Fruit Tissue Injury by Frost to 'York Imperial' Apples[1]

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Abstract. The effects of freeze injury occurring at anthesis upon young developing fruit were studied anatomically. Slight injury was characterized by separation of the hypodermis from the outer cortex during the initial stages of fruit growth. Necrotic tissues were adjacent to the main part of the injury. The internal cortical tissues and vascular bundles in the core line were uninjured. The basin or fruit apex was sensitive to freeze injury resulting in stylar abscission, abortive ovules, and large breaks in the cortical tissue. Growth distortions in the outer cortex were evident 21 days after injury, indicating sensitivity to frost and to the initiation of corking.

Wound healing occurred 1 week after full bloom with the development of callus or proliferated tissues which united the wound areas.

CELLULAR changes that resulted from injury to blossoms and to very young fruits by a late spring freeze, are described anatomically. The tissue development of 'York Imperial' apples from trees with a history of corking disorders was studied sequentially through the 1968 growing season to determine abnormal symptoms contributing to, or precursor to, corking.[4] Some of the blossoms (probably the earliest and weakest) had been subjected to a light freeze, resulting in damage to the outer protective region of the fruits causing them to drop. Since this slight injury, which may be characterized by separation of the hypodermis from the outer cortex, occurred during the initial stages of fruit growth, this study will give additional information concerning wound healing of young tissues.

Dorsey (2) reported that ice formed in flower rudiments when the temperature was near 28°F throughout the dormant season. Rogers (5) described frost damage to 'Cox's Orange Pippin' in full blossom as follows: at 28°F, a layer of ice forms beneath the skin (including the epidermis and hypodermis) and separates it from the cortex. This damage heals readily. At 27°F, damage extends to the base of the style in some flowers after thawing. The damage appears as a brown discoloration, is fatal, and may extend to the placenta and ovules. If the blossom temperature falls to 25 or 26°F, the damage becomes widespread and the crop is reduced. Rogers stated that loss of less than 50 percent of the blossoms does not usually affect the crop. Simons and Lott (7) found that freeze damage 1 month after full bloom caused anomalous fruit development and the presence of large areas of dead cells in fruits which persisted to maturity.

Fruit injury has resulted in frost bands and internal lesions affecting marketability (1, 4, 6, 7). The temperature has been quite specific to these tissues at a critical growth period. An example of specificity of tissue injury has been reported (6) in which the sepal and petal bundles in the outer cortical region were the main sites of injury. Watanabe (9) discussed early frost injury as a cause of russet in apples.

This study describes the effect of frost injury at anthesis upon subsequent development of the 'York Imperial' apple. It illustrates the fruit tissue injured at bloom time and during the stages of fruit development as healing occurs.

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Materials and Methods

Frost-injured fruit samples of the 'York Imperial' apple were collected at the Fruit Research Laboratory, Arendtsville, Pa. during the 1968 season from plots having a known history of corking disorders. Approximately 10 fruits were sampled for anatomical studies at weekly intervals from full bloom through the first stages of fruit development. Full bloom occurred on May 7, with the following temperatures recorded: May 6, 35°F; May 7, 32°F; and May 8, 35°F. Slight injury was observed. The fruits were collected and preserved in a standard formalin-acetic acid-alcohol killing and fixing solution (FAA). Tissues were dehydrated and prepared for embedding using a graded alcohol series through xylol. The samples were then embedded in 'Tissuemat' with a melting point of 52.5°C. Sections were cut on a rotary microtome at 12μ and stained with safranin and fast green.

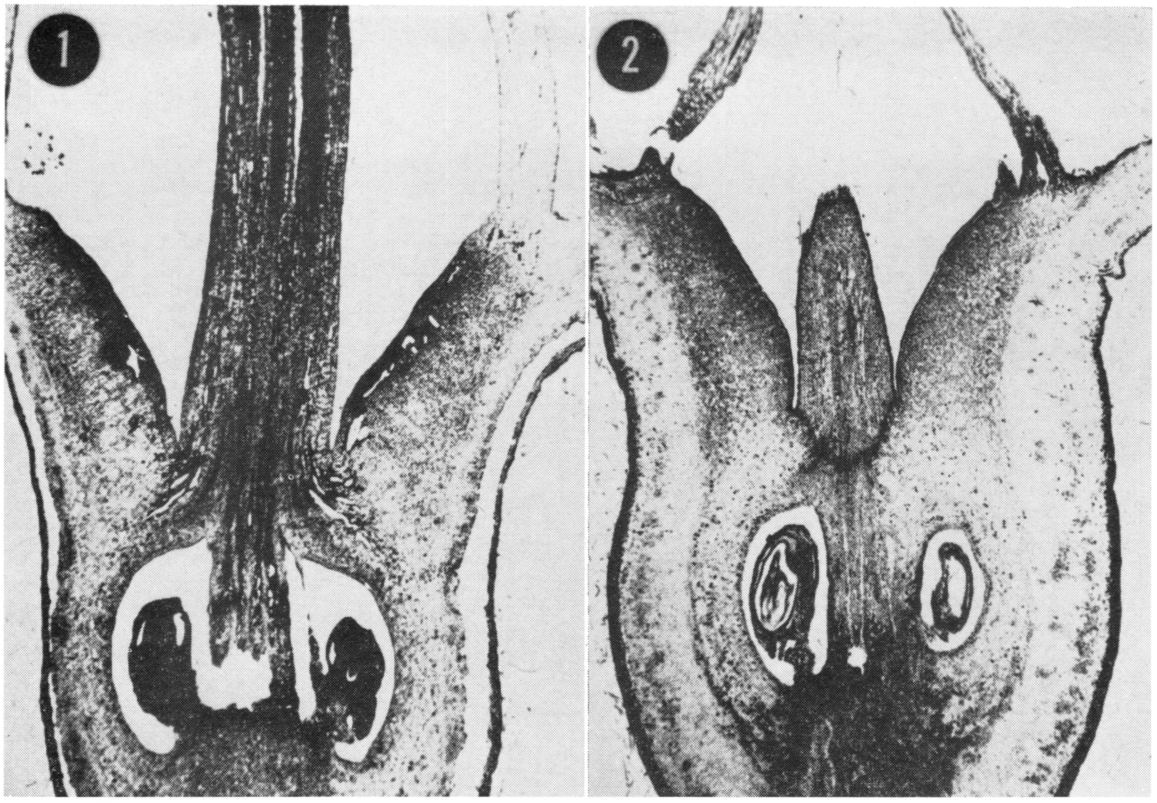
Reference was made to Tukey and Young (8) for fruit terminology and to Esau (3) for anatomical terms. The terms fruit apex and basin have been used interchangeably, and the cavity region or area designates the area of fruit pedicel attachment. "King" fruit denotes the first developed apple within the cluster.

Results

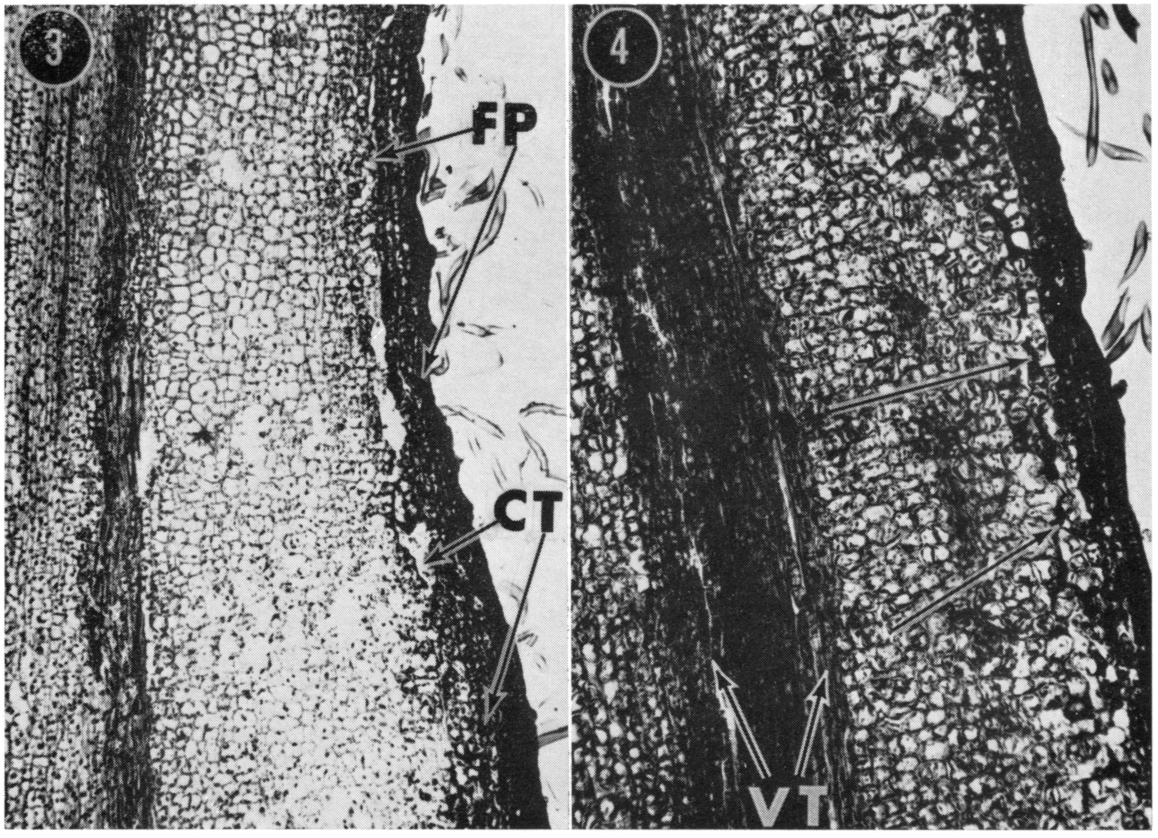
Longitudinal sections of the "king" fruit (Fig. 1) revealed that the outer protective epidermis and hypodermis had separated from the outer cortex. Tissue disruption was observed throughout the basin areas of the fruit and especially at the base of the style. Injury in which the outer protective region has separated from the outer cortex is commonly referred to as "slip skin". This injury could not be detected by macroscopic observations.

A week later the epidermis and hypodermis of uninjured developing fruit were intact with the outer cortical region (Fig. 2). The earlier blossoms seemed to sustain more severe injury, while some of the stronger, late blooms were uninjured. The fruit in Fig. 2 had increased in size, and the outer cortical region and the tissues within the core line had progressed at an equal rate. The sepals and petals had abscised although remnants were still evident. The abscission zone at the base of the style was apparent.

The junction of the outer fruit cortex to the fruit pedicel is shown by an arrow in Fig. 3, in which the tissues have been separated by normal growth. Although similar in appearance this is not the result of frost injury. As the fruit enlarged, these cortical tissues developed into the cavity region. It has been observed in other apple cultivars that the cavity area will be intact, al-



Figs. 1–2. Longitudinal sections of the "king" fruit. *Fig. 1.* Stage of development at full bloom with initial injury. The epidermis and hypodermis have separated from the outer cortex with injury appearing in the fruit apex. *Fig. 2.* Uninjured fruit development 7 days after full bloom with the outer cortex and hypodermis united. Both X 16.

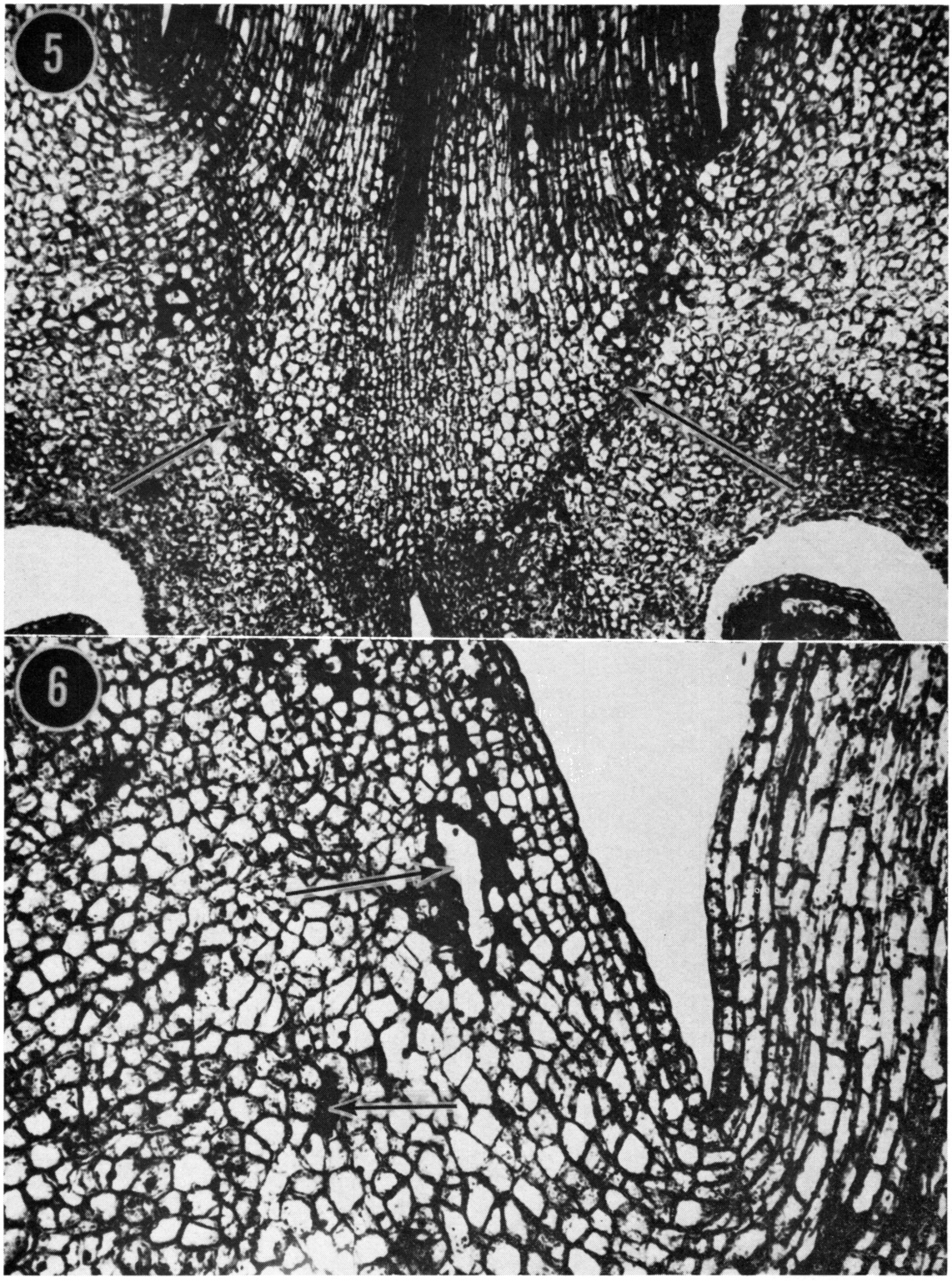


Figs. 3–4. Longitudinal sections showing tissue development 7 days after full bloom. *Fig. 3.* Fruit tissue separation (arrows) where the cortical tissues (CT) are united with the fruit pedicel (FP). At maturity this area will become the cavity. *Fig. 4.* Tissue suberization (arrows) beneath the hypodermis in the equatorial axis of the fruit. VT indicates vascular tissue in the core line. 3, X 63; 4, X 160.

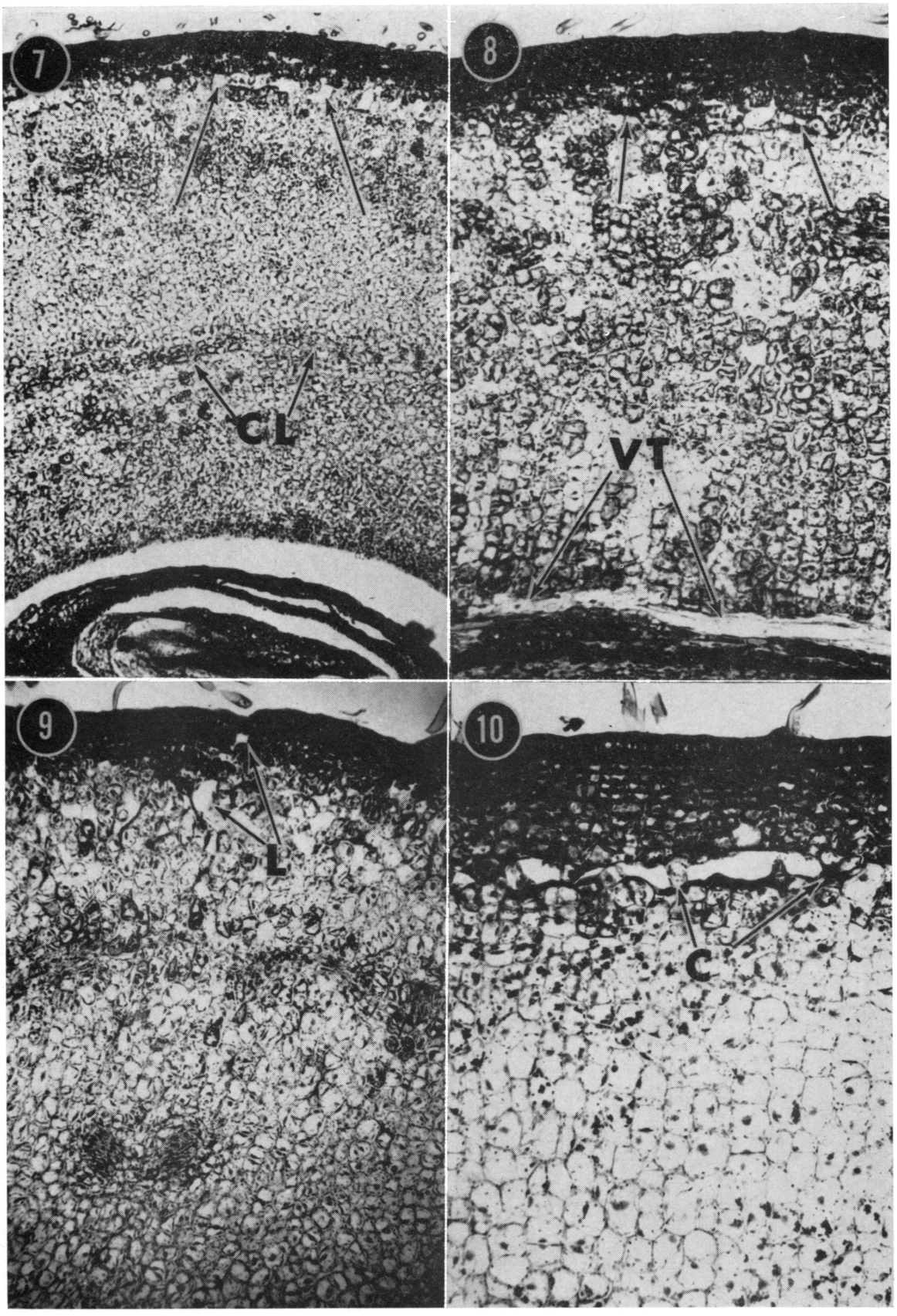
though the cellular structure has changed markedly in that the hypodermal layers have become distinct at the point of fruit tissue and fruit pedicel juncture.

Figure 4 shows tissue suberization, 1 week after full bloom, where the area of injury on the fruit surface occurred near the equatorial axis. Necrosis was apparent just beneath the last formed hypodermal layer. Division of cortical cells resulted in enlargement of the fruit. Anticlinal cell division in the epidermis had occurred, with subsequent stretching to produce a periclinal arrangement in orientation. The vascular tissues of the sepal and petal bundles (VT) in the core line were uninjured.

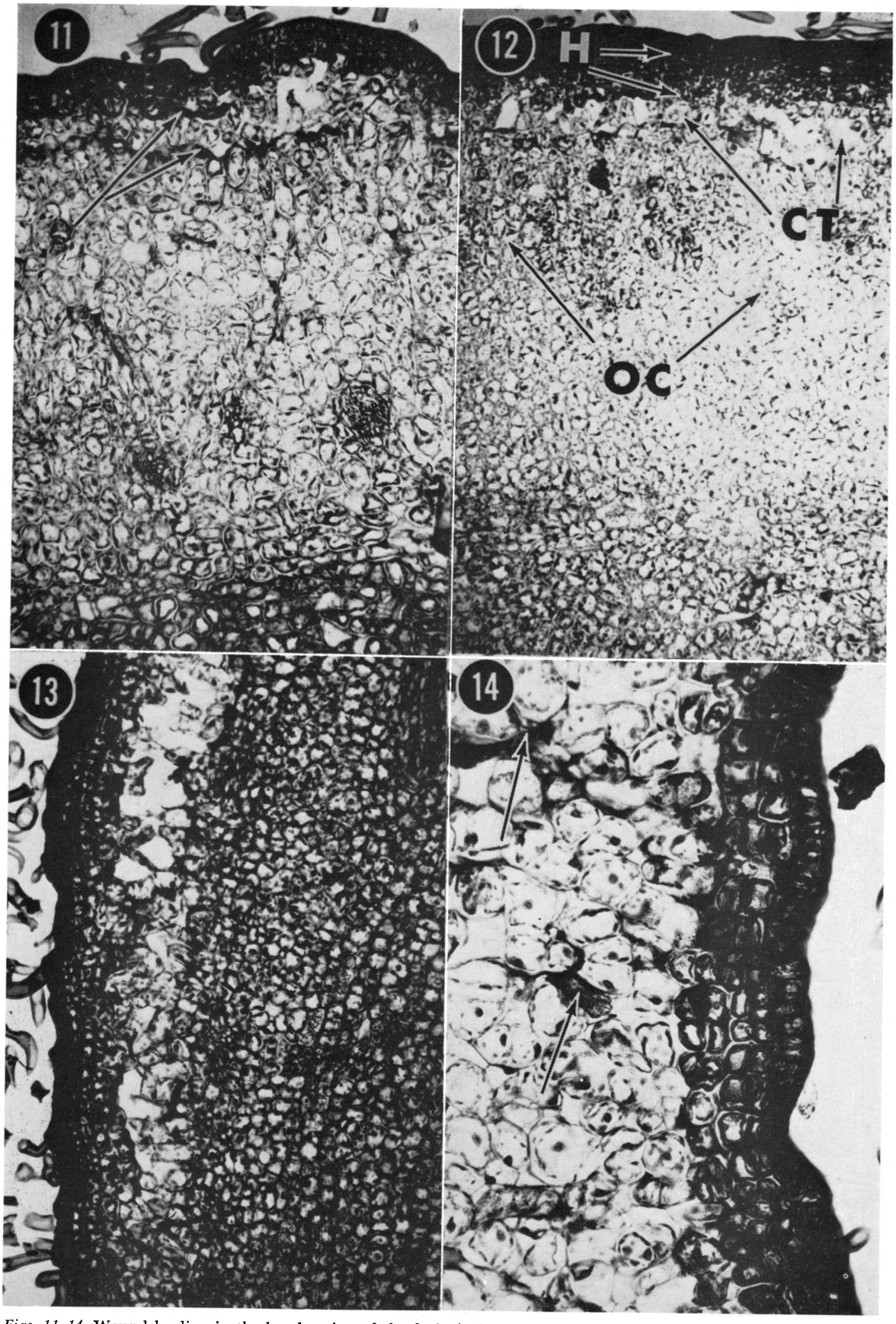
Abnormal tissues in abortive fruit with abnormal



Figs. 5–6. Tissue development in the fruit apex 7 days after full bloom showing injury. *Fig. 5.* Abscission zone (arrows) at the base of the style cutting through the placental tissue to the ovarian locules. The ovules have already aborted. *Fig. 6.* Necrotic tissue (arrows) at the base of the style and continuing into the cortical cells of the fruit apex. Note the large lesion caused by injury. 5, X 63; 6, X 128.



Figs. 7–10. Fruit tissue 7 days after injury. *Fig. 7.* Tissues of the equatorial axis showing relatively little injury extending from the epidermis to the ovules. Arrows in the outer cortex indicate wound healing and CL the core line. *Fig. 8.* Vascular tissue (VT) in the sepallary and petallary bundles are developing and changing in orientation with the inner cortical cells. Arrows indicate necrosis in the outer cortex. *Fig. 9.* Tissue disruption in the equatorial axis with large lacunae (L) beneath the epidermis and outer cortex. Note necrotic tissue (arrows) in this area. *Fig. 10.* A distinct wound injury in the outer cortex at the junction with the hypodermis. Some callus tissue (C) has formed uniting the 2 sides of the wound. 7, X 80; 8, 9, X 100; 10, X 160.



Figs. 11–14. Wound healing in the basal region of the fruit, 7 days after injury. *Fig. 11.* Two layers of necrotic cells (arrows) resulting in uneven growth of the epidermis. *Fig. 12.* Callus tissue (CT) in response to wound healing and uniting outer cortex (OC) with inner hypodermal (H) layers. *Fig. 13.* Callus tissue uniting the hypodermal and cortical cells with much of the injury completely healed. *Fig. 14.* Small areas of necrotic tissue (arrows) and irregular epidermal surface. 11, X 160; 12, 13, X 128; 14, X 250.

ovules are shown in Figs. 5 and 6, 1 week after full bloom. An abscission zone was apparent at the base of the style and extended to the ovarian locules, as indicated by arrows in Fig. 5. The placental tissue adjacent to the ovarian locules had scattered necrotic areas. Necrosis also was apparent in the basin region at the base of the style. Large cavities were surrounded by necrotic tissue and, in many cases, small isolated spots of necrosis were found throughout the basin region (Fig. 6).

Wound healing of the cortical tissues, 1 week after full bloom, within the outer protective region is shown in Figs. 7–10. In Fig. 7 the core line (CL), indicated by arrows, and the major portion of the cortex were uninjured. Tissue healing adjacent to the inner hypodermis had started, with the separated tissues uniting the larger lacunae. Little necrotic tissue was evident in this area. The thick hypodermal region (Fig. 8) shows necrosis in the inner layers and extends through the cortex to the vascular tissues in the core line. However, the other tissues appeared normal. It is apparent that cell orientation between the inner cortical tissues and the core line had been changed and new layers of cells were formed that became part of the vascular tissue network.

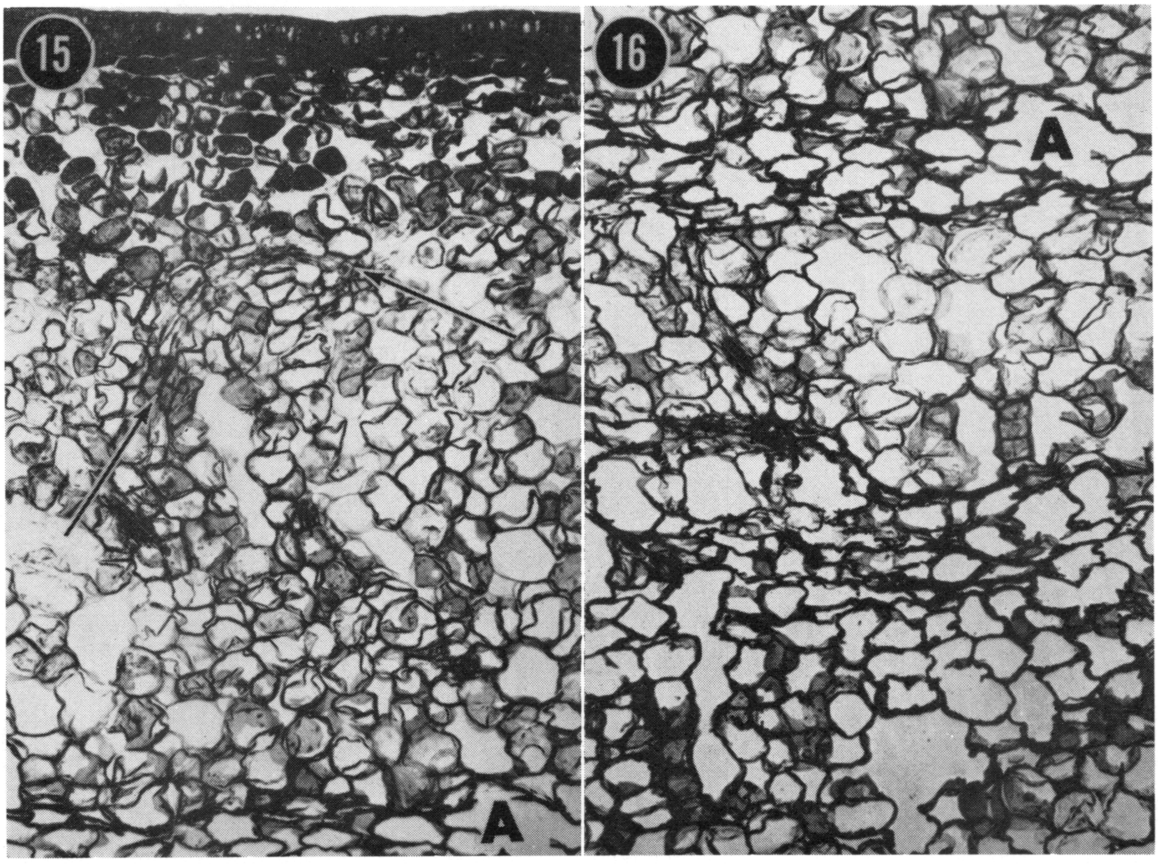
A rough fruit surface was evident in Fig. 9 and large lacunae were evident just beneath the epidermal tissue. Disrupted tissues were adjacent to this area. Some small necrotic spots were evident throughout the outer protective region. The injury was slight and wound healing had already progressed at this time. A distinct breakage of tissues is shown in Fig. 10 between the outer cortical region and the inner hypodermis. Areas of collapsed tissues were united with small callus-like cells. Approximately 8 hypodermal layers were present. The injury persisted along the inner layer adjoining the outer cortex. The interior portion of this large cavity was lined with suberized tissues. The stage of fruit development is indicated by nucleated cortical cells.

Tissue disruption in the basal portion of the fruit is shown in Figs. 11–14. The tissues of this region are particularly susceptible to freeze injury. Although the injured area had become stratified with necrotic tissue, it surrounded dividing cells which had formed proliferated tissues that would unite the 2 sides of the wound. This internal injury beneath the hypodermis was evident and an abnormal, rough epidermal surface was apparent (Figs. 12, 13).

There was scattered necrosis in cortical tissues, indicated by arrows in Figs. 11 and 14. Some cells or groups of cells had completely collapsed while, in others, there was only a slight injury to the cell walls.

Twenty-one days after injury, tissue destruction was apparent throughout the outer equatorial axis and extended deep into the cortical region (Figs. 15, 16).

Figs. 15 and 16 (A) show the extension of the same injured tissues deep into the cortex, (A) being the same in each photomicrograph. Growth distortions in the cortical cells were evident, with changes in orientation around necrotic tissues and large lacunae. Arrows indicate areas which, in older fruit, were found to be sensitive to frost and to the initiation of corking. Some of the vascular bundles had become necrotic. As growth progresses and the fruit enlarges, it will either develop abnormally in shape or abscis. Injured areas such as



Figs. 15–16. Injury in the outer cortical region 21 days after injury extending deep into the cortex. *Fig. 15.* Equatorial axis exterior to injured cortical cells as shown in *Fig. 16.* Area in *Fig. 15(A)* corresponds to *16(A).* This injury has penetrated deep into the cortex. Cell orientation has changed and large lacunae are present. Both X 100.

this develop proliferated tissue characteristic of callus in fruits that survive.

Fruit tissues in the outer cortex at bloom time are particularly susceptible to frost injury. Although injury was slight, the outer cortex separated from the hypodermis and subsequent healing was accomplished by the formation of callus tissue which engulfed the wound area. As the healing of these wounds progressed, a rough epidermal surface developed with pronounced lenticels.

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Comparison of Factors Influencing Fruit Size in Large-Fruited and Small-Fruited Clones of Strawberry[1]

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Abstract. The relative decline in fruit size from primary to secondary to tertiary positions on the inflorescence of large-fruited clones was much greater than for small-fruited clones. Large-fruited clones produced fruit with more achenes and larger achenes than did small-fruited clones. Fruit weight was positively correlated with total achenes per fruit, developed achenes per fruit, mean weight of total and developed achenes, and fruit weight per developed achene. These results lead to the conclusion that fruit size differences among strawberry clones are due to the combined effects of developed achene number, developed achene size, differential activity of achenes in producing growth hormones and differential sensitivity of receptacular tissue in responding to growth hormones.

FRUIT size is one of the most important characters for evaluating or developing strawberry cultivars. Large fruit size is especially important in merchandising fresh-market strawberries, and is also a significant factor in reducing harvest costs. Most strawberry breeding programs have large fruit size as one of their major objectives.

Considerable variability exists in fruit size among strawberry cultivars. Large size differences also exist within individual inflorescences of a cultivar, depending on fruit position on the inflorescence (1, 3, 5, 10, 11). There is a marked decrease in fruit size with each inferior blossom position on the inflorescence. Sherman and Janick (10) reported that the relative decline in size of fruits from the primary berry to inferior flower positions on the inflorescence was approximately the same in all cultivars. Janick and Marshall (6) found that the rate of decrease in fruit size in later pickings was similar for the 9 cultivars studied. Valleau (11), however, presented data which showed that fruit size of inferior positions, calculated as percent of primary size, ranged from 63 to 90% for secondary berries, 35 to 55% for tertiaries, and 25 to 40% for quaternary berries in 3 genetic clones. Valleau's data also showed that the greatest percent decrease in berry size occurred in the clone with the largest primary berry and the least percent decrease in inferior positions occurred in the clone with smallest primary fruit size. Darrow (1) found that the relative size decrease of fruits in inferior inflorescence positions varied greatly among genetic clones and was related to type of inflorescence branching.

Fruit size, number of achenes, and position of the fruit on the inflorescence are closely related within a cultivar (3, 5, 7, 11). Both fruit size and achene number show a decrease with lower flower positions (5, 11). That fruit enlargement in the strawberry is dependent upon hormones produced by the achenes was clearly shown by Nitsch (9).

This study investigates the factors influencing fruit size in large-fruited and small-fruited clones of strawberry.

MATERIALS AND METHODS

The two largest-fruited ('NC 2840', 'Md-US 3082') and the two smallest-fruited ('Blakemore', 'Md-US 3365') genetic clones in a test planting of 57 cultivars and selections were selected for study. Each clone was replicated 5 times in a randomized block design.

During fruiting in 1969, 5 fruits from each of 3 flower positions (primary, secondary, tertiary) were collected at random from each replication of each clone as they matured. The fruits were labeled, placed in plastic bags, and stored in a freezer.

In October, each fruit was weighed and volume measured as amount of water displacement. Individual fruits were macerated in a laboratory food blender. Achenes that floated in water were considered undeveloped and achenes that sank were considered developed (8). Both types of achenes were collected, dried, counted, and weighed.

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