stellen damit eine Funktion der Zeit dar. Die Dicke befallener Blätter ist stark reduziert, was auf eine Abnahme der Zellzahl beider Mesophyllschichten zurückzuführen ist. Zellen, die von Milben angestochen wurden, besitzen keinen Inhalt mehr. Benachbarte, nicht ausgesaugte Zellen, zeigen ein koaguliertes Plasma und als Folgeschäden Abbauprozesse in den Chloroplasten. Somit ist der Fraßschaden von *Tetranychus* nicht nur auf angestochene Zellen beschränkt, sondern auch benachbarte Zellen werden in Mitleidenschaft gezogen. Die verschiedenen Auslöser dieses Sekundärschadens werden diskutiert.

INTRODUCTION

Due to feeding injury on different cultivated plants the phytophagous mites, to which tetranychids belong, are known as an important economic group. Little is known about the histological alterations of plant tissue which may occur as result of feeding by these mites. Biochemical stu-

dies of spider mites feeding injury (AVERY and BRIGGS 1968 a, c, BOULANGER 1958, LEIGH 1963, STORMS 1971, WIESMANN 1968) have led to the conclusion that fundamental metabolic pathways such as dissimilation, assimilation and water balance are disturbed before visible changes in the leaf occur (HALL and FERREE 1975). LIESERING (1960) reported that feeding injury by the two spot-

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ted spider mite, *Tetranychus urticae* Koch is caused by mechanical puncturing of plant cells with the stylets. He found 18-22 injections per minute resulted in 100 totally exhausted cells after 5 minutes. MOTHES and SEITZ (1981 *a*) found that the alimentary canal of *T. urticae* contained cell constituents such as granal thylakoids, storage products and cytoplasmic portions. It was suggested that salivary secretions had partly digested chloroplasts prior to absorption and that whole cells had been exhausted.

Light microscopical investigations of GEIJSKES (1938), BLAIR (1951), AVERY and BRIGGS (1968 *b*) and LIESERING (1960) show flattened epidermal cells, collapsed palisade and spongy parenchyma cells after mite feeding. TANIGOSHI and DAVID (1978), examining apple leaves injured by *T. mcdanieli*, reported coagulated protoplasts, some devoid of any visible contents, in punctured cells. In addition, they showed that apparently unattacked cells were affected, the swollen chloroplasts exhibited cup-shaped thylakoids. Morphological responses of strawberry leaves to infestations of twospotted spider mite were examined by SANCES et al. (1979 *a*). They reported that mite feeding injury at 4.8 mite-days/cm² was restricted to localized areas of the spongy mesophyll and lower palisade mesophyll layer. The present paper describes cytological alterations resulting from feeding of *T. urticae* on bean plant leaves using light and electron microscopy.

MATERIALS AND METHODS

The animals were reared according to the procedure of MOTHES and SEITZ (1980).

Non-infested and infested bean plants were cultured under constant conditions with regard to humidity (50-60 %), temperature (24°C) and amount of light (16 : 8/day : night).

Well differentiated leaves of the same age (non-infested and infested) were used for electron microscopy. Fixation of leaf pieces (Ø 20 mm²) in different stages of mite injury (2d, 6d) and non-infested, symptomless plants was in a solution of 4 % glutaraldehyde, 5 % tannic acid and 2 % sucrose in 0.1 M sodium cacodylate buffer pH 7.2 according to the method described by LOCKE and HUIE (1972, 1975). Fixation time was 12 h under partial vacuum (10-20 torr). Post-fixation followed in buffered 1 % OsO₄ for 30 min. The samples were dehydrated and embedded in Spurr's medium (SPURR 1969). Ultrathin sections were cut with a diamond knife on an LKB ultratome and stained with KMnO₄ and lead citrate (REYNOLDS 1963) using a method modified from SOLOFF (1973). Semithin sections (1-2 µm) were cut on a LKB pyramitome and stained with alcoholic methylene blue. The resin was removed prior to staining by means of a method derived from LANE and EUROPA (1965) and MAYOR et al. (1961). Examination of the ultrathin sections was done with a Hitachi HU 12 A electron microscope, of the semithin sections with a LEITZ Orthoplan microscope.

OBSERVATIONS

1. Control leaves.

Non-infested leaves exhibit one layer of palisade parenchyma cells which could be differentiated from the underlying spongy parenchyma cells (Figs 1, 2, 5). The upper and lower epidermis consist of a single layer of cells in both of which stomata could be observed (Figs. 1, 2). The ave-

rage number of chloroplasts per cell area seen in semi- and ultrathin sections is 5.7 in palisade parenchyma and 2.8 in spongy parenchyma. The thickness of the whole leaf is approximately 180 µm. The cells of both mesophyll layers exhibit a fine granular cytoplasm which encloses chloroplasts, mitochondria, probable peroxisomes and nuclei. The central vacuole is sometimes filled with electron-dense structures (Figs. 5, 7, 8).

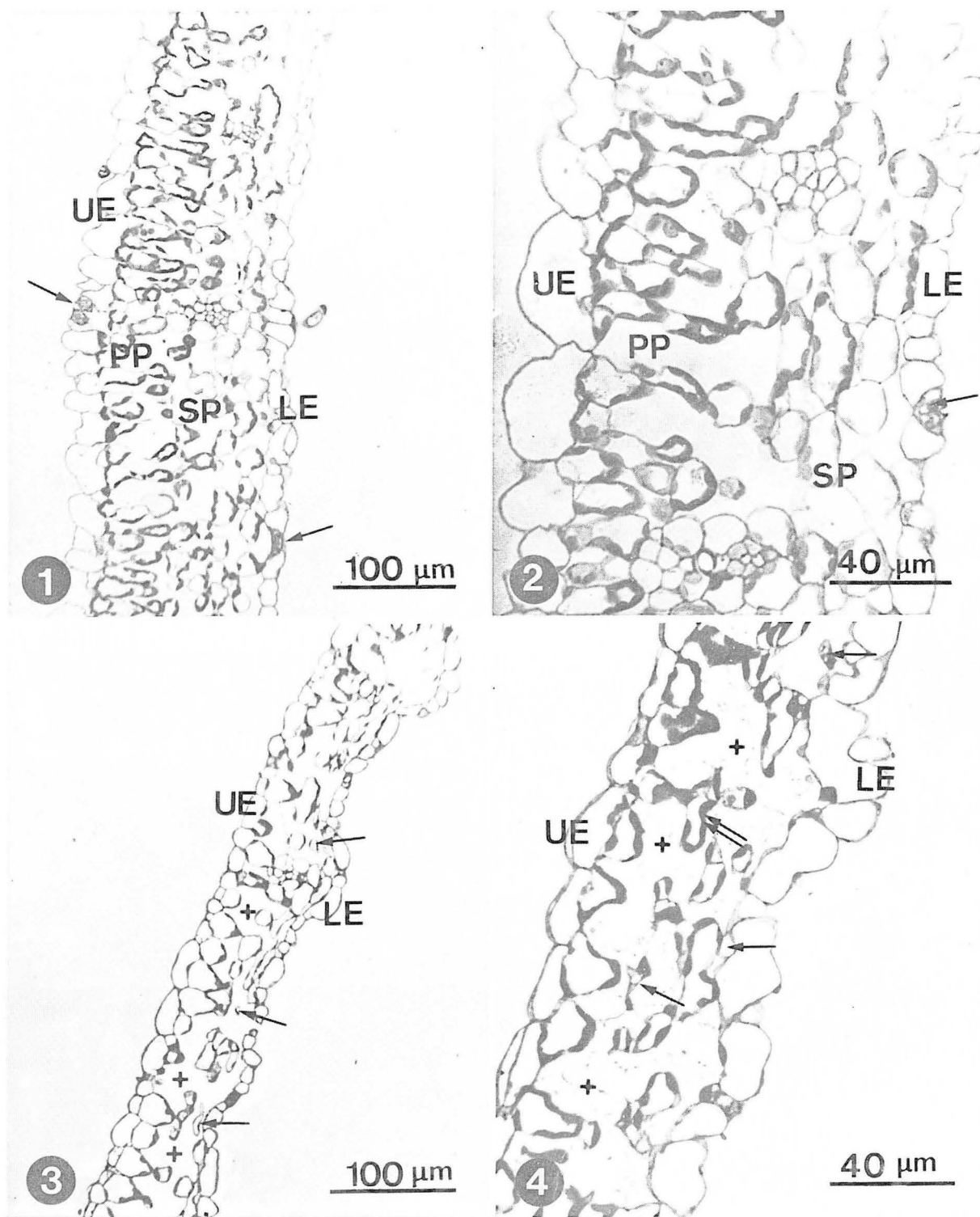


FIG. 1-2 : Unaffected leaf, cross section. Upper site left, lower site right.

The leaves exhibit one layer of palisade parenchyma (PP) cells which could be differentiated from the underlying spongy parenchyma (SP) cells. The upper (UE) and lower (LE) epidermis consists of a single layer of cells. Stomata in both epidermal layers (arrow).

FIG. 3-4 : Infested leaf, cross section. Upper site left, lower site right.

Extensive disruption of all mesophyll layers. Epidermal cells (UE, LE) irregular, empty spaces in the mesophyll layer (+). Punctured cells collapsed (arrow), apparently unattacked cells (double arrow).

2. Infested leaves.

Infested leaves show a strong alteration in their structure (Figs. 3, 4, 6). The thickness is reduced to approximately $85\text{ }\mu\text{m}$. The average number of chloroplasts per cell area decreases to 3.9 in palisade parenchyma and to 2.6 in spongy parenchyma. The number of cells in both mesophyll layers is reduced also. Histological effects of infested leaves are indicated by extensive alterations in all layers of mesophyll cells (Figs. 3, 4). The damage is directly related to the feeding intensity of the mites. The epidermal cells become irregular and large empty spaces open within the mesophyll layers (Figs. 3, 4). Two types of affected cells may be observed : one type is fully collapsed due to puncturing by feeding mites, while the other, which apparently remains unattacked, seems only slightly collapsed (Figs. 4, 6). Fully collapsed cells sometimes exhibit a small margin of coagulated cytoplasm but mostly show no contents at all (Figs. 4, 9). Apparently unattacked cells which have close contact with punctured cells contain distinguishable organelles (Figs. 3, 4, 6, 10), but show damage under higher magnification. The most conspicuous changes occurring within these cells are a coagulation of the cytoplasm (Fig. 10), an alteration in the cell wall structure, and a degeneration of the nucleus and of other cell organelles. Membranes are difficult to detect and may be absent. The degeneration of organelles increases with the number of mites per leaf and mite-days per leaf, and is a function of time. With increasing time during mite feeding, the leaves become bronzed and the degenerative processes in chloroplasts lead to a deposition of weakly stained lipid droplets (Fig. 11) and to an alteration of stromal thylakoids in the chloroplasts. The granal thylakoids remain well distinguishable (Figs. 11, 12) but become larger. Electron-dense particles can be observed near the fragmented tonoplast (Fig. 12).

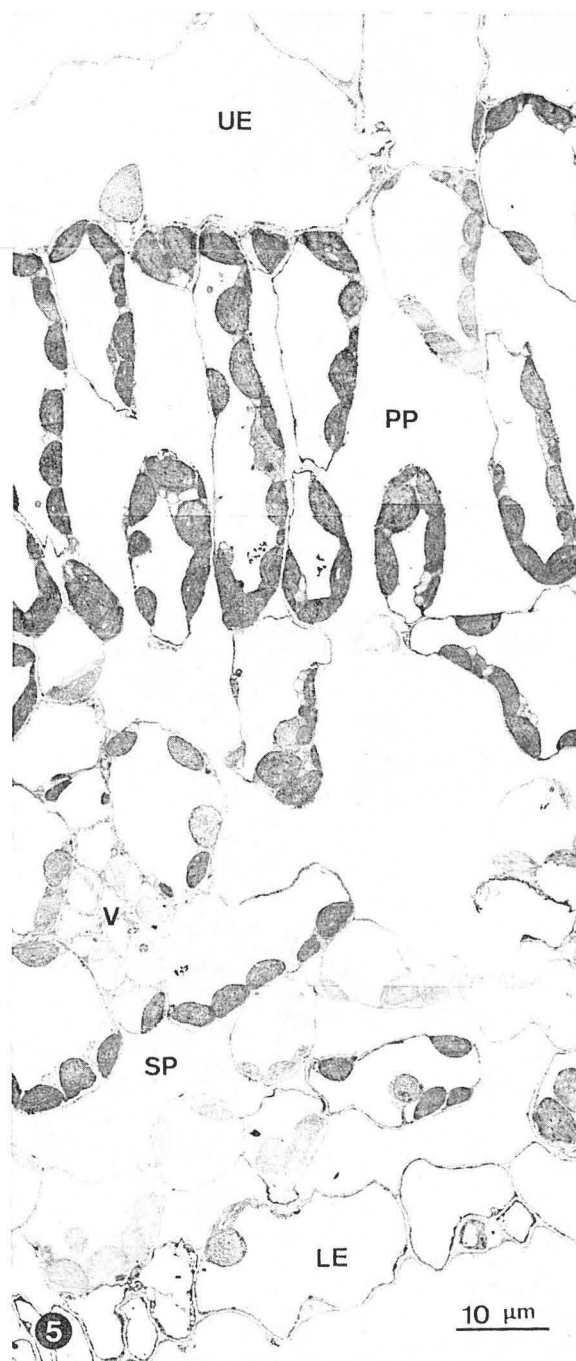


FIG. 5 : Electron micrograph of a non-infested leaf, cross section. Upper (UE) and lower (LE) epidermal cells exhibit a small protoplast. Palisade parenchyma cells (PP) differentiated from the spongy parenchyma cells (SP). Vein of a leaf (V).

DISCUSSION

Our results on the feeding injury of *Tetranychus urticae* on bean plant leaves are in agreement with the descriptions of GEIJSKES (1938), BLAIR and GROVES (1952), AVERY and BRIGGS (1968 *b*) on *Panonychus ulmi* feeding damage on apple foliage, of TANIGOSHI and DAVIS (1978) on *T. mcdanieli* feeding damage on apple foliage and of LIESERING (1960) on *T. urticae* feeding damage on bean plants. However, adjacent to the cells damaged by mite puncturing and drainage, we also observed apparently unattacked cells which showed an alteration in their structure. Cells which have been punctured by the mite do not participate further in the metabolic processes of

the plant. Decrease of leaf thickness corresponds to a reduction in the number of cells. Adjacent unpunctured cells show a reduction of chloroplasts from 5.7 to 3.9 per cell area of palisade parenchyma and from 2.8 to 2.6 per cell area of spongy parenchyma. Such a reduction of photosynthetic active organelles in apparently unattacked cells results in a decrease in the rate of photosynthesis and metabolic activities, although the cells themselves are not injured by mechanical influence of the mites. This is contrary to the results of SANCES et al. (1979 *a*) who suggested that mite feeding injury from lower leaflet surface of strawberry leaves resulted in minimal injury to the uppermost palisade layers where the majority of chloroplasts are located. They demonstrated (SANCES et al. 1979 *b*) that total chlorophyll content was not reduced significantly even by relatively high mite densities. The disproportional high reduction of chloroplasts in apparently unattacked palisade parenchyma cells of bean after *Tetranychus* feeding, and the investigations of BOULANGER (1958) and LIESERING (1960) are not in agreement with this suggestion. Three interpretations of the damage to unpunctured cells may be possible. The first is, that during feeding the mites produce a strong partial vacuum which forces the cells to burst. The second is, that salivary secretions of *T. urticae* may influence adjacent unpunctured cells and the third is, that degenerative processes in unattacked cells are a response of the plant itself. TANIGOSHI and DAVIS (1978) pointed out that many of the histological symptoms resembled the changes in tissues of other plants affected by

1. normal senescens (SHAW and MANOCHA 1965)
2. fungal (MANOCHA and SHAW 1966) and viral infections (GEROLA et al. 1965)
3. cold injury (PLATT-ALOIA and THOMSON 1976)
4. chemical treatment (BERRY et al. 1975)
5. mineral deficiencies (VESK et al. 1966).

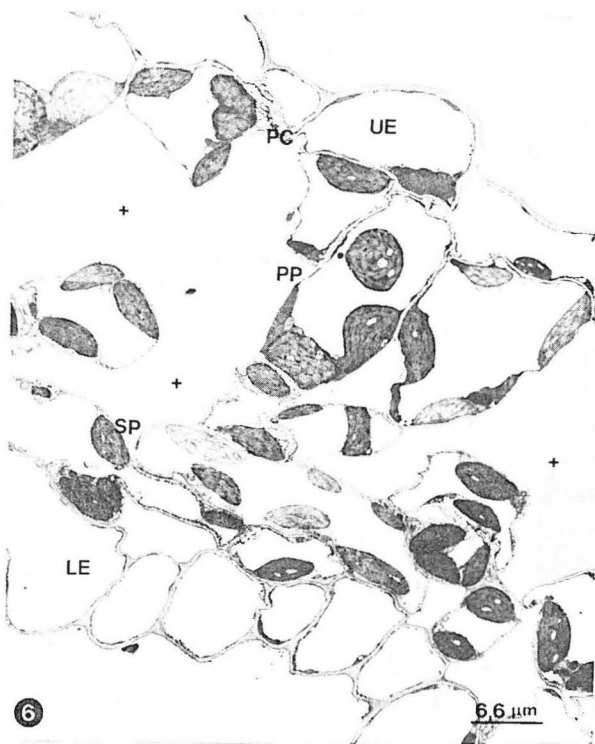


FIG. 6 : Electron micrograph of an infested leaf, cross section. Upper (UE) and lower (LE) epidermal cells irregular. Palisade parenchyma cells (PP) reduced in number and size. Spongy parenchyma cells (SP) reduced in number. Punctured cells (PC) collapsed, other cells apparently unattacked. Large intercellular spaces (+).

As previously described (MOTHES and SEITZ 1981 a) the ventricle of *Tetranychus* is filled with granal thylakoids, starch grana and cytoplasmic portions after food absorption. Decomposition of nuclei, chloroplast stroma, and of other cell organelles must have occurred before absorption by the mites. Thus, salivary secretions may be responsible for the physiological and morphological effects of mite feeding injury on unpunctured cells of bean plants. WIESMANN (1968) reported that salivary secretions of *T. urticae* would dissolve cell contents and increase the permeability of the cell walls. STORMS (1971) showed after autoradiographic tests that saliva of *Tetranychus* was transported into the apical portions of the plants. Because of the active phosphate metabolism of the mites a transport of phosphates occurred into the apical leaves. Another result was that gibberellin content was reduced. On the other hand, LIESERING (1960) was unable to demonstrate salivary secretions in mites although he supposed that enzymes act before food could be absorbed. SANCES et al. (1979 a) suggested that *T. urticae* feeding injury was mechanical and that no toxins were associated with its feeding. Earlier studies on the histology of the salivary glands of *Tetranychus* (MOTHES and SEITZ 1981 b) do not show the nature of the secretions. The morphologically visible response of the bean plant to mite feeding does not offer any solution to this question either.

The cup-shaped chloroplasts which were reported by TANIGOSHI and DAVIS (1978) as an indirect change in apparently unattacked cells, could not be demonstrated by us in bean plant leaves. The sequence of chloroplast damage does not include changes in the arrangement of thylakoids. The first visible reaction seems to be a disruption of the outer membrane. Granal and stromal thylakoids could be distinguished further on, although some differences occurred between control and short-time infested leaves. Swollen chloroplasts with enlarged interthylakoid spaces reported by TANIGOSHI and DAVIS (1978) as a further stage in the degenerative process could only be demonstrated in a few samples. Their demonstration may be dependent on the leaf area which is ex-

amined. AVERY and BRIGGS (1968 b) reported strong damage to veins and adjacent cells, but not to epidermal cells after mite feeding on apple foliage. They measured the length of stylets and found them to be 103 μm (larvae) to 157 μm (adult females). The suggestion was that stylets could penetrate a leaf approximately 70-100 μm . This indicates that differences in the pattern of feeding injury of different mite and plant species depends on the length of stylets and the thickness of leaves (bean plants : 180 μm , apple foliage : 200-265 μm (AVERY and BRIGGS, *op. cit.*), strawberry leaves : 160-182 μm (SANCES et al. 1979)). The depth of penetration directly influences the physiological effects after mite feeding, i.e. whether a reduction of chloroplasts in palisade parenchyma cells occurs or not depends on the thickness of the leaf and the mite species which is feeding on it.

Physiological effects of mite feeding injury on bean plants described by LIESERING (1960) show that the water balance is greatly disturbed, transpiration is highly accelerated and finally leads to the drying out of the leaves, dissimilation is increased, photosynthesis is inhibited, and the composition of the leaf pigments is changed. Some physiochemical effects of mite feeding damage on plants occur before visible changes in the coloration of the leaf surface can be detected (HALL and FERREE 1975). BOULANGER (1958) pointed out that the feeding injury of *Metatetranychus ulmi* is detrimental to the metabolic activities on apple foliage. A reduction in the chlorophyll content may take place before photosynthesis and transpiration are affected.

The present study supports the physiochemical results occurring after mite feeding on different plants. It shows that not only mechanical influences lead to physiological alterations in metabolism but also secondary effects in unpunctured cells contribute to the feeding damage of *T. urticae*. Because of the small size of *T. urticae*, it seems impossible at this time to answer metabolic and energetic questions which arise from mite feeding injury on plants and in the animal itself. Subsequent investigations should combine plant and animal reactions and their mutual effects.

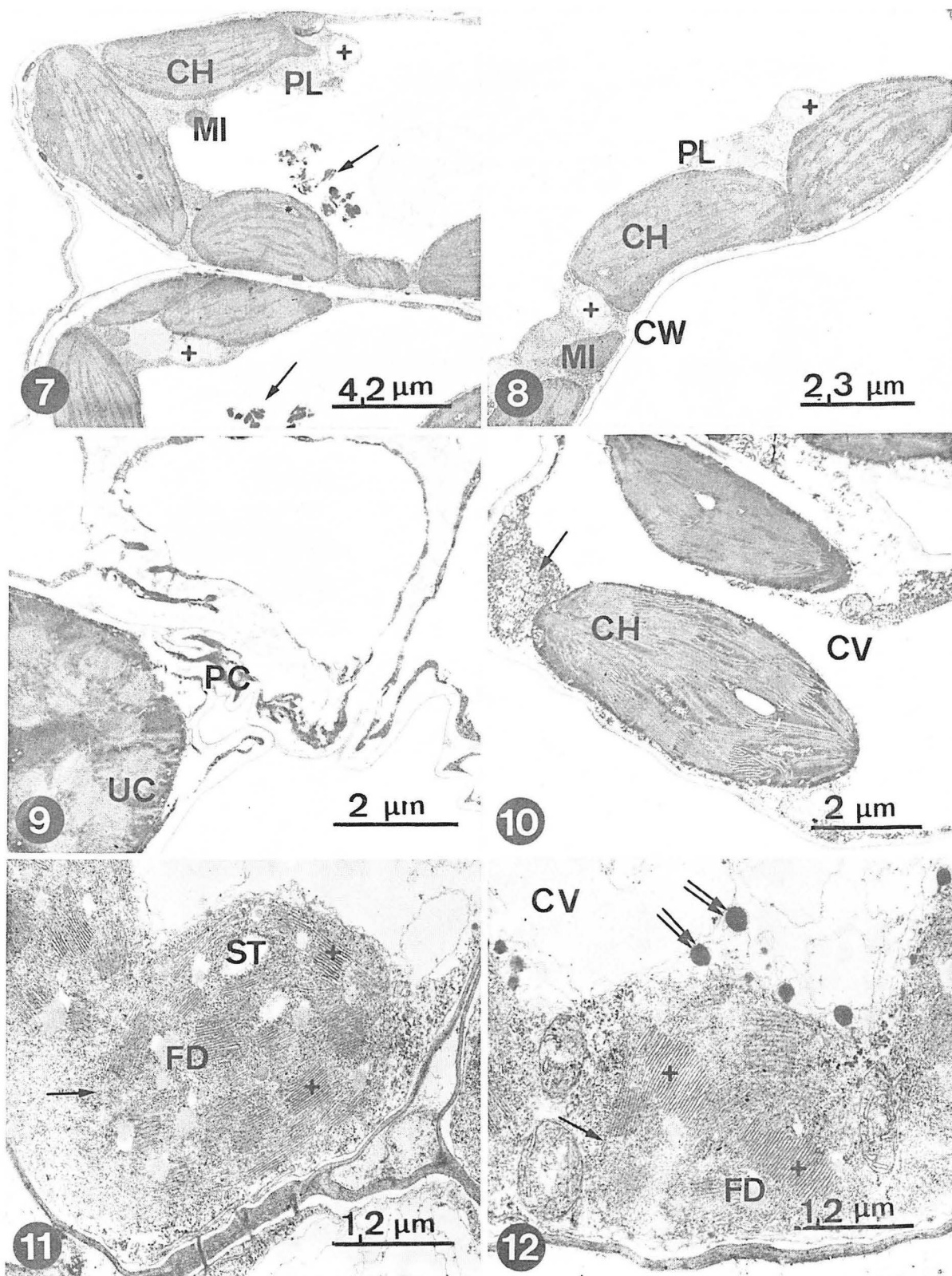


FIG 7-8 : Electron micrograph of a noninfested leaf. Chloroplasts (CH), mitochondria (MI) and peroxisomes (+) surrounded by a fine granular cytoplasm (PL). Some chloroplasts exhibiting starch grana (asterisk). Central vacuole filled with electron dense depositions (arrow). Cell Wall (CW).

FIG. 9-10 : Electron micrograph of an infested leaf. Short time of mite feeding (2 d). Punctured cell (PC) collapsed with only a small layer of residual cytoplasm. Adjacent apparently unattacked cells (UC), but with coagulated cytoplasm (arrow). Chloroplasts (CH) without any visible damage. Central vacuole (CV).

FIG. 11-12 : Electron micrograph of an infested leaf. Extended mite feeding (6 d). Protoplast coagulated. Chloroplast without outer membrane (arrow). Stromal thylakoids altered, granal thylakoids distinguishable and larger than control (+). Former chloroplasts with weakly stained lipid droplets (LD) and starch (ST). Central vacuole (CV). Electron dense grana occurring near the fragmented tonoplast (double arrow).

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