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CUTICLE DEVELOPMENT AND INCIDENCE OF RUSSET ON 'GOLDEN DELICIOUS' APPLE AS INFLUENCED BY SUBCLONE SUSCEPTIBILITY AND SHELTERS

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CUTICLE DEVELOPMENT AND INCIDENCE OF RUSSET ON 'GOLDEN DELICIOUS' APPLE AS INFLUENCED BY SUBCLONE SUSCEPTIBILITY AND SHELTERS

By

Stephen Michael Long

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ABSTRACT

CUTICLE DEVELOPMENT AND INCIDENCE OF RUSSET ON 'GOLDEN DELICIOUS' APPLE AS INFLUENCED BY SUBCLONE SUSCEPTIBILITY AND SHELTERS

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Fruit cuticle characteristics of 'Golden Delicious' (Malus pumila Mill.), Frazier Spur, Smoothee and 'Red Delicious' were examined. Scanning electron micrographs (SEM) indicated that 'Red Delicious' fruit were covered with more projecting wax structures than fruit of the 'Golden Delicious' strains. Total membrane, epicuticular wax, cuticular wax, total wax and cutin matrix (cutin acid plus carbonate soluble material) weights were not different among the 'Golden Delicious' strains 30 days after petal fall, and thin-layer chromatography revealed no differences in wax composition. There were no significant correlations between cuticular component weights 30 days after petal fall and russet severity at harvest.

Cuticle from russeted and non-russeted areas of 'Golden Delicious' and Frazier Spur fruit was examined. SEM revealed massive cuticle disruption in russeted areas, while some epicuticular wax was visible. Russeted areas from both 'Golden Delicious' strains contained less epicuticular and cuticular wax than non-russeted areas. Cold storage reduced the rate of water loss from russeted fruit, but even under these conditions the rate of water loss from 'Golden Delicious' and Frazier Spur fruit was double the rate from 'Red Delicious' fruit.

Shelters were used to eliminate rain, alter light quality, and decrease light quantity on 'Golden Delicious' trees. Black polyethylene shade (8.1% full sun) resulted in 27.3% fruit suitable for the fresh market, while full sun controls resulted in 1.6%. Different colored cellophane reduced russet formation, however no relation between light wavelengths and russet formation was established. Clear plastic rain shelters resulted in 82.7% fruit suitable for the fresh market, while unsheltered controls resulted in 0.3%. Light quality and quantity were found to affect the formation of russet, and to affect the quantities of cuticular components present in fruit. However, no correlation was found between these components and russet severity.

DEDICATION

To Susie, whose love and understanding made it possible.

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INTRODUCTION

'Golden Delicious' is the second most important apple cultivar in the U.S. (18). However, fruit russeting in eastern and midwestern States is a long-standing problem for apple growers (8,9,16,18,65). Consumer preference is for smooth-skinned apples, in spite of the fact that the eating quality of russeted fruit is just as high as that of smooth-skinned (29). Another major problem for russeted apples is their poor storage quality. Russeted fruit lose more water during storage than smooth fruit (2,20,57), and as a result often have shriveled skins, reducing consumer acceptance. However, such water loss is reduced by storage in high humidity with low temperature (2,29,51). The serious problem of consumer preference for smooth fruit remains unsolved (9,10, 12,13,16,25,26,32,50). Methods to eliminate russeting of 'Golden Delicious' apples are desired.

Russet may cover entire fruit, or only small areas of fruit (7). It may occur on fruit throughout the entire tree or be limited to some branches of the tree (46,47,48). Russeting may be influenced by many factors, including: genetic make-up, cultural practices, water relations, pesticide sprays, nutrition, environmental effects, frost and mechanical injury (2,5,16,26,27,39,43,44,65). The present study was initiated to investigate the influence of several of these factors on

russet formation. 'Golden Delicious' cultivars of different russet susceptibilities were compared, as were russet and non-russet tissues. The quantity and quality of light incident on fruit was altered, and fruit were grown with protection from rainfall.

LITERATURE REVIEW

Literature Review

I. The development of russet tissues

Russet is initiated within about 30 days after full bloom (5,6,15,33,45,46,47,48). Cork (periderm) formation has been observed as early as 20 days after fruit set at 10X magnification (47). The greatest increment of tangential growth in apples has also been observed within this early time period (30). The greatest susceptibility to russet formation due to spray injury occurs between pink and about 14 days or more after petal fall (39). Several microscopic studies have followed the earliest histological development of russet tissue.

In 1937 Bell (4,5,6) found epidermal development of 'Golden Russet' to be the same as in other varieties up until full bloom. During and shortly after full bloom, epidermal cells of 'Golden Russet' underwent distinctive tangential divisions which did not occur in other varieties. The cuticle was seen to develop rapidly during early stages of growth, forming a continuous layer over epidermal cells. Most epidermal cells divided to form a 2-4 cell thick layer of epidermis. However, some epidermal cells did not divide, leaving areas of epidermis only 1 cell thick. In the following weeks cuticle was observed to extend down between and under many epidermal cells. An active cork cambium was initiated in lower epidermal regions which underwent rapid cell divisions. Subsequent cork cell development was towards the outermost epidermal cells. Upon maturity the cambial cells were observed to form rows of cork cells.

The original epidermal cells were crushed by the continued outward expansion of cork cambium, with subsequent dispersal of cellular contents. Eventually the cuticle itself ruptured, forming a network of fine cracks considered typical of russeted apples. After the initial formation of a russet layer, cork cells continued to push outward. Sloughing-off of cuticle, dead epidermal cells, and cork cells was observed throughout the growing season. Though some areas of intact cuticle remained, cork or periderm eventually became the dominant protective layer of the russeted apples.

Meyer (33) confirmed Bell's observation that early development of the epidermis in russeted 'Golden Delicious' apples proceeded in the same manner as in non-russeted fruit of other varieties. That is, as fruit began to expand, epidermal cells divided radially while increasing tangentially. However, 30 days after full bloom epidermal cells of 'Golden Delicious' fruit underwent considerable periclinal division not observed in non-russeted fruit. This critical difference resulted in an irregular epidermal layer with frequent underlying air spaces. Cracks were observed in the cuticle, and these widened and extended into epidermal and hypodermal cell layers as the fruit continued to enlarge. Cells adjacent to these cracks were often left unprotected by cuticle or cork, thus allowing excessive water loss and shrinkage of stored fruit.

More recent studies by Simons et al. (44,45,46,47,50,51) indicate a different development of russeted 'Golden Delicious' fruit. Simons noted a lack of periclinal and anticlinal

divisions in the epidermal cell layer at about 35 days after full bloom. The hypodermal layer of russeted fruit had 25% less growth than in non-russeted fruit by 14 days after fruit set. This lack of cell divisions resulted in fruit tissues unable to increase in size without disruption of the outermost protective layers. As the fruit increased in size during normal growth, extensive disruption of the epidermal and hypodermal cell layers occurred. Cork cambium cells began to divide and push outward, eventually rupturing the cuticle. Simons also observed the abnormal appearance of many air spaces in cortical tissues of russeted fruit. Russet development was thereafter observed to continue just as described by Bell and Meyer.

Studies by Skene in 1965 (52) with 'Cox's Orange Pippin' fruit, indicated a close relationship between russet initiation and the occurrence of cracks and dead cells. Skene's count in young fruit showed that 5% of epidermal cells were dead in russeted apples. Cell divisions common to russeting were frequently found directly beneath these dead epidermal cells. And epidermal cracks were often found associated with dead cells. Skene hypothesized that these dead cells could be instrumental in the initiation of russet by their influence on surrounding cells. However, Skene also noted that some russet varieties examined had virtually no dead cells present.

DeVries in 1968 (61) found a "second" cuticle formed beneath outer necrotic russeted tissues late in their development. He also found many invaginations of cuticular material

between disrupted epidermal cells.

In 1972 Pratt (40) studied the anatomical origin of periderm in 'Stark' apples and the russet-forming sport 'Stark 287'. Pratt noted periclinal divisions of epidermal cells and the subsequent death of many epidermal cells. However, epidermal cells in russeted fruit were observed to cease division sooner than in non-russeted fruit. Normal growth of inner tissues then disrupted the epidermis and phellogen layer (active cork cambium). The phellogen layer was observed to originate in hypodermal cells, with some involvement of derivatives of epidermal cells. Pratt's work was in agreement with that of Simons (47) which indicated that russet was initiated by the loss of functioning outer cells, that is, death of epidermal cells triggered the development of periderm.

Electron microscopy studies in 1972 by Gough and Shutak (23) and by Faust and Shear (17) indicated very fine surface cracks throughout the epicuticular wax of 'Golden Delicious' apples. Of several varieties examined, only 'Golden Delicious' had this extensive wax cracking. Faust and Shear took these cracks to be evidence of failure of the wax to expand fast enough to keep up with the growth of internal tissues.

II. Factors influencing russet development

The factors which influence the initiation and development of russet may be divided into two basic groups (16,48, 65). These are:

a) internal factors, including genetic make-up, which affect the susceptibility of fruit to russet formation, and

b) external factors, especially environmental conditions, which promote the initiation and/or development of russet.

Internal factors.

Among apple cultivars, 'Golden Delicious' is one of the most susceptible to russet formation. However, this susceptibility is known to vary between individual 'Golden Delicious' subclones (8,10,18,46,47,49,52,57). Individual trees have been observed to contain branches which produce severely russeted fruit, while other branches on the same tree produce normal-appearing fruit (19,46).

After gamma irradiation, some 'Golden Delicious' buds produced fruit with russet-free sectors. Un-irradiated controls produced no such clean sectors (11). Irradiation of russeted sports 'Stark 287' by Pratt (40) also produced russet-free sectors on fruit. Pratt also reported reversions to non-russeted fruit among controls. Similar work on both irradiated and control 'Sargeant Golden Delicious' showed no reversions to russet-free fruit. It was suggested that the russeted sports may be periclinal chimeras, with a mutation for abnormal development of fruit epidermis in the first layer or layers of the apical meristem.

No subclone of 'Golden Delicious' has yet been established to be completely russet-free. Evaluations of spur strains (Schell, Thompson, Elliot, Thornton, Goldspur, Morrison, Columbia River, Templin, Frazier, Yellowspur and

Starkspur) have shown them to consistently produce lower quality fruit with more russet than regular 'Golden Delicious' (16,18). Russet-resistant sports have been reported, including: Smoothee, Kelly, and Magnolia Gold (49). Of these, only Smoothee has been shown to have a high quality approaching that of regular 'Golden Delicious', while producing fruit with less russet (10,18,49).

Some evidence exists that rootstocks can affect russet formation. Walter (65) mentioned studies in Belgium and Denmark where less russeting was found on more vigorous rootstocks such as: MM 106, MM 104, and M 16. Work by Chandler and Mason (8) indicated that some favorable effects could be gained by grafting unto "low russet" rootstocks. However, no significant data was obtained.

Cuticle thickness and structure are important factors related to the occurence of russet. Apple cultivars with thin cuticles (4), or areas of fruit with thin cuticles (such as the stem-end of Yellow Newtown apples) are more likely to russet (7). Simons (45) found cuticle thickness on normal 'Golden Delicious' apples significantly greater than on russeted beginning at 37 days after full bloom. However, Gough and Shutak (23) found 'Golden Delicious' to have thicker cuticle than non-russeting 'Cortland' and 'McIntosh' varieties.

Epicuticular wax structure of 'Golden Delicious' fruit as well as that of other varieties was examined with scanning electron microscopy by Faust and Shear (17), and by Gough and Shutak (23). 'Golden Delicious' had an amorphous

cuticle with wax platelets embedded in a structureless matrix, while non-russeting varieties had epicuticular wax arranged in free platelets.

No qualitative and only slight quantitative differences were found between cutin acids (fatty acids not removed by boiling in MeOH and $CHCl_3$) from russeted and non-russeted fruit (62,63). The cutin acids from both types of tissue were predominantly fatty acids with chain lengths of 16 and 18 carbon atoms. Deposition of wax above the epidermal cells was retarded in russeted fruit as compared to non-russeted (61).

Cuticle elasticity may also be an important factor in russeting. A direct relationship has been found in tomatoes between the ability of the skin to stretch and the resistance to cracking (3,60). Microscopic examinations by Meyer (33) and Skene (52), as well as electron microscope work by Faust and Shear (17) and by Gough and Shutak (23) all suggested that 'Golden Delicious' cuticle is unable to stretch sufficiently to accomodate the expansion of the growing fruit. The growth rate of fruit will also affect the formation of russet. Many interactions between internal and external factors are involved with these growth rates, including: tree vigor, age, nutritional status, water status, and temperature (16,24,26,65). When conditions are favorable for rapid fruit growth, cultivars with thin, inelastic cuticles and unfavorable wax structure may be induced to russet (17). It is also known that cool climates increase epicuticular wax and humid conditions decrease epicuticular

wax (1). Cuticle cracks (4,5,14,20,21,23,38,52,57) and dead epidermal cells (52) have been associated with russet areas and are apparently due to high tension during growth.

In addition to the over-all fruit growth rate, another factor may be the diurnal fluctuations in fruit size. Apples have been found to decrease in size during the day, and increase in size during the night (14,24,56,58). Eggert (12) found russet to be more severe when trees were given treatments causing a greater diurnal fluctuation in fruit size. It is possible that fruit less subject to this fluctuation would be less subject to cracking of surface waxes and russet formation (17).

External factors

Temperature was first linked to russet formation in relation to spray injury. In 1930 MacDaniels and Heinicke (31) reported that low temperature (about freezing) before or during bloom caused russet that was often mistaken for spray injury. Other studies (27) supported this view of harmful effects of low temperatures. In 1942 work by Chandler and Mason (8) indicated no relationship between temperature and russeting. Faust and Shear (16) in 1972 suggested that russet is less likely to develop in areas with low night temperatures. This view was supported by Taylor in 1975 (55). Slower fruit growth rates are partially a result of lower temperatures, and result in less russet formation. In 1977 Creasy published results (9) which showed that temperature is a critical factor in russet formation from full bloom

until 10 days after full bloom. Higher temperatures during this period resulted in more russet formation.

Chandler (8) found no significant relationship between rainfall and russet formation. However, Palmiter in 1944 mentioned a prevailing theory that wet or humid weather during spraying increased russet (39). Also Montgomery in 1959 (38) observed that high rainfall during June and August coincided with the formation of heavy fruit russet. Work by Hatch in 1975 involved the covering of apple trees with plastic canopies (25). The prevention of rainfall from striking the apples resulted in less russet than that formed on uncovered trees. Studies by Edgerton et al. (12, 13) found high rainfall increased russet also. In 1977 Creasy (9) found precipitation to be a critical factor in russet formation during the period between 10 and 20 days after full bloom. During this period, higher rainfall gave higher russet. The study also found that rain coverings prevented russet.

It is widely accepted that russet of 'Golden Delicious' is more severe in growing areas with high humidity than in areas with low humidity (9,10,16,18,65). Verner's study of fruit cracking (59) indicated that high humidity around fruit would result in increased hydration, which could increase cracking if the cuticle was unable to stretch adequately. It has also been found that when water is allowed to pool atop fruit, a localized, severe russet occurs (57,66). Other work by Tukey (57) and Watanabe (66) indicated that covering apples with moisture-proof materials (beginning 3

weeks and 10 days after petal fall, respectively) resulted in high humidity and high russet. Mink (34) and Creasy (7) discussed the commerical use of water-permeable newspaper coverings in Japan, which results in russet-free apples. In contrast, Hatch (25) recently indicated that apples grown under plastic canopies had less russet than controls, in spite of higher humidity under the canopies (70% vs.40%). Hatch also grew 'Golden Delicious' trees in a greenhouse which produced smooth, almost russet-free fruit.

The over-all water status of the apple tree can also influence russet formation. Both water stress, and an over-abundance of water (from excessive rain or irrigation) can lead to extensive skin cracking and russet formation on fruit (14,21,22).

Some evidence has been obtained concerning the role of sunlight and fruit exposure in russet formation. Verner suggested that shaded fruit may have more elastic cells than those exposed to sunlight. Exposed fruit had rigid cells which were unable to expand during great increases in turgor, and therefore were more likely to burst (59). On the other hand, Tukey (57) observed that russet occurred over entire fruit surfaces, not just those exposed to sunlight. From this he concluded that sunlight was not an important factor. Montgomery (28) believed that a poor foliage cover (due to disease or improper nutrition) exposed fruit to excessive climatic influences, and resulted in greater russeting.

Various paper and plastic materials have been used to cover apples. In addition to restricting gas and water

movements, these coverings also produced a partial shade over the apples (9,34,41,66). Proctor and Lougheed (41) found that covered 'Golden Delicious' fruit were significantly firmer than controls, but gave no data on russet. Tukey (57) reported that apples covered in paper bags and in clear, fairly permeable plastic bags had no russet, and had a good oily and waxy finish. Mink reported that apples grown inside paper bags in Japan had a good finish with no russet, but also had reduced color and storage quality.

The effect of sunlight on russet formation was studied by Watanabe (66) by covering apples with various paper, plastic, and cellophane materials. In addition to reducing russet formation, several types of coverings also significantly altered the cuticles of the apples. Coverings made of newspaper, cellophane, and glass all increased the amount of waxy substance present compared to uncovered apples. Coverings of black vinyl and polyethylene showed no change in the amount of waxy substance present. All of the covering materials resulted in apples with a slightly greater weight of cuticlar membrance weight versus uncovered fruit. Watanabe also exposed 'Golden Delicious' apples to U.V. light at various times throughout the growing season. Apples exposed very early in the season developed localized heavy russet, while those exposed later in the season showed no effects from the U.V. exposure. DeVries (64) indicated that sunlight may exchange the polymerization of cutin acids into an amorphous matrix which is more susceptible to cracking.

Frost damage incurred on immature apples is known to persist as russet bands upon the fruit when they mature (2, 26,27,29,38,43,38,51,65). Similar russet forms as a result of accidental mechanical injury. The injury may be caused by hail striking young fruit, abrasion from wind-blown dust, abrasion from fruit and branches colliding via wind, machinery bumping fruit, and by abrasion from the force of pesticide sprayer (65). Several studies have been done conerning apples injured by frost and by deliberate mechanical means (2,26,29,43,44,61). These studies indicated that such russet formation proceeded in a manner very similar to that of "normal" russet. Outer cells were greatly disrupted, and reversion to meristematic activity occurred to produce the periderm characteristic of russet. However, russet due to frost and mechanical injury generally remains localized and develops rapidly, unlike the slow and dispersed formation of "normal" russet.

Several studies investigating the effects of nutrition on russet indicate that high nitrogen applications led to greater russeting (15,54), presumably because high nitrogen induced cell enlargement (28,61). However, others have shown no relationship between nitrogen and russet (15,25), and a positive relationship between smooth fruit and potassium (15). Similarly, low phosphorous applications resulted in more russet (15), apparently because high phosphorous levels produce correlation exists between magnesium levels and russet formation (15,25,38), while various levels of boron applications did not affect the amount of russeting (8,25).

Levels of calcium, manganese, iron, copper, zinc and sodium were also found to not affect russet formation (25).

Many studies indicated that certain spray materials increased russet formation when applied near the full bloom period. The damage was most severe when adverse weather was involved, namely temperature near or below freezing (27), or excessive moisture (39). Bordeaux mixture, copper, sulfur, lime, lead, and arsenate were the pesticide chemicals found to be harmful to 'Golden Delicious' apples (6,27,35,36, 37,38,39,42,53). Later studies indicated that oil-containing sprays (9) and benzyladenine sprays (55) increased russet when applied during the critical period from petal fall until about 15-20 days after petal fall. Hatch (25) indicated that certain fungicides plus rain produced greater russeting than the same fungicides without rain.

III. Remedies

Russet formation can be reduced to some extent by proper cultural practices, namely: proper nitrogen and phosphorous nutrition (15,38), minimization of water imbalances (21, 22, 65), proper pesticide usage (35,36,37,39,42,53), and selection of russet-free subclones of 'Golden Delicious' (10,18,49 66). While covering the apple fruit with paper bags is known to eliminate russet formation (34,66), this practice is not economically feasible in the United States. Several studies have indicated that the use of Captan reduces russet on 'Golden Delicious' apples (27,38,53). However, as Faust and Shear pointed out (16), no method for effective

reduction or elimination of russet on 'Golden Delicious' apples was available.

Recent work suggests that some reduction in russet formation is associated with certain chemical sprays. Taylor (55) found that applications of GA (4+7) at petal fall and petal fall +7 days reduced russet significantly. As the concentration of GA was increased from 25 ppm to 200 ppm, the reduction of russet was increased. However, Taylor also reported unfavorable side-effects as a result of the GA application. These included a decrease in flower initiation, a decrease in seed number, and an increase in the formation of spindly shoots. Work by Edgerton et al. (12) and by Meador (32) showed that Apasil (a commercial preparation containing silicon dioxide) applied at 2.5% at petal fall and petal fall +7 days, significantly reduced russet formation. No harmful side-effects were observed with the use of Apasil. Additional work by Edgerton and Veinbrants in 1979 (13) showed that a combination of Apasil and GA (4+7) provided better russet control than either compound used alone. The most successful mixtures involved the use of Apasil at 2.5%, and GA (4+7) at 25 and 50 ppm (to avoid harmful side-effects). Exogenous GA apparently affects cell division or elongation, while Apasil probably affects the moisture conditions on fruit surfaces (12).

IV. Conclusions

In spite of significant reductions of russet by the use of Apasil and GA (4+7), the complete control of russeting

on 'Golden Delicious' apples has not yet been accomplished (13). A combination of many of the several factors mentioned may contribute to the occurrence and severity of russet each year in any given orchard (13,16,32). Further research is needed in order to better understand the physiological basis for russet formation, and to improve methods for controlling it.

SECTION I

RUSSET VARIATION AND FRUIT CUTICLE CHARACTERISTICS OF SEVERAL VARIETIES OF DELICIOUS APPLE

RUSSET VARIATION AND FRUIT CUTICLE CHARACTERISTICS OF SEVERAL VARIETIES OF DELICIOUS APPLE

Abstract

Fruit cuticle characteristics of 'Golden Delicious' (Malus pumila Mill.), Frazier Spur, Smoothee and 'Red Delicious' fruit were examined. Scanning electron micrographs indicated that 'Red Delicious' fruit were covered with a greater number of projecting wax structures than fruit of the 'Golden Delicious' strains throughout the growing season. Total membrane, epicuticular wax, cuticular wax, total wax and cutin matrix (cutin acid plus carbonate soluble material) weights were not different among the 'Golden Delicious' strains 30 days after petal fall. However, at harvest Smoothee fruit had greater total membrance, cuticular wax and total wax than 'Golden Delicious' and Frazier Spur fruit, and 'Red Delicious' fruit had more epicuticular wax than those 'Golden Delicious' strains. At harvest correlations between all cuticular components (except epicuticular wax) and russet severity were very significant, however correlations were not significant 30 days after petal fall. The data presented indicates that russet formation is not related to the quantity of cuticular components present in the fruit of the 'Golden Delicious' strains, nor to the composition of the waxes present.

Introduction

Among apple cultivars, 'Golden Delicious' is one of the most susceptible to fruit russet formation. Russet appears as an uneven, rough surface composed of cork-like periderm cells. Susceptibility to russet formation is known to vary among individual 'Golden Delicious' trees, and among 'Golden Delicious' subclones (6,7,11,19,20,21,22,24). Individual trees have been observed to contain branches which produce severely russeted fruit, while other branches on the same trees produce russet-free fruit (14,19). Individual trees and branches also vary from year to year in russet susceptibility (21).

Evaluations of spur strains have shown them to consistently produce lower quality fruit with more russet than regular 'Golden Delicious' (9,11). Russet-resistent sports have been reported, including 'Smoothee', 'Kelly' and 'Magnolia Gold' (21). Of these sports only Smoothee has been shown to have a high quality similar to that of regular 'Golden Delicious', and to produce less russet (7,11,21). However, no sport or subclone of 'Golden Delicious' is completely russet-free.

Fruit susceptibility to russet formation is greatest between petal fall and 30 days after petal fall (4,16,18). Two hypotheses currently exist: firstly, susceptible varieties have thin cuticles (3,24) which are easily damaged by factors

such as frost, rainfall, pesticides and mechanical injury, which cause russet to form as a protective response; secondly, abnormal growth of underlying epidermal cells causes internal pressures to develop which cause cracking of cuticular membranes unable to sufficiently expand. Cell death has been observed underneath cuticle cracks (22). Active cork cambium initiated in nearby cells as a repair mechanism, thus giving rise to cork cells and the appearance of russet (9).

The present study made us of four apple strains with known differences in russet susceptibility: Frazier Spur (subclone of 'Golden Delicious' , severe russet), 'Golden Delicious' (moderate russet), Smoothee (mutation of 'Golden Delicious', light russet) and 'Red Delicious' (no russet). The purpose of the study was to examine fruit cuticle components in relation to russet formation for each of these types of apple.

Materials and Methods

Russet evaluation. Mature fruit (30-100/tree) were harvested at random (9/24/79) from 4 trees each of 'Golden Delicious' (14-year-old, seedling rootstock), Frazier Spur (13 year-old Malling-Merton 111 rootstock), and 'Red Delicious' (14-year-old, seedling rootstock) and from 8 trees of Smoothee (11-year-old, Malling 9 rootstock) grown on the Horticultural Research Center, East Lansing, Michigan. Fruit were evaluated for russet severity using a 1-5 subjective scale by comparing individual fruit with photographs of representative

fruit in each class (12). A russet rating of "1" indicates fruit free of russet, while higher ratings indicate the presence of progressively more russet. Fruit with ratings of "1" and "2" are considered suitable for fresh sales, and fall within the "U.S. Fancy" grade (1). Russet indexes were also calculated for each group of fruit by multiplying the number of fruit in each russet class by the class value (1-5), and then dividing the total by the number of fruit in the group. Both the % of fruit suitable for the fresh market and the russet index give a concise measure of the severity of russet present within a group of fruit.

Cuticular components. Fruit (40-50) were randomly collected from each of the 4 cultivars 30 days after petal fall (6/20/79) and cuticular components were determined using a method similar to that described by Flore and Bukovac (13). Briefly, discs (8 mm diam, approx 1 mm thick) were punched from fruit and randomly assigned to 3 groups of 50 each. Epicuticular waxes were extracted by dipping the discs into 2 successive 50 ml portions of redistilled chloroform for 20 seconds. The extracts were pooled and the solvent was removed under reduced pressure on a rotary evaporator (40°C). The waxes were transferred to tared test tubes and epicuticular wax weight was determined by subtraction. This technique gave similar results to those obtained when wax extraction was performed prior to the punching of cuticle discs (12).

Cuticular membranes were then isolated in a solution of 5.0% (w/v) pectinase (Nutritional Biochem. Corp., Cleveland,

Ohio) plus 0.2% (w/v) cellulase (Nutritional Biochem. Corp., Cleveland, Ohio) buffered at pH 3.2 (dibasic sodium phosphate/ citric acid) under mechanical agitation for approx 72 hours. Fresh enzyme solution was substituted after the first 24-36 hours of digestion. After cuticle isolation the discs were agitated in distilled water for approx 24 hours, and then air-dried to a uniform weight.

Cuticular waxes were Soxhlent extracted (chloroform/ methanol 9:1 vol/vol) for 2 hours at 45°C. Solvent was removed under reduced pressure on a rotary evaporator and waxes were transferred to tared test tubes for weight determination by subtraction. Discs were again air-dried to determine the weight of the remaining cutin matrix, which is composed of cutin acids and carbonate soluble material (13). Cutin matrix was added to total wax to give total membrane weights. Cuticular components for mature fruit were determined using the same procedure with sample discs 12 mm in diameter, approx 1 mm thick. Data are expressed on a weight/ unit area basis ($\mu g/cm^2$), and represent the average of 3 samples from each cultivar.

Thin layer chromatography. Epicuticular and cuticular wax components were separated by TLC using precoated silica gel thin-layer plates (Uniplate, 250 µm, Analtech, Inc., Newark, Delaware), which were prewashed in benzene and dried at 110°C for 30 minutes. The waxes were dissolved in 1:1 (vol/vol) chloroform/ethyl acetate (5 mg/ml 30 days after petal fall, 10 mg/ml at harvest) and spotted (2 µl) onto the plates along with cabbage wax for reference. The plates

were developed in chloroform/ethyl acetate (7:3 vol/vol), and the components were located by charring (160°C) after spraying with 5% K_2CrO_4 in 50% H_2SO_4.

Surface fine structure. Freeze dried fruit cuticle sections were attached to aluminum studs, coated with gold (approx 20 nm), and observed with a JEOL Model JSM 35C scanning electron microscope operated at 15 kV. Sections from 3 fruit/cultivar were examined from several collection dates, and representative sections were photographed and used for detailed observation.

Statistical. Data were subjected to analysis of variance and significance between treatment means was determined by Duncan's multiple range test (23). Regression analysis was performed using cuticular component weights ($\mu g/cm^2$) as the independent variable and the degree of russet at harvest as the dependent variable. A Control Data Corp. 6500 computer and the Statistical Package for the Social Sciences (17) were used to analyze the data.

Results

Russet evaluation. Significant differences in russet severity between strains was evident at harvest with Smoothee being the least susceptible, 'Golden Delicious' intermediate, and Frazier Spur most susceptible (Table 1). No russet was observed on 'Red Delicious'. The relative rankings of these strains is similar to that found from 1975-1978, although the magnitude changed from year to year (12).

Surface fine structure. Fruits of all cultivars were covered with trichomes (similar in size and shape) from petal fall to 9 days after petal fall (Fig. 1A,C). An amorphous wax with occassional granular masses was evident early in the season, while flat droplets and free standing platelets became visible at later sample dates (Figs. 1,2). 'Golden Delicious' surfaces exhibited few projecting wax platelets 9 days after petal fall (Fig. 1D), but none were visible on later sample dates. The wax appeared generally amorphous 17, 30 and 129 days after petal fall while exhibiting small wax droplets (Fig. 2A,B,C).

Smoothee surfaces remained amorphous until 17 days after petal fall when projecting platelets became visible (Fig. 2A). These platelets were more pronounced 30 days after petal fall but an amorphous surface was present 129 days after petal fall (Fig. 2B,C). Frazier Spur surfaces also remained amorphous until 17 days after petal fall when flattened wax platelets were visible (Fig. 2A). Later samples appeared to have the same surface structure as 'Golden Delicious' (Fig. 2B,C). Projecting wax platelets were observed on 'Red Delicious' cuticles 9 days after petal fall (Fig. 1D), and covered the surface extensively 17 and 30 days after petal fall (Fig. 2A,B). 'Red Delicious' surfaces were similar to those of 'Golden Delicious' and Frazier Spur 129 days after petal fall (Fig. 2C).

Table 1. Degree of russet among different strains of 'Golden Delicious' apple fruit at harvest.

	Russet evaluation						
	Fruit in each class (%)[z]					Suitable for fresh market (%)[y]	Index value[w]
	1	2	3	4	5		
Golden Delicious	2.8	21.4	52.2	20.0	3.6	24.2b[x]	2.98
Frazier Spur	0.5	4.9	37.5	46.1	11.0	5.4c	3.63
Smoothee	13.1	45.7	34.8	5.0	1.4	58.8a	2.27

[z] 1= no russet; 5= severe russet

[y] % in classes 1 and 2

[x] Mean separation by Duncan's multiple range test, 5% level

[w] Summation of the number of fruit in each class times the class number, divided by the total number of fruit

Fig. 1 Scanning electron micrographs illustrating epicuticular wax fine structure of different strains of 'Golden Delicious' and 'Red Delicious' apple. Columns from left to right: Smoothee, 'Golden Delicious', Frazier Spur, 'Red Delicious'. Row A: fruit harvested at petal fall (PF), 54X. Row B: fruit harvested at PF, 1000X. Row C: fruit harvested at PF +9 days, 54X. Row D: fruit harvested at PF + 9 days, 1000X.

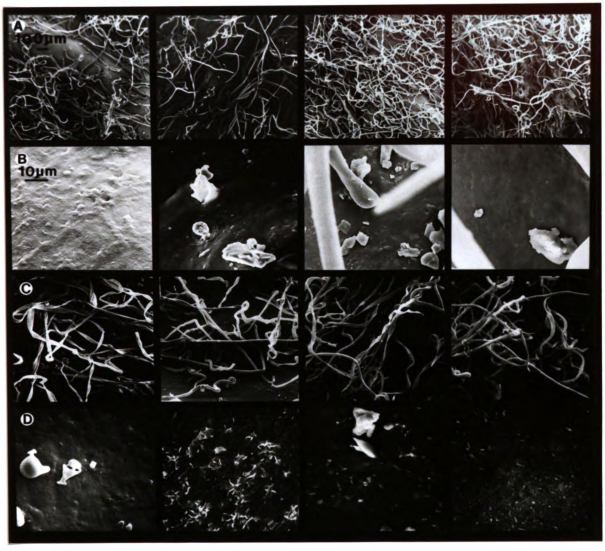
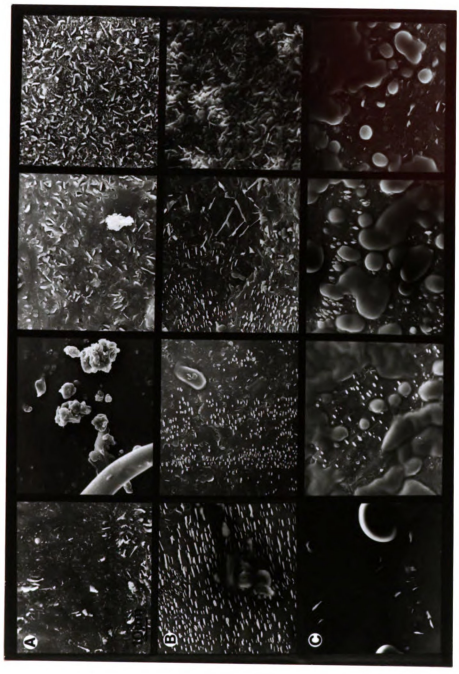

A
100µm
B
10µm
C
D

Fig. 2. Scanning electron micrographs illustrating epicuticular wax fine structure of different strains of 'Golden Delicious' and 'Red Delicious'. Columns left to right: Smoothee, 'Golden Delicious', Frazier Spur, 'Red Delicious'. Row A: fruit harvested at PF + 17 days, 1000X. Row B: fruit harvested at PF + 30 days, 1000X. Row C: fruit harvested at PF + 129 days, 1000X.


A
B
C

Cuticular components. There were no significant quantitative or qualitative differences in the cuticular components between the different strains of 'Golden Delicious' 30 days after petal fall (Table 2, Fig. 3). 'Red Delicious' had significantly less epicuticular wax, total wax and total membrance weight than the 'Golden Delicious' strains. Regression analysis revealed no significant correlation between cuticular components and russet 30 days after petal fall (Table 3).

At harvest 'Red Delicious' had significantly more epicuticular wax than the other samples, and 'Golden Delicious' had significantly more than Frazier Spur, but no Smoothee (Table 4). Smoothee had significantly more cuticular wax than the other sample, and cutin matrix weights greater than those of Frazier Spur. Total membrane weights for Smoothee were significantly greater than those of Frazier Spur and 'Red Delicious', while 'Golden Delicious' had more total membrane than Frazier Spur. Smoothee had significantly more total wax than the other samples, while 'Red Delicious' had more than Frazier Spur. TLC again revealed no differences between the wax compositions of the 'Golden Delicious' strains, but 'Red Delicious' had an additional epicuticular wax component with an R_f value of .84-.88 which co-chromatographed with cabbage wax ketones (Fig. 3).

Very significant positive correlations were found between cuticular wax, total membrane and total wax weights vs.

russet (Table 3). A significant positive correlation was also present between cuticular matrix weights and russet.

Table 2. Cuticular components of different strains of 'Golden Delicious' and 'Red Delicious'

	Cuticular component ($\mu g/cm^2$)				
	Total membrane	Epicuticular wax	Cuticular wax	Total wax	Cutin matrix
Golden Delicious	1978.7a[z]	415.0a	197.8ab	612.8a	1266.0z
Frazier Spur	1913.8a	415.0a	233.6a	648.7a	1265.3ab
Smoothee	2069.0a	416.6a	212.1ab	628.7a	1440.4a
Red Delicious	1594.3b	366.1b	127.3b	493.4b	1100.9b

[z]Mean separation within columns by Duncan's multiple range test, 5% level.

Table 3. Correlation between cuticular components and russet severity of 'Golden Delicious' apple 30 days after petal fall and at harvest.

	Date of Cuticular analysis			
	30 days after petal fall		At harvest	
Cuticular Component	r value	F signi-ficance(%)	r value	F signi-ficance(%)
Total membrane	.20602	59.5	.51572	15.5
Epitucular wax	.27928	46.7	.82976	0.6
Cuticular wax	.51288	15.8	.72238	2.8
Total wax	.38420	30.7	.90419	0.1
Cutin matrix	.22236	56.6	.91171	0.1

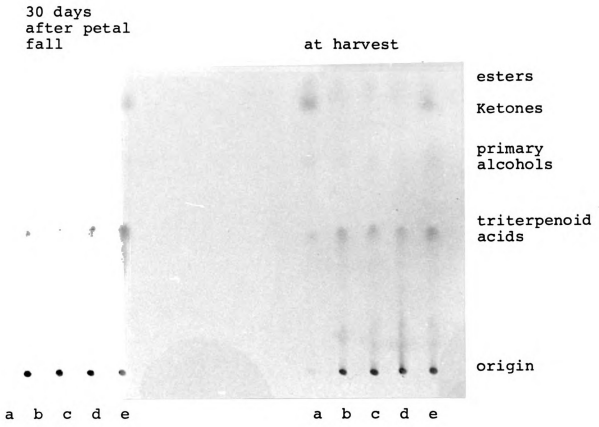


Fig. 3. Thin-layer chromatograms of epicuticular wax (developed in chloroform/ethyl acetate 7:3) Position (a) cabbage wax for reference, (b) 'Golden Delicious' (c) Frazier Spur, (d) Smoothee, (e) 'Red Delicious'.

Table 4. Cuticular components of different strains of 'Golden Delicious' and 'Red Delicious' apple at harvest

	Cuticular component ($\mu g/cm^2$)				
	Total membrane	Epicuticular wax	Cuticular wax	Total wax	Cutin matrix
Golden Delicious	3379.9ab[z]	630.8b	485.6b	116.4bc	2263.5ab
Frazier Spur	2875.8c	564.5c	479.2b	1043.7c	1832.1b
Smoothee	3723.0a	601.8bc	781.6a	1383.4a	2339.6a
Red Delicious	3115.9bc	745.0a	452.2b	1197.2b	1918.7ab

[z]Mean separation within columns by Duncan's multiple range test, 5% level.

Discussion

Russet evaluations confirmed the differing russet susceptibilities of 'Golden Delicious', Frazier Spur, and Smoothee (Table 1). No significant correlations were found between cuticular components 30 days after petal fall and the amount of russet present at harvest (Table 3). TLC revealed no differences in the wax compositions of the 'Golden Delicious' strains (Fig. 3). Since it is generally accepted that russet initiation occurs between petal fall and 30 days after petal fall, it appears that russet formation is not related to the amounts of cuticular components present in the strains, nor to the composition of the waxes present.

Examination of fruit surface fine structure revealed that 'Red Delicious' fruit were covered with projecting wax platelets at 9, and especially 17 and 30 days after petal fall (Figs. 1,2). In contrast, fruit of the 'Golden Delicious' strains exhibited such projecting platelets in smaller quantities and only briefly during this crucial time period. Water repellancy is known to be greatest when wax has a rough surface in the form of projecting rods or other structures (15). The relative shortage of projections on fruit of the 'Golden Delicious' strains may result in more water diffusing into epidermal cells through cracks, causing increased turgor and possible cell rupture and possibly more russet (9). It is possible that an upright arrangement of wax platelets allows the wax of russet-resistent varieties to better accomodate the expansion of underlying fruit

tissues (10). The generally amorphous wax on fruit of all 3 'Golden Delicious' strains may be less able to expand during normal fruit growth. The appearance of the surface fine structure of fruit of the 'Golden Delicious' strains did not indicate possible causes for the differing russet susceptibilities of these strains.

The differences in cuticle component weights at harvest (Table 4) may be due to normal cuticle development throughout the growing season, primarily after russet formation has occurred. This would explain the close correlations between cuticular component weights and russet found at harvest, while no significant correlations were found 30 days after petal fall.

Russet formation could be related to the growth rate of fruit tissue, which may result in cracking of amorphous waxes such as those observed on fruit of the 'Golden Delicious' strains (Figs. 1,2). Russet formation could also be related to the composition of the cutin matrix (not investigated in the present study), or to the cuticular elasticity of different apple varieties. Studies with tomato have shown a relationship bewteen the ability of cuticles to stretch and the occurrence of fruit cracking (2,25). An experiement is presently underway to investigate this relationship in apples.

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SECTION II

CHARACTERIZATION OF CUTICLE FROM RUSSET AND NON-RUSSETED AREAS OF 'GOLDEN DELICIOUS' APPLE

CHARACTERIZATION OF CUTICLE FROM RUSSET AND NON-RUSSET AREAS OF 'GOLDEN DELICIOUS' APPLE

Abstract

Cuticle from russeted and non-russeted areas of 'Golden Delicious' (Malus pumila Mill.) and Frazier Spur was examined. Scanning electron microscopy revealed massive cuticle disruption in russeted areas, however epicuticular wax fine structure was still evident. Frazier Spur fruit cuticle was generally composed of less epicuticular wax, cuticular wax, cutin matrix (cutin acid plus carbonate soluble material), and total membrane weight than cuticle from 'Golden Delicious' fruit. Russeted cuticle from fruit of both 'Golden Delicious' and Frazier Spur contained less epicuticular and cuticular wax than non-russeted cuticl. At room temperature (20°C) russeted fruit lost water at a greater rate than non-russeted fruit. In cold storage (1°C) russeted fruit initially lost water at a greater rate than non-russeted, but the rates were essentially the same during later storage. However, even under cold storage conditions 'Golden Delicious' and Frazier Spur fruit lost water twice as fast as 'Red Delicious' fruit.

Introduction

Histological studies indicate that apple russet results from irregular cell divisions which lead to the formation of an uneven, disrupted layer of epidermal cells (1,2,7,8). Active cork cambium is initiated in lower epidermal layers which divides toward the outermost epidermal cells and eventually ruptures the fruit surface. Russet is observed throughout the remaining growing season as a sloughing of cuticle, dead epidermal cells and cork cells. Russeted fruit are generally not acceptable to consumers, and they are more susceptible to water loss and shriveling in storage than are non-russeted fruit (6,11).

The present study was initiated in order to characterize differences between russet and non-russet cuticles in relation to water loss during storage. Since little quantitative information is available concerning russet and non-russet cuticular components, these were determined for both types of cuticles. 'Golden Delicious' (moderate russet) and its subclone Frazier Spur (severe russet) were used to determine possible differences in russet tissue characteristics when different russet susceptibilities are known to exist.

Materials and Methods

Plant Materials. Mature fruit were harvested from 14-year-old 'Golden Delicious' trees (seedling rootstock) and from 13-year-old Frazier Spur trees (MM 111 rootstock) grown on the Horticultural Research Center, East Lansing, Michigan.

Storage behavior. 'Golden Delicious' and Frazier Spur fruit were evaluated for russet severity as previously described (3,5) and separated into "moderate russet" and "severe russet" groups. The moderate russet group contained fruit rated 1-3, and the severe russet group contained fruit rated 3-5. Samples of 10 fruit each were randomly selected from these groups and the russet index was calculated for each as previously described (5). Fruit samples were placed into cold storage (1°C) and kept at room temperature (20°C). Moderate and severe russet samples of 'Golden Delicious' and Frazier Spur were replicated 5 times in each storage temperature. Five replicate samples of non-russet 'Red Delicious' fruit were also put into cold storage for comparison.

Initial sample weights were recorded and each sample was weighed periodically thereafter. Fruit kept at room temperature were discarded after 5 weeks because of severe deterioration. Fruit kept in cold storage were sampled for 12 weeks. The weight loss during each interval between weighings was divided by the previous sample weight, and then by the number of days between weighings to give the relative weight loss per day for each storage interval.

Surface fine structure. Fruit tissue samples were collected and prepared for S.E.M. examination as previously described (5). Representative examples of russet and non-russet fruit surfaces were photographed for detailed observation.

Cuticular components. Fruit tissues were selected as russeted and non-russeted, and cuticular components were

isolated and analyzed as previously described (5). Three replicate samples of 50 discs (12 mm diam) were analyzed for each type of tissue.

Statistical. Standard deviations were calculated from the relative water loss/day values for each treatment. Correlation coefficients (r) were calculated using relative water loss/day as the dependent variable and the russet index as the independent variable. Cuticular component data were subjected to analysis of variance and significance between treatment means was determined by Duncan's multiple range test (10).

Results

Storage behavior. Cold storage greatly reduced water loss for both moderate and severe russet fruit of the 'Golden Delicious' strains (Figs. 4,5). The rate of water loss for all treatments except 'Red Delicious' were generally highest during the first week of storage and gradually decreased thereafter. In cold storage all 'Golden Delicious' and Frazier Spur fruit lost water at similar rates, however the rate for severe russet fruit was much higher for both from 10/8/-10/12 (7 days of storage) and for Frazier Spur from 10/23-10/30 (27 days of storage) than that observed for moderate russet fruit. The relative water loss rate of 'Red Delicious' in cold storage was much lower than that of all 'Golden Delicious' treatments on all sample dates.

Severe russet caused significantly greater rates of water loss at room temperature storage than those which

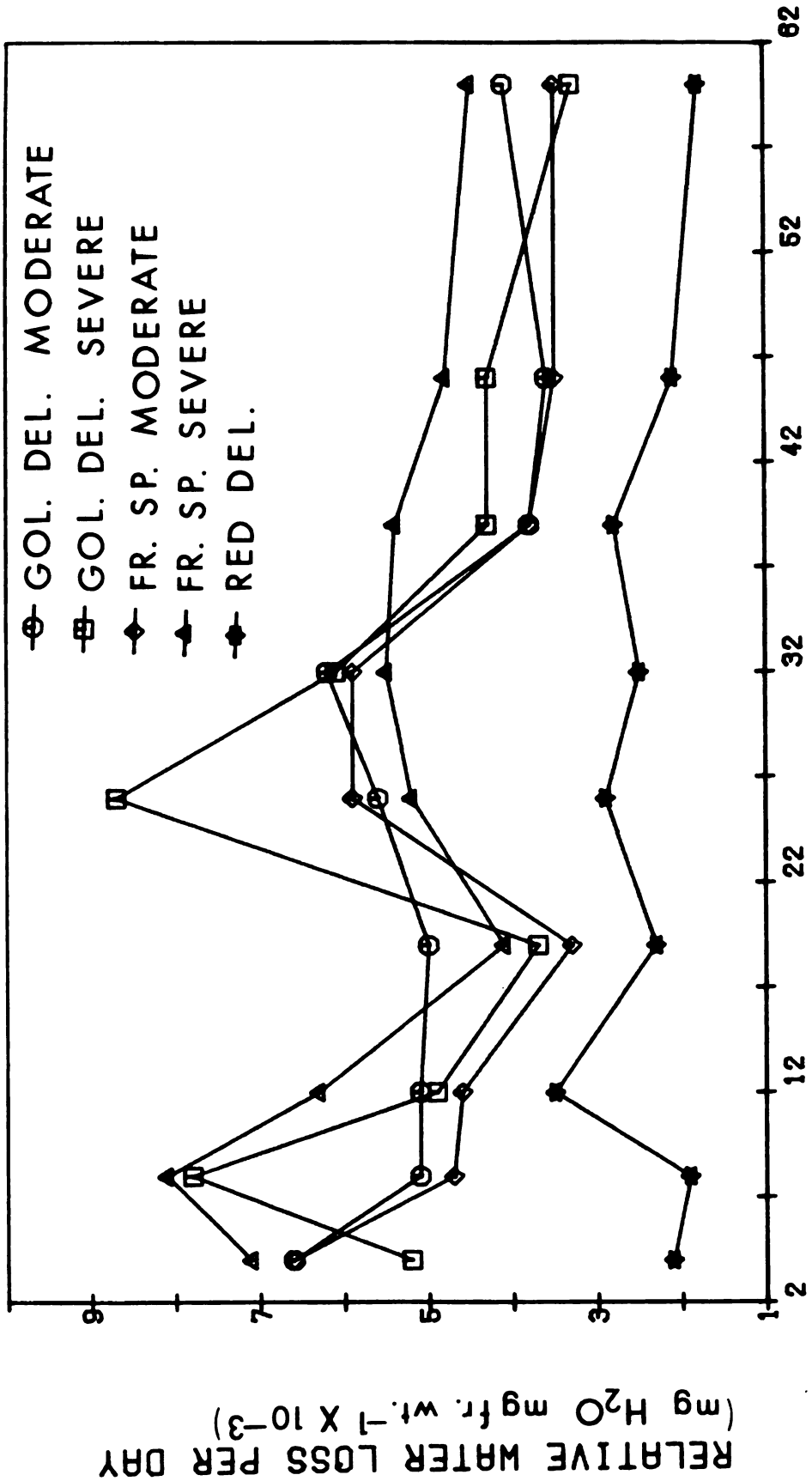

Fig. 4 The effect of russet severity on relative water loss of 'Golden Delicious' Frazier Spur and 'Red Delicious' fruit stored at 1°C.

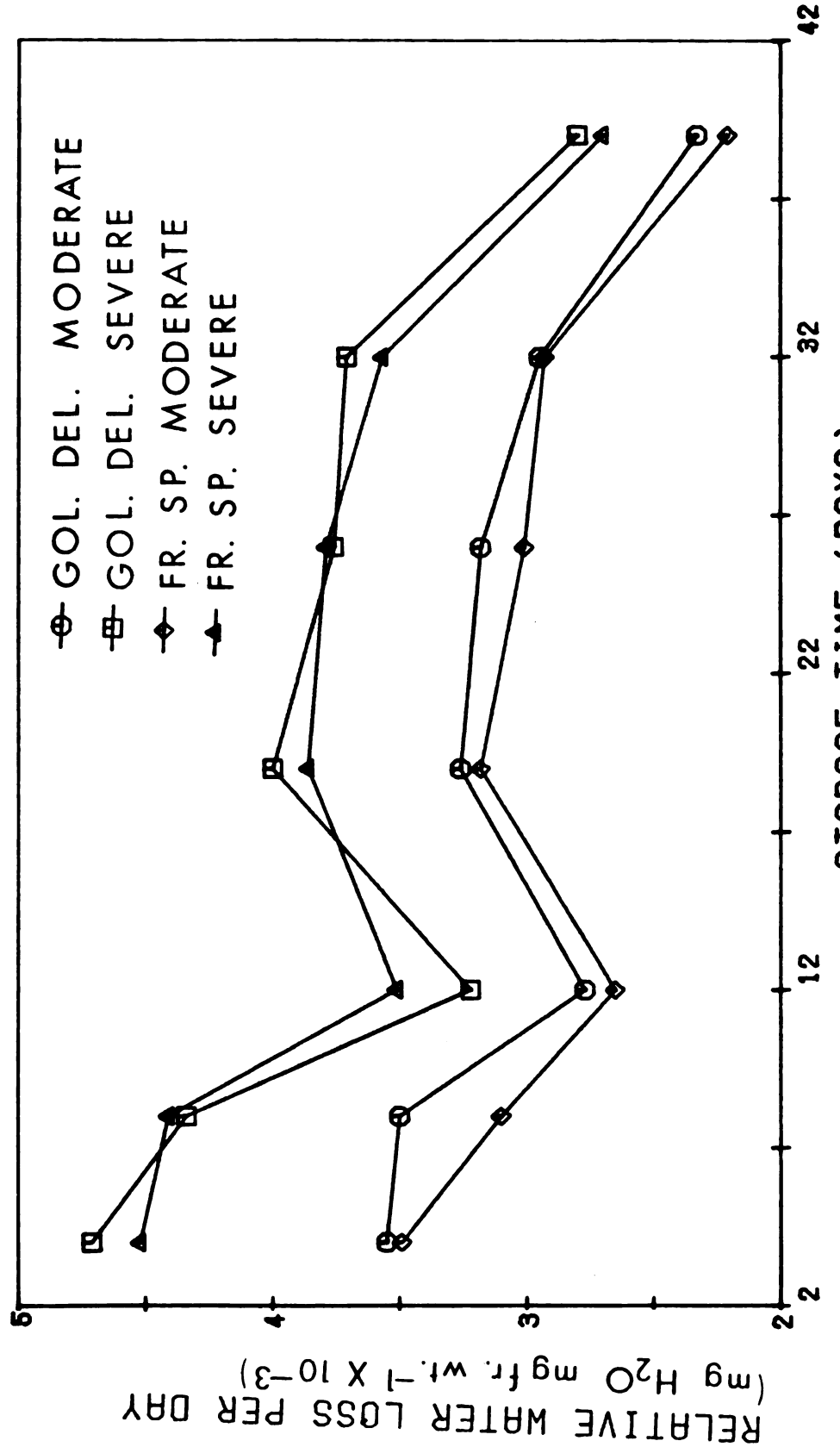

Fig. 5. The effect of russet severity on relative water loss of 'Golden Delicious' and Frazier Spur fruit stored at 20°C.

occurred in moderate russet samples (Fig. 5). Rates for severe russet samples of 'Golden Delicious' and Frazier Spur were not significantly different in most cases, and the same was true for moderate russet samples of the two strains stored at room temperature. A very significant positive correlation (r=0.59) was present between the russet index and relative water loss per day in room temperature storage.

Surface fine structure. Extensive cuticle disruption was observed on russeted tissue sample from both 'Golden Delicious' and Frazier Spur fruit (Fig. 6). While non-russet surfaces were seen to be continuous coverings of epicuticular waxes, the russet surfaces were discontinuous and broken. Underlying epidermal cell outlines were also clearly visible in russet areas. It was evident that internal tissues were still somewhat protected in russet areas since wax deposits were visible. The fruit surfaces of non-russet areas adjacent to russet areas appeared to be identical to non-russet areas present anywhere on the fruit.

Cuticular components. More cuticular matter of every type was present in non-russet 'Golden Delicious' than in non-russet Frazier Spur, and more was present in russet Golden Delicious' than in russet Frazier Spur (Table 5). Although the differences were not all significant, the trend suggests that Frazier Spur fruit normally have less cuticular matter than 'Golden Delicious' fruit. Russet areas contained significantly less epicuticular and cuticular waxes than

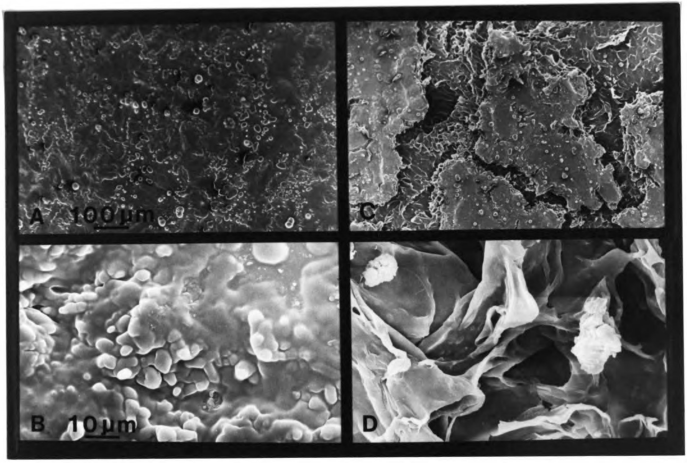

Fig. 6. Scanning electron micrographs illustrating epicuticular wax fine structure of russeted and non-russeted areas of 'Golden Delicious' fruit. (A) non-russeted area, 100X. (B) non-russeted area, 1000X. (C) russeted area, 100X. (D) russeted area, 1000X.

non-russet areas within each 'Golden Delicious' strain. Cutin matrix weights were not significantly different for russet and non-russet 'Golden Delicious', nor for russet and non-russet Frazier Spur. Total membrane weights for both 'Golden Delicious' and Frazier Spur were higher in non-russet than in russet samples but the differences were not significant.

TLC revealed the presence of a component (R_f 0.13-0.15) in the cuticular waxes of russet tissues not present in non-russet tissues. Epicuticular waxes from russet samples of both 'Golden Delicious' strains had a major component (R_f 0.36-0.39) which was present only as a trace in non-russet samples (Fig. 7) and which was identified as triterpenoid acids by the characteristic purple color after charring (110°C for 3 min).

Table 5. Cuticular components from russeted and non-russeted areas of 'Golden Delicious' and Frazier Spur apple fruit.

	Cuticular component ($\mu g/cm^2$)				
Type of cuticle	Total Membrane	Epicuticular wax	Cuticular wax	Total wax	Cutin matrix
Golden Delicious non-russet	3307.4a[z]	585.3a	501.1a	1086.3a	2221.1ab
Golden Delicious russet	3031.7ab	454.0c	312.5b	766.4bc	2265.3a
Frazier Spur non-russet	2963.8ab	519.4b	426.7a	946.1ab	2017.7ab
Frazier Spur russet	2554.1b	380.2d	248.1b	628.3c	1925.8b

[z]Mean separation within columns by Duncan's multiple range test, 5% level.

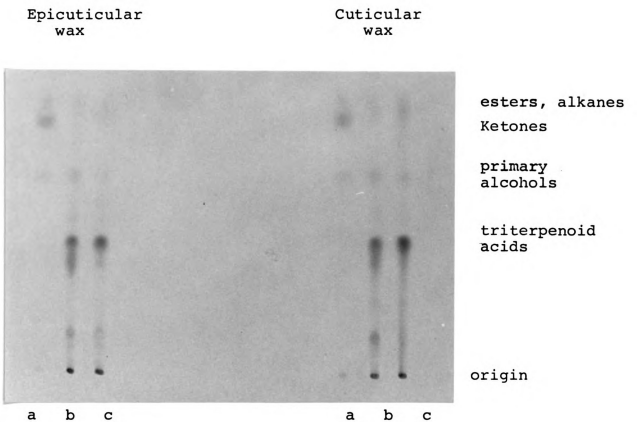

Fig. 7. Thin-layer chromatograms of wax extracted from russeted and non-russeted areas of 'Golden Delicious' fruit (developed in chloroform/ethyl acetate 7:3). Position (a) cabbage wax for reference, (b) russeted area, (c) non-russeted area.

Discussion

The use of cold storage eliminated much of the high water loss due to severe russet which occurred in room temperature storage (Figs. 4,5). However, even in cold storage the rates of water loss from both moderate and severe russet 'Golden Delicious' and Frazier Spur were more than double the rates which occurred in 'Red Delicious' fruit. Greater amounts of epicuticular wax were found on 'Red Delicious' fruit at harvest than were present on fruit of 'Golden Delicious' and Frazier Spur (5), and this may account for the lower water loss of 'Red Delicious' fruit observed. The removal of epicuticular wax deposits from 'Golden Delicious' fruit has been reported to increase the rate of water loss during storage (4), and the cuticular waxes of potato tubers have been found to be the major diffusion barrier to water vapor (9).

The presence of russet may also increase fruit water loss in storage. Russeted areas were seen to have greatly disrupted cuticular membrane surfaces (Fig. 6), which could allow water to escape more easily from underlying fruit tissues no longer protected from the surrounding (relatively drier) environment. Although some epicuticular wax deposits were visible on russeted areas, these areas contain significantly less epicuticular and cuticular waxes than non-russeted areas (Table 5). The presence of less fruit cuticle wax represents a less substantial barrier for diffusion of water, and thus results in greater rates of water loss from

severely russeted fruit than from less russeted fruit. Epiticular and cuticular waxes from russet tissues were found to have components not present in non-russet tissues (Fig. 7), which indicates that some chemical changes in wax do occur during storage. These wax changes could contribute to water loss from russeted fruit by affecting diffusion rates of water. The composition of the cutin matrix may also differ between russet and non-russet cuticles, and thus could be involved in the storage behavior of fruit containing differenct amounts of russet cuticle.

The total membrane weight was not significantly changed during russet formation, but the amount of total wax was decreased (Table 5). This means that russeted cuticles are composed of smaller proportions of waxes than non-russet cuticles. It seems reasonable that the formation of cork-like russet tissue occurs to the exclusion of the normal fruit cuticle, and that wax production is decreased during russet formation process. The stage of fruit development during which the difference in wax quantity and composition occurs in russet cuticles should be the subject of future study.

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SECTION III

THE EFFECT OF SHELTERS ON RUSSET FORMATION AND CUTICLE DEVELOPMENT OF 'GOLDEN DELICIOUS' APPLES

THE EFFECT OF SHELTERS ON RUSSET FORMATION AND CUTICLE DEVELOPMENT OF 'GOLDEN DELICIOUS' APPLES

Abstract

Shelters were used to eliminate rain, alter light quality and decrease the light quantity on 'Golden Delicious' (Malus pumila Mill.) trees. Black polyethylene shade material (8.1% full sun) resulted in 27.3% fruit suitable for the fresh market, while full sun controls resulted in only 1.6%. Different colored cellophane reduced russet formation significantly, however, no relation between incident light wavelength and russet formation was established. Clear polyethylene rain shelters resulted in 82.7% fruit suitable for the fresh market, while unsheltered controls resulted in only 0.3%. Fruit grown under shelters had significantly more epicuticular wax than unsheltered controls 30 days after petal fall, and significantly less at harvest. Light quality and quantity were found to affect the amount of total membrane, cutin matrix (cutin acid plus carbonate soluble material), epicuticular wax, cuticular wax and total wax present on fruit, however, no relationship was found between these components and russet formation.

Introduction

Among apple cultivars 'Golden Delicious' is one of the most susceptible to fruit russet formation. Environmental factors such as humidity, rainfall and sunlight have been implicated as factors which contribute to the occurence and severity of russet. Creasy (2) fround evidence that higher rainfall during the period between 10 and 20 days after full bloom resulted in increased russet formation. The study also found that rain coverings reduced russet formation, as did the work of Hatch (6).

High humidity around fruit a has been widely accepted to be a cause of greater russet formation (2,3,4,19). Studies by Tukey (15) and Watanabe (20) indicated that covering apples with moisture-proof materials resulted in high russet formation. Similar coverings made with moisture-permeable materials resulted in less russet formation. In contrast, more recent research by Hatch (6) indicated that apples grown under plastic coverings (for rain protection) had less russet than uncovered fruit in spite of high humidity underneath the plastic (70% vs. 40% outside the plastic).

Verner (16) suggested that shaded fruit may have more elastic cells than those exposed to sunlight, and thus may be better able to expand or shrink without cracking and russet formation. Watanabe (20) observed changes in wax quantities and russet formation after covering fruit with various shading materials. DeVries (18) indicated that sunlight may change the polymerization of cutin acids into an amorphous

matrix which is more susceptible to cracking and russeting. Tukey (15) concluded that sunlight is not an important factor in russet formation since russet is often found over entire fruit surfaces, not just those exposed to sunlight.

Since russet is a physiological disorder of apple fruit cuticles, and since environmental factors are known to influence russet formation, it seems reasonable that environmental conditions may act directly on fruit cuticles to influence russet formation. The present study was initiated to better determine the effects of light quantity and quality, rainfall and high humidity on russet formation of 'Golden Delicious' apples. The study was also intended to quantify any changes in fruit cuticle compositions which occur as a result of imposed environmental conditions, and to seek possible relationships between these changes and russeting.

Materials and Methods

General. Different shelter treatments were applied to 5-year-old 'Golden Delicious' trees on M 26 rootstock located at the Graham Horticultural Experiment Station, Grand Rapids, Michigan. Russet evaluation (at harvest) and cuticle analysis (30 days after petal fall and at harvest) were performed as previously described (5,9). All shelters were applied at petal fall (5/21/79) and were removed 40 days later (except where noted).

Polyethylene shade material. Black polyethylene shade materials (Chicopee Manufacturing Company, Cornelia, Georgia) of 2 different densities (92%, 55% manufacturer's

designations) were placed over the south and upper part of each tree, thus allowing rainfall to penetrate and air movement to occur in order to avoid temperature and humidity build-ups (Fig. 8). Three trees chosen for uniform size and flowering were covered with each shade density, and 6 adjacent trees were likewise chosen as shadeless controls. The effect of the shade materials on light quantity and quality was determined with a spectroradiometer (ISCO, Model SR) under full sun (FS) conditions (Fig. 9, Table 6). The black polyethylene decreased light to 8.1% (92% manufacturer's designation) and 32.0% (55% manufacturer's designation) of FS, and had no significant effect on light quality. The 2 shade densities are referred to as 8.1% FS and 32.0% FS throughout this paper.

Colored cellophane. Cellophanes of 7 different colors (red, blue, puple, green, yellow, orange and clear) were formed into open-ended cylinders (approx 24" long, 12" diam) which were fitted over individual branches, and which allowed good air movement and some rain penetration (Fig. 8). Four replications of each cellophane were placed randomly throughout 12 trees selected for uniform size and flowering, and branches adjacent to treatments were selected as controls. Spectroradiometer measurements indicated that each cellophane color admitted light wavelengths which roughly corresponded with its own color, and that the different colors caused a wide range of decreases in FS (Fig. 10, Table 6).

Plastic shelter. Clear plastic (4 mil thick polyethylene) was placed to cover the tops and sides of trees while

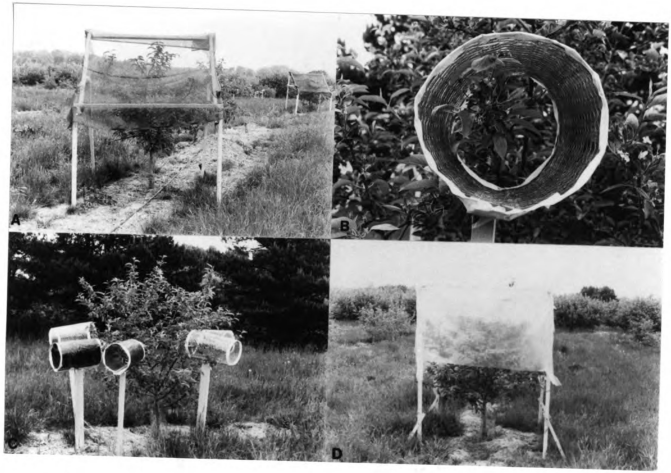

Fig. 8. Shelters: (A) black polyethlylene shade, (B) and (C) colored cellophanes, (D) clear polyethylene rain shelter.

Table 6. The effect of shelter materials on transmittance[z] of sunlight under full sun conditions.

Material	Transmittance (% of full sun)
Clear cellophane	100.0
Purple	80.6
Yellow	75.1
Blue	62.1
Orange	56.9
Green	42.4
Red	37.5
92% black polyethylene[y]	8.1
55% black polyethylene	32.0
Clear polyethylene	93.2

[z]Calculated from data determined by spectroradiometry.

[y]Manufacturer's shade designations, referred to as 8.1% FS and 32.0% FS respectively.

leaving the lower 2-3 feet open for air movement (Fig. 8). Six trees selected for uniform size and flowering were covered while 6 adjacent trees were selected as controls. The plastic shelters were removed from 3 of the trees 40 days after petal fall ("shelter removed") and from the remaining trees at harvest ("shelter kept on"). Spectroradiometer measurements showed that the plastic did not affect light quality, while it decreased FS by 6.8% (Fig. 9, Table 6).

Statistical. Data were subjected to analysis of variance and significance between treatment means was determined by Duncan's multiple range test (14). Regression analysis was performed using cuticular components from the various treatments as the independent variables and the degree of russet at harvest as the dependent variable. A Control Data Corp.

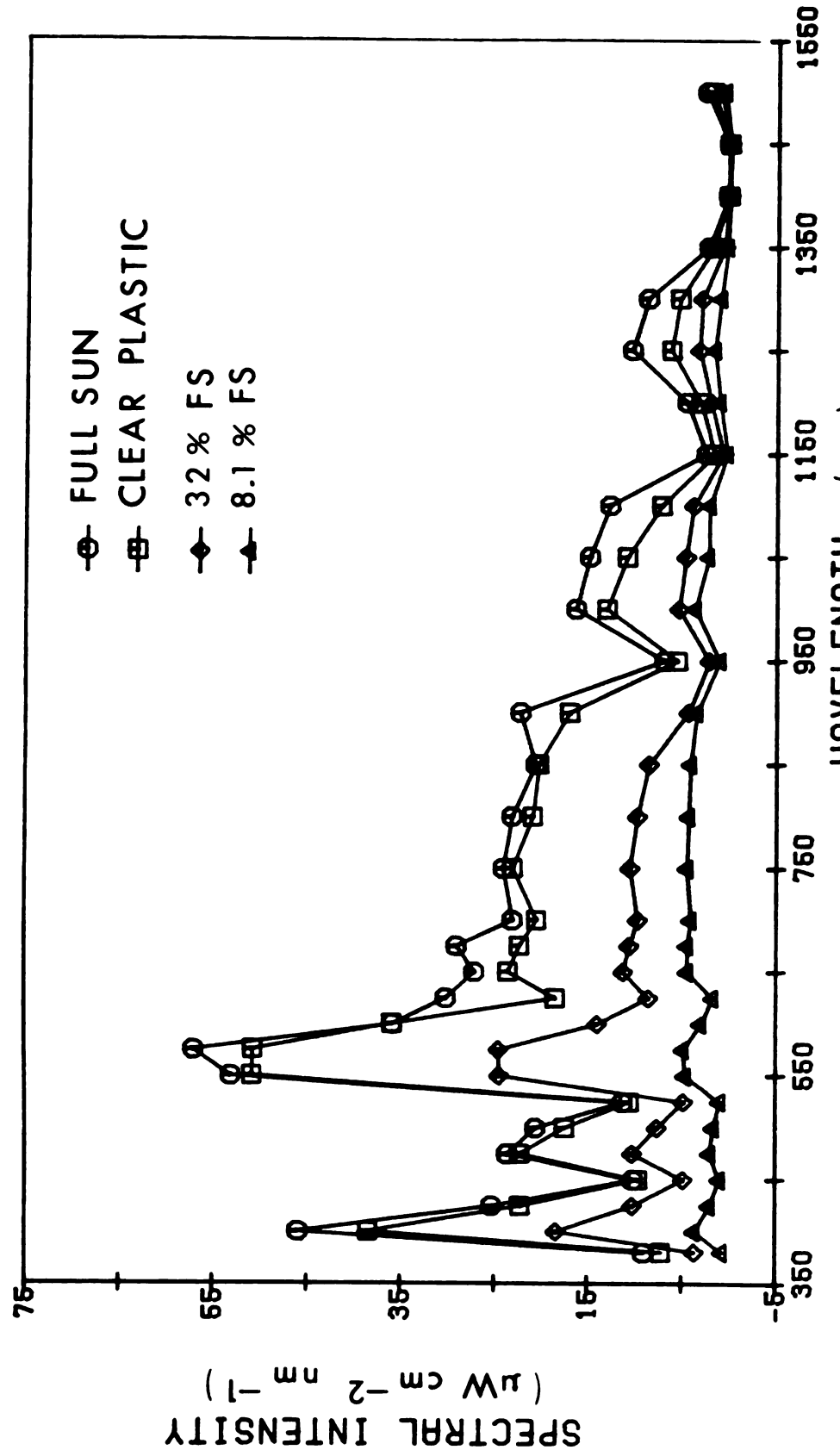

Fig. 9. The effect of black and clear polyethylene materials on light transmittance under full sun conditions.

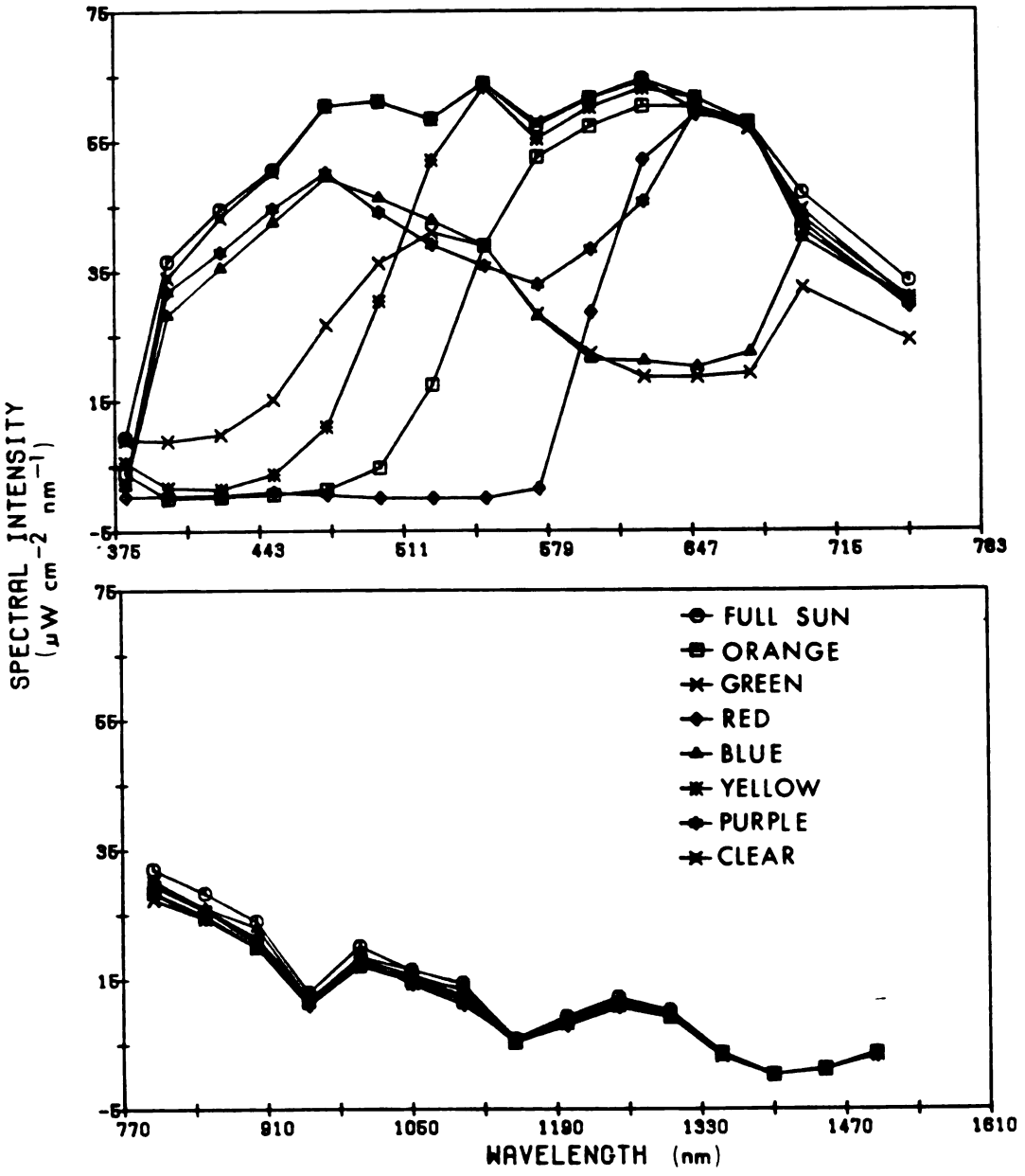

Fig. 10. The effect of colored cellophane materials on light transmittance under full sun conditions.

6500 computer and the Statistical Package for the Social Sciences (8) were used to analyze the data.

Results

Polyethylene shade material. The 8.1% FS treatment significantly increased the % of fruit suitable for the fresh market as compared to untreated fruit (Table 7). The 32.0% FS treatment also improved fruit quality as evidence by 61.1% in russet class 3 and no fruit in russet class 5, compared to 29.5% in class 3 and 17.1% in class 5 for control fruit.

Cuticular component weights of both shade treatments and controls were not significantly different 30 days after petal fall, nor at harvest (Table 8). No significant correlations were detected between cuticular component weights and russet (Table 9). TLC of both epicuticular and cuticular waxes from both sample dates showed no differences between any of the treatments (Fig. 11).

Colored cellophane. Russet formation was significantly reduced by red, blue, purple and clear cellophanes (Table 10). The % of fruit suitable for the fresh market from the blue cellophane was the highest of all the cellophanes, and this treatment also produced no fruit rated in the most severe russet classes (4 and 5).

Clear and blue treated fruit had significantly more epicuticular wax at harvest than those of the other treatments and controls (Table II). Epicuticular wax weights of purple and red treated fruit were not significantly different than controls. Fruit from red, green and yellow treatments had

Table 7. The effect of black polyethylene shade material on the degree of russet on 'Golden Delicious' apple fruit at harvest.

Treatment	Russet evaluation: Fruit in each class (%)[z] 1	2	3	4	5	Suitable for the fresh market[y]	Index value[w]
Full sun	0.0	1.6	29.5	51.9	17.1	1.6b[x]	3.87
32.0%FS	0.0	2.8	61.1	36.1	0.0	2.8b	32.7
8.1%FS	6.1	21.2	54.5	18.2	0.0	27.3a	2.87

[z]1=no russet; 5=severe russet.

[y]% in classes 1 and 2.

[x]Mean separation by Duncan's multiple range test, 5% level

[w]Summation of the number of fruit in each class times the class number, divided by the total number of fruit.

Table 8. The effect of black polyethylene shade material on cuticular components of 'Golden Delicious' apple 30 days after petal fall and at harvest.

Sample Date	Treatment	Cuticular component ($\mu g/cm^2$): Total membrane	Epicuticular wax	Cuticular wax	Total wax	Cutin matrix
30 days after petal fall	Full sun	1712.1[z]	383.2	187.0	570.2	1141.9
	32.0% FS	1752.7	386.0	175.1	561.0	1191.7
	8.1% FS	1661.2	397.9	195.0	592.9	1068.3
At harvest	Full sun	3504.6	802.5	401.4	1203.9	2300.7
	32.0% FS	3390.5	786.9	455.0	1241.9	2148.6
	8.1% FS	3444.3	800.6	422.6	1223.2	2221.1

[z]No significant differences were detected within columns for each sample date by Duncan's multiple range test, 5% level.

Table 9. The effect of black polyethylene shade material on the correlation between cuticular components and russet severity of 'Golden Delicious' fruit at at harvest.

Cuticle component	r value	F significance (%)
Epicuticular wax	.03775	92.3
Cuticular wax	.26534	49.0
Total wax	.18780	62.8
Cutin matrix	.02187	95.5
Total membrane	.12536	74.8

Table 10. The effect of different colored cellophane material on the degree of russet on 'Golden Delicious' apple fruit at harvest.

	Russet evaluation						
	Fruit in each class (%)[z]					Suitable for the	Index
Treatment	1	2	3	4	5	fresh market[y]	value[w]
Control	0.0	0.8	10.9	35.2	53.1	0.8c[x]	4.40
Clear	8.3	37.5	45.8	8.3	0.0	45.8ab	2.63
Orange	12.5	20.8	50.0	16.7	0.0	33.3bc	2.80
Green	11.1	22.2	63.0	3.7	0.0	33.3bc	2.55
Red	23.1	30.8	38.5	7.7	0.0	53.9ab	2.20
Blue	27.8	61.1	11.1	0.0	0.0	88.9a	2.20
Purple	18.2	40.9	31.8	9.1	0.0	59.1ab	2.20
Yellow	11.1	33.3	27.8	27.8	0.0	44.4bc	2.58

[z] 1=no russet; 5=severe russet

[y]% in classes 1 and 2

[x]Mean separation by Duncan's multiple range test, 5% level.

[w]Summation of the number of fruit in each class times the class number of fruit.

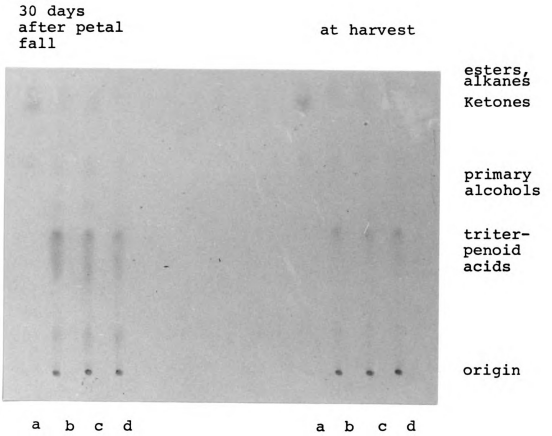

Fig. 11. Thin-layer chromatograms of epicuticular wax extracted from fruit grown under control and black polyethylene shade conditions (developed in chloroform/ethyl acetate 7:3). Position (a) cabbage wax for reference, (b) control (c) 32.0% FS, (d) 8.1% FS.

Table 11. The effect of different colored cellophane on cuticular components of 'Golden Delicious' apple at harvest.

Treatment	Cuticular component ($\mu g/cm^2$)				
	Total membrane	Epicuticular wax	Cuticular wax	Total wax	Cutin matrix
Control	3218.3b[z]	608.7cd	542.4bcd	1151.0ab	2067.3
Purple	3115.7bc	595.2cd	429.9cd	1020.2b	2095.5
Red	3387.5a	561.1d	766.2a	1327.4a	2060.2
Green	3143.5bc	592.9cd	651.3abc	1244.2ab	1899.3
Orange	3097.5c	639.5bc	569.4abdc	1208.9ab	1888.6
Blue	3227.8b	666.0ab	501.7bcd	1167.7ab	2060.2
Clear	3174.8bc	669.6a	365.5d	1065.1b	2109.7
Yellow	3152.0bc	609.6cd	701.5ab	1311.1a	1840.9

[z]Mean separation within columns by Duncan's multiple range test, 5% level.

significantly more cuticular wax than controls, and the red treatment resulted in significantly more cuticular wax than did the blue, purple and clear treatments. No total wax weights from the treatments were significantly from the controls, while those from red and yellow treatments were significantly higher than those from purple and clear treatments. No significant differences were present between cutin matrix weights from treatments and controls. Only the red treatment produced significantly higher total membrane weights than the controls, while the orange treatment produced weights significantly lower than the controls.

TLC revealed no differences in epicuticular wax compositions from any treatments and controls (Fig. 12). Although

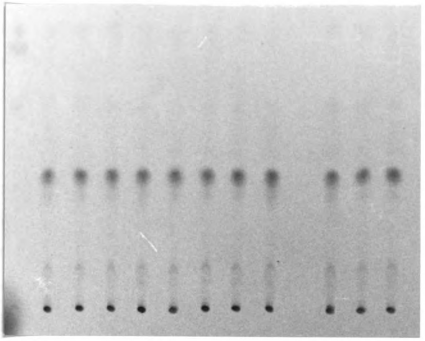

Fig. 12. Thin-layer chromatograms of epicuticular wax extracted at harvest from fruit grown under control, cellophane, and plastic shelter conditions (developed chloroform/ethyl acetate 7:3). Position (a) cabbage wax for reference, (b) control, (c) clear, (d) orange, (e) green, (f) red, (g) blue, (h) purple, (i) yellow, (j) unsheltered control, (k) plastic shelter kept on. (i) shelter removed.

many significant differences in russet formation and cuticular formation and cuticular component weights resulted from the cellophane treatments, no significant correlations were found between these data (Table 12).

Plastic shelter. Both plastic shelter treatments caused a significant increase in the % of fruit suitable for the fresh market as compared to unsheltered controls (Table 13). At harvest a small decrease in fruit quality was observed on the trees from which the plastic shelters were removed 40 days after petal fall. This treatment resulted in fewer fruit suitable for the fresh market than was present when the shelters were left on (68.9% vs. 82.7%, respectively). This difference appears to be due to the larger % of fruit in russet class 3 when the shelters were removed (26.2%) as compared to when the shelters were left on (11.9%).

Fruit grown under plastic shelters had significantly more epicuticular wax than controls 30 days after petal fall (Table 14). Control fruit had significantly higher cutin matrix weights than the treated fruit at this time. At harvest control fruit had significantly higher epicuticular wax and total membrane weights than fruit with the shelters left on. Both control fruit and fruit with the shelters removed had significantly greater total wax weights than fruit with the shelters left on.

TLC revealed no differences in the compositions of epicticular and cuticular waxes on both sampling dates from the shelter treatments and controls (Fig. 12). Correlations

Table 12. The effect of cellophane material on the correlation between cuticular components and russet severity of 'Golden Delicious' fruit at harvest.

Cuticle component	r value	F significance (%)
Epicuticular wax	.16867	35.6
Cuticular wax	.05570	76.2
Total wax	.01094	95.3
Cutin matrix	.06426	72.7
Total membrane	.09317	61.2

Table 13. The effect of clear polyethylene shelter on degree of russet on 'Golden Delicious' apple fruit at harvest.

	Russet evaluation						
	Fruit in each class (%)[z]					Suitable for	Index
Treatment	1	2	3	4	5	fresh market (%)[y]	value[w]
Control	0.0	0.3	9.8	40.8	49.0	0.3b[x]	4.30
Shelter removed	17.2	51.7	26.2	4.8	0.0	68.9a	2.20
Shelter kept on	20.2	62.5	11.9	4.2	1.2	82.7a	2.03

[z]1=no russet; 5=severe russet.

[y]% in classes 1 and 2.

[x]Mean separation by Duncan's multiple range test, 5% level.

[w]Summation of the number of fruit in each class times the class number, divided by the total number of fruit.

Table 14. The effect of clear polyethylene shelter on cuticular components of 'Golden Delicious' apple 30 days after petal fall and at harvest.

Sample date	Treatment	Cuticular component ($\mu g/cm^2$) Total membrane	Epicuticular wax	Cuticular wax	Total wax	Cutin matrix
30 days after petal fall	Control	1819.5	380.8b[z]	124.5	505.3	1314.2a
	Shelter	1802.4	407.0a	179.1	586.1	1216.3b
At harvest	Control	3050.7a	526.6a	483.3	1009.9a	2040.7
	Shelter removed	2995.6ab	522.9ab	492.1	1015.1a	1980.6
	Shelter kept on	2831.2b	502.2b	422.6	924.9b	1906.3

[z]Mean separation within columns for each sample date by Duncan's multiple range test, 5% level.

between cuticular component weights and russet severity were not significant. Previous results (10) showed that russeted cuticles have significantly less epicuticular wax than non-russeted. Thus it is not surprising that a significant positive correlation between epicuticular wax weight and russet reduction was detected when data from all treatments were combined.

Table 15. The effect of clear polyethylene shelter on the correlation between cuticular components and russet severity of 'Golden Delicious' fruit at harvest.

Cuticle component	r value	F significance (%)
Epicuticular wax	.56847	11.0
Cuticular wax	.42928	24.9
Total wax	.53521	13.8
Cutin matrix	.49727	17.3
Total membrane	.58116	10.1

Table 16. The effect of black polyethylene shade, clear polyethylene shelter, and colored cellophane on the correlation between cuticular components and russet severity of 'Golden Delicious' fruit at harvest.

Cuticle component	r value	F significance (%)
Epicuticular wax	.23354	7.5
Cuticular wax	.07516	57.2
Total wax	.06600	61.9
Cutin matrix	.01342	92.0
Total membrane	.03034	82.0

Discussion

Very low incident light (8.1% FS) was favorable for less russet formation, while somewhat higher incident light (32.0% FS) caused a lesser reduction in russet formation (Table 7). While no differences in cuticular components or waxes were found as a result of the shade treatments, it is possible that these treatments produced more elastic fruit cuticles as postulated by Verner (16), and thus were able to resist cuticle cracking and russet formation to some degree. It is also possible that in spite of the structural design used, temperature was increased, or rainfall was somewhat excluded underneath the black shade materials, thus confounding the shade effects.

Russet initiation is believed to occur during the first 30 days after petal fall (3). At the end of this period, fruit grown under clear plastic shelters had significantly more epicuticular wax than unsheltered control fruit (Table 14). However, cuticular wax and total wax from sheltered fruit and all waxes from fruit grown under black polyethylene shade material were not significantly different from waxes of control fruit at this time (Tables 8, 14). At harvest, sheltered fruit had less total wax than control fruit and fruit from which the shelters had been removed. This decrease in wax quantity may have been caused by the humid environment present underneath the plastic shelters throughout the growing season (15). The increase in russet on fruit from which the shelters had been removed (Table 13)

suggests that the severity of russet present at harvest can be affected by the environmental conditions present after the primary russet initiation period has passed.

Factors other than cuticular components may have been responsible for the differences in russet formation which occurred as a result of the various treatments. Rainfall or high humidity may cause pooling of water atop fruit, which presumably causes epidermal cells to absorb water by diffusion, and can lead to over-expansion and bursting of these cells, and thus to russet formation (15, 20). Rainfall striking very young fruit may cause sufficient physical abrasion to damage epidermal cells and induce russet formation as is known to occur by frost or deliberate mechanical injury (1,7,11,13,17). The data presented here also indicate the importance of rain for russet formation. Rain shelters resulted in very little fruit russet when kept over trees throughout the growing season, while removal of the shelters 40 days after petal fall resulted in more heavily russeted fruit (Table 17).

It seems doubtful that the russet reduction obtained under the cellophane treatments was due to the different light wavelengths applied. Clear cellophane produced significantly less russeted fruit (Table 10), yet this cellophane was shown to have no effect on the incident sunlight (Table 6, Fig. 10). The other most successful cellophane colors had very different spectra (Fig. 10), and very different light transmittance (Table 6). Red

cellophane admitted almost no light below 55 nm, and admitted almost all above 650 nm. Both blue and purple admitted at least half of the light below 550 nm, and while purple admitted almost all of the light above 650 nm, blue admitted less than half between and 550 and 675 nm. Clear cellophane transmitted 100% of the incident sunlight, purple transmitted 80.6%, blue transmitted 62.1%, and red transmitted only 37.5% of the sunlight.

The quantity and quality of light transmitted through the various cellophane colors had variable success in russet reduction, and various effects on cuticular component weights. However, no relationship between light quality and quantity and russet formation was established (Table 12). Since all the cellophane treatments did reduce fruit russet (Table 10), it is possible that the success was due to rain sheltering as was observed under the clear plastic shelters. The cellophane treatments were applied in such a manner as to allow as much rainfall penetration as possible, while the plastic shelters were applied so as to totally exclude rainfall. The markedly lower russet reduction which resulted from the cellophane treatments as compared to fruit grown under plastic shelters may have been a result of the cellophanes only partially blocking rain from the fruit.

Artificial shelters did alter the amount of cuticular components present 30 days after petal fall and at harvest (Tables 11,14). However, from the data presented it seems unlikely that either epicuticular wax, cuticular wax, or

membrane weight are associated with russet formation. No significant differences in cuticle component weights were present 30 days after petal fall as a result of black polyethylene shade treatments (Table 8), yet the shade treatments resulted in fruit with less russet (Table 7). Cuticle component weights of fruit grown under cellophanes and plastic shelters were not correlated with russet severity (Tables 12, 15). Only epicuticular wax weight was related (7.5% level) to russet severity when component weights from all treatments were combined (Table 16), and this relationship was shown to be due to the presence of less epicuticular wax on russeted areas of fruit at harvest (10).

Rainfall, or an interaction between rainfall and light may be responsible for the reduction of russet observed. The composition of the cutin matrix may be affected by environmental conditions and may be involved in russet formation (18). Cuticle elasticity may also be an important factor in russet formation in relation to cell growth and expansion, and it is being investigated at the present time.

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APPENDICES

APPENDIX A

Fruit epicuticular wax changes during storage.

Equipment and time limitations cause cuticular component analysis for many samples to be conducted over a period of several weeks. If changes in epicuticular wax weights occur during cold storage of sample fruit, wax weights determined on one date could not be correctly compared to those determined on other dates. Epicuticular waxes were extracted from mature 'Golden Delicious', Frazier Spur and 'Red Delicious' fruit after several periods of cold storage to test for any change .

Whole fruit of the three cultivars were measured for length and width with a caliper, and then epicuticular waxes were extracted. The fruit were immersed two separate times for 20 sec. in fresh redistilled chloroform. Five fruit selected randomly were used for each sample. Samples for each variety and length of storage were replicated 3 times. Extracts for each sample were combined and the solvent was removed under reduced pressure on a rotary evaporator. Epicuticular waxes were transferred to tared test tubes and then were allowed to air-dry to a constant weight. The combined wax weights for each 5 fruit samples were divided by the combined surface area of these fruit (see Appendix II) to give ug/cm^2 of fruit surfact.

The initial extraction was carried out on fruit kept in cold storage (1°C) and on fruit kept at room temperature

(20°C) for 1 month. The following extractions were performed on fruit kept in cold storage for 2 and 3 months.

No consistent change in epicuticular wax weight was observed over the 3 month period of cold storage (Table A1). Fruit stored for 1 month at room temperature had somewhat higher epicuticular wax weight than those kept at cold storage temperatures. Similar results have been observed on 'Cox' (E. A. Baker, et al. 1963. Bristol U. Agr. and Hort. Res. Sta. Ann. Rpt. for 1962:69-76) and 'Sturmer' apples (I.M. Morice and F.B. Shorland. 1973. J. Sci. Fd. Agr. 24: 1331-1339), while increases in epicuticular wax during storage have been observed on 'Bramley', 'Granny Smith' and 'Dougherty' apples.

Table A1. Changes in epicuticular wax for 'Golden Delicious', Frazier Spur and 'Red Delicious' fruit during storage at 1°C.

		Epicuticular wax ($\mu g/cm^2$)							
		Air Temp	Date Taken From Cold Storage			Date/Variety Averages			
Variety/Rep.[z]		11/10	11/18	12/5	1/3	11/10	11/8	12/5	1/3
Golden Del.	1	608.33	624.80	597.17	599.77	668.89	630.31	624.26	593.40
	2	656.86	672.62	651.34	623.46				
	3	741.48	593.50		556.97				
Frazier Sp.	1	673.14	468.07	583.85	614.39	641.45	453.35	592.85	584.35
	2	631.75	482.89	601.84	612.51				
	3	619.46	409.10		526.15				
Red Del.	1		680.68	891.26	735.32		673.12	841.70	747.51
	2		623.58	792.13	838.37				
	3		715.11		668.83				

[z]Each sample was composed of 5 mature fruit.

APPENDIX B

Geometric approximations of fruit surface areas.

The study of Galbreath (1975. New Zealand J. of Agr. Res. 19:543-544) concluded that simple geometric approximations of fruit surface areas were little different than estimates obtained by the use of more complex and tedious methods such as measuring the area of peelings. The present study was initiated in order to determine differences between surface area estimations obtained using two geometric approximations for both large and small apple fruits.

The length and width of fruit harvested at petal fall + 30 days and at maturity were measured with a caliper. Since apple fruit are neither perfect spheres nor perfect ellipses, variations were made in the geometric approximations used. Both the length and width measurements were used as the diameter component within the calculations. Thus five different formulas were used to estimate fruit surface areas as listed below.

Based on the surfact area of an ellipse:

1) 2 x diameter x profile length; width used as diameter.

2) 2 x diameter x profile length; length used as diameter.

Based on the surface area of a sphere:

3) Pi x $(\text{diameter})^2$; width used a diameter.

4) Pi x $(\text{diameter})^2$; length used as diameter.

5) Pi x $(\text{diameter})^2$; average of length and width used as diameter.

Profile lengths were calculated by: $\frac{a^2 + b^2}{2}$,

where a and b are semi-axes of the ellipse, here 1/2 length and 1/2 width.

The results showed little difference between the 5 geometric approximation formulas (Table B1 and B2). The use of lengths as diameters gave slightly larger surface area estimates than did the use of widths (formulas 2 and 4 vs. formulas 1 and 3). The use of length and width averages as diameters gave estimated surface areas of intermediate size (formula 5 vs. formulas 3 and 4).

An examination of the Standard Errors (S.E.) obtained with the different formulas showed that smaller values were obtained when formula 1 was used to estimate the surface area of large fruit. For this reason formula 1 may be preferred when geometric approximations of fruit surface areas are used.

Table B1. Large fruit surface areas (cm^2)[z] ± S.E. as estimated by five geometric approximation formulas.

	Formula Used				
Variety	1	2	3	4	5
Golden Delicious	141.04 ±3.54	141.68 ±5.01	140.84 ±4.39	142.12 ±6.60	141.23 ±3.86
Frazier Spur	131.45 ±5.27	134.40 ±5.31	130.02 ±5.33	135.91 ±5.44	132.93 ±5.26
Red Delicious	155.46 ±7.37	146.18 ±6.84	160.27 ±8.70	141.78 ±7.69	150.61 ±6.67

[z]Each variety sample was composed of 9 fruit, values given are the average for each sample.

Table B2. Small fruit surface areas (cm^2)[z] ± S.E. as estimated by five geometric approximation formulas.

	Formula Used				
Variety	1	2	3	4	5
Golden Delicious	17.28 ±0.62	19.26 ±0.79	16.32 ±0.58	20.27 ±0.90	18.23 ±0.70
Frazier Spur	19.95 ±0.60	22.32 ±0.59	18.80 ±0.69	23.53 ±0.70	21.08 ±0.58

[z]Each variety sample was composed of 12 fruit, values given are the average for each sample.

APPENDIX C

Ethylene production as an indicator of russet formation.

Russet formation occurs as a major disruption of the epidermal cells followed by periderm cell proliferation. Since russet development resembles a wound protection response, ethylene production may be higher within fruit which are developing severe russet than within fruit developing little or no russet. Ethylene production rates by fruit during the critical period of russet initiation (from petal fall to petal fall + 30 days) may be an indication of the severity which will be present at harvest.

In order to test for this relationship, ethylene production of fruit from cultivars known to have differing russet susceptibilities was measured and remaining fruit from the same branches were evaluated for russet severity at harvest. Additional measurements were made on fruit which had been deliberately wounded by the use of copper sulfate sprays and by U.V. light exposure.

A preliminary trial showed that ethylne was being produced by small fruit in sufficient quantities for detection by gas chromatography (Varian Aerograph Series 1400) using standard methods (activated 60/80 mesh alumina column, 80°C). Branches were selected on 3 trees each of 'Golden Delicious', Frazier Spur, Smoothee and 'Red Delicious'. For each ehtylene sample 3 fruit were placed into sealed 10 cc plastic syringes in the field. Once in the lab syringes were kept at a constant temperature (25°C) using a water bath. 1 cc gas

samples were extracted while 1 cc of the sample volume was pushed out. Three selected 'Golden Delicious' branches were sprayed with a copper sulfate solution at petal fall + 10 days. Fruit on 3 selected Frazier Spur branches were exposed to U.V. light for 5 mintes, and on 3 branches for 10 minutes at petal fall + 20 days.

Ethylene production rates over a 24-hour period were measured at petal fall + 11 days. (Table C1). The highest rates were produced by Smoothee and copper-treated fruit, while Frazier Spur and 'Red Delicious' fruit had the lowest ethylene production rates at all times. 'Golden Delicious' fruit had intermediate rates.

Table C1. Average ehtylene production at petal fall + 11 days (µg/kg/hr).

	Sample Time				
Sample	11 A.M.	1 P.M.	4 P.M.	7 P.M.	9 A.M.
Golden Del.	2.898	2.016	1.147	0.925	0.741
Copper	3.508	3.008	2.173	1.501	1.414
Smoothee	3.664	2.205	1.216	0.916	0.556
Red Del.	1.263	0.768	0.469	0.398	0.407
Frazier Spur	1.077	1.115	0.750	0.585	0.577

Ethylene production rates 3 hours after sample collection were measured at petal fall + 21 days (Table C2). The highest rates were present in U.V. treated fruit (10 min. exposure), 'Golden Delicious' and copper-treated fruit. Smoothee and 'Red Delicious' fruit had the lowest ethylene production rates, while those of Frazier Spur and U.V. treated (5 min. exposure) had intermediate rates.

Smoothee fruit had very little russet at maturity, while Frazier Spur produced few clean fruit and 'Golden Delicious' had fruit of intermediate quality (Table C3). No fruit from the copper treatment were suitable for the fresh market due to extensive russet formation. While the U.V. light exposure was seen to produce severe localized russet, nearby control fruit were also severely russeted (naturally). For this reason no difference in russet ratings was apparent between the U.V. treatments and controls.

The copper treatment gave severe russet, yet no real difference in ethylene production rates was seen between these fruit and untreated 'Golden Delicious' fruit. A comparison of the ethylene production rates of Smoothee, 'Golden Delicious' and Frazier Spur on both dates gave no indication of the large differences in russet severity which existed between the cultivars at harvest. This evidence suggests that no predictive ability exists from early season ethylene production rates for the severity of russet at harvest. However, it must be realized that no russet evaluation was possible of the fruit actually sampled for ethylene production. So it is not known if the measured fruit actually differed in russet severity. Since branches from which ethylene samples were taken did develop fruit with differences in russet severity, it seems reasonable to expect that sampled fruit would have differed in both russet severity and ethylene production if a relationship did exist between the two.

Table C2. Average ethylene production at petal fall + 21 days (µl/kg/hr).

Sample	
Golden Delicious	1.133
Copper	1.078
Smoothee	0.639
Red Delicious	0.605
Frazier Spur	0.842
U.V. - 5 min.	0.846
U.V. - 10 min.	1.183

Table C3. Mature fruit russet evaluations from branches earlier sampled for ethylene production.

Treatment	# of Fruit	% of Fruit in Each Russet Class[z] 1	2	3	4	5	% Fruit Suitable for fresh Market
Copper	46	0.0	0.0	0.0	8.7	91.3	0.0
Golden Del. (also copper cont.)	60	15.0	46.7	38.3	0.0	0.0	61.7
Smoothee	59	16.9	57.6	22.0	1.7	1.7	74.5
Frazier Spur	229	1.7	17.0	51.5	26.7	3.1	18.7
U.V. - 5 min.	6	0.0	0.0	0.0	1.7	83.3	0.0
U.V. - 5 min. (cont.)	8	0.0	0.0	0.0	50.0	50.0	0.0
U.V. 10 min.	9	0.0	0.0	0.0	33.3	66.7	0.0
U.V. 10 min.	16	0.0	0.0	37.5	37.5	25.0	0.0

[z]1= no russet; 5= severe russet.

APPENDIX D

Fruit Growth Rates.

Ten clusters of 3 fruit each were selected over 3 trees of 'Golden Delicious', Frazier Spur, Smoothee and 'Red Delicious'. The diameters of these fruit were measured throughout the first month after petal fall at 7 a.m. and 2 p.m. on selected days, and at 2 p.m. only on other days. Fruit drop reduced the number of fruit being measured to about 10/ variety by 30 days after petal fall.

The fruit diameters were measured using a photographic method. A piece of white cardboard with a ruler attached was held directly behind each cluster, which was then photographed (35 mm, Tri-X film). The film negatives were developed using standard procedures. The negatives were projected using a film enlarger. A ruler was used to measure the magnified (projected) size of the photographed ruler increments, and to measure the magnified diameter of the photographed fruit. The proportion of the magnified ruler increments to the actual ruler increments was used to calculate the actual fruit diameter from the magnified fruit diameter measured.

Results. The fruit growth rates observed were very similar for the different apple varieties (Fig. D1). Diurnal fluctuations in fruit size were detected by the photographic method on all days when 2 measurements were taken: 10, 15, 24 and 29 days after petal fall. However, no significant differences were found between varieties in the % change in diameter on any given day and for all dates combined.

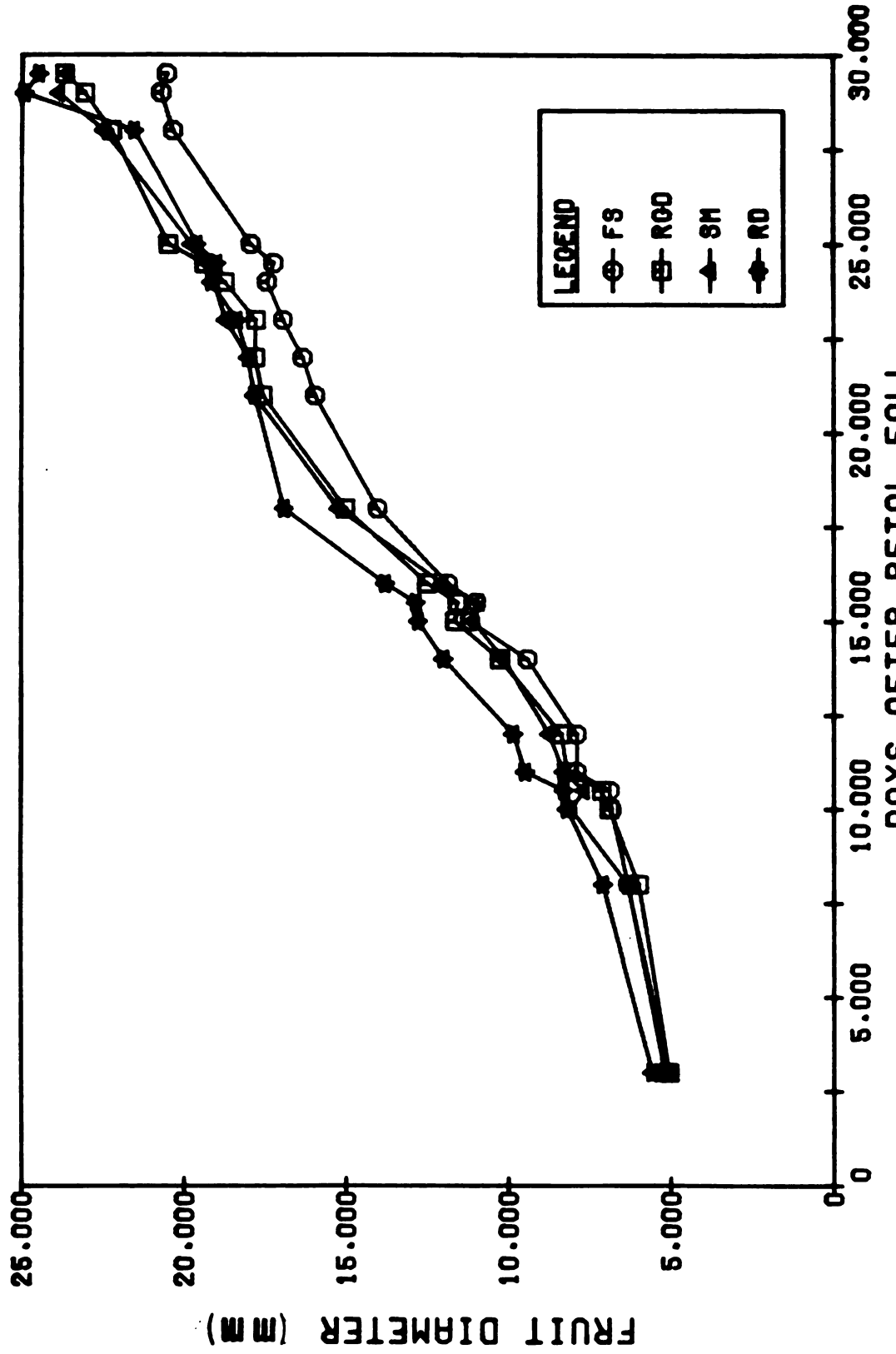

Fig. D1. Fruit growth rates of 4 apple strains as measured by the change in fruit diameter.

Correlations between the average % change in diameter vs. the average % of fruit suitable for the fresh market were not significant.

Neither the rate of fruit growth, nor the amount of diurnal size fluctuation appeared to be related to the different russet susceptibilities of the 4 apple varieties. However, since only 9-11 fruit/variety were measured through petal fall + 30 days, it is possible that not enough fruit were measured to detect possible differences. Since individual trees and branches differ in russet susceptibility, it is also possible that the fruit measured were not representative of russeting and non-russeting fruit.

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