Table 3. Cup counts of 'Early Black' cranberries receiving preharvest sprays of ethephon, SADH, and malathion.

Treatment*	Year+ 1968	1969	1970	Mean 1968-70
	No. berries/standard cup			
Check	139	114	118	124
4 lb./A SADH	138	116	116	124
8 lb./A SADH	141	114	116	123
1 lb./A ethephon	142	115	120	126
2½ lb./A malathion	138	118	118	124

+$LSD_{(.01)}$ between years = 16.
*No significant differences between treatments within a given year.

increased the anthocyanin content of 'Early Black' cranberry, although not to the extent that ethephon did. The malathion rate used in this color study is the same rate cleared for use on cranberries as an insecticide. Both ethephon and malathion significantly increased anthocyanin levels in cranberries when the data were combined and analyzed over the 3-year period. SADH did not influence anthocyanin content in 'Early Black' cranberry.

An increase in the anthocyanin level was not accompanied by reductions in yield or by smaller fruit size (Tables 2 and 3). This was of some concern, since the use of malathion for insecticidal purposes on cranberries during the growing season caused phytotoxicity, and reduced yields.[4] Yields were highest in 1968, and were not significantly different between 1969 and 1970. The yields obtained were well above the avg production of 53 barrels per acre in New Jersey in 1969.[5]

The application of either ethephon or malathion is a practical method of increasing the anthocyanin content in early harvested cranberry fruit. Whether the mode of action in anthocyanin enhancement is the same for the 2 materials is not certain. An ethylene stimulus of anthocyanin production may be operative in both treatments. In the case of ethephon, the ethylene is produced by chemical decomposition of (2-chloroethyl)phosphonic acid within the plant[6]; in the case of malathion, ethylene may be produced as the indirect result of tissue injury since bronzing of the leaves is usually associated with the fall application of malathion. It would be useful to be able to determine whether combined treatments of ethephon and malathion would be additive or synergistic with respect to anthocyanin content of the cranberry.

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An Anatomical and Histochemical Study of Abscission in Maturing Sweet Cherry Fruit

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Abstract. Abscission of maturing sweet cherry fruit (*Prunus avium* L. cv. Windsor) occurred at 2 different abscission zones, depending on the stage of fruit development. Immature fruit abscised at the upper zone between the pedicel and peduncle; mature fruit abscised at the lower zone between the fruit and receptacle. Separation in the abscission layer began directly above the stony pericarp and resulted in the formation of a cavity. Later separation occurred at the fruit:pedicel indentation and extended through the abscission layer toward the vascular bundles. Abscission involved the fracturing of cell walls as well as wall separation. There was no evidence of change in pectins, cellulose or other polysaccharides in the cell walls of the abscission layer prior to or during fruit separation. No starch accumulation in the abscission zone or lignification of tissue adjacent to the abscission layer was observed through fruit maturity.

Numerous studies and review articles have been published on the anatomical and physiological aspects of leaf abscission (1, 3, 4, 8, 12, 16, 17, 23). These have been conducted on both herbaceous (16, 17, 23) and woody species (4, 8, 12). In contrast to the extensive work on leaf-fall, the anatomy of fruit abscission is not well documented.

The abscission of immature apple (9, 10, 13, 14, 15) and cherry fruit (19) occurs at the juncture of the pedicel and spur. In these fruits secondary cell division preceded the formation of an abscission layer, which was histochemically indexed by loss of cell-wall constituents (13, 15, 19).

Anatomical changes associated with the abscission of mature fruit are not as well-defined as those for leaf-fall and the fall of immature fruit. In apple, abscission of mature fruit occurred between the pedicel and spur; however, separation was not preceded by cell division and the abscission layer was not restricted to any defined layers of cells in the zone (13, 14, 15). Conversely, abscission of mature avocado, mango (2), orange (24), and sour cherry fruit (20, 21) occurred at the juncture of the pedicel and fruit. The abscission layer in mature avocado and mango comprised the area between the fruit and receptacle

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[2]Bednarz, R. M. 1970. The changes in anatomy and fine structure as related to the physiology of abscission in the lower pulvinus of bean (*Phaseolus vulgaris* L.). Ph.D. Thesis, Michigan State University.

[3]Wittenbach, V. A. 1970. Morphological and physiological aspects of cherry fruit abscission with reference to 2-chloroethylphosphonic acid. M.S. Thesis, Michigan State University.

tissues (2). No cell division was observed prior to or during abscission.

Recent studies established that separation of mature orange (24) and sour cherry fruit (20, 21) proceeded through an abscission layer characterized by a loss of cell-wall constituents and containing cells anatomieally different from those in the adjacent receptacle and fruit tissues. Cell division was not an essential aspect in abscission of either fruit.

There is a marked difference in the fruit removal force (FRF) of sour and sweet cherry fruit at maturity (5, 6). The greater force required to effect separation of sweet cherry fruit has been an obstacle to machine harvesting and has resulted in greater bruising and tearing of fruit tissue at harvest. This investigation was undertaken to characterize the abscission of maturing 'Windsor' sweet cherry fruit to further our knowledge of fruit abscission and to provide a basis for future work on the regulation of fruit abscission.

Materials and Methods

Anatomy. Samples of sweet cherry (*Prunus avium* L. 'Windsor') fruit were collected weekly from bloom and every other day from the start of stage III of fruit growth (22) through maturity. Fruits were detached above the upper abscission zone and immediately killed and fixed in FAA (formalin:acetic acid:alcohol) (11). Segments of tissue containing the upper or lower abscission zone were removed, dehydrated in a gradient TBA (tertiary butyl alcohol) series (18) and embedded in Tissuemat (melting range 56-58°C). Tissue sections were cut at 12 μm and affixed to glass slides with Haupt's adhesive, using 4% formalin to flatten and expand the sections on a warming plate (11). The sections were passed through an alcohol series to water and stained with iron haematoxylin. Abscission zones were sampled from 15 fruit for each collection date.

Histochemistry. Changes in pectins, cellulose and other polysaccharides, lignin, and starch were followed histochemically in the abscission zone during fruit maturation. FAA fixed tissue of the various stages of abscission was sectioned at 15 μm. The following histochemical reactions, as outlined by Jensen (11), were used: periodic acid-Schiff's reagent (polysaccharides), ruthenium red and hydroxylamine-ferric chloride (pectin), phloroglucinol-HCl (lignin), and iodine potassium iodide (starch). Changes in cellulose orientation in cell walls of the abscission zone were studied using plane-polarized light. All determinations were performed at least twice.

The distribution of water-insoluble Ca and Mg across the abscission zone was followed using an electron microprobe (Applied Research Laboratories Model EMX-SM). Paraffin sections cut at 15 μm were mounted on quartz slides using 4% formalin to flatten the sections. The tissue was incinerated by increasing the temp from 21° to 350°C over a period of 5 hr and held at 350°C for 6 hr in a muffle furnace. The gradual increase in temp minimized the spattering of paraffin[2].

After incineration, the remaining stable white ash was coated with a layer of carbon (approx 200 Å thick) and the specimen was examined using 25 kv electron accelerating potential and a sample current of 0.05 μa. The abscission zone just below the indentation between the fruit and receptacle was scanned. Similar distribution patterns were obtained for both Ca and Mg, however, the level of Mg was far below that of Ca. Only the results for Ca are presented.

Terminology. The terms *abscission zone* and *abscission layer* have been variously defined in writings on fruit abscission. We have chosen to use Esau's definitions (7) modified (shown in parenthesis) to take into account (a) that the abscission zone may not always occur at the base of the fruit, and (b) that the protective layer may or may not play a role in fruit abscission. These definitions are well-established, sufficiently broad, and applicable to abscission of mature fruit.

Abscission zone. "Zone (generally) at base of leaf, or fruit, or flower, or other plant part, that contains the abscission layer and the protective layer, both of which (may) play a role in the separation of the plant part from the plant."

Abscission layer. "In abscission zone. Layer of cells the disjunction or breakdown of which separates a plant part, such as leaf, fruit, flower, etc., from the plant. Syn. separation layer."

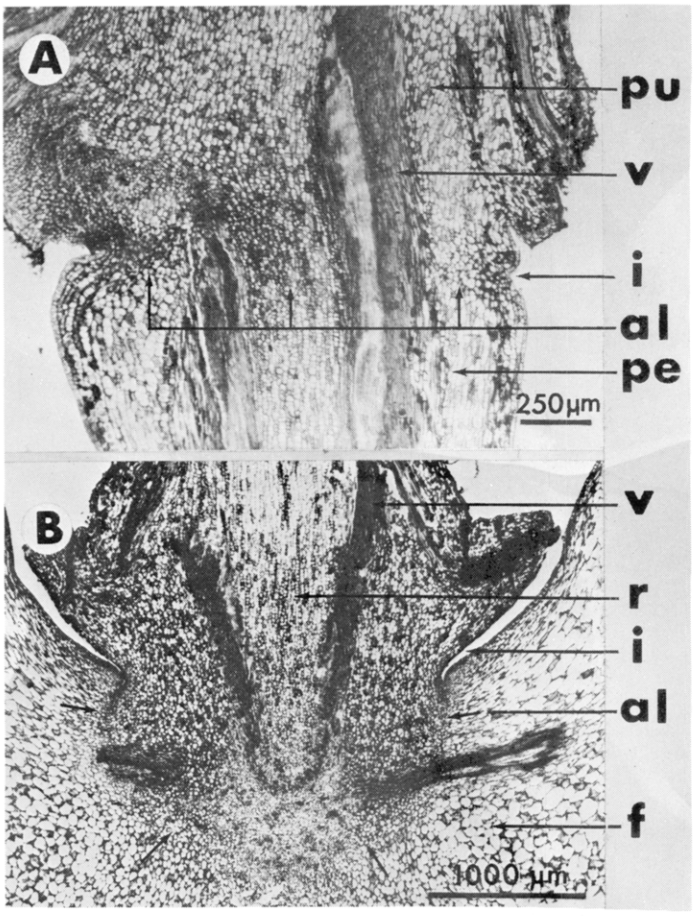

Fig. 1. Photomicrographs of longitudinal sections illustrating the upper (A) and lower (B) abscission zones of 'Windsor' sweet cherry fruit approx 18 days prior to maturity. pu – peduncle, v – vascular tissue, i – indentation, al – abscission layer, pe – pedicel, r – receptacle, f – fruit.

Results

Anatomy of the abscission zones. Two abscission zones were observed in maturing 'Windsor' sweet cherry fruit, hereafter referred to as the upper and lower abscission zone (Fig. 1). The upper zone represented the transition region between the pedicel and peduncle (Fig. 1A) and was denoted externally by an indentation at the juncture of the two tissues. Anatomically, this zone was characterized by a gradation in cell size, from larger, periclinally-elongated cells of the peduncle to smaller, isodiametric cells of the abscission layer (8-10 cells wide) and then to the larger, periclinally-elongated cells of the pedicel. The vascular bundles traversing the upper zone were often interrupted by branching of a vascular trace to another fruit or

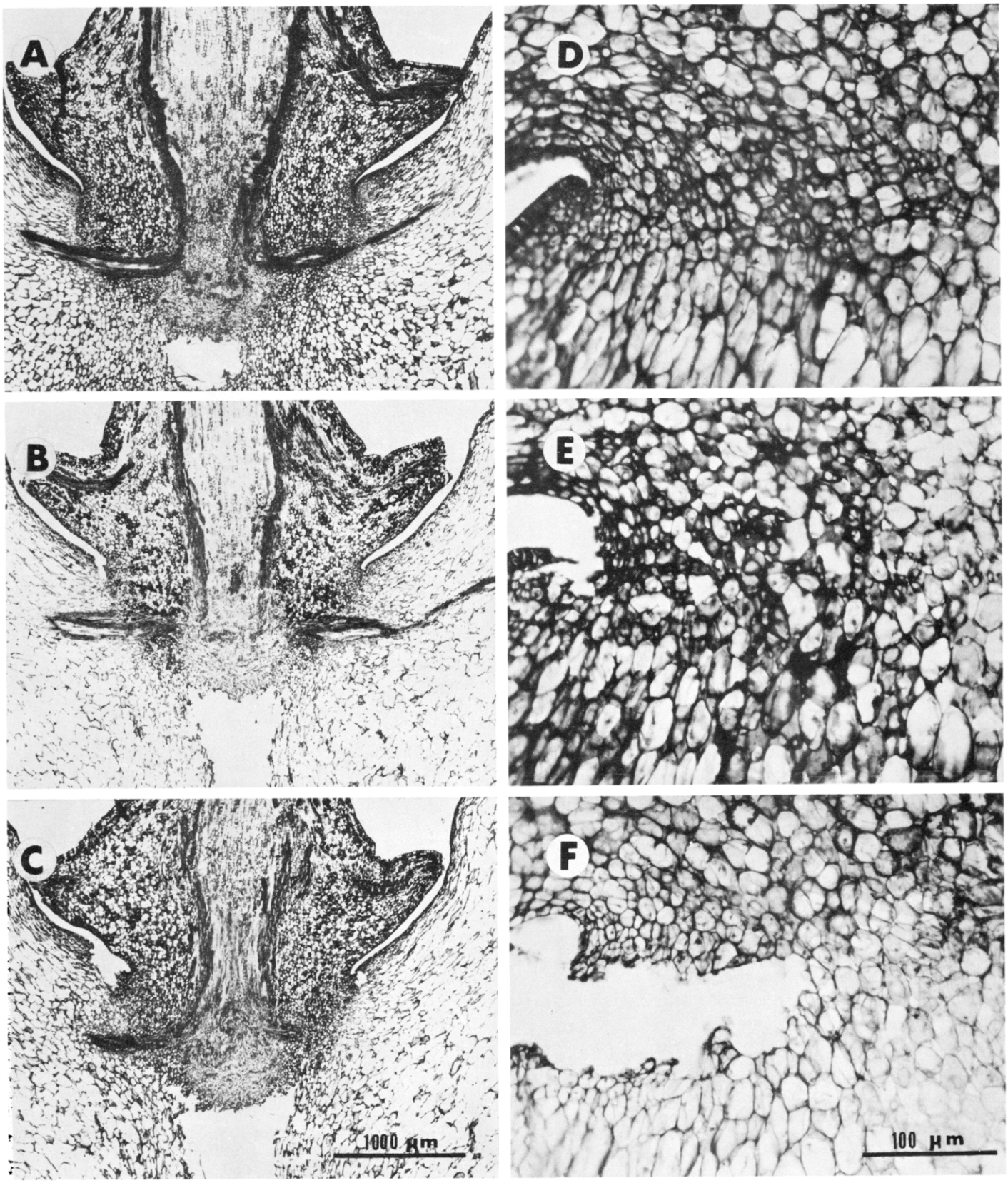

Fig. 2. Photomicrographs of longitudinal sections illustrating anatomical changes in the lower abscission zone (A, B, C) and the details of separation in the abscission layer between the indentation and vascular bundles (D, E, F) of 'Windsor' sweet cherry fruit. A and D, B and E, and C and F are representative of fruit 12, 6, and 0 days prior to maturity, respectively.

spur leaf. The vascular tissue did not exhibit a gradation in cell size through the abscission zone, and the abscission layer was not continuous across the vascular bundles. Abscission at this zone occurred during the fall of immature fruit and after detachment of the fruit or removal of the fleshy pericarp experimentally during fruit development.

The lower zone, which denotes the transition region between the fruit and receptacle (Fig. 1B), was delineated externally by the fruit-receptacle indentation. Near the time of fruit maturity the zone was characterized anatomically by progressively smaller cells from the receptacle and fruit sides toward the juncture of the two tissues. The juncture was further delineated by small isodiametric cells of the receptacle (proximal) and angular, expanded cells of the fruit (distal). This line of contiguity between the 2 defined the abscission layer, and separation at maturity occurred along this juncture or just proximal to it through the small isodiametric cells. Differentiation of the abscission layer began near the beginning of stage II of fruit development as these cells stopped enlarging. The adjacent cells of the receptacle continued to enlarge, for a short time, and cells of the fruit underwent rapid expansion during the 3rd growth stage. Near maturity, the abscission layer represented a structurally weak connection between the thin-walled, rapidly-expanding cells of the fruit and the thick-walled, fully-developed cells of the receptacle. The vascular cylinder supplying the fruit was branched in the receptacle near the abscission layer and entered the fleshy pericarp as 10 to 12 individual bundles. These bundles exhibited no evidence of an abscission layer but were weakened by the absence of sclerenchyma tissue associated with the vascular cylinder in the pedicel and receptacle.

The anatomically defined lower abscission layer at maturity extended from a point above the stony pericarp to the indentation and was interrupted by the radiating vascular bundles. The distal portion of the layer extended to within approx 10 cell layers of the stony pericarp. The cells between the distal portion of the abscission layer and pit enlarged only during the terminal phase of fruit development and even 15-20 days prior to maturity were only slightly larger than the cells of the abscission layer (Fig. 1B).

Anatomical changes during fruit abscission. No significant anatomical changes were observed in the upper abscission zone during the 15-20 day period preceding fruit maturity. In contrast, with applied force fruit separation occurred at the lower zone at maturity. The remainder of the data will describe abscission at this zone only.

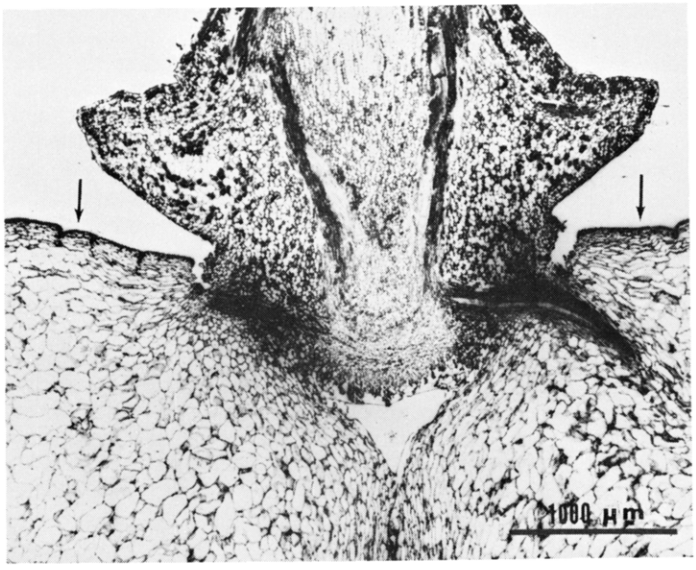

Fig. 3. Photomicrograph of a longitudinal section showing collapsed fruit lobes (note arrows) and cell separation 7 to 10 days past maturity.

The first visual evidence of abscission was the separation of cells between the distal portion of the abscission layer and the stony pericarp approx 15 days before maturity. This separation and possible degradation of some cells led to the formation of a cavity above the stony pericarp (Fig. 2A). Tissue adjacent to the cavity became stressed and collapsed in toward the cavity (Fig. 2B, C), and appeared to result in the collapse of the fruit lobe tissue surrounding the pedicel (Fig. 3). Concomitant with development of the cavity, separation extended toward the vascular bundles through the abscission layer (Fig. 2B, C). Separation occurred between the receptacle and fruit at the indentation (Fig. 2D, E, F). This separation seldom extended more than half the distance to the vascular bundles (Fig. 2F) Even when the fruit was mechanically protected and kept on the tree 7 to 10 days past maturity, separation never extended completely to the vascular bundles (Fig. 3). Separation involved fracturing of cell walls and pulling apart of intact cells along the middle lamella. There was no evidence of abscission across the vascular bundles, which were broken in response to mechanical force. Some random cell division was observed in the abscission zone near the indentation and proximal to the abscission layer (Fig. 2D); however, divided cells did not appear to be involved in separation.

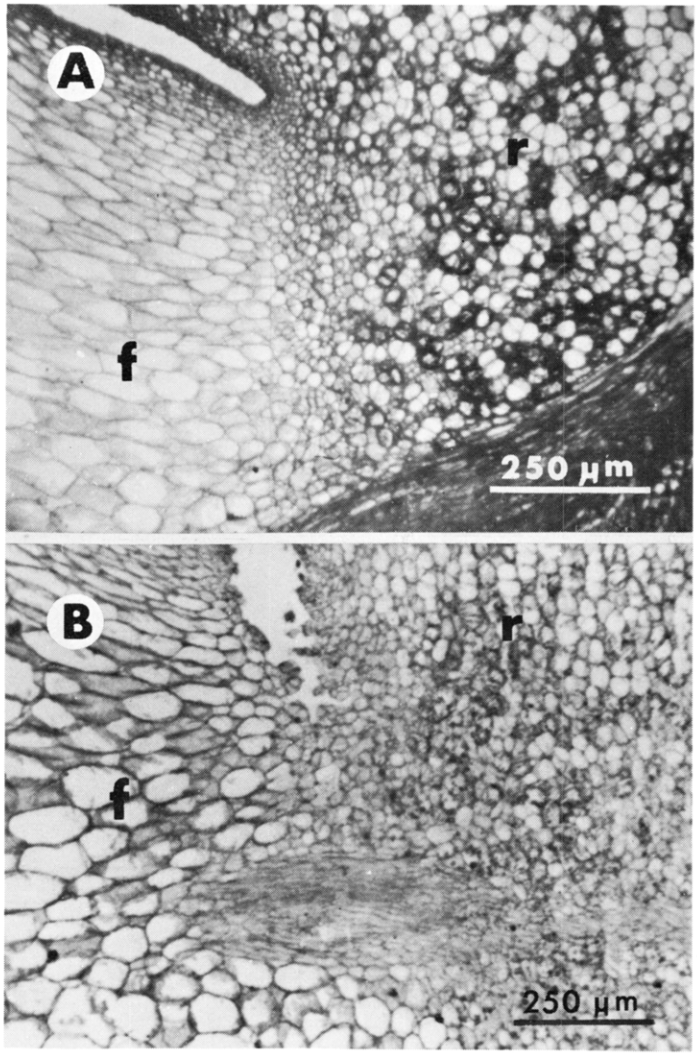

Fig. 4. Photomicrographs of longitudinal sections of the lower abscission zone between the indentation and vascular bundle stained for polysaccharides with periodic acid – Schiff's reagent just prior to separation (A) and stained for pectins with ruthenium red during separation (B). r – receptacle, f – fruit.

Histochemical changes during fruit abscission. Localization data for non-cellulosic polysaccharides (Fig. 4A), pectins (Fig. 4B), and cellulose in the abscission zone revealed no change in these cell-wall constituents in the abscission layer prior to or during cell separation in comparison to the adjacent fruit and receptacle tissue. Nevertheless, separation consistently occurred through the naturally weak abscission layer where the thick-walled, small cells of the receptacle abutted the expanding, thin-walled cells of the fruit (Fig. 4B).

Localization of starch during abscission indicated a large number of grains in the cells of the receptacle. There was less starch in the fleshy pericarp, and at maturation the number of starch grains in the fruit tissue had declined. The level of starch in the cells of the receptacle was relatively high even at maturity. Little or no starch was evident in the abscission layer. No lignification of cells on either side of the abscission layer was observed through fruit maturity. Only fiber and vascular bundle cells gave a positive reaction for lignin.

More water-insoluble Ca was present in walls of cells in the abscission zone than in the fruit. During cell separation there was a loss of Ca from the abscission layer (Fig. 5). This loss appeared to be associated with the rupturing of cell walls (Fig. 5). Hence, the water-insoluble Ca remaining appeared to be almost completely associated with the cell walls and as these walls were fractured the Ca was either lost or it remained in the cell-wall fragments in the abscission layer (Fig. 5).

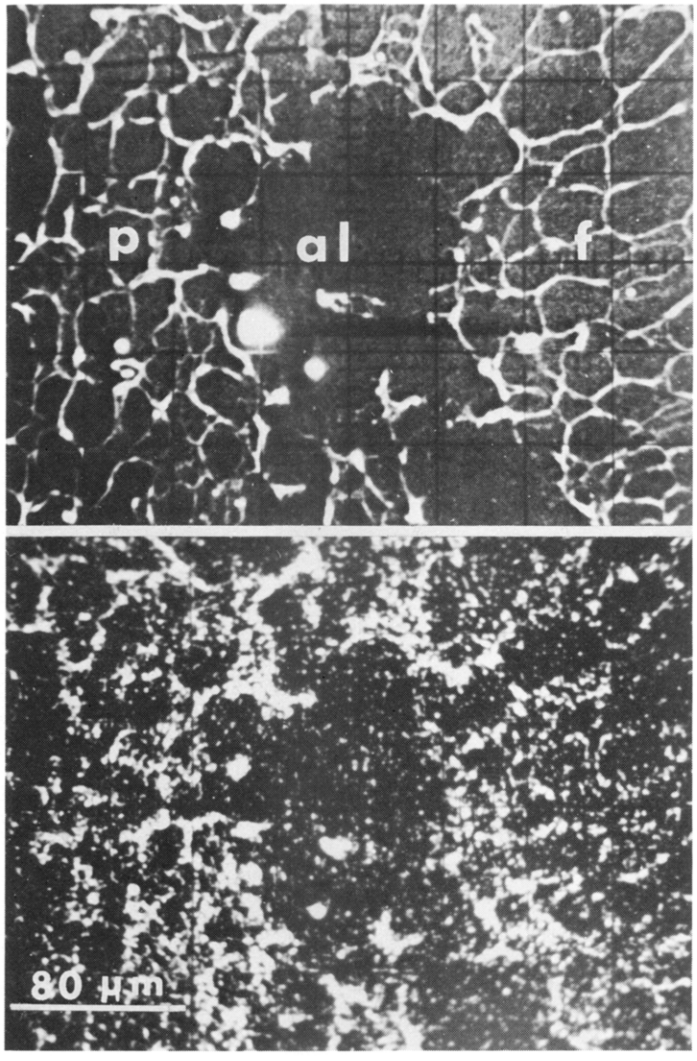

Fig. 5. Secondary electron micrograph (top) of a tissue section (longitudinal) from the abscission zone between the fruit-receptacle indentation and the vascular bundles, and a corresponding calcium x-ray micrograph (bottom) illustrating the distribution of Ca in the tissue section. p – pedicel, f – fruit, al – abscission layer.

Discussion

There are two potential sites of abscission for 'Windsor' sweet cherry fruit during development. Abscission of immature fruit -- those that failed to set or develop ("June drop" or damaged fruit) -- occurred with the pedicel attached, indicating the potential for layer development in the upper zone. The force required to effect separation at this zone changes in relation to fruit development (6). Lower FRF values were recorded at the end of stage I and at the beginning of stage III, than during stage II or late stage III. As the fruit approached maturity there was an apparent strengthening of tissue, resulting in a rise in FRF.

The abscission layer in the upper zone was differentiated early in fruit development (stage I) and was characterized by small, isodiametric cells at the juncture of the peduncle and pedicel (19). It resembled the abscission layer observed in apple (13, 15) and in the upper zone of avocado, mango (2), and sour cherry fruit.[3] It was also similar anatomically and histochemically to the layer commonly associated with leaf abscission (1, 12, 16, 23). Cell division and loss of cell-wall constituents from the abscission layer preceded separation (19). Hence, the abscission layer in the upper zone was well-defined anatomically, histochemically, and as indexed by FRF, but abscission occurred at this site only during the fall of immature fruit or after damage or detachment of the fruit or removal of the fleshy pericarp during development.

Fruit abscission at maturity occurred at the lower zone as evidenced by a pronounced reduction in FRF, which started as the fruit entered stage III and reached a min FRF at maturity (6). Separation consistently occurred where the fruit and receptacle tissues were contiguous. Compared to the abscission layer in the upper zone and to that observed for leaves, the abscission layer in the lower zone was less well-defined anatomically and histochemically. It was not composed of distinct layers of cells, but rather was delineated by the juncture of the fruit and receptacle tissues. Further, no specific layer or layers of cells were observed (histochemically) to undergo differential cell-wall changes in pectin, polysaccharides, or cellulose prior to separation as has been demonstrated for the upper zone of sweet cherry (19), for the upper and lower zone of sour cherry (20), and for leaf abscission (12, 16, 17, 23). We do not imply that physiological events are not involved, but rather that abscission of sweet cherry fruit differs from the sour cherry as indexed by these parameters. In fact, Poovaiah (unpublished) has associated phosphatase, peroxidase, and dehydrogenase activity with fruit separation in the sweet cherry.

Fruit abscission in sweet cherry was characterized initially by the formation of a cavity between the tissue of the receptacle and fruit just above the stony pericarp. Differential stress, probably caused by rapid expansion of the fruit cells and little or no expansion of the receptacle cells, appeared to cause enlargement of this cavity. Adjacent cells collapsed in toward the cavity and these cellular changes later resulted in the collapse of the fruit lobe. Mechanical stress, prior to the collapse of the lobe, may also be responsible for separation at the indentation where rupture of cell walls and separation of entire cells along the middle lamella were common. Separation between the receptacle and fruit tissues in the abscission layer was limited to areas above the stony pericarp and at the periphery near the indentation.

The localized nature of separation and the extensive fracturing of cells suggest that physical forces were involved in abscission. Such forces could result from the wt and movement

of the fruit (by wind) and the rapid and differential expansion of cells of the receptacle and fleshy pericarp during stage III of fruit growth. Further, a significant component in the abscission of mature 'Windsor' cherry fruit may be a manifestation of fruit ripening rather than the result of a specific abscission process *per se*. Barnell (2) concluded that separation of mature avocado and mango fruit was the result of histological and biochemical changes similar to those observed during ripening of the fleshy pericarp. In 'Windsor' we also observed no change in cell-wall constituents in the abscission layer during separation when compared to the adjacent fruit tissue. Perhaps in this sweet cherry cultivar as well as in avocado and mango, a specific mechanism of abscission has not evolved or has not developed to the same extent as in the sour cherry fruit (20, 21) and in leaf abscission in general (1, 12, 17, 23).

The marked decrease in FRF during stage III was typical of all sweet cherry cultivars investigated and may be explained by the observed localization of cell separation in the abscission layer. To what extent these observations on 'Windsor' apply to other sweet cherry cultivars and the physiological nature of abscission is currently being investigated.

The question of appropriate definitions of abscission zone and layer for maturing fruit is critical to avoid confusion. Stösser et al. (20) reported that there was no evidence of an abscission layer between the fruit and pedicel in 'Windsor'. This conclusion was based on the absence of a well-defined layer or layers of cells anatomically different from those in the adjacent receptacle or fruit tissues and on the absence of differential staining of the cells in this zone with haematoxylin. Within this restricted definition, one would conclude that there is no abscission layer in 'Windsor'. However, if one recognizes the broader definition of Esau, that is, "Layer of cells the disjuncture or breakdown of which separates a plant part ... from the plant," then, as shown in this paper, one can conclude that there is an abscission layer in 'Windsor'. Because of the degree of restrictiveness authors have imposed on a given definition, the lack of consensus among authors on one suitable definition, and that often in maturing fruit, abscission may not be similar to that considered classical for leaf abscission, we have suggested that the definitions of Esau, as pointed out under Methods, be adopted wherever possible in publications on fruit abscission.

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