Anatomical and Histochemical Modifications Associated with Abscission of Cucumis Fruits¹

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Abstract. Anatomical changes which take place in conjunction with abscission of cantaloupe fruits (Cucumis melo L. cv. Powdery Mildew Resistant No. 45) include cell separation and cell collapse. Structural modifications in abscission zone cell walls are accompanied by histochemical changes which include losses of pectins and insoluble polysaccharides. Development of a separation cavity is consistently correlated with physiologically defined stages of fruit maturity, and the time of abscission coincides with the peak of the respiratory climacteric. Anatomical and histochemical changes similar to those in cantaloupe also take place in honeydew fruits (Cucumis melo L. cv. Honeydew). However, the abscission zone of honeydews is not structurally well defined, changes are limited to certain parenchyma cells, and the honeydew fruits do not normally abscise. It is suggested that the most desirable growth regulator for use on cantaloupes to accelerate and unify the time of fruit ripening would be one which did not coincidentally accelerate abscission.

In 1970, Kasmire, Rappaport, and May (7) reported that applications of 2-chloroethylphosphonic acid (ethephon) to field grown cantaloupes resulted in apparent ripening of immature melons and increased marketable yields. The prospect of enhanced, simultaneous ripening of large numbers of melons subsequently stimulated interest in the feasibility of developing a mechanical harvester for the crop. It also provoked concern about abscission of cantaloupes, specifically about early abscission, which is induced in conjunction with ethephon treatment. The extent to which early abscission is desirable is somewhat problematical since precocious separation of the fruit from the vine signifies cessation of soluble solids accumulation within the fruit at what may be a less-than-desirable level.

There are no reports in the literature on anatomical or histochemical aspects of abscission in cantaloupes or other muskmelons. The synchronization of abscission and ripening has been alluded to in studies of the respiratory climacteric in cantaloupes (10, 18) and abortion of young cantaloupes has been studied in connection with fertilization, seed development and fruit set (11). It seems appropriate and timely in view of current concerns about application of growth regulators, possible development of mechanical harvesting, and about fruit abscission in general to investigate the nature of abscission in muskmelons.

Materials and Methods

Seeds of cantaloupe (Cucumis melo L. cv. Powdery Mildew Resistant No. 45) and honeydew (Cucumis melo L. cv. Honeydew) muskmelons were sown in the field in late spring. Blossoms were tagged with the date of opening and fruits of known age were subsequently collected at regular intervals during the growing season. Tissue samples approximately 2 cm in length were excised to include the potential region of abscission, located at the juncture of the fruit and pedicel, and equal contiguous portions of pedicel and fruit.

For anatomical and histochemical study, material was fixed in cold formalin-acetic acid-alcohol (FAA), dehydrated with tetrahydrofuran (9), embedded in Tissuemat and sectioned at 10 μ. Sections were stained for anatomical observation with safranin and fast green or safranin and haematoxylin (6). For histochemical studies, fresh-cut and embedded sections were stained “purified” redudium to distinguish regions of pectin localization (27) and with the periodic acid-Schiff's (PAS) reagent for detection of insoluble polysaccharides (5). Samples of fresh material cut on a sliding microtome at 30 μ were stained with iodine-potassium iodide (IKI) to observe starch distribution (5) and with phloroglucinol and hydrochloric acid for lignin detection (6).

Results

The muskmelon is a large pepo, i.e., a berry-like fruit derived from an inferior ovary and having a leathery external layer and a fleshy internal region. Some pepos, such as the cantaloupe, regularly abscise, and others, such as the honeydews, do not. However, both the cantaloupe and honeydew differentiate an abscission zone early in ontogeny. About one week after anthesis the zone is conspicuous externally as a narrow, dark green area at the juncture of the pedicel and fruit (Fig. 1). Internally, the abscission zone is structurally similar in cantaloupe and honeydew. It is a compact cellular region comprised of 15–25 tiers of densely protoplasmic, thin-walled, isodiametric parenchyma cells (Fig. 2) which are distinguishable from adjacent fruit and pedicel cells by their smaller size and by the minimal affinity of their walls for stain. Fast green or haematoxylin-stained abscission zone cells display pale and fuzzy-looking walls (Fig. 3); during ontogeny the walls never stain with the sharpness and clarity characteristically associated with haematoxylin and demonstrable in the fruit cell walls (Fig. 3).

Vascular bundles which traverse the melon pedicel are arranged in a double circle. They diverge and flare into the fruit at the base of the abscission zone. Vascular cells in the abscission region are not modified in size or shape nor do they show any unique characteristics in the abscission region; rather, they resemble the contiguous vascular cells of the fruit and pedicel.

Anatomical changes related to abscission of cantaloupes. Cantaloupe fruits mature and ripen on the vine for approximately 42 days after anthesis, at which time they abscise. The anatomical changes which culminate in abscission of the fruit from the pedicel take place during the 10-day period prior to abscission (from 32–42 days after anthesis).

Structural changes are initiated internally in parenchyma tissue of the abscission zone. The critical anatomical change is that of cell separation, the nature and magnitude of which determine the manner and extent to which the fruit and pedicel ultimately split apart. Barnell (1) and others have described 2 types of cell separation in fruit, one involving lateral cleavage along the middle lamella between 2 adjacent rows of cells and another involving breakdown of the entire middle lamella surrounding a cell. In the abscission zone of cantaloupe the latter mode of cell separation prevails. It is manifested by relatively simultaneous separation of small numbers of contiguous parenchyma cells at several different places within the abscission zone. Cells

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separate as a result of modifications of the middle lamella and so appear isolated from each other as single cells each surrounded by an intact primary wall (Fig. 3).

Neither the number or position in the abscission zone of eventual areas of cell separation can be predicted. There is evidence of some cell wall swelling prior to visible cell separation but swelling occurs so randomly in abscission zone cells that it cannot be related with certainty to cell separation.

Disjunct parenchyma cells are apparently non-viable, and they soon collapse (Fig. 4). This results in the appearance of several small cavities within the abscission zone. Cavities are separated from each other by intact parenchyma and vascular tissue. They are enlarged as contiguous intact parenchyma cells separate from each other and subsequently break down (Fig. 5). In such manner small cavities gradually broaden and finally coalesce to give rise to a single extensive separation cavity in the abscission zone.

It is interesting to note that cell separation followed by cell collapse is characteristic of cortical parenchyma cells of the abscission zone. Epidermal cells and vascular cells ultimately collapse, but do not separate from each other beforehand.

The external evidence of separation is a crack between the pedicel and fruit, first evident about 38 days after anthesis. Depending on the extent of the crack, the cantaloupe fruit is variously designated as being in the “forced slip”, “half slip”, or “full slip” stage of ripeness (15, 21). “Full slip” usually occurs 42 days after anthesis; at that time abscission is complete.

Figs. 1-5. Anatomical characteristics of the abscission zones of cantaloupe and ‘Honeydew’ melons. Fig. 1. Seven-day-old cantaloupe fruit. Arrow denotes region of abscission at juncture of pedicel and fruit. Fig. 2. Photomicrograph of a longitudinal section illustrating the region of abscission (between arrows) of a 4-day-old ‘Honeydew’. Note apparent lack of structural modification of vascular tissue in the abscission region. F—fruit, P—pedicel, VT—vascular tissue. Fig. 3. Photomicrograph showing separation of cells (arrows) within the abscission zone of melon. Fig. 4. Photomicrograph of a longitudinal section of the abscission zone (AZ; double arrow) of a 35-day-old cantaloupe. Note relatively lighter stain in walls of the abscission zone cells compared to intensity of stain in walls of contiguous tissue. Single arrow denotes small separation cavity within the zone. Fig. 5. Photomicrograph of a longitudinal section of the abscission zone of a 39-day-old cantaloupe. Note cell separation and extensive cell collapse.
Cell collapse involves extensive alterations in the integrity of walls and cell contents and it ultimately results in a condition of complete cytolysis in the abscission region. The most conspicuous modifications related to cell degradation include loss of cell wall rigidity, loss of membrane integrity, and deterioration of mitochondria, plastids, nuclei, and other cell inclusions.

Some vascular cells in the abscission region remain intact until the "full slip" stage of fruit-pedicel separation is reached. Such cells are visible as taut, slender strands in the separation cavity. Their persistence throughout the period of separation cavity development is significant in relation to the application of growth regulators which modify the normal time of abscission.

**Histochemical changes in the abscission zone of cantaloupe.** Anatomical observations suggest that chemical changes in the walls of abscission zone cells might accompany structural changes. Staining with ruthenium red to observe pectins in the middle lamella and cell walls indicated the following: bright red (positive) stain in lamella and walls of parenchyma cells prior to visible structural changes; blue-red stain in swollen wall areas; pink stain in primary walls of isolated parenchyma cells; and no stain in collapsed cell walls. Staining with the PAS reagent for indication of the presence of insoluble polysaccharides during abscission gave the following results: bright red (positive) stain in cell walls and blue stain in the middle lamella in intact parenchyma cells of the abscission zone; less intense red stain in the walls of separated cells; and faint pink or no stain in collapsed cell walls.

Staining with IKI indicated a paucity of starch grains in the abscission zone, with relatively fewer grains in internal parenchyma than in outer mesocarp parenchyma cells. No localization of starch was noted as abscission progressed.

Staining with phloroglucinol-hydrochloric acid for lignin gave positive results in xylem walls until the separation cavity developed in the region of the vascular strands. Thereafter the staining reaction was negative.

**Anatomical changes associated with 'Honeydew' abscission.** Under field conditions, 'Honeydew' melons have a prolonged period of horticultural maturity prior to ripening. For commercial distribution they are severed from the vine when mature but unripe and are subsequently treated with ethylene to induce ripening.

During growth and maturation of the fruit the abscission zone of 'Honeydew' tends to become structurally less distinct. The small, closely packed parenchymatous cells which typically constitute the abscission zone are often interspersed with vertical tiers of angular, more loosely arranged cells similar to those which comprise the fruit and pedicel (Fig. 6). In tissue samples taken from melons ranging in age from 16 to 52 days, a general lack of consistency in the relative distinctiveness and form of the abscission zone was observed to be the rule rather than the exception.

Cellular changes related to abscission take place to a limited and varied extent in those fruits of 'Honeydew' which maintain some semblance of an abscission zone. The pattern of structural changes in the zone is similar to that observed in cantaloupes: small groups of contiguous parenchyma cells separate from each other and subsequently collapse, giving rise to several cavities within the abscission zone (Figs. 6, 7). The extent of separation and collapse is limited and cellular changes are confined to the small parenchyma cells of the zone. Cavities remain separated from each other by larger parenchyma cells and by intact vascular and epidermal cells. There is no correlation between the limited structural changes and the degree of fruit maturity.

Some 'Honeydew' melons abscise. Even in these melons there is no correlation between abscission and maturity; sometimes abscission is observed in insect infested or diseased melons.

**Histochemical changes in the abscission zone of 'Honeydew'.** In most 'Honeydew' melons, histochemical staining in the abscission zone prior to and during cell separation gave inconsistent and erratic results. This may be partly attributable to the mixture of typical small and atypical large parenchyma cells in the zone. The most consistent results were as follows: small parenchyma cells (those which might ultimately separate and collapse) stained red or pink with ruthenium red and red with the PAS reagent. Staining with either reagent was negative during the period of cell collapse. Larger parenchyma cells stained positively for both pectins and insoluble polysaccharides.

Staining with IKI indicated the presence of a few starch grains in cells of the abscission zone, and no significant change in starch localization in connection with cell separation or collapse. Vascular cells reacted positively to phloroglucinol and hydrochloric acid prior to, during and following parenchyma cell collapse.

Figs. 6-7. Photomicrographs of longitudinal sections showing anatomical characteristics of the abscission zone of 'Honeydew' melons. Fig. 6. Cell degradation and cavity formation (arrows) within the abscission zone. Fig. 7. Separation and collapse of small parenchyma cells of the abscission zone. Note larger, intact parenchyma cells (upper right) which appear unmodified although structural changes are evident in contiguous cells.
Discussion

Fruit abscission has not been studied as extensively nor in as great detail as leaf abscission, but a number of similarities exist between them. Detachment of both fruits and leaves is facilitated by development of an abscission zone. Retention of both varies with species and environment and can frequently be modified by treatment with growth regulators. Abscission of both fruits and leaves is often correlated with aging and senescence. Many anatomical changes related to abscission are also common to both fruit and leaves; these include enlargement, separation and breakdown of cells in the abscission zone. Finally, similar histochemical reactions, particularly in abscission zone cells, have been reported for fruit and leaves. Comparable changes in pectic and cellulosic components of the walls are among the most commonly noted.

The critical anatomical modification in cantaloupe and ‘Honeydew’ abscission zones is separation of parenchyma cells. Cell separation is also an important structural change in abscission zones of leaves of some woody dicotyledons, in stems of Psoralea and in some fruits, including Malus, Prunus, Mangifera, and Persea (1, 2, 8, 13, 23). Meristematic activity precedes cell separation in abscission zones of woody dicotyledonous leaves, Psoralea stems and immature Malus fruit. It does not occur in conjunction with cell separation in cantaloupe, ‘Honeydew’, Prunus, Mangifera or Persea. Early workers in the field of abscission, including von Mohl (26) and Tison (25), regarded mitosis as a prerequisite for separation. This view has been modified primarily as a result of studies of abscission of fruits, in which cell division is exceptional rather than commonplace in abscission zones. It is relevant to note that meristematic activity in melon fruits ceases early in ontogeny (e.g., 3–5 days after anthesis in ‘Honeydew’) and further growth of the fruit is attributable to cell enlargement (11, 12).

Separation of parenchyma cells is followed by cell breakdown in cantaloupe and ‘Honeydew’ abscission zones. The collapse of the cells is total, resulting in a condition of complete cytolysis similar to that detailed by Pfeiffer (16) and Weinheimer and Woodbury (28) in Coleus and Solanum. The nature of cell wall breakdown in melons is reflected in results of histochemical observations which indicate pectin dissolution (related primarily to the cell separation phase) and polysaccharide changes (related primarily to the cell degradation phase). These changes generally accord with observations of cell wall modifications both in leaves and in other fruit (8, 19, 20, 29).

Some of the most interesting contemporary research on fruit abscission is being carried out by Bukovac and his associates (3, 17, 22, 23, 24). Wittenbach and Bukovac (29) recently directed attention to an interesting perspective of fruit abscission, promulgated in part some time ago by Barnell (1), namely that the most significant component in [sweet cherry] fruit abscission may be ripening of the fruit itself rather than some specific abscission process per se. Barnell based his view on histological and biochemical similarities between ripening avocado fruits and developing separation layers, and Wittenbach and Bukovac suggested that a specific mechanism of abscission may not have developed or evolved in certain fruits. These observations are significant in relation to abscission of cantaloupes, in which consistent correlations can be demonstrated between stages of fruit maturity and development of a separation cavity. The time of fruit abscission coincides with the peak of the respiratory climacteric, which is attained when processes of fruit ripening are well underway (18). Such correlations are not demonstrable in ‘Honeydew’ melons. The extent of detachment of ‘Honeydew’ melons from the vine is variable, although localized cell separation and cell collapse in the abscission zone are frequently observed. The limited extent of abscission may be partly attributable to the lack of a distinct abscission zone in maturing ‘Honeydew’ fruits. It is well established that detachment of plant parts is facilitated by development of a unique abscission zone development. The zone is characteristically comprised of cells anatomically and physiologically different from those adjacent to it. The abscission zone of maturing ‘Honeydew’ melons is a ductile mixture of parenchyma cells, including thin-walled, closely packed, uniformly small cells (typical of abscission zones in general) and loosely arranged, large, more angular cells, similar to those of the fruit. A relationship between structural distinctiveness of the abscission zone and extent of natural separation appears to exist in some leaves and some other fruits. For example, Nicotiana, Eupatorium and Parthenium leaves never do develop abscission zones; they also normally never abscise (4). Fruits of Prunus avium L. do not have a structurally distinct abscission layer; they are mechanically removed from the tree with difficulty and limited success (23).

Ethylene involvement in certain fruit ripening phenomena of cantaloupes has been reported by Lyons et al. (10) and ethylene is also known to hasten the ripening of preclimacteric but mature muskmelons (14). These observations provided the basis for the experiments by Kasmire et al. (7) using ethephon to enhance ripening of field-grown cantaloupes. In general, ethylene stimulation of ripening would not be considered particularly beneficial for most melon cultivars since the fruits are self-ripening. A combination of ethephon does appear to be advantageous in unifying ripening of the cantaloupe crop, however, and it may thus enhance the possibility of mechanistically harvesting the crop. The major drawback to successful use of ethephon on cantaloupes arises from the fact that it promotes abscission, and in so doing terminates the fruit-to-vine transport system and the resultant accumulation of soluble solids in the fruit. The nature of abscission zone cell wall changes reported here are typical of those frequently associated with ethylene application to both leaf and fruit abscission zones. On the basis of these observations it appears reasonable to suggest that the most desirable growth regulator for use on cantaloupes would be one which promotes uniform ripening and concomitantly delays abscission until a desirable fruit quality is attained.

Literature Cited

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Reduced Translocation of Carbon and Nitrogen from Leaves with Symptoms of Pear Curl

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Abstract. With normal pear leaves, soluble carbohydrates (sorbitol, sucrose, glucose, fructose) and starch were higher in green than in abscising, yellow senescent ones. Considerable accumulation of these components occurred in curl leaves which were about to abscise. Greatly reduced transport of photosynthetically fixed $^{14}$C from the leaves occurred in curl-affected ones. Normal leaves photosynthesized and exported $^{14}$C even when abscission was imminent as long as chlorophyll was present. Curl-affected leaves abscised earlier while considerable photosynthetically potential remained. As normal leaves approached senescence their $N$ content decreased dramatically. Curl-affected leaves abscised when $N$ levels were still high. Curl deprives the tree of potentially usable carbohydrate and $N$ resources through inhibition of translocation and premature abscission. These effects of curl may explain reduced vigor and productivity as well as difficulties in establishing young trees. It is postulated that curl and decline are not solely graft union problems.

In 1963 a condition called pear leaf curl was described (10) in which the laminae curled downward longitudinally toward the petiole, sometimes touching the midrib. A more striking symptom is that leaves also become much more intensely colored in autumn, red to purple instead of yellow. Affected leaves usually abscise several weeks before normal ones. This condition most often affects young trees, but is not restricted to them. Reduced growth and yields of some trees showing curl symptoms have been reported (3, 4, 11). Curl may be associated also with difficulties experienced in establishing young trees (2). Afflicted trees are often smaller and have a less extensive root system than normal ones. Seedlings and most of the cultivars grown commercially in CA can be affected and the presence of a bud-union is not a prerequisite for symptom expression (3, 4, 10).

A close association between curl and pear decline seems evident. Both diseases are transmitted by pear psylla (3, 4, 7) and symptoms of curl may precede gradual debility or collapse of pear trees on decline susceptible rootstocks (2, 11, 16). Mycoplasma-like organisms (MLO) have been observed in phloem of decline affected trees (6) and recently reported to be in phloem of leaves with curl symptoms (11). It has been suggested also that decline symptoms are expressed by trees with susceptible rootstocks such as Pyrus serotina or P. ussuriensis whereas curl symptoms appear with decline-tolerant P. communis stocks (15). With pear decline, the collapse or decline of trees without bud-unions (15) and the browning of leaf veins (22) suggests that phloem and translocation phenomena may be affected elsewhere than at the bud-union. If both decline and curl are caused by the same MLO then the transport mechanism in trees with curl might be similarly affected. Development of red foliage, especially in autumn is considered to be associated with accumulation of carbohydrates and anthocyanin synthesis (8). The intense red to purple color of pear leaves affected by curl suggested that similar phenomena had occurred. Thus, we wished to determine whether carbohydrates accumulated in curl-affected leaves, and if translocation of photosynthate was restricted. Inhibition of translocation could have an impact on the nutrition of the tree. Nitrogen was also of concern because of its solublization and return to the stem during autumnal senescence of leaves (20).

Materials and Methods

Except as noted, 4- to 6-year-old trees of P. communis L., 'Bartlett' on 'Winter Nelis' or 'Bartlett' seedling rootstocks were used. For determination of carbohydrates and $N$, normal and curl leaves with wide differences in color were obtained. Leaves were selected for uniformity within each category and 1 sample of each type was taken. Each sample consisted of 10 leaves obtained from several trees.

Samples of leaves were weighed, cut finely, and placed in Soxhlet extraction thimbles. The thimbles were then put into large test tubes, sufficient boiling water was added to cover the tissue, and the mixture allowed to simmer for 10 min in a water bath. Each thimble and initial extract was transferred to a Soxhlet apparatus and further extracted for 18 hr. After removal of the methanolic extract, the residue was extracted with distilled water for 4 hr. The methanolic and aqueous extracts were reduced in volume in an air stream, combined, and made to a known volume.

The amounts of individual soluble carbohydrates were determined gas chromatographically as their trimethylsilyl ether derivatives (19).

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