HISTOLOGICAL AND GROSS STUDIES OF FRUIT RUSSETING IN THE APPLE (*MALUS DOMESTICA* BORK.) AS RELATED TO FUNGICIDES AND FREEZING AIR TEMPERATURES

By

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AN ABSTRACT

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ABSTRACT

An investigation was conducted in 1956 and 1957 at East Lansing, Michigan to determine the possible interrelationship of freezing air temperatures occurring near bloom and the use of certain fungicides as affecting fruit russeting of apples (Malus domestica Bork.).

In controlled temperature experiments, bearing trees of the varieties Jonathan and Golden Delicious growing on Malling VII rootstocks were exposed to, or protected from freezing air temperatures. The trees were pruned to divide the bearing surface of each tree into two distinct halves so that two fungicide treatments could be evaluated in the same environment.

At harvest in 1956, it was found that the fruit from the glyodin treatment on the Golden Delicious variety following exposure to freezing air temperatures were 84.5 percent heavily russeted, as compared to 24.4 percent for the captan treatment. The fruit from the tree protected from freezing air temperatures and receiving the glyodin treatment were 27.8 percent heavily russeted, while the portion treated with captan had no fruit so affected. In 1957, where glyodin was used at half-strength (one pint) plus one-fourth pound of Phygon, it was found that the fruit were less severely russeted than where glyodin was used alone at one quart per 100 gallons.

Of the harvested fruit of the Jonathan variety receiving the glyodin treatment in 1957 and protected from exposure to temperatures below 32° F,

2. 94 percent were heavily russeted and 48. 53 percent lightly russeted. For those treated with captan 2. 44 percent were heavily russeted, and 19. 51 percent lightly russeted. Exposures to minimum temperatures of 27. 5°, 30. 5°, 31. 5° and 30. 5°F near bloom where captan was used, resulted in 15. 52 percent of the fruit heavily russeted and 46. 55 percent lightly russeted as compared to 67. 07 percent heavily russeted and 28. 05 percent of the fruit lightly russeted from the use of glyodin.

Field studies were conducted at Grand Rapids, Michigan on bearing Jonathan, and McIntosh apple trees comparing ferbam, glyodin, thiram and captan in 1956, and wettable sulfur, glyodin, thiram and captan in 1957, as affecting fruit russeting. In 1956, a 30-minute period of 30. 5°F was experienced prior to bloom and no differences were observed between treatments. In 1957, temperatures of 31. 5°, 31. 0°, and 31. 5°F were recorded near bloom and significant differences of heavily russeted fruits were observed between treatments on the Jonathan variety. The trees treated with captan were located in three different areas of the planting, and the percentage of fruit heavily russeted from these locations ranged from 12. 1 to 21. 6 percent. This compared to 12. 4 percent for the sulfur treatment, 18. 0 percent for the thiram treatment, and 29. 5 percent for the glyodin treatment. The captan treatment having the 21. 6 percent heavily russeted fruit was lower in

elevation than the other treatments. The excessive russeting could be associated with lower air temperatures in the less favorable elevation.

The same weather conditions and spray treatments on McIntosh resulted in no significant differences between treatments. However, in 1957 more russeting was observed for all the treatments made on both varieties than in 1956.

Histological studies were conducted in 1956 to determine the differences in development of the cuticle and epidermal tissue of the varieties Golden Delicious, Jonathan and McIntosh; and to study the development of russeted tissue of Golden Delicious when certain fungicides are used prior to second cover.

The first microscopic signs of russet formation on Golden Delicious were observed three weeks after full bloom as a small number of irregularly proliferating cells of the outer cortical layer. Four weeks after full bloom, these proliferating cells had ruptured the epidermis and cuticle. As growth of the fruit continued, more sub-epidermal proliferation occurred and at harvest time, a very evident phellogen was formed below the russeted area. The initial ruptures of the epidermal layer could not be associated with the presence of either trichomes or stomata.

Microscopic studies of the development of the protective layers of the fruit revealed that at the time the flowers emerge from buds, the receptacle is not covered with a cuticle. However, some fatty substances were found within the exposed cell-walls of the epidermal cells prior to bloom. Cuticularization occurred first at the bases of the trichomes. Ten to fourteen days after full bloom, the cuticle was complete on the fruit surface.

The epidermis of McIntosh, Jonathan and Golden Delicious was similar until approximately 30 days after full bloom. At this time the epidermal cells of Golden Delicious appeared to separate and separation increased as the fruit continued to enlarge. At maturity, no well-defined epidermal layer could be found. In contrast, the epidermal layer of the McIntosh remained very evident during growth and maturity, and no separation of epidermal cells was observed. The epidermal layer of Jonathan, although discernable, was not as regular as that of the McIntosh.

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TO MY PARENTS - FRED AND ANN KRETCHMAN

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INTRODUCTION

During the past ten years, Michigan apple growers have been utilizing many of the new organic pesticides in various types of spray programs under diverse climatic conditions and have experienced wide variations in their effects on fruit russeting. They accepted the russeting as cold injury. Mitchell (35), studying the influence of fungicides on fruit finish, found that trees sprayed with a certain fungicide produced fruit with a very favorable finish one year, whereas the following year, using the same chemical, the fruit was very severely russeted. However, other fungicides included in the study were found to cause very little russeting of the fruit.

Data from other workers (21, 29, 43, 46) have indicated that fruit russeting attributed to spray injury seemed to be more severe when "spring frosts" had occurred near the blossoming period. However, no attempt was made to correlate the combined effects of "spring frost" with pesticide chemicals on the amount of russeting.

It was evident from preliminary reports (34, 35, 38) that not all varieties were russeted from the occurrence of freezing temperatures close to bloom even though the same pesticide chemicals were used. This sug-

gested that a possible dis-similarity of the protective layers of the fruit exists between some varieties. Histological studies of normal and russeted tissues have been described by earlier investigations (1, 2, 3, 4, 24, 32, 55, 57, 58, 60, 64) but the possible cause for varietal differences in susceptibility to fruit russeting was neglected. However, mention was made of certain specific varietal characteristics relative to the outer tissues of the fruit (24, 32, 55, 57, 60).

Since early fruit russeting is generally not observable without the aid of a lense until approximately six weeks after full bloom (6), it was believed that spray chemicals alone caused the injury (21, 24, 43). This premise could account for the oversight of a possible interaction of freezing air temperatures and the use of certain pesticide chemicals as affecting fruit russeting.

It was suggested by Bell (2) that the apple flower receptacle is not completely covered with a cuticle until shortly after full bloom. Because of the protective nature of plant cuticle, its development may be related to fruit russeting.

Previous histological investigations of russeted fruit (4, 24, 29, 60) have indicated an association with trichomes and stomata of the fruit epidermis. However, casual field observations have resulted in a questioning of this premise with respect to freezing temperatures and organic pesticide chemicals.

Therefore, this investigation was undertaken to determine:

1) The effects of freezing temperatures and organic fungicide chemicals on fruit russeting and the interacting effects of these two factors.

2) The relationship of cuticle development as it may influence the factors affecting fruit russeting. 3) The manner of russet development and its association with the trichomes and stomata of the fruit epidermis.

4) The possible reason for varietal differences of russet susceptibility.

REVIEW OF LITERATURE

"Frost" has long been considered a primary cause of fruit russeting of apples. One of the earliest recordings of this premise was made by Dorsey (9) in 1918. Referring to the condition of the apple a week after being exposed to a temperature of 27° F occuring at full bloom[1/], he said "The pistils, seeds, and core were brown--that is killed--and the skin separated from the young apple in patches or entirely. There were some variations found in the degree of killing. In some cases the pistils were not killed and in others the seeds were not. In all cases where the pistil, seeds, and core were killed, the apple fell a few days afterward. Where these were not killed, and the skin only was affected by being broken away, these apples 'set' and matured. The skin being killed entirely or only part way around soon dried and turned brown, and with further growth of the apple, was torn away, leaving the surface underneath exposed. It was now necessary to form a new surface to this area left bare by the removal of the skin or epidermis."

Similar conditions were reported by MacDaniels and Heinicke (29) and by Rosen (53) following freezing temperatures occuring near bloom.

[1/] See Appendix A for an explanation of terminology.

The type of fruit russeting occuring from cold injury of this nature described by many authors (1, 9, 10, 11, 14, 15, 16, 20, 24, 29, 55), might best be illustrated by the words of Hedrick (24), "Frost russeting on fruit nearly always appears in bands or zones of greater or less width running around the fruit midway between base and apex". Other kinds of russet patterns were reported by Gourley et al. (20), "Frost injury may take the form of russeting the fruit, occuring either in bands, in patches about the basin or cavity, or in spots on the surface of the skin". This latter type of russeting has usually been associated with milder freezing temperatures.

Cold injury, severe enough to cause bands of russeted tissue on the fruit as described by Hedrick (24) was not necessarily related to the use of spray chemicals because it was observed in orchards following freezing temperatures close to bloom that received no sprays during the growing season (29). When freezing temperatures occurred close to bloom, MacDaniels and Heinicke (29) postulated that an explanation for the occurrence of the russeting in a rather localized manner on some fruit and diffused over the fruit on others might be due to a slight difference in the temperature which killed the cells of the epidermis and adjacent tissues. Air movement could influence this variation in temperature. They also stated, "Where the freeze occurs before the flowers of the blossoming clusters separate, the injury has been observed to be confined

to the definite sectors of the fruit, touching each other at that time. Contact of the young ovaries of the blossoms with each other or adjacent leaves may also have something to do with the frost rings that are frequently formed".

For many years the copper fungicides, bordeaux and fixed copper, lime-sulfur mixtures and later elemental sulfur were the principal fungicides used on apple trees (Malus domestica Bork.) in this country. The reports of injury to the finish of the fruit during this period are quite numerous (1, 11, 15, 18, 24, 43, 47, 51, 52, 63). Dutton (11) has stated, "The russeting of the fruit, following the use of bordeaux in early summer applications, is a limiting factor in the use of this spray on apples".

Morse (43), using liquid lime-sulfur as the fungicide and lead arsenate as an insecticide, reported a higher percentage of russeting in the sprayed plots than in the unsprayed plots. Baker (1) noted that "sulfur burning" occurred late in the season during hot weather and generally resulted in russeting of the fruit. These authors (1, 43) associated a blotchy or "net-like" russeting with spray injury. This is quite different than the "russet rings" or streaks described earlier (24) as characteristic of frost injury. It should be noted also from these earlier reports on fruit finish, that frost was probably related to the severity of russeting, although not

recognized as a factor. For example, Morse (43) observed that during the year when the spring was wet, cold and "frosty", more russeting was noted at harvest than in the following year using the same spray chemicals when more favorable weather conditions occurred in the spring.

Hamilton and Palmiter (21) observed that the fruit from a lower elevation of a block of Golden Delicious had more russeting than fruit from the higher portion of the planting. This, they stated, indicated that air drainage is a factor in russet injury. These authors were interested in air drainage as it influenced drying conditions rather than the possible association of freezing temperature and russeting at these lower elevations.

Since the introduction of ferbam[1/] in 1939 (19, 25), many organic fungicides have been found which have less unfavorable physiological effects on apple trees and fruits than the earlier conventional chemicals. Many of these materials have had wide-spread use in commercial apple orchards with varying results. Palmiter (46) reported that less fruit were russeted from the use of ferbam with lead arsenate than wettable sulfur and lead arsenate. He also observed more russeting on Golden Delicious with the use of ferbam than on Red Delicious. Garman (17, 18) found similar results with the same materials on other varieties of apples.

[1/] See Appendix B for the chemical nature of the spray compounds.

Sharvelle (54) noted that the use of captan produced a higher proportion of russet-free fruit than when bordeaux, wettable sulfur or ferbam were used in the spray program on Golden Delicious.

A statement made by Mitchell (33) in 1951 is of interest, "With the weather information now available, growers can avoid a certain amount of russeting by using spray chemicals appropriate to the weather conditions. During some seasons almost any of the spray chemicals may be used with little effect on finish. It is the occasional year that selection of spray chemicals is so important". The occasional year he refers to is one in which freezing temperatures occur close to bloom. This premise is the first indication since the introduction of organic pesticide chemicals, that weather conditions are playing a role in the effects of certain of these new fungicides on fruit russeting.

Since this early generalization, Mitchell (36, 37, 38, 39) has reported further observations on fruit russeting supposedly caused by fungicide chemicals closely related to freezing temperatures near full bloom. He stated (36), "No spray chemical can be expected to substitute for location and weather conditions when these two factors may be the reason for fruit russeting. For example, in 1953 captan was used on bearing Golden Delicious trees in two locations of the Michigan State University Horticultural

farm, East Lansing. The same spray mixtures of captan were used on each block of trees. However, at harvest time the fruits from one location were severely russeted while the finish of fruits from the second location was excellent. Cold, humid conditions existed on or about the time of full bloom. This condition, coupled with the lack of air drainage in one location, resulted in severe fruit russeting of the Golden Delicious. Nevertheless, the fruit of McIntosh, Cortland, Northern Spy and Wealthy grown in this same location had very good finish. Thus, it is clear that under certain environmental conditions fruit russeting of some varieties will occur regardless of spray chemical used".

Mitchell has reported that glyodin has caused russeting of Golden Delicious (37). He also observed the increased incidence of russeting on Jonathan from the use of ferbam (38) when a low of 29° F occurred at the time of pink and a low of 31° F at full bloom. This same material, however, reduced the amount of russeting on Red Delicious and McIntosh when compared to the use of wettable sulfur. He noted in years when the air temperature remained above 33° F, there was very little difference in the fruit finish of these three varieties resulting from the treatments, wettable sulfur versus ferbam.

Investigations on fruit finish of the Bartlett pear by Harris and

Griggs (22, 23) revealed russeted fruit in locations that had experienced freezing temperatures near bloom. In areas that did not have these cold temperatures, very little russeting was observed.

Hedrick (24) suggested in 1907 that the amount of injury from bordeaux sprays varied greatly with: (1) The structure of the skin due to the great variations in the skins of apples of different varieties. (2) The condition of the skin as to the age of fruit and the succulency or tenderness from weather conditions. He observed that from four to six weeks after blossoms drop, the young apple was covered with a felt of fine hairs. He stated, "Examined under the microscope, one sees upon the epidermis of the young apple many stomata. In time the hairs and most of the stomata are lost, the former dropping off, leaving a distinct scar". Hedrick explained that bordeaux probably caused injury to these hairs and adjacent epidermal cells as well as to cells adjacent to the stomata. The result being that these cells were unable to, in his words, "bear their share of the surface tension" as the fruit grows, causing a tear in the skin of the fruit. He further stated, "It is the dead cells and the healing of these lacerations, the cicatrization of wounds, that causes the corky, russeted layer on fruits which we call bordeaux injury". He made another important observation that bordeaux injury probably does not occur after the hairs are shed,

or when the stomata have changed to lenticels and after a waxy coating is formed.

Morse (43) observed in 1916, that when the pink-stage spray of liquid lime-sulfur and lead arsenate was omitted, nearly 13.5 percent more merchantable apples were obtained due to a greater freedom of russeting.

More recent studies by Palmiter (46) and by Mitchell (33, 36, 37, 38, 39) have indicated that the critical period of fruit development when injury may occur is from pre-pink to approximately two weeks after full bloom.

This could be related to the type of flower and fruit of the apple. Black (5) described the apple as a re-enforced or composite fruit consisting of one to several drupe-like fruits embedded in a fleshy torus and is called a pome. The torus is the receptacle of an epigynous flower and by excessive growth produces the major fleshy or edible portion of the fruit.

By this description of an epigynous flower, it may be assumed that as soon as the receptacle of the flower is exposed to external conditions, any environmental factors affecting the flower will subsequently affect the appearance of the resulting fruit.

Histological Studies

Bell (2) has divided the protective layers of the apple into four regions: (a) coating of epidermal hairs; (b) the cuticle; (c) the epidermis; and (d) the hypodermis. He observed that at the time the flowers emerge from the winter buds, the flower receptacle was covered with a thick coating of hairs. Three weeks before full bloom cuticle started to form at the base of the hairs and by about thirty days after full bloom, it had completely filled in around the base and generally any movement would cause the hair to break off. Concerning the cuticle, Bell stated, "At the time the flower emerges the cuticle is hard to define, but by using Sudan IV, it can be seen as patchy on the surface and by the end of the second week after full bloom, the cuticle is complete except for stomata or young lenticels where it shades off to nothing. During August the cuticle starts to invade the epidermal and in some cases even the hypodermal layer of cells and it is difficult to measure its thickness". He approximated its thickness to be about 23 microns.

Tukey and Young (59) reported that the cuticle appears in cross-section as scarcely more than a line at full bloom. Meyer (32), using Sudan III as a specific stain for fatty materials (27), noted that the outer tangential walls of the epidermal cells took a small amount of Sudan III indicating a

deposition of waxy materials. He also observed a slight increase in the cuticle which occurred from full bloom onward, particularly around the hair bases. However, he stated that the cuticle was not distinct and measurable on Golden Delicious until approximately eighteen days following full bloom after which it increased in thickness to 13.18 microns.

Priestly (50), who worked on stems of angiosperm plants stated, "From the outset the essential first stage of a cuticle is present over the apex as a continuous layer of fatty substances overlying the cellulose walls of the outermost cells". He indicated that plant tissues exposed to the atmosphere were covered with a continuous film of oil or fat that could be termed cuticle. Esau (13) presented a similar description of the cuticle coating of exposed plant tissue. These generalizations are in distinct contrast with observations (2, 32, 59) made on apple fruit development. Furthermore, Whaley, Mericle and Heimisch (62) and Mericle and Whaley (31) reported that in the meristematic apex of the shoot of corn (Zea mays L.) they could find no positive indication of the presence of fats in the walls of the meristematic cells. These reports of varied observations still leave room for debate on cuticle development of apple fruits.

The epidermis of McIntosh and Twenty Ounce as studied by Tukey and Young (59) was described as a uniseriate layer of palisade-like cells

which could be identified even one month before full bloom. They observed the cells to be elongated radially until full bloom when the cells began a tangential elongation which continued until maturity. They found that division of epidermal cells continued until even 85 days after full bloom.

According to Bell (2) cell division ceases in the variety McIntosh in Nova Scotia during the middle of June when full bloom was June 2. Tetley (58) reported division in Bramley Seedling on June 12 in material collected near Cambridge, England. The findings of Bell (2) and Tetley (58) were based on observation of mitotic divisions, whereas Tukey and Young (59) made counts of numbers of epidermal cells at various stages of development.

Reports relating to the cell structure of the epidermis give the indication that in certain varieties, the epidermis may remain intact until maturity, while other varieties may have the epidermis interrupted by the cuticle. In the latter case, individual cells or groups of cells of the epidermis may be completely surrounded by cuticle. There may also be interceding variations between these two extremes.

Meyer (32), studying the Golden Delicious, observed that one month after full bloom the epidermis became irregularly interrupted by cuticle which was distinctly different from the regular epidermis of the

Winesap variety at this stage. Verner (60) stated, "In all varieties examined, the cells of the epidermis had pulled apart into small groups and the epidermis had remained intact only by virtue of increase in the cuticle, which had been projected into the interstices formed between the radial walls of the cells that had been separated. In many cases, cells of the epidermis were completely imbedded in cutin and obviously were incapable of further growth. The development of this condition of the epidermis probably is attributed to premature cessation of division and growth in the cells of this layer, with a subsequent separation of these cells as the fruit enlarges". These observations were made on mature fruit. Hedrick (24) and Tetley (57, 58) noted similar conditions.

Work by Simons (55) revealed that the epidermis of Golden Delicious on September 10 in Illinois was very irregular and that the cuticle extended deeply into the epidermal and hypodermal layers. On the Jonathan variety at the same date and in the same location, he observed a continuous layer of uninterrupted cells in the epidermis, and the sub-epidermal cortical cells were much more orderly arranged than in the Golden Delicious. He reported the average cuticle thickness at this date as 10.0 microns for Jonathan and 24.3 microns for Golden Delicious.

The fourth and final region in the protective layers considered

by Bell (2) is the "hypodermis". Tukey and Young (59) describe the hypodermal layer as follows: "The hypodermal layer is part of the cortex immediately beneath the outer epidermis, characterized in the mature fruit by thick-walled collenchyma in which the protoplastic contents have become granular in nature and are less densely pigmented. Together with the epidermis it forms the skin of the fruit". The authors (59) observed cell walls of the outer cortical cells one or two cell-layers below the epidermis as becoming thickened by full bloom with this thickening increasing as the fruit develops. Furthermore, they state that these cells are small and easily defined but the numbers of cell-layers in thickness is not easily determined. Bell (2) also had difficulty in determining the numbers of cell layers in the hypodermis due to the noted irregularity.

Various histological descriptions of russeted tissue and its development have been advanced. Zschokke (64) was the first to examine microscopically, cork formation on apple fruits. Although his work was primarily concerned with lenticel formation, he did observe that the formation of cork on the normally russeted varieties of apples started about six to eight weeks after flowering. He commented on the presence of epidermal hairs and in some instances cork formation was observed at the bases of the hairs. This he classified as a type of lenticel formation

but these lenticels were not like typical lenticels. Nevertheless, he considered that they were functionally comparable. Typical lenticels, as observed by Zschokke (64), were considered to originate from numerous stomata on the surface of a young apple fruit and which became sealed by the subsequent formation of cork below the stomatal orifice.

Hedrick (24) examined russeted tissue caused by bordeaux injury. He observed that early russeting usually started at the basal cells of the trichomes (epidermal hairs) which cover the young fruit or at the stomata which are interspersed between the hairs. He postulated that the trichomes, through some peculiarity of structure, permit the entrance of the toxic substance of the bordeaux mixture to a greater extent than do other cells while the stomata give an almost unobstructed entrance. Hedrick (24), like Zschokke (64), observed the injury on the fruit approximately six weeks after petal fall. Hedrick (24) also found the cuticle proper and many of the cells of the epidermal layer destroyed and it appeared to him that the toxic substance entered the cells themselves and effected only those in which the toxic compound entered. He concluded that cork formation or russeting was a healing process below these injured cells similar to russeting which occurs when a fruit is rubbed against a branch.

The diffused type of russeting found on the Grimes apple by Baker (1) was attributed to possible spray injury, high atmospheric humidity,

cool weather at blossoming time, frost injury, sulfur-dioxide in the air or a combination of these factors. He described russet formation as developing when the epidermis or the cuticle is injured with the mature cells immediately below the epidermis (hypodermal cells) reverting to meristematic activity. This is followed by the formation of a phellogen or cork cambium which subsequently gives rise to the russeted or corky area.

Histological studies of cork formation were made also by Tetley (57) in England. She, too, observed cork formation beginning at the base of the trichomes in the sub-epidermal cells. She felt that the hair bases evidently represented weak places on the surface of the apple for any crack in the skin nearly always began in this region. However, she noted under certain weather conditions cracks occurred with no special reference to lenticels or bases of hairs. These conditions, she reported, were associated with a long dry, cold period when the fruit was setting which was followed by a warm, rainy period when the apple was ready to swell. This cold period, according to Tetley (57), produced a comparatively thick and unelastic type of cuticle with the result that the epidermis was unable to resist the rapid swelling of the cells within during the warm rainy period. Thus, subsequent cracking resulted.

Studies reported by Bell (3) on the origin of russeting of

the Golden Russet apple, indicated that periclinal divisions occur in the epidermis of this variety near full bloom and subsequently, an epidermal layer of 2 to 4 cells in thickness results. Early in July, the innermost layer of these cells continues meristematic activity forming a very active cambium which in turn forms cork centrifugally, giving the typical russeted skin of this variety.

Bell later (4) studied russeting of the McIntosh Red variety resulting from bordeaux spray applied at about the time of full bloom. He concluded that the first apparent injury occurred from 10 to 15 days after the spray application, which was identified as a browning of the epidermal cells at the base of the trichomes. Because continued growth of these cells was inhibited, cracking resulted from subsequent fruit enlargement which in turn exposed adjacent hypodermal and cortical tissue. This initiated an immediate series of periclinal divisions in the hypodermal cells beneath the trichome elevating the injured tissue above the remainder of the epidermis.

As the fruit continued to enlarge, he related (4), the cracks multiplied, extended tangentially and deepened, resulting in further browning of cells and a deeper formation of meristematic activity in the cortex. Eventually, a cork cambium became evident. He postulated that the final scurf-like patches of scar tissue were a mixture of dead epidermis, hypodermis, cortex, cork, and cork cambiums and therefore were not true cork but "rhytidome".

MacDaniels and Heinicke (29) indicated that the cork comes from a true cork cambium which originates sub-epidermally after lethal freezing of the epidermal cells.

Studies on mature russeted fruit by Verner (60) lead one to believe that russeting occurs following injury to the cuticle. He stated, "A layer of cork cambium assumes the position normally occupied by the epidermis, cutting off to the outside several or many tangential layers of cork cells which constitute the scurf-like russet. No phelloderm could be observed inside the cork cambium and the hypodermis appeared normal below this meristematic region".

Very recently, Simons (55) conducted studies of russeted tissue of Golden Delicious caused by severe freezing temperatures. His earliest observations were made on sections cut approximately 5 weeks after full bloom. He noticed some disruption of epidermal cells and some periclinal divisions below the injured area. One month after this date, meristematic activity had taken place between the normal and injured tissues producing a "periderm" several cell-layers thick. "Periderm" continued to be formed throughout growth and maturity of the fruit. Simons (55) stated that the cuticle varied in thickness from 8.5 to 25.6 microns in July, reached a variable thickness of 12.1 to 29.7 microns

by September, after which time it decreased to a thickness varying from 8.3 to 25.9 microns at maturity.

As noted by the review of literature, a number of studies have been made on russeting of fruit and formation of cuticle and corky tissue. No studies were found that clearly showed the interaction of cold injury and the use of certain pesticides on fruit russeting as suggested by Mitchell (33). Also, no published information was available explaining how russeting occurs following the use of certain fungicides after a drop in air temperature to 32° F or lower close to the time of bloom. There are questions as to where and how rapidly the cuticle develops on the fruit of the apple and the varietal differences that may be present with respect to this protective coating. Therefore, a study was initiated to develop a better understanding of some of these points in question.

MATERIALS AND METHODS

Experiment 1. Controlled temperature studies to determine the relationship of freezing air temperatures and fungicides on fruit russeting.

In April of 1956, wooden frames of two by four inch lumber were constructed around bearing, four-year-old apple trees growing on Malling VII rootstocks located at the Michigan State University Horticultural farm, East Lansing, Michigan (Figure 1). The tops of the cages were covered with 0.002 inch polyethylene[1/] fixed permanently in place with wood-lath strips. The sides were fitted with removable panels covered with the same type of polyethylene. Two trees each of the varieties Jonathan and Golden Delicious were enclosed and a remaining tree of each variety was exposed to unaltered environmental conditions to serve as a control. All trees were in good bearing vigor growing in a trashy cultivation type of soil management.

One cage enclosing each variety was equipped with a thermostatically controlled refrigeration unit. Each unit was composed of an eight-ounce Tecumseh type F-1 compressor, operated with a three-fourths horsepower motor, a product of the Tecumseh Manufacturing Company, Tecumseh,

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[1/] 0.002 inch polyethylene obtained through the courtesy of the Bakelite Division of Union Carbide Corporation.

Figure 1. Polyethylene cages with removable panels enclosing apple trees growing on dwarfing rootstocks.

Michigan. The coil units were Model WJ45 of the Bush Manufacturing Company of Hartford, Connecticut and contained a large fan for air circulation. The thermostates were type T491A1X3 "Air Switch", manufactured by Minneapolis-Honeywell Regulator Company, Minneapolis, Minnesota. Small electrical, fan equipped, General Electric space heaters were available for each cage when heat was necessary. The refrigeration units were designed to lower the night temperature in the cages to a desired temperature below 32° F when necessary and the heaters were used to prevent the temperature within the cages from dropping lower than was desired. The removable side panels were placed on the cages only during those nights when temperature treatments were being made, or when the air temperature was predicted to drop to 32° F or lower. In the latter case, one tree of each variety was enclosed and heated while the other tree remained exposed to the freezing temperatures. The trees were exposed at other times during the growing season.

The trees were pruned to divide the bearing surfaces into two distinct halves so that two fungicide treatments could be evaluated in the same environment.

Because of unfavorable pollination weather during the bloom period of Jonathan, only Golden Delicious, which blossomed later during more favorable conditions, was used in the studies in 1956.

The treatments expressed as amounts per 100 gallons of spray mixture were as follows:

Three applications of Phix at four ounces plus wettable sulfur at three pounds were made uniformly on all trees through delayed dormant. Beginning with early pre-pink, eight applications of the comparison treatments of captan at two pounds and glyodin at one fluid quart were made on one-half of each of the three Golden Delicious trees. The captan served as the control treatment. Beginning at petal fall, two pounds of methoxychlor was used uniformly in the last four fungicide applications. Five additional sprays of two pounds of methoxychlor were necessary in July and August for insect control. A separate single application of 25 percent wettable malathion at two pounds plus four fluid ounces of Triton B-1956 as a wetting agent was made on June 19 between the second and third cover sprays to control green apple aphids (Aphis pomi, DeGeer).

The three applications prior to early pre-pink were made with a John Bean No. 40 Speed Sprayer (the John Bean Division of Food Machinery and Chemical Corporation, Lansing, Michigan). All subsequent applications were made with a Myers Silver Prince motor-powered wheelbarrow sprayer (F. E. Myers Brothers Company, Ashland, Ohio) operating at 150 pounds pressure equipped with an adjustable nozzle hand gun. A canvas curtain was used to separate the halves of each tree while applying the fungicide

treatments to avoid contamination from drift.

Maximum and minimum temperatures were recorded inside the cages with Taylor thermometers (Taylor Instrument Company, Rochester, New York) placed in the central portion of the tree. Continuous recording of temperature and humidity was obtained with a Friez model 594 Hygro-thermograph (Friez Instrument Division, Bendix Aviation Corporation, Baltimore, Maryland), which was placed in each cage near the edge of the tree approximately five feet from the ground.

At harvest time, all the fruits from each treatment were picked and the finish of the fruit was evaluated and rated as free of russeting, lightly russeted, or heavily russeted. Fifty to seventy-five fruits were evaluated from each half-tree treatment.

Light russeting refers to the amount of russeting allowable in the grade of U. S. No. 1, or higher, and as described by the Michigan Apple Marketing Law Act 132, P. A. 1937: Any amount is permitted in the stem cavity or calyx basin which cannot be seen when the apple is placed on a flat surface except when excessively rough or barklike or when the appearance is materially affected. Amounts permitted outside cavities:

1. Smooth, netlike; total area of 25 percent of surface, if pattern and color show no marked contrast with background color.

2. Smooth, solid; total area 25 percent of surface, if pattern and color show no marked contrast with background.

3. Slightly rough; total area one-half inch in diameter.

4. Rough; one-quarter inch in diameter.

Heavy russeting disqualifies the fruit for the grade of U. S. No. 1 or higher. Free of russeting being self-explanatory, Figure 2 gives an example of light and heavy russeting.

To further evaluate the effects of glyodin and captan on russeting of Golden Delicious under field conditions, a thirty-year-old tree located approximately 500 feet from the cage trees, was divided into two equal halves for the two spray treatments.

The spray chemicals, the amounts used and the dates of application were the same as for the cage trees. However, an additional separate application of EPN at one pound per 100 gallons of spray mixture was necessary in August to control two-spotted spider mite (Tetranychus bimaculatus, Harvey) and European red mite (Paratetranychus pilosus, Canestrini and Fanzago).

The applications were made using an adjustable nozzle gun attached to a John Bean No. 26 Speed Sprayer equipped with a 15-gallon per minute pump operating at 400 pounds pressure. They were made on days when the wind was relatively calm and the tree was sprayed to prevent drift from one treatment to the other.

Figure 2. Apple fruits representing light and heavy russeting. The apple on the left shows the maximum allowable amount of russeting that can be termed "light russeting". The apple on the right illustrates a heavily russeted apple.

At harvest time, fruit from the central portion of each one-half of the tree sprayed with the separate fungicides were picked and evaluated for russeting as previously described. Approximately 700 apples were examined from each treatment.

Experiment 2. Field studies to evaluate the effects of fungicides and freezing air temperatures on fruit russeting of apples.

A mixed block of sixteen-year-old apple trees at the Michigan State University Graham Experiment Station, Grand Rapids, was selected for field studies in 1956. The block included Golden Delicious, Jonathan and McIntosh, and was divided to provide five single tree replicates of each variety for four pesticide treatments. The fungicides used as the treatments were (1) thiram, (2) glyodin, (3) captan, and (4) captan followed by glyodin after first cover. The spray program expressed as amounts per 100 gallons of spray mixture was as follows:

Two applications of liquid lime-sulfur at eight quarts were made uniformly on all trees prior to pre-pink. Two succeeding treatments before full bloom consisted of captan at two pounds, thiram at two pounds, and glyodin at one quart. After bloom, seven sprays of captan at one pound, thiram at one pound, and glyodin at one pint were made on the treatment plots. Phygon at four ounces was included with each of the treatment fungi-

cides in the petal fall, first, and third cover sprays. One pound of parathion was used in the second and third cover sprays with the third cover receiving in addition, one pound of DDT. On June 28, all treatments received an extra spray of aramite at one pound, Ovotran at one-half pound, and DDT at one and one-half pounds with no fungicides being used. The last three cover sprays included with the fungicide treatments, lead arsenate at two pounds with the last cover receiving in addition, DDT at one and one-half pounds. When lead arsenate was included with glyodin, ferrous sulfate at one-half pound was added to prevent possible arsenical injury.

Another block of seven-year-old apple trees was divided into three plots. Each of these plots was divided into sub-plots so as to contain five trees of each of the two varieties, McIntosh and Jonathan. One sub-plot of each plot was treated with captan and the remaining sub-plots of the plots included glyodin, thiram and ferbam as the spray treatments. The amounts and times of application of spray materials used in these plots were the same as in the block of sixteen-year-old trees, except for the added ferbam treatment. Ferbam was used at one and one-half pounds per 100 gallons prior to bloom, and was decreased to three-fourths of a pound beginning with petal fall.

The spray materials of all treatments at the Graham Experiment Station were applied with a Robinson air-blast attachment on a Hardie sprayer

equipped with a thirty gallons per minute pump operating at 600 pounds pressure.

Air temperatures were obtained from thermograph records of the station located near the test orchards. The elevation of the spray plots and recording instrument did not vary greatly.

At harvest time a bushel of fruit was picked randomly from each single tree replicate in each treatment. This fruit was examined and russeting records were taken, as described in Experiment 1.

Experiment 3. Controlled temperature studies in 1957 to further evaluate the influence of fungicides and freezing air temperatures on fruit russeting.

The same trees and cages as described in Experiment 1 were used in these studies except, due to unfavorable weathering of the polyethylene, the tops of the cages had to be replaced. A clear plastic film .010 inch thick of "Polyflex II" produced by Plax Corporation, Hartford, Connecticut was used to recover the tops. The removable side panels remained covered with the .002 inch polyethylene. The refrigeration and heating units were the same as previously described.

The trees were pruned to divide the bearing surfaces into two distinct halves so that two fungicide treatments could be evaluated in the same environment.

The Jonathan and Golden Delicious had sufficient fruit on the trees to be used for fungicide treatments. The amounts of fungicides used in the treatments on Golden Delicious expressed as amounts per 100 gallons of spray mixture were as follows:

At the green-tip stage, Phygon at one-half pound was applied uniformly on all trees. Wettable sulfur at three pounds plus four ounces of Phix was used at delayed dormant. Starting with the pink stage of development, one-half of each of the three trees was sprayed with glyodin at one quart. The remaining one-half of each tree was sprayed with glyodin at one pint, plus four ounces of Phygon. These amounts were continued for eight applications (through fourth cover), after which the trees were sprayed uniformly for two more cover sprays with glyodin at one pint. Methoxychlor at two pounds was included uniformly in all mixtures starting with first cover. Two additional sprays of malathion at two pounds plus methoxychlor at two pounds were necessary for insect control after the fungicide applications were completed. A fourth Golden Delicious tree outside of the cages was sprayed with captan at the same time as these three trees just discussed. This served as a control treatment to be compared with the results of Experiment 1 conducted in 1956.

The treatments on the three Jonathan trees expressed as amounts per 100 gallons of spray mixture were as follows:

At the green-tip stage, Phygon at one-half pound was used uniformly on all trees. Wettable sulfur at three pounds plus four ounces of Phix was used on all trees at delayed dormant. Starting with pre-pink, half of each of the three trees was sprayed with glyodin at one quart and the other half with captan at two pounds. These treatments were continued for eight more sprays or through fourth cover. In the fifth and sixth cover sprays, captan was reduced to one pound and glyodin to one pint. Methoxychlor at two pounds was added uniformly to all treatments starting with first cover for insect control. Two additional sprays of methoxychlor at two pounds and malathion at two pounds without the fungicides were necessary during July and August for insect control.

The two applications prior to pre-pink on both varieties were made with a John Bean No. 26 Speed Sprayer. All subsequent applications were made with the motor-powered wheelbarrow sprayer described in Experiment 1. Here also a canvas curtain was used to separate the halves of each tree while applying the fungicide treatments to avoid contamination from drift.

Temperatures were recorded with the use of a thermocouple placed within a blossom cluster in the central portion of each tree.

A continuously operating Minneapolis-Honeywell recorder was connected to the thermocouples so that temperatures could be recorded

continuously from each tree throughout the period of delayed dormant through second cover. A Friez hygrothermograph was also used in a small weather house among the trees to keep a seasonal record of air temperatures. As a further check, Taylor maximum-minimum thermometers were operated within the center of each tree of the study.

At harvest time, the fruit from each treatment was picked and evaluated for the presence of surface russeting, as described in Experiment 1.

Experiment 4. Further field studies to determine the effects of fungicides and freezing air temperatures on fruit russeting.

The same block of young apple trees of Jonathan and McIntosh at the Michigan State University Graham Experiment Station that was used in 1956 was again used in 1957 for further evaluations. The main block was again divided into three plots. These plots were divided into two sub-plots of five single-tree replications of each variety. One sub-plot of each plot received captan as a control treatment and the three other sub-plots were sprayed with thiram, glyodin and wettable sulfur as the fungicide treatments.

The spray program expressed as amounts per 100 gallons of spray mixture was as follows:

Two applications of liquid lime-sulfur at eight quarts were made uniformly on all trees prior to pre-pink. Starting at pink, the treatments were thiram at one and one-half pounds, glyodin at one quart, wettable sulfur at six pounds, and captan at two pounds. This rate was used for four applications (through second cover) after which the amounts were reduced by one-fourth for five additional sprays. Phygon at four ounces was added to all treatments at petal fall, and Phix at four ounces was used uniformly at first cover. Starting with second cover, parathion at one pound, and DDT at one pound was added to all treatments.

A separate single application of DDT at one and one-half pounds was made in August for insect control. Lead arsenate at two pounds was used in the fifth and sixth cover sprays in place of parathion. When lead arsenate was used in the glyodin treatment, ferbam at one-half pound was added to prevent possible arsenical injury.

All spray applications were made with a Robinson air-blast attachment on a Hardie sprayer equipped with a thirty-gallons per minute pump operating at 600 pounds pressure.

Air temperatures were obtained from the thermograph records of the station located near the test block. The elevations of the spray plots and the recording instrument were similar.

At harvest time, a bushel of fruit was picked randomly from each single tree replication in each treatment and examined for possible russeting as described in Experiment 1.

Histological Investigations

Experiment 5. Histological investigations to study the development of the protective layers of Golden Delicious, Jonathan, and McIntosh with special attention given to the cuticle, and to study the development of russeted tissues of the fruit.

Flowers and fruits selected for this study were taken from thirty-year-old bearing Golden Delicious, Jonathan, and McIntosh apple trees located at the Michigan State University Horticultural farm, East Lansing. All trees received a uniform spray treatment of captan, DDT and parathion throughout the growing season. Blossom clusters from which samples were collected were from spurs on vigorous three- or four-year-old wood located on the south side of the tree at a height of five to seven feet. Four complete flower clusters were taken twice weekly from each variety from the first stage of bud swelling to pre-pink. After pre-pink the central blossom of four clusters were collected twice weekly from each variety until one month after full bloom. The succeeding samples were collected weekly until harvest time.

Immediately after removal from the tree, the plant material was placed in labeled bottles containing a killing and fixing solution of five milliliters of formalin, five milliliters of glacial acetic acid and ninety milliliters

of seventy percent ethyl alcohol. The material was then placed in a large evacuator and held under vacuum for approximately three hours. The vacuum placed on the material was increased gradually to prevent damage to the tissue structure. After the material was evacuated, the original solution of formalin-acetic acid-alcohol was replaced with a fresh solution. The material was stored for varying periods of time before embedding in tissuemat.

Other samples of fresh flowers and fruits, selected in the manner just described, were placed in labeled polyethylene bags, sealed, and stored at 0° F for future studies of fresh material using the freezing microtome.

The killed and fixed material was dehydrated using the tertiary butyl alcohol method as described by Johansen (27) and embedded in Fisher tissuemat having a melting point of 54-56° C. After embedding, the material was stored in the freezing compartment of a refrigerator for two days to prevent crystallization of the paraffin. The material was then sectioned using a Model 820 American Optical Company rotary microtome. The sections were cut six to ten microns in thickness, depending upon the age of the plant material and the relative size of the cells. Both transverse and longitudinal sections were made of the developing fruit or flower receptacles. Serial sections were then affixed to standard microscope slides using Haupt's adhesive (27).

The paraffin was removed from the sections with xylol and the sections were stained with Safranin and Fast Green as outlined by Johansen (27). The sections were mounted in clarite, a synthetic resin, and allowed to dry for two days prior to microscopic examination.

The frozen material was removed from the 0° F storage and placed into a saturated absolute ethyl alcohol solution of Sudan IV for 10 minutes. The excess stain was then rinsed off with 95 percent ethyl alcohol and the plant material was placed immediately in a ten percent gelatin solution on the stage of a Model 880 American Optical Company freezing microtome. The plant material was then refrozen and transverse sections cut at six microns in thickness.

The sections were mounted in water on standard microscope slides, a cover-slip placed on them, and the excess water blotted off. The edges of the cover-slip were then sealed with clear fingernail polish and allowed to dry for approximately fifteen minutes before examination.

All microscopic examinations were made with a Zeiss 250190 microscope. The permanently prepared material was examined as serial sections to obtain a true prospective of the protective layers of the fruit.

Photomicrographs were taken with an Exakta VX camera with a microscope adapter. A type 31-33-26 Bausch and Lomb Optical Company microscope light was used as the light source including a ground glass

filter (39375) and a blue glass filter (39370) between the light source and sub-stage mirror of the microscope. Kodak Plus-X film was used for taking black and white photomicrographs and Kodachrome (daylight type) film was used for taking colored photomicrographs.

Comparative observations were made on the three varieties studied relative to cuticle development, trichome structure and method of trichome elimination from the fruit surface, type of epidermal and hypodermal layers, and the progressive changes that occur in these tissues as the fruit enlarges.

The fruits used in the histological study of the development of russeted tissues were collected from the thirty-year-old Golden Delicious apple trees receiving the glyodin treatment as described in Experiment 1. Samples were collected weekly starting two weeks after full bloom and continuing until harvest maturity. The procedures for preparing the material for microscopic examination were the same as previously described for the permanently mounted sections. Photomicrographs were taken also, as previously described.

Observations were made relative to time of the earliest recognizable russet development, the actual formation of russeted tissue and the final type of tissues resulting in the russeted areas.

To more fully evaluate the role of injury of trichomes in fruit

russeting, macroscopic studies were conducted on large thirty-year-old Golden Delicious and Jonathan apple trees growing on the Michigan State University Horticultural farm. A minimum of ten flowers at each of the stages, pink, full bloom, and petal fall were selected for this study. With the aid of a hand lense and sharp-pointed tweezers, the fine mat of trichomes on each flower receptacle was removed in the form of distinct narrow bands running parallel to the long axis of the flower. Every precaution was made to prevent the tweezers from actual contact with the epidermis of the receptacles.

Another similar series of flowers had portions of the trichome mat touched with a block of dry ice (solid carbon dioxide) to a point that the tips of the trichomes became curled and brown in color. The dry ice did not come in contact with the epidermis so that only injury to the outer portion of the trichomes occurred.

The flowers in each cluster that were not treated were removed and the fruiting spur was properly tagged as to type of treatment, stage of blossom development at the time of treatment, and date of treatment.

The fruits were examined weekly for the first six weeks and then monthly thereafter for signs of russet development. A final check was made at fruit maturity.

The trees were sprayed with two pounds of captan plus one pound of DDT plus one pound of parathion per 100 gallons of spray mixture for insect and disease control throughout the growing season.

RESULTS

Experiment 1. Controlled temperature studies to determine the relationship of freezing air temperatures and fungicides as affecting fruit russeting.

The various air temperatures to which each Golden Delicious tree on Malling VII rootstocks was exposed are given in Table I. One tree was enclosed during periods when air temperatures of 32° F or lower were expected and the tree heated to prevent exposure to freezing air temperatures while the other tree was exposed. The third tree was entirely exposed to unaltered environmental conditions. During the early morning hours of May 16, 20, and 24 freezing temperatures of 31.7°, 31.5° and 28.0° F respectively, were recorded on thermometers and thermographs placed within the fruiting portion of the exposed trees (Table I). The tree selected for protection from these temperatures was enclosed during the nights when these temperatures occurred and heated with electrical space heaters. The air temperatures of the enclosed tree did not drop below 32.5° F (Table I). The 28° F recorded on May 24 lasted for approximately 45 minutes while the other freezing temperatures recorded were for shorter durations.

Records of russeting of the harvested fruit were obtained from the total numbers of fruits from each half-tree treatment which varied from 50 to 75 fruits. The data of the fruit russeting resulting from the two factors, fungicides and freezing air temperatures are presented in Table II.

TABLE I

Minimum Air Temperatures Recorded Within Fruiting Area of Protected and Exposed Trees of Golden Delicious - 1956

Date	Exposed Trees	Protected Trees
	Minimum Temperature (Degrees F)	Minimum Temperature (Degrees F)
May 16 (Pink)	31.7	32.5
May 20 (Early Bloom)	31.5	34.5
May 24 (Early Petal Fall)	28.0	33.0

TABLE II

Russeting of Golden Delicious Apples as Related to the Use of Certain Fungicides and Exposure to Freezing Temperatures or Protected from Freezing Air Temperatures. East Lansing, 1956

Tree	Treatment	Free of Russeting (Percent)	Light Russeting (Percent)	Heavy Russeting (Percent)
Cage tree,	Exposure to above 32.5° F			
	Captan	56.8	43.2	0.0
	Glyodin	0.0	72.2	27.8
Cage tree,	Exposure to below 32° F			
	Captan	2.7	72.9	24.4
	Glyodin	0.0	15.5	84.5
30-year-old,	Exposure to below 32° F			
	Captan	9.9	64.6	25.5
	Glyodin	0.0	3.5	96.5

The combination of glyodin and freezing temperatures resulted in 84. 5 percent heavily russeted fruit, while on the same tree the use of captan resulted in only 24. 4 percent heavily russeted fruit. All of the remaining fruit from this tree, except 2. 7 percent of the fruit free of russeting from the captan treatment, were lightly russeted, 15. 5 percent for the glyodin treatment, and 72. 9 percent for the captan treatment. It should be re-emphasized that the fruit classified as lightly russeted were acceptable for U. S. No. 1 and Fancy grades.

Of the fruit from the tree protected from the freezing air temperatures and receiving the glyodin treatment, 27. 8 percent were heavily russeted and 72. 2 percent were lightly russeted. The captan treated portion had no heavily russeted fruits, 43. 2 percent were only lightly russeted, and 56. 8 percent were entirely free of russeting.

A relative comparison of the fruit of the two fungicide treatments from trees exposed to freezing temperatures is illustrated in Figure 3. Examples of fruits from the protected tree receiving the same two treatments are shown in Figure 4.

The third Golden Delicious tree exposed to unaltered environmental conditions had insufficient fruits for evaluation.

The records of russeting of the harvested fruit of the thirty-year-old bearing Golden Delicious tree which was divided into two equal halves,

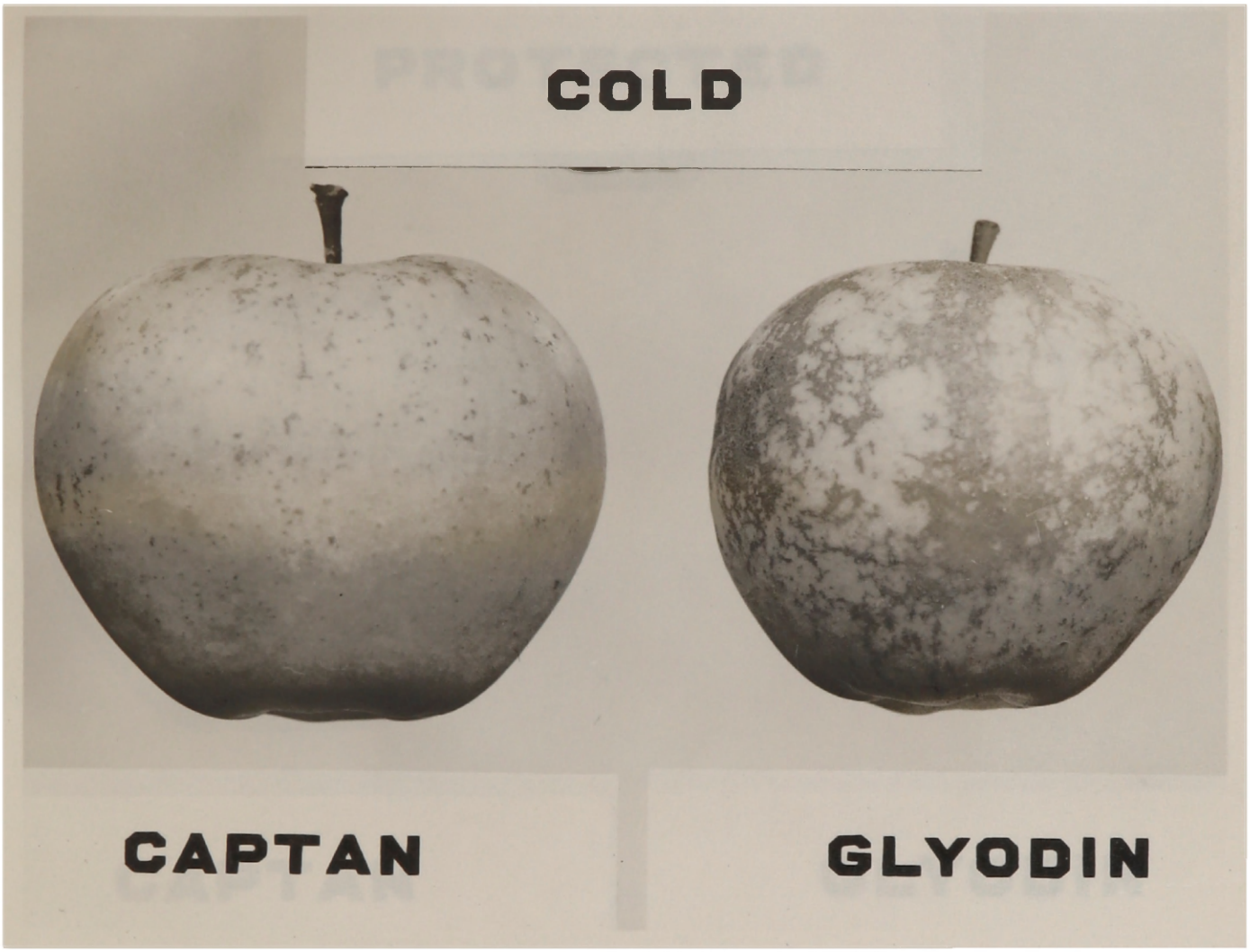


Figure 3. A representative sample of apples taken from the trees exposed to freezing air temperatures and sprayed with captan and glyodin.

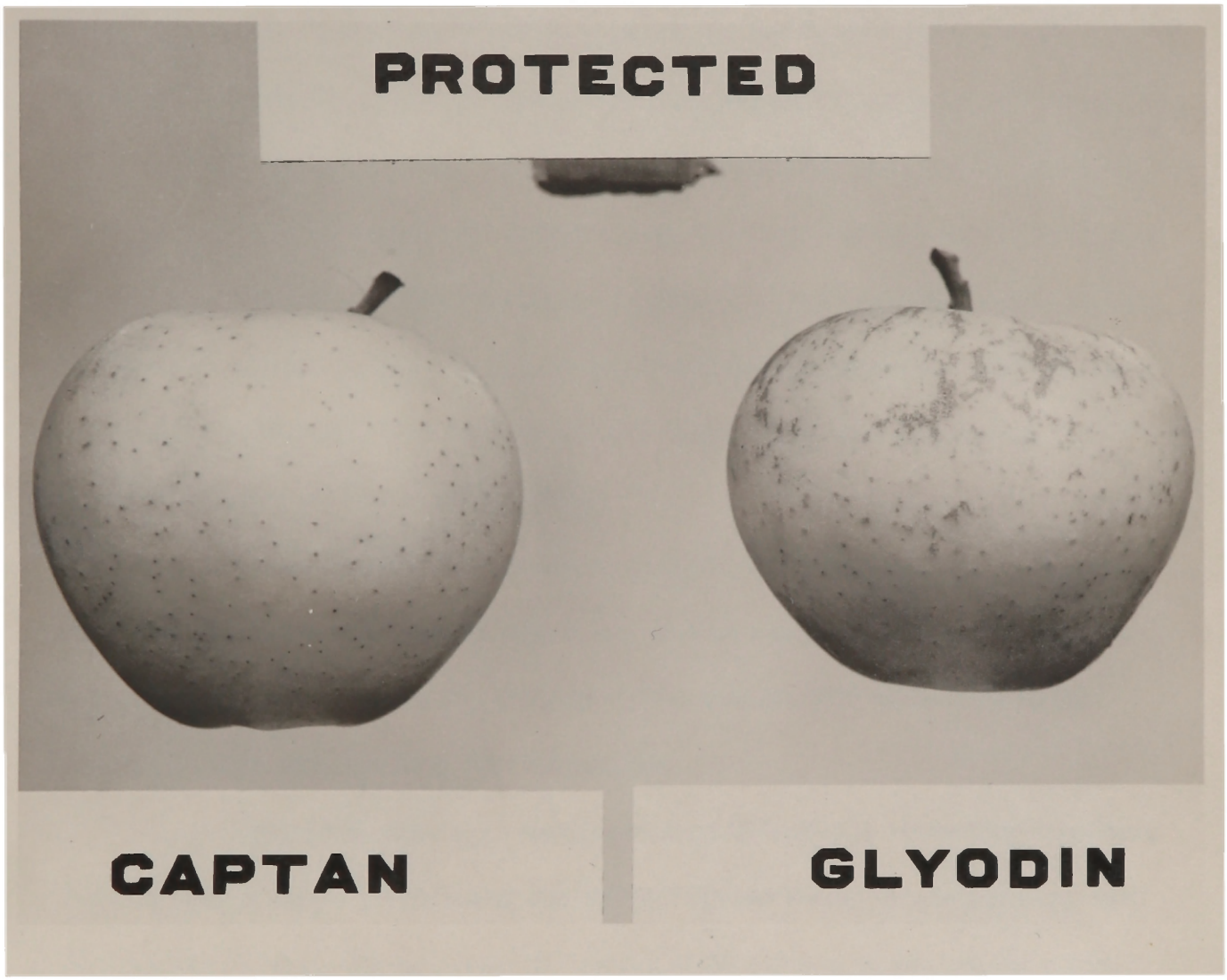


Figure 4. A representative sample of apples taken from the tree protected from freezing air temperatures and sprayed with captan and glyodin.

one-half sprayed with glyodin and the other captan, are presented also, in Table II. These data agree closely with the results obtained from the exposed tree on Malling VII rootstocks and receiving the freezing air temperatures (Table II). In a seven-bushel sample of the harvested fruit from the portion of the tree treated with glyodin, 96.5 percent were heavily russeted and 3.5 percent were lightly russeted, while in a seven-bushel sample from the captan treatment, only 25.5 percent were heavily russeted and 64.6 percent lightly russeted. Furthermore, the captan treated portion had 9.9 percent of its fruits free of russeting.

Because of the close proximity of this tree to the cage trees and since the elevation was nearly identical, it was assumed that freezing temperatures of very close to 31.7° F, 31.5° F, and 28.0° F occurred in the location of this tree on May 16, 20 and 24.

From these data it is demonstrated that slight russeting resulting from the occurrence of freezing air temperatures early in the growing season, during or near bloom, may be increased to heavy russeting of a major economic importance by the use of certain pesticide chemicals.

Experiment 2. Field studies to evaluate the effects of fungicides and freezing air temperatures on fruit russeting of apples.

The findings of the treatments thiram, captan, glyodin, and captan followed by glyodin after first cover, made in the block of sixteen-year-

old Jonathan, Golden Delicious, and McIntosh apple trees are given in Table III. In this study, five single tree replications of each variety were included in each treatment.

A single freezing temperature of 30.5° F of a thirty-minute duration occurred on May 16 when the blossoms were in the early-pink stage.

The results from examinations of the harvested fruit, given in Table III, revealed no significant differences of fruit russeting between treatments. The use of captan through first cover followed by glyodin was an attempt to determine if glyodin would cause russeting if used on Golden Delicious after first cover. However, as no differences were noted between any of the treatments and the use of glyodin did cause russeting on Golden Delicious as shown in Table II, it was conjectured that method of application of the chemicals probably played a very important role as to whether or not a pesticide chemical was phytotoxic.

The results of fruit russeting from the treatments of glyodin, thiram, captan, and ferbam, in Blocks I, II and III at the Graham Experiment Station are given in Table IV. There were no significant differences in heavy russeting between treatments for any of the varieties. There were significant differences, however, in light russeting from the various spray treatments in Block I between the use of captan and ferbam on Jonathan, 11.54 percent for the captan treatment as compared to 5.8 percent for the ferbam

TABLE III

Russeting of Jonathan, Golden Delicious, and McIntosh from the Use of Certain Fungicides after the Occurrence of 30.5° F for 30 Minutes. Graham Experiment Station, 1956

Variety	Chemical Treatment	No Russeting (percent)	Light Russeting (percent)	Heavy Russeting (percent)
Jonathan	Captan	97.85	0.95	1.20
	Thiram	97.35	1.33	1.32
	Glyodin	95.55	2.78	1.67
	Captan followed by Glyodin	96.71	2.12	1.17
Golden Delicious	Captan	96.86	3.14	0.00
	Thiram	96.73	2.39	0.88
	Glyodin	95.90	2.54	1.56
	Captan followed by Glyodin	98.67	0.83	0.50
McIntosh	Captan	90.94	4.67	4.39
	Thiram	93.97	2.93	3.10
	Glyodin	91.72	3.37	4.91
	Captan followed by Glyodin	90.52	3.86	5.62

No significant differences between treatments.

TABLE IV

Russeting of Jonathan and McIntosh Varieties from the Use of Certain Fungicides Following the Occurrence of 30.5° F for Thirty Minutes. Graham Experiment Station, 1956

Variety	Block	Chemical Treatment	No Russeting (percent)	Light Russeting (percent)	Heavy Russeting (percent)
Jonathan	I	Captan	80.74	11.54* †	7.72†
		Ferbam	90.05	5.80*	4.15
	II	Captan	96.54	1.05	2.41
		Glyodin	95.86	2.17	1.97
	III	Captan	94.37	2.89	2.74
		Thiram	94.91	3.10	1.99
McIntosh	I	Captan	95.70	1.48	2.82
		Ferbam	96.83	1.90	1.27
	II	Captan	96.61	1.79	1.60
		Glyodin	97.57	1.09	1.34
	III	Captan	97.70	1.27	1.03
		Thiram	96.59	1.40	2.01

*Differences between treatments significant at the 5 percent level.

†Captan treatment of Block I significantly higher than Captan treatments of Blocks II and III of the Jonathan variety.

treatment. However, it should be pointed out that the portion of Block I receiving the captan treatment was lower in elevation than that portion receiving the ferbam treatment or the other blocks in this study. It is very probable that in this area a lower temperature than the recorded 30.5° F could have occurred, which may account for the higher amount of russeting of the Jonathan variety in the captan treatment. The amount of both light and heavy russeting found on Jonathan in the captan treated portion of Block I was significantly higher than that found in the captan treated portions of Blocks II and III (Table IV).

Experiment 3. Controlled temperature studies in 1957 to further evaluate the relationship of fungicide and freezing air temperatures on fruit russeting of Jonathan and Golden Delicious.

The data on fruit russeting obtained from the half-tree treatments of captan and glyodin on Jonathan exposed to different air temperatures near bloom are given in Table V. Variations in fruit russeting on Golden Delicious from half-tree treatments of glyodin at full strength (one quart) and of glyodin at half-strength (one pint) plus one-fourth pound of Phygon are presented in Table V.

Tree number one of the two varieties was exposed to only one hour of a low of 32.0° F for the Jonathan and 31.0° F for the Golden Delicious, while tree number two of both varieties was exposed to a period of four

TABLE V

Russeting of Jonathan and Golden Delicious Apples Treated with Certain Fungicides Following Exposure to Varying Amounts of Freezing Temperatures. East Lansing, 1957

Variety	Tree No.	Chemical Treatment	No Russeting (percent)	Light Russeting (percent)	Heavy Russeting (percent)
Jonathan	1	Captan	78.05	19.51	2.44
		Glyodin	48.53	48.53	2.94
	2	Captan	80.39	15.69	3.92
		Glyodin	45.28	47.17	7.55
	3	Captan	37.93	46.55	15.52
		Glyodin	4.88	28.05	67.07
Golden Delicious	1	Glyodin	0.00	3.70	96.30
		Glyodin-Phygon	0.00	60.00	40.00
	2	Glyodin	0.00	25.71	74.29
		Glyodin-Phygon	0.00	26.09	73.91
	3	Glyodin	0.00	0.00	100.00
		Glyodin-Phygon	0.00	0.00	100.00
	4	Captan	0.00	4.00	96.00

hours and twenty minutes of freezing temperatures ranging from 29.5° F to a minimum of 27.5° F. Tree number three was exposed on four nights when minimum air temperatures of 32° F or lower were recorded. The stages of flower and fruit development at the time each freezing air temperature was recorded are given in Table VI.

An added single Golden Delicious tree was sprayed with captan and was also exposed to the four nights of freezing air temperatures given in Table VI.

The portions of the three Jonathan trees receiving the glyodin treatment had a progressively increasing amount of fruits exhibiting heavy russeting, 2.94 percent, 7.55 percent, and 67.07 percent, respectively, which corresponded with the progressively increasing amounts of exposure to freezing air temperatures near bloom. The percentage of fruits exhibiting heavy russeting recorded from the use of captan increased also with increasing exposure to freezing air temperatures. However, the percentages of russeted fruit from the captan treatments were smaller than from the glyodin treatments. To illustrate, from the tree exposed to one night of 32.0° F, only 2.44 percent of the fruit from the captan treatment and 2.94 percent of the fruit from the glyodin treatment were heavily russeted. The tree exposed to the minimum temperature of 27.5° F had 3.92 percent of the fruit from the captan treatment heavily russeted as compared to 7.55

TABLE VI

Minimum Air Temperatures Recorded Within the Fruiting Area of the Trees of Jonathan and Golden Delicious Growing on Dwarfing Rootstocks. East Lansing, 1957

Date	Tree No.	Jonathan Minimum Temperature (Degrees F)	Golden Delicious Minimum Temperature (Degrees F)
May 3	1	32.0	31.0
(Early Pink)	2	27.5	27.5
	3	27.5	27.5
May 4	1	38.5	37.0
(Early Pink)	2	37.0	39.0
	3	30.5	30.5
May 5	1	34.5	34.5
(Pink)	2	36.5	40.5
	3	31.5	31.5
May 6	1	37.5	38.0
(Pink)	2	37.5	40.0
	3	30.5	30.5

percent from the glyodin treatment. The greatest difference occurred from the exposure to the four periods of freezing air temperatures, 15.52 percent of the fruits were heavily russeted when captan was used, as compared to 67.07 percent of heavily russeted fruit from the use of glyodin.

There were no russet free Golden Delicious fruits harvested from any of the spray treatments, Table V. The treatments, glyodin at one quart (full strength) as compared to glyodin at one pint (half strength) plus four ounces of Phygon, were designed with the thought that a reduction in concentration of glyodin from one quart to one pint and include one-fourth of a pound of Phygon would decrease the amount of russeting. This did hold true to some extent as the amount of heavy russeting from the use of glyodin at one quart with the tree exposed to a 31° F minimum air temperature for one night was 96.30 percent as compared to 40.00 percent for the glyodin-Phygon treatment. However, there was no comparable decrease in heavy russeting on the tree exposed to 27.5° F minimum temperature even where the reduced concentration of glyodin was used. Another observation of interest, although the fruits exposed to the 31.0° F temperature were heavily russeted, the russeting was much less heavy than when the exposure was 27.5° F. Also, the heavily russeted fruit from the glyodin-Phygon treatment and exposed to 27.5° F were much less severely russeted than the fruits from the glyodin treated portion of the tree. This same observation

was noted of the fruit on the tree exposed to the four periods of freezing temperatures although 100 percent of the fruits from both treatments were heavily russeted.

The tree receiving the captan treatment following exposure to four periods below 32° F had 96 percent heavily russeted fruit and 4 percent lightly russeted. This does not compare with 24. 4 percent of the fruit heavily russeted and 72. 9 percent lightly russeted recorded for the same treatment in 1956. However, only three periods of air temperature below 32° F were recorded in 1956 (Table I) and these were less severe than the four minimum temperatures recorded in 1957 (Table VI).

The types of light and heavy russeting that occurred on the Jonathan variety in 1957 are illustrated in Figure 5. The russet pattern on Golden Delicious in 1957 was similar to that occurring in 1956, and presented in Figure 3.

Experiment 4. Further field studies to determine the effects of fungicides and freezing air temperatures on fruit russeting.

The treatments at the Graham Station were made only in Blocks I, II and III and only the varieties McIntosh and Jonathan were evaluated for fruit russeting in 1957. The fungicides studied were thiram, glyodin, and wettable sulfur with captan being used in each block as a control treatment.

Figure 5. Samples of russeted Jonathan apples occurring in East Lansing studies, 1957. The fruit on the right is heavily russeted, and the fruit on the left is lightly russeted.

The data from five one-bushel samples of the replicated trees are given in Table VII.

During the early morning hours of May 3, when the flowers were at early pink, a minimum of 31.5° F was recorded near the test plots. Two days later, when the blossoms were at full pink, a minimum temperature of 31.0° F was experienced. During petal fall on May 16, a third minimum temperature of 31.5° F was recorded.

At harvest time, when the samples were examined, much more russeting was observed than was present the previous year on the Jonathan and McIntosh varieties (Table VIII).

The quantity of heavily russeted Jonathan apples harvested from the captan treatment in Block I was significantly higher, 21.60 percent, than the 12.40 percent for the sulfur treatment. However, the plot receiving the captan treatment was at a lower elevation than the other test plots. Therefore, it is reasonable to assume that lower temperatures may have occurred in this plot than was experienced in the other treatments which could perhaps account for this higher percentage of heavily russeted fruits (21.60 percent) than occurred from the use of captan in the other blocks, 17.30 percent and 12.10 percent.

The use of glyodin on Jonathan resulted in 29.50 percent of the fruit heavily russeted at harvest time, as compared to 17.30 percent for

TABLE VII

Russeting of the Jonathan and McIntosh Varieties from the Use of Certain Fungicides Following the Occurrence of Freezing Air Temperatures. Graham Experiment Station, 1957

Variety	Block	Chemical Treatment	No Russeting (percent)	Light Russeting (percent)	Heavy Russeting (percent)
Jonathan	I	Captan	51.20	27.20	21.60** †
		Sulfur	63.50	25.10	12.40**
	II	Captan	56.10	26.60	17.30*
		Glyodin	46.40	24.10	29.50*
	III	Captan	62.00	25.90	12.10*
		Thiram	58.60	23.40	18.00*
McIntosh	I	Captan	89.28	7.59	3.13
		Sulfur	87.20	8.19	4.61
	II	Captan	86.11	10.32	3.57
		Glyodin	85.14	8.43	6.43
	III	Captan	88.75	5.96	5.29
		Thiram	84.59	9.42	5.99

**Difference between treatments significant at the 1 percent level.

*Difference between treatments significant at the 5 percent level.

†Captan treatment of Block I significantly higher than captan treatment of Block III of Jonathan variety.

TABLE VIII

Comparative Russeting of the Jonathan and McIntosh Varieties of Apples from the Use of the same Spray Chemicals During Two Seasons and Exposed to Different Amounts of Freezing Air Temperatures. Graham Experiment Station, 1956 and 1957

Variety	Chemical Treatment	Year	No Russeting (percent)	Light Russeting (percent)	Heavy Russeting (percent)
Jonathan	Captan	1956	90.55	5.16**	4.29**
		1957	56.40	26.60**	17.00**
	Glyodin	1956	95.86	2.17**	1.97**
		1957	46.40	24.10**	29.50**
	Thiram	1956	94.91	3.10**	1.99**
		1957	58.60	23.40**	18.00**
McIntosh	Captan	1956	96.68	1.51**	1.81**
		1957	87.96	7.98**	4.06**
	Glyodin	1956	97.57	1.09**	1.34**
		1957	85.14	8.43**	6.43**
	Thiram	1956	96.59	1.40**	2.01
		1957	84.59	9.42**	5.99

**Differences between years significant at the 1 percent level.

the captan treatment. This difference is significant. It should be noted that the differences in amount of heavy russeting in Block III, 12.10 percent for the captan treatment, as compared to 18.00 percent from the use of thiram, is also significant.

No significant differences in either light or heavy russeting were found between treatments made on the McIntosh variety in 1957.

Since the fungicides used through first cover were the same for the treatments in the years 1956 and 1957 and the method of application was the same, the data of fruit russeting were evaluated to determine if the effects of freezing air temperatures were significantly different for the two seasons under field conditions. A single freezing temperature of 30.5° F occurred at early pink in 1956, whereas three periods of temperatures below 32° F were recorded in 1957.

In every case the percentage of both light and heavy russeted fruits in 1957 was higher than in 1956, with the same treatment (Table VIII) and the differences were highly significant, except for the heavy russeting of the thiram treatment. Here the variation between trees was greater than the difference between the treatments made in 1956 and 1957 so no significance resulted even though the average of heavy russeting in 1957 was higher than in 1956.

Interaction of the use of certain fungicide chemicals and occurrence of freezing air temperatures near and during bloom on fruit russeting of Golden Delicious, Jonathan and McIntosh apples.

Graphic presentations of the interaction resulting from the use of certain fungicides and the occurrence of freezing air temperatures (32° F and below) near and during bloom are given in Figures 6 to 10.

Golden Delicious: In 1956 at the Graham Experiment Station, a single recorded minimum air temperature of 30.5° F occurred when the blossoms were in the early pink stage. The fungicides, captan, thiram, glyodin and captan followed by glyodin after first cover, were used as the treatment sprays with insecticides used uniformly throughout all treatments. No significant differences were observed between numbers of russeted fruits harvested from the trees of the fungicide treatments (Figure 6). In this study, the spray materials were applied with an air blast attachment mounted on a high pressure sprayer.

During the same year in East Lansing where glyodin and captan were used as half-tree treatments on the same tree and the tree was protected from freezing air temperatures, no heavy russeting occurred from the captan treatment, but 27.8 percent of the fruit was heavily ruseted from the glyodin treatment. In this case, applications were made with an adjustable nozzle hand gun.

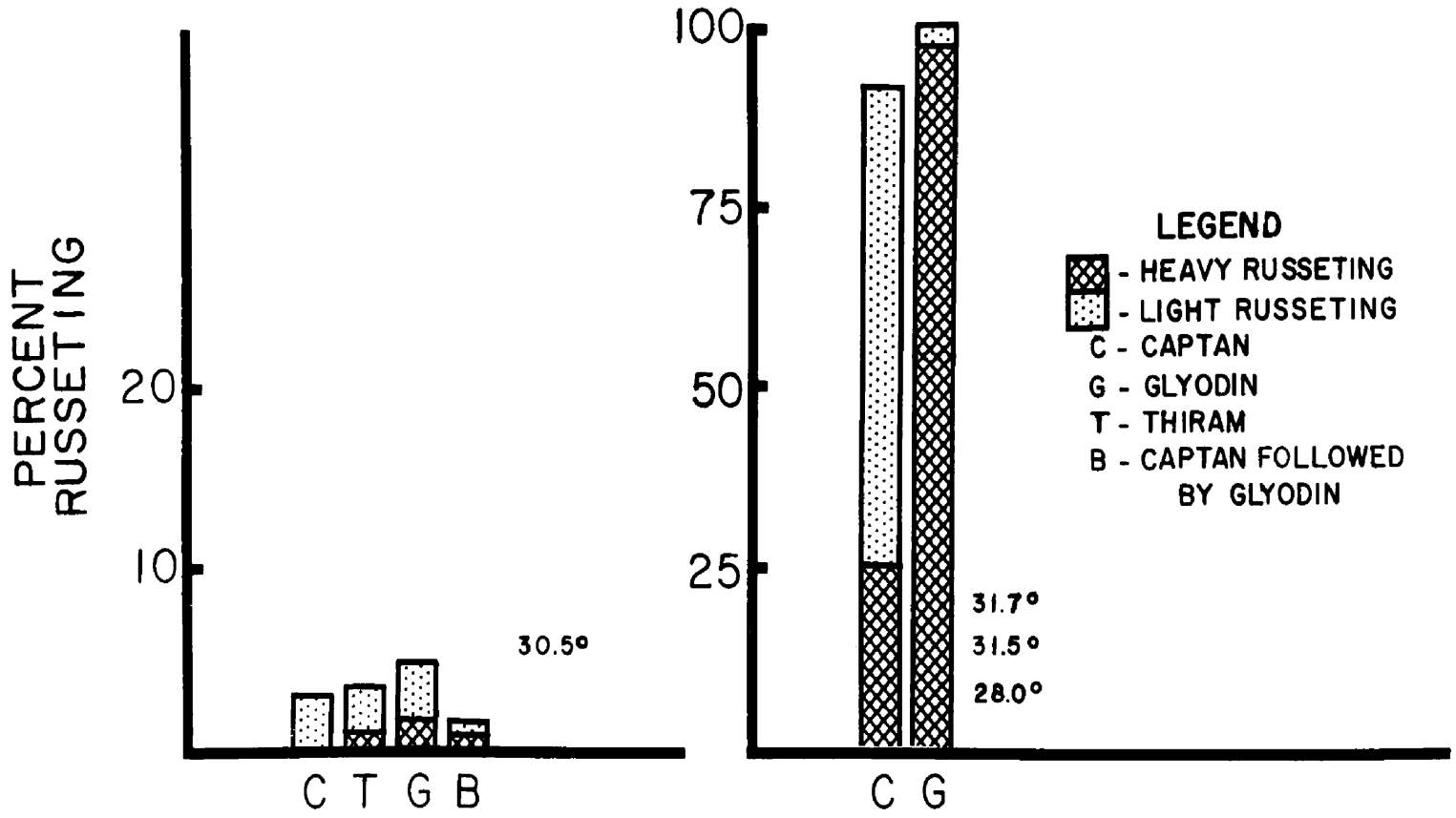


Figure 6. The effects of fungicides and various freezing air temperatures occurring near or during bloom on russeting of Golden Delicious apples under field conditions. Left - Graham Experiment Station, Grand Rapids; Right - East Lansing, 1956. The minimum air temperatures experienced in each case are given in degrees Fahrenheit to the right of each set of treatments.

The amount of fruit russeting on Golden Delicious in the controlled temperature studies and in the field studies carried on in East Lansing in 1956 using the same spray chemicals is very similar (Figures 6 and 7). It is assumed that the reason for very little russeting of Golden Delicious from the use of glyodin in plots at the Graham Experiment Station was related to the method of application as well as freezing air temperatures. It is apparent that mechanical injury to the epidermal tissue from gun spraying makes it possible for the glyodin or a component of glyodin to pass through the epidermal layer and contact the outer cells of cortical tissue. This, in turn, may cause proliferation of the outer cortical cells rupturing the epidermal layer (Figure 25). The epidermal cells apparently were not injured from air blast applications and thus the epidermal cells functioned as a protective layer against the absorption of glyodin.

The russeting of Golden Delicious apples in 1957 was very severe (Figure 7). However, a reduction of russeting by decreasing the concentration of glyodin in the spray mixture was observed when the tree was exposed to the 31.0° F minimum temperature. Also, the exposure of 27.5° F did not appear to cause any difference in amount of russeting between treatments. The fruits from the glyodin and reduced strength glyodin plus Phygon treatments following exposure to four periods of freezing temperatures were 100 percent severely russeted. Even the captan treatment

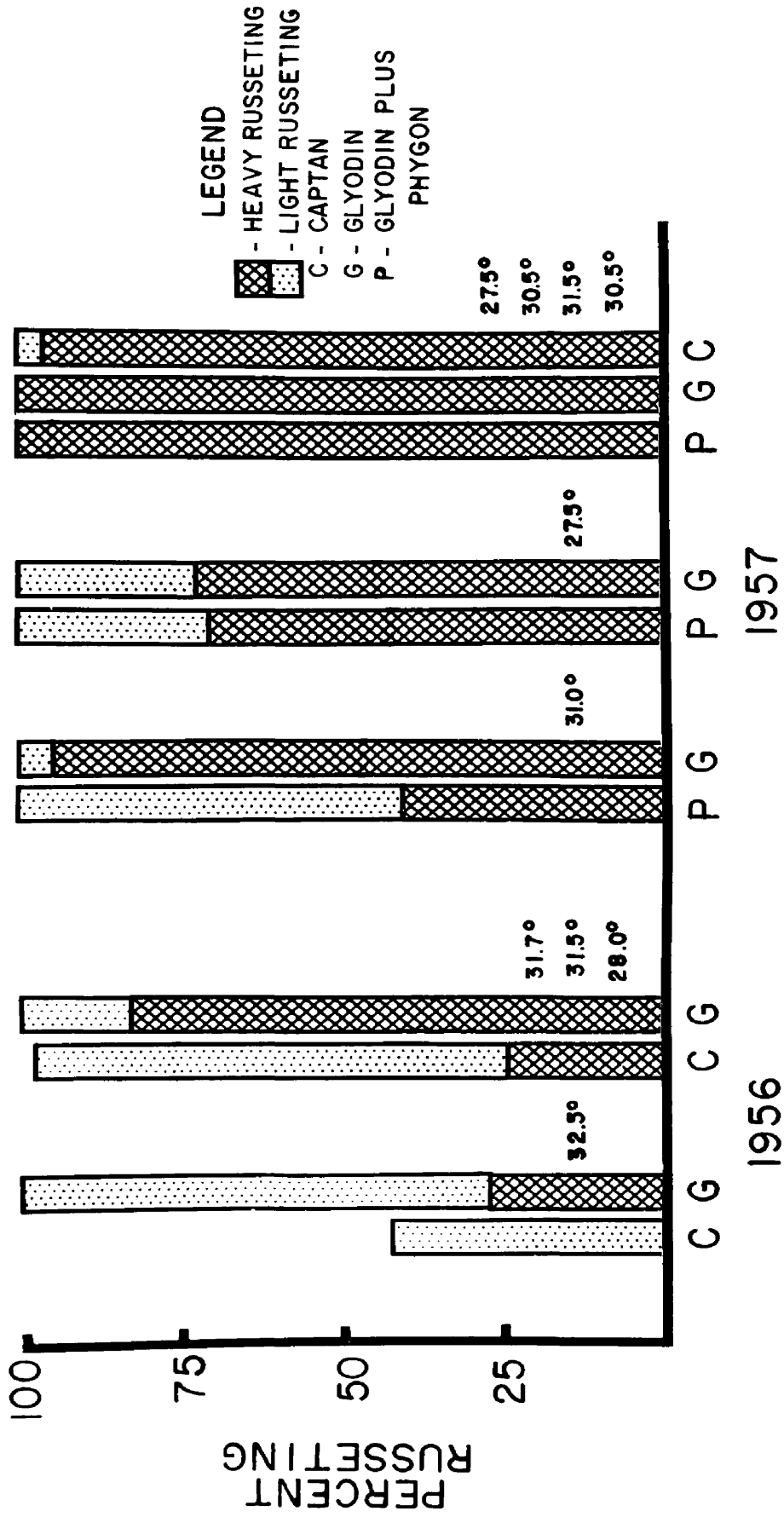


Figure 7. The effects of fungicides and various freezing air temperatures occurring near or during bloom on russeting of Golden Delicious apples, East Lansing, 1956 and 1957. The minimum air temperatures experienced in each case are given in degrees Fahrenheit to the right of each set of treatments.

exposed to these four periods of freezing air temperatures had 96 percent heavily russeted fruit. It was observed in all treatments, however, that the heavily russeted fruit from the reduced strength glyodin plus Phygon treatment were less severely russeted than the fruit receiving full strength glyodin.

In comparing the glyodin treatments in the controlled temperature studies of 1956 with the same treatment in 1957 and with the two captan treatments (Figure 7), it is very evident that the amount of heavy russeting was greater in 1957 than in 1956. Also, the recorded minimum temperatures in 1957 were lower, 27.5°, 30.5°, 31.5° and 30.5° F than in 1956, 31.7°, 31.5° and 28° F (Tables I and VI), and they occurred prior to bloom in 1957 (Table VI), whereas the 28.0° F low recorded in 1956 was after bloom (Table I).

Jonathan: A comparison of the field studies with those conducted under controlled conditions (Figures 8 and 9) showed that as the hours of freezing temperatures increased, the amount of russeting increased correspondingly. Nevertheless, the amount of heavy fruit russeting observed in 1957 in both the field studies and those under controlled conditions when freezing temperatures occurred near bloom was considerably less from the use of captan than from the use of glyodin.

A greater percentage of russeted fruits resulted under the more

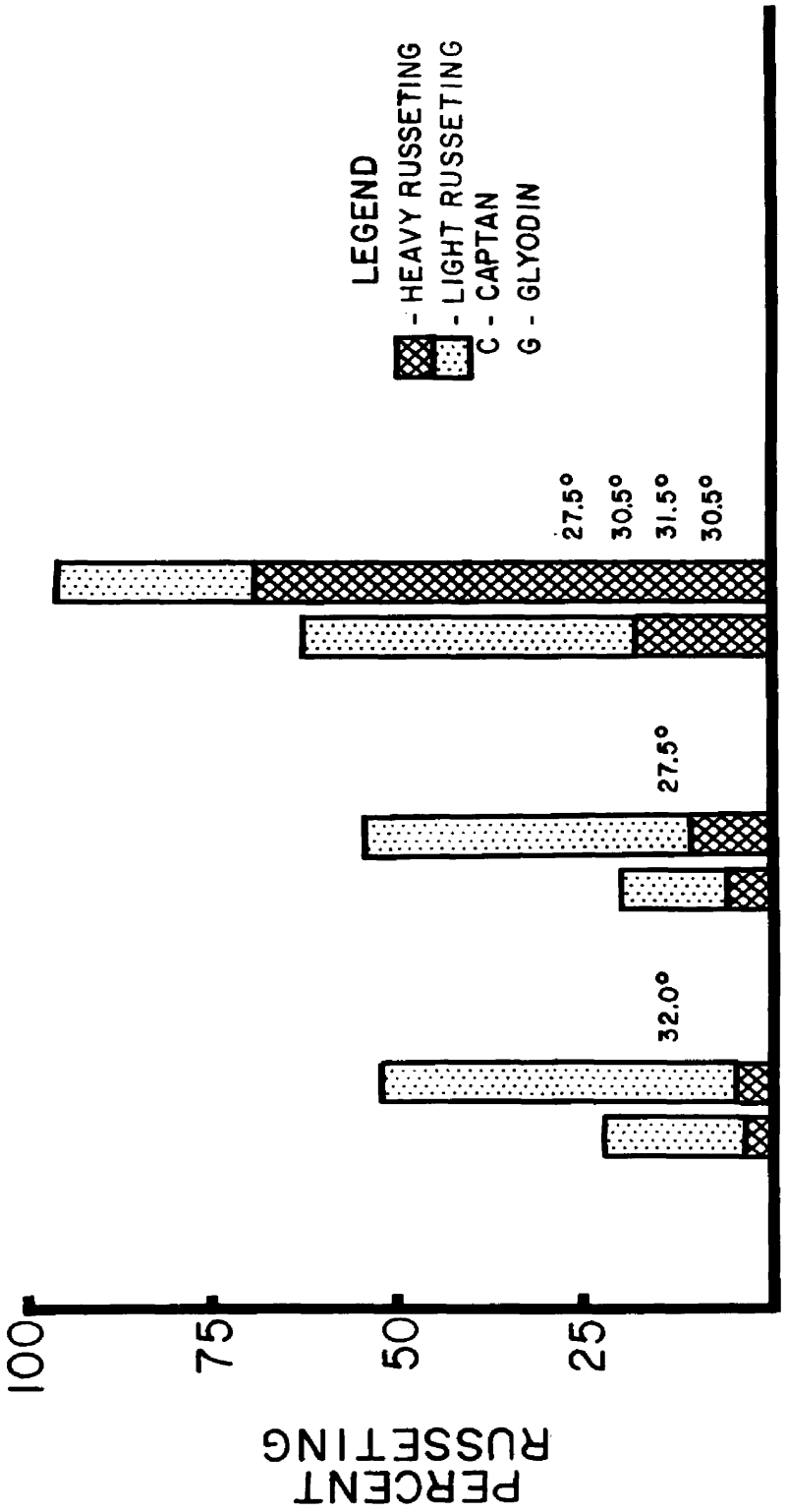


Figure 8. The effects of fungicides and various freezing air temperatures occurring near or during bloom on russeting of Jonathan apples, East Lansing, 1957. The minimum air temperatures experienced in each case are given in degrees Fahrenheit to the right of each set of treatments.

Figure 9. The effects of fungicides and various freezing air temperatures occurring near or during bloom on russeting of Jonathan apples under field conditions, Graham Experiment Station, Grand Rapids, 1956 and 1957. The minimum air temperatures experienced in each case are given in degrees Fahrenheit to the right of each set of treatments.

severe freezing temperatures of the controlled studies than was observed in the field studies. Here again, it should also be noted that the field applications were made with an air-blast sprayer, while the applications in the controlled temperature studies were made with an adjustable nozzle hand gun.

McIntosh: Fruit russeting, in general, on the McIntosh variety (Figure 10) was less than on the other varieties for the same fungicide treatments and weather conditions, and no results were significantly different. However, it was observed that the larger number of freezing air temperature periods near full bloom in 1957 increased the amount of both light and heavy russeting in all treatments over that found in 1956 (Table VIII).

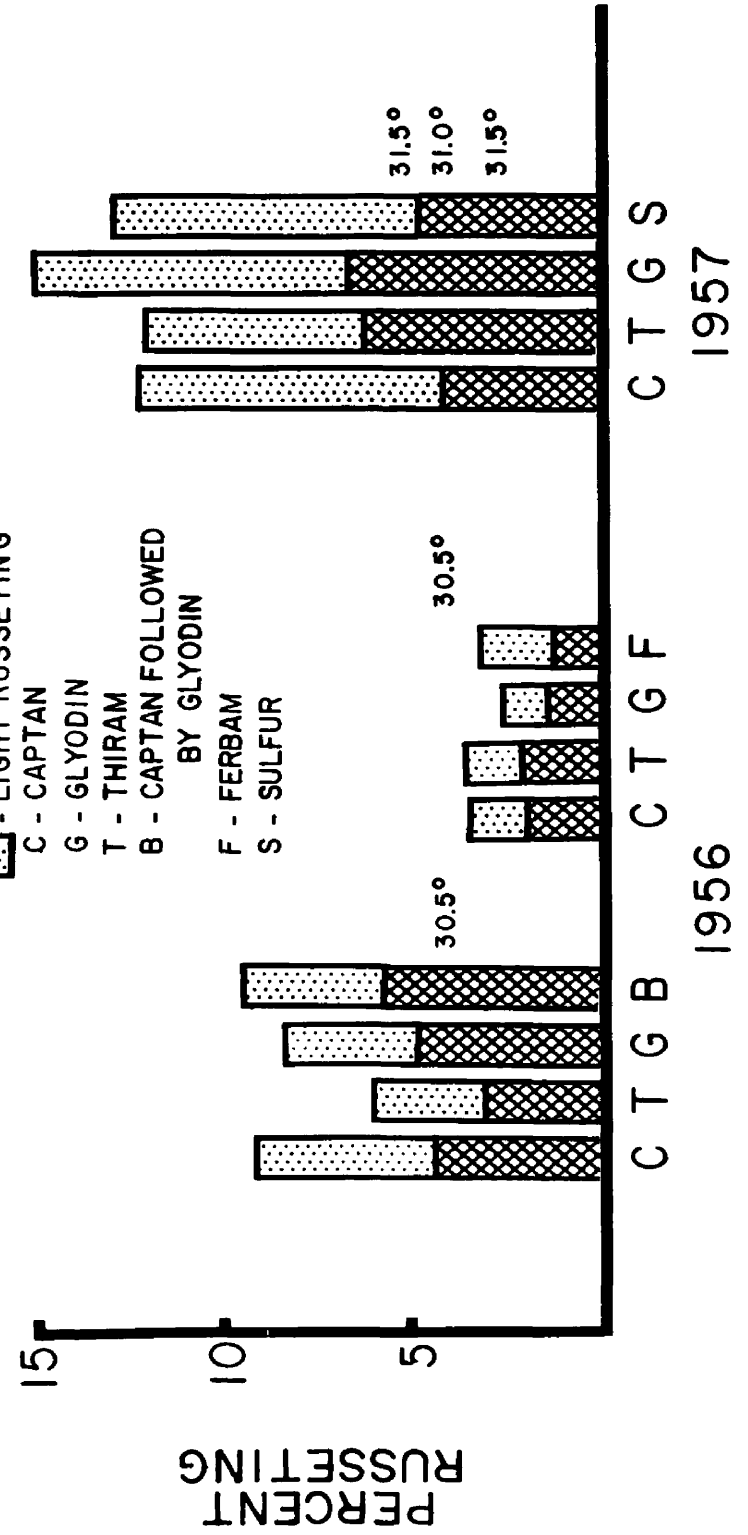


Figure 10. The effects of fungicides and various freezing air temperatures occurring near or during bloom on russeting of McIntosh apples under field conditions, Graham Experiment Station, Grand Rapids, 1956 and 1957. The minimum air temperatures experienced in each case are given in degrees Fahrenheit to the right of each set of treatments.

Histological Investigations

Experiment 5. Histological investigations to study the development of the protective layers of Golden Delicious, Jonathan, and McIntosh with special attention given to the cuticle and to study the origin and growth of russeted tissues of the fruit.

The receptacular portion of flowers before bloom and the developing fruits after bloom of Golden Delicious, Jonathan and McIntosh apples were examined microscopically to study the development of the epidermal layer and the formation of cuticle. Serial sections of killed and fixed material were examined from the stages of full bloom to maturity. Sections of frozen material were examined from the pre-pink stage to petal fall. The development of the protective layers of Golden Delicious is illustrated in Figures 11 through 17. As no apparent differences could be observed between the developing cells and tissues of the three varieties until approximately 30 days after full bloom, the illustrations of the Golden Delicious, Figures 11 through 15, represent also the McIntosh and Jonathan.

As the flowers emerge from the winter bud and when the floral receptacle became first exposed to environmental conditions at the time of pre-pink, the receptacle was covered with what appears to be a dense mat of trichomes. Microscopic examination of the three varieties revealed that

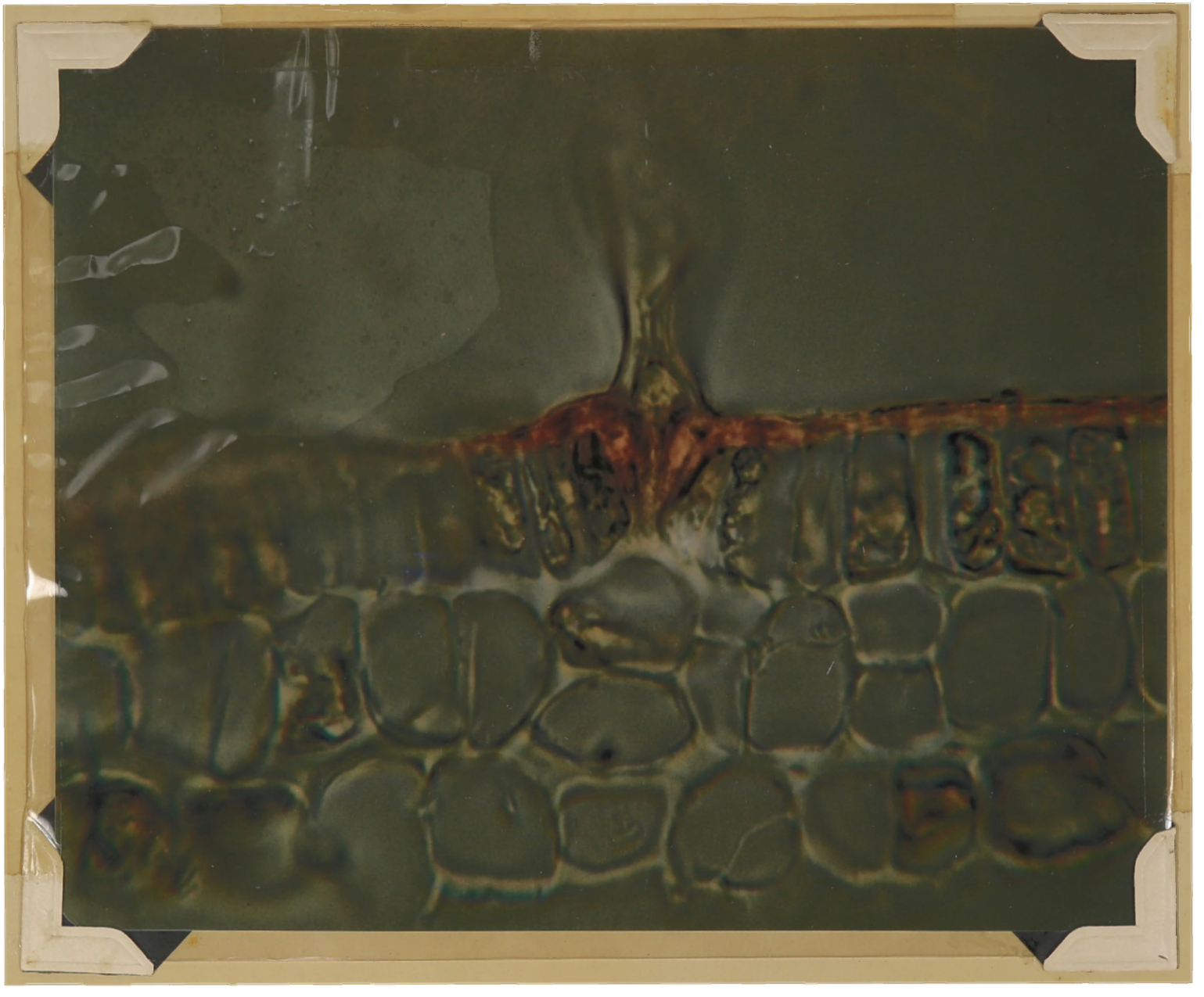
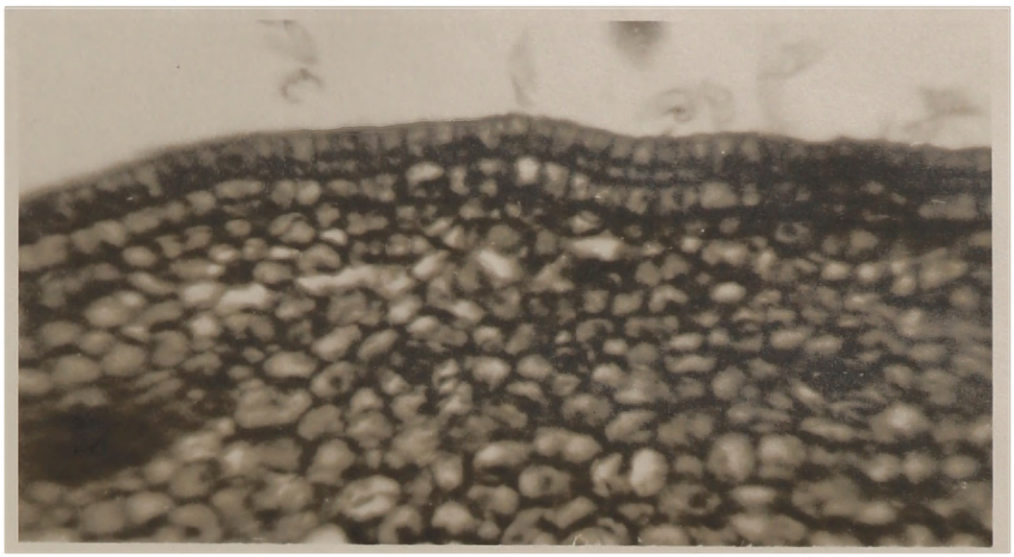


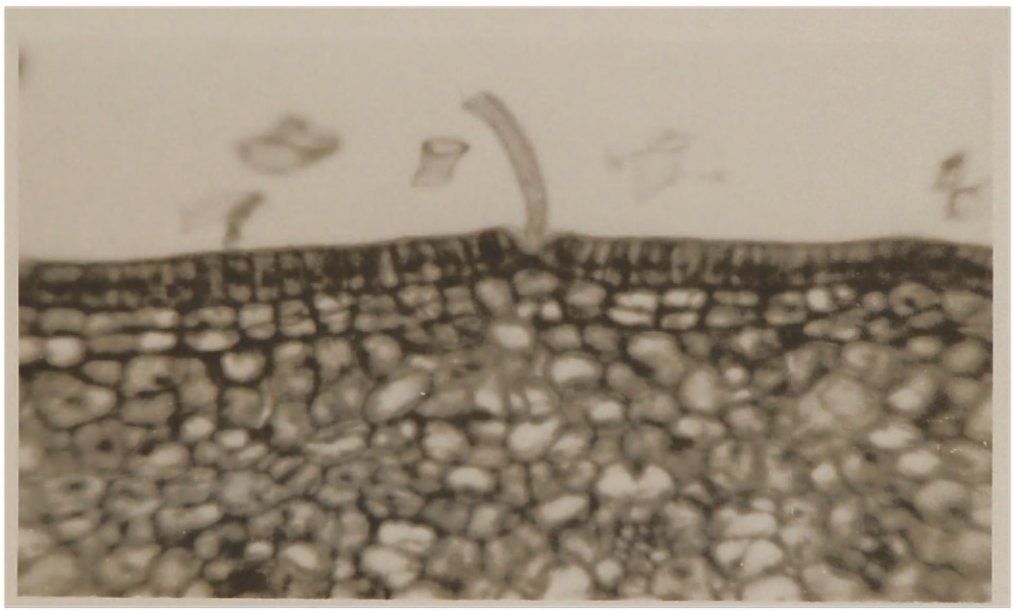
Figure 11. A transverse-section of the floral receptacle of Golden Delicious at full bloom sectioned with the freezing microtome at six microns and stained with Sudan IV (X1750).

Figure 12. Transverse-section of the flower receptacle of Golden Delicious at full bloom showing absence of cuticle on the fruit surface (X350).

Figure 13. Transverse-section of the fruit of Golden Delicious at petal fall showing the earliest deposit of cuticle occurring at the base of the trichome. The outer cortical cells are starting to show thickening of the cell walls (X350).

Figure 14. Transverse-section of the fruit of Golden Delicious, seven days after petal fall (first cover) showing deposit of cuticle at the base of the trichome and a complete cuticle on the fruit surface (X350).





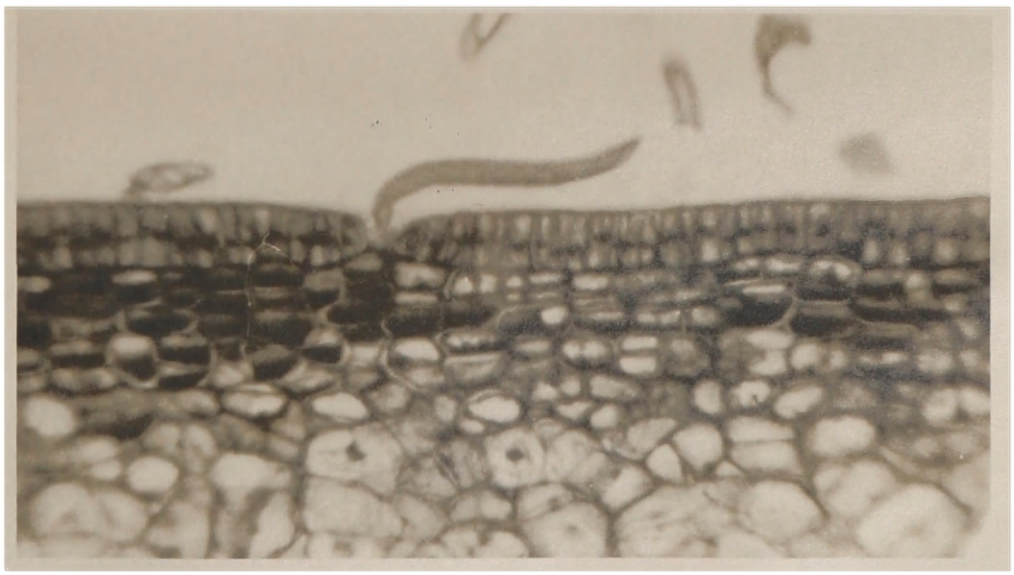
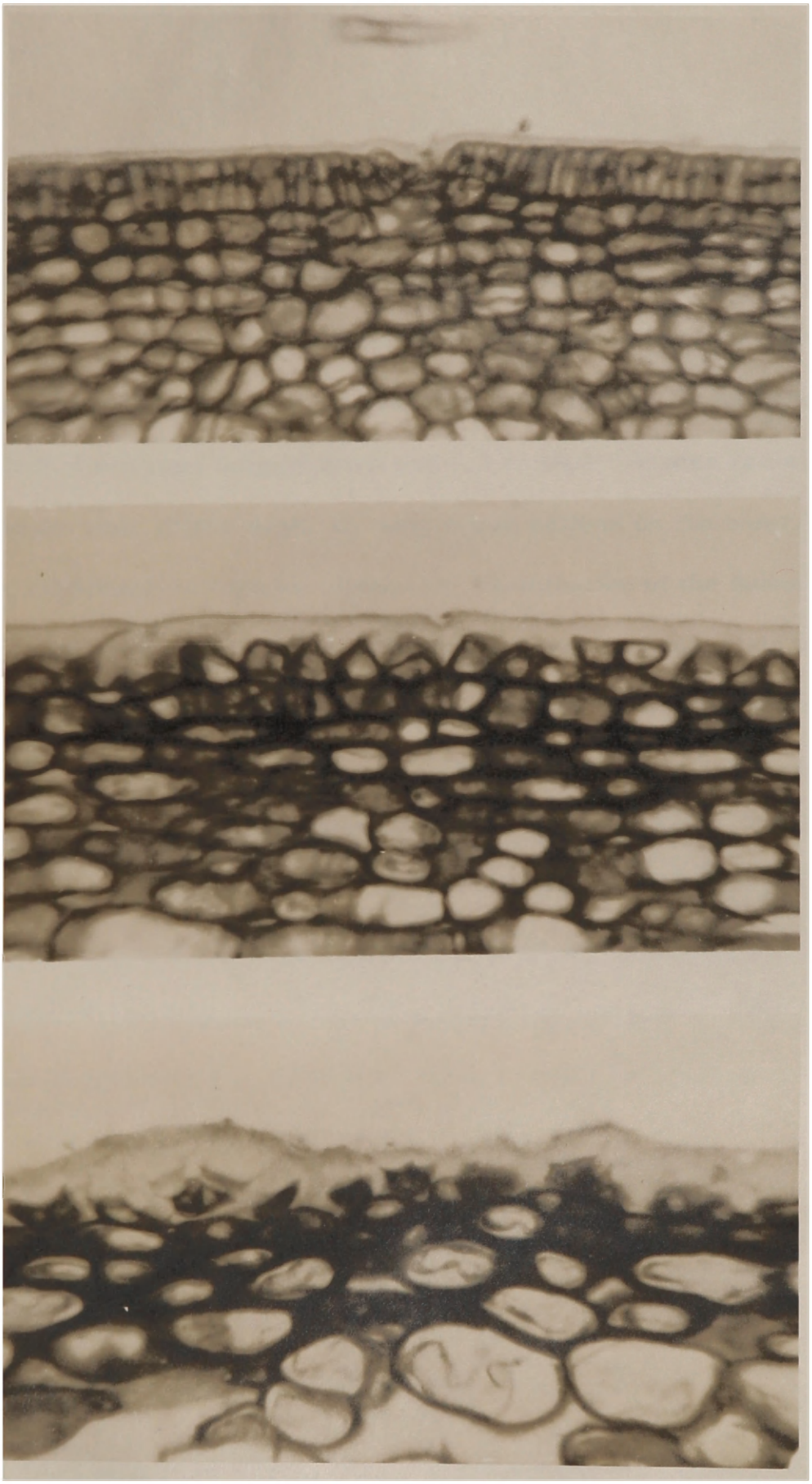


Figure 15. Transverse-section of the fruit of Golden Delicious, three weeks after full bloom (second cover) showing the results of the sloughing off of a trichome and the completeness of a cuticle on the fruit surface (X350).

Figure 16. Transverse-section of the fruit of Golden Delicious, two months after full bloom showing separation of the epidermal cells and the formation of cuticular pegs (X350).

Figure 17. Transverse-section of the fruit of Golden Delicious at maturity showing irregular cuticle, indistinguishable epidermal layer and cell separation of outer cortical cells with cuticle filling these gaps (X350).



the actual numbers of modified epidermal cells forming the trichomes were relatively few. Because they were very long and entangled, these long unicellular trichomes formed a matted mass that covered the receptacle. The epidermal layer at this stage consisted of radially elongated, densely packed, brick-shaped cells. The dimensions of these cells for the three varieties were 3.6 to 5.4 microns tangentially, and 7.2 to 10.8 microns radially.

At the time of pre-pink, no cuticle was evident on the outer surface of the epidermal cell walls. However, examination of the Sudan IV stained sections of frozen material revealed that some orange stain was present within the outer cell wall of some epidermal cells. According to Johansen (27), waxes and other fatty materials stain a dull red with Sudan IV. The stained portion seemed to be arranged as radial bands within the cell wall of the exposed portion of the cell. Many cells were not stained at all. There seemed to be no specific pattern as to where the cells that were receptive to stain were located on the flower receptacle. The Jonathan floral receptacles exhibited the deepest stain in the epidermal cell walls. The epidermal cells of the McIntosh stained somewhat lighter. The Golden Delicious was observed to have only an occasional exposed wall of an epidermal cell with a slight orange tinge.

As the flowers continued to develop to full bloom, the intensity of the Sudan IV stain increased for all varieties, but continued to be within the

exposed wall of epidermal cells. At the pink stage the orange stain appeared to be most intense in the epidermal cell walls of the Golden Delicious. Nearly all of the outer walls of the epidermal layer of the three varieties showed some degree of intensity of orange stain, although some areas in the exposed wall of some cells were not stained.

The intensity of orange stain continued to increase within the exposed cell walls of the floral receptacle as the flowers continued to approach full bloom, and at full bloom, all the exposed cell walls of the receptacle of the flowers of the three varieties were clearly stained by the Sudan IV. A deep orange stain was observed at the base of the trichomes extending from the epidermal layer as illustrated by Figure 11. Upon close examination, some externally stained material could be observed accumulating in the cavities between certain trichomes and the adjoining epidermal cells. This was especially true with the McIntosh variety, while only an occasional deposit of this nature was observed for the Jonathan and Golden Delicious varieties. An occasional trichome of the McIntosh was dislodged during the sectioning process and in every case, a small, very thin, heavily stained film was attached to the base of the trichome, indicating that an external deposit of cuticle was present. No similar deposit was observed on the dislodged trichomes of the varieties Jonathan and Golden Delicious.

No external cuticle could be observed on the serial sections of

permanent preparations (Figure 12) made of the three varieties at this stage. This could be due to the possible solubility of this early deposit of cuticle in the materials used for preparing these permanent sections, such as the ethyl alcohol and xylene. Apple cuticle in later stages has been considered to be insoluble in most organic solvents (7).

At petal fall, three to six days after full bloom, depending upon the variety, a distinct cuticle was evident at the base of the epidermal hairs (Figure 13) of prepared sections of the fruit. However, no cuticle could be detected on the surface of the epidermal cells except that deposited around the base of the trichomes. Examination of the frozen material stained with Sudan IV revealed that a cuticle was present in other areas as well as the base of the trichomes. The deposit was not continuous over the entire surface of the fruit and was less than one micron thick in the area of the heaviest deposit.

The dense mat of trichomes was still present on the fruit at the petal fall stage. The epidermal layer of all three varieties still consisted of tightly packed, radially elongated cells with very dense cytoplasm. At this stage the outer cortical layer of cells appeared to show signs of differentiating into a tissue somewhat different from the other cortical cells (Figure 13). The cell walls of this layer also appeared thicker than inner cortical cells and some periclinal division of cells was evident.

As the fruit continued to develop, more cuticle was deposited on the surface of the epidermal layer and by seven days after petal fall, considered as the time of first cover, the deposit of cuticle was complete and the outer walls of the epidermal cells were covered with a waxy coating (Figure 14). This was true of the Jonathan, Golden Delicious and McIntosh varieties. The cuticle was observed to have filled in around the base of the trichomes so that the trichomes appear to be separated from the epidermal layer. The epidermal cells were similar to the description given for petal fall and the outer cortical cells had become increasingly dense, the cell walls had thickened, and there was evidence of both anticlinal and periclinal divisions.

Three weeks after full bloom, many of the trichomes had sloughed off due to the deposit of cuticle at their bases (Figure 15). The epidermal layer of the fruits of all three varieties was similar to the description previously given, (illustrated by Figures 15, 19 and 22) and no differences could be observed between varieties. The distinguishable outer cortical layer had increased one to three cells in thickness and the cell cytoplasm remained quite dense and the cell walls thick.

At approximately 30 days after full bloom, the cells of the epidermal layer of the Golden Delicious appeared to be separating with cuticular pegs or extensions developing between the anticlinal cell walls. As the fruit

continued to grow, the separations became more evident (Figure 16), and by maturity many cells or groups of cells appeared in cross-section to be completely suspended in the cuticle with the cuticle in contact with the sub-epidermal cells of the outer cortex (Figure 17).

By comparison, the epidermal cells of the McIntosh appeared isodiametric until about six weeks after full bloom, when they were found to be stretched and flattened in cross-section (Figure 19). However, at maturity (Figure 20) the cells of the epidermal layer were still intact and the layer complete, even though some cuticular flanges were observed to extend partially between the anticlinal cell walls of some epidermal cells. The outer cortical cells of the McIntosh fruit were very dense and had very thick cell walls. The number of cells in this layer varied considerably from one area to another.

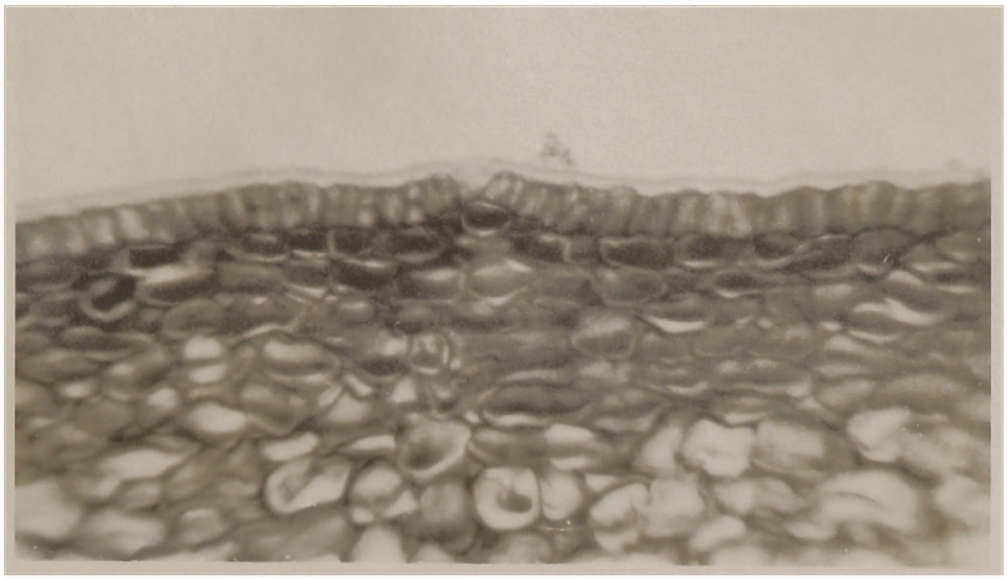
The development of the epidermal layer of Jonathan was found to be more similar to the Golden Delicious than the McIntosh, but the presence of cuticle within and below this cell layer was not as extensive as for the Golden Delicious (Figures 22 and 23). The outer cortical layer of cells was more intact than in Golden Delicious, similar to the McIntosh. The outer cortical cells were small, very dense and thick-walled.

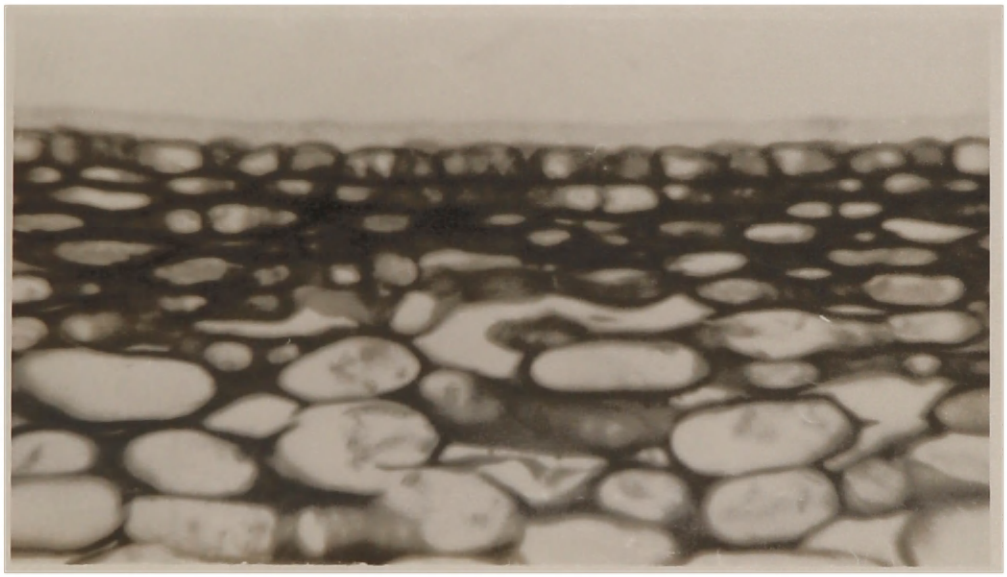
A relative comparison of the development of cuticle of the three varieties, McIntosh, Jonathan and Golden Delicious, is illustrated in Figure 24.

Figure 18. Transverse-section of the fruit of McIntosh, three weeks after full bloom (second cover) showing the area where a trichome had sloughed off, the complete cuticle and regular epidermal layer (X350).

Figure 19. Transverse-section of the fruit of McIntosh, two months after full bloom showing a very regular epidermal layer with only slight indentations between cells that have been filled-in with cuticle (X350).

Figure 20. Transverse-section of the fruit of McIntosh at maturity showing the very regular epidermal layer with only slight indentations between cells that have been filled-in with cuticle (X350).





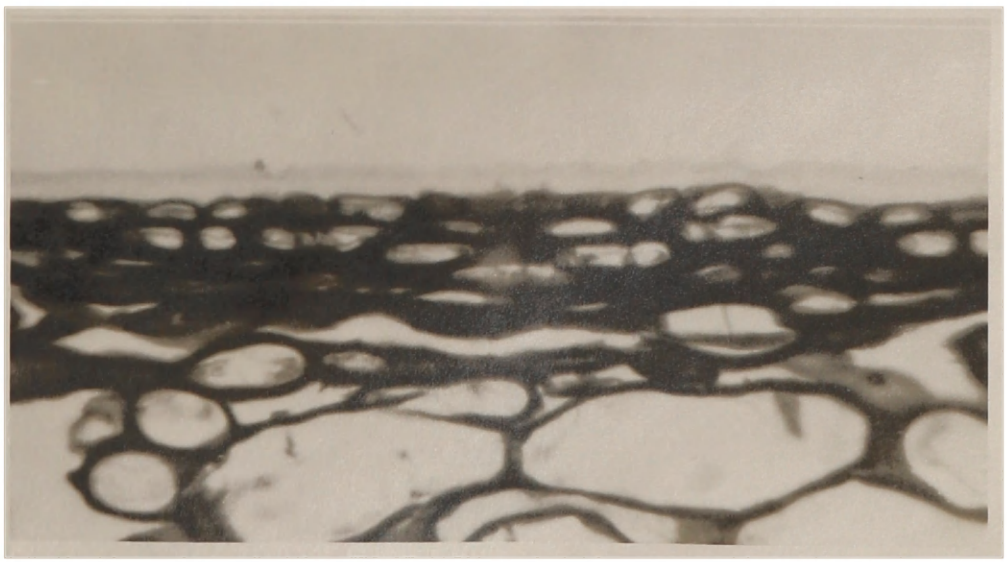
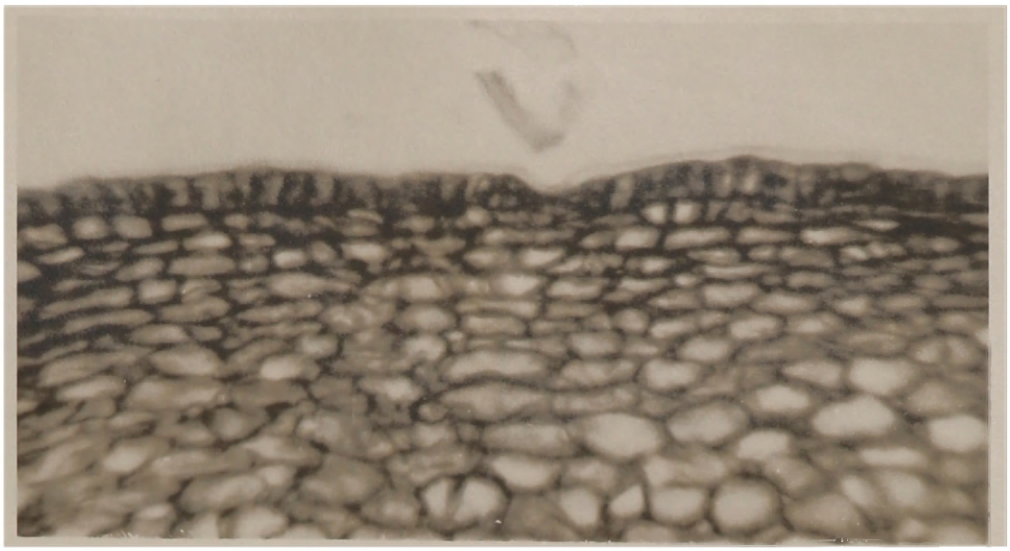
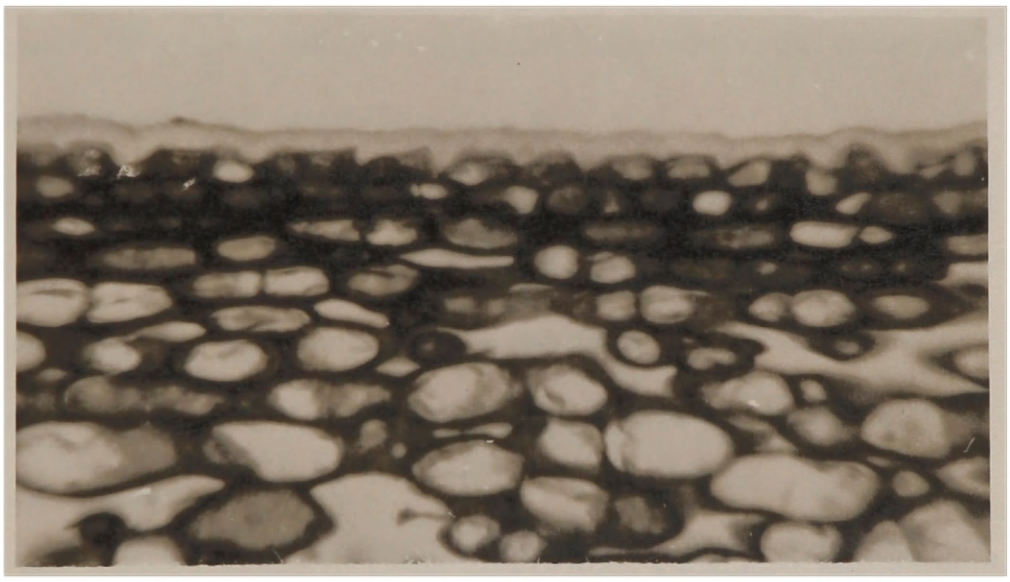


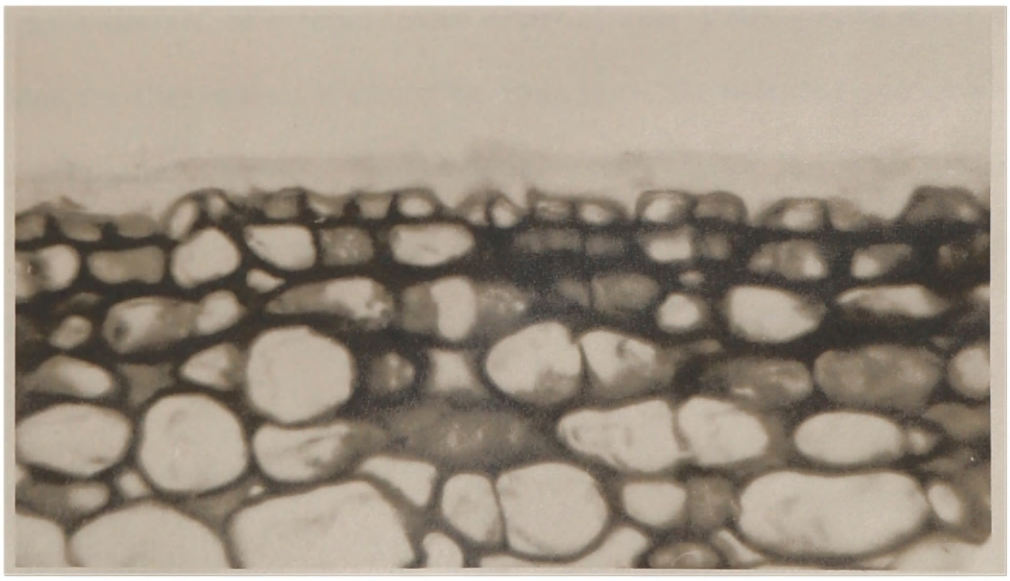
Figure 21. Transverse-section of the fruit of Jonathan, three weeks after full bloom (second cover) showing trichome being sloughed off, complete cuticle and regular epidermal layer (X350).

Figure 22. Transverse-section of the fruit of Jonathan, two months after full bloom showing some evidence of cell separation of the epidermal layer and the formation of cuticular pegs in these breaks (X350).

Figure 23. Transverse-section of the fruit of Jonathan at maturity showing slight separation of cells of the epidermal layer with cuticle filling in these areas (X350).







The thickness of the cuticle for each time of observation for each variety is the average of ten measurements. The measurements were taken from the extremity of the outer epidermal cell wall to the outer portion of the cuticle and did not include the cuticular penetration of the epidermal or outer cortical layers. Measurements earlier than two weeks after full bloom (second cover) were considered undesirable as the cuticle was incomplete until this time, and the relatively thin areas that could be observed and measured were only in isolated patches.

Histological studies of Golden Delicious apples from the Horticultural farm, East Lansing, in 1956 sprayed with glyodin have revealed why russeting in this variety may result from a glyodin-freezing temperature interrelationship. Figures 25 through 28 are photomicrographs illustrating this russet formation.

The first evidence of russet on Golden Delicious apples could be observed with the aid of a hand lense approximately six weeks after full bloom. Shortly thereafter it could be seen with the unaided eye and appeared as small, irregular, brown, scaly spots, scattered on the fruit surface. As the fruit matured, these spots increased in both size and irregularity. At the time of harvest, scales were easily dislodged from the russeted areas.

In studies of the prepared histological sections of russeted tissues,

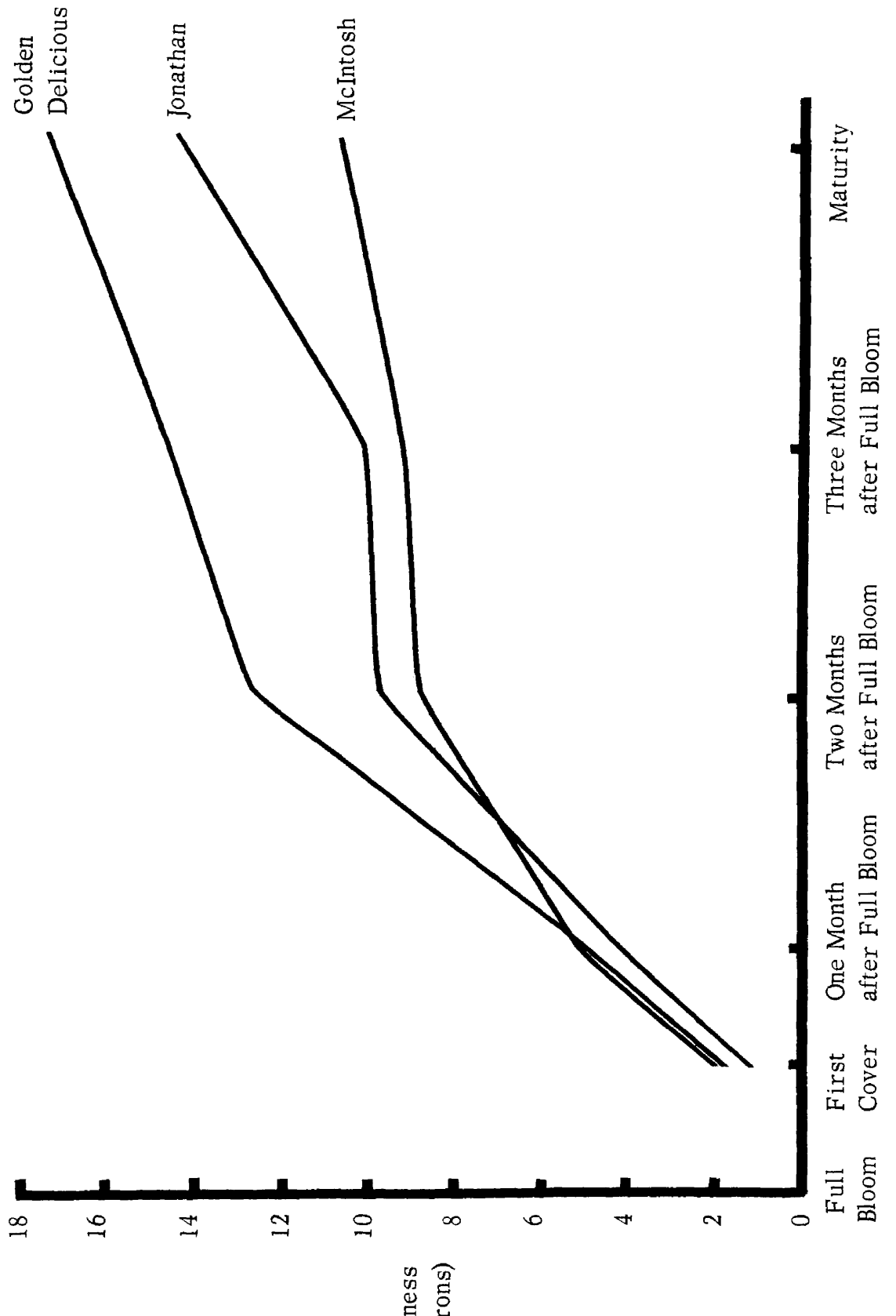
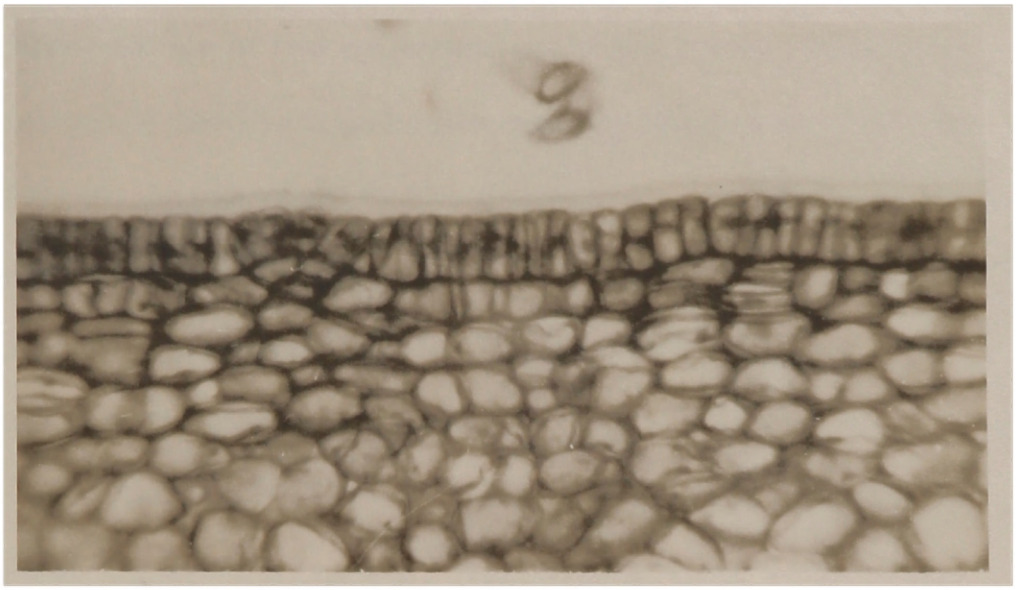
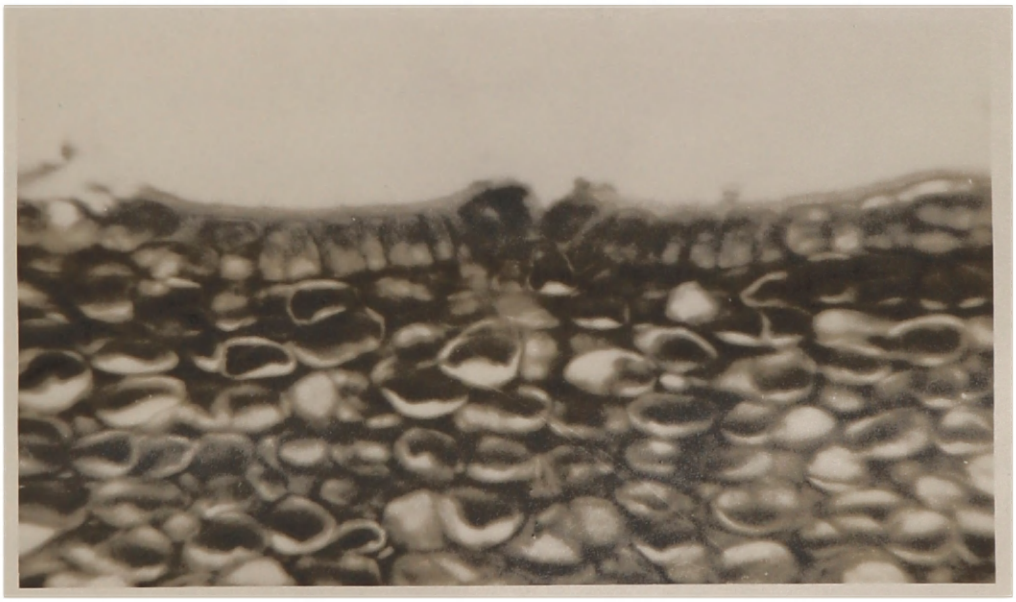


Figure 24. Increase in cuticle thickness during growth and maturity of Golden Delicious, Jonathan and McIntosh apples, East Lansing, Michigan. 1956.

Figure 25. Transverse-section of the fruit of Golden Delicious three weeks after full bloom (second cover) showing first signs of proliferation of sub-epidermal cells (X350).

Figure 26. Transverse-section of the fruit of Golden Delicious four weeks after full bloom showing rupture of the cuticle and epidermal layer due to proliferation of the sub-epidermal cells (X350).





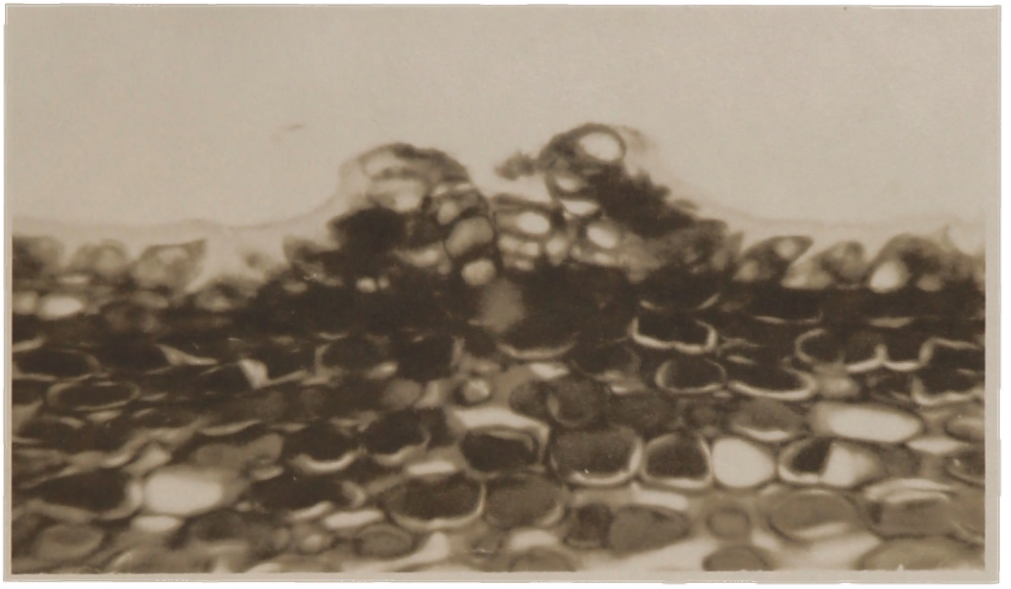
the first abnormality of the epidermal layer of the Golden Delicious was observed microscopically approximately four weeks after full bloom. It appeared as a rupture of two epidermal cells and cuticle apparently caused by proliferation of outer cortical cells directly beneath the ruptured cells (Figure 26). No cuticle could be observed on the newly exposed surfaces of the ruptured cells. In many areas there were numerous breaks in the epidermal layer, with ruptures occurring at intervals of five or six cells. In every instance the breaks appeared as a fissure of two epidermal cells caused by proliferation of the outer cortical cells directly under the epidermal layer.

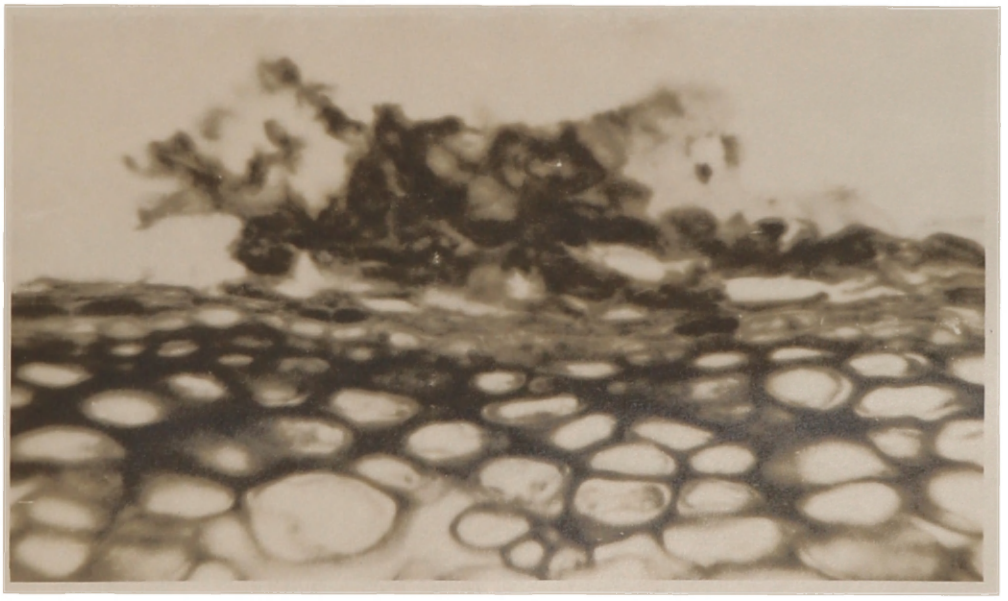
Examination of samples collected three weeks after full bloom (second cover) revealed some small areas of sub-epidermal cell proliferation (Figure 25) but no break in the epidermis and cuticle was found. The proliferation of cells had caused only a slight rise of the epidermal layer and cuticle of the fruit.

At six weeks after full bloom more proliferation of sub-epidermal cells was observed and quite significant ruptured areas of the fruit surface were noticeable in cross-section (Figure 27). The cells near the outer portions of the fissure had become somewhat crushed, and collapse of these cells was evident.

Figure 27. Transverse-section of the fruit of Golden Delicious six weeks after full bloom, showing sub-epidermal cell proliferation and further enlargement of the russet lesion (X350).

Figure 28. Transverse-section of the fruit of Golden Delicious at maturity showing the large mass of russet tissue formed from a meristematic layer of cells (phellogen) below the russeted area (X350).





As the fruit continued to enlarge, the ruptured areas enlarged also, and the cells filling the ruptured areas were small and brick-shaped which are characteristic of meristematic cells. The covering of the russeted areas consisted of large masses of heavily stained, crushed cells which had been ruptured and crushed by the dividing cells beneath them.

At maturity, a well defined meristematic layer of cells was observed below the russeted areas (Figure 28). This meristematic layer appeared to have developed new cells centrifugally in a manner similar to phellem formation of woody stems. Great masses of crushed cells were present in transverse-section above the russeted areas, which probably were the scales observed macroscopically on the fruit surface in these russeted areas.

The results of the field studies to evaluate the role of the trichomes in fruit russeting indicated that injury to the trichomes, even to their removal, did not promote russeting, because no russeting was observed at maturity on the fruit of any treatment of either variety.

DISCUSSION

Earlier investigations of apple fruit russeting have indicated that either spray chemicals (1, 11, 15, 18, 24, 43, 47, 52, 63) or freezing temperatures (1, 9, 11, 14, 15, 16, 20, 26, 29, 53, 55) had caused this injury. Recent reports (36, 37, 38, 39, 48) have suggested the possibility of slight cold injury to the epidermal cells of the fruit when the air temperature dropped to 32° F or lower near or during the period of full bloom. This slight cold injury, in turn, was increased to severe injury in the form of russeting by the use of certain pesticides after the occurrence of the freezing temperature. There was also an indication that no russeting of the fruit occurred from the use of the same pesticide chemicals in seasons when the air temperature remained above 32° F (36).

Since the fruit of the Golden Delicious and Jonathan varieties have been reported very sensitive to both pesticide chemical and freezing temperatures (21, 24, 34, 38, 40, 46, 54, 55), this investigation was initiated to determine the relationship between the injury from freezing temperatures and the use of certain pesticide chemicals on fruit russeting. The fruit of the McIntosh has been reported (24, 29, 35, 38) to be less sensitive to injury from these two factors and thus, was included in this study in the hope of establishing a possible reason for these reported differences of

russeting between varieties as affected by chemicals or freezing temperatures.

It has been previously reported (38) that the use of glyodin on Golden Delicious during the period "pre-pink" through "second cover" resulted in an excessive number of russeted fruits even in seasons when the air temperature remained above 32° F. It was also reported (37) that in some years even though captan was the only fungicide used throughout the season, russeting occurred on Golden Delicious. This happened in those seasons that had air temperatures below 32° F either just before or just after full bloom. The russeting was thought to be the result of cold injury to the fruit and not to the use of captan. Under the controlled temperature conditions of Experiment 1, it was found that the use of captan on the tree protected from freezing temperatures, resulted in no heavily russeted fruit. However, on the tree exposed to temperatures of 31.7°, 31.5° and 28.0° F, 23.7 percent of the fruit were heavily russeted (Tables I and II). These findings would appear to substantiate the premise that freezing temperatures near bloom could cause russeting on Golden Delicious.

The results of Experiment 1 tend to reveal some of the factors responsible for fruit russeting of Golden Delicious. As all treatments were made on half-trees, the environmental conditions were as similar

as possible between treatments. The half-tree treatments would also tend to eliminate any inherent variations that might exist between trees. The portions of the trees sprayed with the glyodin treatment and protected from possible cold injury still produced fruit with some degree of russeting (Table II). Moreover, the use of glyodin on the fruit exposed to air temperatures lower than 32°F for three nights during the blossoming period resulted in an even larger number of fruits exhibiting both light and heavy russeting (Table II).

Comparing fruits from the same Golden Delicious trees sprayed with different fungicides of Experiment 1, carried on in 1956, the use of captan resulted in a much smaller percentage of fruits either lightly or heavily russeted and a larger percentage completely free of russeting than was found from the use of glyodin (Table II). In fact the percentage of heavily and lightly russeted fruits of the captan treatment and exposed to the freezing temperatures given in Table II is very similar to those of the glyodin treatment of the tree protected against exposure to freezing temperatures (Table II).

In 1957, when glyodin, used at full strength (one quart per 100 gallons), was compared to glyodin at one pint plus four ounces of Phygon per 100 gallons, the percentage of heavily russeted fruit was appreciably lower for the glyodin-Phygon treatment, Table V. Phygon has been

reported favorable for producing unblemished fruit on Golden Delicious (35).

The temperature conditions in 1957 were different than in 1956 (Tables I and VI). In 1957 one tree was exposed to a single low temperature of 31.0°F. Tree number 2 experienced a minimum temperature of 27.5°F. Tree number 3 and the control tree treated with captan were exposed to minimum temperatures of 27.5°, 30.5°, 31.5° and 30.5°F. These recorded temperatures occurred during the early pink and pink stages of development.

The results (Table V) indicate that although a large percentage of fruits were heavily russeted, a reduction in severity of heavy russeting was observed where glyodin was used at reduced strength. However, as the exposure to freezing temperatures increased, the magnitude of the difference in heavy and light russeting was less. The fact that 96.0 percent of the fruits from the captan treatment were heavily russeted indicated that freezing temperatures were a major cause of russeting on the trees exposed to the four periods of temperatures below 32°F.

The use of captan resulted in much less russeting on this variety in 1956 than glyodin following freezing temperatures (Table II); but, when numerous freezing periods occurred near bloom in 1957, even the fruits of the captan treatment were severely russeted (Table V). This agrees with previous observations (38).

The findings of studies conducted at the Graham Experiment Station in 1956, Experiment 2, comparing glyodin and captan on Golden Delicious, indicated that the use of glyodin did not cause a significantly greater amount of either heavy or light russeting than the use of captan. The near absence of russeting of Golden Delicious in this study (Table III) even when glyodin was used, although a temperature of 30.5° F was recorded at early pink, has a possible explanation. The stage of floral development was such that the receptacle was partially protected from freezing injury by the floral bracts and the young leaves surrounding the flower cluster. Also, the 30.5° F temperature was recorded for slightly less than thirty minutes just before sunrise, which could have been too short a period of time to cause freezing injury.

The method of application may have been another factor. In Experiment 1, an adjustable nozzle gun was used to make the applications. Even though the operating pressure was only 150 pounds per square inch, the gun was operated within two feet of the leaves and fruit. Thus, the droplets could have hit the flowers and fruit at a greater force than when the air-blast attachment was used to make the applications in Experiment 2. This possible mechanical injury has been supported by observations by Mitchell and Kretchman (41).

In 1957, the same pesticide chemicals used on Golden Delicious in 1956, were applied as half-tree treatments on Jonathan under controlled

temperature conditions (Experiment 4). Three trees were exposed to various freezing temperatures: (1) 32.0°F minimum; (2) 27.5°F minimum; and (3) 27.5°, 30.5°, 31.5° and 30.5°F minimum temperatures (Table VI). The magnitude of difference of russeting of this variety was not as great as with the same materials on the Golden Delicious variety. Nevertheless, the general pattern was somewhat similar.

The amount of heavy fruit russeting on Jonathan was nearly equal for both glyodin and captan on the tree exposed to a 32.0°F minimum temperature. However, the amount of light russeting from the glyodin treatment was greater, 48.53 percent, as compared to 19.51 percent from the captan treatment (Table V). At a lower minimum temperature (27.5°F) the amount of heavy russeting from the captan treatment was only one-half the amount resulting from the glyodin treatment, 3.29 percent and 7.55 percent, respectively. The light russeting from both the glyodin and captan treatments did not vary greatly from that of the fruit exposed to 32.0°F. The greatest difference between spray treatments occurred on the tree exposed to the four periods of freezing temperatures, 27.5°, 30.5°, 31.5° and 30.5°F (Table V). The portion receiving the captan treatment had 15.52 percent of the fruit heavily russeted compared to 67.07 percent heavily russeted fruit from the glyodin treatment. The portion of the harvested fruits free of russeting was 37.93 percent for the captan treat-

ment and only 4.88 percent for the glyodin treatment. Field studies (Tables IV and VII) using the same materials, supported these findings of the controlled temperature studies (Table V). This appeared to substantiate previous observations (35) that certain chemicals may cause some degree of russeting on Jonathan, but these same materials may accentuate the amount of russeting if applied after a cold period near bloom.

The amount of russeting from the glyodin treatment in field studies on Jonathan was less than found in the controlled temperature studies. Here again, the period of exposure to freezing temperatures was less in the field investigations. Also, the application in the field being made with an air-blast sprayer, could have caused less mechanical injury than resulted from the use of an adjustable nozzle gun in the controlled temperature experiments (41).

It was significant to note that in the plot receiving the captan treatment under field conditions, resulted in heavy russeting that was significantly higher than that from the use of sulfur in the adjacent plot (Table VII). Although the captan plot was slightly lower in elevation, this would appear to substantiate previous findings (35, 38) to the effect that the use of wettable sulfur on Jonathan may result in a more favorable finish when freezing temperatures occur near bloom.

It should be mentioned that Phygon was used in all treatments at the Graham Experiment Station in 1957. In field studies not a part of this investigation, made on Jonathan in grower's orchards in 1957, it was found that fruit from the use of Phygon plus captan following the occurrence of an air temperature below 32° F, had a greater amount of russeting than that resulting from the use of wettable sulfur plus a mercurial compound. As the use of wettable sulfur on Jonathan at the Graham Experiment Station gave less russeting than captan, it may be deducted that Phygon could be unfavorable to use on Jonathan following the occurrence of freezing temperatures close to bloom. A second field study in a grower's orchard carried on in the years 1955, 1956 and 1957 has shown that the use of glyodin on Jonathan resulted in no more heavily russeted fruit than the use of captan. Based on these observations, there is a possibility that the high wetting properties of glyodin (27) enhanced the capacity of Phygon to increase the amount of heavily russeted fruit at the Graham Experiment Station in 1957.

No small trees of McIntosh were available for controlled temperature studies. Nevertheless, the field studies support previous observations (24, 29, 35, 38) that McIntosh fruits are much less sensitive to the effects of freezing temperatures and pesticide chemicals on fruit russeting (Tables III, IV, VII). No significant differences were noted for the fungicides

used on this variety regardless of the exposure to varying amounts of freezing temperatures as experienced at the Graham Experiment Station in 1956 and 1957.

The comparison of the findings of 1956 with those of 1957, Experiments 2 and 4 at the Graham Experiment Station, using the same pesticide chemicals for the two years on the same trees and applied with the same equipment, indicated that significantly more russeting occurred on the Jonathan and McIntosh varieties in 1957 than in 1956. The only apparent difference between the two seasons was duration of the temperatures below 32° F. A single low of 30.5° F occurred in 1956 just before bloom, whereas in 1957, periods of 31.5°, 31.0° and 31.5° F were recorded during the blossoming period.

To summarize, the knowledge of interrelationships between freezing temperature and use of certain fungicides tend to reveal some of the factors responsible for russeting. From the findings where the air temperature was controlled, Tables II and V, and from studies made under commercial orchard conditions, Tables III, IV, VII and VIII, it may be stated that air temperatures of 32° F or below may cause some fruit russeting on the varieties Jonathan, Golden Delicious and to a lesser extent on McIntosh. This russeting may be increased by the use of certain fungicides. Thus, it is evident that newly introduced pesticides designed for use near and during bloom should be studied on the so-called sensitive apple varieties to determine their effect on fruit russeting.

Histological Studies

The method of initiation and the development of russeted tissues of the apple have been discussed by various workers (1, 3, 4, 24, 55, 57, 58, 60, 64). The general consensus of opinion was that the bases of the trichomes and the stomata represent weak spots in the epidermal layer. They suggested that injury to the trichomes by freezing temperatures, or entry of a pesticide chemical through the gap left after the trichomes sloughed off, or through stomatal openings results in killing of epidermal cells adjacent to these openings. As the fruit developed, these areas of dead epidermal cells were unable to continue meristematic activity and thus the epidermal surface split and exposed sub-epidermal cells. The russet was thought to develop from meristematic activity of the underlying cells as a normal healing process of this crack or break in the epidermal surface.

The results of the histological investigations of the development of russeting on Golden Delicious made in this study were not in accord with these previously reported observations. Microscopic studies of the fruit of the Golden Delicious treated with glyodin and exposed to freezing temperatures (Experiment 5) revealed that the numbers of breaks in the cuticle and epidermal layer of fruit were evident at four weeks after full bloom (Figure 26). This was not found on the developing fruit protected from freezing temperatures and sprayed with captan (Figure 15). Further-

more, these breaks were not associated with the presence of trichomes or stomata, Figure 26.

Secondly, newly exposed surface of the lesions or crevices were not covered with a cuticle (Figure 26) as was found when the trichomes sloughed off (Figure 15). In this study, the first evidence of cuticularization was at the base of the trichomes (Figure 13) and as further cuticle deposition occurred, the trichome appeared to be actually forced out of the epidermal layer by the developing cuticle (Figures 14 and 15). This suggests that if the observed rupturing of the epidermal surface found four weeks after full bloom, Figure 26, was related to location of trichomes, the newly exposed internal surface of the lesions or crevices would be covered with a cuticle (Figure 15). This was not observed, Figure 26.

To further confirm the fact that russeting may not be associated with removal or injury to trichomes, trichomes were removed mechanically from the floral receptacles and young fruits in distinct strips at various stages of development. At the same time, the tips of the trichomes of other flowers and fruits were injured with dry ice. This was done on Golden Delicious and Jonathan trees exposed to freezing temperatures and sprayed with captan (Experiment 5). Since no russeting occurred on these injured fruits, the fruit russeting resulting from the fungicides used in this study could not be associated directly with injury to the trichomes on the fruit.

The earliest apparent development of fruit russeting was shown in Figure 25. Certain sub-epidermal cells in the outer cortical layer divided periclinally and anticlinally resulting in a small group of cells which, as this proliferation continued, ruptured the epidermal layer and cuticle. This was illustrated in Figure 26. As the fruit continued to enlarge, the lesions or crevices became wider. Proliferation of cells continued, and the internal pressure caused the outer cells to collapse and become crushed and eventually the crushed cells sloughed off. As cell development continued, a distinct layer of small, brick-shaped, non-vacuolated cells having the characteristics of a cambium, was formed below the lesion (Figure 28). This cambial-like layer has been termed a phellogen (1, 3, 4, 29, 55, 60). It continued to form phellem centrifugally in the same manner as a phellogen in a woody dicotyledonous stem (13).

The later stages of actual russet formation observed in this study tend to agree with previous findings (1, 3, 4, 24, 29, 55, 57, 60, 64) but the reasons for fruit russeting as associated with injury to trichomes and cells adjoining stomata were not evident in this investigation. Injury and resultant russeting in these studies appeared as meristematic activity of outer cortical cells which, in turn, forcefully ruptured the epidermal layer. There was no collapsed or dead epidermal cells or trichomes to bring about meristematic activity of outer cortical cells. This meristematic

activity apparently was induced either by the fungicide glyodin or by the breakdown products of glyodin or by adjuvants included in the formulation of glyodin, which passed through the epidermal layer and came in contact with the outer cortical cells. Whether translocation took place between the epidermal cells or through the epidermal cells is not known. It does seem apparent, however, that exposure of the receptacle of the flowers or fruits to a freezing temperature of 32° F or lower near or during bloom, increases the ease by which the fungicide may pass through the epidermal layer. This premise is based on the fact that the percentage of Golden Delicious fruits heavily and lightly russeted was greater from the use of glyodin when temperatures below 32° F occurred near bloom in 1956 than from the use of glyodin when the air temperature was not allowed to fall below 32.5° F (Table II).

Studies of the protective layers of the three varieties, McIntosh, Golden Delicious and Jonathan, gave some insight into the reasons for the differences between varieties relative to their susceptibility to russeting. It also offered a possible explanation of the interrelationship between freezing temperatures and certain pesticide chemicals as they affect fruit russeting.

Studies by Modlibowska and Rogers (42) on the freezing of plant tissues under the microscope revealed that ice usually formed in the inter-

cellular spaces first. However, if no intercellular spaces were present, as in meristematic tissues, they observed that in some cases actual freezing of the cell walls occurred, which in some instances resulted in the longitudinal splitting of the cell walls along the middle lamella, leaving the separated cells undamaged. Their studies were made on Tradescantia staminal hairs, receptacles of apple flowers, and moss leaves (Pterygophyllum lucens).

It was also observed by these authors (42) that the cuticle, the cell wall and the ectoplasm together formed an effective barrier against ice inoculation. They observed that an undamaged cuticle protected both the epidermal hairs and the epidermal cells from ice inoculation. Because the epidermal cells were frequently small and thick-walled, the underlying layer of thin-walled cells were inoculated with ice before the epidermal cells and thus they were the first to freeze and, in turn, inoculated the overlying epidermal cells with ice.

Studies of the development of the cuticle, Experiment 5, revealed that no cuticle was present on the external surface at the pre-pink stage of flower development when the receptacle is usually completely exposed. However, some cutinization of the exposed wall of the epidermal cells was taking place. As the flower continued to develop, more cutinization occurred and at full bloom a small amount of external cuticle could be observed near

the base of the trichome (Figure 9). At petal fall, a distinct external cuticle was present and surrounded the base of the trichomes, as illustrated by Figure 13, and approximately seven days after petal fall (first cover), the cuticle was complete (Figure 14).

Thus, it was apparent that for the three varieties included in this study that the receptacle of the flowers and the young fruits was not completely protected by cuticle between the time the receptacle was first exposed to environmental conditions (pre-pink) and until shortly before first cover.

This provides a possible explanation for the fungicide-variety-temperature interrelationship developed from this series of studies. If freezing temperatures occur during or near full bloom when the fruit is not protected by a cuticle, injury from ice inoculation to sub-epidermal cells may take place without apparent injury to the epidermal cells. The subsequent healing of these injured areas of cells may cause cell proliferation that may eventually rupture the epidermis. Subsequent russeting may develop on the varieties more susceptible to inoculation of ice in sub-epidermal areas, such as Jonathan and Golden Delicious, and not the McIntosh. Since the floral receptacle and very young fruit is very meristematic during this early stage as was observed by various workers (2, 57, 58, 59), there may be little, if any, intercellular spaces in the cortical

tissues and the epidermis, so that freezing temperatures may, as was found by Modlibowska et al. (42) cause a temporary separation of the cells of the epidermal layer. If a certain pesticide chemical is applied following this injury and prior to the formation of a complete cuticle, the chemical may actually penetrate the epidermis into the outer cortical cells causing meristematic activity of these cells as illustrated in Figures 25, 26 and 27. This, in turn, would increase the number of ruptured areas and increase the degree of fruit russeting beyond that normally occurring from freezing temperatures alone (Table II). However, this separation of the cells of the epidermal layer, described by Modlibowska (42) was not observed in the material studied in this investigation.

The severity of fruit russeting appeared to be correlated with the relative time freezing temperatures occurred and the development of cuticle on the young fruit. In 1957, Trees 1 and 2 of Golden Delicious treated with glyodin (Table V) were exposed to freezing temperatures occurring at early pink. In 1956, the exposed tree receiving the glyodin treatment was subjected to three periods of freezing temperatures at pink, early bloom and early petal fall (Table I). However, the amount of russeted fruits was very similar (Tables II and VI). The lack of cuticularization of the flower receptacle at the earlier stage of flower development would tend to make the tissues more susceptible to ice inoculation from freezing temperatures

(42) which, in turn, would result in more fruit russeting than if freezing temperatures occurred later in the fruit development.

Since most fungicides probably have some phytotoxic properties (63), this possible explanation of the fungicide-freezing temperature interrelationship does not account for the differences in fruit russeting resulting from the use of the various chemicals. Glyodin used in this investigation has been reported (28) to have surface active properties; that is, it will decrease the surface tension of a water mixture so that a surface which is difficult to wet, may become wetted with the spray mixture. The receptacles of the apple flower in this study were covered with a dense mat of trichomes which were difficult to wet when sectioning, using the *freezing microtome technique.*

Therefore, because of its surfactant property, glyodin may be able to wet the trichomes of the flower receptacle, penetrate this mat and come in contact with the epidermal layer of cells of the receptacle. This wetting property would facilitate the entry of the chemical into the sub-epidermal layer of cells by passing between the anticlinal walls of the cells of the epidermis.

Another possible explanation of the variation in fruit russeting from the use of different fungicides, may be the degree of solubility of the compound in a spray mixture. Certain pesticide materials are wettable

powders and are completely insoluble in water; whereas, others may be partially soluble or a part of the molecular structure may be released into solution as a soluble material (8, 49). The solubility or partial solubility of the compound would facilitate the entry into or through the epidermal tissue of the fruit, causing russeting of certain varieties. However, the scope of this investigation was not sufficiently extensive or so designed to determine this supposition.

The slight anatomical differences observed between varieties early in the growing season when fruit russeting was first initiated, make it very difficult to explain why some varieties russet more easily from cold injury and chemical than others. However, the histological studies (Figures 15 through 24) support the observations by Bell (2) and others (24, 32,55, 57, 58, 60) that apparent variations in epidermal layers of various varieties do exist as the growing season progresses.

The results of the fruit russeting experiments in this investigation indicated that Golden Delicious was the most sensitive to external conditions that promote russeting and the McIntosh least sensitive with Jonathan being intermediate. This appeared to be correlated with the variation in the epidermal layers of the three varieties. The McIntosh having the most complete epidermal layer at maturity (Figure 20) was less susceptible to russeting; whereas, the Golden Delicious with the least distinguishable

epidermis (Figure 17) was the most susceptible. Jonathan (Figure 23) was intermediate in both respects.

From this study, it appeared that visible differences in the epidermal layer of Golden Delicious, Jonathan and McIntosh were not distinguishable until approximately four weeks after full bloom. However, it was apparent that exposure of the receptacle of the flower or fruit to freezing temperatures near or during bloom resulted in more fruit russeting at harvest time from the use of some chemicals. This apparent change in the protective capacity of the epidermal layer following exposure to freezing air temperatures could be a result of a change in the chemical nature of the intercellular substances of the epidermal cells, this chemical change occurring more readily in the epidermal layer of one variety than another. The results of this series of studies do suggest such a possibility.

SUMMARY

1. An investigation was conducted in 1956 and 1957 at the Michigan State University Horticultural farm, East Lansing, to determine the possible interrelationship between the occurrence of freezing temperatures near or during bloom and the use of certain pesticide chemicals as affecting fruit russeting of apples (Malus domestica Bork.). Bearing trees growing on Malling VII rootstocks, of the varieties Golden Delicious and Jonathan were exposed to or protected against freezing air temperatures of 32° F or lower by enclosing the trees with polyethylene covered cages and using supplemental electric heat. The trees were pruned to divide the bearing surface of each tree into two distinct halves, so that two fungicide treatments could be evaluated in the same environment.

(a) Half-tree treatments of captan and glyodin were made on Golden Delicious in 1956. At harvest time, the portion of the tree protected from freezing air temperatures and treated with glyodin had 27.8 percent of the fruit heavily russeted, while the portion treated with captan had no fruit so affected. On the tree exposed to temperatures of 31.7°, 31.5°, and 28.0° F near bloom, 84.5 percent of the fruit treated with glyodin were heavily russeted as compared to 24.4 percent for the captan treatment.

(b) Treatments of glyodin at one quart, and glyodin at one

pint plus four ounces of Phygon per 100 gallons were made on the half-trees of Golden Delicious in 1957 and the trees were exposed to varying amounts of freezing temperatures near bloom. At harvest no fruits from any treatment were free of russeting, but the severity of the russeting was less from the use of glyodin at one pint plus Phygon than when glyodin was used at full strength.

(c) Treatments of full strength captan and glyodin were made on half-trees of Jonathan in 1957. At harvest, 2.44 percent of the fruit treated with captan and exposed to 32° F were heavily russeted, and 19.51 percent lightly russeted. The glyodin treatment had 2.94 percent of the fruit heavily russeted and 48.53 percent lightly russeted. An exposure of 27.5° F increased very slightly the amount of heavily russeted fruit from the use of glyodin treatment as compared to an exposure of 32° F. Exposures to minimum temperatures of 27.5°, 30.5°, 31.5° and 30.5° F near bloom resulted in 15.52 percent of the fruit heavily russeted, and 46.55 percent lightly russeted for the captan treatment, and 67.07 percent of the fruit heavily russeted and 28.05 percent lightly russeted from the use of glyodin.

2. A mixed block of 16-year-old Golden Delicious, Jonathan and McIntosh apple trees located at the Michigan State University Graham Experiment Station, Grand Rapids, was used to evaluate the effects of captan, thiram, glyodin, and captan followed by glyodin after first cover on fruit russeting.

The treatments were applied with a commercial sprayer with an air-blast attachment. A low of 30.5° F occurred at the early-pink stage of development. There were no significant differences of fruit russeting between treatments.

3. A second block at the Graham Experiment Station containing 7-year-old McIntosh and Jonathan apple trees was divided so as to compare the influence of ferbam, glyodin and thiram with that of captan on fruit russeting. In 1957, the study was repeated, except that wettable sulfur was substituted for ferbam. All treatments were applied with a commercial sprayer with an air-blast attachment.

(a) In 1956, a minimum temperature of 30.5° F was recorded at the time of early pink. At harvest, no significant differences of heavy russeting were noted either between treatments or between varieties. However, one group of trees of the captan treatment was lower in elevation than the other trees, and these trees did have a larger amount of lightly russeted fruit than the other plots.

(b) In 1957, minimum temperatures of 31.5°, 31.0° and 31.5° F were recorded near this experiment. Significant differences for heavily russeted fruits did occur between spray treatments used on Jonathan. There were significant differences in the amount of heavily russeted fruits between the groups of trees treated with captan, the range being 12.1 percent

to 21.6 percent. This is compared to 12.4 percent heavy russeting for the sulfur treatment, 18.0 percent for the thiram treatment, and 29.5 percent for the glyodin treatment. The captan treatment having the 21.6 percent heavily russeted fruit was lower in elevation than the other treatments. The excessive russeting could be associated with lower air temperatures in the less favorable elevation. There is some evidence, not a part of this series of studies, to indicate that the excessive heavy russeting from the use of glyodin could have resulted from the use of Phygon with the glyodin after the occurring freezing temperatures, and not to glyodin alone. No significant differences were found between treatments with respect to light russeting.

(c) The same weather conditions and spray treatments on McIntosh resulted in no significant differences between treatments.

4. Comparing the results of the two seasons for the varieties McIntosh and Jonathan when the same fungicide treatments and method of application were used, there was a significantly higher amount of russeting in 1957 than in 1956. Temperatures of 31.5°, 31.0° and 31.5° F were recorded near bloom in 1957, whereas a minimum of 30.5° F was recorded prior to bloom in 1956.

5. Histological studies were conducted in 1956 for the purpose of (1) determining the differences in development of the cuticle and epidermal

tissue of the varieties Golden Delicious, Jonathan and McIntosh; and (2) determining how and when fruit russeting of Golden Delicious develops when certain pesticide chemicals are used prior to second cover. Frozen sections stained with Sudan IV and killed and fixed material differentially stained with safranin and fast green were used for these studies.

(a) The first microscopic signs of russet formation on Golden Delicious were observed three weeks after full bloom as small numbers of irregularly proliferating cells in the outer cortical layer. At four weeks after full bloom, these proliferating cells had ruptured the epidermis and cuticle. This initial proliferation was presumably stimulated either by injury to the outer cortical cells by freezing temperatures, the use of certain fungicide chemicals which, in turn, came in contact with the outer cortical layer of cells, or a combination of the two factors.

As growth of the fruit continued, more sub-epidermal proliferation occurred and at harvest time a very evident phellogen was found below the russeted areas.

(b) The initial ruptures of the epidermal layer could not be associated with either the trichomes or stomata because the fissures were more numerous than the total numbers of trichomes and stomata. Also, the inner exposed surfaces of the initial ruptures were not covered by a cuticle. Actual removal or injury of the trichomes with dry ice at various stages of flower development did not cause russet formation.

(c) Microscopic studies of the development of the protective layers of the Golden Delicious, Jonathan and McIntosh varieties revealed that at the time the flowers emerge from buds, the receptacle was not covered with a cuticle. However, some fatty substances were found within the exposed cell-walls of the epidermal cells prior to full bloom. Cuticularization occurred first at the bases of the trichomes and ten to fourteen days after full bloom, the cuticle was complete on the epidermal surface of the fruit.

(d) The development of the protective layers of the varieties Golden Delicious, Jonathan and McIntosh was similar until approximately 30 days after full bloom at which time cuticular pegs were found to be filling the cavities in the epidermal layer of Golden Delicious created by the separation of epidermal cells as the fruit enlarged. This was less evident in the Jonathan and McIntosh varieties. At harvest maturity, a distinct, intact epidermal layer could not be found in Golden Delicious. By contrast, the epidermal layer of the McIntosh was very evident and no separation of epidermal cells was observed. The epidermal layer of Jonathan, although discernable, was not as regular as in the McIntosh.

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APPENDIX A

Explanation of Terminology

Certain terminology used in this paper has been used in cited literature with any of several different connotations. Therefore, for clarification as to their meaning, a listing of terms and a brief description of their more accepted use will follow:

A. Terminology used in sequence of fruit development of apple, taken from MacDaniels et al. (30) and Mitchell et al. (40):

1) Dormant - fruiting buds completely enclosed by bud scales.

2) Delayed dormant - bud scales and several scale-like leaves are shed and basal spur leaves are flat in the bud enclosing the receptacle of the flower.

3) Early pre-pink or cluster bud stage - corolla completely covered by sepals and the receptacle still somewhat enclosed by expanding leaves.

4) Pre-pink - spur leaves mostly unrolled and blossoms held together by entangling of the trichomes in a cluster with the receptacle exposed.

5) Pink - individual blossoms separated within a cluster and all blossoms having exposed, but closed petals.

6) Full bloom - a minimum of three-fourths of the blossoms in all clusters on the tree unfolded with all parts exposed.

7) Petal fall - three-fourths of the petals fallen from all the clusters on the tree.

8) First cover - seven to ten days after petal fall.

9) Second cover - three weeks after full bloom.

B. Terms relative to anatomy of the protective layers of the fruit as described by Orgell (45) and Eames and MacDanield (12) and have been misinterpreted in past literature:

1) Cuticle - consists of one or more layers of semi-lipoidal material exterior to the outer epidermal cell wall that includes cutin layers and wax encrustations.

2) Cuticularization - the process of cuticle formation.

3) Cuticular skirts or flanges - the projections of the cuticle between the anticlinal epidermal cell walls. These flanges are often called "pegs" when observed in cross-section.

4) Cutin - the semi-lipoidal polymer which constitutes the matrix and usually the bulk of the cuticle. Its chemical nature is poorly understood and it may be impregnated or overlain with wax of various forms.

5) Cutinization - the process of cutin impregnation of cell walls; particularly the epidermal cell walls.

6) Phellogen - the meristematic cork cambium.

7) Phellem - corky tissue produced centrifugally from the pellogen.

8) Phelloderm - the thin-walled cells produced internally by a phellogen.

9) Periderm - the collective term applied to the phellogen, phellem and phelloderm.

APPENDIX B

Chemical nature of spray materials discussed in this investigation.

1) Aramite - 2-(p-tert-butylphenoxy) isopropyl-2-chloro-ethyl sulfite.

2) Captan - N-trichloromethylmercapto-4, 4-cyclohexene-1, 2-di-carboximide.

3) DDT - 1, 1, 1-trichloro-2, 2-bis(p-chlorophenyl) ethane.

4) EPN - O-ethyl-O-p-nitrophenyl benzene ethiophosphate.

5) Ferbam - ferric dimethyldithiocarbamate.

6) Glyodin - 2-heptadecylglyoxalidine acetate.

7) Malathion - S-(1, 2-dicarboxyethyl), O, O-dimethyl dithio-phosphate.

8) Methoxychlor - 1, 1, 1-trichloro-2, 2-bis (p-methoxyphenyl)-ethane.

9) Ovotran - p-chloropehnyl-p-chlorobenzenesulfonate.

10) Parathion - O, O-diethyl, O-p-nitrophenyl thiophosphate.

11) Phix - Phenylmercuric acetate.

12) Phygon - 2, 3-dichloro-1, 4-naphthoquinone.

13) Thiram - tetramethylthiuram disulfate.

14) Triton B 1956 - a phthalic glycerol alkyd resin. (used as a surfactant only).