ABSTRACT

MORPHOLOGICAL AND HISTOLOGICAL DEVELOPMENT OF THE GOLDEN DELICIOUS APPLE (*MALUS DOMESTICA* BORK.), AS INFLUENCED BY LEAF NITROGEN

by Wilfred F. Wardowski II

A study was conducted at East Lansing, Michigan in 1964 and 1965 to determine the morphology and histology of Golden Delicious fruits as influenced by the leaf nitrogen levels.

The percent russeted fruit was significantly greater in 1964, but not 1965, for the 4-pound sodium nitrate ($NaNO_3$) treated trees than for the treatment receiving no supplemental nitrogen.

The $NaNO_3$ treatment disregarding years had significantly larger values for leaf nitrogen, calcium, magnesium and copper, and significantly smaller values for leaf potassium, phosphorus and zinc as compared with the no nitrogen treatment. The only difference between years was a significantly higher leaf nitrogen value for the no nitrogen treatment in 1964 than in 1965.

Measurements taken both seasons at 5 a.m. and noon revealed that fruits often lost size during the daylight hours, but differences between treatments were not noted.

A histological study was carried on during 1965 comparing the pith, cortex, hypodermis and epidermis of developing fruits from the $NaNO_3$ and no nitrogen treatments.

There were no consistent differences in cell size between treatments for the pith and cortex. However, the cortex cell walls of the fruits of the $NaNO_3$ treatment were noticeably more wrinkled and irregular than those of the control for samples taken July 13 and later. No differences were noted between treatments for the sizes of pith and cortical tissues until the June 15 sampling when the samplings from the $NaNO_3$ treatment had nearly three times as much pith and one-fifth less cortex than the samplings of the no nitrogen treatment. And, after June 15, the pith was larger in every sample from the $NaNO_3$ treatment measured, while the cortex was smaller, except for those samples collected in September.

The hypodermis of fruits of the no nitrogen treatment had fewer layers of cells and was more sharply defined from the inner portion of the cortex than in the $NaNO_3$ treatment. At harvest the hypodermal cells appeared to be crushed radially, but not in the no nitrogen treatment.

The epidermis of each treatment appeared to be similar until two weeks after bloom, when the epidermal cells of the no nitrogen treatment exhibited tangential as well as radial

divisions, while those of the $NaNO_3$ treatment were dividing radially and only occasionally tangentially. Two weeks later, there was less evidence of epidermal cell division for the $NaNO_3$ treatment and the cells were larger than those from the no nitrogen trees.

As the season progressed, the epidermal cells of both treatments became separated with cuticle filling the created cavities. Some cells were completely surrounded by the cuticle. Cells so isolated were radially collapsed as the fruits matured. Separation, isolation and collapsing of epidermal cells occurred first in the fruits of the $NaNO_3$ treatment. A similar development for the fruits of the no nitrogen treatment was noted two to four weeks later. The areas of collapsed epidermal cells were greatest at harvest for the fruits of the $NaNO_3$ treatment.

The appearance of russeted tissue was similar on fruits from the $NaNO_3$ treatment, of corked over stomates of fruits from both treatments and of the russeted portion of the fruit with a sectional chimera. A phellogen originating from cells of the hypodermis produced crushed cork cells to the outside forcing the epidermis and cuticle off.

An X-ray microprobe was used to analyze the druse and rhombic crystals which were concentrated in the carpels and vascular strands at the time of bloom, and which were less frequent as the season progressed.

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By

Wilfred F. Wardowski II

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TO MY PARENTS -- MERNA AND ALFRED WARDOWSKI

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TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

APPENDIX

INTRODUCTION

The Golden Delicious cultivar of the apple (Malus domestica Bork,), although grown throughout the world, has one major fault under certain conditions, the formation of epidermal lesions giving the fruits a russeted appearance.

A study was undertaken to reveal some of the physiological aspects and the internal structure of russeted and nonrusseted fruits. Such information should add to that previously reported and enhance understanding of the phenomenon of the formation of epidermal lesions of the fruits.

Written descriptions of the internal structure of pome fruits utilize terminology from either of two theoretical origins. The appendicular theory presented by McDaniels (33) pictures the pome fruit as a central core of carpellary tissue surrounded by a floral tube which phylogenetically consists of fused bases of floral parts. In contrast, the receptacular theory described by Kraus (26) regards the outer bulk of the fruit as an extension of modified receptacle.

In the course of this study, the structures observed seemed to be most nearly described by the receptacular theory. Thus, the terminology employed herein is that of the receptacular viewpoint utilized by Tukey and Young (57).

REVIEW OF LITERATURE

The problem of epidermal lesions and subsequent russeting of the apple is widespread. Stem end russeting was recorded by Blodgett (8) as occurring in Israel, Europe, New Zealand, South Africa and the United States. There are an array of causes or conditions which enhance epidermal lesions formation.

Freezing temperatures occurring near full bloom are reported to result in russeting of apples (14, 34, 38, 42, 43, 48, 52).

Rogers (42) described the lifting of epidermis and hypodermis from the cortex of the fruit of Cox's Orange Pippin by the formation of ice at 28°F. Studying the receptacle of an apple flower, Modibowski and Rogers (38) stated, "This split between cells of the cortex and the hypodermis occurred in a similar way to that observed in the moss leaf under the microscope. Ice formed between the cell walls, withdrew water from the surrounding tissue several cells deep, and thus protected them from freezing." Healing was then attributed to the subsequent formation of callus cells. This healing process was similar in appearance to that described by Dorsey (14) for injury from the use of certain chemicals.

Small splits in the cuticle caused by a light frost were credited by Sorauer (52) as the stimulant to cork russeting in apples as well as in pears, plums and grapes.

MacDaniels and Heinicke (34) suggested and illustrated that some cases of russeting commonly called "spray burn" were in reality freezing injury. Well illustrated reports by Simons (43, 44) and Simons and Lott (48) substantiated this.

A separation of the epidermal and hypodermal layers from the cortex of McIntosh in the bud stage was described by Gilbert (19) to be similar to separations previously attributed to "frost" injury. However, he reported that during the period of his study no frost occurred and the separations he observed were due to structural differences between the tissues.

Many workers have reported apples being russeted by pesticide chemicals (5, 28, 37, 41, 53). Bell (5) described the development of russeting in McIntosh Red caused by Bordeaux spray. The fact that spray induced russeting of Delicious and Golden Delicious was increased by slow drying conditions has been documented by Palmiter (41). Stiles, et al. (53) reported that russeting and cracking of Staymen apples were not significantly altered by sprays of urea as

compared to soil applications of nitrogen. Mitchell, et al. (37) gave specific recommendations for pesticide chemicals resulting in the desired Golden Delicious finish for Michigan conditions.

The interrelationship of freezing air temperatures and fungicides as it relates to Jonathan and Golden Delicious fruit finish was investigated by Kretchman (28). Fruits exposed to freezing air temperatures near full bloom were more heavily russeted when treated with glyodin than when treated with captan. When the fruits were protected from the cold, less russeting was found.

Nutrition has been suggested as a possible contributor to russeting (15, 63) and to cracking (39) of apples. Russeting of Golden Delicious was shown by Eggert (15) to be associated with high nitrogen treatments. High iron content of the water used for spraying Golden Delicious was suggested as a cause of russeting by vanBelle (63). Montgomery (39) noted more cracking in Cox's Orange Pippin with both high nitrogen and high potassium treatments.

High relative humidity has been suggested as a cause of russeting in the Golden Delicious and Rome Beauty by Tukey (58). He enclosed fruit in plastic bags and allowed the relative humidity to remain at or near 100 percent. Longer periods of exposure resulted in greater amounts of russeting.

Other factors which have been reported to influence russeting include russet sports as reported by Simons (45, 46) and Chandler and Mason (10) mechanical injury by Simons and Aubertin (47), sulphur dioxide in the air by Baker (2), drought by vanBelle (63), and rootstock, interstock or soil by Chandler and Mason (10).

Bell (7), Gardner and Christ (18) and Meyer (35) noted that russeted fruits lost moisture more readily than nonrusseted fruit.

Many workers have followed the size increase and periodicity of growth of apples and other fruits (1, 7, 12, 13, 15, 21, 32, 36, 51, 57, 59, 60, 62, 64, 66).

The seasonal growth pattern of the apple fruit is well established as a fairly steady increase in size until the fruit approaches maturity at which time a decrease in the rate of growth is noticeable (12, 13, 57, 64, 66). Tukey and Young (57) noted that the early maturing varieties did not possess the characteristic of a decreased growth rate near maturity.

Mitchell (36) indicated that the size increase of the Bartlett pear was similar to that of the apple. He did note, however, that there was a period, nine weeks after full

bloom, when the rate of increase in size was noticeably reduced at the time of rapid enlargement of the embryo.

The diameter enlargement of Stayman Winesap apples as reported by Verner (64) was greatest at times of lowest atmospheric evaporation and smallest when the atmospheric evaporation was high.

Size increase in the apple fruit occurs initially by cell division which is completed about four weeks after pollination, and is then due to cell enlargement (1, 32, 51, 57). Denne (13) noted that in Miller's Seedling, cell division continued for about twelve weeks after full bloom.

The time of fruit enlargement was recorded by Harley and Masure (21) to have a daily periodicity with the maximum enlargement occurring predominantly during the dark hours and the minimum growth between four and six in the afternoon. Tukey (62) utilized a continuous recording instrument to measure the expansion and contraction of fruits. He reported (62) fruit shrinkage as part of diurnal fluctuations for apples, peaches and sour cherries, and suggested that this was due to moisture stress. The growth cycle for apples was indicated by Tukey (60) to be a loss in size from sunrise until noon and then a gradual enlargement until the next sunrise, attaining a new maximum diameter.

Eggert (15) utilized an instrument called a volumeter (16) to record the volume of intact fruits of Golden Delicious to estimate their growth patterns. He noted that shrinkage of the fruit occurred more often where trees had been treated with nitrogen than for those without.

Tukey (61) reported that light was a more important factor in the diurnal fluctuation of Golden Delicious fruits than temperature.

The formation of lenticels has been discussed in detail (11, 54, 67) and their location has been traced to the bases of trichomes, stomata and other cracks in the surface of the young fruit.

Russeting, cracking and epidermal lesions of apples have been described anatomically and histologically (2, 4, 5, 9, 15, 28, 43, 44, 45, 46, 47, 54, 65). Russeting was presented by Tetley (54) as an extension of cork cambium activity similar to that which forms lenticels, but covering a larger area.

On the subject of the origin of russeting in the Golden Russet apple, Bell (4) states, "The cambium cell is initiated always in a cell of epidermal origin and never in a cell of sub-epidermal origin." He explained that the epidermal cells of the young fruit divide one or more times

to form a multi-epidermis, the inner layer of which initiated the cork cambium. When describing the origin of russeting from Bordeaux sprays in the McIntosh Red, Bell (5) reported that cork cambium is formed from cortex cells. Baker (2) observed that the collenchyma cells below the cork cambium were of normal structure, with the abnormal structures being confined to the region originally occupied by the epidermis and cuticle.

Kretchman (28) noted that the first cork cambium cells initiated by the combination of freezing air temperatures and spray chemicals were, to quote, "a small number of irregularly proliferating cells in the outer cortical layer." Similar findings were recorded by Eggert (15) for fruits of Golden Delicious trees which were high in nitrogen. Eggert set the time of rupturing at seven weeks after full bloom, and he also noted that ruptures appeared to be caused by internal stress rather than by hypodermal cell proliferation.

Verner (65) attributed cracking in apples to the inability of the epidermis and hypodermis to keep pace with the enlargement of the fleshy portions of the fruit.

More severe stem end russeting of the Yellow Newton apple was reported by Brown and Koch (9) for lateral fruit

than for terminal fruit. Similar findings were noted by Eggert (15) for russeting of the Golden Delicious, and by Lotter and VanZyl (31) for stem end russeting of the Ohenimuri variety of apple.

The anatomical and morphological characteristics of russeting of the Golden Delicious have been reported numerous times by Simons (43, 44, 45, 46) and by Simons and Aubertin (47).

The protective layers of the apple fruit were discussed by Bell (3) in their functional order, using his terminology, "the coating of epidermal hairs, the cuticle, the epidermis and the hypodermis." Whereas the apple's first external protection from the environment after emergence from the winter bud was considered to be epidermal hairs, it is appropriate to consider it first in discussing the protective layers.

Epidermal Hairs

When the flower bud emerges, it is covered with a dense mat of tangled hairs. Bell (3) described each hair as a modified epidermal cell which is living and active, the surface not being easily wetted. The hairs formed a layer that enclosed myriads of minute and more or less enclosed air spaces,

which provided efficient protection for the young ovary. This dense mat of hairs was reported by Bell and Facey (6) to be a real obstacle to the sectioning of winter buds.

Tetley (54) and Bell (3) described the gradual wide separation of the hairs to be due to the anticlinal division of epidermal cells between the hairs. Tetley (54) indicated that broken hairs represent weak places in the apple's surface. However, she mentioned that cracks may occur which have no reference to hair bases or to lenticels. Cracks related to the epidermal hairs were not found by Bell (3) in studying the McIntosh Red. Lenticels formed at the base of epidermal hairs have been reported by Zschokke (67), Clements (11) and Gilbert (19).

Cuticle

The first cuticle laid down on an angiosperm shoot is described by Lee and Priestly (30) as migrating out of the cells along the cell walls and being more or less even in distribution over the surface. "In the McIntosh apple", Bell (3) stated, "the first deposition of cuticle could be described as almost 'patchy', with the thicker patches bearing no relation to the cell structure of the epidermis, and there was no evidence of thickenings over the ends of the

radial wall. Also the cuticle at this stage is thicker at the bases of the hairs into which it extends." He further stated that the cuticle replaced the epidermal hair as an effective protective layer and may be detected in McIntosh Red as early as three weeks before full bloom, being thicker at the hair bases than elsewhere. Bell wrote, "The deposition of cuticle continues and by the middle of June the space formerly occupied by the hair base is completely closed."

Kretchman (28) did not find a cuticle covering the receptacle of McIntosh, Jonathan or Golden Delicious apples at the time the flowers emerged from the buds. He did find some fatty substances within the exposed cell walls of the epidermal cells prior to bloom. Cuticularization occurred first at the bases of the trichomes, and 10 to 14 days after full bloom the cuticle was complete on the fruit surface.

According to Bell (3), the cuticle increased in thickness with a relatively smooth outer surface and irregular inner surface for the McIntosh Red. However, the Golden Delicious has been reported to have epidermal cells completely surrounded by the cuticle (7, 15, 28, 35, 46). Tetley (55) reported this also for the fruits of Bramley's Seedling.

The importance of cuticle to water loss has been the object of several studies (7, 20, 22). Bell (7) suggested that the uneven cuticle with its cracks could contribute to the greater water loss from Golden Delicious fruits when compared with other cultivars. Horrocks (22) demonstrated the importance of cuticular wax in the apple to water permeability using discs of skin from Golden Delicious and Granny Smith fruits. The leaves of white clovers (Trifolium repens) demonstrated increased transpiration rates according to Hall and Jones (20) when the cuticular wax was disrupted by brushing with a camel-hair brush.

Skene (50) studied the structure of the surface of several varieties of apples, pears and plums with the electron microscope. He reported differences in the structure of each apple variety as great as differences between apples and pears.

The cuticle of the Bramley's Seedling, reports Tetley (55), was thinner on the red side of the fruit than on the green side.

The leaf cuticles of 14 species of ornamental plants were studied by Sitte and Rennier (49) using a polarizing microscope. They found that all exhibited positive birefringence, and that cuticular waxes could not be stained by Sudan dyes. In contrast, Jensen (23) indicated that these waxes are stained by Sudan dyes as are the fats and oils.

Epidermis

The epidermis of the McIntosh Red at the time flowers began to emerge from the winter buds, according to Bell (7), was a layer of active thin-walled isodiametric cells. Speaking of the full bloom stage, he continued, "The epidermis is then a closely packed layer of columnar cells and remains as such for two or three weeks." At this stage he noted that the radial measurement was about twice the tangential measurement and cell division was on a radial plane. Tukey and Young (57) described "palisade-like" epidermal cells for all the apple varieties studied which were distinguishable one month before full bloom. For the epidermal cells of Golden Delicious Bell (7) reported a radial measurement of nearly three times that of the tangential measurement one week after full bloom.

Meyer (35) noted that for the Golden Delicious fruits with a diameter of 1.7 centimeters, periclinal divisions of the epidermis were conspicious along with the anticlinal divisions which prevailed in the fruits of Winesap. He suggested that this may be the reason for an irregular Golden Delicious epidermis at maturity.

The next stage in the development of epidermal cells, as reported by Bell (3), was the cessation of division and vacuolation. Normal stomata were present two weeks after full bloom, and by six weeks after full bloom tangentially elongate cells were present in the epidermis with more pronounced vacuolation. Later in the season he found spaces between the epidermal cells extending to the hypodermis which was filled with cuticle.

Kretchman (28) stated, "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."

At maturity the epidermal cells of Golden Delicious have been reported to be completely surrounded by cuticle (7, 15, 28, 35, 46).

Stomatal development and its significance in apple fruits has been investigated (11, 54, 67). In 1897 Zschokke (67) noted the occurrence of stomata on the surface of the apple and cork formation below the stomata giving rise to lenticels as the fruit matures. Clements (11) and Tetley (54) concurred that this is one way in which lenticels may

be formed. Tetley (54) also described the stages of formation of a stomate in the Court Pendu Plat apple.

Kretchman (28) reported, "The initial ruptures of the epidermal layer could not be associated with the presence of either trichomes or stomata."

Hypodermis

"One or more layers of cells beneath the epidermis in leaf, stem and root may be morphologically and physiologically distinct from the deeper-lying ground tissue." This description by Esau (17), even though it does not pertain to fruit, agrees with that by Bell (3) for the developing flowers of the McIntosh Red in that there was a two-layered hypodermis at the time the flowers began to emerge from the winter buds. Radial divisions, noted Bell, allowed the hypodermal cells to maintain their size and shape as the fruits enlarge, and subsequent tangential divisions brought the average thickness to four layers at blossom time.

Hypodermal cells of the apple are described as having a thickening of the cells walls (3, 7, 57).

Gradual disorganization of the hypodermis follows as the cells elongate tangentially, lose their identify as layers, and finally appear to be crushed (3, 7).

The foregoing discussion makes it apparent that russeting of the Golden Delicious has been widely studied and can be partially controlled. However, the causes of russeting are not completely understood and control is not complete. Thus, the study reported herein was made in an effort to verify some of the findings of Eggert (15) and to bring to light some possible reasons not yet reported for the susceptibility of this cultivar to russeting.

MATERIALS AND METHODS

Nitrogen Nutrition Study

An investigation was conducted in 1964 and 1965 to evaluate the effect of two levels of nitrogen on the finish of Golden Delicious fruits. This study in part was a modification of the work reported by Eggert (15). The randomized block experimental design included four replications, and independent variables of (a) two levels of sodium nitrate, (b) two years and (c) two rootstocks.

Sixteen 9-year-old trees growing on East Malling (EM) VII and EM XVI rootstocks were used as plant material. The trees were a part of a rootstock-variety orchard growing in sod at Michigan State University Horticultural Farm, East Lansing, Michigan.

Treatments of the sample trees included no supplementary nitrogen and four pounds of sodium nitrate annually. The sodium nitrate was applied on April 20, 1964 and April 16, 1965 as a broadcast treatment under the tree from the drip line to within one foot of the trunk. Identical treatments had been made to these trees each of the three previous springs (15).

Four other trees received eight pounds of sodium nitrate in 1964 and 1965 and four received no nitrogen. For the three years prior to this all eight trees had received eight pounds of sodium nitrate annually (15). These trees continued under observation but were not included in the statistical analysis. Observations were made on the fruits and records were kept throughout the study.

Air temperatures were recorded daily for the period of bloom through two weeks following petal fall using two maximum and minimum thermometers placed in the orchard at heights of three and five feet.

Observations on Harvested Fruit

A one bushel sample of fruit was taken at random from each tree at the time of harvest. When the yield of a tree was less than one bushel, the entire yield was taken as the sample. The fruits were graded each season for degree of russeting and size. Yields were recorded as pounds of fruit per tree.

An analysis of variance with unequal frequencies was used to test the major effects influencing the percent of russeted fruits and size of fruits. For clarity, the frequencies were unequal in that the values were considered

to be missing when the yield of a tree was less than 20 pounds.

For statistical analysis, the fruits from each tree were classified in two groups, (a) less than 2-3/4 inch diameters, and (b) 2-3/4 inch and greater. Henceforth, these two groups will be termed "small fruit" and "large fruit" respectively.

The fruits from each tree were graded into four groups for russeting: (1) russet free, (2) lightly russeted, (3) moderately russeted, and (4) heavily russeted (Figure 1). The Michigan Apple Marketing Law, Act 132, P.A. 1937 described the amount of russeting allowed on U.S. No. 1 grade fruit. The russet free and lightly russeted fruits met the specifications for U.S. No. 1 and the moderately and heavily russeted exceeded these amounts. The russet free and lightly russeted fruits were treated in this study as non-russeted and the moderately and heavily russeted as russeted.

Leaf Analysis Study

A sample of 50 leaves was collected from each tree on July 21, 1964 and July 29, 1965. The leaves selected were mature and located approximately midway on the current seasons growth and were free from damage by insects or diseases. The leaves of each sample were washed, rinsed, air dried and then ground with an intermediate Wiley mill using a 20 mesh screen.

Figure 1. Degrees of russeting on the Golden Delicious apple.

a. Russet-free fruit

b. Lightly russeted fruit

c. Moderately russeted fruit

d. Heavily russeted fruit

Figure 1

Analyses were made for nitrogen by the Kjeldahl-Gunning method (40), and for potassium with a Bechman Model B Spectrophotometer[1] (40). Further analyses were made for phosphorus, sodium, calcium, magnesium, manganese, iron, copper, boron, zinc and aluminum with a photoelectric spectrometer[2] (25).

Fruit Growth and Diurnal Fluctuation Study

In 1964 fruit growth and diurnal fluctuation of tagged fruit were recorded using the volumeter (16). Measurements were taken of 13 fruits on each of 16 trees at weekly intervals beginning on June 10 and continuing through September 23, 1964. Fruit volumes were determined also at 5 a.m., and noon, from noon of June 22 through June 26, in an effort to evaluate diurnal fluctuation.

In 1965 diameter measurements of fruits were made, rather than volume measurements, as certain parts of the volumeter had worn excessively and replacements were not available. The correlation of fruit diameter with fruit volume has been shown by Eggert (15) to be 0.90 and greater.

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[1] Product of Beckman Instruments, Inc., South Pasadena, California.

[2] Product of Applied Research Laboratories, Inc., Glendale, California.

The maximum transverse diameters were taken in a standard manner on the tagged fruits with a direct-reading caliper gage[1] graduated to 0.1 millimeter. All measurements were taken in a north-south direction.

On May 26, 1965, one week after bloom, 50 fruits on each of four trees, two receiving zero nitrogen and two treated with 4-pounds of sodium nitrate, were tagged with numbered paper tags. The tagged fruits were the central fruit of each cluster, the others having been carefully removed by hand. The tag was attached to the shoot or spur so as not to damage the pedicle or the fruit itself.

Measurements were taken at 5 a.m. and noon from May 30, through June 12, 1965, and at noon on weekly intervals from May 28, through September 24, 1965. In all cases, measurements given are for fruits which adhered until harvest.

Gross Fruit Development Study

To study morphological development, fruits were selected at weekly intervals from full bloom until harvest in 1965. These fruits were from a tree growing on EM VII rootstock and

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[1] Product of Federal Products Corp., Providence, Rhode Island (Model 49P-172-R1).

bearing an abundant crop of fruit. For each sampling two fruits were selected whose transverse and longitudinal diameters measured the mean of 10 tagged fruits on the same tree.

The fruits were subjected to several measurements using vernier calipers. These measurements were the following:

(a) Transverse diameter: the mean greatest cross-sectional diameter,

(b) Longitudinal diameter: the mean length of the fruit,

(c) Pith: the mean diameter of an area limited by the five sepal bundles and the five petal bundles in median cross-section of the fruit,

(d) Core diameter: the mean diameter of a circle drawn through the five dorsal carpellary bundles in median cross-section of the fruit,

(e) Carpel blade width: the mean largest measurement of the cartilaginous portions of each of the five carpels from dorsal suture to ventral suture,

(f) Carpel blade length: the mean of the length of the cartilaginous portions of five carpels in median longitudinal section,

(g) Seed length: the mean length of ten normal appearing seeds,

(h) Embryo length: the mean length of ten embryos.

A transverse and a longitudinal slice, each approximately one-quarter inch thick, were taken each week from the two fruits used to make the gross measurements. These slices were placed in a clearing solution consisting of equal parts by weight of phenol, lactic acid, glycerin and distilled water. After a period of one week in the clearing solution, the sections appeared translucent. Their outlines and vascular systems were traced by projecting the images on the ground glass back of a camera.

Histological Study

Beginning on March 25, 1964 and March 26, 1965, samples of developing fruits were collected at weekly intervals throughout the growing seasons for histological study. In addition, samples were collected twice a week from March 26, through June 8, 1965. Representative developing fruits were collected each year from the trees treated with zero and four pounds of sodium nitrate.

The plant materials were immediately placed in a killing-fixing solution of 5 parts formalin, 5 parts glacial acetic

acid and 90 parts 70 percent ethanol. Penetration of the solution into the samples was enhanced by holding them overnight under a vacuum. The vacuum in the evacuator was gradually increased so as to not injure the plant material. Following evacuation, and prior to storage of the samples, the killing-fixing solution was decanted off and a fresh supply added.

Dehydration of selected killed and fixed developing fruits was accomplished using the tertiary butyl alcohol method as described by Johansen (24). They were embedded in Fisher tissuemat with a melting range of 56 - 58°C.

Serial sections were cut at 10 microns on a Model 820 American Optical Company rotary microtome and then affixed to microscopic slides using Haupt's adhesive (24).

The paraffin was removed from the sections with xylene.

Staining of the 1964 material was accomplished with hematoxylin or with safranin and fast green as described by Johansen (24). The length of staining time varied a great deal with the age of the tissue. Generally the younger tissues required less time.

The cover slips were applied using piccolyte, a synthetic resin, thus making the slides permanent.

The 1965 slides were prepared in an identical manner except that the staining was eliminated. The paraffin was removed with xylene and the coverslips immediately applied.

Evaluations were made also using hand-cut razor blade sections of fresh and preserved plant materials. Additional fresh sections were cut on a Model CTD Cryostat[1] and either not stained, or stained with Sudan IV.

A Wild M 20 microscope[2] with a built-in light source, equipped with phase contrast, polarizing discs and a Photoautomat MKa4 35 millimeter camera[2] attachment was used for the various observations, measurements and photographs. A didymium contrast filter was used in the photography work. Unstained sections were viewed with the phase contrast microscope more clearly than stained slides. Bright field and polarizing discs were used also for certain observations.

Measurements of cortex intercellular spaces and of the tissue and cell size of pith, cortex, hypodermis and epidermis were made in transverse sections of the fruits. In each case

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[1] Product of International Equipment Company, Needham Heights, Massachusetts.

[2] Product of Wild Heerbrugg Ltd., Heerbrugg, Switzerland.

the cross-section was taken midway through the carpels. Attention was also directed toward the epidermal hairs, cuticle, stomata, lenticels and russeting whenever they were present. All measurements of cells represent the mean of 20 normally appearing cells.

The electron Microprobe X-ray Analyzer[1] was used to analyze the crystals which are readily seen with either phase contrast, or polarizing light. Tousimis (56) suggests the use of this instrument for the analysis of certain biological specimens.

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[1] Product of Applied Research Laboratories, Inc., Glendale, California.

RESULTS

Nitrogen Nutrition Study and Observations on Harvested Fruit

The randomized block experimental design was used to study the effects of leaf nitrogen on the russeting of Golden Delicious fruits. The results of this study are presented in Tables 1 through 6, and Figure 2.

The effects of year and of rootstock on the percent russeted fruit were not significant. The fruits on the trees treated with four pounds of sodium nitrate were more russeted than fruits from the trees receiving no sodium nitrate. When the percent russeted fruit of the sodium nitrate treatments are classified as to year, the differences in russeting were significant between treatments in 1964 but not in 1965.

For the period of bloom through two weeks following petal fall the lowest recorded air temperature was 33°F. in 1964 and 34° F. in 1965.

Data on fruit size were taken on the harvested fruit to determine the effect of sodium nitrate treatment (Table 1).

Table 1. Percent russeted fruit and percent large fruit from 9-year-old trees as influenced by sodium nitrate treatments, by year and by East Malling rootstocks

Independent Variable	Percent Russeted Fruit	Percent Large Fruit (2-3/4")
Treatment		
No Nitrogen		
1964	2.6 a[1]	67.1
1965	5.1 a	55.8
		NS
4 lbs $NaNO_3$		
1964	18.6 b	62.4
1965	10.6 ab	59.3
		NS
Year		
1964	11.2	67.8
1965	8.1	57.8
	NS	NS
Rootstock		
EM VII	12.8	51.6 a
EM XVI	7.7	71.5 b
	NS	

[1] Values followed by different letters differ significantly at the 5 percent level.

The use of 4 pounds of sodium nitrate had no effect on fruit size, nor did years (Table 1). However, fruits from trees with East Malling (EM) XVI rootstocks were found to be larger than those from trees with EM VII rootstocks.

The mean values of yield in pounds per tree are presented in Table 2. The 1964 yields were significantly higher than those of 1965. The effects of sodium nitrate treatments, of EM rootstocks and of the first order interactions for the independent variables on yield were not significant.

The mean values of leaf nitrogen, potassium, phosphorus, calcium and magnesium (expressed as percent dry weight) are presented in Table 3. Similar values for leaf sodium, manganese, iron, copper, boron, zinc and aluminum (expressed as parts per million) are given in Table 4.

Leaf nitrogen, calcium, magnesium and copper were greater for the treatment of four pounds sodium nitrate, while potassium, phosphorus and boron were greater for the zero nitrogen treatment. The differences were not significant for the remaining elements (Tables 3 and 4).

The values for leaf nitrogen, copper and boron were higher in 1964 than in 1965, with only aluminum higher in 1965. The effect of year on the remaining elements was not significant (Tables 3 and 4).

Table 2. Mean values of the yield expressed as pounds per tree from 9-year-old trees as influenced by sodium nitrate treatments, by year and by East Malling rootstocks

Independent Variable	Pounds Yield
Treatment	
No Nitrogen	
1964	166
1965	51
	NS
4 lbs $NaNO_3$	
1964	212
1965	118
	NS
Year	
1964	189 a[1]
1965	84 b
Rootstock	
EM VII	109
EM XVI	164
	NS

[1] Values followed by different letters differ significantly at the 5 percent level.

Table 3. Mean values of leaf nutrients for 9-year-old trees as influenced by sodium nitrate treatments, by year and by East Malling rootstocks

Independent Variable	Nitrogen	Potassium	Phosphorus	Calcium	Magnesium
	Percent dry weight				
Treatment					
No Nitrogen	1.79	1.67	0.31	1.20	0.33
4 lbs $NaNO_3$	2.28	1.17	0.15	1.41	0.47
	**	**	**	**	**
Year					
1964	2.11	1.33	0.26	1.29	0.38
1965	1.96	1.51	0.20	1.32	0.41
	**	NS	NS	NS	NS
Rootstock					
EM VII	1.99	1.44	0.23	1.28	0.35
EM XVI	2.08	1.40	0.23	1.33	0.44
	NS	NS	NS	NS	**

** Significant differences at the 1 percent level.

Table 4. Mean values of leaf nutrients for 9-year-old trees as influenced by sodium nitrate treatments by year and by East Malling rootstocks

Independent Variable	Sodium	Manganese	Iron	Copper	Boron	Zinc	Aluminum
			Parts per million				
Treatment							
No nitrogen	124	99	149	9.2	26.3	25	411
4 lbs $NaNO_3$	154	102	145	10.6	23.0	24	368
	NS	NS	NS	**	*	NS	NS
Year							
1964	144	106	146	10.3	30.0	23	231
1965	135	94	148	9.4	19.4	26	548
	NS	NS	NS	*	**	NS	**
Rootstock							
EM VII	145	97	137	10.3	24.3	23	387
EM XVI	133	104	157	9.5	25.1	26	392
	NS	NS	NS	NS	NS	NS	NS

* Significant differences at the 5 percent level.
** Significant differences at the 1 percent level.

The magnesium leaf values were higher for EM XVI than for EM VII. The influence of rootstock on the remaining elements was not significant (Tables 3 and 4).

First order interactions of the independent variables on the 12 nutrient elements evaluated by leaf analysis were not significant with two exceptions. These are presented in Tables 5 and 6, and Figure 2. The percent of leaf nitrogen of the control treatment which received no supplemental nitrogen was lower in 1965 than in 1964, while the nitrogen levels from the four pound sodium nitrate treatment were practically the same for both years.

The leaf nitrogen level of the control was lower for EM VII rootstock than for EM XVI, while the leaves of the trees from the 4-pound sodium nitrate treatment had about the same percentage of nitrogen for both rootstocks.

Table 5 includes the leaf values of potassium, phosphorus and magnesium for sodium nitrate treatments and years. For each year the values for potassium and for phosphorus were significantly higher and that for magnesium was lower for the no nitrogen than for the 4-pound sodium nitrate treatment.

Table 5. Leaf nutrient values for 9-year-old trees in relation to the interaction of sodium nitrate treatments and years

Treatment	Year	Nitrogen	Potassium	Phosphorus	Magnesium
		Percent dry weight			
No Nitrogen	1964	1.92 a[1]	1.57 a	0.35 a	0.30 a
	1965	1.66 b	1.77 a	0.27 a	0.35 a
4 lbs $NaNO_3$	1964	2.29 c	1.09 b	0.17 b	0.46 b
	1965	2.27 c	1.25 b	0.13 b	0.48 b

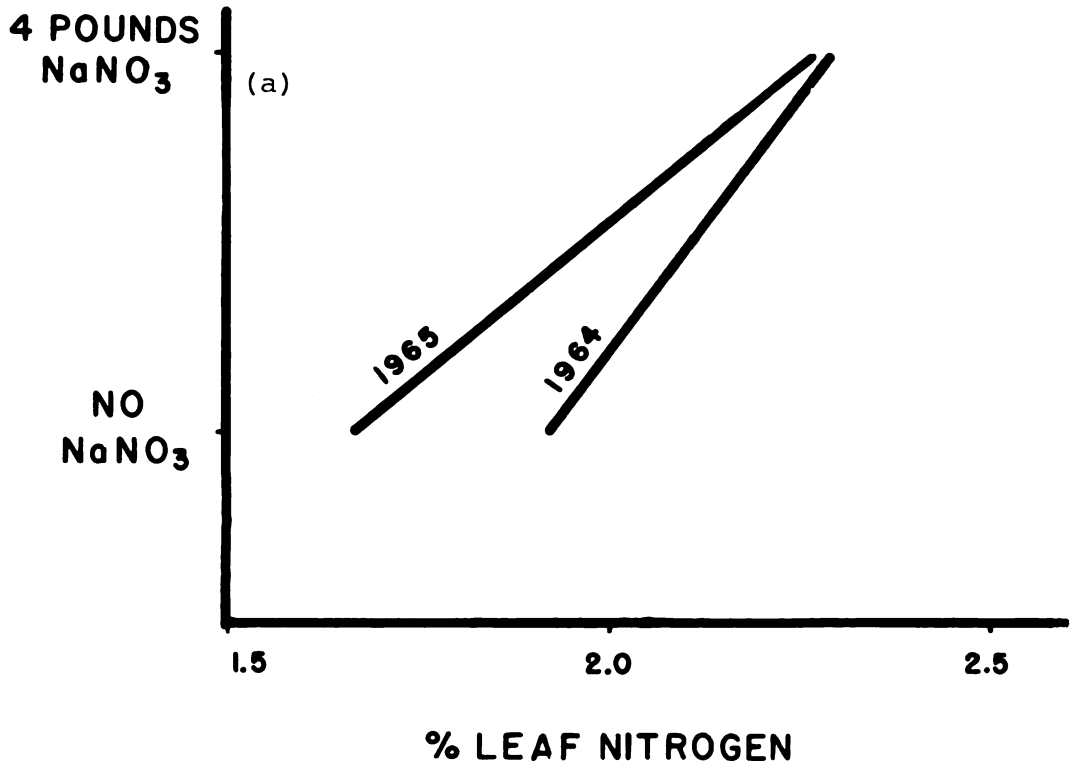
[1] Values followed by different letters differ significantly at the 5 percent level.

Table 6. Leaf nitrogen values for 9-year-old trees in relation to the interaction of sodium nitrate treatments and East Malling rootstocks for the years 1964 and 1965

Treatment	EM VII	EM XVI
	Percent dry weight	
No Nitrogen	1.68 a[1]	1.90 b
4 lbs $NaNO_3$	2.31 c	2.25 c

[1] Values followed by different letters differ significantly at the 5 percent level.

Figure 2. Leaf nitrogen values expressed as percent dry weight for 9-year-old trees as influenced (a) by sodium nitrate treatments and years, and (b) by sodium nitrate treatments and East Malling rootstocks for the years 1964 and 1965


4 POUNDS
NaNO3
(a)
1963
1964
NO
NaNO3
1.5
2.0
2.5
% LEAF NITROGEN

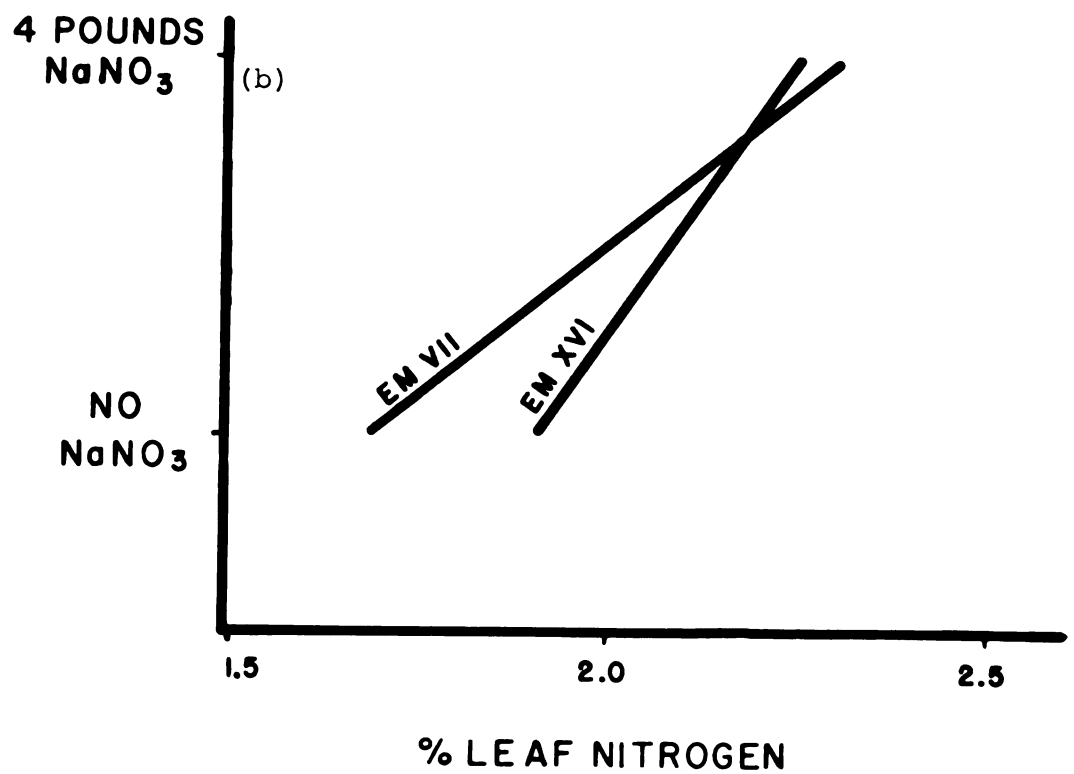

4 POUNDS
NaNO3
(b)
EM VII
EM XVI
NO
NaNO3
1.5
2.0
2.5
% LEAF NITROGEN

Figure 2

Fruit Growth And Diurnal Fluctuation Study

The results for fruit growth and diurnal fluctuation are presented only for those fruits which persisted on the trees at the time of harvest. Eighty-one of these fruits were still on the trees at harvest in 1964 for the no nitrogen and 62 for the 4-pound sodium nitrate treatments, while in 1965 the number persisting was 66 and 83, respectively.

The curves reflecting rate of fruit enlargement for each treatment by years are presented in Figures 3 and 4, and the mean values of the fruits measurements are listed in Appendix Tables 10 and 11.

In 1964 the fruits of the zero nitrogen treatment were initially slightly larger than those of the 4-pound sodium nitrate treatment, and in 1965 the situation was reversed. This was the result of random selection. In each year as the season progressed, the size of the fruits from the trees treated with the sodium nitrate gradually became greater than those of the control, with the result that the mean values of the fruits of the nitrogen treatment were larger at the end of the season.

The values obtained from measurements taken diurnally at 5 a.m. and 12 noon are presented in Figures 5 and 6 and

Figure 3. Mean volumetric values in cubic inches of fruits from the no nitrogen treatment (81 fruits) and the 4-pound sodium nitrate treatment (62 fruits) recorded at weekly intervals in 1964

Regression equations for curves:

(x=1 June 10, x=2 June 17)

No nitrogen

$y=0.257 + 0.07138(X^2) - 0.003415(X^3) + 0.0000453(X^4)$

4-pound sodium nitrate

$y=0.331 - 0.1177\ (X) + 0.11105(X^2) - 0.006839(X^3) + 0.0001389(X^4)$

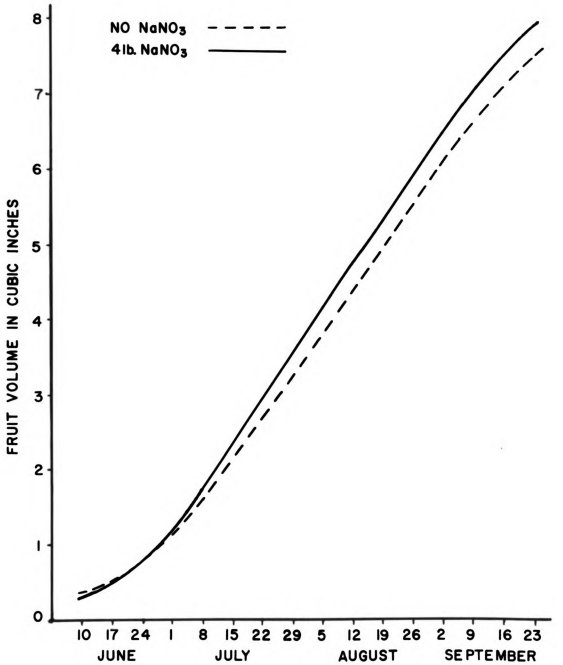

NO NaNO3
4 lb. NaNO3
FRUIT VOLUME IN CUBIC INCHES
0
1
2
3
4
5
6
7
8
10 17 24 1 8 15 22 29 5 12 19 26 2 9 16 23
JUNE
JULY
AUGUST
SEPTEMBER

Figure 3

Figure 4. Mean transverse diameter values in centimeters of fruits from the no nitrogen treatment (66 fruits) and 4-pound sodium nitrate treatment (83 fruits) recorded at weekly intervals in 1965

Regression equations for curves:

(x=1 May 28, x=2 June 4)

No nitrogen

$$y = -0.054 + 0.8337(X) - 0.052371(X^2) + 0.002129(X^3) - 0.0000379(X^4)$$

4-pound sodium nitrate

$$y=0.135 + 0.7561(X) - 0.02889(X^2) + 0.0000235(X^4)$$

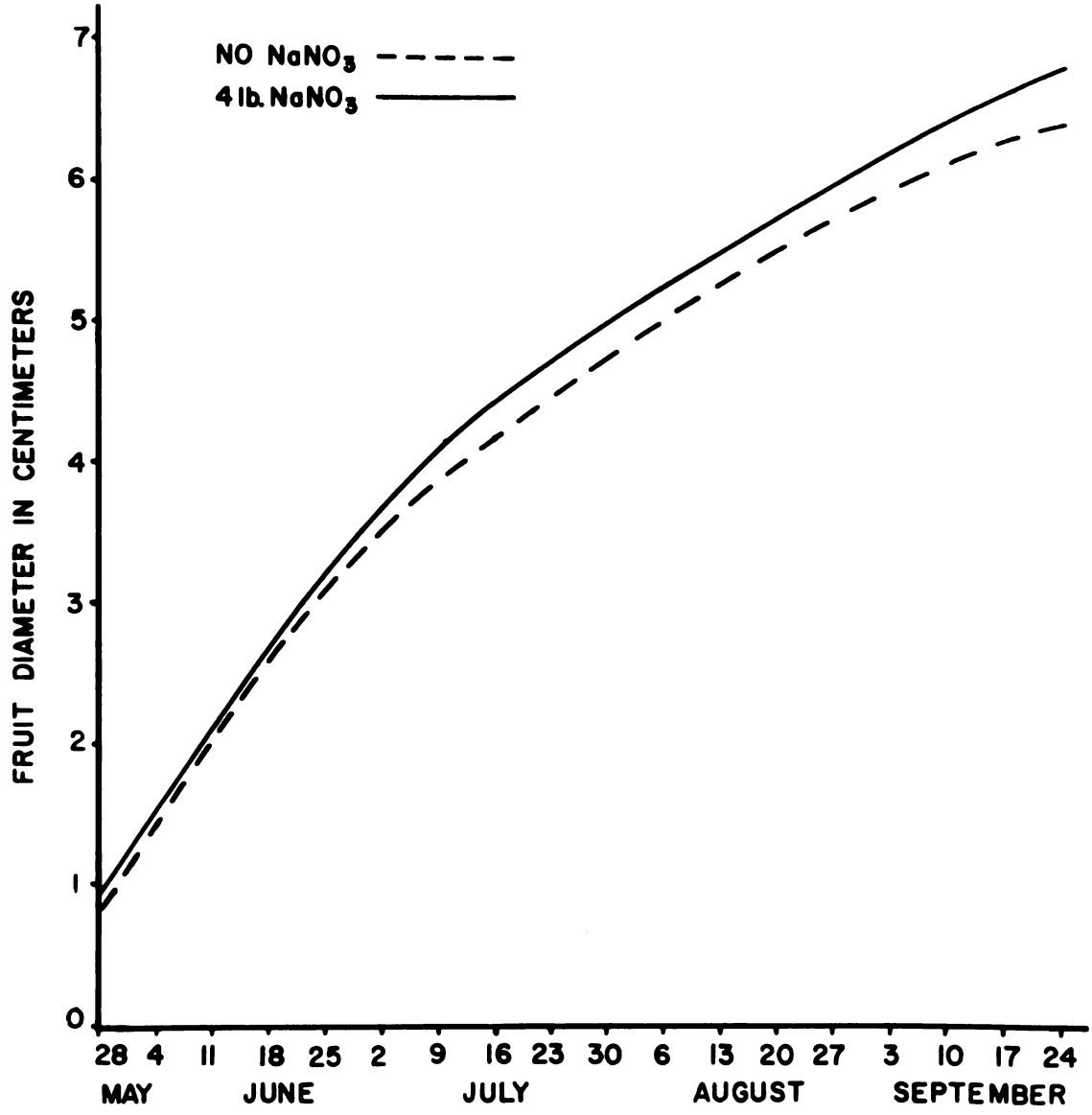

NO $NaNO_3$
4 lb. $NaNO_3$
FRUIT DIAMETER IN CENTIMETERS
0
1
2
3
4
5
6
7
28 4 11 18 25 2 9 16 23 30 6 13 20 27 3 10 17 24
MAY
JUNE
JULY
AUGUST
SEPTEMBER

Figure 4

Figure 5. Mean diurnal volumetric changes in cubic inches of fruits from the no nitrogen treatment (81 fruits) and the 4-pound sodium nitrate treatment (62 fruits) recorded at 5 a.m. and 12 noon, June 23 through June 26, 1964

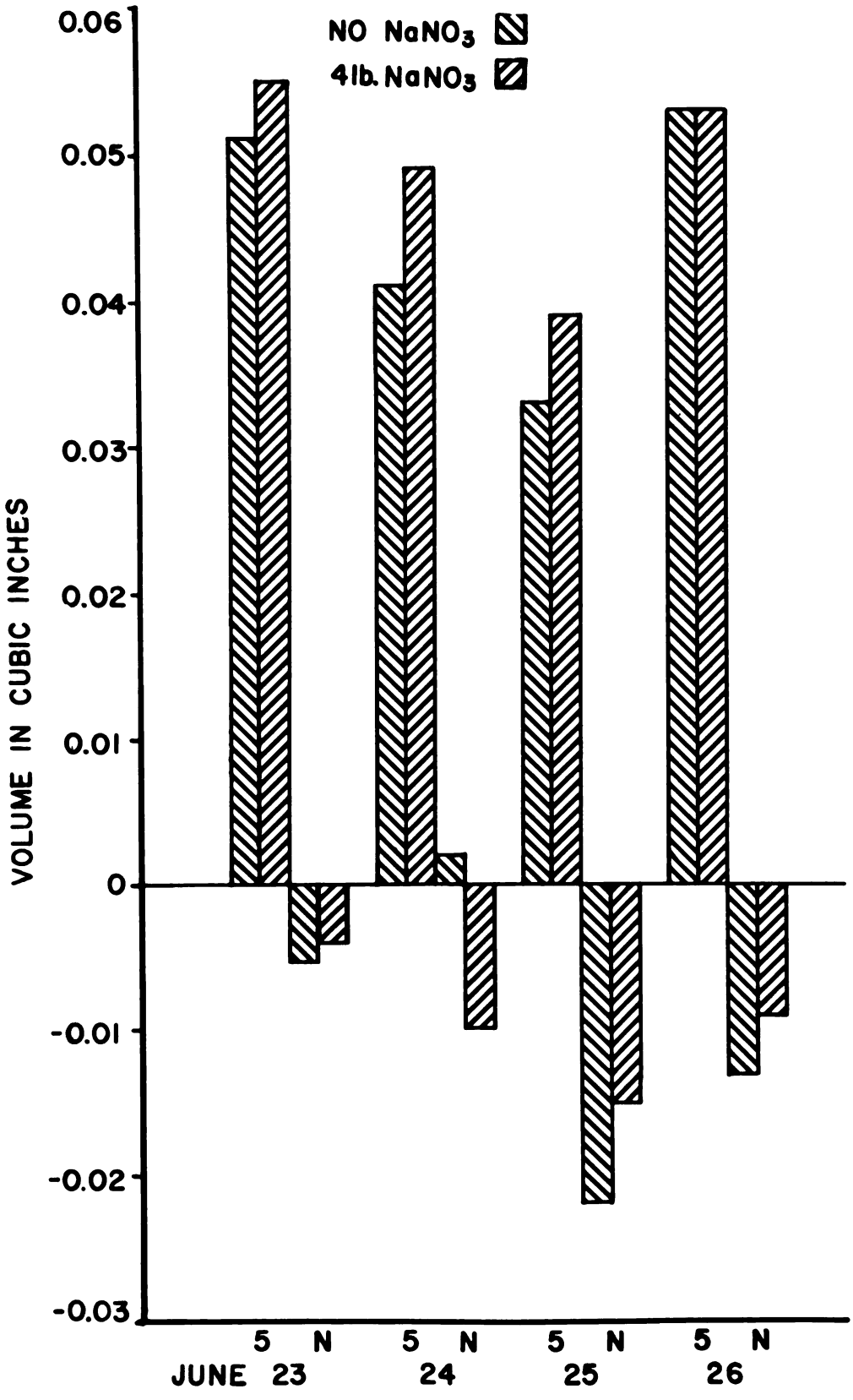

NO NaNO3
4lb. NaNO3
VOLUME IN CUBIC INCHES
0.06
0.05
0.04
0.03
0.02
0.01
0
-0.01
-0.02
-0.03
5 N 5 N 5 N 5 N
JUNE 23 24 25 26

Figure 5

Figure 6. Mean diurnal diameter changes in centimeters of fruits from the no nitrogen treatment (66 fruits) and the 4-pound sodium nitrate treatment (83 fruits) recorded at 5 a.m. and 12 noon, May 31 through June 12, 1965

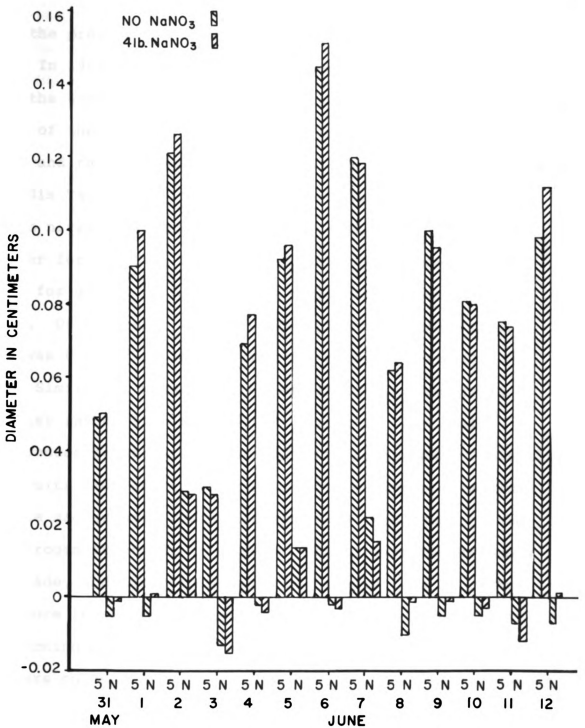

NO $NaNO_3$
4 lb. $NaNO_3$
DIAMETER IN CENTIMETERS
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0
-0.02
5 N 31 MAY
5 N 1
5 N 2
5 N 3
5 N 4
5 N 5
5 N 6
5 N 7
5 N 8
5 N 9
5 N 10
5 N 11
5 N 12
JUNE

Figure 6

Appendix Tables 12 and 13. The values are the mean differences from the previous measurement.

In 1964 the overnight increases in fruit enlargement from the 4-pound sodium nitrate treatment, when compared with those of the control, were greater for three of the four days, and the increase was identical for the fourth (Figure 5, Appendix Table 12). The morning shrinkages were less for the no nitrogen treatment in three of the four days, and greater for one day, but these differences between treatments for degree of diurnal volumetric change were very small. Of interest, the only precipitation during this time was 0.10 of an inch during the night of June 23.

Similarly, measurements made in 1965, using fruit diameter rather than volume also reflected only small differences between treatments (Figure 6, Appendix Table 13). The fruits from the trees treated with 4-pounds of sodium nitrate had greater overnight increases than those of the no nitrogen treatment in eight of the 13 days measurements were made, and the morning shrinkages were less or gains were more in six of 13 days. No differences occurred for the morning of June 5. Precipitation for approximately 18 hours on June 2 amounted to 1.53 inches, while 0.50 of an inch fell during the afternoon of June 6, and 0.28 in the evening of June 8. Some of the largest measured increases occurred during and immediately following precipitation.

Net daily increases in fruit size were greater for the 4-pound sodium nitrate treatments in three of four days for 1964 and nine of 13 days in 1965.

The differences shown for diurnal fluctuations between treatments were very small, and for 1964 only one difference was as great as 0.01 cubic inch -- on the morning of June 24 when the fruits of the 4-pound sodium nitrate treatment had greater shrinkage. For 1965 the differences were as great as 0.01 centimeter only twice -- for overnight growths of June 1 and June 12 when the fruits from trees treated with 4-pounds of sodium nitrate grew more.

Gross Fruit Development Study

Outline drawings of longitudinal and transverse sections of developing fruits are presented in Figures 7 and 8. The drawings represent samples taken in 1965 two weeks prior to bloom, at bloom (May 18, 1965), and two, four, six, eight, 12, 16 and 19 weeks after bloom.

The sepal and petal bundles are represented as dotted lines in the longitudinal sections and as heavy dots in the transverse sections. Dotted lines in the transverse sections represent the exterior limit of the core diameter or fleshy

Figure 7. Outline drawings of longitudinal sections of developing fruits in 1965 for the dates indicated. Dotted lines represent sepal and petal bundles

a. May 4
b. May 18
c. June 1
d. June 15
e. June 29
f. July 13
g. August 10
h. September 7
i. September 28

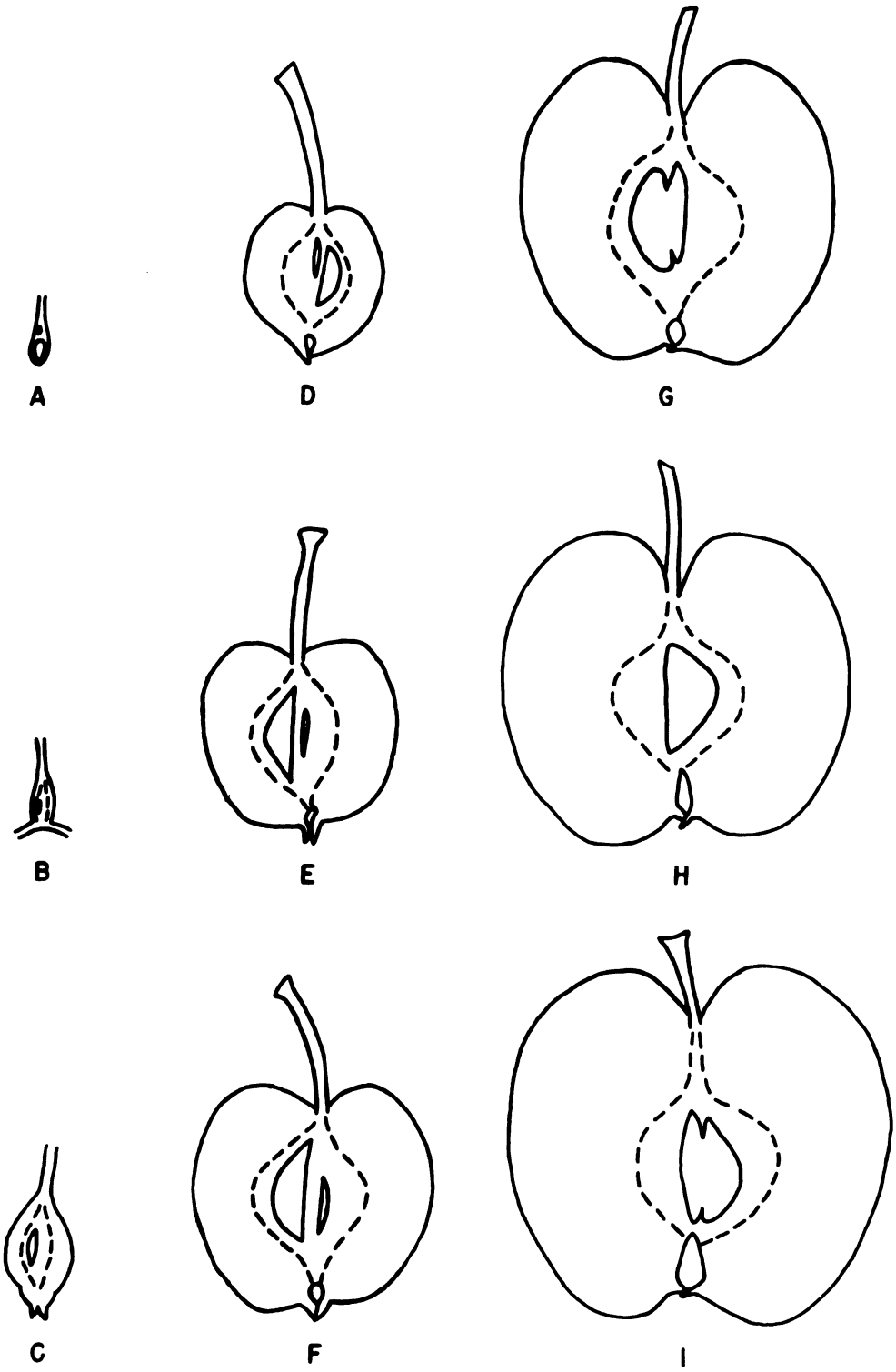


Figure 7

Figure 8. Outline drawings of transverse sections of developing fruits in 1965 for the dates indicated. Heavy dots represent sepal and petal bundles and dotted lines represent the exterior limit of the fleshy carpel

a. May 4
b. May 18
c. June 1
d. June 15
e. June 29
f. July 13
g. August 10
h. September 7
i. September 28

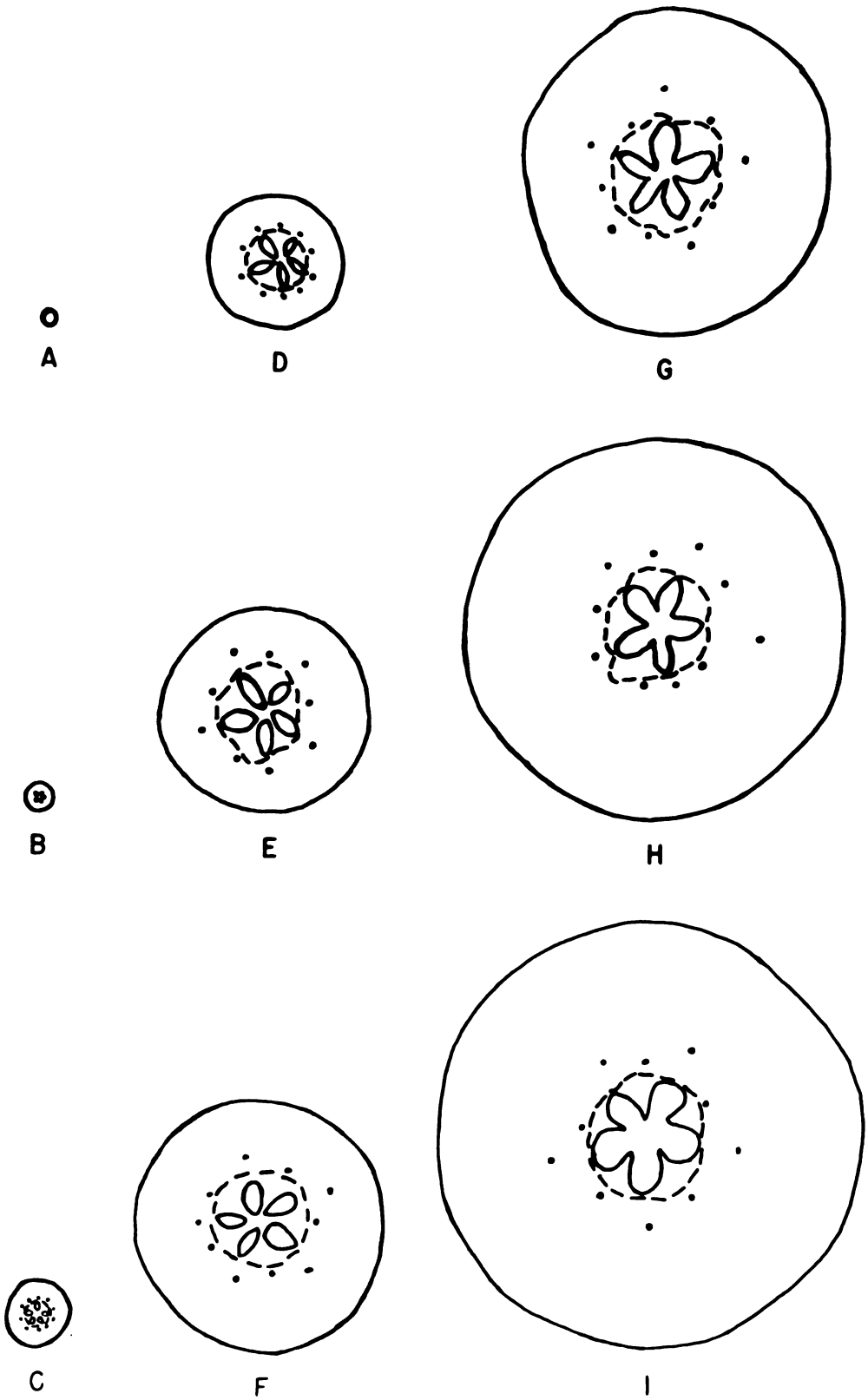


Figure 8

carpellary tissue. This interpretation differs from that of Tukey and Young (57) and Kraus (26) for other apple cultivars. Photographs of a cleared cross-section and a cleared longitudinal section of fruits are presented in Figure 9.

The measurements taken are presented numerically in Table 7 and Figure 10.

The longitudinal diameter was found to be greater than the transverse diameter until June 22 when both measured 3.2 centimeters, and after which time the transverse diameter became the greater. Different from the transverse and longitudinal diameter, the core diameter and pith increased until the end of July and then increased at a relatively slow rate.

The embryos increased in size for a four week period beginning about June 15 and then remained the same size until harvest. During the period of rapid growth of the embryo, the overall growth of the fruit continued at a rapid rate. This agrees with Tukey and Young (57) for apples, but not with Mitchell (36) for the Barlett pear.

Figure 9. Cleared (a) transverse section and (b) longitudinal section of mature fruits

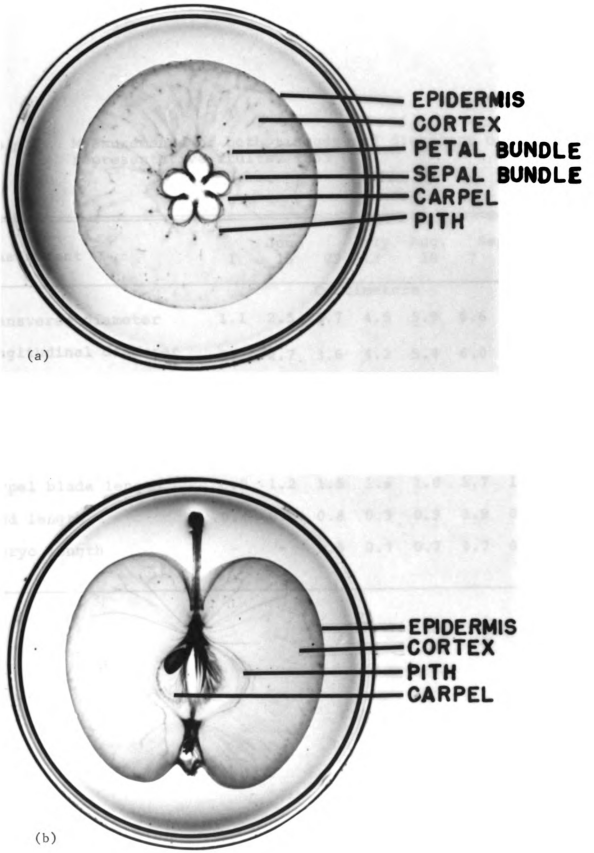

EPIDERMIS
CORTEX
PETAL BUNDLE
SEPAL BUNDLE
CARPEL
PITH
(a)
EPIDERMIS
CORTEX
PITH
CARPEL
(b)

Figure 9

Table 7. Measurements of both tissues and diameters of representative fruits, 1965

Measurement	June 1	June 15	June 29	July 13	Aug. 10	Sept. 7	Sept. 28
	Centimeters						
Transverse diameter	1.1	2.5	3.7	4.5	5.9	6.6	7.1
Longitudinal diameter	1.3	2.7	3.6	4.2	5.4	6.0	6.4
Pith diameter	0.6	1.3	1.9	2.1	2.4	2.5	2.7
Core diameter	0.5	1.1	1.6	1.6	1.8	1.9	2.2
Carpel blade width	0.2	0.5	0.7	0.7	0.7	0.7	0.7
Carpel blade length	0.5	1.2	1.5	1.6	1.8	1.7	1.8
Seed length	0.4	0.8	0.8	0.9	0.9	0.9	0.8
Embryo length	-	-	0.5	0.7	0.7	0.7	0.7

Figure 10. Periodic measurements of the transverse diameter, longitudinal diameter, pith diameter, core diameter and embryo plus cotyledon in centimeters of representative fruits in 1965

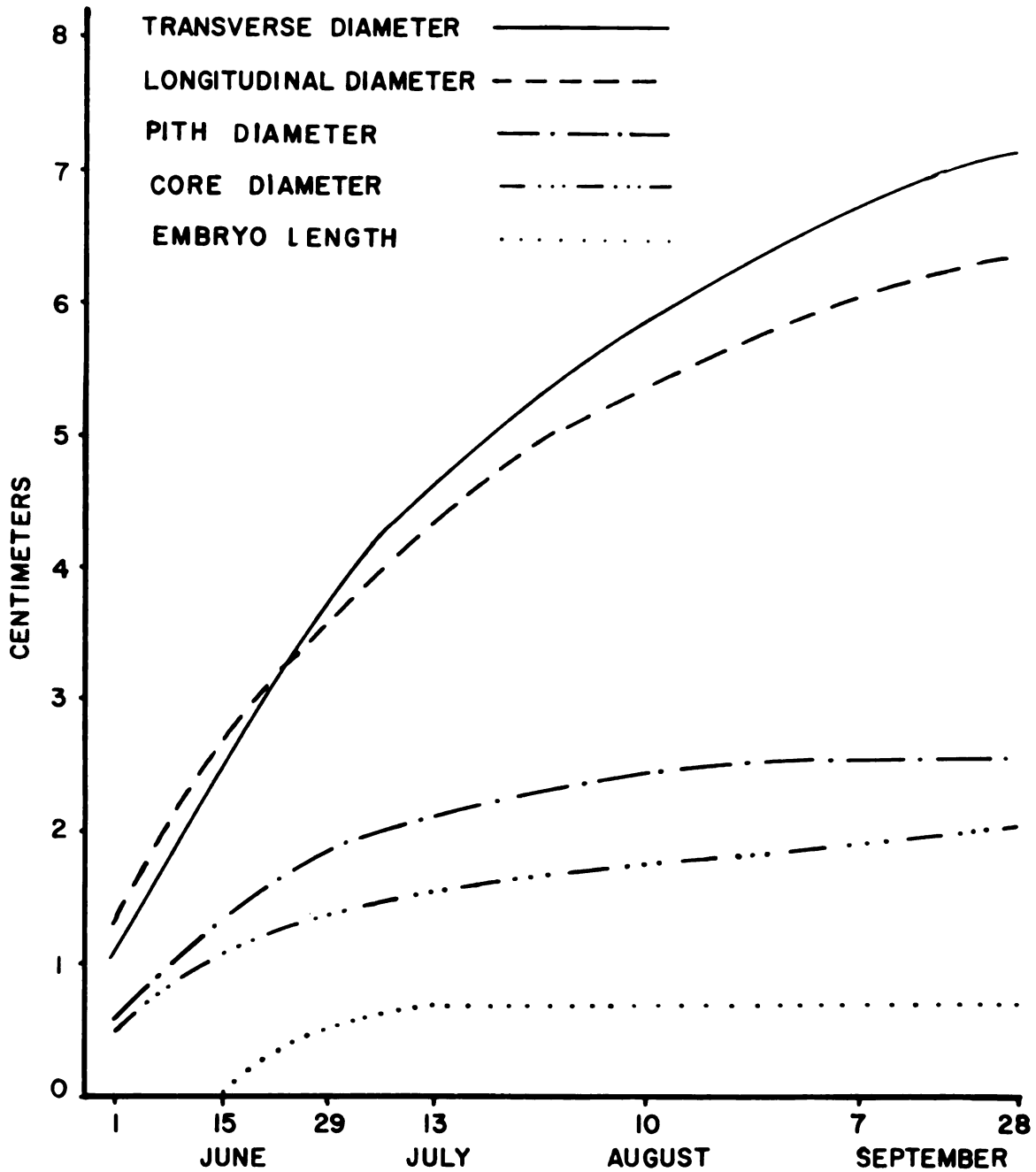

TRANSVERSE DIAMETER
LONGITUDINAL DIAMETER
PITH DIAMETER
CORE DIAMETER
EMBRYO LENGTH
CENTIMETERS
0
1
2
3
4
5
6
7
8
1
15
29
13
10
7
28
JUNE
JULY
AUGUST
SEPTEMBER

Figure 10

Histological Study

Cells and Tissues

Measurements of the cells of the epidermis, hypodermis, pith and cortex of developing fruit from the treated trees are presented in Table 8. The radial measurements of the cells of the pith and cortex and the number of cells found in cross-section median through the carpels for these tissues are given in Table 9. The dates for which these observations were made are two weeks prior to bloom, the period of bloom (May 18, 1965), and two, four, six, eight, 12, 16 and 19 weeks following bloom.

The size of the cells of the epidermis and hypodermis were found to be approximately the same for the developing fruits from the trees of the two nitrogen levels under study until mid-June when the cells of these two tissues were larger for the fruits of the sodium nitrate treatment than those from the zero nitrogen control. However, as illustrated in Figures 11 and 12, a difference did exist between the epidermal cells of the two treatments on June 1, two weeks prior to the time of noticeable measured differences. A striking difference in the epidermis was noted wherein tangential cell division was much more frequent at this time for the zero sodium nitrate control.

Table 8. *Diameters of cells for the tissues of the developing fruits of (a) zero nitrogen and (b) 4-pound sodium nitrate treatments, 1965. All measurements were made from cross-sections median through the carpels and represent the mean of 20 cells*

Tissue	Treatment	May 4	May 18	June 1	June 15	June 29	July 13	August 10	September 7	September 28
		Microns								
Epidermis[1]	a	13x8	15x8	18x13	13x15	15x15	22x23	13x25	10x23	16x22
	b	13x8	15x8	19x13	17x20	16x22	15x24	13x22	16x22	13x25
Hypodermis[1]	a	10x15	9x20	14x19	10x20	16x31	16x33	21x44	22x50	31x64
	b	8x13	7x20	14x22	13x35	13x25	15x29	19x40	19x45	19x40
Cortex[2]	a	13	15	38	60	80	61-153	75-214	101-227	113-252
	b	12	15	41	50	76	51-163	88-176	76-252	88-252
Pith[2]	a	10	13	20	50	88	112-244	88-215	131-378	88-252
	b	10	25	31	50	62	92-163	126-202	150-366	100-352

[1] Radial x tangential.

[2] Radial.

Table 9. Number of cells in cross-section of the cortex and pith and the width of each tissue for developing fruits of (a) zero nitrogen, and (b) 4-pound sodium nitrate treatments, 1965. All measurements were made from cross-sections median through the carpels

Tissue	Treatment	May 4	May 18	June 1	June 15	June 29	July 13	August 10	September 7	September 28
Cortex No. of cells	a	8	12	56	107	129	135	123	111	147
	b	8	11	65	84	118	93	109	138	122
Cortex Width (microns)	a	110	200	2100	5100	8200	10954	12370	13248	16710
	b	80	186	2331	3975	7490	8264	11340	16380	17010
Pith No. of cells	a	8	8	8	8	8	9	9	13	24
	b	8	8	8	19	15	17	14	27	25
Pith Width (microns)	a	63	145	163	316	675	880	1008	2394	3024
	b	68	153	189	918	1125	1683	1842	4910	4180

Figure 11. Longitudinal sections of developing fruits from (a) the no nitrogen treatment and (b) the 4-pound sodium nitrate treatment showing portions of the epidermis and hypodermis in 1965 for the dates indicated. Note more frequent tangential divisions in the epidermis of the no nitrogen fruit of June 1 than in that of the sodium nitrate treatment. Phase contrast, 550X.

1. May 18
2. June 1
3. June 15
4. June 29

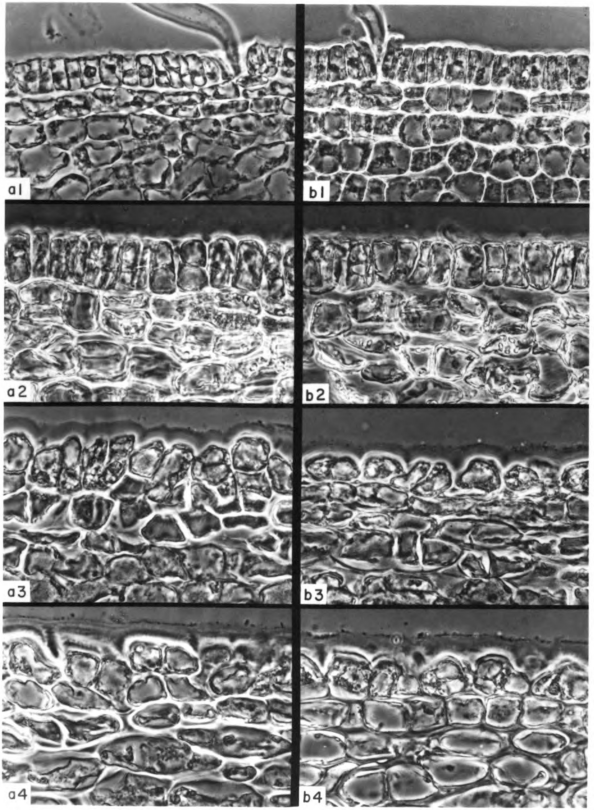


Figure 11

Figure 12. Longitudinal sections of developing fruits from (a) the no nitrogen treatment and (b) the 4-pound sodium nitrate treatment showing portions of the epidermis and hypodermis in 1965 for the dates indicated. Note more cellular extensions into the cuticle, less association between cells and earlier collapsing of cells of the epidermis for the sodium nitrate treatment. Phase contrast, 550X.

1. July 13
2. August 10
3. September 7
4. September 28

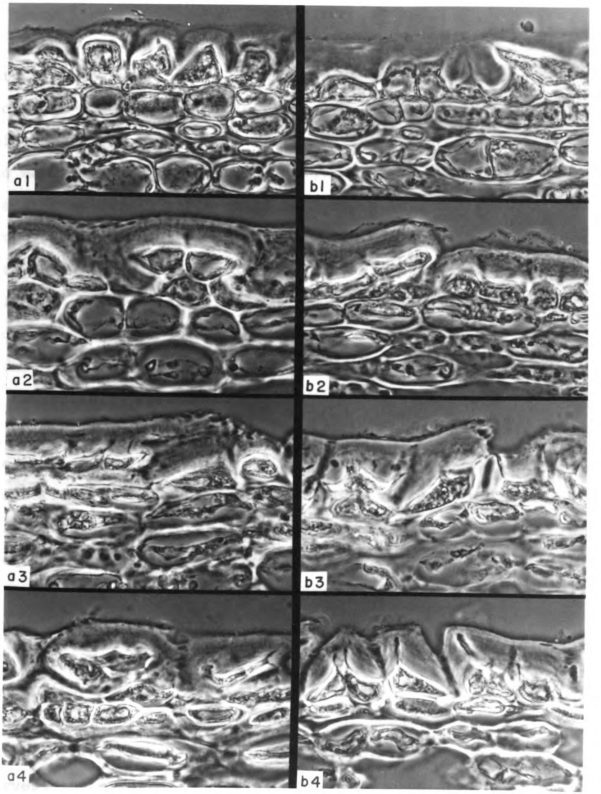


Figure 12

By July 13 the epidermal cells for the fruits of both treatments (Figure 12 , a1, b1) became separated from each other with cuticle filling in between. Eventually epidermal cells became completely isolated from other cells and were surrounded by cuticle (Figure 12, a4, b4). The isolated epidermal cells could be found singly and in small groups. Measurement of epidermal cells became quite difficult, in that one could not always tell which cells belonged to the epidermis and which were of hypodermal origin. And, as the fruits approached maturity, some epidermal cells became radially narrow and appeared to be radially collapsed or tangentially stretched.

In the July 13 sampling, the cells of the epidermis in the case of the sodium nitrate treatment had narrow projections extending into the cuticle. These projections were found also in later samplings, but were much less frequent for the zero nitrogen treatment (Figure 12, a4, b3, b4). Eggert (15) described these projections as "funnel-shaped extensions."

The successive stages of separation, isolation and collapsing appeared sooner and to a greater degree in the fruits from the trees treated with four pounds of sodium nitrate than in those of the zero nitrogen treatment. At harvest the epidermal cells of both treatments appeared

similar, except that the nitrogen treated samples had a larger number of cells which appeared collapsed.

As illustrated in Figure 12, the cuticle portion of the fruits from August 10 to maturity was smoother for the fruits of the zero nitrogen trees than for those receiving sodium nitrate. The cuticle of the fruits of the 4-pound sodium nitrate treated trees had more cracks and crevices, and generally presented a rougher surface.

The hypodermis of the fruits from the nitrogen treatment had more cell layers and was not as clearly defined from those of the cortex than was the hypodermis of the fruits of the zero nitrogen trees.

At harvest, the hypodermal cells and the epidermal cells of the fruits from 4-pound sodium nitrate treated trees had the appearance of being crushed (Figure 12, b4). Measurements of the hypodermal cells reflect this (Table 8). Also, hypodermal cells of the 4-pound sodium nitrate treatment consistently measured a little smaller radially on June 29 and on later dates.

Illustrations of some of the developmental changes for cortex cells are shown in Figure 13 and for pith cells in Figure 14.

Measurements of the pith and cortex cells revealed no consistent differences between the two treatments (Table 8).

Figure 13. Transverse sections of developing fruits from the no nitrogen treatment in 1965 for the dates indicated showing a portion of the cortical tissue with its prominent intercellular spaces in the later stages of development. The area in the May 18, full bloom sample, between the arrows is cortex. Note the small spherical starch bodies in the samples of June 29 through September 28. Phase contrast, 125X.

a. May 18
b. June 1
c. June 15
d. June 29
e. July 13
f. August 10
g. September 7
h. September 28

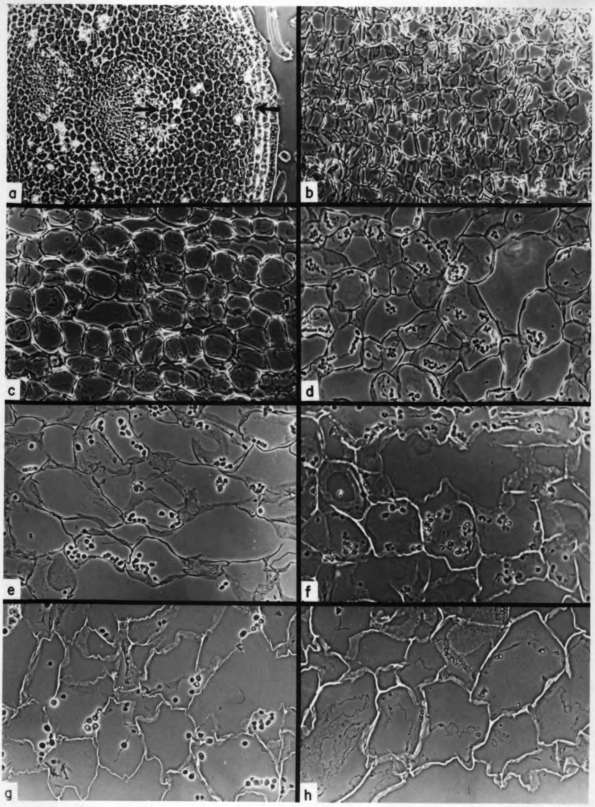


Figure 13

Figure 14. Transverse sections of developing fruits from the no nitrogen treatment in 1965 for the dates indicated showing a portion of the pith tissue. The area in the May 18, full bloom sample, between the arrows is pith. Note the small spherical starch bodies in the samples of June 29 through September 28. Phase contrast, 125X.

a. May 18
b. June 1
c. June 15
d. June 29
e. July 13
f. August 10
g. September 7
h. September 28

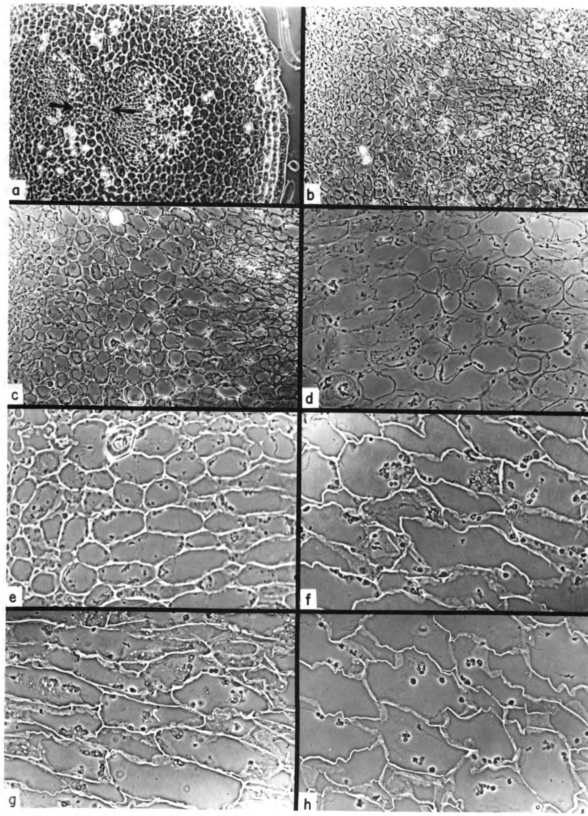


Figure 14

Because of large variations in the sizes of these cells as they enlarged, size ranges are presented for the July 13 and later samples. The cells of the cortex in fruits sampled July 13 and later from the 4-pound sodium nitrate treated trees had less regular shapes and more wrinkled cell walls when compared to samples of the trees receiving no nitrogen.

The number of cortex cells in a radial direction was less and the radial measurement of the cells was smaller for those from the 4-pound sodium nitrate treatment than the no nitrogen treatment, from June 15 through August 10 (Table 9).

The intercellular spaces of the cortex were noticeable on June 15 for both treatments and they continued to increase in size until fruit maturity. They were predominantly radially elongated when observed in cross-section of the fruit, and varied greatly in size. Representative radial measurement ranges found were 88 to 315 microns on June 15, and 163 to 408 microns on September 28 for the control; and 75 to 378 microns, and 266 to 630 microns, respectively, on the same dates for the 4-pound sodium nitrate treatment. Because of this large variation in size, differences were difficult to determine. However, the samples from the 4-pound

sodium nitrate treated trees in each case appeared to have larger intercellular spaces. Bell (7) suggested that the many intercellular spaces in the Golden Delicious fruit could contribute to the susceptibility of this cultivar to moisture loss in storage.

The pith cells when measured radially were considered to be those between the dorsal carpellary bundle and the sepal bundle. Cells in this location tended to have a narrower tangential diameter than those found in the areas between the sepal and petal bundles. But, upon checking, it was dtermined that the radial cell measurements were similar throughout the pith of each sample.

The area of the pith of the fruits from the trees receiving four pounds of sodium nitrate was larger by mid-June than those of the control, and continued so until harvest, although the differences were not as great as earlier. The number of cells across the pith in cross-section was also correspondingly greater for the samples from the trees treated with sodium nitrate. It was noted that differentiation of the cells of the pith and carpel was much more distinct in the case of the 4-pound sodium nitrate treatment than for the control. For the fruits of the zero nitrogen

treatment, the cell size in the outer carpel portion was larger and more nearly approached the size of the pith cells.

The photomicrographs in Figures 13 and 14 illustrate numerous small starch grains present in the pith and cortex cells from the samplings of June 29 through September 28.

The pith and cortex cell walls on June 1, and later refracted plane polarized light, and consequently, were birefringent when viewed under plane polarized light. This indicated that the cellulose molecules have formed crystalline lattices. Esau (17) illustrated both primary and secondary walls which were birefringent.

Crystals

At the time of bloom many crystals were present in the flower which made sectioning very difficult, and resulted in tearing of the tissue. The crystals associated with the vascular tissues were predominately rhombic in shape, while those associated with the carpels were predominantely druse. Druse crystals were also found in the pith and cortex, but were not as numerous as in the carpels.

The crystals seemed to be less numerous as the fruit enlarged. This could have been due (a) to fruit enlargement and consequent spreading, (b) to metabolic utilization, or (c) to a combination of these factors.

Sections of flowers sampled on May 18, 1965 were mounted on carbon and copper discs and subjected to analysis by an electron microprobe X-ray analyzer. Results of this technique are illustrated in Figure 15.

Photographs of the calcium electrons as viewed on a phosphorus screen revealed large concentrations of calcium in druse and rhombic shapes. Adjacent serial sections mounted on glass slides revealed similarly shaped crystals in the same areas.

Counts of the calcium electrons from a number of crystals made it obvious that the concentration of calcium was not identical for all the crystals. These crystals appeared to be calcium oxylate and calcium carbonate. Further analysis should be made to better characterize the compounds present in these crystals.

As can be seen in Figure 15 (a and c) prominent druse and rhombic crystals are found to nearly fill cells in which the nuclei are readily visible.

A Russet Chimera

While evaluating the fruits on the trees, a chimera fruit with a sectional russeted area was found and studied. Photographs from this study are shown in Figure 16. Surface views revealed that the russeted area was generally rough

Figure 15. Longitudinal sections of flowers sampled on May 18, 1965. 800X.

a. Area of carpel with druse crystals present. Clear area in bottom of photograph is the carpel cavity. Phase contrast.

b. Calcium electron picture of adjacent serial section to (e) with druse shapes evident.

c. Area of developing vascular strand with rhombic crystals present. Phase contrast.

d. Calcium electron picture of adjacent serial section to (c) with rhombic shapes evident.

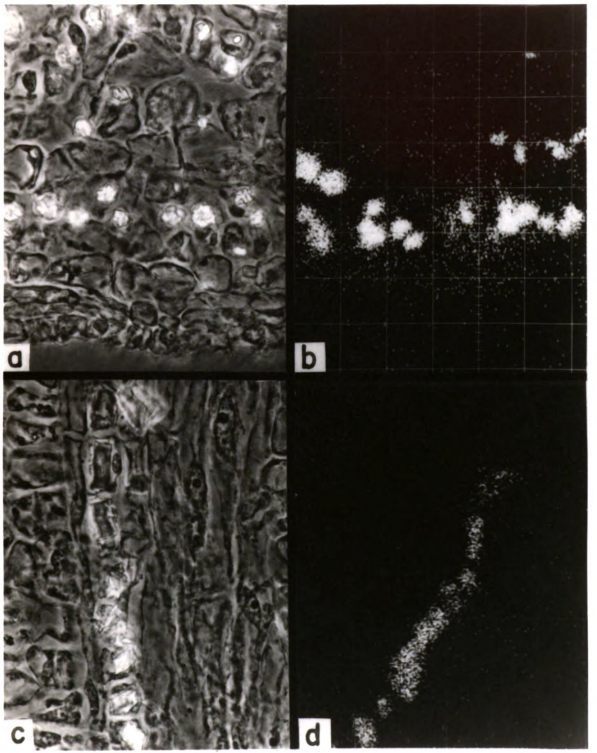


Figure 15

Figure 16.

a. A chimeral Golden Delicious fruit with a russeted section.

b. Surface view of an area of the chimera where the russeted area (right) meets the non-russeted area (left), 50X.

c. Transverse section of the chimera, prepared with the cryostat at a thickness of 4 microns, illustrating the russeted portion (R) and the non-russeted portion (N). Polarized light, 125X.

d. Transverse section of a non-russeted portion of the chimera, prepared on the cryostat at a thickness of 4 microns, with the cuticle surrounding some of the epidermal cells. Note birefringence of the outer wax layer of the cuticle and the cell walls. Polarized light, 550X.

e. Transverse section of a russeted portion of the chimera, prepared on the cryostat at a thickness of 4 microns, with a small piece of cuticle and epidermal cells still attached. Note the highly birefringent cell walls of the cork cells. Polarized light, 550X.

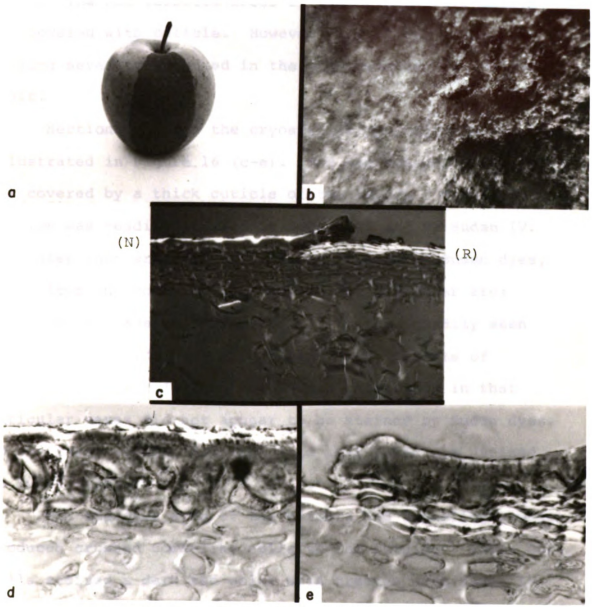


Figure 16

with saucer-shaped pieces lifting from the surface at the edges. The non-russeted areas of the chimera were smooth and covered with cuticle. However, occasional splits of varying severity appeared in the non-russeted area of the fruit.

Sections made on the cryostat at four microns are illustrated in Figure 16 (c-e). The non-russeted area was covered by a thick cuticle of two layers. The inner portion was readily stained by Sudan III and by Sudan IV. The outer thin area was not visibly stained by Sudan dyes, even after one month in the dye. Also, the outer area did refract plane polarized light. This is readily seen in Figure 16 (c,d) as a bright band. The results of Sitte and Rennier (49) coincide with this study in that cuticular waxes did not appear to be stained by Sudan dyes. In contrast, Jensen (23) indicated that waxes are stained by Sudan dyes.

The russeted area of the chimera had a phellogen which produced crushed cork-like cells to the outside. These cork cells stained a dark red color when treated with Sudan IV.

Epidermal Lesions

Epidermal lesions in various stages of development are presented in Figure 17.

Figure 17. Epidermal lesions of fruits in 1965 for the dates indicated. Phase contrast, 125X.

a. June 29 sample with a proliferation of cells beneath the surface which nearly ruptured the surface in two locations.

b. June 29 sample with a break in the surface above a proliferation of cells.

c. July 13 sample with cork cells covering a lenticel-like lesion.

d. August 10 sample with tissue tearing from the right hand margin of a lesion.

e. September 7 sample with a small corked over lenticel.

f. September 7 sample with a large corked over lenticel.

g. September 28 sample with a small lesion (left) and cracks in the cuticle (right).

h. September 28 sample with a proliferation of non-cork cells filling a lesion.

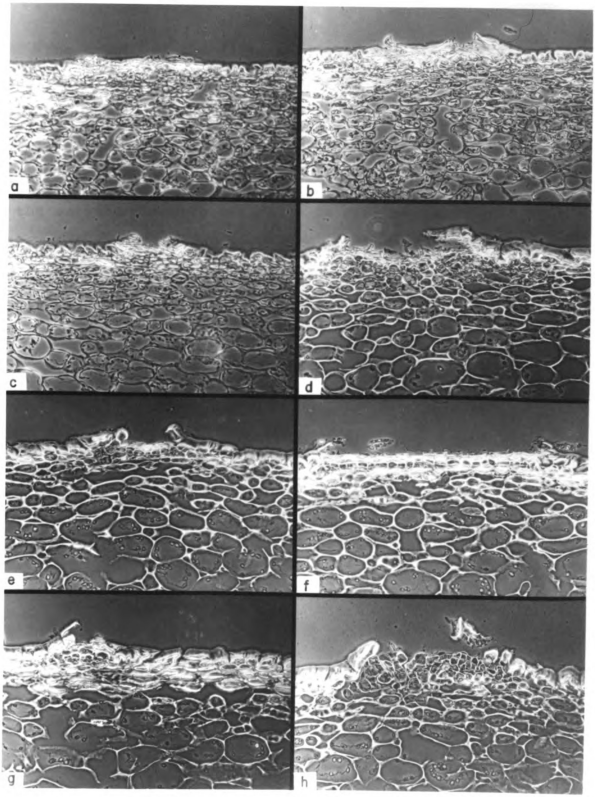


Figure 17

The origin of the first phellogen was not easily established, but it did appear to arise from cells of the outer hypodermis.

Phellogen activity appeared to produce a few phelloderm cells to the inside, but most of the new cells were oriented to the outside and were cork-like in appearance. These cork cells were suberized and appear to be crushed. The cuticle and outer cells were pushed outward, cracked and ultimately flaked off.

DISCUSSION

Russeting of apple fruits has been studied by numerous researchers and many causes have been reported. Some of the most widely studied of these causes are, (a) freezing air temperatures at or near bloom, (b) the affects of pesticide chemicals, (c) the nutrient status or vigor of the plant, (d) high levels of relative humdity, and (e) the inherent characteristics of a plant or mutant portion of a plant. The Golden Delicious is a highly susceptible cultivar to russeting, particularly in eastern apple growing areas; and hence, many studies of apple russeting have focused on it.

Except for Eggert (15), no reports were found relating fruit russeting of this cultivar to the level of nitrogen in the leaves or to tree vigor. And, it should be stated that from the time of bloom for the remainder of the growing season no freezing air temperatures were recorded for the two years (1964 and 1965) reported herein. Also, the pesticides included in the spray program were the same as reported by Eggert (15) and all applications were made with airblast equipment to avoid induced russeting from pesticides or application.

Contrary to the findings of Eggert (15), in 1965 the differences in percentage of russeted fruits for the no nitrogen treatment, 5.1, and for the 4-pound sodium nitrate treatment, 10.6, were not significant. The differences in the percentage of russeted fruits for these two treatments were significantly different in 1964, 2.6 and 18.6 respectively (Table 1).

This difference in the findings between 1964 and 1965 cannot be attributed to leaf nitrogen as the leaf nitrogen level for the sodium nitrate treatment was nearly the same for both years, 2.29 in 1964 and 2.27 in 1965, and the values for the no nitrogen treatment were 1.92 in 1964 and 1.66 in 1965 (Table 5). In both years the leaf nitrogen values were favorable for good finish of the fruit of the no nitrogen treatment. However, in 1964 the precipitation for May and June was 4.49 and 3.44 inches while in 1965 the precipitation for these two months was 1.34 and 2.89 inches. The reduced precipitation for May and June in 1965 could have influenced the rate of enlargement of the fruit by influencing cell divisions, as the cell numbers for the pith and the cortex in cross-section of representative fruits of both treatments were similar for May and until June 15, 1965 (Table 9). During this period fruit enlargement is accomplished by cell division and cell enlargement.

Of interest, cell size for the pith and cortex was found to be very similar for both treatments in May and through June 15, 1965 (Table 8). It may be that this uniformity in cell number and cell size for the two treatments did not exist in 1964 when more rainfall occurred in May and June.

The part played by potassium in fruit russeting of Golden Delicious is not understood. As pointed out by Eggert (15), the treatment highest in leaf potassium also had the smallest number of russeted fruits. This was also true for this study. In 1964 the potassium level in the leaves was 1.57 percent for the no nitrogen and 1.09 percent for the 4-pound sodium nitrate treatment. In 1965 these levels were 1.77 and 1.25 percent respectively (Table 5). For the 4-pound sodium nitrate treatment, the leaf potassium level was 1.09 percent in 1964 and 1.25 percent in 1965, while the percent of russeted fruits for this treatment was 18.6 in 1964 and 10.6 in 1965. The increase in potassium level in 1965 also could have influenced the decrease in the percentage of russeted fruit in 1965. All the trees in this experiment received a supplemental broadcast treatment of muriate of potash at the rate of 265 pounds per acre as the potassium level of certain trees was considered low in 1964.

Potassium is considered low in the leaves of apple trees in Michigan when the percentage of potassium is below 1.00.

Of added interest, one tree of the 4-pound sodium nitrate treatment in 1964 had 58.5 percent russeted fruit. The nutrient content for all elements evaluated in the leaves was approximately the same as for the remaining trees of this treatment except that the potassium level was 0.42 percent, the lowest of the eight trees in this treatment, while the magnesium was 0.69 percent, highest of the eight trees. In 1965 following the application of muriate of potash the leaf potassium level of this tree was 1.00 percent and the magnesium level 0.55 percent. Also, in 1965 the percent of russeted fruit from it dropped to 15.5. The relationship of available potassium to fruit russeting of Golden Delicious is worthy of continued study. The other elements evaluated did not follow specific trends as did nitrogen and potassium (Table 5).

Morphological measurements of developing fruits revealed that the transverse diameter was initially smaller than the longitudinal diameter two weeks after bloom, but increased at a faster rate, becoming equal approximately five weeks after bloom and greater six weeks after bloom (Figure 10). The rate of enlargement for both the transverse

and longitudinal diameters decreased as the growing season progressed. This agreed with the findings of Tukey and Young (57) for late maturing varieties of apples. The growth patterns for the developing pith, core diameter and embryo (Figure 10), also conform to those reported by Tukey and Young (57) for apple cultivars.

The interpretation of the line of division of the pith and fleshy portions of the carpel varies among workers depending upon the apple cultivars under study. In this work the core diameter is considered to be a circle drawn through the dorsal bundles of the five carpels. For the cultivar Twenty Ounce used by Tukey and Young (57) to illustrate the tissues and vascular strands of the fruit in a cleared cross-section, the fleshy portion of the carpel was referred to as the area within the anastomosed vascular strands surrounding the bony carpel blades with these vascular strands separating the carpel and the pith. Kraus (27) presented cleared cross-sections of 157 apple cultivars other than the Golden Delicious and did not give clear cut lines of demarcation for the tissues. As MacDaniels (33) used the appendicular theory of interpretation for the apple fruit, the so-called fleshy carpel and pith were not identified or a line of demarcation between them given. The cleared cross-sections of the Golden Delicious apples appeared

to conform with the receptacular theory, as labeled tissues in Figure 9 indicate, with no precedent set in the designation of tissues for this cultivar. As viewed on both the tissue and cellular levels, the distinction between cortex and pith was not clear for the Golden Delicious, but as interpreted herein, a line of demarcation occurred between the pith and fleshy carpel.

An histological investigation was conducted to determine if differences existed between the tissues and cells of the developing fruits from the zero nitrogen and 4-pound sodium nitrate treated trees.

Microscopic evaluations comparing the cells and tissues of the pith and cortex in cross-section, median through the carpels, revealed that the histological development of these two structures was similar for the fruits of both treatments through the June 1 sampling. However, the sample of 4-pound sodium nitrate treatment of June 15 had nearly three times as much pith tissue as the sample from the no nitrogen treatment (Table 9). On subsequent dates a similar pattern of difference prevailed with the fruits of the nitrogen treated trees having approximately twice the amount of pith tissue as the no nitrogen samples of June 29 through September 7, and one-third more at the time of the last sampling, September 28.

At the time the difference in pith tissue of the two treatments was first detected, the measurements of the cortex tissue revealed that this tissue of the nitrogen treated fruits was smaller than that of the fruit of the control treatment and remained so for each sampling through August 10. After this time, on September 7 and 28, this relationship was reversed. On these dates the fruits from the 4-pound sodium nitrate treated trees had a larger cortex than the fruits of the control (Table 9).

Of interest, while there was a large difference between treatments in the development of the pith and cortex of the fruits, the overall morphological enlargement of the fruits for each treatment appeared to continue at a similar rate. When the pith tissue of the fruit was found to be larger in the fruits of one treatment the cortical tissue seemed to "compensate" by being smaller, giving a similar overall increase for the fruits of both treatments.

Eggert (15) noted epidermal lesions which appeared to be brought about by physically pulling apart epidermal cells by internal stress as the result of rapid swelling of tissues rather than proliferation of cells beneath the epidermis. Epidermal lesions caused by internal stress as

reported by Eggert were not found in 1965. However, the diurnal fluctuation in size of fruit was not as great in 1965 (Figure 6, Appendix Table 13) as reported by Eggert for 1962 and 1963.

No differences between treatments were noted for the epidermis or the hypodermis in the fruit samples taken in May. However, in the sampling of June 1, 1965 the epidermal cells of the fruit from the zero nitrogen treatment were dividing tangentially as well as radially, while the epidermal cells of those from the sodium nitrate treatment exhibited tangential divisions only occasionally. Meyer (35) noted tangential divisions of epidermal cells of Golden Delicious at this approximate stage of development and suggested that this might account for the susceptibility of this cultivar to russeting. This does not agree with the findings herein, for the samples exhibiting tangential divisions were from the treatment with the least amount of russeting.

On June 15 and 29 the cells of the epidermis were smaller for the fruit samples from the control trees when compared to those from the nitrogen treatment. The epidermis of the fruit from the no nitrogen trees appeared to be two layers in thickness in places while that of the nitrogen treated fruit had only one layer of cells.

The epidermal cells of the fruits from the sodium nitrate treated trees appeared collapsed radially at an earlier date, July 13 and August 10, and to a greater degree than the fruits of the trees receiving no nitrogen. The epidermal cells of these fruits from the no nitrogen treatment did not appear crushed until the September sampling.

No histological differences in the nature of russeted lenticels and lesions were noted for the fruits of the nitrogen and no nitrogen treatments. The pattern of development of these lesions has been described by a number of researchers (2, 4, 5, 9, 11, 15, 28, 43, 44, 45, 46, 54, 65, 67), as phellogen cells initiated either from the cells of the epidermis or the outer cells of the cortex. They indicated that the phellogen produces cork cells centrifugally which isolate and rupture epidermal cells and cuticle.

The nature of all epidermal lesions appeared to be the same for the fruits of both treatments, but the frequency of the lesions was less for the no nitrogen treatment.

Russeting of fruit has been reported to be a response to cracks or breaks in the cuticle exposing the outer tissues to air (52, 54). Although it is difficult to rule out this possibility because of the three-dimensional nature

of the samples, it was not seen in this study. The earliest phellogen appeared to exist with no epidermal lesion.

Epidermal hair bases and stomata have been reported as the locations at which phellogen was later found (11, 19, 54, 67). Many epidermal hair bases and stomata were examined with this in mind, but phellogen formation was not found under or near them.

The pronounced differences in tissue development as found in June, 1965 do not appear to explain the response of more fruit russeting of Golden Delicious in the nitrogen treatment as compared to the fruits from the trees receiving no nitrogen. The differences in the epidermal and hypodermal tissues do, however, appear to be directly related to the appearance of the surface of the fruit. The reduced rate of division at an earlier date for the fruits of the nitrogen treatment causing greater stretching and stress on individual epidermal cells could account for a greater number of areas of collapsed epidermal cells and a greater frequency of epidermal lesions. This could be a possible explanation for a larger percentage of russeted fruits for the 4-pound sodium nitrate treatment in 1964 and 1965 under the conditions of this study.

An X-ray microprobe analysis of the druse and rhombic crystals present in the fruits at the time of bloom and later revealed that the crystals were calcareous, probably calcium oxylate and calcium carbonate. The crystals seemed less numerous as the fruits enlarged. This could have been due to (a) fruit enlargement and subsequent spreading, (b) to metabolic utilization, or (c) to a combination of these factors. The possibility of these crystals being metabolically utilized as the fruits enlarge is worthy of additional study. Esau (17) indicated that such crystals are waste products of plant metabolism.

SUMMARY

1. An investigation was conducted in 1964 and 1965 to determine the morphology and histology of Golden Delicious apples as influenced by leaf nitrogen levels. Bearing trees growing on East Malling (EM) VII and EM XVI rootstocks received annual soil applications of four pounds of sodium nitrate ($NaNO_3$). Trees of the control received no nitrogen fertilizer.

2. Evaluations of fruit russeting, fruit size and yield were made each season of the study.

(a) The percent russeted fruit for the $NaNO_3$ treatment was significantly greater in 1964, but not in 1965, than the zero nitrogen treatment. No differences in russeting were found which could be attributed to rootstocks.

(b) Fruit size was not found to vary significantly with the $NaNO_3$ treatment or with years. However, trees growing on EM XVI rootstocks had a significantly larger percent of large fruit than trees growing on EM VII when evaluated for the 2-year period.

(c) The yield did not vary significantly with the $NaNO_3$ treatment or with the EM rootstocks. Yield was shown to be higher in 1964 than in 1965, a factor of biennial bearing.

3. The $NaNO_3$ treatment disregarding years had significantly larger values for leaf nitrogen, calcium, magnesium and copper, and significantly smaller values for leaf potassium, phosphorus and zinc as compared with the no nitrogen treatment. The only difference between years was a significantly higher leaf nitrogen value for the no nitrogen treatment in 1964 than in 1965.

4. Measurements taken both seasons at 5 a.m. and noon revealed that fruits often lost size during the daylight morning hours, but differences between treatments were not noted. In both years the fruits of the $NaNO_3$ treatment grew a little more rapidly throughout the season than the fruits of the control.

5. The pith and cortex of the Golden Delicious fruits had no distinct separation, while there was a line of demarcation between the pith and fleshy carpel.

6. A histological study carried on during 1965 comparing the pith, cortex, hypodermis and epidermis of developing fruits from the two treatments revealed the following:

(a) There were no consistent differences in cell size between treatments for the pith and cortex. However, the cortex cell walls of the fruits of the $NaNO_3$ treatment were noticeably more wrinkled and irregular than those of

the control for samples taken July 13 and later. No differences were noted between treatments for the sizes of pith and cortical tissues until the June 15 sampling when the samplings from the $NaNO_3$ treatment had nearly three times as much pith and one-fifth less cortex than the samplings of the no nitrogen treatment. And, after June 15, the pith was larger in every sample from the $NaNO_3$ treatment measured, while the cortex was smaller, except for those samples collected in September.

(b) The hypodermis of fruits of the no nitrogen treatment had fewer layers of cells and was more sharply defined from the inner portion of the cortex than in the $NaNO_3$ treatment. At harvest the hypodermal cells of the $NaNO_3$ treatment appeared to be crushed radially, which was not the case for the no nitrogen treatment.

(c) The epidermis of each treatment appeared to be similar until two weeks after bloom, when the epidermal cells of the no nitrogen treatment exhibited tangential as well as radial divisions, while those of the $NaNO_3$ treatment were dividing radially and only occasionally tangentially. Two weeks later, there was less evidence of epidermal cell division for the $NaNO_3$ treatment and the cells were larger than those from the no nitrogen trees.

As the season progressed, the epidermal cells of both treatments became separated with cuticle filling the created cavities. Some cells were completely surrounded by the cuticle. Cells so isolated were radially collapsed as the fruit matured. Separation, isolation and collapsing of epidermal cells occurred first in the fruits of the $NaNO_3$ treatment. A similar development for the fruits of the no nitrogen treatment was noted two to four weeks later. The areas of collapsed epidermal cells were greater at harvest for the fruits of the $NaNO_3$ treatment than for those of the no nitrogen treatment.

7. Although seemingly not related to tree vigor as reflected by leaf nitrogen, a study was made of the many crystals present in the fruits at the time of bloom and thereafter.

(a) The crystals associated with the vascular tissues were predominantly rhombic in shape, while those associated with the carpels were predominantly druse. Druse crystals were found in the pith and cortex, but were less numerous than in the carpels. Using histological techniques it was not possible to determine if crystals were metabolically utilized or appeared less frequent due to growth of the fruits.

(b) X-ray microprobe analysis showed that large amounts of calcium were present in the crystals.

8. The appearance of russeted tissue was similar on fruits from the $NaNO_3$ treatment, of corked over stomates of fruits from both treatments and of the russeted portion of the fruit with a sectional chimera. A phellogen originating in the hypodermis produced crushed cork cells to the outside which forced the epidermis and the cuticle off. A few phelloderm cells were produced to the inside, but most of the new cells were oriented outside the phellogen.

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APPENDIX

Table 10. Mean values for volumetric enlargement in cubic inches of fruits of the no nitrogen treatment (81 fruits) and the 4-pound sodium nitrate treatment (62 fruits) as recorded at weekly intervals in 1964

Date		No Nitrogen	4 Pounds $NaNO_3$
		Cubic inches	
June	10	0.34	0.31
	17	0.51	0.50
	24	0.84	0.84
July	1	1.14	1.18
	8	1.64	1.73
	15	2.12	2.31
	22	2.71	2.98
	29	3.27	3.58
Aug.	5	3.92	4.26
	12	4.42	4.79
	19	4.95	5.30
	26	5.55	5.97
Sept.	2	6.12	6.53
	9	6.68	7.08
	16	7.03	7.49
	23	7.52	7.96

Table 11. Mean values for diameter enlargement in centimeters of fruits of the no nitrogen treatment (66 fruits) and the 4-pound sodium nitrate treatment (83 fruits) as recorded at weekly intervals in 1965

Date		No Nitrogen	4 Pounds $NaNO_3$
		Centimeters	
May	28	0.80	0.95
June	4	1.32	1.42
	11	2.00	2.11
	18	2.55	2.71
	25	3.15	3.20
July	2	3.45	3.68
	9	3.91	4.15
	16	4.20	4.47
	23	4.49	4.76
	30	4.74	4.99
Aug.	6	5.05	5.28
	13	5.31	5.48
	20	5.53	5.72
	27	5.76	5.97
Sept.	3	5.93	6.18
	10	6.13	6.43
	17	6.29	6.62
	24	6.41	6.80

Table 12. Mean volumetric diurnal changes in cubic inches of fruits of the no nitrogen treatment (81 fruits) and the 4-pound sodium nitrate treatment (62 fruits) recorded daily at 5 a.m. and 12 noon in 1964

Date	No Nitrogen		4 Pounds $NaNO_3$	
	5 a.m.	Noon	5 a.m.	Noon
	Cubic inches			
June 23	0.051	-0.005	0.055	-0.004
June 24	0.041	0.002	0.049	-0.010
June 25	0.033	-0.022	0.039	-0.015
June 26	0.053	-0.013	0.053	-0.009

Table 13. Mean diameter diurnal changes in centimeters of fruits of the no nitrogen treatment (66 fruits) and the 4-pound sodium nitrate treatment (83 fruits) recorded daily at 5 a.m. and 12 noon in 1965

Date	No Nitrogen		4 Pounds $NaNO_3$	
	5 a.m.	Noon	5 a.m.	Noon
	Centimeters			
May 31	0.049	-0.005	0.050	-0.001
June 1	0.090	-0.005	0.100	0.001
June 2	0.121	0.029	0.126	0.028
June 3	0.038	-0.013	0.028	-0.015
June 4	0.069	-0.002	0.077	-0.004
June 5	0.092	0.013	0.096	0.013
June 6	0.145	-0.002	0.151	-0.003
June 7	0.120	0.022	0.118	0.017
June 8	0.062	-0.010	0.064	-0.001
June 9	0.100	-0.005	0.095	-0.001
June 10	0.081	-0.005	0.080	-0.003
June 11	0.075	-0.007	0.074	-0.012
June 12	0.098	-0.007	0.112	0.001