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Histochemical and Ultrastructural Studies of Fruit Abscission in the Olive after Treatment with 2-Chloroethyl-tris-(2-methoxyethoxy)-silane[1]

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Abstract. **Following the application of 2-chloroethyl-tris-(2-methoxyethoxy)-silane (CGA 13586), an ethylene-releasing compound, to olive (*Olea europaea* L.) shoots, 2 abscission zones were observed. One occurred at the proximal, the other at the distal end of the pedicel. Actual separation occurred mostly at the distal end of the pedicel. Changes observed during the development of the abscission zone included: cell plasmolysis, cell wall and middle lamella dissolution, starch grain accumulation and a general breakdown of cells at the abscission zone.**

Mechanical harvesting of olives has been hampered by the high attachment force of the fruits in relation to their mass, particularly for fruit being harvested at the immature, green to straw-color stage as required for the Spanish green and California black-ripe and green-ripe processed olives. The location of the fruits on long, willowy shoots accentuates this problem.

Sprays of various chemicals have been tested to induce separation of olive fruits from the shoots. Ethylene-releasing compounds have shown greatest promise for this purpose (5).

Anatomical and cytological aspects of natural and ethylene-induced abscission have been studied in bean leaves (16, 17, 18, 19), tobacco and tomato flower pedicels (3, 14, 15) and various fruit crops (1, 8, 12, 13, 21, 22). Anatomical aspects of abscission zone development and the point of fruit separation varied among the different fruit species studied.

The study reported here was undertaken to determine the histochemical and ultrastructural changes occurring in the abscission zone of olive fruits following application of 2-chloroethyl-tris-(2-methoxyethoxy)-silane (CGA 13586), an ethylene-producing compound, sold under the trade name of Alsol[2].

Materials and Methods

Two branches on each of 3 'Manzanillo' olive trees with heavy crops were sprayed with 0 and 1,500 ppm CGA 13586 with 0.3% Regulaid added as a surfactant. Sprays were applied the third week of Dec., 1973, the second week of March, 1974, and the second week of Nov., 1974 when the olives were either straw-green or cherry red in color. Ten samples of peduncle: pedicel and pedicel:fruit junctions, including a small segment of adjacent peduncle, pedicel and fruit tissue, were collected at each time interval (1, 2, 3, 4, 6, 9 and 11 days after spray treatment) for histochemical and ultrastructural studies.

Fruit removal force measurements. The force required to remove the olive fruit from its pedicel after spraying was determined with a Chatillon dial push-pull gauge[3] using fruits on the same branches from which the samples for histochemical studies were collected.

Anatomical and histochemical studies. Tissues were fixed in formaldehyde-acetic acid-alcohol (FAA), dehydrated through a *t*-butylalcohol series, and embedded in paraplast. Ten μm sections were prepared with a rotary microtome and mounted on slides with Haupt's adhesive (4). For general anatomical observations, sections were stained with tannic acid-ferric chloride-safranin-fastgreen and Heidenhain's iron-hematoxylin-safranin. Histochemical stains used included: hydroxylamine-ferric chloride for pectic substances (9) and periodic acid-Schiff's base (PAS) for insoluble polysaccharides (4). Free-hand cross sections of the pedicel were used in phloroglucinol-HCl stain for lignin (7).

Ultrastructural studies. Tissues were fixed in 4% glutaraldehyde (in 0.2 M phosphate buffer, pH 7.2) at 4° for 2.5 hr, followed by 17–20 hr at 4° in 2% osmium tetroxide (in 0.2 M phosphate buffer, pH 7.2), dehydrated through alcohol series, and embedded in Spurr's plastic. Approximately 1 μm sections were cut and stained with toluidine blue for light microscopy. A Zeiss EM-9 was used for electron microscopic studies. Materials were sectioned with glass knives on a Porter-Blum MT-1 ultramicrotome and stained with 1% uranyl acetate (10 min) and lead citrate (10 min) (10).

Results

Fruit removal force measurements. CGA 13586 at 1,500

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[2]Manufactured by CIBA-GEIGY Co., Greensboro, N.C.

[3]Model DPP-1 KG, John Chatillon & Sons, Kew Gardens, N.Y. 11415.

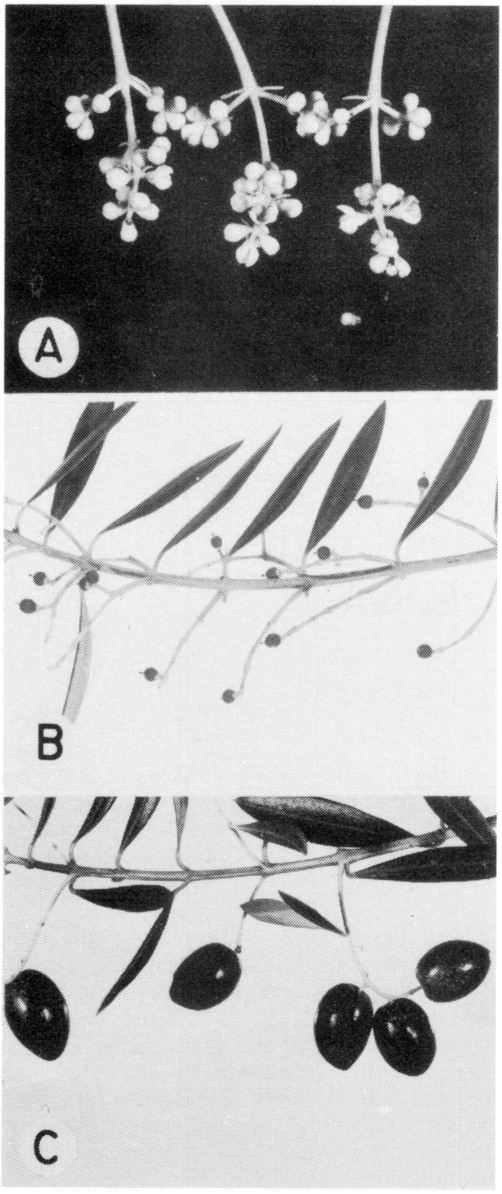


Fig. 1. Three stages in olive fruit development: (A) inflorescences (May 15); (B) young fruits (June 1); (C) mature black olives (Dec. 15).

ppm caused a 35% reduction in fruit removal force of olives in 3 days and an 80% reduction 9 days after spraying.

Anatomical and histochemical observations of the abscission zones. Three stages in the development of peduncle, pedicel, and olive fruits are shown in Fig. 1. From each compound inflorescence only one or a few fruits persist to maturity. The mature peduncle, therefore, includes one or more branch segments (pedicels) of the original inflorescence.

The point of separation of olive fruits after CGA 13586 treatment occurred most frequently at or near the distal end of the pedicel (Fig. 2A, B, C). A small section of the pedicel frequently remained attached to the fruit after its separation. The abscission zone consisted of 15 to 20 layers of cells which had no distinctive anatomical features prior to the formation of the abscission zone (Fig. 2B). The inner pith cells in the pedicel, proximal to the abscission zone, were thick-walled and highly lignified, with lignification decreasing towards the junction between fruit and pedicel. There was a layer of fiber cells around the stele and the number of these fibers also decreased toward the distal end of the pedicel.

Occasionally, the separation of fruits occurred at the junction between the pedicel and peduncle (Fig. 3A, B, C). This abscission zone consisted of 15 to 20 layers of cells and its position was denoted by an indentation at the outer cortical tissue just below the scar of the pedicel bract (Fig. 3A). The cells of the abscission zone were isodiametric, and smaller than the slightly elongated cells of the adjacent pedicel and peduncle (Fig. 2B).

One or more abscission zones may occur along the pedicel. Abscission zone formation is commonly initiated both at the junction of pedicel:peduncle and towards the distal end of the pedicel. Multiple abscission sites have also been reported in bean leaves (19) and in maturing cherry fruits (21). Thus, the point of olive fruit detachment is determined by the relative stage of development of the abscission zones.

One of the most prominent features distinguishing the cells of the abscission zone was cell plasmolysis (Fig. 2C and 3C). This occurred originally in the pith cells and the cortical cells of the pedicel, and extended from both directions towards the phloem. No xylem vessel disruption was found.

At the more advanced stages of abscission zone formation, a general loss of pectic substance and cell wall polysaccharides was observed in cells of this zone. This was evident from the decreased color intensity at the middle lamella and cell wall region following staining with hydroxylamine-HCl and PAS. At the final stage before fruit abscission the walls of some of the cells had completely disintegrated and were no longer recognizable (Fig. 4A).

Large quantities of starch grains were typically present in the cells of the abscission zone following application of CGA 13586 (Fig. 4B).

No cell division was found at or near the abscission zone.

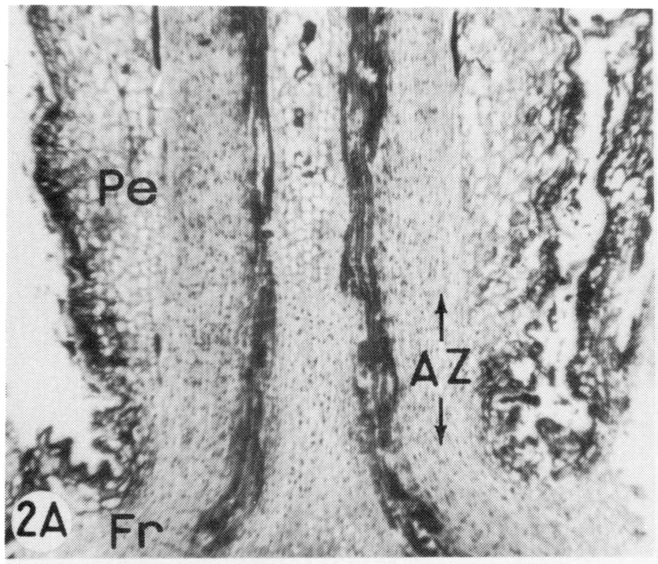
Ultrastructural observations. Plasma membrane invagination was observed in cells during the early stage of abscission zone development (Fig. 5). As the abscission zone development progressed, severe plasmolysis and collapse of cells became obvious. In these severely plasmolyzed cells structural details of the cytoplasm were sometimes lost (Fig. 6). At the late stage of abscission zone development and before total plasmolysis, the cytoplasm appeared to become more electron dense, usually starting from the margins of each cell and extending towards the center (Fig. 6). In this darkened area, the details of the cytoplasmic organelles such as mitochondria, chloroplasts and nucleus frequently became less clear, and the cytoplasm showed signs of coagulation. This is similar to observations made by Valdovinos et al. (15) in ethylene-induced tobacco flower abscission. The cause of this change is not known. The cells of the abscission zone contained noticeably fewer mitochondria than did normal cells, and these mitochondria had fewer cristae.

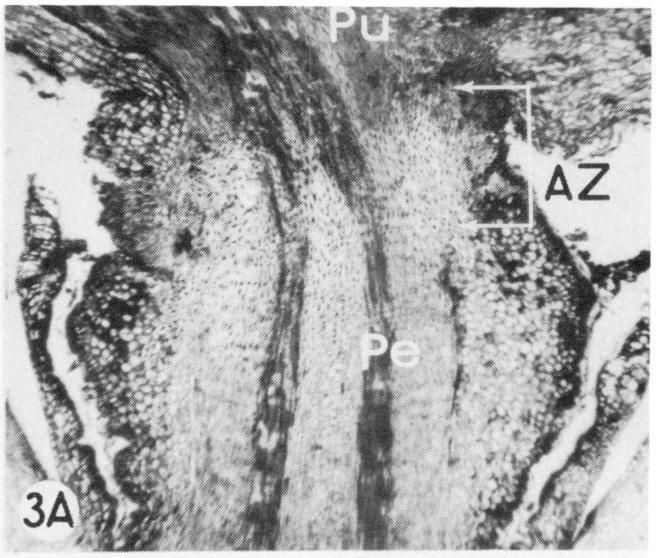
Disintegration appeared to occur both in the middle lamella and in the cell wall. Clearing of cell wall material and the vesicular formation at the middle lamella region are shown in Fig. 7. This is similar to the changes reported to occur during tobacco pedicel abscission (14).

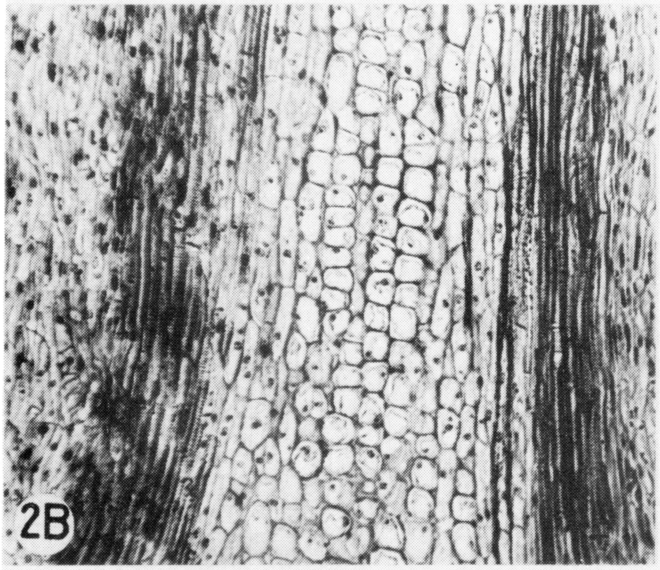
Discussion

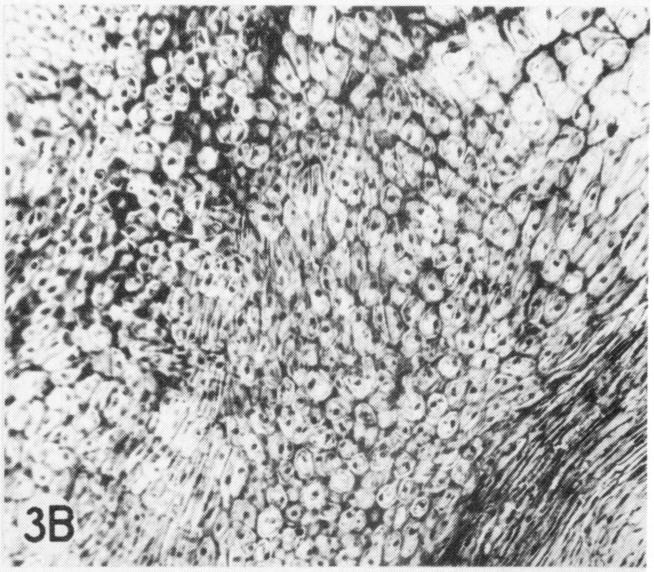
The olive has no well-defined period of natural fruit abscission. Fruits remain attached until full maturity in winter then, if not harvested, drop over an extended period, lasting sometimes until the blooming period the following spring. Olive fruits do not drop naturally at the time of year they are harvested for Spanish green and for the California black and green-ripe style product.

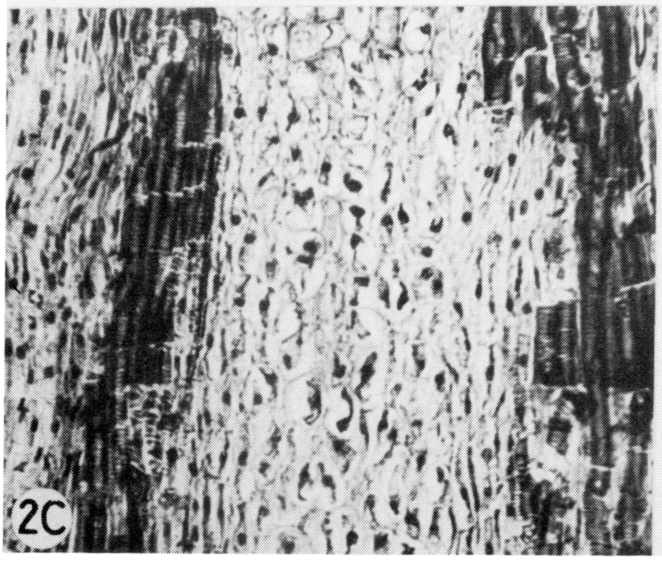
The location of abscission zone formation varies widely in different fruit species and during different periods of natural fruit drop or different periods following chemical treatments (13). The present study showed that after CGA 13586 treatment, abscission zone formation in the olive could be found both at the junction of pedicel:peduncle and near (or at) the distal end of the pedicel. The former zone is well-defined











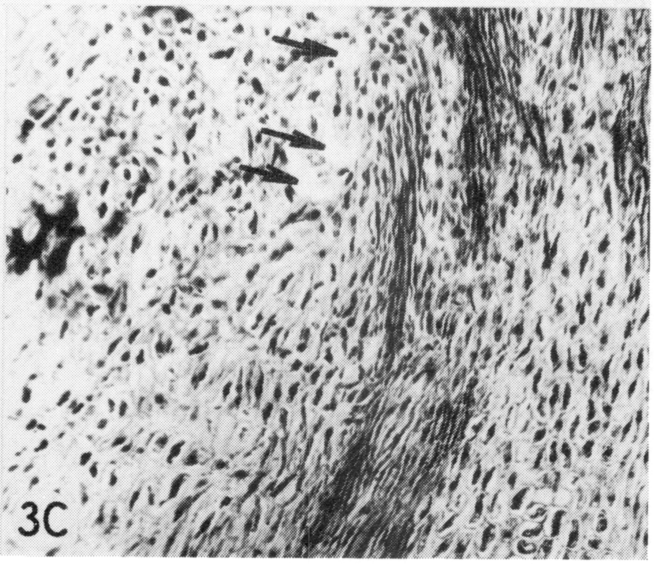


Fig. 2. Photomicrographs of longitudinal sections of olive abscission zone at the distal end of the pedicel: (A) 11 days after treatment with 1,500 ppm CGA 13586. (52×). (B) pith cells of the untreated control at the pedicel where future abscission zone would be expected. (200×). (C) pith cells of treated sample (11 days after spray). (200×). Note the cell plasmolysis and lack of cell wall distinction at the abscission zone as compared to (B). Abscission zone – AZ; fruit – Fr; pedicel – Pe.

Fig. 3. Photomicrographs of longitudinal sections of olive pedicel: peduncle junction. (A) 9 days after the treatment with CGA 13586. (52×). (B) the transition region between pedicel and peduncle of an untreated sample (200×). (C) cortical cells at the transition region of a treated sample (9 days after spray). Note the development of an abscission zone, in which cells were plasmolyzed, and cell walls were either less clear or dissolved, leaving cavities (arrows). (200×). Abcission zone – AZ; pedicel – Pe; peduncle – Pu.

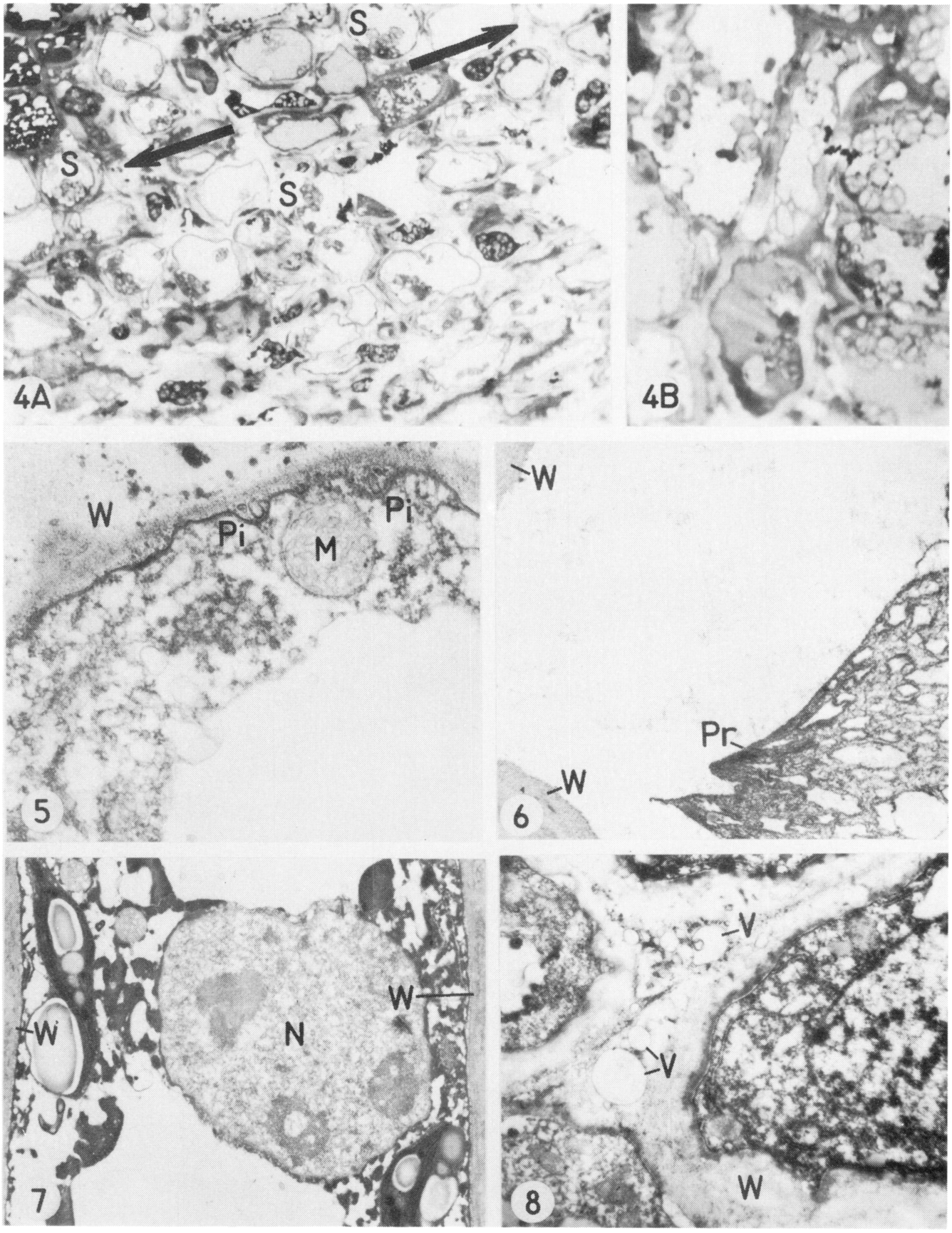


Fig. 4. Longitudinal section of olive pedicel, showing cells just above the line of rupture in the abscission zone. (A) arrows denote the proximal margin of the abscission zone. (550×). Starch grain – S. (B) starch grain accumulation in cells of the abscission zone (1,000×). Fig. 5. Membrane invagination in olive pedicel 6 days after CGA 13586 treatment. (34,000×). Mitochondria – M; plasmalemma invagination – Pi; cell wall – W. Fig. 6. Cell plasmolysis in olive pedicel 11 days after CGA 13586 treatment. The cytoplasmic details are unrecognizable. (13,500×). Protoplast – Pr; cell wall – W. Fig. 7. Changes in cytoplasm in olive pedicel 11 days after CGA 13586 treatment. Note the darkening of cytoplasm progressing from the periphery to the center of the cell. (12,500×). Nucleus – N; cell wall – W. Fig. 8. Cell wall breakdown in olive pedicel 6 days after CGA 13586 treatment. Note the vesicular formation and clearing at the cell wall. (6,300×). Vesicular formation – V; cell wall – W.

anatomically and histochemically, similar to that of the naturally occurring upper abscission zone in cherry (13). The abscission zone near the distal end of the pedicel, however, does not have distinctive anatomical features prior to the abscission zone formation. This zone is different from the lower abscission zone in cherry (13) and in mango and avocado fruit (1). Important changes at the cellular level associated with mango and avocado fruit-fall were observed in the fruit tissue adjacent to the pedicel. The abscission zone in cherry occurs between the receptacle and the fruit, whereas in the olive the abscission zone involves only the pedicel tissue, leaving the fruit tissue unaltered. This is similar to the situation for tomato and tobacco flower abscission at anthesis (3).

In olive there was considerable variability in the rate of abscission zone development in response to CGA 13586 treatment, and this precluded the establishment of a detailed sequence of events leading to abscission.

The histochemical and cytological changes associated with abscission zone formation in olive are, in general, similar to those observed in other plant species and organs (8, 16, 22). Among these are: cell plasmolysis, dissolution of middle lamella and cell wall, and accumulation of starch grains.

Cell plasmolysis in the abscission zone has also been reported for other species (18, 20). Studies with the electron microscope presented here and by other workers (3, 18) have shown that plasmalemma invagination occurs frequently. This may be associated with the observed plasmolysis (18).

Accululation of large quantities of starch in the cells of the abscission zone has also been reported in orange fruit (6, 20) and in bean leaves (2, 17). The role of starch grains in the process of abscission is not clear. According to Wilson and Henderschott (20), starch accumulation may merely be a manifestation of a particular metabolic state when cells are approaching maturity, rather than being actively involved in the process of abscission.

Cell wall dissolution was obvious in some abscission zone cells. This involved not only degradation of the middle lamella, but also the breakdown of cell wall material as was also indicated in unpublished data cited by Rufener and della Pieta (11) in olive fruit abscission after CGA 13586 treatment. This type of change, involving both middle lamella and cell wall, is similar to that described for abscission of tobacco and tomato flowers (14), bean leaves (18) and sour cherry fruits (13).

Abscission zone formation induced by CGA 13586 appears to be the same for olives both in the straw-green and in cherry-red stage of maturity.

Preliminary studies of olive abscission induced by cycloheximide (60 ppm) and ethephon (2500 ppm), both ethylene-inducing compounds, indicated that the events which occurred in the process of abscission zone development are identical with those reported here for CGA 13586-induced abscission.

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