Tissue Response of Young Developing Apple Fruits to Freeze Injury[1]

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Abstract. Morphological and anatomical development of young fruit were studied in relation to the effects of a late spring freeze. 'Lodi' and 'Duchess' apples were frozen 16 days after full bloom. Samples were collected for anatomical study on 4 subsequent dates following the freeze. The secondary vascular tissues in the outer cortex were affected, resulting in lesions in those tissues where adjacent parenchyma cells had collapsed. The main vascular strands in the outer cortex were killed, the injury extending from fruit base to apex. Separation of the hypodermis from the outer cortex occurred in the fruit apex where tissues were injured most severely. Also injuries were evident contiguous to the core line. Internal phellogen, phellem, and phelloderm in the parenchyma had formed within 18 days after injury. In some cases, cell proliferation and dedifferentiation of cortical parenchyma was noted 46 days after injury.

Introduction

Young developing apples are often subjected in many areas to freezing temperatures late in the spring season. Tissues of young fruits have been studied in their responses to injury and their ability to recover.

Groves (6) reported freeze damage to young apples that were a half-inch in diameter. A large number of frozen fruit dropped from the tree, but many remained and developed to maturity. The regions of killed cells, apparent as light brown areas, were rendered obscure by subsequent growth of uninjured cells. Fruits were often deformed as a result of the internal injury, although there was no frost russet on the fruit. Gardner (4) states that frost occurring after the time of fruit set may occasionally arrest the further development of seeds and still permit the fleshy tissues to develop and mature, giving rise to fruits abnormal in size and shape.

Rogers (8) has found that the tissues commonly supercool to about 28 or 29°F before any ice formation takes place. If supercooling persists and no ice has formed, the plant is endangered, even by severe frost. At full bloom in the 'Cox's Orange Pippin', a temperature of 28° resulted in formation of a layer of ice beneath the skin (including the epidermis and hypodermis) which was thereby lifted from the cortex. This damage heals readily. At 27°, damage occurs at the base of the style. This damage appears as brown discoloration after thawing, may spread to the placenta and ovules, and is often fatal. If the temperature falls to 25 or 26°, the damage becomes widespread and the crop is reduced. Rogers also found that most apples are more susceptible to frost in the fruitlet stage than at full bloom.

Freezing of the fruit has many implications for its development to maturity and for its susceptibility to storage disorders. In instances where the injury is not severe, growth will continue. This may be one source of the physiological disorders found in apples such as watercore and cork spot. Abnormal development of mature apples injured by a late spring freeze has been discussed in a previous report (12). Another type of freeze damage already reported is the occurrence of superficial injuries, i.e. frost rings and the sequential development of injured tissues (9, 11).

This study recorded the effects of a late spring freeze on the morphological and anatomical development of very young fruit in its early stages of development.

Materials and Methods

A freeze in the early morning of April 24, 1967 in western Illinois, 16 days after full bloom for 'Lodi' and 'Duchess' apples, caused severe damage to fruit tissues. The lowest temperature for the area was 27°F.

In each sampling date, 12 representative injured fruits were collected for anatomical study on April 28, May 12, May 23, and June 9. Additional samples were made for the X-Sections of the fruit base and apex. Approximately 100 fruits were observed in obtaining each morphological observation for the 2 cultivars. These morphological observations were made 4 and 46 days respectively for 'Lodi' and 29 days after injury for 'Duchess'. These observations were recorded as growth anomalies appeared macroscopically.

Anatomical studies were aimed toward determination of specific tissue responses to be found within the various parts of the apple. Specimens of injured tissue were preserved immediately in a standard formalin-acetic acid-alcohol killing and fixing solution. After a brief aspiration they were carried through an alcohol-xylol dehydration series and then infiltrated and embedded in par-

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affin. Longitudinal and transverse sections were cut 12μ thick and stained with 50% safranin as described by Johansen (7). Photomicrographs were prepared to illustrate tissue injury and response in the fruit of cultivars 'Lodi' and 'Duchess'. Injury in the young developing 'Lodi' fruit is recorded in Fig. 1–22 and Fig. 23–30 for 'Duchess'.

Results and Discussion

'LODI'

Morphological characteristics.

Four days after injury (20 days after full bloom), macroscopic observations showed that injury to the 'Lodi' apple was not evident at this time, and there was no evidence of tissue damage to the outer protective region. However, the small fruit (1.1 cm across the equatorial axis) was severely injured in the basin region with dark, brown streaks occurring along the sepallary and petallary bundles. These injured areas extended into the very prominent calyx lobes and much of the tissue underlying the base of the calyx lobes was killed. These injured areas continued from the base of the calyx lobes to the region of greatest transverse diameter or the equatorial axis of the fruit which averaged .8 cm. Another type of typical injury was a distinct brown line separating the carpellary and cortical tissues which would indicate extreme sensitivity to low temperatures in this area.

Forty-six days after injury (52 days after full bloom), lesions were in evidence under the calyx lobes and extended to the greatest transverse diameter of the fruit with the sepallary and petallary bundles killed. Browning of the carpellary tissues was present and breaks in the outer cortical tissues were observed macroscopically. The fruit base continued to enlarge with apparent depression of the apex by unequal growth of the tissues. This resulted in an abnormal shape that would not be characteristic of the cultivar.

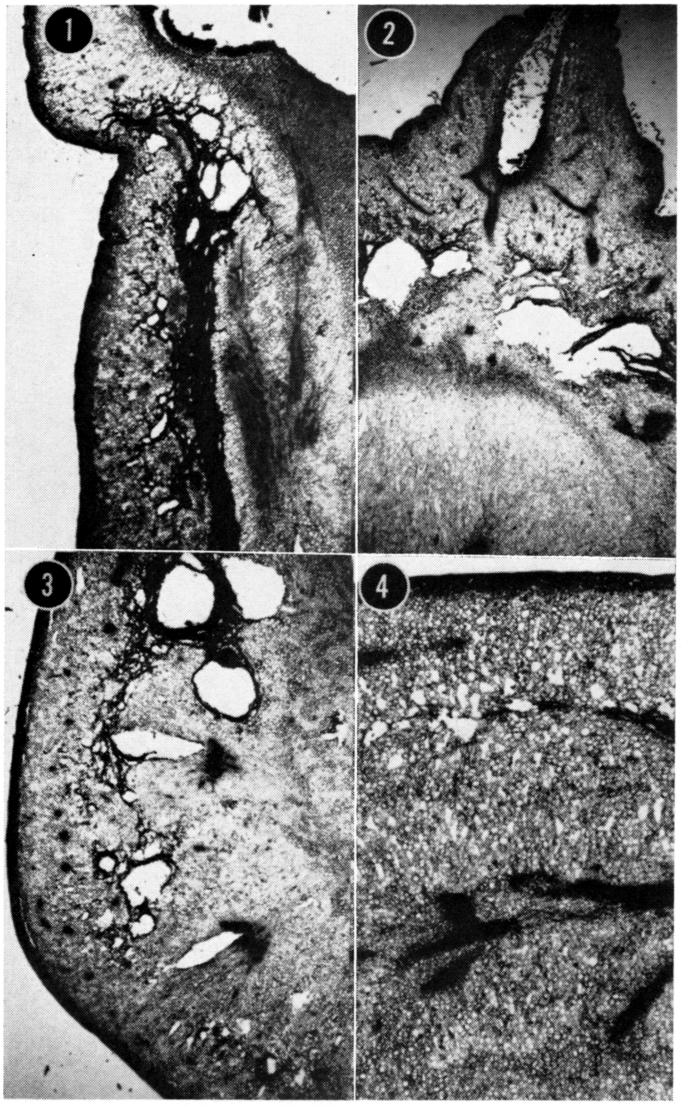

Fig. 1–4. Longitudinal (Fig. 1 and 2), and X-Sect. (Fig. 3) and transverse section (Fig. 4) of a 'Lodi' apple 4 days after freeze injury. Note vascular tissue injury in the sepallary and petallary bundles extending to the apex (Fig. 1), and the tissue destruction extending from the core line outward to the calyx lobes (Fig. 2). Fig. 3. X-Sect. of injury extending from the dorsal carpellary bundles outward through the cortex, with separation occurring between the hypodermis and the outer cortex. Fig. 4. Tissue response 18 days after injury with some cell proliferation occurring in the wounded areas of the outer cortex. Fig. 1, × 13.6; Fig. 2 and 3, × 11.1; Fig. 4, × 13.6.

Anatomical characteristics.

Four days after the freeze, damage was prevalent in the secondary vascular tissues in the outer cortex (Fig. 1–3). This damage extended from the fruit apex to base. The main vascular strand in the outer cortex was killed (Fig. 1) with the secondary strands resulting in large holes in the tissue where the adjacent parenchyma cells had collapsed. This same pattern of injury was observed in the vascular bundles in the outer cortex. Separation of the hypodermis from the outer cortex occurred in this fruit. Small isolated lesions in the tissue were prevalent throughout the outer cortex.

The fruit apex was the most severely damaged (Fig. 2). Injury in this area was intense and continuous to the core line. Since these fruits were injured in the outer protective region (epidermis, hypodermis and outer cortex) many of them continued to grow and develop shapes uncharacteristic of the cultivar. Similar results have been previously reported (12).

Eighteen days after injury, the fruit had increased in

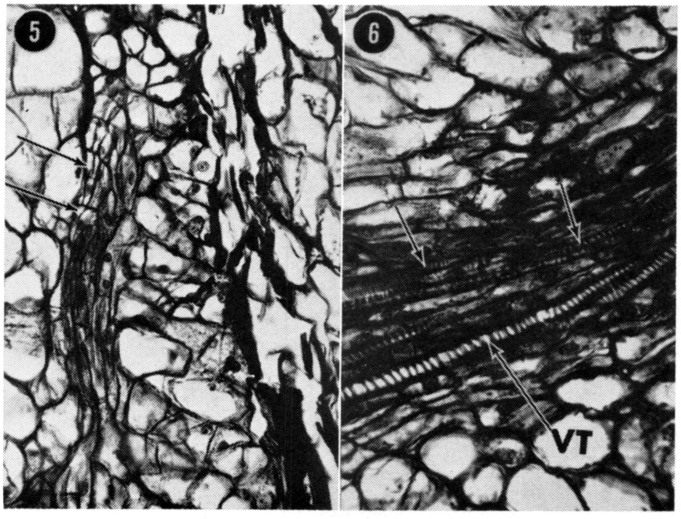

Fig. 5, 6. Cortical tissue response in the 'Lodi' apple 18 days after freeze injury. Fig. 5. Meristematic activity of cortical tissues in response to injury showing nucleated cells forming a phellogen layer several cells away from the injury. Arrows indicate the area of phellogen origin. Fig. 6. Dedifferentiation of cortical parenchyma into vascular tissues (VT) as a result of contiguous injury. Arrows at top indicate developing vascular tissue. Fig. 5, × 190; Fig, 6, × 305.

diameter, with large air spaces occurring adjacent to the vascular tissues in the outer cortex (Fig. 4).

The internal wound meristem of the injured parenchyma tissues is recorded in Fig. 5, 18 days after injury. That fruit parenchyma tissue continues to develop regardless of injury has been demonstrated at various stages of fruit development (11, 12). However, in the present study, cell death resulted in the formation of an internal wound meristem several cells away from the original injury (Fig. 5). Although this layer of phellogen had formed in response to the original injury, other layers would probably have formed as the fruit continued to grow. Cells located between the phellogen layers and the injured tissue were all nucleated and meristematic. However, those located beyond this point in the cortex were normal parenchyma cells. It appears that formation of the phellogen tissue began at the upper left of Fig. 5 (indicated by arrows) where the parenchyma tissue had partially collapsed in a narrow band and some cells between the injuries became meristematic thus producing a phellogen contiguous to the more intensely injured areas and indicating a greater meristematic activity in response to injuries at this point.

Dedifferentiation of cortical parenchyma to vascular tissue occurred in some damaged areas (Fig. 6). Slight injury of the parenchyma tissue resulted in the random formation of vascular strands through otherwise normally developing tissue. Pre-existing vascular tissues were in evidence as indicated by (VT) at the bottom of Fig. 6. However, redifferentiation has produced fragments of vascular tissue as indicated by arrows. Cell wall thickening was apparent throughout the tissues in this area.

Freeze damage of the outer protective region of the fruit resulted in only a slight discoloration of tissues. However, injury between the outer cortex and the hypodermis was extreme, with complete tissue separation (Fig. 7). At this point, the hypodermis was well defined, with cell division in the epidermis changing from radial to tangential and thus coinciding with fruit enlargement. Further instances of localized injury were observed in the lenticel 18 days after injury, with formation of an internal phellogen progressing through the hypodermis into the outer cortex (Fig. 8). Though the epidermal and hypodermal tissues were disrupted, the cuticle remained intact except for the lenticel opening. The cortical tissue adjacent to the wound meristem appeared normal.

Tissue development 46 days after injury is shown in Fig. 9–22. Injury of the vascular tissues in the outer cortex is shown in Fig. 9 and 10. Fruit diameter had increased by this time and large longitudinal splits in the injured areas of the outer cortex had appeared. An enlargement of typical injury from the equatorial axis to the fruit apex is shown in Fig. 10. Tissue orientation on

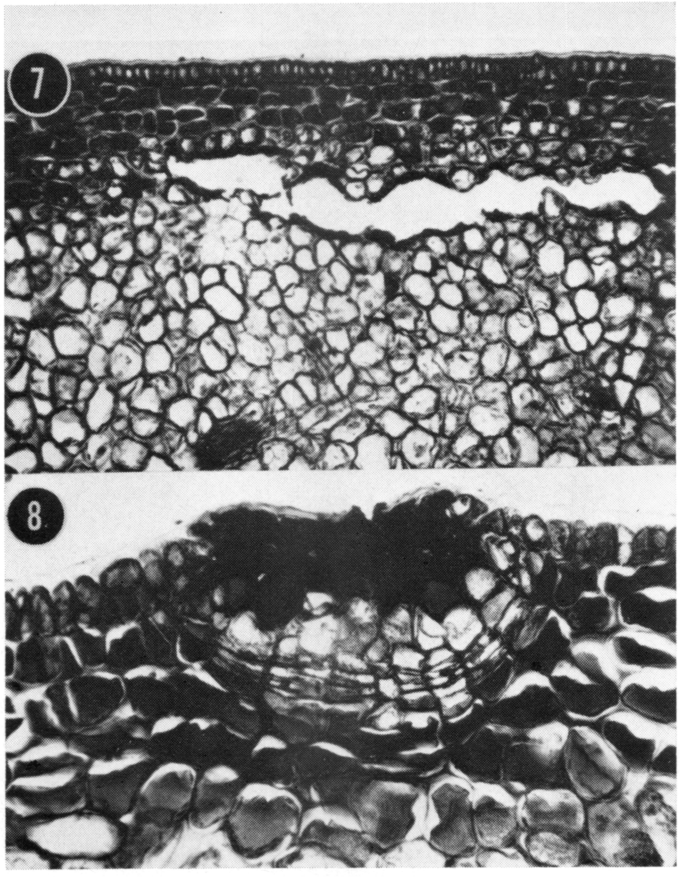

Fig. 7, 8. Transverse section of 'Lodi' apple. Fig. 7. Separation between the outer cortex and the hypodermis 4 days after injury. Fig. 8. Epidermal tissue response 18 days after injury showing phellem, phellogen and phelloderm layers with cuticle remaining partially intact over the surface. Fig. 7, × 65; Fig. 8, × 127.

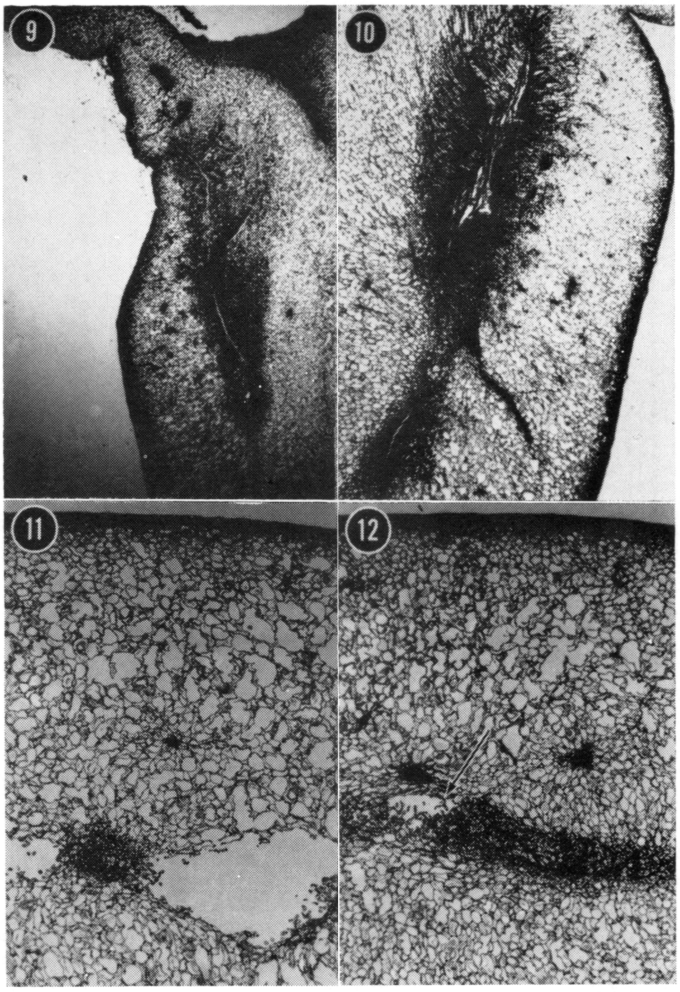

Fig. 9–12. Recovery response in the 'Lodi' apple 46 days after injury. Fig. 9–10. Longitudinal sections showing healing of the internal wounds may be compared with Fig. 1–3, 4 days after injury. Fig. 11–12. Cell proliferation is apparent throughout the injured areas. Note the proximity of the injury to the vascular bundles (indicated by arrow). Fig. 9, × 7.8; Fig. 10, × 10; Fig. 11, 12, × 12.5.

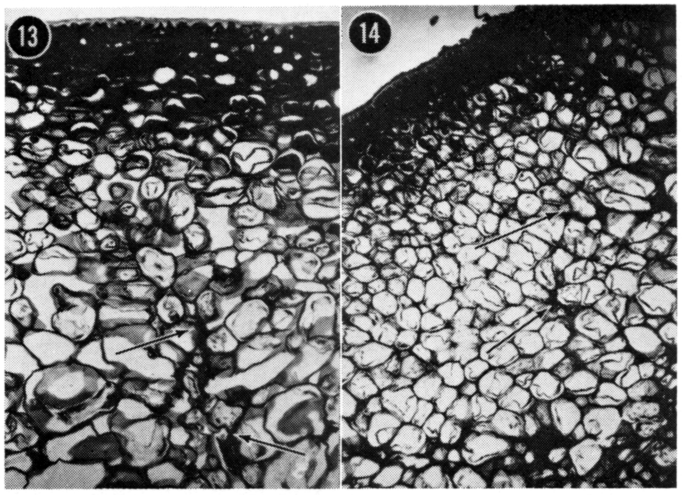

Fig. 13, 14. Tissue response 46 days after injury showing development of slight necrosis in isolated areas throughout the cortex (indicated by arrows). Fig. 13. The equatorial region of the fruit. Fig. 14. The fruit apex. Fig. 13, 14, × 77.3.

both sides of the wound was evident, with meristematic activity appearing contiguous to these areas.

Early stages of cell proliferation and callus formation were evident in injured areas of the outer cortex extending from the equatorial axis to the fruit apex (Fig. 11, 12). The split regions show chains of callus cells extending into the cracks. Outward to the epidermis were both isolated and concentrated areas of collapsed cells. The vascular bundles were disrupted, producing large areas of cell disintegration, Fig. 11 and 12. The small injured area, as indicated by an arrow in Fig. 12, may become completely filled with proliferated tissue at fruit maturity.

Localized injury as evidenced by occasional necrotic cells, appeared from the equatorial axis (Fig. 13) to the fruit apex (Fig. 14). Isolated cells or groups of cells in the outer cortex of the equatorial axis were observed 46 days after injury (Fig. 13). Although no internal phellogen had formed at this time, one would expect to find more severe injury and erratic tissue response in underlying tissue. Isolated necrotic tissue and associated proliferated callus tissues have formed in small areas with little meristematic activity around the wound. The apex of a less severely injured fruit is shown in Fig. 14. There are isolated injured spots over the entire area, extending from the epidermis deep into the cortex.

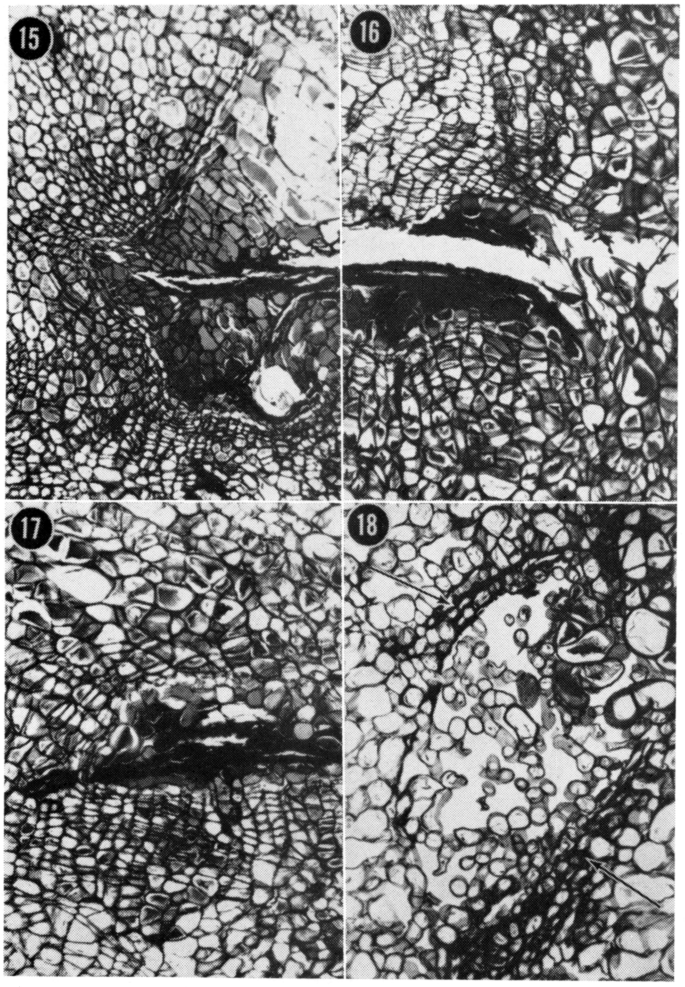

Fig. 15–18. Varied responses of cortical tissue in the 'Lodi' apple 46 days after injury. Fig. 15, 16 and 17 show extreme meristematic activity, with formation of large areas of phellem and a distinct phellogen layer. Cortical cells a great distance away from the original injury have been affected. Fig. 18. Cell proliferation and callus tissue between two separate layers of injury (indicated by arrows). Fig. 15, × 46.4; Fig. 16, 17, 18, × 77.3.

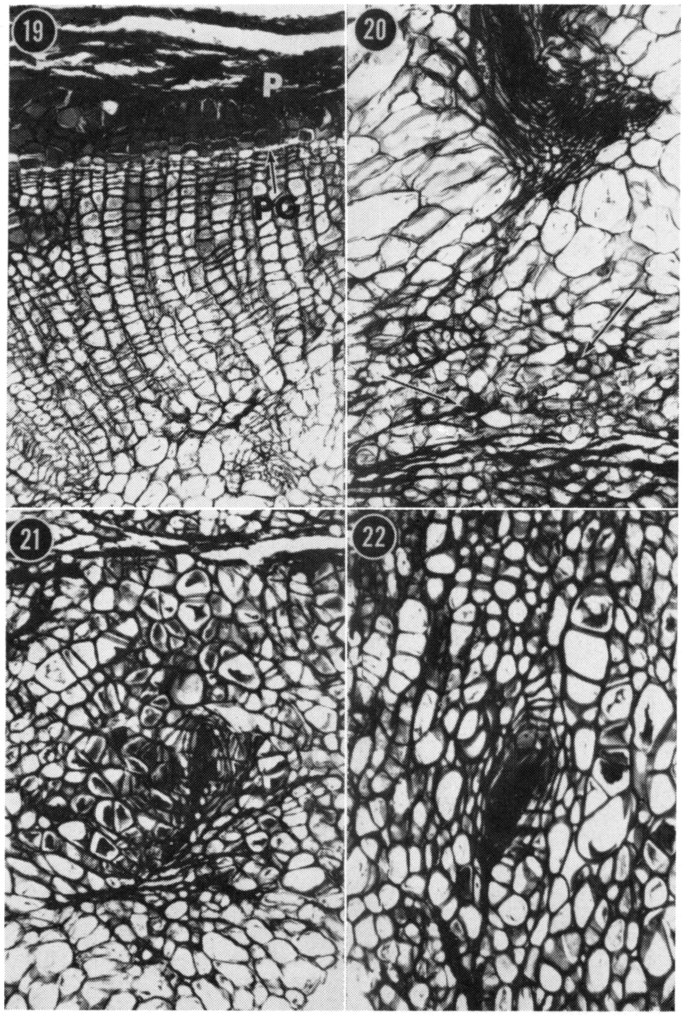

Fig. 19–22. Varied responses in cortical tissue of the 'Lodi' apple 46 days after injury. Fig. 19. Large internal wound with phellem (P) a distinct layer of phellogen (Pg) and phelloderm, and a continued response to the injury by the formation of serial rows of parenchyma cells. Fig. 20. Injured tissues at edge of vascular tissue (indicated by arrows). Fig. 21, 22. Isolated areas of necrotic tissue with surrounding cortical cells, orientating in response to the injury. All figures × 77.3.

Various patterns of internal wound meristem occurring in the fruit apex 46 days after injury are shown in Fig. 15–22.

Phellogen development was active during this interval after injury. Cell activity near the phellem decreased while intense meristematic activity continued beyond the phellogen layer with a serial alignment in the meristem zone (Fig. 15, 16, 17). The phellogen did not develop periclinally to the injury. In many cases it formed in a 45° angle away from the injury. This may be explained by the variation in severity of cell injury. In some fruits several layers of phellem developed (Fig. 15, 16, 17). It would appear that the greater the formation of phellem, the further away from the injury the meristematic activity continued. Illustrations of this separation of phellem 46 days after injury, with large lesions (Fig. 15–18) show the changes since the samples of injured fruit 4 days after the freeze (Fig. 1–3). Although growth may have compressed these tissues, they will never come together. Fig. 18 shows development of less severely injured tissues than those illustrated in Fig. 15, 16 and 17, and the beginning of cell proliferation and callus formation in response to injury at two locations (indicated by arrows). Some cell collapse, suberization and death was noted.

A large area of parenchyma tissue influenced by injury is shown in Fig. 19. A wide band of phellem was formed, with contiguous cell layers dividing in radial rows. These in turn formed a newly active phellogen layer. As growth progresses, observations have shown that new layers of phellogen will continue to form deeper into these parenchyma tissues producing new layers of phellem. The wound tissue was scattered throughout this area, but the greatest influence upon the radial division occurred from the most severely injured area, as shown at the top of Fig. 19.

The proximity of injury to the vascular bundles is shown in Fig. 20. Scattered layers of suberized cells were found throughout the parenchyma tissue adjacent to vascular strands. This injury was evident in some thin-walled cells adjacent to the bundle.

Localized tissue response has been shown in Fig. 21 and 22. Small areas deep within the cortex have been injured, but large areas around the injury show changes of cell orientation with absence of meristematic activity in the adjoining cells. Such injuries as these will be a deterrent factor in producing high-quality fruit at harvest time because of the presence of internal cork tissues.

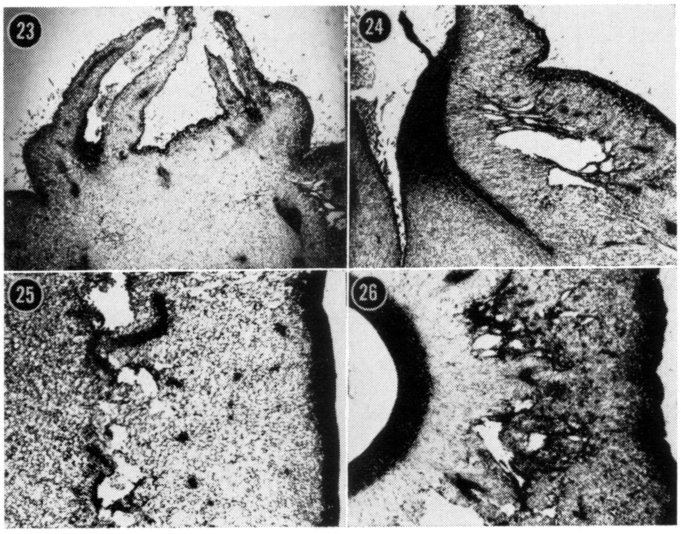

Fig. 23–26. Tissue injury to the apex of 'Duchess' apples. Fig. 23, 24 (longitudinal sect.) and 26 (transverse sect.) show fruit development 18 days after injury. Fig. 25. Transverse section 28 days after injury. Fig. 23, × 6; Fig. 24, × 7.7; Fig. 25, × 9.6; Fig. 26, × 6.

'Duchess'

Morphological characteristics.

Twenty-nine days after injury, ridges extended from fruit base to apex with some longitudinal cracking present. The fruit was hypertrophied at the apex, with prominent calyx lobes. There was a heavy first drop of fruit containing dead ovules. Such fruit was abnormally dark red. Injured fruits were beginning to develop into 'flat' apples uncharacteristic of the cultivar. Ruptured or cracked areas were present close to the pedicel at the fruit base and on the equatorial axis. Some of the outer protective region at the fruit apex had been ruptured and subsequently healed. Internal cavities were present in the cortex.

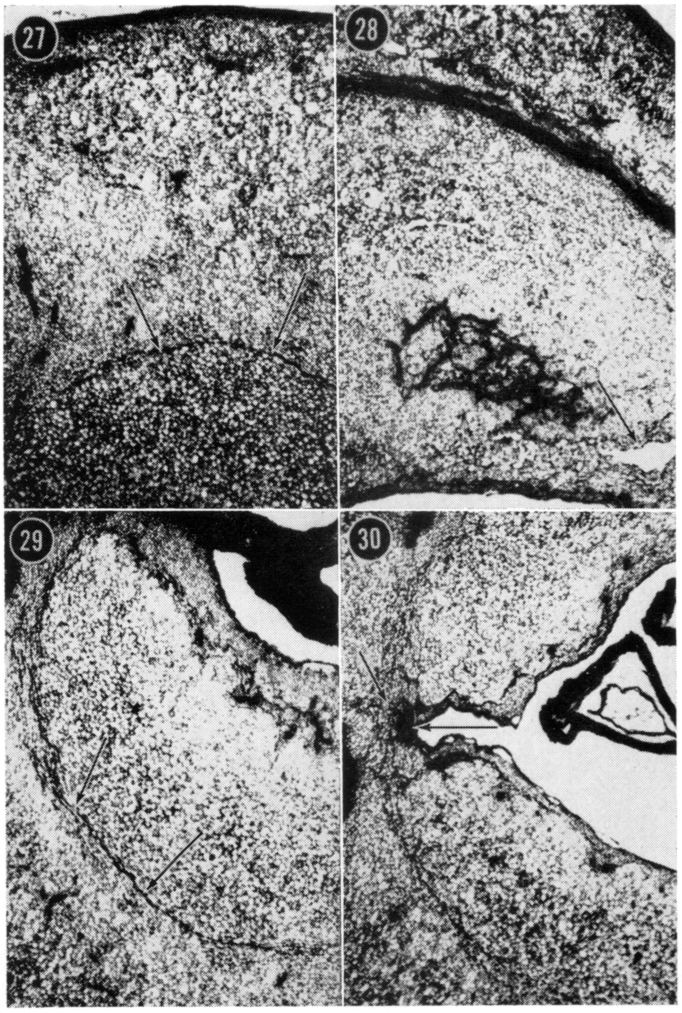

Fig. 27–30. Tissue injuries affecting ovule development of 'Duchess' apples, 28 days after injury. Fig. 27. Necrotic tissue along the core line (indicated by arrows). Fig. 28. Necrotic tissue along the edge of the ovarian locule. Injury was scattered throughout the cortex. Fig. 29. Necrotic tissue present along the core line and extending into the placental area (indicated by arrows). Fig. 30. Necrotic tissue surrounding the ovarian locule with all tissues killed at the funicular base (indicated by arrows). All figures × 12.3.

Anatomical characteristics.

The same general types of injuries were present in this cultivar as those in 'Lodi'. The influence of injury on fruit development 18 days after injury has been recorded in Fig. 23–26. Injured tissues were present within and underlying the calyx lobes. This type of injury caused a depression around the base of the calyx lobes and, in many instances, hypertrophy. Tissue destruction in the sepallary and petallary bundle areas is shown in Fig. 24. The fruit continued to grow abnormally until near maturity. The vascular bundles and adjoining areas in the outer cortex were seriously affected by the low temperatures.

Destruction of tissues within the cortex of the equatorial region was similar to that for 'Lodi' (Fig. 25). A X-section of the fruit apex (Fig. 26) shows the extent of injury that may be localized or may occur throughout the fruit in the outer protective areas, with a hypertrophied epidermis. The core line was not injured in this instance.

The diverse types of tissue injury in the 'Duchess' apple are shown 28 days after injury (Fig. 27–30). The tissues are apparently normal except for a small area of dead tissue occurring along the core line and isolated necrotic areas throughout the outer cortex indicated by arrows (Fig. 27, 29). A greater area of affected tissue is shown (Fig. 28) with a large lesion occurring near injured vascular tissues within the inner cortex, indicated by arrow. Small areas of tissue were adversely affected throughout the cortical tissues.

Extent of the injury upon 'drop' fruit is shown in Fig. 29 and 30. The outer cortex ceased to grow, with injury showing along the core line into the ovarian locule. The placental tissue supporting the ovules was killed. The whole carpel area was injured, with a sharp line of collapsed cells denoting the edge of the carpel blade. Injury to the funicular base of the ovule in a 'drop' fruit is shown in Fig. 30. The ovules aborted as a result of this injury and the fruit had completely stopped growth. The thin parenchyma cells of the ovary wall were destroyed.

A growth crack found in the outer cortex of a 'drop' fruit penetrated almost to the carpel cavity. The tissue affected beyond this break extended to the carpel cavity. In these injured areas there were well developed layers of phellem, phellogen and phelloderm. These new layers were formed periclinally to the newly broken surface, and at 90° angle to the normal fruit surface.

Eighteen days after injury, irregular growth of the fruit apex was noted. The sepallary and petallary bundles, where most of the injuries have occurred, remained intact. However, some cell necrosis was present and injury occurred in the epidermal, hypodermal and outer cortex.

Fruit finish was adversely affected, with many areas of the outer protective region being destroyed. In the equatorial axis, phellem formation 28 days after injury progressed rapidly, while many cells were replaced with a new phellogen layer. These injured areas were not continuous but were in sufficient quantity to seriously affect fruit finish. It is assumed that these growth characteristics would be representative for other cultivars of apples in the same stages of development.

Discussion

Variable types of freeze injury to young developing apples have produced a large number of tissue responses. Recovery from injury appears to be rapid since this is a period of cell division accompanied by cell enlargement.

Injuries occurred in the outer cortical region, producing large breaks in the tissue along the sepallary and petallary bundles. Also, the outer carpellary tissues along the core line were particularly sensitive to low temperatures. In some instances, large areas of the tissue in the outer cortex were stratified. These consisted of many layers of injured and non-injured tissues, contiguous to each other. Cortical tissues throughout the fruit were affected by this with varying intensity.

Response in the recovery areas of the outer cortex may be classified as typical callus tissue. Bloch (1) quotes Ulrich in reporting that "callus was formed best in fruits during rapid growth". Esau (3) has cited wound healing in *Hibiscus* stem as an example of callus tissue development in contrast to the callus development found in the apple, which has more cell proliferation and less cell organization, Fig. 18. This would be typical of the callus tissue defined by Esau (2) who describes "a tissue composed of large thin-walled cells usually developing as a result of injury". In this study on 'Lodi' and 'Duchess' apples, a typical phellogen layer was found in many areas of the outer cortex which produced an initial layer of cork tissues as illustrated in Fig. 15. The quantity of cell proliferation and callus tissue formation was dependent upon the severity of the injury.

Bloch (1) also states that "typical dedifferentiation has been frequently described for plants. Plants may lose, not only in wound and regenerative tissues but also during the course of normal development, their differentiated character becomes more embryonic and less specialized. Resumption of cell division and wall growth are frequently followed by functional and metaplastic changes which indicate that the cells have undergone a process of redifferentiation". Tissue development as described by Bloch may be seen in Fig. 11, 12 and 18 in which dedifferentiation has been followed by redifferentiation. Tissue development such as this coincides with that of hail injury occurring to young apples (10). Gautheret (5) has discussed the aspects of cell proliferation as contrasted to differentiation and dedifferentiation in various tissue culture studies.

According to Bloch (1), the cell division in a wound meristem is usually parallel to the surface of the wound. Other directions may also occur, especially in the later stages of wound tissue formation and in special locations. The areas of meristem development for the apples of this study are shown in Fig. 15, 16, 17, 18 and 19. The rapid tissue response to wound injury during these stages of development of the fruit are noted.

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Absorption of Phosphorus by *Chrysanthemum morifolium* Cuttings Propagated Under Nutrient Mist[1]

J. A. Wott[2] and H. B. Tukey, Jr.[3]

Abstract. Chrysanthemum morifolium, Ram. cuttings were rooted in special containers so that the stems and foliage were exposed to intermittent nutrient mist at the same time that the basal ends of the stems and developing roots were irrigated with ^{32}P-labeled nutrient solution. Analyses of the radioactive and non-radioactive P contents of the cuttings revealed that almost all of the P absorbed by the cuttings during propagation was absorbed by the stems and foliage from the nutrient mist and either utilized in new growth of the foliage or translocated into the developing roots. Absorption of P from the rooting medium was of importance only during the last 2 days of propagation when new roots were well developed, and even then, was less than foliar absorption. Applications of nutrients to herbaceous and softwood cuttings are made more efficiently through nutrient mist than to the rooting medium directly.

Introduction

Nutrients can be applied in water mist to cuttings during propagation (3, 10, 11, 15, 16) to replenish leached nutrients and to provide nutrients for new growth occurring during propagation (4). Cuttings of several species of ornamental plants, both softwood and herbaceous types, have been shown to exhibit large increases in growth, both linear stem length and dry weight, and in nutrient content when propagated under nutrient mist (15, 16). In many species propagated under nutrient mist, cuttings have developed a higher quality root system with more and longer roots, have made more linear growth from lateral buds, and have continued to grow at a faster rate after propagation than have cuttings propagated under water mist without nutrients. Hardwood cuttings have also responded favorably to nutrient mist, but the response has not been as great as with fast-growing softwood and herbaceous cuttings (13, 14).

Nutrients have also been applied to cuttings through the root medium, but with only indifferent and erratic results (6, 8, 17). This suggests that the method of application and subsequent uptake of nutrients may be critical.

It is well known that nutrients are absorbed effectively by both roots and above-ground plant parts, including stems and foliage (12). Less well appreciated is the fact that substances can also be taken up through the cut basal ends of the stems of cuttings (9). The following experiment, using radioactive phosphorus, has evaluated the contribution of nutrient absorption through the foliage versus absorption through the basal ends of the stems and developing roots.

Methods and Materials

Four hundred *Chrysanthemum morifolium,* Ram. 'Giant No. 4 Indianapolis White' cuttings, each with the same leaf number, approximate size, and approximate fresh weight, were inserted through the lids of Freezete polyethylene containers (20 cuttings/container) 4 cm into a rooting medium of quartz sand. Plastic caps insured a water-tight seal around the cutting stems, and the lids were carefully sealed. The cuttings and containers were placed beneath intermittent mist containing a soluble fertilizer (Ra-Pid-Gro, analysis 23-19-17) at the rate of 4 oz./100 gal of water. The misting interval was controlled by a time clock at 10 sec of mist every 10 minutes from 7:00 AM to 7:00 PM. The nutrient mist bathed the foliage and the upper portion of the stems of the cuttings, but did not contact the stems or the rooting medium within the containers.

The rooting medium was uniformly irrigated with a similar nutrient solution to which high specific activity radioactive phosphorus (^{32}P) was added at the rate of 5 mc/80 cuttings. The radioactive nutrient solution was dispensed by gravity from 5-gal plastic carboys, and after irrigation of the rooting medium was collected for disposal.

Thus, the above-ground parts of the cuttings, i.e. the foliage and upper portion of the stems, received non-radioactive nutrient mist, whereas the rooting medium, the basal ends of the stems, and new roots as they developed received ^{32}P-labeled nutrient solution.

On the second day after insertion of the cuttings into the rooting medium, and every second day thereafter until the end of the propagation period, 2 containers of 20 cuttings each were removed. These were severed at the "soil line" approximately 4 cm from the base of the cuttings, washed for 15 sec in distilled water and dried at 70°C in a forced air drying oven. Any callus and root growth were noted.

After determining the dry weight, a 0.25-g sample of tissue was ashed and the total P content including both radioactive and non-radioactive P was determined colorimetrically (7). These results were compared with similar analyses of cuttings before propagation.

The remaining portion of dried plant material from each cutting was then ashed in a muffle furnace at 525°C for 17–18 hr. After cooling, several drops of concentrated HNO_3 were added and the samples were re-ashed for 2 hr. After ashing, 5 ml of 20% HCl were added and the samples were evaporated to dryness on a hot plate. Five ml of a 1% HCl solution were added, from which 1-ml aliquots were pipetted onto planchets, dried, and analyzed for radioactivity with a Nuclear-Chicago Model D47 Gas Flow Detector. The total P and ^{32}P content of the nutrient solution applied to the rooting medium was determined in a similar manner as the tissue analyses.

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