Phloem Tissue Development in Response to Freeze Injury to Trunks of Apple Trees

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Abstract. A subfreezing temperature caused trunk splitting in 13-year-old apple trees of the cultivars 'Golden Delicious' and 'Starking' growing on EM VII rootstock. 'Starkrimson' and 'Golden Delicious' on a seedling rootstock and 'Jonared'/EM VII were unaffected by the freeze. No splitting occurred below the graft union of any tree. Phloem, 8 months after the initial injury, showed extreme variation in the development of periderm throughout the injured tissues. The sieve elements, sieve plates, and phloem-ray cells in the functioning phloem were distorted and necrotic depending upon severity of injury. Cellular structure showed evidence of injury a considerable distance beyond the wound and some of the injured areas had subsequently healed. In severe but closed wounds parenchyma was the only wound tissue evident. In some cases, this tissue developed into a narrow band of phloem capable of translocation. Callus tissue formed in areas adjacent to the periderm. Dilution tissues were evident within split phloem-ray cells. In some areas the phloem-ray cells were killed and translocatable metabolites accumulated in the sieve-plate area. Some of the injured tissue was necrotic and translocation appeared impossible.

Freeze damage to trees has been a common problem throughout many of the fruit-producing areas of the United States. The extent of injury varies with losses ranging from the killing of fruit buds to trunk girdling. Large portions of the conducting tissues may be killed, resulting in loss of the tree.

Literature on frost killing and winter injury is extensive. The freeze during the winter of 1955–56 was reported by Anthony (2), Burkholder (6), Rawlings (12), Tingley (17, 18), and Waring (19). Other types of injury such as bud and twig injury were reported by Brierley (5) in which hardy varieties were severely injured in Minnesota. This injury resulted from the combined effects of previous crop reduction by frost, and delayed maturity resulting from heavy crop, drought, and unusually mild temperatures accompanied by rains in late September and early October. This warm weather was followed by temperatures of −7°F on November 30.

Varying degrees of trunk injury have been reported for 80 winters in Michigan orchards by Bradford and Cardinell (4). They described a frost crack on a seedling tree that resembles the trunk injury in this report. Similar conditions were found by Anthony (2) in which injured tissues matured slowly. He also reported that where excess fertilization, heavy pruning, late cultivation, thin soil, spray injury, or heavy bearing were present, the freeze damage to the trees appeared to be more severe. Trunk splitting on 8-year-old 'Golden Delicious' trees as described by Sudds and Marsh (15) was more extreme than that reported in this paper. The Agricultural Research Service (1) has provided a description of various disorders occurring to plants that have been exposed to severe freeze damage. Also a histological evaluation of low-temperature injury to apple trees was made by Steinmetz (16).

The purpose of this paper is to give an evaluation of the phloem response of the scion during the immediate growth period after injury. Symptoms of many of these disorders may appear a long time after the initial injury. The wound response of phloem tissues will give specific information concerning healing and recovery of these wounds.

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Materials and Methods

Injury occurred in early January 1968 and samples of the bark were obtained from the 'Golden Delicious' /EM VII in late August after a season's growth, 8 months after injury. These were obtained by using a 'Jones patch budder' to remove the bark from the tree. Three samples, each 14 x 11 cm in size, were obtained from both sides of the injured area of 10 trees and were cut for microscopic examination both longitudinally and radially. The longitudinal sections were obtained from the exterior portion of the bark and continued through to the cambial zone.

Immediately after collection, the tissue was preserved in distilled water until the time of sectioning, which was approximately 24 hr. The sample was then frozen in 2–3 min and sectioned on a freezing microtome at 20 μ and at a temperature of −10°C.

After the sections were placed on a glass slide, they were allowed to dry, then immediately placed in a safranin stain as prescribed by Johansen (11) for 15–20 hr. They were then rinsed in distilled water for 5 sec and placed in 95% and absolute ETOH for 40 sec each, before placing into 3 changes of xylol. After this was accomplished, they were made into permanent slides using 'Permount' as a mounting medium. Photomicrographs were obtained with a Zeiss photomicroscope. Terminology for the various phloem tissues was made in reference to Batjer and Schneider (3), Esau (7, 8, 9), Evert (10), Schneider (13), and Simons (14).

Results and Discussion

The low point of temperature fluctuations early in January 1968 caused trunk splitting in 'Golden Delicious' and 'Starking' growing on the EM VII rootstock. 'Starkrimson' and 'Golden Delicious' growing on seedling rootstocks and 'Jonared' on the EM VII rootstock were unaffected by the freeze. All of the injured trees were part of large blocks in commercial production.

Mild temperatures in the 50°F range were recorded throughout western Illinois in December 1967. January 1 temperatures were recorded at −10°F to −12°F. Another temperature drop from −11°F to −15°F was recorded on January 7. This continued on January 8 with −8°F as the low point.
Fig. 1-4. Trunk injury (indicated by arrows) to 13-year-old 'Golden Delicious'/EM VII rootstock. Fig. 1. Longitudinal splitting of the trunk extending from the main scaffold area to the graft-union area. Extreme ridging of the trunk resulted from the stock-scion combination. Fig. 2. Longitudinal split in the area below a main scaffold branch. Fig. 3. Extreme splitting extending from the main scaffold branches to the stock-scion union. Fig. 4. Tree that has recovered from a previous injury with the longitudinal cracks in evidence to the graft union line.
lowest temperature. The major part of the injury to the trees occurred on January 1.

Fig. 1–4 are examples of the injury as it appeared at the time of sampling. However, immediately after the trunks split in January, wide cracks were evident. After the trees thawed, the wounds closed somewhat. In many cases, the cracks were so large that they would not heal, as indicated in Fig. 2 and 3. An example of wound healing from a previous injury is shown in Fig. 4 and is indicative of the type of recovery made by most of these injured trunks. The injury persisted on the main part of the trunk and extended to the graft-union area between the scion and stock. No splitting occurred below the graft union even in the EM VII rootstock.

Longitudinal splitting occurring in the main part of the tree trunk and extending into the crotch angle of the scaffold branches was most common, Fig. 3. Trunk splitting in the distal portion of the scion also occurred with evidence of injury between the scaffold branches which would indicate immature tissues in these areas. When splitting appeared on a lateral branch, the bark was pulled away from the wood and the phloem failed to unite or heal.

A significant result of the scoring performed 3 years previous to the freeze is shown by the longitudinal cracking which consistently appeared below the lowest area where the tree had been scored. In cases where splitting was less severe, strands of tissue connected the 2 sides of the injury.

Tissue growth response of injured ‘Golden Delicious’ trees on EM VII rootstock is shown in Fig. 5–20. A transection of the existing injury, as shown in Fig. 5, illustrates the depth and width of the injury to these tissues. Tissues uniting the wound at the maximum point of injury grew approximately 1 cm in thickness as contrasted to 4.5 cm in the apparently uninjured areas. The effect of injury by the split to the epidermis and outer cortex, as indicated by arrow at the top of Fig. 5, ranged 2.9 to 4.0 cm in width. This included the phellem tissues to a point where the epidermis was not disrupted. Phellem was evident in large quantities in the areas of non-functioning phloem, with an active phellogen layer. These growth sequences, with decreased quantity of phellem, continued also into the functioning phloem where the tissues were barely united and where growth was impeded. Dilated rays were evident adjacent to the wound.

Fig. 5. A transverse section showing recovery of the tissues 8 months after injury. The outer cortex in the non-functioning area has produced a large amount of phellem as contrasted to the thin layer at the maximum depth of injury. Note the injury to the epidermis at the edge of the wound (arrow). Details: p, periderm; fp, functioning phloem. × 16.
area in the outer cortex and in the non-functioning phloem.

The epidermis was separated from the outer cortex, and this injury continued to have a disruptive effect upon the functioning and non-functioning phloem, cutting transversely across the fiber sclerids, through the cambium and into the xylem tissues. An increase in dilation tissue was found in the non-functioning phloem and continued into the functioning phloem. The formation of these tissues was evident approximately 4.5 cm away from the point of breakage, and developed within the phloem-ray cells. There was tissue separation throughout the phloem, and a distinct phellogen line appeared along the main tissue break with intense meristematic activity of adjacent tissues which included a distance of approximately .35 cm. Evert (10) found in normal phloem that rays become distorted in the outermost part of the phloem but do not become dilated in relation to the increase in circumference of the axis resulting from secondary growth. However, Esau (7) has illustrated the dilated rays occurring in the secondary phloem of *Tilia* that was characteristic of dilation found in these injured apple phloem tissues.

The deepest point of the injury constituted the area of functioning phloem, with much injury evident throughout these remaining live tissues. However, the phloem-ray cells and sieve tubes were disarranged from the normal pattern of growth, with much necrotic tissue appearing within this area. Dilation rays had formed throughout the secondary phloem which would be the exterior portion of this deepest wound area.

The conducting tissue was impaired throughout the interior portion of the wound, but 8 months after injury there was sufficient recovery to promote callus formation and subsequent healing. The exterior surface of the functioning phloem adjacent to the cambial layer contains phellem, phellogen, and phelloderm tissues contiguous to parenchyma cells, characteristic of callus.

A pattern of regrowth with a periderm forming within the injured tissues resulted in the formation of large masses of callus tissue. Gaps within this callus tissue indicated incomplete tissue unification. Necrosis, interspersed between these parenchymatous cells, was apparently due to growth stress within the wound areas.

A transection of the sieve tubes and phloem-ray cells revealed that a definite phellogen had formed as a result of the injury. It also severed the phloem-ray cells and sieve tubes, with a complete encirclement of the fiber sclerids. Phloem-ray cells adjacent to the wound area split and a small amount of proliferated parenchyma was evident within the split area. Sieve tubes were necrotic throughout this general area adjacent to the periderm.

The injurious effect upon the various phloem tissues as much as 1 cm. away from the tissue break is illustrated in Fig. 6–8. Fig. 6 shows the tissue near the outer cortex, with splits occurring within the ray cells. These ray cells were completely killed near the break, but were gradually distorted in the contiguous areas away from the initial injury. Sieve tubes, though not killed, were injured throughout this area. It was in the immediate break area that the tissues were killed. Deeper in the tissue (Fig. 7) the ray cells were distorted and dilation tissues were formed. Splitting of the ray cells continued near the break and many were necrotic.

The functioning phloem in Fig. 8 showed evidence of necrosis in the sieve tubes even though it was not close to the injury. This illustration was taken from the apparent uninjured phloem tissue that had the greatest thickness adjacent to the wound area. This would indicate that some damage occurred to other portions of the tree trunk and could be recorded microscopically.

Examples of the connecting strands between the 2 sides of the wound are illustrated in Fig. 9–12 with a periderm surrounding both sides of the wound. Much variation existed within tissues in the wound area. Fig. 9, a longitudinal section, indicated by arrow, shows phloem-ray cells (in these strands uniting the wound), which are growing at a right angle when compared to normal orien-

Fig. 9-12. Longitudinal sections of wounded tissues 8 months after injury and the longitudinal orientation is indicated by a complete arrow. Fig. 9. Connective tissues between the two sides of the wound with large areas of tissue destruction surrounded by necrotic tissues. The live phloem in these strands is capable of conduction but is much smaller than normal functioning phloem tissues. Fig. 10. Another area of the injured connective tissues with much scattered necrosis within the living tissues. Note the proliferated parenchyma (pp) within the injured area that was surrounded with necrotic tissues. Fig. 11. A more severe destruction of tissues in which they were completely killed. Arrows indicate a longitudinal strand in which the phloem was killed adjacent to the main point of injury. Fig. 12. The layers of living cells remaining intact between the two sides of the wound. Fig. 9, × 10; Fig. 10, × 12; Fig. 11, × 40; Fig. 12, × 160.
Fig. 13-16. Proliferated parenchyma and callus formation within injured tissues. Fig. 13. Proliferated parenchyma located between two sides of the injury in which necrotic phloem-ray cells were present. Fig. 14. Necrotic phloem-ray cells and two of them completely disrupted with proliferated parenchyma. Fig. 15. Unification of callus tissues across the injury surrounded by necrotic tissues. An active phellogen exists on both sides of the wounded area. Fig. 16. Injured phloem-ray cell filled with proliferated parenchyma which is characteristic of callus. Details: PP, proliferated parenchyma; P, phellogen; C, callus. Fig 13, 14, × 100; Fig. 15, 16, × 160.
Fig. 17–20. Various degrees of degeneration appearing in the phloem tissues some distance away from the main tissue break. Direction of arrows indicates longitudinal orientation. Fig. 17. Necrotic phloem-ray cells with an accumulation of metabolites. Fig. 18. Necrotic phloem-ray cells with proliferated parenchyma (pp) arising from the sieve tube and sieve plate area. Fig. 19. Necrosis of phloem-ray cells and sieve tubes. Fig. 20. Degeneration and disruption of phloem-ray cells into non-functioning tissues. Also note metabolites that have accumulated in the sieve tube area. Fig. 17, 19, X 128; Fig. 18, 20, X 100.
tation. Necrotic tissues were interspersed throughout this injured area. Although the injury would callus and heal, with an eventual uniting of the cambium of the 2 sides, a considerable portion of this area would contain corky tissues resulting from formation of periderm tissues in response to the injury.

A large connective strand with split and necrotic phloem-ray cells surrounded by phellem and periderm is shown in Fig. 10. Callus parenchyma filled large holes completely encircled by necrotic tissue. When injury was more severe (Fig. 11), extensive phellem surrounded the exterior and interior of large cavities. Phellogen present in the lower portion of the connective strand was the only live tissue in this area. The phloem-ray cells adjacent to this wound changed into parenchyma tissues, with the subsequent formation of phellogen and phellem. The functioning phloem in this area was interspersed with necrotic tissues, appearing as a narrow longitudinal strip with large adjacent dead areas. It would be expected that such a severe injury would require 2-3 years to completely heal.

The persistence of these tissues to live and unite is illustrated in Fig. 12. Two strands of living cells were the only connecting units between the two sides of the wound, although a great distance separated them. Proliferated parenchyma formed adjacent to these strands.

Formation of proliferated parenchyma and dilation tissue is shown in Fig. 13-16. Examples of these tissues were found in the outer cortex and within the wound areas that were not completely killed. Fig. 13 is a longitudinal section through the outer cortex of the wound area. The dilation tissue formed throughout the wound which was located between phloem-ray cells. These cells were necrotic on both sides of the wound. Distorted phloem-ray cells (Fig. 14) became meristematic and were engulfed by proliferated parenchyma, resulting in an increase in width of the original cells. These cells at the outer edge were necrotic. Other adjacent phloem-ray cells were necrotic with no meristematic activity present in this area, which indicates variations in tissue response to the low temperatures.

Callus tissues resulting from the immediate wound area, with the presence of phellogen and the formation of phellem, are shown in Fig. 15. The 2 sides of the wound tissue united in a small area and continued production of callus tissue would completely cover the wound and facilitate translocation. However, many large spaces existed where no tissues were present and this would prohibit complete tissue unification.

Proliferated parenchyma cells within an injured phloem-ray cell are shown in Fig. 16. All of the cells in the adjacent area were necrotic, with the exception of some scattered proliferated parenchyma cells. This particular cell type was not entirely killed, resulting in cell proliferation and formation of tissues characteristic of callus. This injury was apparent throughout the less injured phloem tissues a short distance away from the 'bark split', thus indicating some phloem tissue injury existed throughout the conducting system of the tree.

Longitudinal sections of the functioning phloem in Fig. 17-20 show necrosis in most of the phloem-ray cells and sieve tubes. An apparent injury to the sieve tubes and sieve plates caused metabolite accumulation (Fig. 17). This could possibly lead to further physiological disturbances of the tissues in this area. Additional injury (Fig. 18) caused complete disruption and formation of callus tissue within the sieve element and plate area, with necrosis of these tissues. This was the only evidence of regenerative growth in this area.

Evidence of sieve-tube necrosis and phloem-ray cell injury is presented in Fig. 19. The sieve elements and plates are necrotic (indicated by arrows). Adjacent phloem-ray cells were disrupted and though they were not killed, injury was evident throughout the cell-wall area.

A more extreme case of tissue injury is illustrated in Fig. 20. The phloem-ray cells were completely killed and the translocatable metabolites accumulated in the sieve-plate area. Complete splitting of the phloem-ray cells is shown at the top of Fig. 20, with the connecting strands of fibers and the sieve tubes still evident. In some areas of this tissue, there was so much necrosis that translocation appeared impossible.

**Conclusions**

Trunk splitting occurred in scion tissues of 'Starking' and 'Golden Delicious' growing on EM VII rootstock, but 'Starkrimson' and 'Golden Delicious' on seedling and 'Jonared' on EM VII rootstock were unaffected. Severity of injury was influenced by the cultivar-rootstock combination.

Phloem tissues have the ability to unite and heal, even though severely injured. When this type of injury is present, care should be taken in weed control so that herbicides will not injure the young tissues in the breaks. Fertilizer applications should be adjusted in order to improve the vigor of the injured trees and speed the healing processes.

**Literature Cited**

Fluctuations in the Cold Resistance of Apple Twigs During Spring Dehardening

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Abstract. The relationship of environmental temperature to the cold resistance of apple bark tissue was studied on mature orchard trees in the field during natural spring dehardening and on 3 and 4 year old trees in the containers which were dehardened under controlled conditions. Field studies showed day to day fluctuations in dehardening and rehardening during the spring in each of 2 years. These short term changes in cold resistance were closely related to the air temperatures of the preceding day. In controlled studies, hardy plants during the winter dehardened as much as 15°C in one day in a warm greenhouse, and rehardened 15°C in 3 days when they were held at —15°C. The dehardening process was only partially reversible. Once dehardening began, the bark did not reharden beyond a certain base level. This base level raised with each successive day of dehardening. The base level usually corresponded to the minimum killing temperature on the day preceding the final day of dehardening.

Trees in the temperate zone are exposed to a variety of environmental stresses in nature to which they must adapt or die. The living bark of ‘Haralson’ apple, which is killed at —6°C in the summer resists —50°C in the winter (5). Fully acclimated tissues may be killed in midwinter by low temperatures, but many plants are particularly vulnerable to frost injury in the fall and spring during the transitional periods of hardening and dehardening. Autumn cold acclimation (1, 3, 4, 5, 10, 12) and mid-winter hardiness (1, 2, 6, 9) have been subjects of extensive study but relatively little is known about spring dehardening.

It has been suggested that rest period may prevent premature growth and concurrent dehardening in plants (1, 2, 9) during unseasonably warm spring weather. Recent work on raspberries (7) showed, however, that dormant canes which were deep in the rest period became less hardy than dormant non-resting canes following a warm period.

While rest period could be a protective factor in early winter before chilling requirements are satisfied, the rest period of most northern species is over long before spring dehardening. Mechanisms other than rest period must protect plants from fluctuating temperatures during the dehardening process. Proebsting (9) has shown that the day to day hardiness of dormant peach flower buds fluctuated a few degrees during this period and that hardiness was closely correlated with the minimum air temperature of the previous day. Flower buds that dehardened appeared to reharden in response to low temperatures.

The purpose of this study was to examine the relationship of air temperature to the hardiness of apple bark during the spring dehardening period.

Materials and Methods

‘Haralson’ apple, Pyrus malus L., twigs were collected twice weekly during the late winter and spring of 1967 from mature orchard trees near St. Paul, Minnesota. Maximum-minimum air temperatures and phenological events were recorded in the orchard, and the hardiness of excised 1 year old twigs was determined at each sampling date during natural spring dehardening. A similar study was conducted in 1968 except that sampling was done on a weekly basis. In both field experiments the experimental design consisted of 3 completely randomized replicates. Evaluation of differences between sampling dates was accomplished via the statistic K (11).

Hardiness determinations. The hardiness of the living bark was determined in all studies by subjecting excised 1 year old twigs to a controlled freezing stress. In each case test branches from 3 replicates were collected, cut into 5 cm lengths, labelled, and placed immediately into a series of thermos flasks which were then cooled in a deep freeze at approximately 10°C per hour. A 26 gauge thermocouple inserted in the pith of one stem section in each flask monitored sample temperature. Sample flasks were removed from the freezer at 5°C intervals, and allowed to warm slowly to room temperature. After warming, twigs were placed in a humid chamber at room temperature and held for 5 days. Previous work (6) has shown that twig samples which have firm green bark 5 days after freezing were alive and capable of forming callus within 20 days. During the 5 day incubation period undamaged bark remained green and firm while damaged bark first became water soaked, then darkened, and ultimately became soft and was invaded by saprophytic fungi. On the fifth day individual samples were dissected and examined for injury. The seasonal pattern of hardiness was plotted as killing temperature. This is the highest temperature at which samples were killed at each sampling date.

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