AN ABSTRACT OF THE THESIS OF

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Title: STRUCTURE OF THE ROYAL ANNE CHERRY CUTICLE WITH SPECIAL REFERENCE TO CUTICULAR PENETRATION

Abstract approved: Dr. Robert F. Cain

The structure of the Royal Anne cherry cuticle (Prunus avium L.) and its penetration by a SO_2-calcium bisulfite brine was determined. The structural features of the cuticle are interpreted in the light of its possible significant to cuticular penetration.

The morphology of the cuticle was determined by standard histological and histochemical techniques. The surface structure of the cuticle was studied with a scanning electron microscope. Enzymatically isolated cuticles were used to evaluate the effect of the cuticle on the penetration of the various components of brine.

The surface of the cuticle was found to have a smooth to granular sheet or layer of surface wax which when removed revealed a porous sponge-like surface. The surface wax was found to be intermittently birefringent which increased as the fruit matured. Ectodesmata (MP) were found to occur over anticlinal walls and in guard cells on both sides of the fruit with more on the side opposite

the suture. Both sides were stomatous with more occurring on the suture side. Secondary bleaching was found to alter the structure and permeability of the cuticle.

Removing the wax was found to increase percent weight loss as well as increasing cuticular penetration. Ion penetration was found to be generally greater from the outside to the inside than the inside to the outside surface and was greater from the side opposite the suture than the suture side. Potassium from potassium bitartrate was found to penetrate rapidly while SO_2, Ca^{++}, and H^+ were delayed.

It was concluded from this study that calcium penetration entered by a different route than SO_2; the major penetrating species of SO_2 in water and brine would seem to be molecular SO_2 and bisulfite ion; stomatal pores were not the sites of entry; ectodesmata were probably the sites for polar entry; the guard cells were more important for potassium penetration while ectodesmata in astomatous regions were more important for SO_2 penetration; and one of the major factors involved in penetration of brine is the waxy layer on the cuticle. Causes, preventative measures and recommendations for the structural defect known as "solution pockets" are discussed in light of the structure and penetration data.

Structure of the Royal Anne Cherry Cuticle with Special Reference to Cuticular Penetration

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INTRODUCTION

The production of sweet cherries in the United States was 215,000 tons in 1969. Of this production, 46 percent was preserved as "brined" cherries, 39 percent sold fresh and 16 percent canned. The principal producing states are Oregon, Washington, California, and Michigan. In Oregon, 60 percent of the average annual production of 34,000 tons are brined.

The Royal Anne variety of cherry, sometimes known as Napoleon, is the principle cherry of the brined trade. It is a lightly blushed, firm fleshed variety which is highly adapted to the process called brining. In this process, the fresh cherries are cured in a "brine" consisting of a sulfur dioxide-calcium bisulfite solution. Normally, a water brine consisting of 1.25 percent SO_2 will be used with the addition of 0.8-1.0 percent calcium in the form of $Ca(OH)_2$ or $CaCl_2$. The fresh cherries are kept in this brine for a period of curing during which the cherries are bleached of their anthocyanin pigments, the calcium binds with the pectin and hardens the fruit and the SO_2 preserves the fruit against attack of microorganisms. The result of the brining process is a light yellow (carotenoid pigmented)

firm fruit, devoid of its initial red color and flavor, which can subsequently be manufactured into the maraschino cocktail cherry, the cherries for fruit cocktail, glacé cherries or colored cherries for the bakery, confection or ice cream outlets.

As with all agricultural products, certain "defects" which detract from the grade and quality of brined cherries occasionally appear. One of their defects, known as "solution pockets" has occurred sporadically in recent years. As Cameron and Westwood (1968) and Beavers et al. (1971) noted, the effect of the pockets is to weaken the cherry structure so that, when pitted, the cherry flesh tears and and the fruit collapses rendering it unfit for further manufacture.

A number of theories with respect to the development of solution pockets have been proposed. Attempts have been made, with only partial success, to relate the occurrence of solution pockets to: the effect of various sprays or fertilizers; bruising of the cherries; the various types and amounts of pectic constituents; a change in osmotic concentration and with the movement of water within the cherry tissues.

The cuticular covering of leaves has long been recognized as the first barrier to penetration of its surface. Thus, the cuticle of the cherry would be expected to have an effect on the penetration of brine. However, while many studies have been made and reviewed on leaf cuticle thickness, waxiness, structure and penetration by chemicals

(Van Overbeek, 1956; Foy, 1964; Yamada et al, 1964b, 1965a, b; Franke, 1967; Norris and Bukovac, 1968; Martin and Juniper, 1970), very little attention has been given to the structure of the cherry cuticle or its permeability to the exchange of solutes and solvents. The previous studies were made on intact cherries which only permits indirect approaches to the mechanisms of cuticular penetration. Therefore, the object of this research was to elucidate the structure of the Royal Anne cherry cuticle and the role of its cuticular membrane in the exchange of SO_2, Ca^{++}, and H^+ ions as the tissues are killed by the brining solution. Enzymatically isolated cuticular membranes were used for the penetration studies and compared to whole cherries in order to get more direct information on cuticular penetration. Information derived from this study may help to give a better understanding of the mechanism by which the quality defect known as "solution pockets" are formed.

REVIEW OF LITERATURE

Definition of Solution Pocket

A solution pocket has been found to be an internal rupture of the cherry flesh, which may extend from the pit to the epidermal cells and exhibits itself as a translucent pocket (Cameron, 1966; Cameron and Westwood, 1968; Beavers et al., 1971) (Figure 1). The cuticle of the cherry is not broken as is the case in "brine cracks." This internal pocket contains a solution of ruptured cell contents and brine solution (Cameron and Westwood, 1968; Beavers et al., 1971). In some instances this pocket may fill with gas, in which case it is called a "gas pocket." Beavers et al. (1969) found that the gas in some solution pockets was carbon dioxide.

Solution pockets are most commonly found at the suture side of the cherry, and its size may vary from large to very small (Cameron, 1966; Cameron and Westwood, 1968; Beavers et al., 1971).

Theories of Solution Pocket Formation

A number of theories have been proposed for the development of solution pockets. Some of the theories are as follows:

1. Effect of sprays or fertilizers.
2. Bruising of the cherries.
3. Various types and amounts of pectic constituents.

Figure 1. Normal and solution pocketed brined Royal Anne cherry.

1-1. Normal brined Royal Anne cherry.
1-2. Solution pocket in brined Royal Anne cherry.
1-3. Cross-section of normal brined Royal Anne cherry.
1-4. Cross-section of solution pocket in brined Royal Anne cherry.


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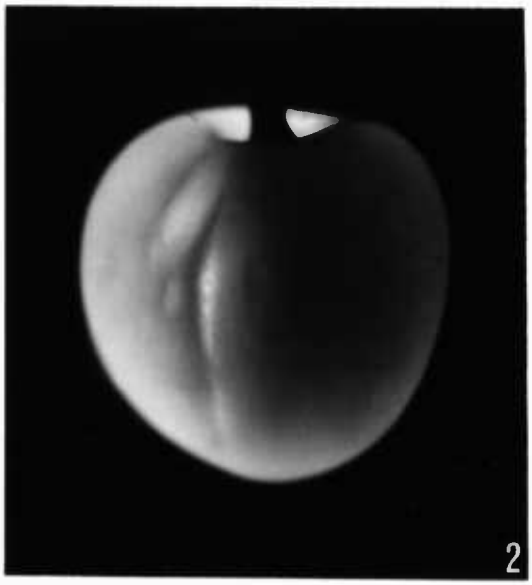
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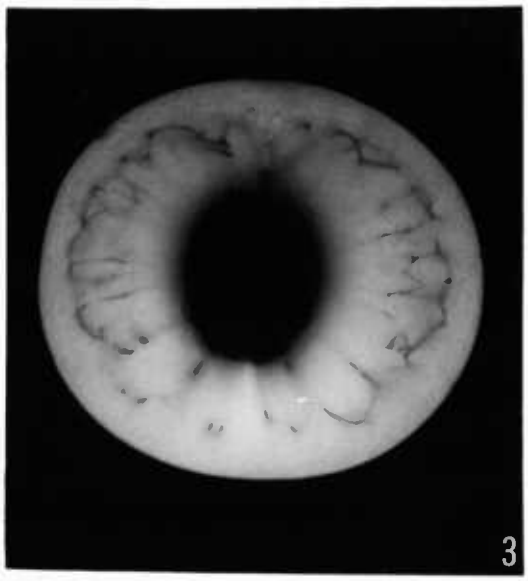
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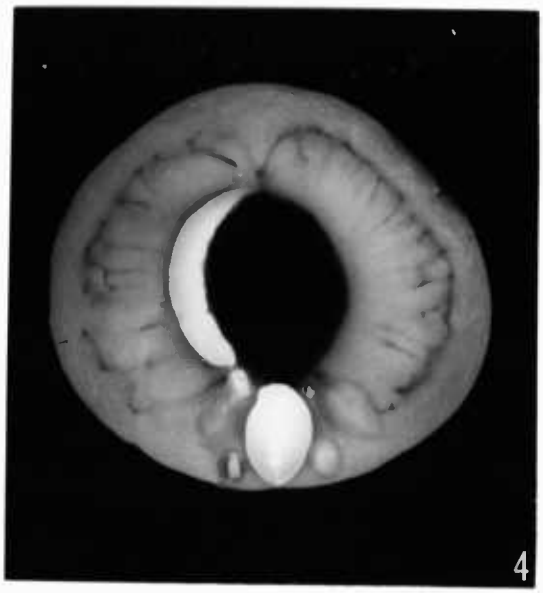
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4. Changes in osmotic concentration.

5. Movement of water within the cherry tissue.

However, the attempts that have been made to prove these theories have met with only partial success.

As solution pocket occurrence has only been noticed in the past 15 years, it was hypothesized that the use of new sprays and fertilizers might cause this defect. However, Cameron (1966) and Cameron and Westwood (1968) found that there was no correlation between incidence of solution pockets and topographic location, soil type, various sprays or high nitrogen fertilizer levels.

It was thought that bruising of the fruit might be responsible for solution pocket formation. Bruising of the cherry flesh might cause a localized increase in soluble solids and an osmotic differential, which would cause a localized imbibition of water resulting in a solution pocket. However, Cain (1971) in 1968 found that even though the localized bruised area had a relatively high soluble solids reading, localized solution pockets (within the bruised area) were not observed. He attributed this to the 48 hour holding period between bruising and brining, which allowed partial desiccation of the fruit thus decreasing the turgor pressure. The cherries in this flaccid condition allowed the brine-water uptake without the formation of solution pockets.

Another theory of solution pocket formation was that they were formed by various types and amounts of pectin or pectic constituents.

This was indicated due to the swelling-power of pectin upon imbibition of water (Doesburg, 1965) which might be a factor in cell rupture and solution pocket formation. Wilson (1969) attempted to show this, but failed to find any correlations between solution pockets and the various pectin fractions from brine equilibrated samples.

The current and best supported theory of solution pocket formation is a combination of osmotic concentration and water movement. It is believed that solution pockets are related to a rapid increase in turgor pressure, within the fruit, from the movement of water into living cells with a high sugar content (Cameron, 1966; Cain and Smith, 1968; Camerón and Westwood, 1968; Beavers et al., 1971). The skin does not crack in this case, as in rain cracked (checked) cherries (Westwood and Bjornstad, 1970), as the epidermal cells have been killed by the penetration of SO_2 (H_2SO_3). The data supporting this theory are as follows:

1. A positive correlation was found between solution pockets and increasing maturity (i.e., as maturity increased, so did incidence of solution pockets)(Cameron, 1966; Cain and Smith, 1968; Cameron and Westwood, 1968; Beavers et al., 1971).
2. A strong correlation was found between solution pockets and the water-sugar content of the fruit (i.e., high sugar, high incidence of solution pockets) (Cameron and Westwood, 1968).

3. A high degree of correlation between the occurrence of solution pockets and desiccation has been found by a number of investigators as follows:

   a. A high degree of correlation (P = .01) between solution pockets and delay prior to brining (a delay in brining of at least 12 hours) significantly reduced solution pockets due to loss of turgor (Cain and Smith, 1968; Beavers et al., 1971).

   b. Immediate brining increased solution pocket formation in Royal Anne cherries (Cain and Smith, 1968) presumably due to high turgor pressure.

   c. Correlation between solution pockets and air temperature (i.e., the cooler the air temperature at harvest, the higher the incidence of solution pockets) (Cameron, 1966; Cameron and Westwood, 1968).

   d. Pre-brining the cherries in a sugar, salt (NaCl), salts ($CaCl_2$ + NaCl) or alcohol solutions, in order to desiccate the tissue, was found to give a marked reduction in solution pockets (Cameron, 1966; Cain and Smith, 1968; Cameron and Westwood, 1968; Beavers et al., 1971).

General Constitution of the Cuticular Layer

There has been no previous work on the cuticular layer of Royal

Anne cherries. The following pertinent information has been derived from that reported with other plant fruits and leaves.

The cuticle has been found to cover all aerial parts of the plant, including leaves, stems, fruits, petals, barks of trees and ordinary and glandular tricomes (Esau, 1965; Prasad et al., 1967; Martin and Juniper, 1970). The cuticle varies in thickness and lies on top of, and merges with, the epidermal cell walls of the plant. This is even true of stomata, as suberisation or cutinisation of air-exposed surfaces, results in a covering of the substomatal cavities (Scott et al., 1948; Scott, 1950; Jyung and Wittwer, 1965; Norris and Bukovac, 1968; Hull, 1970; Martin and Juniper, 1970).

The cuticle is multilayered and is composed of (1) a cuticular membrane (denoting the tissue lying above the pectinaceous layer), (2) the cuticle proper (denoting the outer layer of the membrane composed chiefly of cutin which may have wax on its surface), and (3) the cuticular layer (denoting the inner layer of the membrane composed of cutin incrusted on cellulose) as defined by Martin and Juniper (1970).

In leaves (Figure 2) the simplified structure of the surface is typified by a layer of pectin (P) continuous with the anticlinal walls of the epidermal cells. Outside of this is the cutinised layer (CL) in which pectin and cellulose are bound in cutin. Then there is assumed to be a pure layer of cutin (C) with a thin layer of wax (W) sometimes

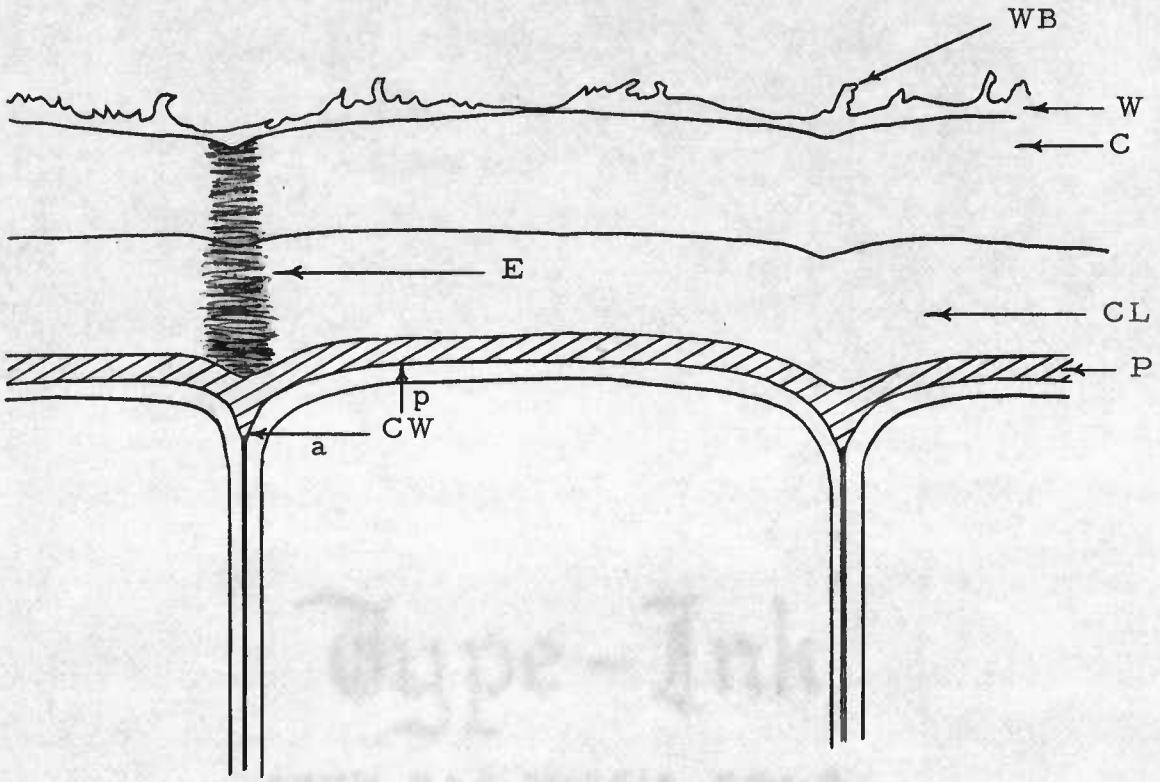


Figure 2. Diagramatic simplified structure of an upper leaf cuticle. C = cutin; CL = cutinised layer; CW = cell wall epidermal cells (a = anticlinal, p = periclinal); E = ectodesmata; P = pectin; W = wax; WB = waxy bloom (modified after Eglinton and Hamilton, 1967; Franke, 1967; Kolattukudy, 1970a).

occurring on top of this as well as in the cutinised layer (Van Overbeek, 1956; Eglinton and Hamilton, 1967; Kolattukudy, 1970b; Martin and Juniper, 1970). There may also be fine structures that extend through the cuticle, but do not perforate it, to the lamella of the epidermal cells which are called ectodesmata (E) (Franke, 1967).

Most fruits have a well-developed cuticle that forms and thickens as the fruit matures (apples), while in others (plums, grapes) the waxy bloom is not developed until the fruit reaches maturity (Martin and Juniper, 1970; Mazliak, 1970).

Waxes

The wax has been found to help preserve the water balance of the plant as well as minimizing mechanical damage to leaf cells and acts as a defense against pathogenic microorganisms and insect attack (Eglinton and Hamilton, 1967; Kolattukudy, 1970a). Martin (1964) gives a number of observations of the wax being toxic to pathogens. Cuticular wax has been shown, by many workers, to play an important role in conserving water by regulating cuticular transpiration (Denna, 1970; Martin and Juniper, 1970). Removal of the wax is reported to cause a several fold increase in water loss (Martin and Stott, 1957; Hall and Jones, 1961; Horrocks, 1964; Hall, 1966; Grncarevic and Radler, 1967).

On leaves, wax is synthesized early and continues to be synthesized during leaf expansion (Juniper, 1960; Hallam, 1970; Martin and Juniper, 1970). In fruits the wax is synthesized after the initial fruit

formation and the quantity of total wax increases during growth following a S-shaped curve (Mazliak, 1970). During this growing period, saturated fatty acids are preferentially synthesize with an accumulation of unsaturated fatty acids on maturation (Mazliak, 1970).

The wax evidently passes through the cuticle (Esau, 1965) and is deposited on the surface of the cuticle. Two views are held of the pathway of wax deposition: (1) that the lipoid droplets move through the epidermal cell wall to the outer surface by diffusion, and (2) that wax channels exist that allow the passage of the wax (Hall, 1967; Martin and Juniper, 1970).

Kolattukudy (1965) found that the site of synthesis or biosynthetic pathway of surface wax differed from that of the internal lipids in Brassica oleracea. The biosynthesis of alkanes from fatty acids is believed to be either an elongation followed by decarboxylation or by head to head condensation between two biochemically dissimilar fatty acids followed by specific decarboxylation of one of them (Kolattukudy, 1970a, b). The biosynthesis of other wax components is also discussed in the above articles.

The surface wax deposits on plant leaves and fruit have been examined by many investigators using the electron microscope with different techniques; and various forms of wax such as granules, rods, nets, plates have been found (Mueller et al., 1954; Schieferstein and Loomis; 1956, 1959; Eglinton and Hamilton, 1967; Martin and Juniper, 1970).

Wax effects the wettability of the plant because it prevents the contact of the liquid with the plant surface. Smooth surfaces are easy to wet while rough surfaces are harder to wet because the liquid is held away from the plant surface (Silva Fernandes, 1964, 1965a; Martin and Juniper, 1970; Rentschler, 1971). The wettability of the surface has been found to depend not only on structure, but on distribution and the chemical composition of the wax (Silva Fernandes, 1964, 1965a; Hall, 1966; Holloway, 1969; Martin and Juniper, 1970; Rentschler, 1971). Thus, the surface wax is of considerable interest in connection with agricultural sprays.

The chemical composition of the cuticular wax has been reviewed by a number of investigators: Eglinton and Hamilton (1967), Mazliak (1968), Kolattukudy (1970a, b), Martin and Juniper (1970). The most common compounds of plant wax are hydrocarbons, wax esters, and free fatty alcohols and acids. Aldehydes, diols, ketones, secondary alcohols and terpines are also found.

The hydrocarbon fraction usually represents a mixture of n-alkanes from C_{25} to C_{35} with the odd numbered chains predominating. Ninety percent of the hydrocarbon fraction is usually C_{29} to C_{31}; however, longer and shorter chains have been found.

The wax esters are usually composed of n-alkanoic acids and n-alkan-1-ols with the majority being even numbered chains in the range of C_{12} to C_{32}. The free fatty acids and fatty alcohols are

usually similar to the wax esters while ketones are more similar to the alkanes.

Triterpine compounds, such as ursolic acid and oleanolic acid ($C_{30}H_{48}O_3$), occur as major components of some fruits as apple (Huelin and Gallop, 1951) and grape (Radler, 1965; Radler and Horn, 1965). Markley and Sando (1937) found that n-nonacosane ($C_{29}H_{60}$), ursolic acid ($C_{30}H_{48}O_3$) and fatty acids were present in the skins of Bing cherries (Prunus avium L., var. Bing).

Grncarevic and Radler (1967) found in model studies that the alcohol, hydrocarbon and aldehyde fractions are the active components in the grape cuticle which prevent water loss. Holloway (1969) in examining the water repellent properties of various leaf wax constituents found that the alkanes are the most hydrophobic, but esters, ketones and secondary alcohols were almost as unwettable. Triterpenoids and sterols were also found to be hydrophobic, which he attributes to a predominantly hydrocarbon surface. However, Grncarevic and Radler (1967) found that the triterpine oleanolic acid from grape wax did not effect evaporation in their model system.

Cutin

Cutin is believed to consist of a network of polymerized cross esterified hydroxy fatty acids (Eglinton and Hamilton, 1967; Kolattukudy, 1970a, b; c). The study of the exact structure is difficult

to accomplish, as depolymerization (usually by saponification) must be carried out to determine its constituents (Hulme, 1970; Kolattukudy, 1970b).

Baker and Holloway (1970) found that the most abundant constituent acid of 24 angiosperm leaves and fruits was 10, 16-dihydroxyhexadecanoic acid with 9, 10, 18-trihydroxyoctadecanoic acid occurring frequently and 16-hydroxyhexadecanoic acid less frequently. They also found mono- basic acids which they believe are important constituents of cutin. A wide variation occurred between species, within species, and between leaves and fruits of the same species (Baker and Holloway, 1970; Holloway and Baker, 1970).

The cutin is believed to migrate to the surface in the form of procutin (Crafts and Foy, 1962; Foy, 1964; Franke, 1967; Robertson and Kirkwood, 1969; Salisbury and Ross, 1969; Kolattukudy, 1970a) where it is oxidized and polymerized to form a spongelike framework (Baker and Martin, 1963; Leopold, 1964; Franke, 1967; Baker, 1970) which on aging becomes "varnish-like" (Foy, 1964). The molecules are believed to orient themselves with their polar groups in the water phase, and their non-polar hydrocarbon chains toward the outside (Foy, 1964; Foy et al., 1967). Thus, cutin has both polar and apolar properties. The hydrophilic groups would be -OH and -COOH groups and the lipophilic being $-CH_2-$ and $-CH_3$ groups (Foy, 1964; Franke, 1967). The presence of the hydrophilic groups explains the ability of

the cutin to swell in water and permit transpiration (Crafts and Foy, 1962; Foy et al., 1967; Franke, 1967). The cutin has been found to possess an overall negative charge which is believed to result in selective cation permeability (Foy, 1964; Middleton and Sanderson, 1965; Franke, 1967).

Once the cutin is formed, it is insoluble in polar and apolar solvents, resistant to cold strong acids, to natural decay, and treatment with potassium hydroxide leaves an unsaponifiable residue (Crafts and Foy, 1964; Esau, 1965; Martin and Juniper, 1970).

While the structure of cutin is not completely elucidated, the biosynthesis has been merely speculative. Heinen and Brand (1963) proposed a model that included three oxidases: steric, oleic acid oxidases and lipoxidase. In this model, cutin is supposed to be built of a few hydroxy-C_{18} fatty acids combined by ester and peroxide bridges. This would then polymerize to form a three dimensional network by further linkages.

Crisp (1966) found ester, alkylperoxide and ether linkages in the cutin from Agave americana L. in an estimated ratio of 7:2:0.12. He also found that ultraviolet radiation enhanced the polymerization of procutin into cutin, especially through peroxide linkages. Thus, instead of an enzymatic polymerization of cutin (Heinen and Brand, 1963) this would indicate that an autoxidative polymerization process stimulated by ultraviolet radiation plays a role in cutin synthesis.

Recently, Kolattukudy (1970d) found that the experimental sequence of cutin biosynthesis in Vicia faba (broad bean) was palmitic acid ⟶ 16-hydroxy palmitic acid ⟶ 10,16-dihydroxy palmitic acid ⟶ cutin. Kolattukudy (1970c) also found that cutin synthesis is under strict physiological control, in that the cutin is synthesized most rapidly in rapidly expanding leaves of Vicia faba, and that cutin synthesis virtually stops when the leaf reaches full maturity. He believes that either the synthesis of cutin synthesizing enzymes or their activity is blocked as the leaf reaches full expansion. As hydroxylation reactions seem to be the key to cutin synthesis, the hydroxylation enzymes and possibly the esterifying enzymes may be involved in the control of cutin synthesis (Kolattukudy, 1970c).

Kolattukudy (1970b) observed that the precursors of the cutin acids could be from the typical fatty acid complement of plants, such as palmitic, stearic, oleic, and linoleic acids, due to the structure of the hydroxy acids found in cutin. It was also observed that the hydroxy acids have ω-hydroxy groups which would seem to indicate the involvement of an ω-oxidation in the cutin synthesis (Eglinton and Hunneman, 1968; Kolattukudy, 1970b).

Suberin

Suberin and cutin have been long confused in the literature. Suberin is the substance that appears to give cork tissue or periderm

its special properties, particularly its ability to be impermeable to water (Esau, 1965; Martin and Juniper, 1970). It is chemically related to cutin in that upon hydrolysis of suberin, similar hydroxy fatty and fatty acids are obtained, but some of the acids characteristic of suberin are of greater chain length than those of cutin (Martin and Juniper, 1970). Suberin also has phellonic acid (22-hydroxydocosanoic acid ($CH_2OH(CH_2)_{20}$ COOH) which has not been detected in the cutin examined (Baker and Holloway, 1970; Martin and Juniper, 1970).

Ectodesmata

Ectodesmata are believed to be fine structures that extend through the cuticle, but do not perforate the surface, to the lamella of epidermal cells (Franke, 1967, 1969). Franke (1969) concluded from comparisons of ion binding sites and ectodesmata locations that the binding sites in the cuticle lie on top of ectodesmata and that they together form a combined pathway of favored penetration or excretion of aqueous solutions.

Ectodesmata have been found predominantly in special sites, such as along anticlinal walls, in some hairs, basal cells of hairs, epidermal cells surrounding hairs, above, beneath and on both sides of veins and in guard cells (Franke, 1961a, 1967).

Schönherr and Bukovac (1970) found that the ectodesmata-like

structures or mercury precipitates (MP) were not specific cell-wall structures. The distribution pattern was found to be dependent upon the permeability of the cuticle rather than the cell wall. Thus, the mercury precipitates were a result of existing sites in the cuticle permeable to mercury chloride (and undoubtedly other polar compounds) and not due to localized reducing substances in specific structures.

Cuticular Penetration

Cuticular penetration has been found to be influenced by such parameters as molecular structure and size of the penetrant, solvent and additives used in its formulation, type of spray (emulsion, solution, suspension), pH, vapor pressure, type and concentration of surfactant, leaf waxiness, temperature, humidity, illumination, and water stress among others (Van Overbeek, 1956; Currier and Dybing, 1959; Juniper, 1960; Hughes and Freed, 1961; Crafts and Foy, 1962; Darlington and Circules, 1963; Silva Fernandes, 1965a; Prasad et al., 1967; Hallam, 1970; Martin and Juniper, 1970; Still et al., 1970; Lopez, 1971; Rentschler, 1971).

The cuticle is the primary barrier to the penetration of polar compounds (Van Overbeek, 1956; Currier and Dybing, 1959; Crafts and Foy, 1962; Norris and Bukovac, 1969). Penetration through this barrier may be classified as either cuticular and/or stomatal. The

relative importance of either as routes of entry has been debated at length.

The abaxial (lower) surface of a leaf is usually more penetrable than the adaxial (upper) and this is usually accounted for by the larger number of stomata and the thinner cuticle on the abaxial surface (Currier and Dybing, 1959; Franke, 1961a; Foy, 1964; Horrocks, 1964; Franke, 1967; Hull, 1970). However, even though the stomata look like obvious "holes" through the cuticle for passage of aqueous solutions, it has been found that surface tension forces prevent infiltration of water (Martin and Juniper, 1970). Middleton and Sanderson (1965) also discounted stomatal entry of solutions.

Currier and Dybing (1959) found that fluorochrome dye penetrated the stomata of leaves provided an effective wetting agent was incorporated into the solution. In order for the stomata to become routes of penetration, liquids of low surface tension, oils or solutions with surfactants must be used. This would allow the solutions to enter the stomatal cavity. However, once there the solution still has to penetrate the internal cuticle of the stomatal chamber (Martin and Juniper, 1970).

More than likely, the important role of stomata will be to provide preferential sites of entry through the surfaces of their guard cells and subsidiary cells (Franke, 1967; Martin and Juniper, 1970).

Penetration of ions and organic compounds through the cuticle

has been found to follow a mathematical equation of diffusion (Darlington and Circules, 1963; Jyung and Wittwer, 1965; Franke, 1967; Bayer et al., 1968). Darlington and Circules (1963) found that penetration was directly proportional to concentration, and increased with increasing temperature and with the higher lipophilic character of the applied compounds.

However, while diffusion is the main process for penetration of many ions and organic compounds, urea penetrates much faster than can be accounted for by diffusion (Yamada et al., 1965a, b). These investigators found the penetration of urea is 10 to 20 times greater than ions and is independent of concentration. The increase in permeability for urea also occurs when urea is applied with other compounds such as Rb^+ and Cl^- (Yamada et al., 1965a). Yamada et al. (1965b) proposed that the urea penetration of the cuticle was a facilitated diffusion process, as penetration of urea through a dialyzing membrane corresponded to a first order reaction. They concluded from this and the finding of nonradioactive oil droplets in their maleic hydrazide penetration experiment that the urea or maleic hydrazide altered the ester, ether or diether bonds in the structure of cutin.

The polarity of the cuticle and of the penetrant is an important factor in the penetration of the cuticle (Martin and Juniper, 1970). The undissociated water soluble molecule has been found to penetrate

the cuticle more readily than the dissociated ion, perhaps because they partition more readily into the lipophilic cuticular membranes (Van Overbeek, 1956; Leopold, 1964; Bukovac and Norris, 1966; Hull, 1970). However, there may still be penetration of ions at pH levels that would cause dissociation due to dissociation of the cuticular components (Van Overbeek, 1956; Leopold, 1964; Robertson and Kirkwood, 1969). Simon and Beevers (1952) found that at the pk value of an organic acid or lower, the undissociated acid would account for the activity, but at pH levels higher than the pk value the ions would contribute to the biological activity. Jyung and Wittwer (1965) noted that it is possible to enhance penetration at higher pH's by adding certain inorganic ions. Bukovac and Norris (1966) found that the half dissociation point for the free carboxyl groups in the cuticular surface of pear leaves was in a pH range of 2.8 to 3.2. Crowdy (1959) also found that cutin had an acidic pk below pH 5. Thus at low pH values the acids would tend to be undissociated and more permeable to anions than at higher pH levels where the acids dissociate and become more permeable to cations.

A mechanism of cation exchange has been postulated for the preferential penetration of cations over anions (Franke, 1967; Martin and Juniper, 1970). Cutin and cellulose are weak cation exchangers with the pectin layer acting as either a polyelectrolyte or an ion exchanger (Martin and Juniper, 1970). Thus, as mentioned

earlier, at low pH the exchanger is in its undissociated form and cations would not be accepted. However, as the pH was raised, the carboxylic groups not bound in linkages would ionize, and the hydrogen ions would be exchangeable with cations. However, the presence of the waxy layer would hinder this process. The above theory would seem to fit into the proposed gradient of polarity (Crafts and Foy, 1962; Foy, 1964) with the more polar groups on the inside of the cuticle and the hydrophobic groups on the outside of the cuticle.

Yamada et al. (1964b) found that the penetration of Ca^{++}, Rb^{+}, $SO_4^{=}$ and Cl^{-} was greater from the outer surface of enzymatically isolated tomato fruit and green onion leaf cuticles than from the inner surface. The differences were found to be greater through the astomatous tomato fruit cuticle than through the stomatous green onion leaf cuticle. Yamada et al. (1965b) found that the same situation existed for the penetration of maleic hydrazide, N, N-dimethylaminosuccinamic acid, and urea through tomato fruit cuticles. However, the penetration was approximately the same with onion leaf cuticle. Schieferstein et al. (1959) found that the permeability of the Hedera helix (ivy) cuticular membrane to water was greater in the inward direction than the outward. Yamada et al. (1964a,b) found that the rate of penetration of the ions was directly related to the binding of the ions on the (negatively charged) surface opposite to the site of entry (i. e., ion binding was greater on the

inside of the cuticle than the outside. Yamada et al. (1966) also confirmed that ion binding on the onion leaf cuticle was more pronounced on the inner surface. It was also found that binding of calcium was more pronounced than chloride and that both were bound to a greater extent than urea. The calcium, chloride, and urea were not localized on either the inner or outer surfaces of the astomatous tomato fruit cuticle, but calcium and chloride binding on green onion leaf cuticles was found to be closely associated with stomatal cavities, adjacent to stomata and periclinal cell walls. They point out that this finding is of interest in connection with the occurrence of ectodesmata.

Numerous sites of polar absorption have been found, as mentioned in an earlier section. Anticlinal walls tend to leave a slight depression on the surface of the cuticle (Linskens, 1966), where dew, rain or sprays may adhere to the leaf. Ectodesmata have been found in tight rows along the anticlinal walls (Franke, 1960, 1961a, b). Recently, Yamada et al. (1966) found that radioactive ions and urea were bound along the anticlinal walls in isolated cuticles. The anticlinal walls were also the site of droplet excretion in onion bulb scales (Franke, 1961b).

Guard cells have been found to be favored sites for the exchange of aqueous substances, instead of through the stomatal pores which function for gaseous exchange (Franke, 1967). This has been

demonstrated by a number of investigations (Dybing and Currier, 1961; Sargent and Blackman, 1962; Moorby, 1964; Jyung et al., 1965; Middleton and Sanderson, 1965). The edges of the guard cells have been found to be favored sites of exhalation of tritiated water vapor (Maercker, 1965), of binding sites of radioactive ions in isolated cuticles (Yamada et al., 1966) and a large number of ectodesmata (Schnepf, 1959; Franke, 1961a, b, 1964a, b; Lambertz, 1964).

Franke (1967) believes that polar absorption as well as polar excretion and cuticular transpiration are localized in special sites and that these sites very probably correspond to ectodesmata.

Schonherr and Bukovac (1970) express some doubt that ectodesmata (Franke, 1969) participate in polar absorption. This is due to their finding that the mercury precipitates (MP) reflect areas of permeability in the cuticle rather than specific cell wall structures. However, they do believe that the presence of such a pathway in the cuticle would be expected to play an important function in penetration and loss of polar compounds.

Bukovac and Norris (1966) found that the naphthaleneacetic acid (NAA) and naphthaleneacetamide was bound more on the abaxial surface of isolated and intact pear leaves and that NAA penetration (Norris and Bukovac, 1969) was greater for the abaxial surface. This is of interest not only for the stomata present on the abaxial surface, but that the cuticular membrane on the abaxial surface is thicker than on the upper adaxial surface. Thus the penetration could not be explained on the basis of thickness alone.

Norris and Bukovac (1968) found that the lower abaxial surface had a less continuous birefringent wax layer than the upper adaxial surface. Thus the lower cuticle might be expected to be more permeable if the birefringent waxes are relatively impermeable compared to the non-oriented waxes. They suggest that the physical arrangement and composition of the lower epicuticular wax might make the lower surface more permeable than the upper surface. Skoss (1955) noticed that when the wax was removed from the leaves of Hedera helix, the cuticle became highly permeable to 2, 4-D (dinitro-o-sec-butylphenol), silver and chloride ions which had been unable to penetrate when the wax was present. Darlington and Berry (1965) found that isolated apricot cuticle became more permeable to N-isopropyl-α-chloroacetamide after soaking in chloroform. Penetration of 3-CP (3-chlorophenoxy-α-propionic acid) in peach leaves was increased 25 and 34 percent after the upper and lower surfaces were brushed with a camel's hair brush (Bukovac, 1965). Hall and Jones (1961) found that brushing removed wax from the surface, thus increasing cuticular permeability to water and enhancing wettability. Bukovac (1965) also found that immature leaves absorbed significantly more 3-CP than either fully expanded or mature leaves. This inverse relationship had been found earlier by Schieferstein (1957) using ivy leaves and 2, 4-D. He found that the older leaves had more wax and a thicker cuticle than the immature leaves. Bukovac and Norris (1967) found

that sorption of naphthaleneacetic acid (NAA) was increased on removal of the wax from isolated pear leaf cuticles and that plating known quantities of wax onto the dewaxed surfaces decreased penetration. Similarly, it has been found that removal of the wax results in an increased water loss (Martin and Stott, 1957; Horrocks, 1964; Hall, 1966; Grncarevic and Radler, 1967). Thus the chemical composition, physical structure and overall quantity of the cuticular wax appears to be important in cuticular penetration.

The water repellency of the wax deposition causes many problems in the penetration of chemicals and pesticides (Silva Fernandes, 1964, 1965a, b; Martin and Juniper, 1970). Surfactants have been found to assist chemical sprays and solutions in wetting and penetrating the waxy layer of plants (Dybing and Currier, 1961; Hughes and Freed, 1961; Sargent and Blackman, 1962; Foy, 1964; Darlington and Berry, 1965; Prasad et al., 1967; Martin and Juniper, 1970; Lopez, 1971).

MATERIALS AND METHODS

Raw Materials

Royal Anne cherries were obtained from two established cherry trees (Prunus avium L., var. Royal Anne), grown at the Oregon State University Botany and Plant Pathology Field Laboratory near Corvallis, Oregon.

The cherries were hand picked with stems attached and sorted into three maturity classifications on the basis of color and soluble solids.

Maturity	Percent soluble solids	Colors
Immature	13.0-16.0	yellow
Mature	16.0-20.0	yellow and red
Overmature	20.0-25.6	predominantly red

The cherries that were not used immediately were divided into two lots and stored for future use. One lot was placed in glass jars and stored at 1.1 C (34 F). The other lot was placed on cloth lined wire trays, blast frozen at -28.9 C (-20 F) and then stored in glass jars at -17.8 C (0 F).

Histological Studies

Preparation

Segments of fresh immature, mature and overmature cherries,

approximately 4 mm square, were cut from the suture and the opposite-suture sides. They were then treated as follows:

1. Infiltrated under vacuum, with a 1.5 percent (w/v) solution of Reten 205, a cationic water-soluble polymer (Hercules Powder Company, Inc., Wilmington, Delaware) (Norris and Bukovac, 1968).
2. Fixed without infiltration in:
   a. formalin, acetic acid, alcohol (FAA)
   b. formalin
   c. Craf (chromic acid, glacial acetic acid, neutral formalin (a, b, c - Johansen, 1940)
   d. formalin-calcium (Baker, 1944, 1946; Jensen, 1962)
3. Placed in Gibson Solution for demonstration of ectodesmata as proposed by Schnepf (1959).

Segments of brined (Payne et al., 1969) and secondary bleached Beavers and Payne, 1969; Beavers et al., 1970a) cherries were cut and infiltrated in Reten 205, as given above.

Sectioning

Two methods were used to obtain sections for staining and birefringent studies. Previously fixed sections were dehydrated, embedded in paraffin and sectioned according to the technique of Johansen (1940). These sections were used for staining only. In the

second method, the cryostat was used to obtain sections (Microtone-Cryostat Handbook, 1964). Cherry sections that had been previously infiltrated or that were infiltrated after fixation were frozen into blocks of Reten 205 and sectioned using an International Cryostat, Model CTI, operating at -15 to -18 C (5 to -0.4 F). The sections were cut from 6 to 8 μ thick and were transfered to a cold slide with a camel hair brush or teasing needle. In order to minimize curling and folding of the sections the slides were rapidly brought to ambient temperature by placing the forefinger under the section and removing the slide from the cryostat. No additional adhesive was necessary.

Staining

Staining procedures used for the sections were as follows:

1. Paraffin section (Johansen, 1940)
    a. Safranin and anilin blue in clove oil
    b. Safranin and chlorazol black E
    c. Safranin and fast green
    d. Hematoxylin and safranin

    (a, b, c, and d for general morphology)
2. Cryostat sections
    a. Aqueous ruthenium red for pectins (Norris and Bukovac, 1968)
    b. Hydroxylamine-ferric ion for localization of pectin

(Gee et al., 1959; Reeve, 1959)

c. Sudan IV in ethylene glycol for total lipids (Chiffelle and Putt, 1951; Jensen, 1962)

Birefringence Study

Birefringence was observed in non-stained sections by using polarizing lens. A polarizing, analyzing first order red, compensating filter (Wild Heerburg Inc., WH 183381) was inserted in the light path to differentiate between the positive birefringence of cellulose and the negative birefringence indicative of oriented wax molecules (Norris and Bukovac, 1968). The filter provided a means of separating the birefringence on the basis of color, negative being blue and positive yellow, on a magenta background. The colors can be reversed by rotating the filter 180°. The filter also permitted simultaneous photography of cell detail and birefringent areas.

Ectodesmata Study

Royal Anne cherry sections that had been previously placed (see Preparation section) in Gibson solution (Schnepf, 1959) were left for 8-16 hours at 38 C (100 F). At the end of this period, it was found that the epidermis could not be stripped from the fixed section as described by Schönherr and Bukovac (1970). Therefore the epidermis was carefully cut 1 to 2 mm below the cuticle with a stainless steel

razor blade. The potassium iodide treatment with pyoktannin staining was omitted as both Franke (1961) and Schönherr and Bukovac (1970) established that this shortened procedure was equally effective in demonstrating ectodesmata.

The distribution of the mercury precipitates (MP) demonstrating ectodesmata was assessed by surface and cross-sectional views. The sections were infiltrated in Reten 205 and sectioned on the cryostat to obtain cross-sections. The surface sections were observed without further preparation.

Surface Replicates

Surface replicates were made of both the suture and also the opposite sides of cherries of the three maturity classifications. The silicone rubber/cellulose acetate replicate procedure of Zelitch (1961) was used. Observations of these replicates were made to show epidermal cells and stomata, as well as the density and number of stomata present. Examination was made of a specific surface area under a Bausch and Lomb microscope at a magnification of 100 to 150 X.

Photomicrographs

All photomicrographs were made using a Zeiss Model GFL microscope equipped with a Zeiss "Attachment Camera I." A "Science

and Mechanics" light meter was used for optimum light exposure settings. Kodak "Professional II" film was used for color slides and Kodak "Kodachrome X" for color prints.

Black and white photographs were made of normal and solution pocketed cherries with a Polaroid MP-3 camera using type 55 P/N film. A Bausch and Lomb Illuminator (B and L No. 31-33-53) was placed behind the cherries to omit cuticular reflections and gain greater detail.

All photographs and photomicrographs were made on representative samples.

Surface Wax and Lipid Determinations

Surface Wax

Determination of the quantity of surface wax from cherries in the three maturities was made by dipping them in analytical reagent grade chloroform. Chloroform was used, as it has been found to extract the major portion of wax in a short period of time (Martin, 1960; Chambers and Possingham, 1963; Purdy and Truter, 1963; Silva Fernandes, 1964a, 1965b; Kolattukudy, 1965; Norris and Bukovac, 1968; Martin and Juniper, 1970). Each cherry of similar diameter was then individually dipped to the base of the stem and gently rotated for 30 seconds in each of four successive tared beakers containing 80 ml portions of chloroform at ambient temperature. Five

cherries dipped, in this manner, were considered a single treatment and the treatment was replicated five times for each cherry maturity. After dipping, the cherries were placed suture side up on petri dishes and used in a weight-loss experiment. The beakers were covered with cover glasses and the chloroform was allowed to evaporate. When no choloroform was visible in the beakers, they were placed in an anhydrous calcium chloride desiccator to finish drying at ambient temperature. The dried beakers were then weighed to obtain the quantity of wax. (All beakers were treated in the same manner to obtain their tare weights.) The surface of the cherries was calculated using the following formula:

$$A_s = 4\ \pi\ r^2 = 12.57\ r^2 = 12.57\ (\frac{d}{2})^2$$

where

$$r\ (\text{radius}) = \frac{d\ (\text{diameter})}{2}$$

The amount of surface wax per unit area was then calculated by dividing the amount of wax (in μg) by the surface area (in mm^2).

Internal Lipid

The amount of internal lipid was determined by three methods: (A) the method of Bligh and Dyer (1959); (B) soxhlet extraction with chloroform (Joslyn, 1970); and (C) by difference: Total lipid - Surface wax = Internal lipid. In method A the cherry cuticles were

cut off using a stainless steel razor blade, the pits and stems were removed, and the remaining cherry flesh used in the procedure.

Method B consisted of preparing the cherry flesh as given in method A and then extracting with chloroform for 72 hours. The chloroform was removed by using a vacuum rotary evaporator and then the flat bottomed flasks were dried at ambient temperature in a desiccator. After weighing, the flasks were washed with chloroform, aspirated and dried as above, to obtain a more accurate tare weight.

Total Lipid

The amount of total lipid was determined by two methods: (A) by using freeze-dried crushed cherries in a soxhlet chloroform extraction (Joslyn, 1970); and (B) by addition: Total lipid = Internal lipid + Surface wax fraction.

In method A, frozen cherries from the three maturities were placed on metal screen racks and the cuticle on each cherry was then broken in numerous places by making surface cuts with a stainless steel razor blade. The racks were then placed in a Hull Corporation freeze-dryer under the following conditions: 10 μ vacuum, 29.4 C (85 F) drying temperature and -73.3C (-100 F) condensor plates for three days. It was observed that the cherries were actually vacuum dried rather than freeze-dried, due to the cuticular barrier. The cherries were then stored in glass jars for future use. The stems

were removed and the vacuum-dried cherry cuticle and flesh were cut from the pit with stainless steel scalpels. This material was then placed onto a layer of Whatman 18.5 cm filter paper, folded, refolded into another layer and wrapped with aluminum wire (all previously tared) to ensure that none of the crushed material would escape during extraction. It was then placed in the tared extraction thimble, weighed, and extracted for 48 hours in chloroform in a soxhlet extractor. Replicates were made of each cherry maturity. Chloroform was removed and the flasks weighed and tared as given in the previous subsection.

Thickness of Surface Wax

The thickness was measured using a micrometer on the microscope when viewing the stained and non-stained sections obtained earlier. The thickness was also determined by calculation, using the amount of wax per surface area. In this calculation, the density of the surface wax was assumed to be that of paraffin and that the wax was uniformly distributed on the cherry surface.

Effect of Removal of Wax on Cherry Weight Loss

Cherries of the three maturities were weighed and then dipped in chloroform, as in the previous section, and put into tared open petri dishes. These tared samples were placed along with tared

untreated controls into a room at 38 C (100 F) at 27 percent relative humidity. The loss in weight was followed by weighing as well as with color photographs. The photographs were made with a Asahi Pentax 35 mm camera using Kodak Professional II color film with General Electric Photo PXC 500 watt high intensity lights.

Preparation of Isolated Cuticles

The cuticular membranes were isolated enzymatically from Royal Anne cherry halves by a modification of the procedure initially reported by Orgell (1955). A series of different cellulase and pectinase enzymes in varying concentrations and conditions were evaluated to find the optimum separating conditions as shown in Table 1. In all cases there were three cherry halves immersed in approximately 50 ml of the enzyme solutions. It was observed in trial runs that the metal screens that Orgell (1955) used to hold the halves under the solutions damaged the cuticle and were not used in the above study. The use of a shaker was also found to be ineffective. It was also observed that the "disinfectant" sodium ethylmercurithiosalicylate (Yamada et al., 1964b) was not needed. The modified method was similar to that technique used by Norris and Bukovac (1968).

In all cases, the cuticle was never completely separated from the other cell constituents. Therefore a gentle stream of deionized, distilled water and a soft camel hair brush were carefully used to

Table 1. Composition of varying cellulase and pectinase solutions for isolation of the Royal Anne cherry cuticular membrane.

A.

Solution No.	% Pectinase (NBC)	% Cellulase (NBC)	% PVP-40
1	2.0	0.1	1
2	2.0	0.1	2
3	2.0	0.1	3
4	2.0	0.1	4
5	2.0	0.1	5

Incubated in light at 38 C (100 F) at a pH of 3.8*
NBC = Nutritional Biochemicals Corporation
PVP = Polyvinylpyrrolidone (Sigma)

B.

Solution No.	% Various pectinases	% Cellulase (NBC)	pH*
1	2 Pectinol R10 (R & H)	-	3.8
2	10 Pectinol	-	3.8
3	2 Pectinol	-	5.5
4	10 Pectinol	-	5.5
5	2 Pectinol	0.1	3.8
6	2 Pectinol + 2 pectinesterase (GB)	-	3.8
7	2 Pectinol + 2 pectinesterase	0.1	3.8
8	2 Pectinase (NBC) + 2 pectinesterase (GB)	0.1	3.8
9	2 Pectinase	0.1	5.5
10	10 Pectinase	0.1	3.8
11	2 Pectinase	10.0	3.8
12	2 Pectinase	0.1	3.8

Incubated in dark at 38 C (100 F)
R & H = Rohm and Haas
GB = General Biochemical

C.

Solution No.	% Pectinase (NBC)	% Cellulase (NBC)	pH*
1	2	0.1	3.8
2	10	0.1	3.8
3	2	1.0	3.8
4	2	10.0	3.8

Incubated in light at 38 C (100 F)

*All pH buffers were sodium acetate/HCl solutions (Norris and Bukovac, 1968).

remove as much as possible of the remaining cell wall constituents. A thin layer of material still remained, even after the above treatment or returning the cuticles to a fresh enzyme solution.

Solution B-11 was found to be the most effective, in that a minimum of two weeks incubation was needed for a usable separation. However, solution B-11 left the remaining cell constituents black in color. Solution A 1 to 5 left the cuticle in its natural color, but separation was not noticeable after two months of incubation. Therefore solution B-12, which was slower than B-11 in speed of separation but left the remaining constituents a light brown color, was used.

After the enzyme treatment and removal of excess cell wall constituents, the cuticle halves were washed several times and placed in a 100 ml beaker of deionized, distilled water. They were then either turned inside out or left as they were to obtain the correct orientation for the ion penetration studies. It had been found, in previous trials, that a cherry halve shaped backing was needed to remove the cuticle halve from the beaker. If this was not done, the cuticle halve would lose its natural shape by collapsing in upon itself like static charged plastic film. The backing also had to be non-stick so that the dried cuticle halve would not be torn or cracked when the backing was removed. The silicone-rubber replicates, from the replicate study, were found to have the above characteristics. The replicate was submerged in the beaker and brought up under the

cuticle halve and both were removed from the beaker. The cuticle halve was then straightened and the excess water removed by gentle blotting with "Celluwipes." The cuticle halves were then allowed to air dry in a well ventilated covered petri dish.

Electron Microscope Studies

Fresh, frozen, thawed chloroform dipped, brined (Payne et al., 1969) and secondary bleached (Beavers and Payne, 1969; Beavers et al., 1970) Royal Anne cherries and their respective isolated cuticles were used in electron microscopy studies.

The surface wax was removed on some of the whole cherries by a 30 second dip in chloroform before cutting out the sections.

Fresh, frozen, thawed chloroform dipped, brined, and secondary bleached cherry sections, 3 mm thick by 8-12 mm diameter dome-shaped sections, were cut from the suture or opposite the suture sides of the whole cherry. These pieces were mounted on specimen stubs with either double adhesive surface tape (Scotch brand) or conductive aluminum paste and examined uncoated. In some cases, samples were allowed to partially air dry on the stub before being placed in the scanning electron microscope.

Isolated cuticles were obtained enzymatically and air dried on Dow Corning Silastic 583 RTV silicone rubber domes, to help preserve their natural shape. The domes were made by filling a

serological plate with the mixed silicone rubber and its catalyst.

After the cuticles had air dried on the silicone rubber domes, they were then fastened on specimen stubs and gold coated in a Varian-Mikros Model VE-10 vacuum evaporator. A 200 nm thick coat was deposited on the samples by vacuum evaporation of gold wire, while the samples were simultaneously rotated with respect to the horizontal and vertical axes.

The samples were located 10-12 cm from the evaporative source and the pressure in the chamber during evaporation was between 0.2 and 0.5 μ of mercury.

All of the above samples were examined using a Cambridge Stereoscan Mark II-A scanning electron microscope. All samples were examined with the instrument operating at 3-10 Kv accelerating potential, 180-200 microamps beam current and with magnification from 42 to 50,000 X.

Photomicrographs were made with a Polaroid camera attachment with black and white Polaroid type 55 P/N film.

Penetration of Brine Components Through Isolated Cherry Cuticles

Definition of Terms

In order to clarify some of the abbreviations used in the tables and discussions, the following definition of terms is given:

Brine or regular brine: SO_2-calcium bisulfite brine

K-brine: regular brine plus potassium bitartrate

IM: immature classification

M: mature classification

OM: overmature classification

S: suture side of cherry

OS: opposite side (in relation to suture) of cherry

O-I: outside (airside) of isolated cuticle going to inside (fleshy side)

I-O: inside (fleshy) of isolated cuticle going to outside (air)

c: control

t: treated

Example: Calcium (K-brine) penetration IM_s O-I, which means: The penetration of calcium derived from a SO_2-calcium bisulfite brine with potassium bitartrate added through an immature cherry cuticle from the suture side of the cherry going from the outside to the inside of the isolated cuticle.

Apparatus and Procedure

The following cuticular membranes were enzymatically isolated, as described previously, to be used in the penetration studies:

Maturity	Side	Orientation
IM	S	O-I
IM	S	I-O
IM	OS	O-I

Maturity	Side	Orientation
IM	OS	I-O
OM	S	O-I
OM	S	I-O
OM	OS	O-I
OM	OS	I-O

A standard dialyzing membrane (NO. 70160-1 Central Scientific Co.) was used as a control.

The apparatus and procedure for the penetration studies was the same as that of Yamada et al. (1964b), as illustrated in Figure 3. A large test tube (inside diameter = 35 mm) containing the outer solution (30 ml deionized distilled water receiver) was suspended into a water bath maintained at 24 C (75.2 F). Into this tube a small tube (inside diameter = 15 mm) was suspended with the cuticular membrane affixed over the tube opening. (Each mounted isolated cuticle was critically inspected for holes, cracks or ruptures before use.) Three ml of the following solutions were added to this small tube (donor) for the various penetration studies:

a. $CaCl_2$ at 8000 ppm Ca^{++}

b. 1.25 percent SO_2-calcium bisulfite brine at pH 2.8-3.0 (Payne et al., 1969)

c. 1.25 percent SO_2-calcium bisulfite brine at pH 2.8-3.0 plus 0.3 percent potassium bitartrate

d. 1.25 percent SO_2 in deionized distilled water

The meniscus of the inner solution was always adjusted to that of the outer solution, which equalized the hydrostatic pressure on the cuticle. A "Silent Giant" aquarium pump was used to supply air to the aeration tubes (inside diameter = 3 mm) in each large test tube

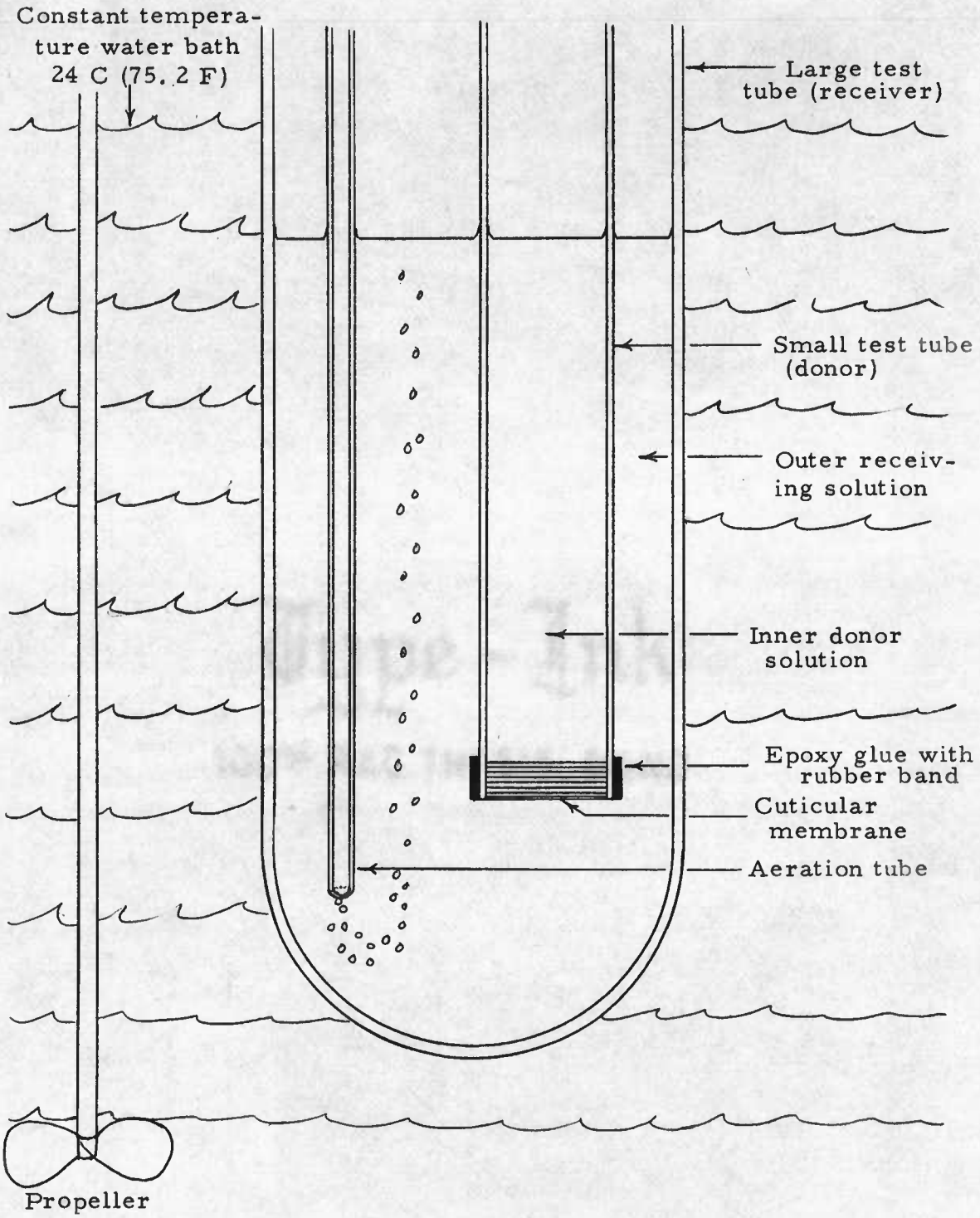


Figure 3. Apparatus for measuring the permeability of a cherry cuticular membrane to cherry brine component (Yamada, Wittwer and Bukovac, 1964b).

(receiver) in order to enhance solution mixing. However, these tubes were not used in the various SO_2 penetration studies, due to the excess volatilization of SO_2.

Sampling

At predetermined intervals, 2 ml aliquots were drawn from the receiver tube of the SO_2 penetration studies and the pH was determined. One ml was then placed into a 1/2 dram vial with a screw top lid, with the remainder being returned to its particular receiving solution. Of this 1 ml aliquot, 1/2 ml was used in the SO_2 determination with the remaining 1/2 ml for the calcium determination.

One ml aliquots were drawn from the calcium (in H_2O) studies and placed into 1/2 dram vials. The full 1 ml was used for the calcium determination.

After the final samples were removed from the receiver solution, 1.0 ml of FD & C Red No. 4 (Ponceau SX) dye solution was mixed into the donor solution. Red 4 was used instead of gentian violet (crystal violet) dye (Norris and Bukovac, 1969) because the brine decolorized the gentian violet dye. The Red 4 has been used commercially in the manufacture of maraschino cherries for some years because it is not decolorized by the brine (Yang et al., 1966). Appearance of the dye in the receiver solution indicated the presence of a rupture in the cuticle, and all data for such penetration were

discarded. The dye was not added with the donor, as Norris and Bukovac (1969) reported lower penetration rates when this was done.

Ion Determination

Determination of free sulfur dioxide was made using the Association of Official Agricultural Chemists method as modified by Payne et al. (1969). A 0.001 N iodine solution was used instead of 1.56 N iodine and a 0.5 ml sample was diluted in 20 ml of deionized distilled water instead of the 5 to 10 ml sample into 100 ml of water as Payne et al. (1969) suggested. The pH was determined by using a Beckman Model G pH meter with microelectrodes.

Calcium and potassium ion determinations were made using a Perkin-Elmer Model 303 atomic absorption spectrophotometer. These metals were determined by direct aspiration of the sample, suitably diluted with 0.1 N HCl. The samples did not need to be ashed or centrifuged prior to analysis, as there was no suspended material present. The following procedure was used:

1. A 0.5 ml sample was placed in a 25 ml volumetric flask.
2. Three ml of SrCl (70 g Sr Cl in 2 l of 0.1 N HCl) were added.
3. 0.1 N HCl was used to fill to volume.
4. Standard 0, 1, 2, 3, 4, 5 ppm calcium and potassium solutions were made from stock 1000 ppm solutions (2.5110 g $CaCO_3$ /l 0.1 N HCl:$CaCO_3$ = 99.45 percent;

1.9074 g KCl/l 0.1 N HCl).

5. A calcium hollow cathode lamp was used for the calcium determination under the analytical operating conditions given in the instrument's instruction manual (1964) with the following exception: Air-acetylene ratio 1:1 with a needle value reading on the Perkin-Elmer Burner regulator of 9. This was done to obtain a more stable flame.
6. A potassium (OSRAM) lamp with a Perkin-Elmer "source power supply" was used for the potassium determinations under the analytical operating conditions given in the instrument's instruction manual (1964).
7. Standards were aspirated first and then the samples were aspirated.
8. The absorption was read from the instrument, changed to percent absoprtion and converted to absorbance using the table given in the instrument's handbook (1964). The standard values were plotted and the sample concentration values were then obtained from this graph.

Ion Binding

To determine if calcium used the ectodesmata for their penetration route, cuticles from the calcium and SO_2-calcium bisulfite penetration studies were stained with alizarin red S to locate binding

sites. A modification of the staining procedure of McGee-Russell (1958; Barka and Anderson, 1965) was used as follows:

1. Cuticles from the calcium and SO_2-calcium bisulfite studies were carefully cut off their respective donor tubes.
2. The cuticles were then washed in deionized distilled water to remove excess unbound calcium.
3. One side of the cuticle was then stained with 2 percent aqueous alizarin red S that had been previously adjusted to pH 4.1 to 4.3 with dilute ammonia using a pH meter. (The deep iodine colored solution obtained is stable (McGee-Russell, 1958).)
4. The endpoint for the staining was chosen empirically by observing the development of the stain microscopically under polarized light. The calcium was indicated by an area covered and surrounded by a birefringent orange-red precipitate on a faint pink background.
5. Excess stain was allowed to drain off. (Other techniques were tried but they were found to be unsatisfactory.)

A visual comparison with a light microscope was then made between the calcium binding sites and the mercury precipitates (MP) sites from the ectodesmata study.

RESULTS AND DISCUSSION

Structure

In this study, it was found that the paraffin method removed the cuticle from the cherry sections (Figures 4 and 5) and for this reason, the paraffin sections were only used to show internal morphology. The sections obtained using the cryostat gave much better cuticles, but unless a hardening agent (FAA, SO_2, Reten 205, Formalin) was used, the fleshy pericarp was destroyed on sectioning (Figures 6, 17, 23-26). It was also found that care must be taken to avoid curling of the section and folding of the cuticle.

General Morphology

The general morphology of the cherry can be seen in Figure 4. The safranin stained the outer layer of epidermal cells and hypodermal layer. The safranin also stained the vascular bundles. The fast-green stained the thin walled parenchyma cells of the fleshy pericarp. The same general morphology can be seen in the unstained sections in the literature review (Figure 1-3).

The safranin staining in Figures 4 and 5 not only show morphology of the cherry fruit but also the presence of cutin, suberin or lignin in the epidermal cell walls. This would indicate that cutinisation or suberisation of these cells has taken plane (Johansen, 1940;

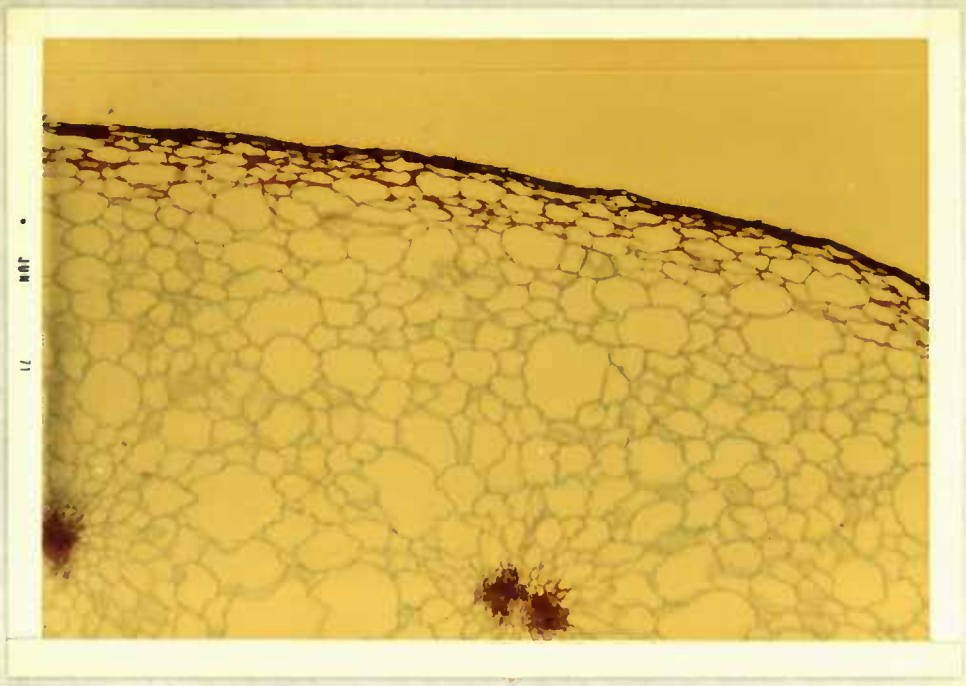


Figure 4. The general morphology of a cherry fruit. (Paraffin section: Safranin and fast green.) 20X.

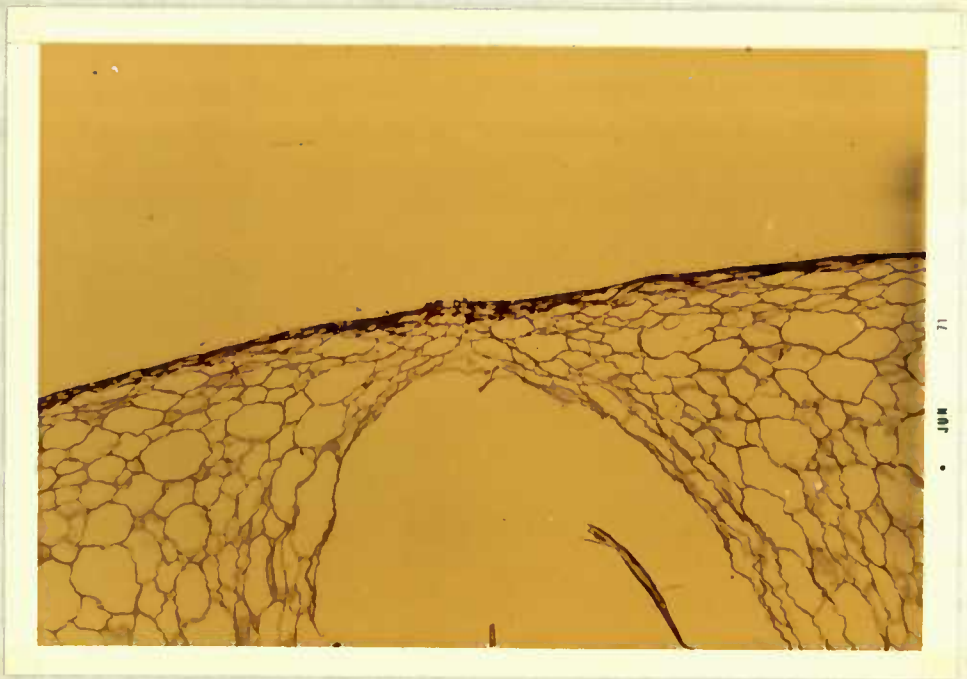


Figure 5. Change in morphology caused by a solution pocket. (Paraffin section: Safranin and chlorazol black E.) 20X

Jensen, 1962). This internal cutinisation would give added strength to the cells as well as being an added barrier to penetration. It could also hinder the enzymatic isolation of the cuticle.

The change in the cellular morphology of the cherry after the occurrence of a solution pocket can be seen in Figure 5. The solution pocket extends through the fleshy pericarp parenchyma cells (black in photo due to staining with chlorazol black E) into the hypodermal layer, the rupture of the epidermal cells is an artifact of the technique. A similar picture of a solution pocket before and after slicing can be seen in the literature review (Figure 1-4).

Epidermis. The outer epidermis in the immature cherry is a row of compact cells (Figure 6) which becomes more disorganized and flattened as they mature to the overmature classification (Figure 8). These observations are very similar to those made on the sour cherry (Prunus cerasus L., var. Montmorency) epidermis (Tukey and Young, 1939). They also state that their drawings are similar to those of peach and sweet cherry diagrams of earlier workers. A number of investigators have observed that the epidermal cells of cherries increase in number during the pre-bloom stage and the first part of the rapid development stage, but after that they only increase in size and wall thickness in the tangential direction (Tukey and Young, 1939; Leopold, 1964; Chaplin, 1971).

Stomata. The epidermis of the Royal Anne cherry is covered

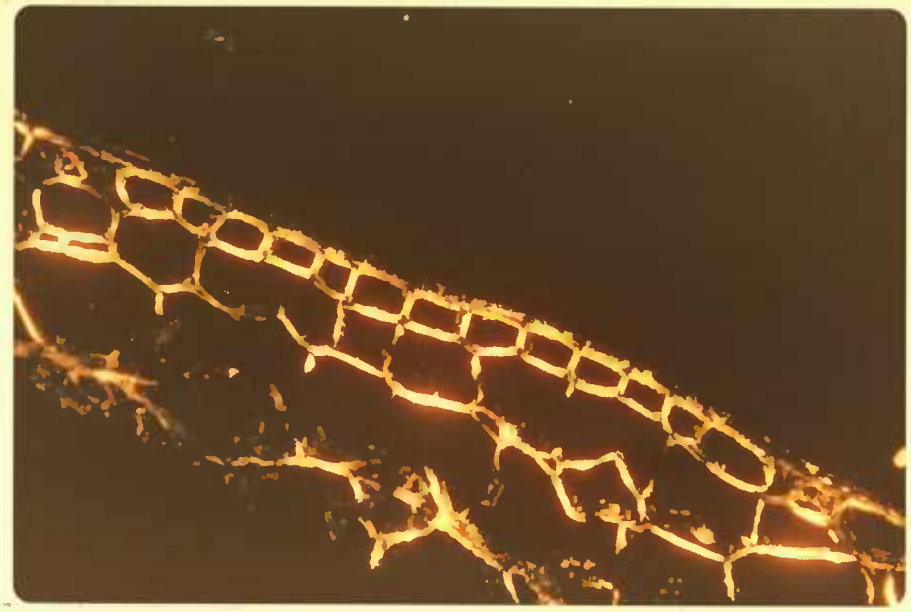


Figure 6. Immature suture cherry section under polarized light. 128X

Figure 7. Immature suture cherry section under polarized light with a first order red compensating filter in the light path. 128X

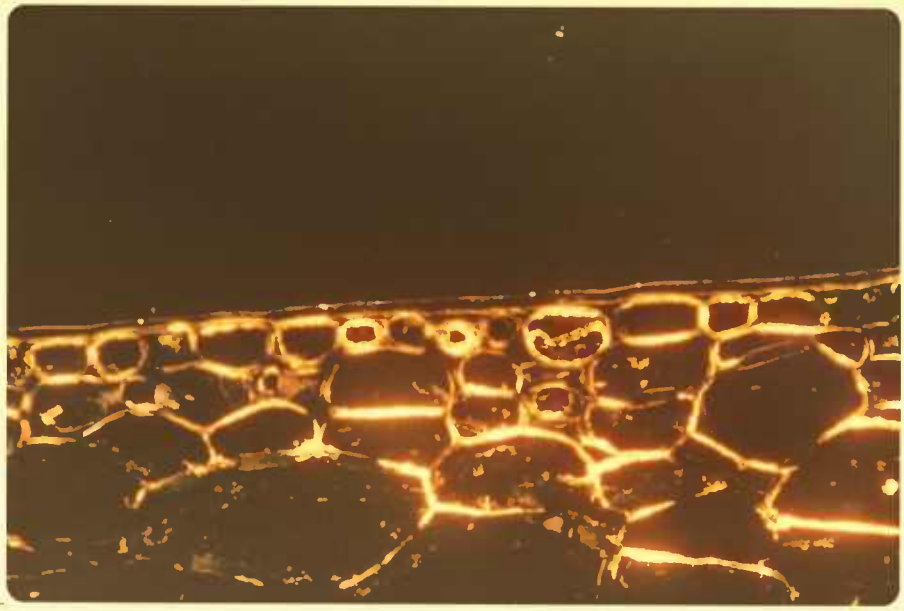


Figure 8. Overmature suture cherry section under polarized light. 128X

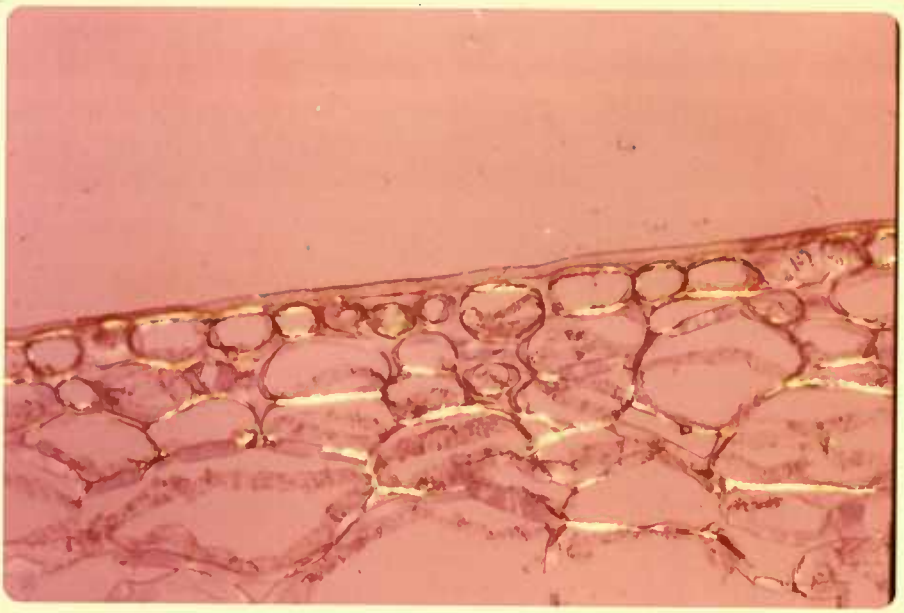


Figure 9. Overmature suture cherry section under polarized light with a first order red compensating filter in the light path. 128X

with a continuous cuticle which is interrupted by stomata as shown by the silicone rubber/acetate replicate (Figure 10). Tukey and Young (1939) found that the stomata in the sour cherry are fully differentiated 18 days before full bloom and that no more stomata are formed afterwards. This situation would seem to hold for the Royal Anne cherry as an analysis of the number of stomata per unit area for immature and overmature fruits showed that there was no change in the number of stomata per unit area from the immature to overmature stage (Table 2). However, there were approximately twice as many stomata on the suture side (0.220 to 0.229 /mm^2) of the cherry than the opposite side of the cherry (0.114 to 0.116 /mm^2) for both maturity classifications. In comparison, the frequency range of stomata on the adaxial (upper) and abaxial (lower) surfaces of a variety of species of leaves are: 0 to 137 /mm^2 for adaxial, 37 to 545 /mm^2 for abaxial or 37 to 545 /mm^2 of the combined surfaces (Martin and Juniper, 1970). Thus, in general the cherry fruit cuticle has a much smaller number of stomata per unit area than most leaves. The occurrence of more stomata on one side over the other could have a direct effect upon penetration of chemicals, as many investigators have found increased penetration of chemicals through stomatous cuticles over astomatous cuticles (Martin and Juniper, 1970). This increased penetration could be explained by the prevalence of ectodesmata (MP) in the guard cells (Franke, 1967; Schönherr and Bukovac, 1970).

Table 2. Number of stomata per unit area in the immature and overmature cherry classifications.

Replicate no.	No. stomata/unit area (mm^2) Classification			
	IM_{os}	IM_{s}	OM_{os}	OM_{s}
1	0.091	0.253	0.116	0.220
2	0.124	0.254	0.118	0.250
3	0.118	0.223	0.094	0.236
4	0.127	0.210	0.114	0.229
5	0.120	0.232	0.127	0.209
6	0.112	0.201	0.126	0.202
7	0.097	0.192	0.118	0.213
8	0.099	0.234	0.122	0.188
9	0.122	0.245	0.104	0.213
10	0.126	0.240	0.124	0.242
$\overline{X}$	0.114	0.229	0.116	0.220
s	0.013	0.020	0.013	0.019

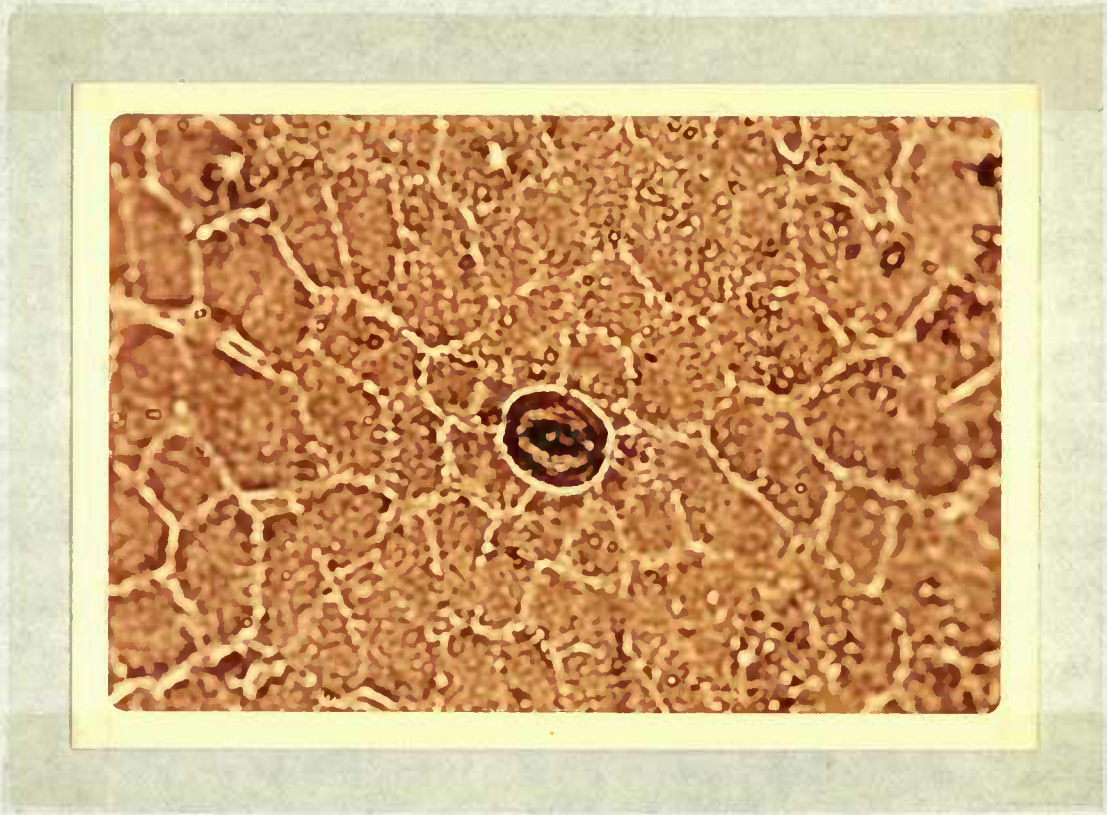


Figure 10. Silicone rubber/acetate replicate of the surface of a mature cherry. 128X

The typical appearance of an immature stomata is shown in the scanning electronmicrographs (Figures 11-1, 2); note that the stomata are in a depression. The appearance of the guard cells changed with increasing maturity, as seen in Figure 12-1, 2. This was also observed in a number of silicone rubber/acetate replicates examined. The number of atypical stomata greatly increased from none in immature to a few in mature to many in the overmature classification. The stretching and rupturing of the stomatas could also be the beginning of lenticel formation. Lenticels are a loose arrangement of cells that protrude above the surface through a fissure in the periderm which usually arise beneath the stomata (Esau, 1965). While lenticels occur on the cherry tree (Prunus avium) (Leopold, 1964; Esau, 1965), Tukey and Young (1939) did not find any lenticels or any stretched or ruptured stomata in sour cherries, even after 60 days past commercial harvest. No true lenticels were observed on any Royal Anne cherries examined in this study. This stretching is undoubtedly the result of the increasing size of the cherry as maturity and overmaturity is reached.

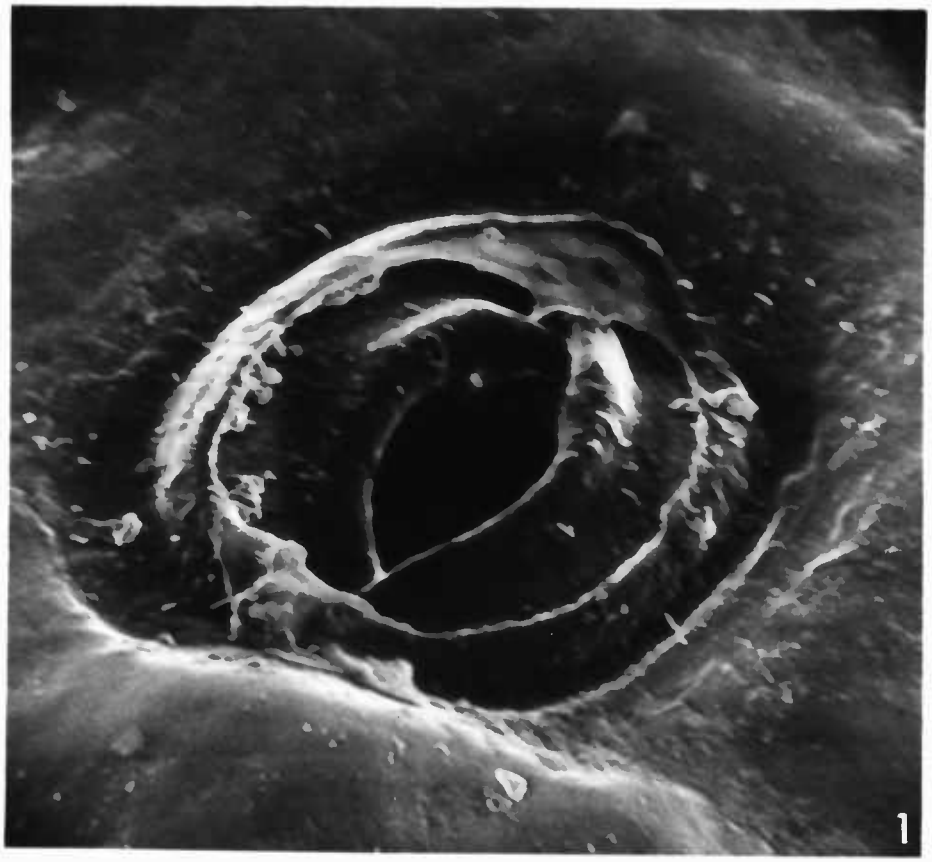
The stomata were found to be unevenly distributed over the surface of the cherry. On the suture side no stomata was found on the suture proper, but some were close to the suture (Figure 13-1, 2). The majority of the stomata were on either side of the suture with a few being near the stem end and more being on the apex. The stomata on

Figure 11. Scanning electronmicrographs of a typical immature cherry stomata.

11-1. 2288.5X. (1 μ = 23 mm)
Note the wax where it has started to flow or extrude onto the guard cells.

11-2. 978X.

11-3. Area near the above stomata.
1904.5X (1 μ = 1.9 mm)
Note smooth and granular structure of the surface.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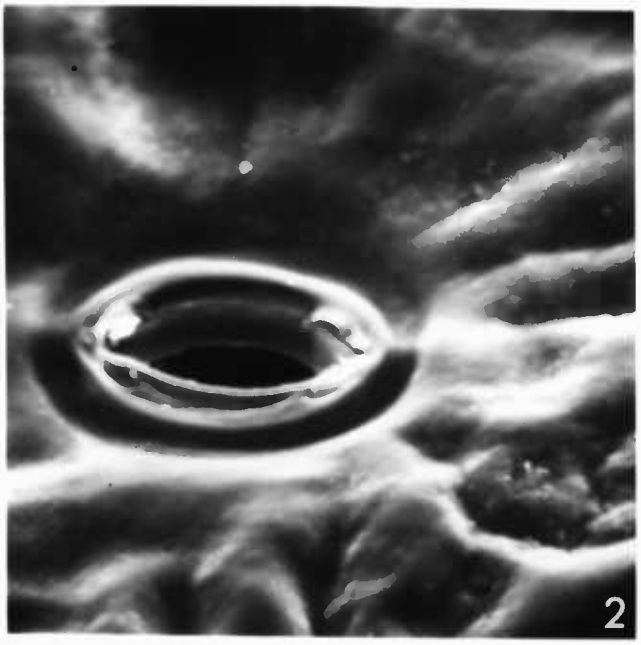
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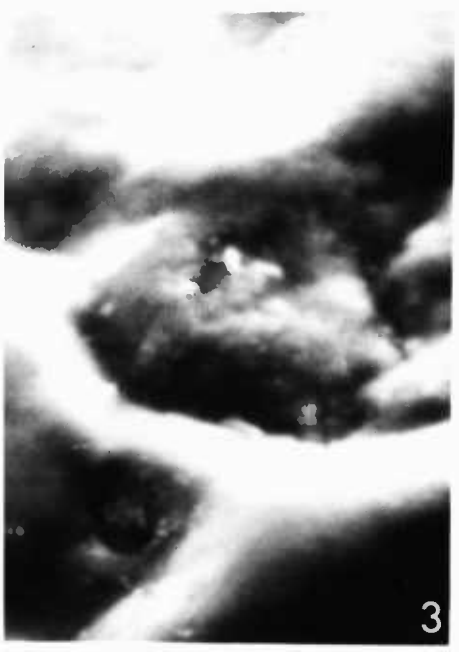
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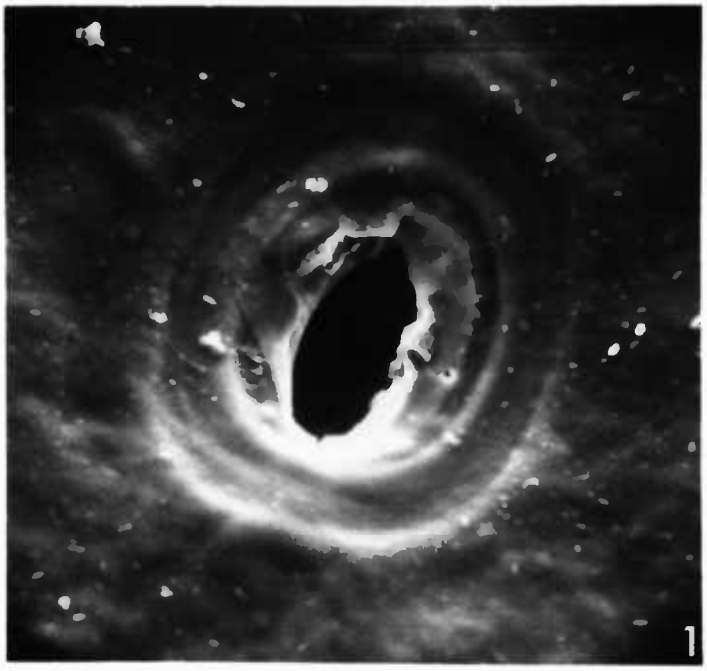
Figure 12. Scanning electromicrographs of a typical overmature cherry stomata.

12-1. Typical brined overmature cherry stomata. 1148X

12-2. Typical fresh overmature cherry stomata. 1041X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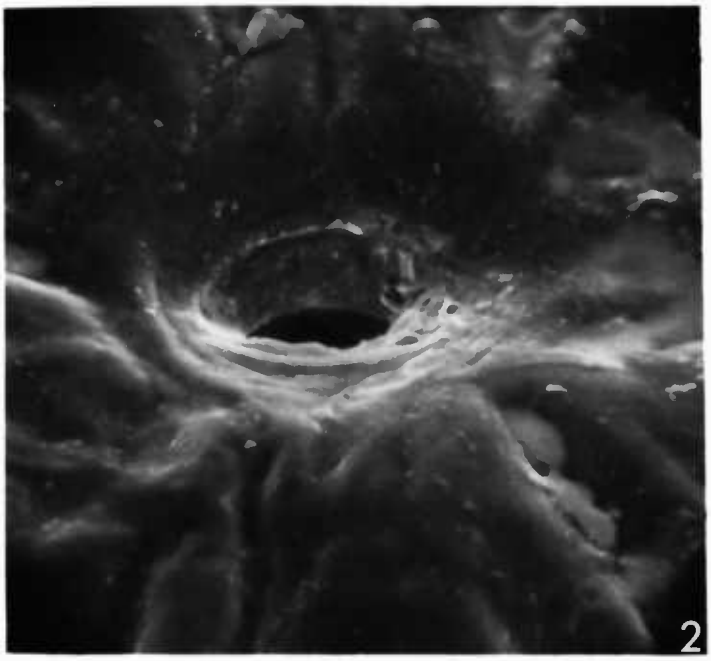
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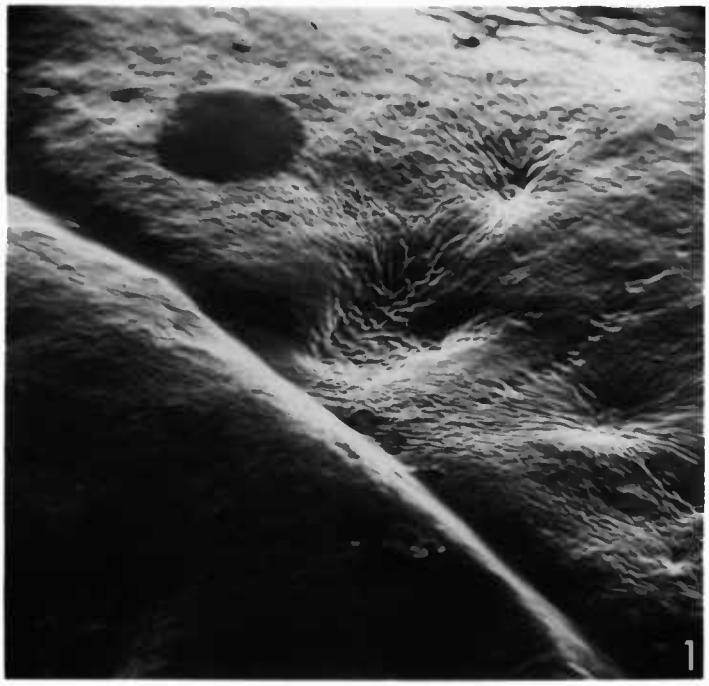
Figure 13. Scanning electronmicrographs of the suture side of a cherry.

13-1. Immature cherry. 42X Note that the stomata are in a depression.

13-2. Suture of an immature cherry. 126.5X

13-3. Suture of an overmature cherry. 102X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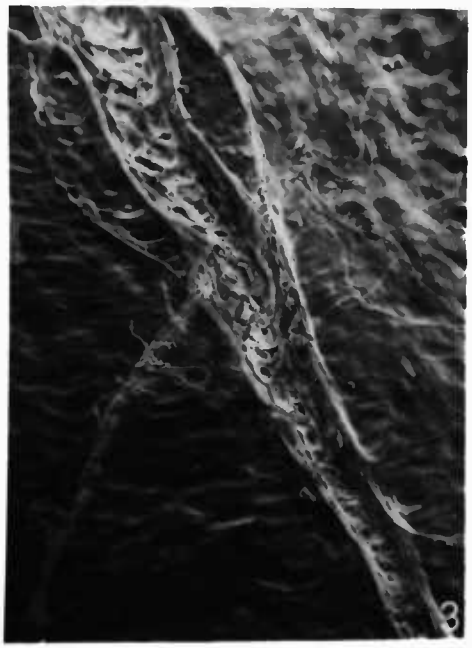
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the opposite suture were unevenly distributed over the entire surface (Figure 14-1, 2).

Pectin. Pectic substances (definition, see Kertesz, 1951, and Doesburg, 1965) occur between the cuticle-cell wall surface and were presumed to be stainable by ruthenium red (Doesburg, 1965; Norris and Bukovac, 1968). However, ruthenium red has been found to stain materials other than pectin (Doesburg, 1965). Recently, Sterling (1970) determined the crystal structure of ruthenium red and its stereochemistry in a pectin stain. He found that the staining site is stereospecific for any environment that has two negative charges 4.2A apart and has enough space to accommodate the staining site. He also found that pectic acid would be stained by ruthenium red, as such a site is formed between each monomer galacturonide and its adjoining neighbor. Thus, the stain will stain deeper for de-esterified pectic materials and less intense for esterified pectic materials.

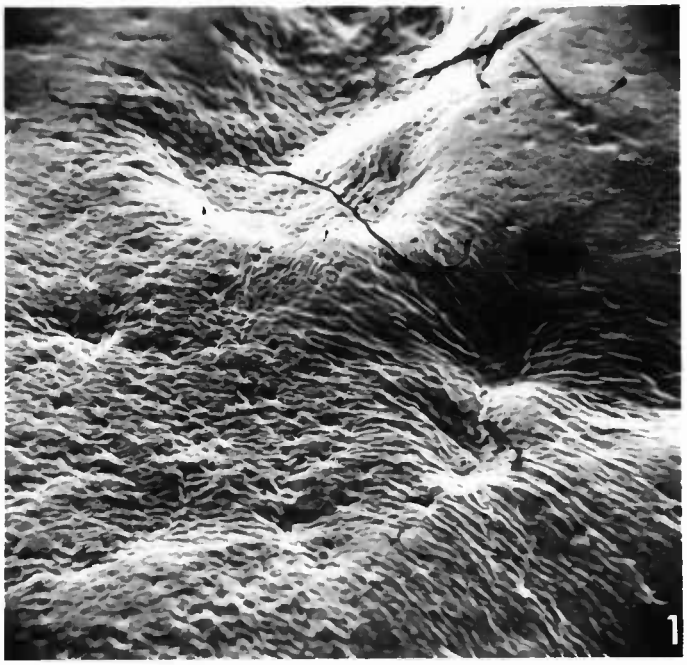
An immature cherry section was stained with ruthenium red and it stained everything except the cuticle (Figure 15). The stain was darker in the cuticle-cell wall interface and between cell interfaces.

The specific hydroxylamine-ferric chloride reaction for the localization of pectin was also used to determine the location of pectic substances in the cherry sections. The reagents and procedure were validated using a known pectin, and they gave a positive very dark red color. Freshly picked sectioned and stained immature fruit showed a

Figure 14. Scanning electronmicrographs of the surface opposite the suture of an immature cherry.

14-1. 42X

14-2. Close-up from above opposite suture. 85X Note that the stomata are in a depression.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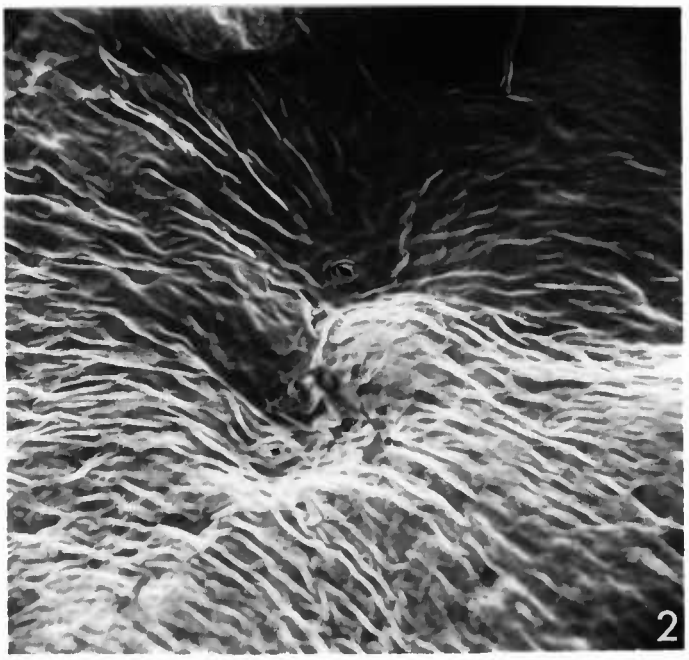

2

light to medium red intensity in the same areas as the dark areas in the ruthenium red stain. An overmature section gave much lighter staining. However, in a stored cherry section (Figure 16) only faint pink areas are present in the cuticle-cell wall interface and cell wall-cell wall interface. This would indicate that the pectin after storage is in its de-esterfied form (i. e., pectic acid) as the stain darkens with increasing esterification (Reeve, 1958).

The degree of esterification of the pectin in Royal Anne cherries was found to be about 50 percent (Hildrum et al., 1971). They also found that the enzymatic demethylation took place even after three months in SO_2 brine, causing a gradual decrease in esterification from about 50 percent to about 25 percent.

The cherries sectioned and stained for pectin in this study were not killed and were stored before sectioning and staining. Thus, pectin methylesterase activity, which is present in the fresh fruit (Hildrum et al., 1971), could account for the de-esterification of the pectin into the pectic acid form. Doesburg (1965) reported from a number of investigations that a striking decrease of the degree of esterification occurs during ripening in pears, tomatoes, cherries and avocados. These pectin stains would then tend to support the de-esterification of the cherry pectin on storage.

Cuticle. Thickness - The cherry cuticle can be distinguished from the underlying cells by staining with Sudan IV (Johansen, 1940;

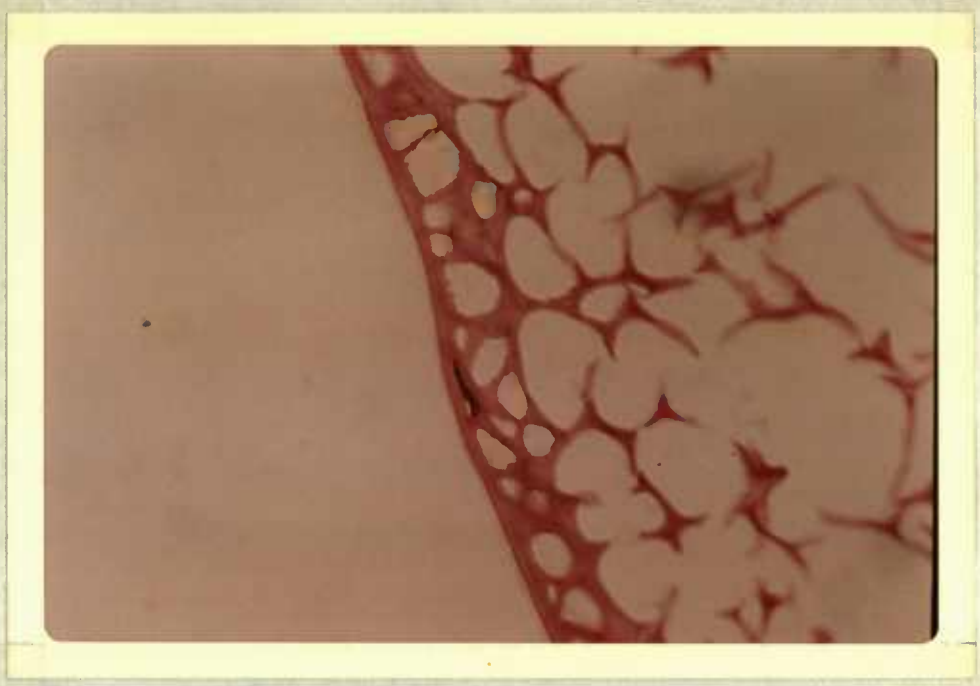


Figure 15. Ruthenium red staining of an immature cherry section. 128X

Figure 16. Hydroxylamine-ferric chloride staining for localization of pectins. 320X

Jensen, 1962; Norris and Bukovac, 1968). The cuticle on the suture side of the cherry is very similar to that of the opposite suture (Figures 17 to 20). The cuticles vary in thickness on the same side while the staining within a cuticle is fairly uniform (Figures 19 and 20). This would indicate that the cutin matrix is homogeneous, without embedded or layered wax as found in a number of other cuticles (Norris and Bukovac, 1968).

The cherry cuticle has only a slight thinning over periclinal walls and thickening inward where the anticlinal walls meet (Figures 6 to 10; 16 to 20). This is in contrast to most leaf cuticles (Baker et al., 1962; Baker and Martin, 1963; Norris and Bukovac, 1968; Martin and Juniper, 1970). The surface of the cuticle seems to conform to the outline of the underlying epidermal cells as seen in the silicone rubber / acetate replica (Figure 10), and in a number of the scanning electronmicrographs (Figure 13).

The cuticle lining the stomatal cavity ruptured during sectioning; thus it could not be shown (Figures 30 and 31) as it has been reported for other species (Martin and Juniper, 1970).

The cuticle varied in thickness between immature and overmature maturity classifications, as was to be expected. The immature cuticle was from 3.0 to 6.1 μ thick while an overmature cuticle was from 6.1 to 8.1 μ thick (Table 3). However, as mentioned earlier, there is a variation within a single cuticle, as shown in Figure 9, and projections

Table 3. Thickness of the Royal Anne cherry cuticle.

Cuticular location	Thickness* of cuticle	Thickness* of wax	
	measured	measured	calculated
IM_s	3.0 to 6.1	0 to 0.1	-
IM_{os}	3.0 to 6.1	0 to 0.1	-
Average IM	3.0 to 6.1	0 to 0.1	0.125 to 0.149
M_s	-	-	-
M_{os}	-	-	-
Average M	-	-	0.143 to 0.161
OM_s	6.1 to 8.1	0 to 0.2	-
OM_{os}	6.1 to 8.1	0 to 0.2	-
Average OM	6.1 to 8.1	0 to 0.2	0.169 to 0.202

*Data in μ

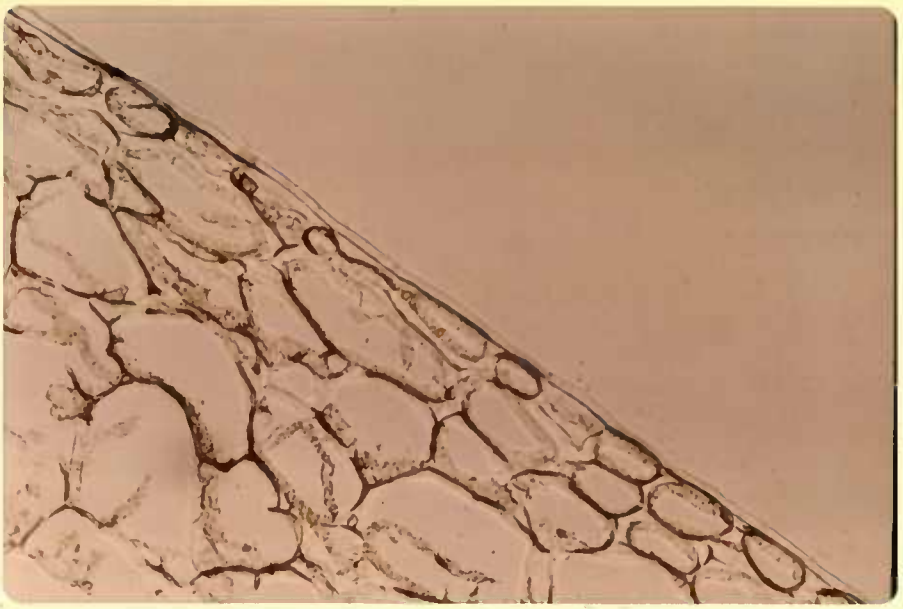


Figure 17. Immature suture side of a cherry section under normal light. 128X

Figure 18. Section opposite the suture of an over-mature cherry viewed under normal light. 128X

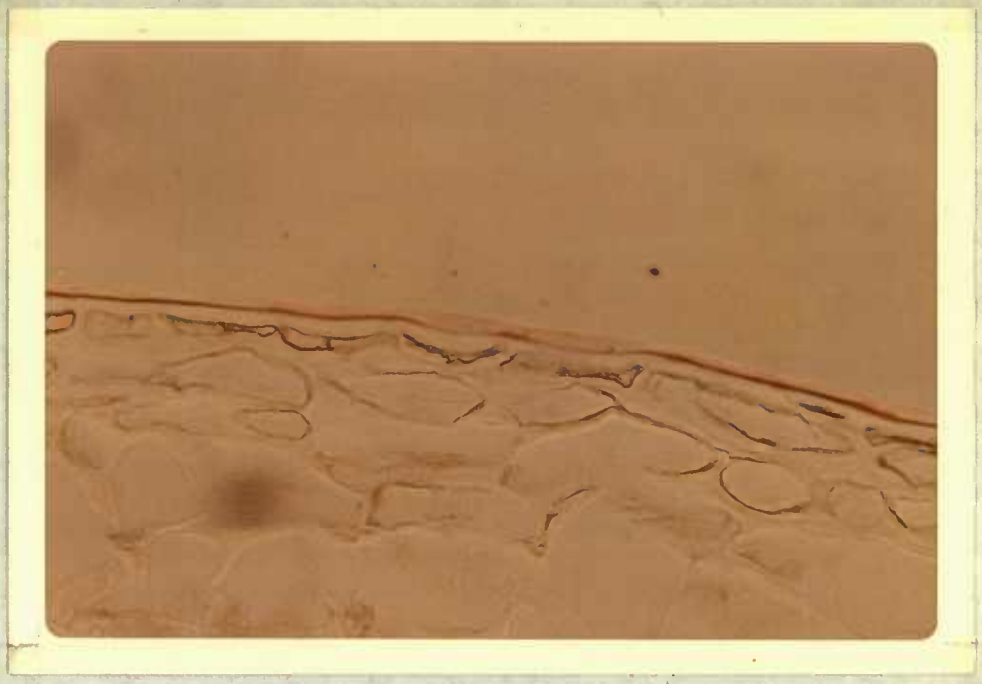


Figure 19. Suture side of an immature cherry section stained for the cuticle (Sudan IV). 128X

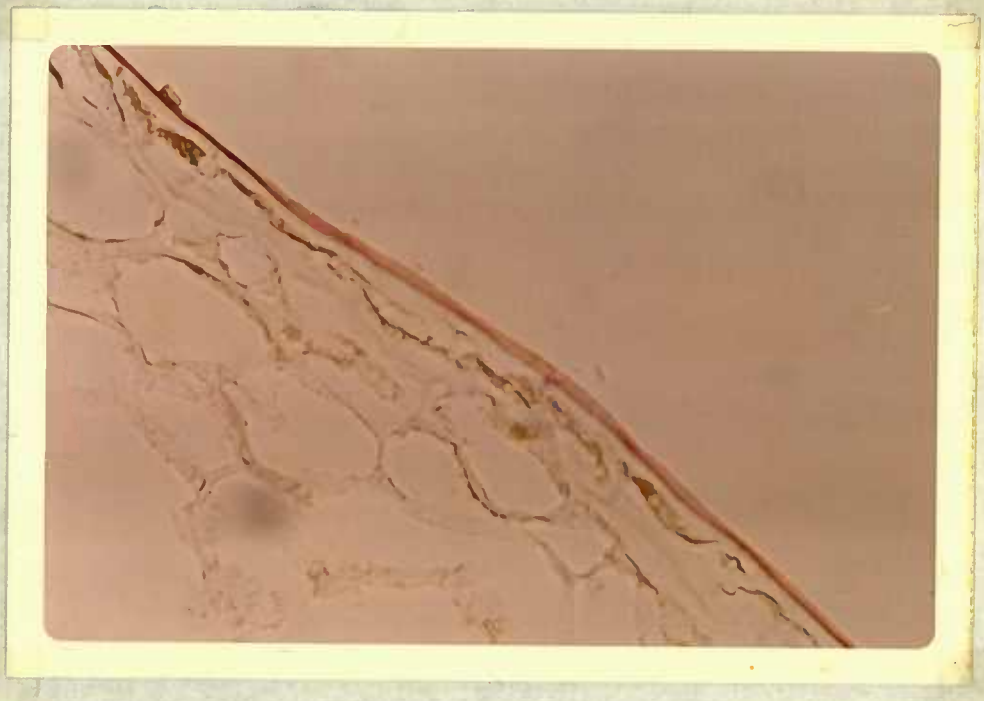


Figure 20. Chloroform stripped immature cherry section stained for the cuticle (Sudan IV). 128X

of the cuticle outward were occasionally found. In comparison, the pear leaf cuticle thickness is from 2.2 to 8.1 μ on the upper surface and 3.2 to 6.6 on the lower surface, periclinal and anticlinal, respectively (Norris and Bukovac, 1968); and the thickness of the cuticular layers in ten different leaves ranged from 0.7 to 13.5 μ (Martin and Juniper, 1970).

Birefringence - When a cuticle is observed under polarized light, areas that are crystalline or semi-crystalline will exhibit themselves as birefringence (Mazliak, 1968). Negative birefringence is indicative of oriented wax molecules while positive birefringence is observed for cellulose. The identification of these two birefringent areas can be accomplished by using a first order red compensating filter, which separates the birefringence on a color basis (Norris and Bukovac, 1968). Negative birefringence is indicated by blue and positive birefringence by yellow in this study. A reversal of the colors can be made by rotating the lens 180°.

The cherry cuticle has a discontinuous surface layer of negative birefringent wax (Figures 6 and 8). Figure 21 shows a surface view of an isolated immature suture cuticle under polarized light with the filter in the light path. The discontinuous deposits of cuticular wax are denoted by the blue areas, the yellow is the epidermal cellulose, while the red is the background. The two large yellow spots surrounded by black are two artifacts of isolation, as discussed in a later section.

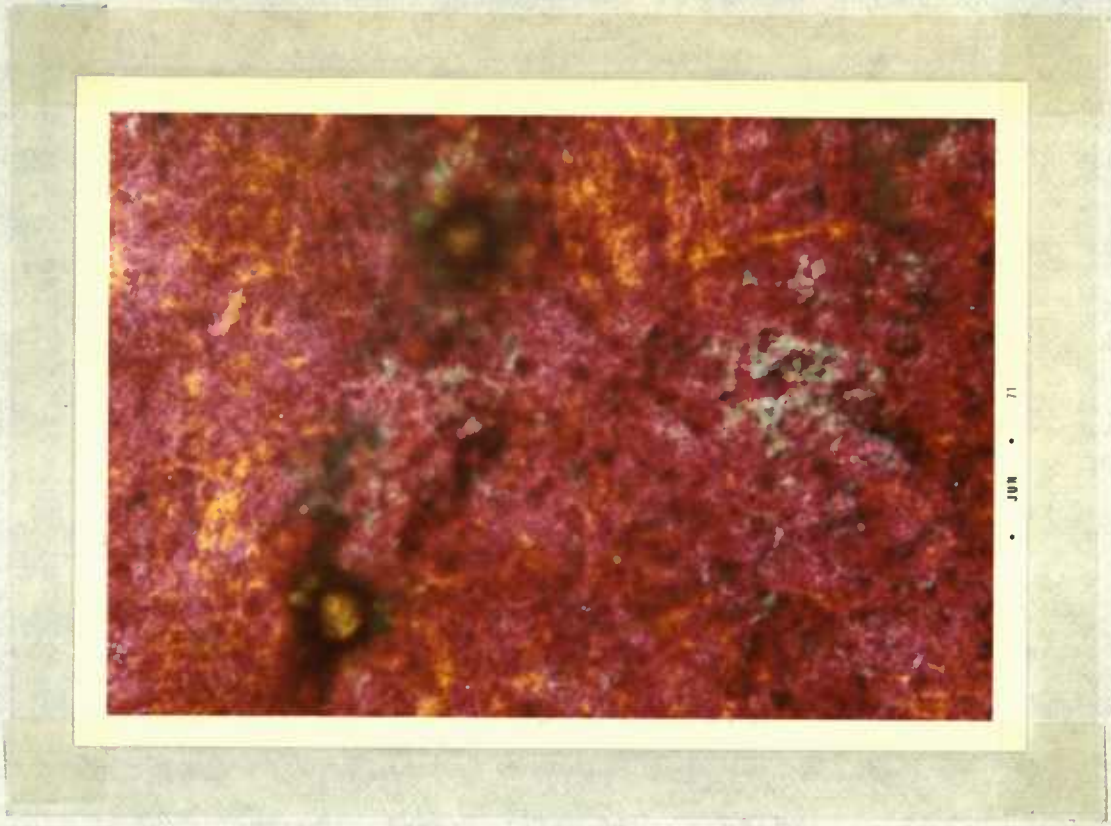


Figure 21. Surface of an isolated immature cherry cuticle under polarized light with a first order red compensating lens in the light path. 128X
Blue = wax; yellow = cellulose; red = background. Two large birefringent artifacts of isolation are located left of center.

It was observed that dipping in chloroform completely removed the negative birefringence thus confirming the removal of the wax. Similar results were found by Norris and Bukovac (1968) using a pear leaf cuticle. The amount of birefringence increased with increasing maturity as can be seen in Figures 6 and 8. The nonbirefringent (isotopic) area between the wax and the cellulose of the epidermal cells is cutin (Mazliak, 1968; Norris and Bukovac, 1968).

The cherry fruit cuticle did not have any embedded birefringent wax in the cutin (Figures 6 to 9) which also differs from the pear leaf cuticle. However, Figure 6 shows one area where the birefringent cellulose of the epidermal layer is embedded in the cutin. The binding of cellulose in the cuticle is also exhibited in the isolation of the cuticular membranes.

Surface Wax and Lipids. Surface wax - The thickness of the birefringent wax layer was found to be approximately 0 to .2 μ thick by direct measurement using a micrometer (Table 3 and Figure 31). The thickness of the surface wax was also determined by dipping in chloroform. These data are shown in Table 4. The cherries were dipped in chloroform only four times as any more were found to contain extracted yellowish internal lipid material, while the surface wax is an opaque white color after extraction. The amount of internal lipid extracted would be small due to the forming of emulsions within the cellular tissue (Joslyn, 1970). The amount of wax increased with

Table 4. Amount of surface wax per Royal Anne cherry as a function of increasing maturity.

Replication[1]	Diameter (mm)	Amount of wax/cherry (μg)	Amount of wax/unit surface area (μg/mm^2)
	A. Immature Classification		
1	25	1080	0.137
2	25	940	0.120
3	25	880	0.112
4	25	980	0.125
5	25	940	0.120
$\overline{X}$	-	960	0.123*
s		70	±0.012
	B. Mature Classification		
1	25	1080	0.137
2	25	1140	0.145
3	25	980	0.125
4	25	1080	0.137
5	25	1120	0.142
$\overline{X}$	-	1080	0.137
s		60	±0.008
	C. Overmature Classification		
1	25	1320	0.168
2	25	1520	0.193
3	25	1140	0.145
4	25	1280	0.163
5	24	1300	0.170
$\overline{X}$	-	1310	0.170*
s		140	±0.018

[1]Each replication = 5 cherries

*Significant at .05 level.

maturity from 0.123 $\mu g/mm^2$ for the immature cuticle to 0.170 $\mu g/mm^2$ for the overmature cuticle. Assuming that the cherries are perfect spheres, that the density of the wax is that of paraffin, and that the wax is uniformly distributed, the thickness of the wax increased from 0.137 μ to 0.189 μ with increasing maturity (Table 3). Thus, the calculated values and the measured values are in fairly close agreement. In comparison, Lopez (1971) found 0.486 $\mu g/mm^2$ of wax or a layer of wax 0.5 μ thick, if compacted, in two week old bean leaves; apple cuticle may have from 4 to 25 $\mu g/mm^2$ depending on the variety and maturity (Baker et al., 1963; Silva Fernandes et al., 1964b; Martin and Juniper, 1970); of 24 species from a wide range of mono- and di-cotyledonous plants the weight of surface wax varied from less than 0.10 to 0.41 $\mu g/mm^2$ for leaves and 0.10 to 4.1 $\mu g/mm^2$ for fruits (Holloway and Baker, 1970). The plum variety "Giant Prune" has 1.2 $\mu g/mm^2$ of surface wax (Martin and Juniper, 1970); different varieties of grapes had from 1.07 to 1.78 $\mu g/mm^2$ of waxy substances (Dudman and Grncarevic, 1962); the pear leaf has from 0.71 to 1.32 $\mu g/mm^2$ of surface wax on the upper and lower cuticle, respectively (Norris and Bukovac, 1968); and Silva Fernandes et al. (1964b) found that the pear fruit had from 1.0 to 2.0 $\mu g/mm^2$ of surface wax depending on the variety tested.

From this information it would appear this is one of the major factors involved in the less efficient protective abilities of the cherry cuticle toward splitting and solution pocket formation.

Lipids - The total lipids of the Royal Anne cherry was determined in addition to the amount of surface wax. The freeze-dried cherry chloroform extraction procedure was found to be the more reproducible, as the water soluble sugars were not extracted as with other techniques. The amount of total lipids and internal lipids (as determined by difference) is given in Table 5. The total value is low compared to the literature values given by Watt and Merrill (1963) and Joslyn (1970). However, these values are an average for all sweet cherry varieties and maturities.

Table 5. Lipid content of the Royal Anne cherry.

Maturity	Total lipid (mg/100 g flesh)	Surface wax (mg/100 g flesh)	Internal lipid (mg/100 g flesh)
Immature	99.74	13.8	85.94
Mature	79.94	14.3	65.64
Overmature	76.09	18.4	57.69
$\bar{x}$	85.26	15.5	69.76
Literature value $\bar{x}$	300		

The lipid material was found to be yellowish in color, which is probably due to extraction of the lipid soluble carotenoids. The total and internal lipids were found to decrease in weight with increasing maturity while the amount of surface wax increased with maturity (Table 4,5). The thickness of the wax as well as the cuticle were also

observed to increase in thickness with increasing maturity (Table 3). This would indicate that the deposition of cutin and wax kept slightly ahead of surface expansion and the subsequent weight increase of the maturing cherry. Similar results have been found for other fruits and some leaves (Martin and Juniper, 1970).

During the attempted freeze-drying of the cherries for the lipid determinations, it was found that if the cuticle was not checked in numerous places the cherry would swell, rupturing the contents of the cells without breaking the cuticle. This resulted in a hollow sphere larger than the original cherry, with empty space around the pit. Even after "checking" the cuticle, drying took over 72 hours, and the cherries appeared to be vacuum dried rather than freeze dried. This again illustrates the cuticle as a barrier to transpiration. It was also interesting to note that the first place for the cells to pull away from the cuticle leaving an air pocket was at the suture, thus indicating a structural weakness at that point.

Surface Structure. The surface of the cherry cuticles was viewed using a scanning electron microscope and are presented in Figures 11 to 14, 22 and 27.

The suture was found to be a smooth depressed valley in the immature cherry (Figures 13-1 and 13-2) that became stretched nearly flat with stretch marks evident in the overmature cherry (Figure 13-3). As mentioned earlier, the same was found in the

stomata of the two maturity classifications (Figures 11 and 12).

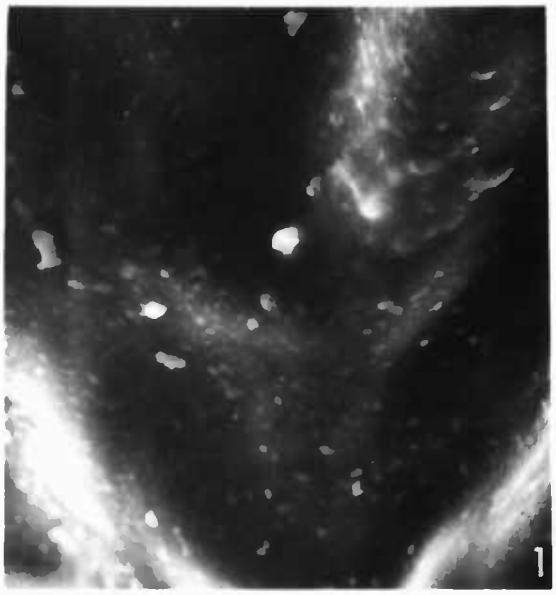
The surface wax does not seem to cover the guard cells in the immature fruit (Figures 11-1 and 11-2), but it can be seen where it has started to extrude or flow over the edge onto the left guard cell in Figure 11-1. In the overmature fruit, the wax covers the guard cells completely, as illustrated in Figure 12-1 and 12-2, and in some cases partially plugged the stomatal aperature.

The surface wax between and around the stomatas appears to be a smooth to granular sheet or layer (Figures 11-1, 2 and 22). These areas were observed from a magnification of 42 to 50,000X without finding any rods, spires, nets or plates, as found on other cuticles (Schieferstein and Loomis, 1956, 1959; Martin and Juniper, 1970). Thus, this surface would be expected to be easily wetted (Silva Fernandes, 1964, 1965a; Martin and Juniper, 1970).

Figure 22-3 and 22-4 shows a cuticle that has been chloroform stripped before observation. The cuticle has become a porous, granular structure reminiscent of a sponge. Whether this porous structure extends through the cuticle is not clear as the cellulose was not completely isolated from the isolated cuticular membranes. This removal of the surface wax would allow the cuticle to swell and absorb water and chemicals, thus enhancing cuticular penetration. This was found in later penetration studies with the Royal Anne cherry cuticle as

Figure 22. Scanning electronmicrographs of the surface of an overmature cherry in an astomatous area before and after chloroform dipping.

22-1. 2040X
22-2. Surface in the upper right hand corner of Figure 22-1. 4692X
22-3. Chloroform dipped. 935X
22-4. Surface in the lower right hand corner of Figure 22-3. 1870X
Note the granular to smooth appearance of the surface before treatment and the porous sponge-like surface after treatment.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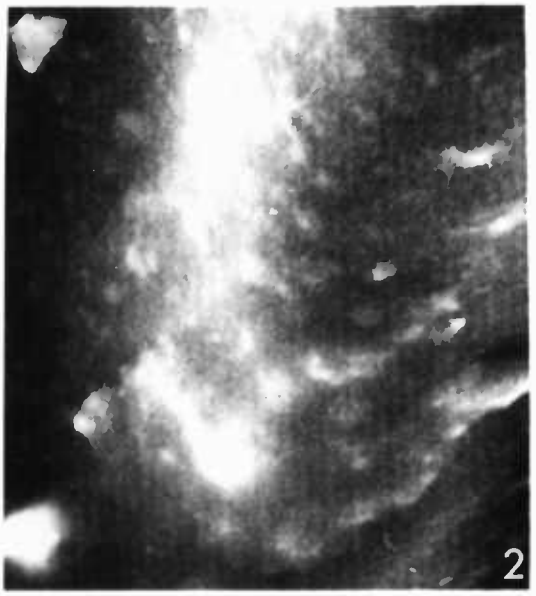
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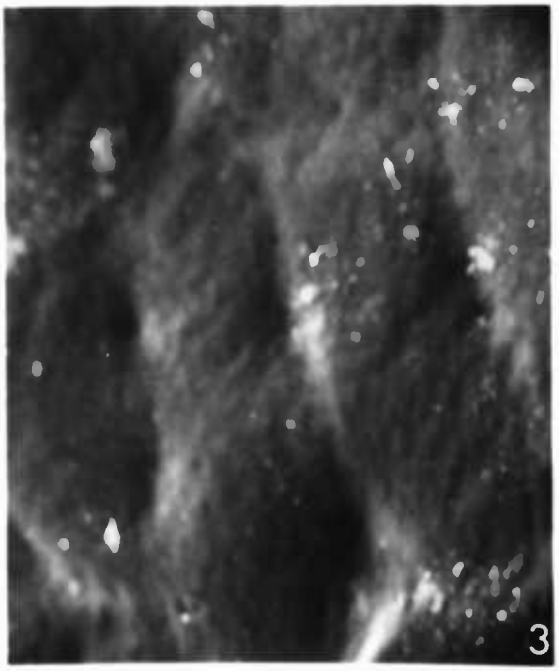
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well as in earlier reports in the literature (Martin and Juniper, 1970).

The effect of SO_2-calcium bisulfite brine on the cuticle was to remove part of the wax and part of the cuticle, as observed in Figures 23 to 25. The brined cherries were much easier to section and gave better sections due to the hardening effect of the brine (Figures 24 and 25). For this reason it is possible to see two different epidermal and hypodermal cell structures (Figures 24 and 25). Figure 25 also has some internal cutinisation as noted by the Sudan IV staining in the hypodermal layer. This may have been an injury to the fruit which caused cutinisation and the atypical cell differentiation. The surface of the brined cherry under the scanning electron microscope is presented in Figure 27-1 and 27-2. In these figures it can be seen that the anticlinal walls of the underlying epidermal cells are much more prominent than the un-brined samples. A number of brush-like marks are visible but they did not penetrate the remaining cuticle. They might have been caused by brushing other fruit in the brine, mechanical handling or pumping. However, the brined samples were the only ones with the brush-like appearance.

The secondary bleached cherry also had a reduced cuticle thickness, that was devoid of wax as illustrated in Sudan stained sections (Figure 26). This was to be expected because 1) the cherries have been brined before the secondary bleach, and 2) the chlorite is a very

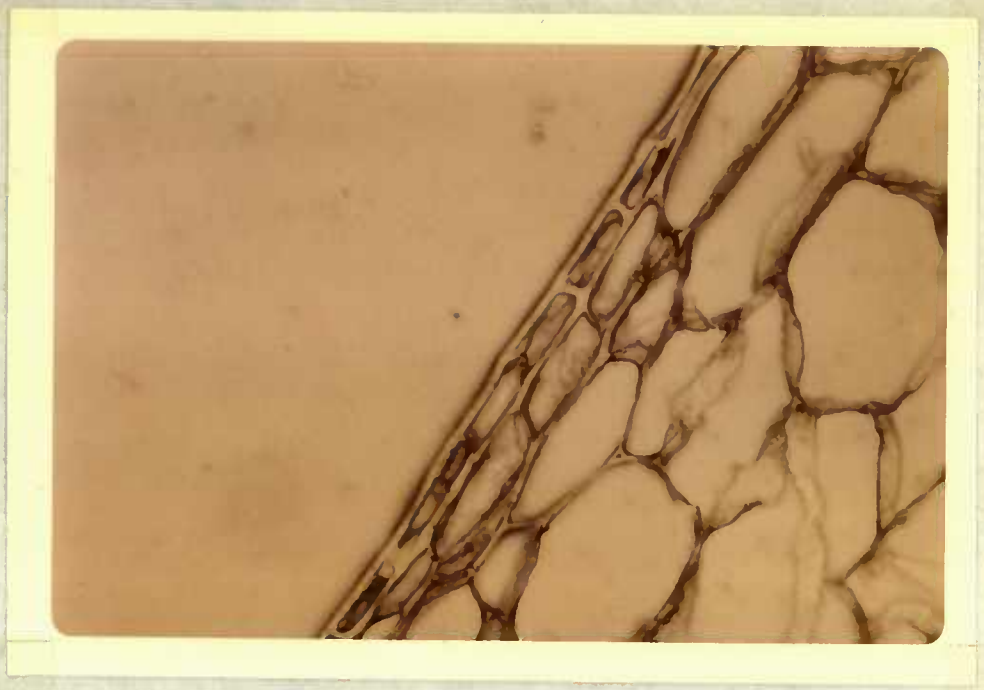


Figure 23. SO_2-calcium bisulfite brined cherry section before staining. 128X

Figure 24. SO_2-calcium bisulfite brined cherry section stained for the cuticle showing typical epidermal cell structure (Sudan IV). 128X

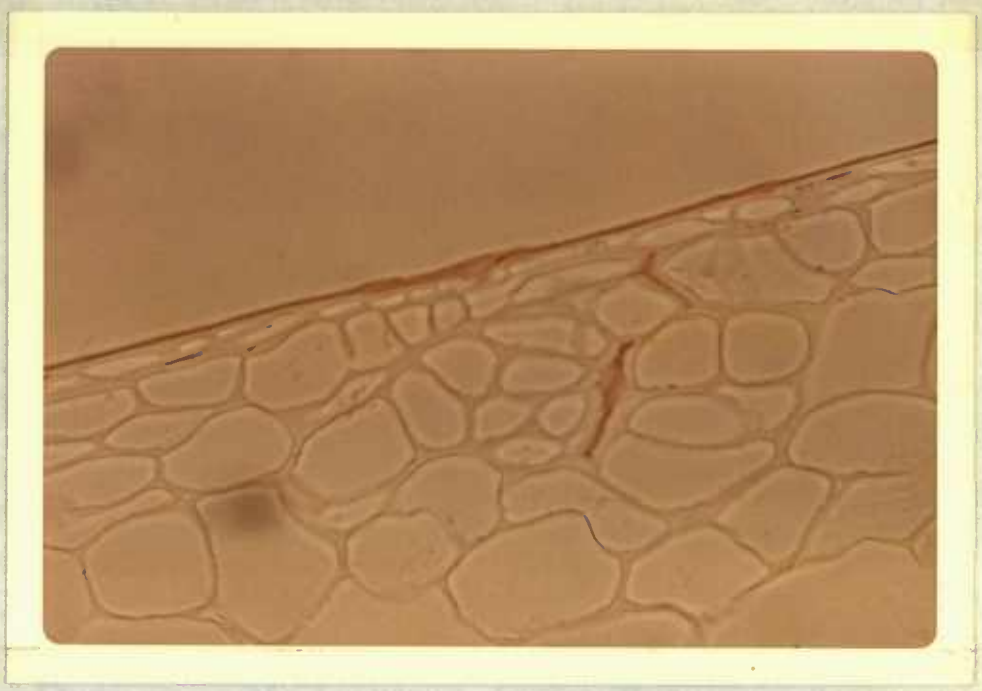


Figure 25. SO_2-calcium bisulfite brined cherry section stained for the cuticle showing atypical epidermal cell structure (Sudan IV). 128X

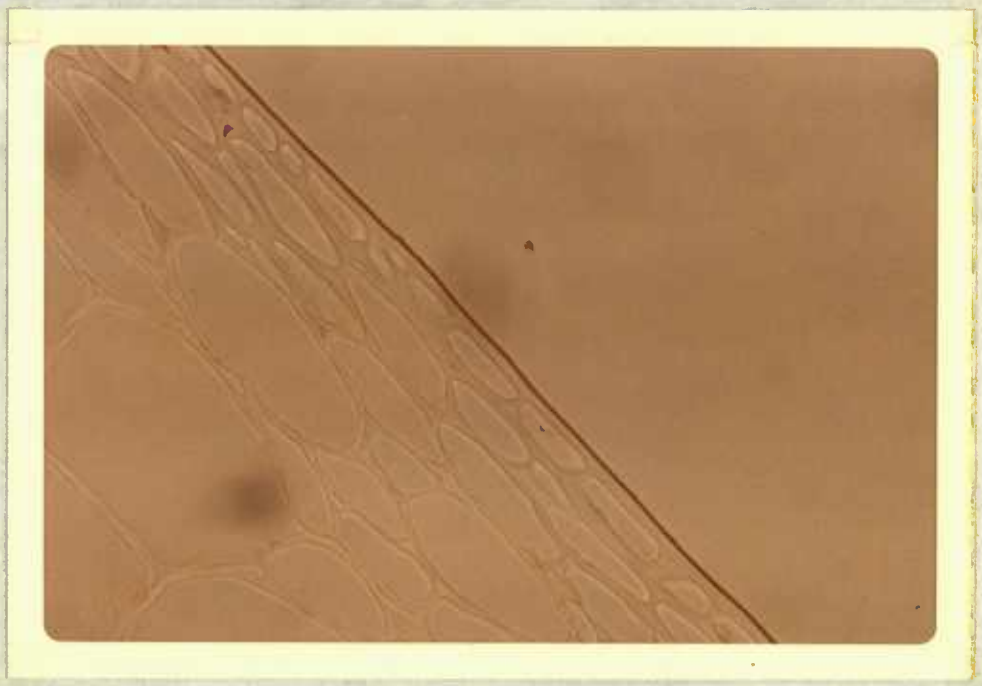


Figure 26. Secondary bleached brined cherry section stained for the cuticle (Sudan IV). 128X

Figure 27. Scanning electronmicrographs of a SO_2-calcium bisulfite brined cherry and a secondary bleached cherry.

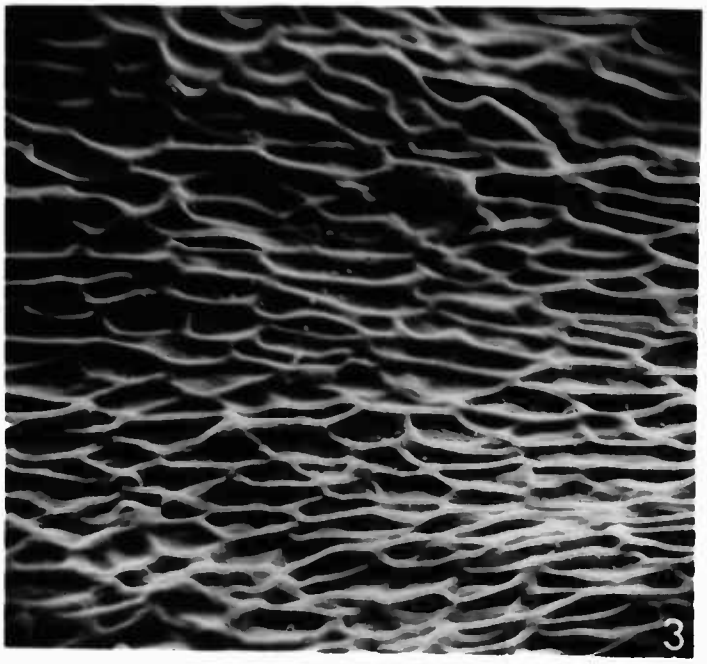
27-1. Surface of a SO_2-calcium bisulfite brined cherry. 58X

27-2. Surface of the upper right hand corner of Figure 27-2. 162.5X

27-3. Surface of a secondary bleached brined cherry. 213X. Note the difference of the surface from the other untreated and treated cherries.


1


2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strong oxidizing agent. The secondary bleach hardened the tissue even more than the brined samples which allowed even the largest thin walled parenchyma cells to withstand sectioning (Figure 26). The surface of the cuticle is very different from any of those examined earlier (Figure 27-3). The periclinal walls have collapsed leaving the anticlinal walls protruding. Part of this effect is undoubtedly an artifact of the vacuum needed for electron microscopy, but the majority is real. The cuticle has been reduced enough in size and strength that periclinal walls are allowed to collapse (this did not happen in untreated or brined samples).

The effect of the secondary bleach on the surface wax and cuticle would then be expected to make the cuticle less of a barrier to penetration than the brined samples or the fresh samples. This was found to happen in the penetration studies.

Ectodesmata. The surface distribution of MP in the astomatous areas of the cuticle was found to be generally random with more over the anticlinal walls than the periclinal walls (Figure 28). However, the number of MP's was less than that found in onion bulb scales (Franke, 1969).

The distribution of MP in stomatous areas was found to be over and around guard cells (Figure 29). The irregularities in focus are due to focusing on the depressed stomata. Cross-sectional views through the stomata confirmed the localization of MP in the guard cells

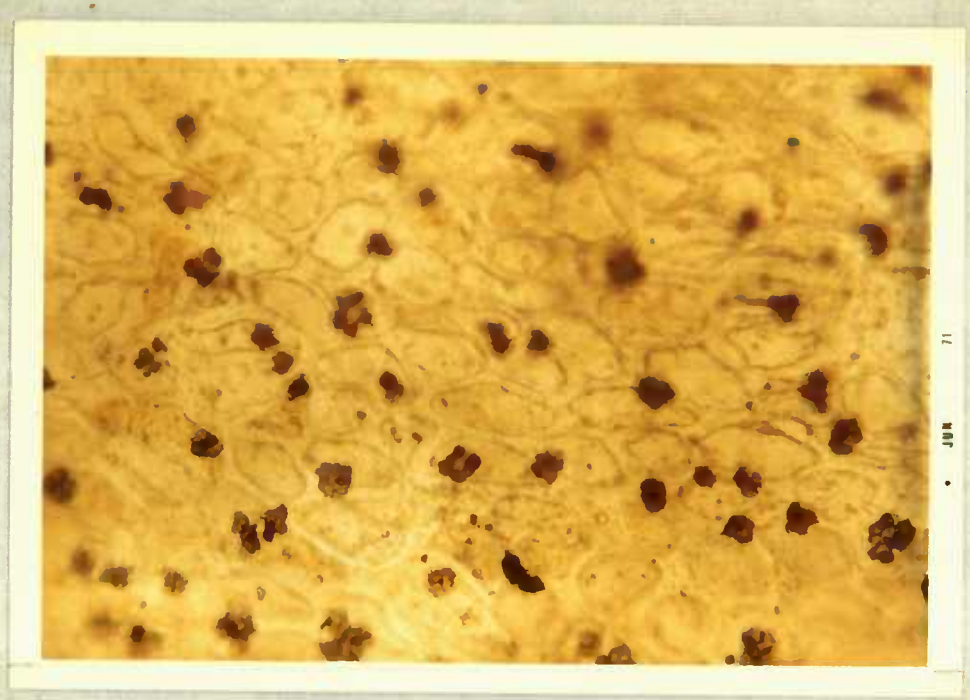

Figure 28. Mercury precipitates (MP) on the surface of an immature cherry opposite the suture. 128X Note MP's over anticlinal walls.

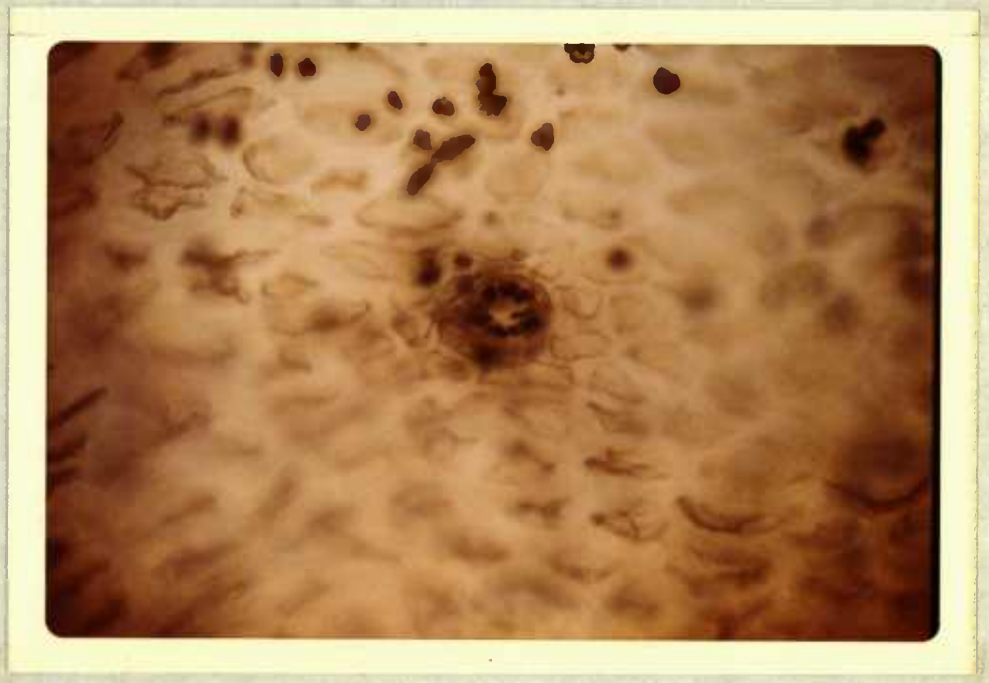

Figure 29. Mercury precipitates (MP) on and around a stomata on the cuticle from an immature suture cherry. 128X. Note MP's not in stomatal aperture.

(Figures 30 and 31). Figure 31 shows birefringence in the guard cells under polarized light with the first order compensating filter in the light path. The cross sections through the cuticle failed to show any MP through the cuticle except in guard cells (Figure 30). The MP bound on the surface were displaced during sectioning. The overall distribution pattern of MP seemed to be more intense on the opposite suture than on the suture side of the cherry.

It was observed that the number of MP greatly increased where the surface wax and cuticle were disturbed. This was also observed by Schönherr and Bukovac (1970). This again points to the significant role played by the wax in the penetration of water and chemicals.

The presence of the MP pathways in the cherry cuticle reflect areas that are undoubtedly permeable to polar compounds and have a marked influence in brine penetration.

Effect of Wax Removal on Weight Loss

The data obtained from the wax removal experiments are presented in Tables 6 to 8 (Figure 34 to 37). Figure 32 shows a typical treated sample and control for the 70-hour period. The chloroform dipping treatment was found to cause rapid browning (Figure 35), undoubtedly due to cellular extraction of lipids and allowing enzymes and substrates to mix. This was also observed by Ponting and McBean (1970). An analysis of regression (Snedecor and Cochran, 1967) of the

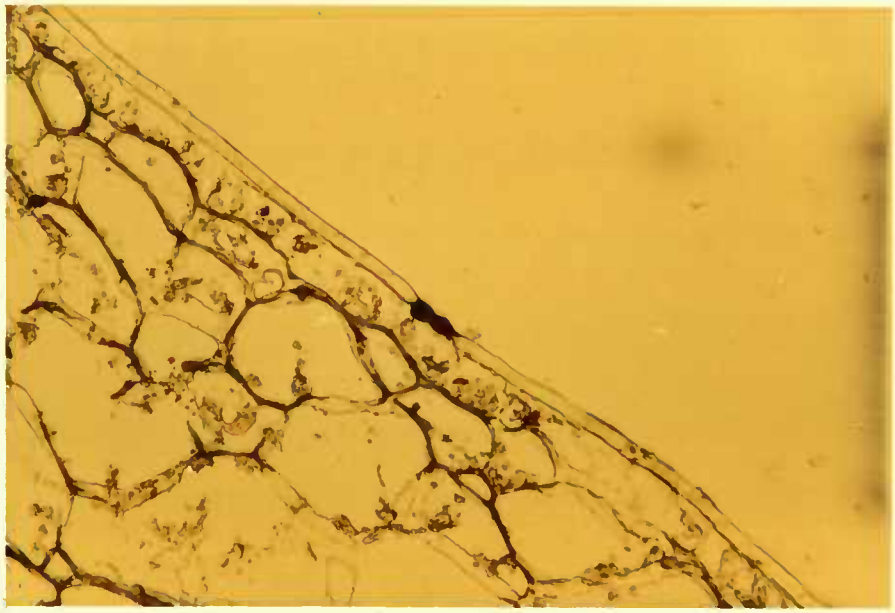

Figure 30. Mercury precipitates (MP) in the guard cells of an overmature cherry (normal light). 128X

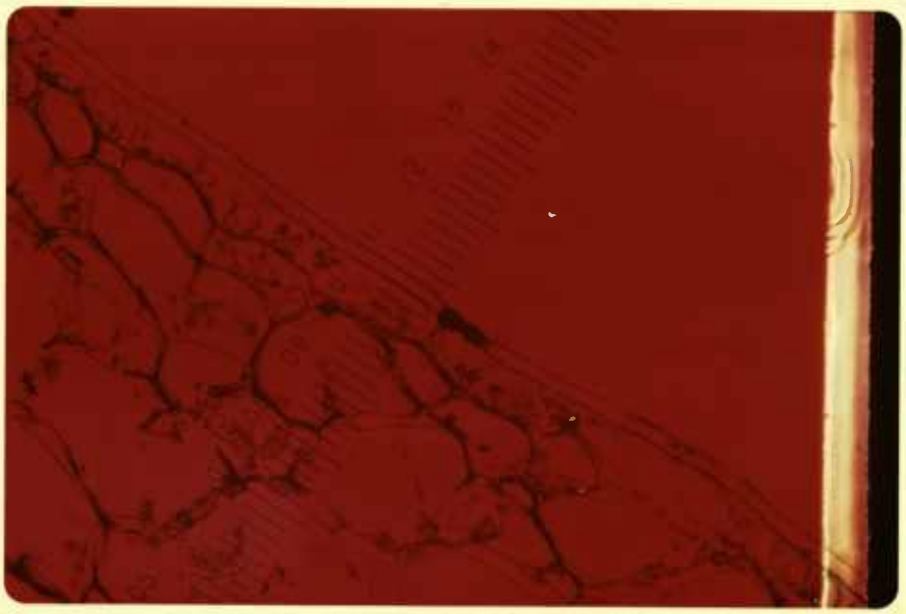

Figure 31. Mercury precipitates (MP) in the guard cells of an overmature cherry under polarized light, with a first order red compensating filter in the light path. 128X. Note birefringence in guard cells from MP's present. Each division of micrometer equals 8.1 μ.

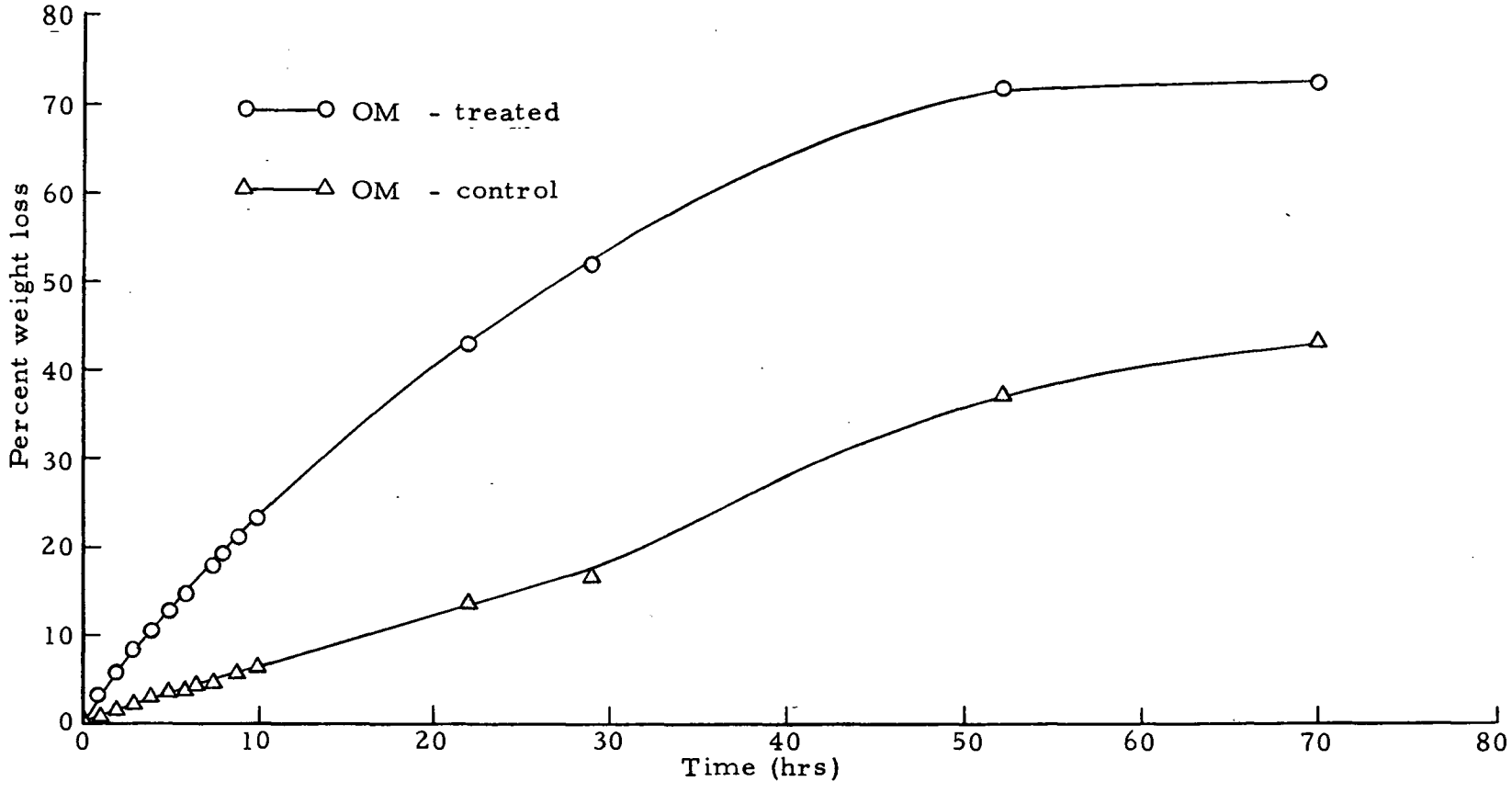

Figure 32. Percent weight loss for chloroform dipped and control overmature cherries. 38 C (100 F), 27 percent R. H.

Table 6. Percent weight loss of chloroform treated and control overmature cherries.

Time (hrs)	Percent weight loss (5 cherries per replicate)				
	A. Chloroform Treated				
	A	B	C	D	E
0	0	0	0	0	0
1.00	3.354	4.706	4.539	3.884	3.874
2.00	5.854	7.267	--	--	--
3.00	8.230	9.848	--	--	--
4.00	10.459	12.304	10.323	9.870	10.284
5.00	12.616	14.638	12.604	12.139	12.873
6.00	14.744	--	14.781	15.358	15.376
7.00	17.859	18.456	17.016	16.473	17.620
8.00	19.015	19.769	--	--	--
9.00	21.072	21.904	--	--	--
10.00	23.059	23.949	23.616	22.966	24.442
11.00	--	--	25.861	25.034	26.593
12.00	--	--	28.5489	27.671	29.501
22.00	42.909	44.166	44.534	42.525	45.239
23.00	--	--	45.981	43.935	46.702
24.00	--	--	47.379	45.297	48.084
28.00	51.798	52.020	52.105	49.660	53.162
30.00	--	--	54.196	51.729	55.562
36.50	--	--	60.415	57.025	61.977
47.00	--	--	67.301	64.150	68.307
48.00	--	--	67.737	64.6050	68.666
52.00	71.544	68.582	--	--	--
70.00	72.358	69.335	--	--	--

(Continued on next page)

Table 6. (continued)

Time (hrs)	Percent weight loss (5 cherries per replicate)				
	B. Control				
	AA	BB	CC	DD	EE
0	0	0	0	0	0
1.00	0.783	0.881	1.282	1.048	1.341
2.00	1.438	1.525	--	--	--
3.00	2.058	2.173	--	--	--
4.00	2.662	2.868	3.318	2.848	2.983
5.00	3.258	3.506	4.079	3.461	3.584
6.00	3.846	--	4.812	4.084	4.179
7.00	4.7432	4.525	5.554	4.666	4.740
8.00	5.002	4.878	--	--	--
9.00	5.594	5.468	--	--	--
10.00	6.135	6.054	7.828	6.556	6.501
11.00	--	--	8.556	7.122	7.028
12.00	--	--	9.457	7.914	7.761
22.00	13.979	13.077	15.744	13.257	12.677
23.00	--	--	16.304	13.732	13.133
24.00	--	--	16.895	14.213	13.576
28.00	16.4969	16.806	19.156	16.110	15.515
30.00	--	--	20.262	17.071	16.422
36.50	--	--	24.068	20.185	19.426
42.00	--	--	29.645	25.259	24.070
48.00	--	--	30.1284	25.674	24.467
52.00	37.095	39.434	--	--	--
70.00	43.339	46.650	--	--	--

Table 7. Percent weight loss of chloroform dipped and control mature cherries.

Time (hrs)	Percent weight loss (5 cherries per replicate)				
	A. Chloroform Treated				
	A	B	C	D	E
0	0	0	0	0	0
1.75	4.313	6.760	6.013	4.998	5.083
3.25	6.579	9.141	9.267	7.832	7.716
4.75	10.018	11.708	12.190	10.731	11.163
6.75	12.810	15.753	15.666	14.205	14.762
8.75	16.564	19.974	19.589	17.792	18.270
10.75	20.072	22.233	23.701	21.176	21.579
12.50	23.590	25.069	27.520	24.604	24.705
22.50	42.168	41.554	43.181	39.176	40.711
69.50	74.670	72.383	73.435	73.428	74.087
	B. Control				
	AA	BB	CC	DD	EE
0	0	0	0	0	0
0.75	0.513	--	--	--	--
1.50	1.593	1.520	1.480	1.330	1.214
3.50	3.083	3.131	3.122	2.982	2.700
5.50	4.487	4.554	4.705	4.622	4.136
7.50	5.712	5.945	6.222	6.149	5.467
9.25	6.857	7.229	7.577	7.539	6.721
19.25	12.681	13.808	14.525	14.476	12.928
66.50	36.506	39.932	41.589	41.407	38.602

Table 8. Percent weight loss of chloroform dipped and control immature cherries.

Time (hrs)	Percent weight loss (5 cherries per replicate)				
	A. Chloroform treated				
	A	B	C	D	E
0	0	0	0	0	0
1.75	5.014	4.958	4.707	5.554	4.226
3.00	7.998	7.853	7.873	7.995	6.400
4.00	10.788	10.613	10.563	10.477	9.067
5.50	13.942	14.152	14.340	13.562	12.146
7.00	17.546	17.913	17.779	16.862	15.568
9.00	22.393	22.613	22.082	21.075	19.852
11.00	26.392	27.045	26.625	24.912	24.130
13.00	30.625	31.454	30.693	28.687	28.099
24.75	50.866	52.967	51.546	48.539	48.841
72.00	78.662	78.110	79.158	79.445	78.929
	B. Control				
	AA	BB	CC	DD	EE
0	0	0	0	0	0
0.75	0.666	0.647	0.705	0.583	0.504
2.25	1.625	1.604	1.703	1.590	1.422
4.25	2.966	2.934	3.043	3.004	2.722
6.25	4.214	4.186	4.291	4.306	3.883
8.25	5.670	5.374	5.469	5.520	4.990
10.00	6.451	5.823	6.572	6.674	6.022
20.00	11.977	12.059	12.132	12.429	11.295
67.00	34.764	35.680	35.165	35.703	33.029

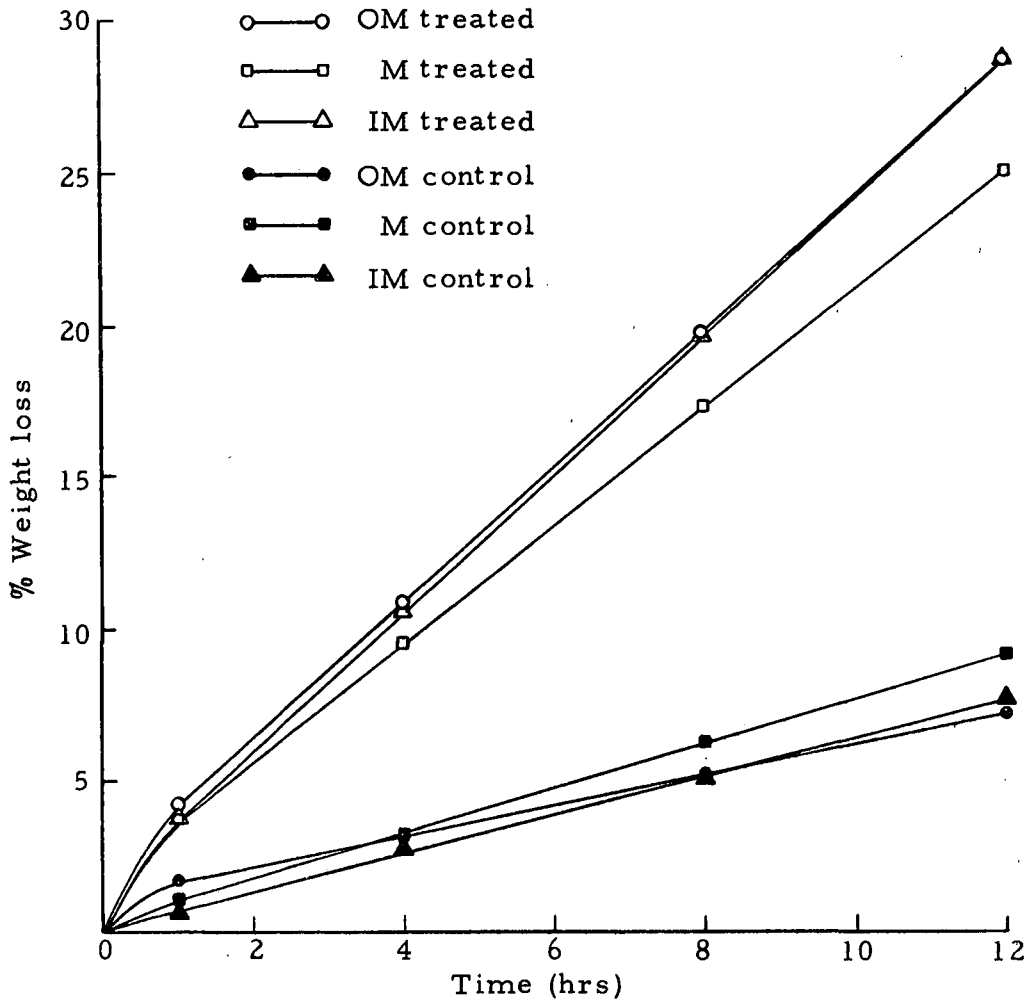

Figure 33. Weight loss of chloroform dipped and control cherries of different maturity classification stored 12 hours. 38 C (100 F), 27% R.H.

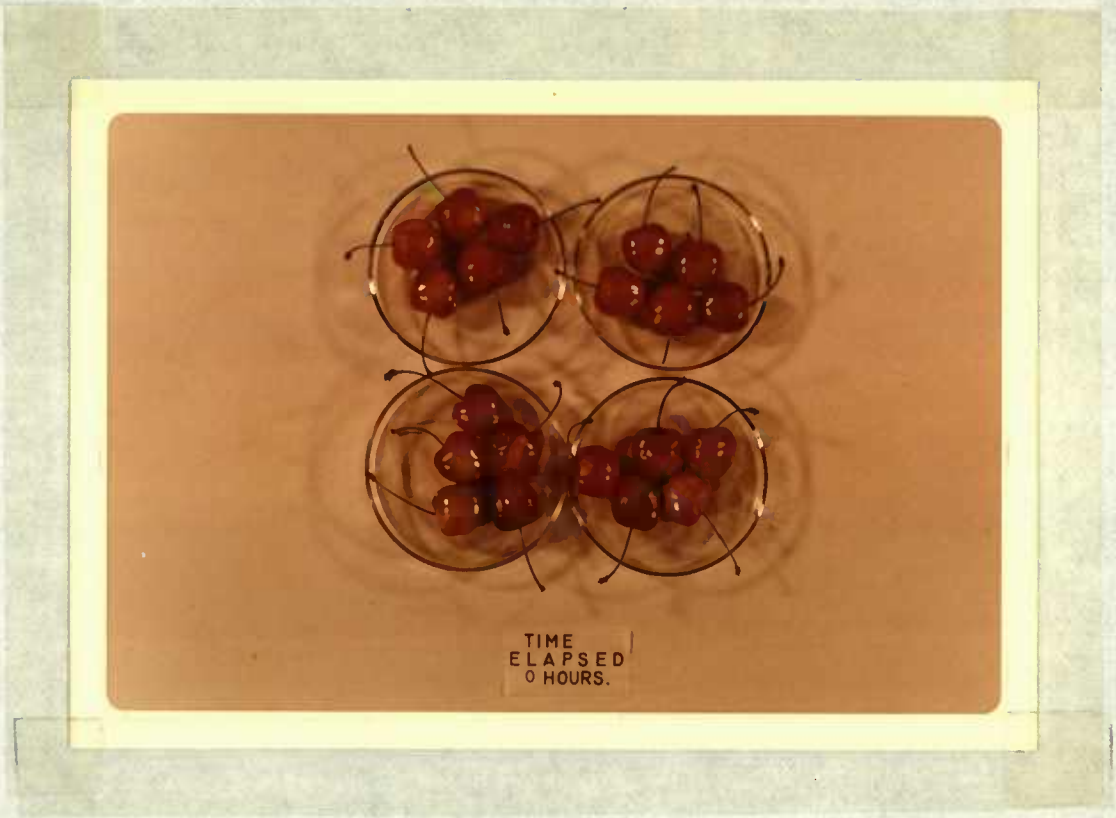

Figure 34. Control cherries before dipping in chloroform in weight loss study.

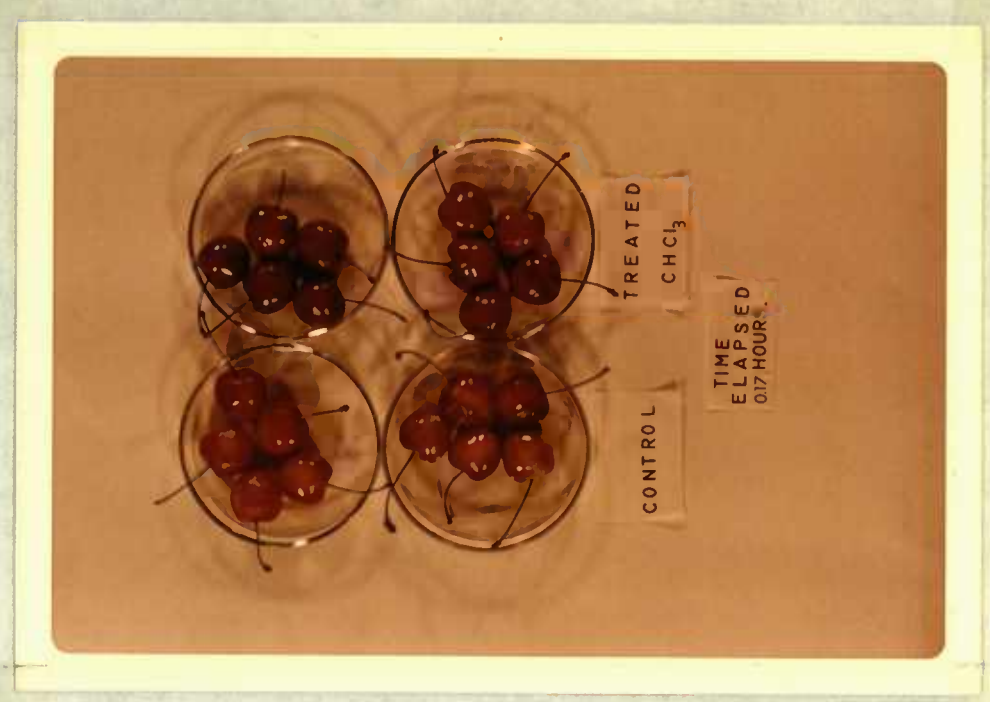

Figure 35. Cherries 0.17 hours after dipping and storage. 38 C (100 F), 27 percent relative humidity.

Figure 36. Cherries 24 hours after dipping and storage. 38 C (100 F), 27 percent relative humidity.

Figure 37. Cherries 48 hours after dipping and storage. 38 C (100 F), 27 percent relative humidity.

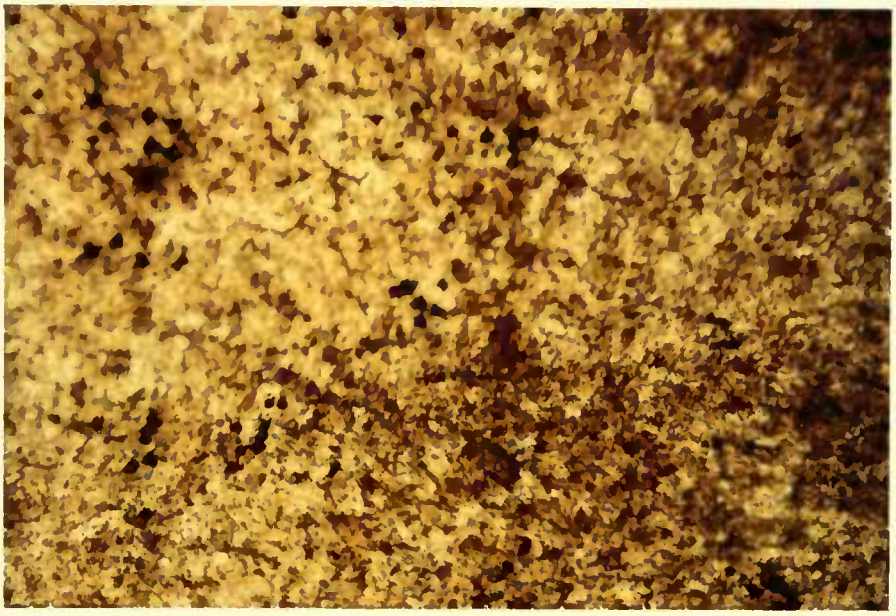

Figure 38. Artifacts under the cherry cuticle after enzymatic isolation (under normal light). 20X

first 12 hours and the statistical values are shown in Tables 9 to 11. Each treatment and control for each maturity was found to have linear relation that was significant at the 1 percent level for the first 12 hours (Figure 33).

A comparison of the treatment and control regression coefficients within each maturity was made (Table 12). This test showed that in all cases the treatment regression coefficient was significantly different from its respective control regression coefficient at the 1 percent level. The percent weight loss for the de-waxed samples is from 2.84 to 4.00 times greater than for the untreated controls during the first 12 hours. Micke (1966) also found that a rapid moisture loss occurred during the first eight hours (5 percent) at 26.7 to 30 C (80 to 86 F) after harvest in Bing cherries. This shows the importance of the cuticular wax in regulating transpiration in the Royal Anne cherry. It is also interesting to note that Levin et al. (1959) found a 5.38 percent increase in weight of Bing cherries soaked in a water solution at 29.5 F (85 C) after five hours.

Isolated Cuticles

In addition to the data in the procedure section on the enzymatic isolation of cherry cuticles, a number of other observations were made.

It was found that light inhibited the isolation of the cuticles,

Table 9. Analysis of regression of chloroform treated and control overmature cherries.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Level of significance P=.05	Level of significance P=.01
		Treatment			
Total	40	2027.9			
Regression	1	1983.9	1983.9	S	S
Error	39	44.0	1.13		
Regression coefficient (slope): 2.224					
Intercept: 1.960					
Regression line: Y = 2.224X + 1.960					
		Control			
Total	40	170.50			
Regression	1	93.64	93.64	S	S
Error	39	76.06	1.95		
Regression coefficient: 0.4956					
Intercept: 1.2143					
Regression line: Y = 0.4956X + 1.2143					

Table 10. Analysis of regression of chloroform treated and control mature cherries.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Level of significance P=.05	Level of significance P=.01
		Treatment			
Total	31	6830			
Regression	1	6790	6790	S	S
Error	30	40	1.33		
Regression coefficient (slope): 1.925					
Intercept: 1.877					
Regression line: Y = 1.925X + 1.877					
		Control			
Total	25	590.3			
Regression	1	586.6	586.6	S	S
Error	24	3.7	0.154		
Regression coefficient: 0.7364					
Intercept: 0.3030					
Regression line: Y = 0.7364X + 0.3030					

Table 11. Analysis of regression of chloroform treated and control immature cherries.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Level of significance P=.05	Level of significance P=.01
		Treatment			
Total	34	9026.9			
Regression	1	9000.2	9000.2	S	S
Error	33	26.7	0.810		
Regression coefficient: 2.883					
Intercept: 1.473					
Regression line: Y = 2.883X + 1.473					
		Control			
Total	29	490.9			
Regression	1	488.7	488.7	S	S
Error	28	2.2	0.0786		
Regression coefficient: 0.6187					
Intercept: 0.2485					
Regression line: Y = 0.6187X + 0.2485					

Table 12. Statistical significance of regression of weight loss between chloroform dipped and control cherries as a function of maturity.

Comparison	$\overline{MSE}$	t	Degrees of freedom	Level of significance	
				P=.05	P=.01
IM_c vs IM_t	0.474	61.38	54	S	S
M_c vs M_t	0.809	31.76	61	S	S
OM_c vs OM_t	0.411	17.57	78	S	S

which would indicate a light sensitive site on the enzyme. A histidine residue might be implicated as they have been found to be light sensitive active sites in other enzymes (Dixon and Webb, 1964).

A comparison of the isolating power of the enzyme solutions B-11 and B-12 was made using tomato, apple, plum and cherry fruit cuticles. Both solutions yielded a completely isolated tomato and apple fruit cuticle, thus showing that the enzyme solution B-12 was comparable to that used by Yamada et al. (1964b). However, the cherry and plum fruit cuticles (which are of the same genus) were never completely separated from their constituents.

Inactivation of plant enzymes by phenolics, tannins, quionones of the polyphenoloxidase system has been found in many plant species when the tissue is disturbed allowing contact of the phenols with the polyphenoloxidase (Loomis and Battaile, 1966; Anderson, 1968). However, as both apple and cherries have this system and the apple cuticles were isolated, it would indicate that while this mechanism will have an effect of slowing isolation it does not account for the incomplete isolation of the cherry cuticle. Further, it was interesting to note that when soluble polyvinylpyrrolidone (PVP) was added to the enzyme solutions, that the browning reaction was stopped in the cherries. However, unfortunately, so was the enzymatic isolation. The latter observation may have been caused by using too much PVP thus diluting the solution, as PVP has been found to be relatively

inert with respect to possible reaction with protein (Loomis and Battaile, 1966). These observations would indicate 1) the enzymes are unable to penetrate to the area beneath the cuticle; 2) the cellulose of the epidermal cell walls is embedded in the cuticle (as noted in the structure study) where it cannot be reached by enzymatic attack; and 3) the incomplete isolation may be a genetic characteristic as it occurred in both species of Prunus tested.

The enzymatically isolated cherry cuticles were found to contain an artifact due to the isolation procedure (Figures 21 and 38). This has not been observed before by other investigators: the structure of the pear leaf cuticle (Norris and Bukovac, 1968) was found to be little effected by isolation (Bukovac and Norris, 1966). Norris and Bukovac (1969) found that the permeability of a tomato cuticle was not altered by the enzymatic isolation when compared to a mechanically isolated cuticle.

These artifacts are birefringent crystalline objects in the cellulose layer near the cuticle (Figures 21 and 38). They were close enough to the cuticle that their removal by brushing damaged the cuticle. They were visibly unaffected by the penetration of brine and its components. The birefringent pattern differed from that of starch. It is very possible that the artifact was due to the incorporation of cellulose materials into the cuticular layer thereby protecting it from enzyme degradation. It appears that the artifact is

characteristic of the Prunus species tested as there were no artifacts in the tomato or apple cuticles isolated.

Scanning electronmicrographs of the isolated cuticle showed a somewhat similar surface as found on the unisolated cuticle. However, there were artifacts produced from the drying of the cuticle over the irregular shaped layer of cellulose on the inner surface. Thus it was not possible to see if the inner surface of the cherry showed secondary cutinisation and deep ridges, pegs or pitting as found in fruit membranes or the fine ridges and thick V-shaped ridges of leaf membranes (Lange, 1969; Baker, 1970).

Penetration of Brine Through Isolated Cherry Cuticles

The cellulose debris that remained after isolation may have an effect on penetration. However, Norris and Bukovac (1969) found that there was no significant change in penetration rate due to an "isolated" cuticle and an isolated cuticle with the cellulose removed with zinc chloride/HCl. This would indicate that their penetration of NAA through the pear cuticle was the rate limiting step rather than being effected by the cellulose debris.

However, care must be made when comparing penetration of ions through isolated cuticles that have used other methods for the isolation process. Norris and Bukovac (1969) found that isolation techniques using ammonium oxalate/oxalic acid (Baker et al., 1964) caused

greater permeability in the isolated cuticle than either the mechanically or enzymatically isolated methods. Thus care must be taken in interpreting the results from ammonium oxalate/oxalic acid isolated cuticle penetration data.

The results on penetration of the membranes to Ca^{++}, H^{+}, SO_2, and K^{+} are summarized in Figures 39 to 54. In general, SO_2 was found to penetrate more rapidly than Ca^{++} (Figures 46 and 51). However, this may be due to the concentration difference between the two ions. A comparison of the penetration of SO_2 and Ca in the brine through a standard dialysing membrane gave a two fold increase in penetration of SO_2 over that of Ca.

Penetration studies through the isolated overmature suture cherry cuticles (OM_s) from the outside to the inside (O-I) were not obtained in all cases due to damaged cuticles. They became brittle "varnish like" on drying, which caused them to crack very easily.

It has been found that the major chemical species of sulfur dioxide in water depends on the concentration used (Schroeter, 1966). At 0.1M the major chemical species is molecular SO_2 in water (SO_2 + H_2O) and the bisulfite ion, as in the following equation:

$$SO_2 + H_2O \rightleftharpoons HSO_3^- + H^+ \qquad a)$$

while at a concentration of 1M the bisulfite ions and the hydrogen pyrosulfite ions ($HS_2O_5^-$) would be present as in the following equation:

$$HSO_3^- + SO_2 \rightleftharpoons HS_2O_5^- \qquad b)$$

DeMaeyer and Kusten (1963) found that the hydration of sulfur dioxide (a) appears to be one of the most rapid hydrolytic reactions known.

In cherry brine the concentration is very near 0.1M and as the chemical species present is also dependent on pH, the equilibrium equation for a sulfur dioxide-calcium bisulfite brine as modified from Schroeter (1966) and Payne et al. (1969) is as follows.

$$SO_2 + H_2O \rightleftharpoons HSO_3^- + H^+ \xrightleftharpoons{Ca(OH)_2} Ca(HSO_3^-)_2 \xrightleftharpoons{Ca(OH)_2} CaSO_3\downarrow + H_2O$$

$$SO_2 \uparrow\downarrow$$

$$HS_2O_5^- + H^+$$

Thus, the distribution of free SO_2 in pH range of 2.8 to 3.0 is approximately

9.0 to 6.0%	$(SO_2 + H_2O)$ or H_2SO_3
90.3 to 93.0%	HSO_3^-
0.7 to 1.0%	$SO_3^=$ (Payne et al., 1969)

Therefore the major species in brine at this pH range would be calcium bisulfite ($Ca(HSO_3^-)_2$); bisulfite (HSO_3^-) and molecular SO_2 in water ($SO_2 + H_2O$).

When the cherry is immersed in water or brine, the cuticle takes up water which fills the spaces within the cutin network as the polymer swells. This allows the carboxyl groups not involved with internal

linkages to be free for ionization, which gives the cuticle the properties of a weak ion exchanger.

As the cuticle tends to be acidic in its reactions (Crowdy, 1959) a large portion of the carboxyl groups present in the pH range of 2.8 to 3.0 would be in their undissociated form. This would allow for three possible routes of penetration through the cherry cuticle.

The first route would be the penetration of the undissociated $Ca(HSO_3^-)_2$ and molecular SO_2 species by partitioning through the lipophilic areas of the cuticle. This mode of entry has been supported by a number of investigators (Simon and Beevers, 1952; Leopold, 1964; Bukovac, 1965) who found that at the pk value or lower, an undissociated acid would account for biological activity. This, in itself, would give a penetration ratio of SO_2 to Ca^{++} in a 2:1 ratio or greater.

The cuticle in its undissociated form can also give two polar routes of penetration. One would be that the cuticle in this form is more permeable to anions, as the cuticle would have a slight positive charge, whereas at higher pH values the cuticular carboxyl groups would dissociate giving the cuticle a negative charge. This is supported by Orgell and Weintraub (1957) who found better absorption of 2, 4-D occurred at low pH in the absence of cations. However, absorption at neutral or alkaline pH was aided by the presence of cationic wetters and cations, which neutralized the charge on the

cuticle. In brine, the negatively charged HSO_3^- species would be attracted to the positive charges on the cuticle, allowing penetration to occur.

However, even at this low pH some of the carboxyl groups could still dissociate and be free to bind the positively charged Ca^{++}, H^+ and/or other available cations. Thus, the cation could be bound and penetrate the cuticle. The gradient of polarity suggested by the structure of the cherry cuticle would readily allow the cations to follow the gradient toward the relatively lower concentration on the inside of the cuticle. While occurring at a limited extent at this pH level, this type of penetration would be favored at higher pH levels where the anions would be repelled by the negatively charged cuticle. Yamada et al. (1965b) found that cations were preferentially absorbed over anions. This also explains why there would be greater binding and penetration of cations over anions (Wittwer, 1964).

Penetration through the cuticle of the individual ions from water solutions was greater than the penetration of the individual ions from the normal brine (Figures 44, 49 and 50). This can be explained not only by competition for binding sites, but by the change in ion exchange properties of the cuticle. Calcium would be expected to penetrate faster at pH 5.8 where the cuticle would have more of a negative charge due to dissociation that at the lower brine pH. This would allow the cuticle to bind more cations and allow them to penetrate

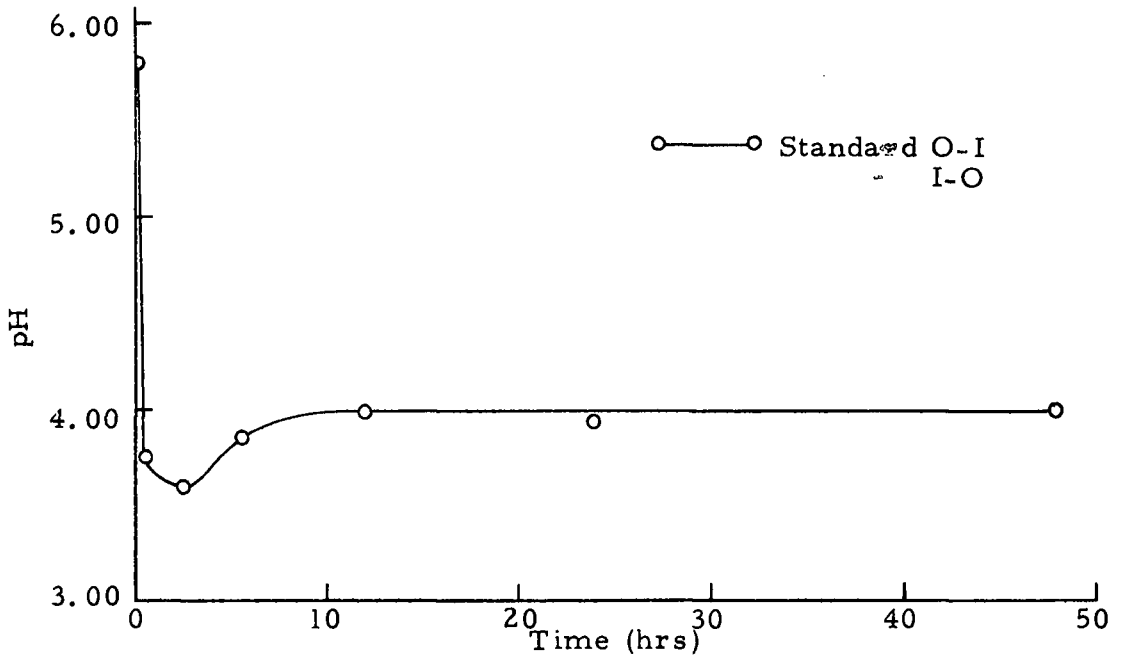

Figure 39. Hydrogen ion (brine) penetration through a standard dialyzing membrane.

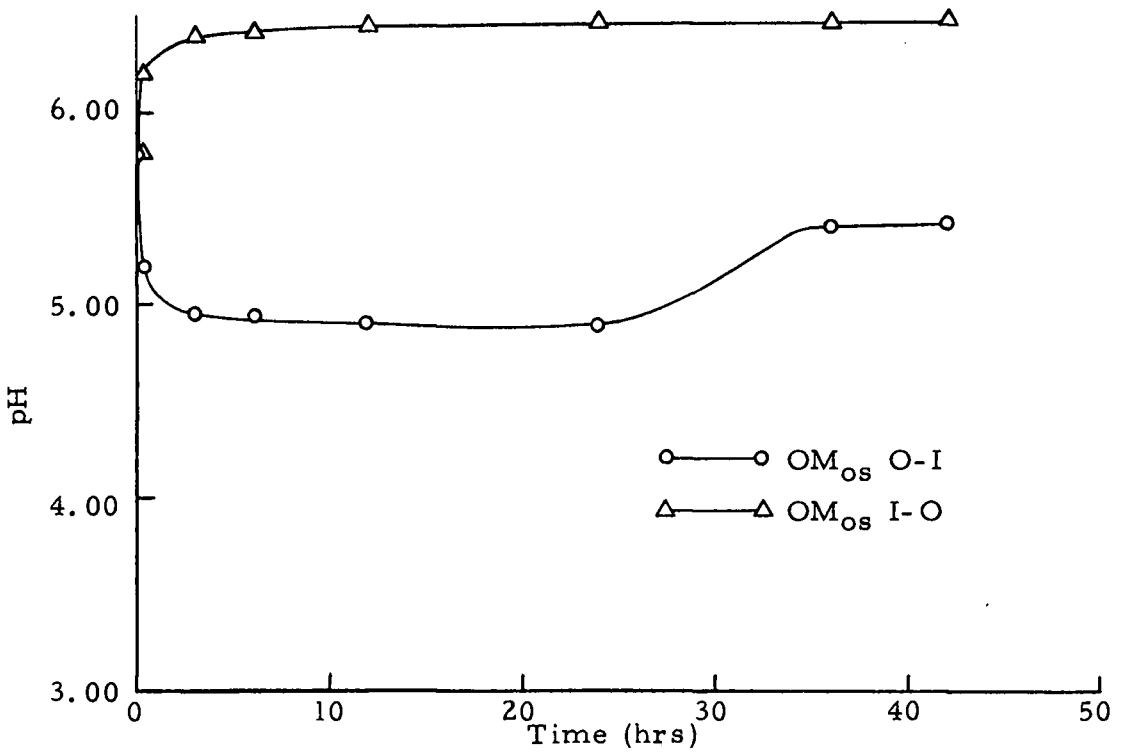

Figure 40. Hydrogen ion (brine) penetration through isolated over-mature cherry cuticles.

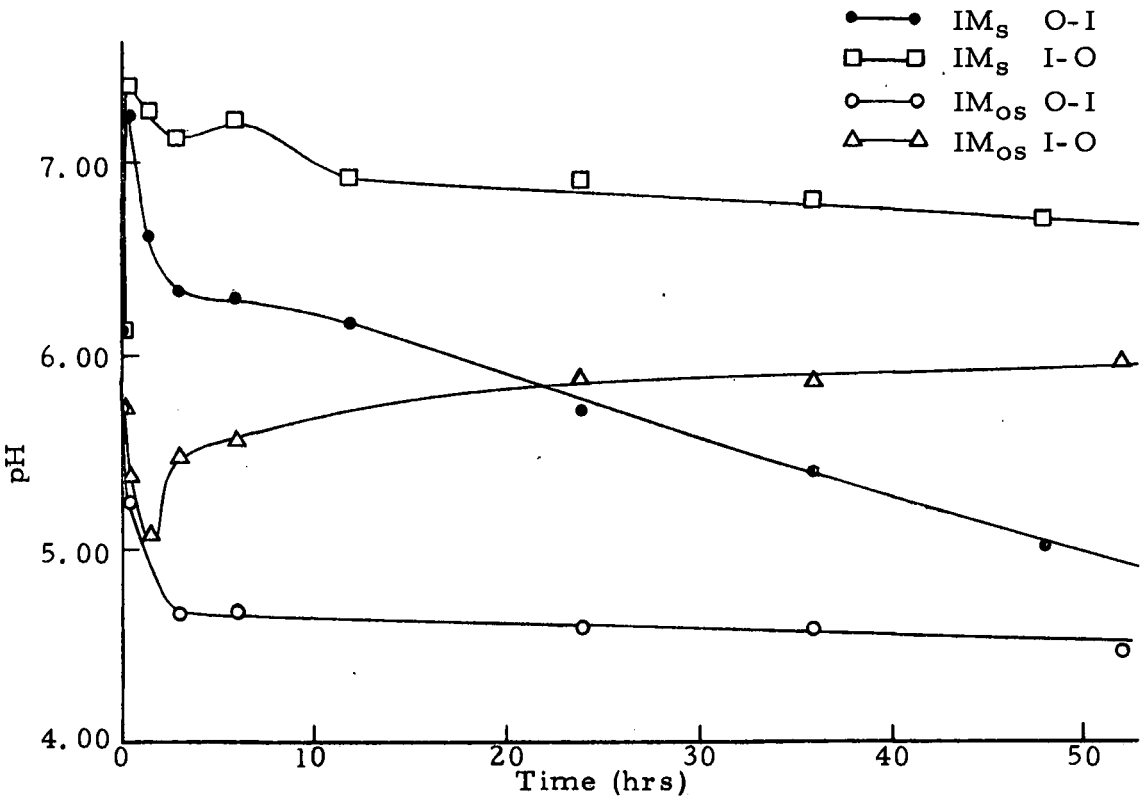

Figure 41. Hydrogen ion (brine) penetration through isolated immature cherry cuticle.

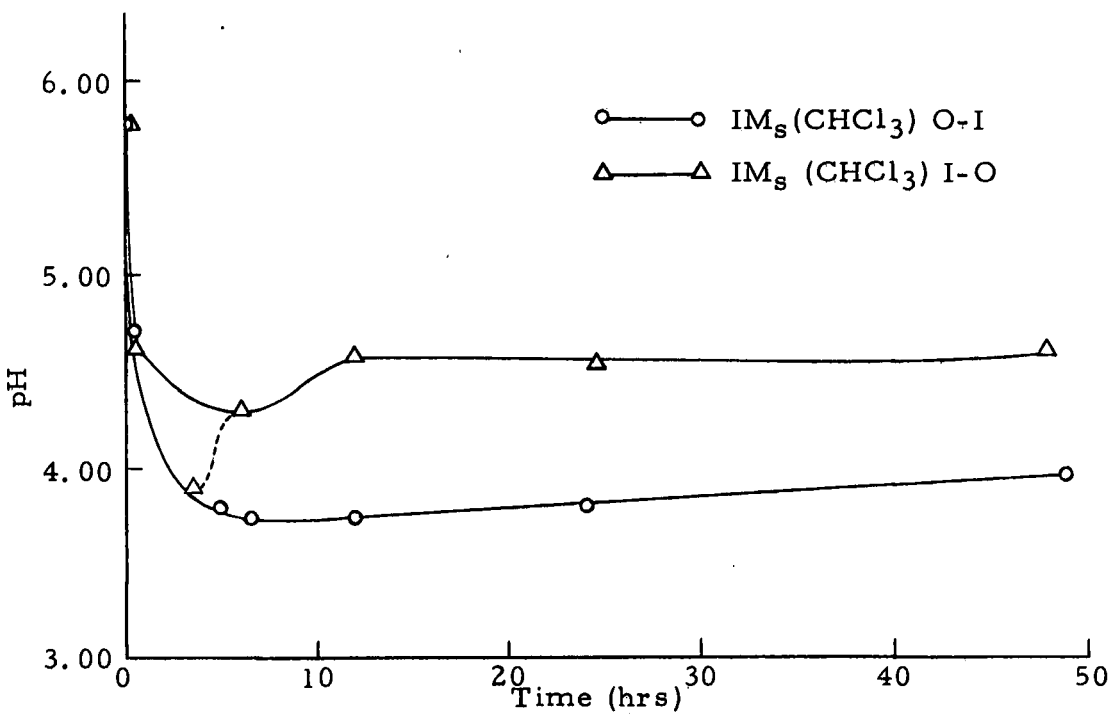

Figure 42. Hydrogen ion (brine) penetration through isolated chloroform dipped cherry cuticles.

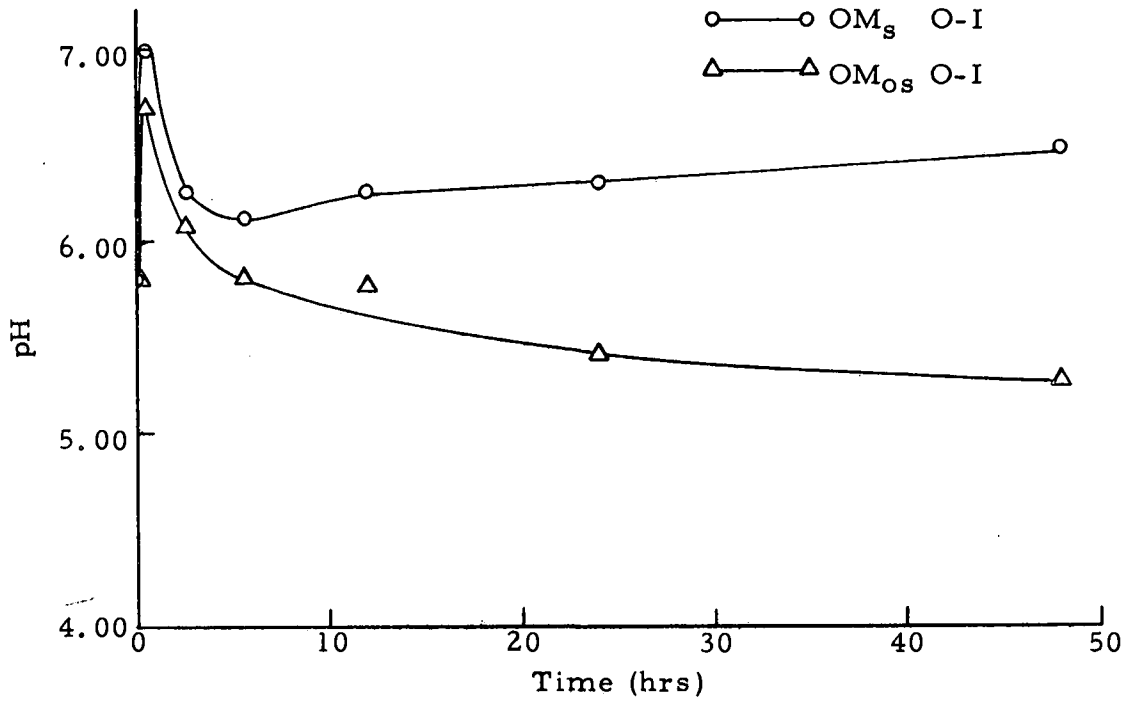

Figure 43. Hydrogen ion (potassium-brine) penetration through isolated overmature cherry cuticles.

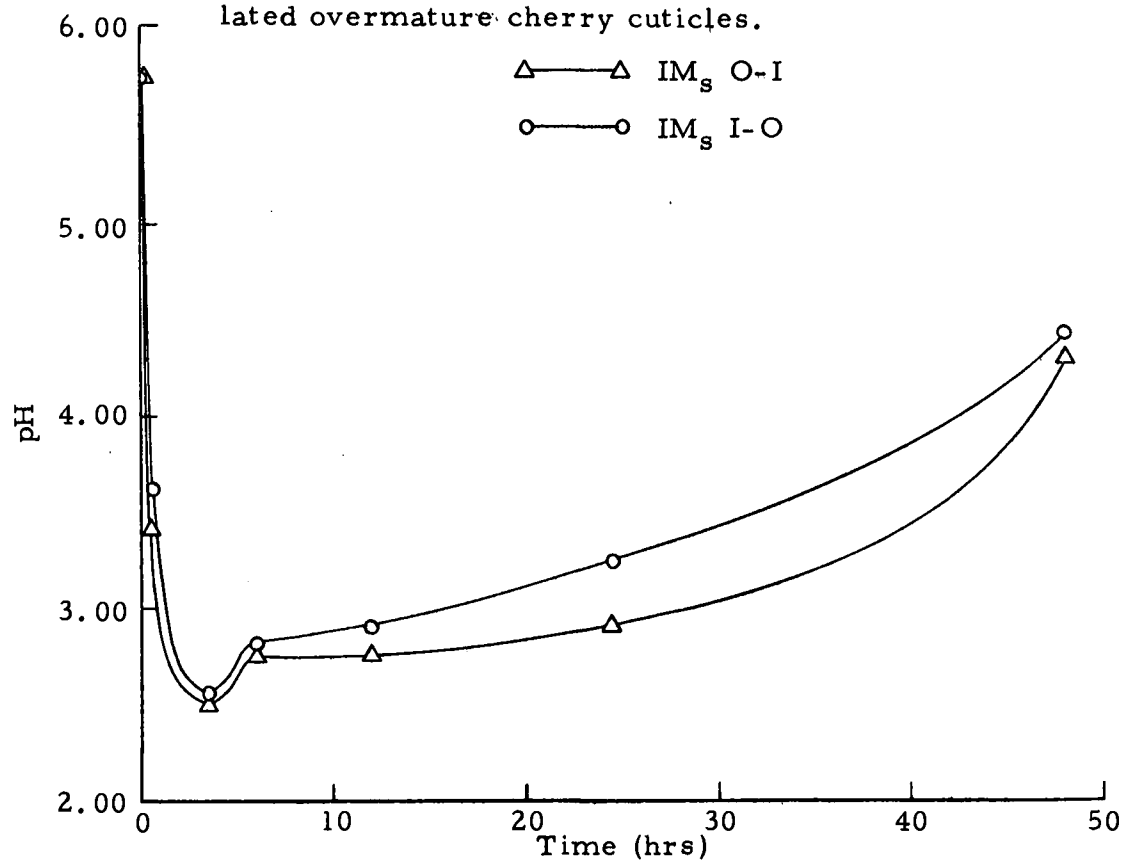

Figure 44. Hydrogen ion (SO_2-H_2O) penetration through isolated immature cherry cuticles.

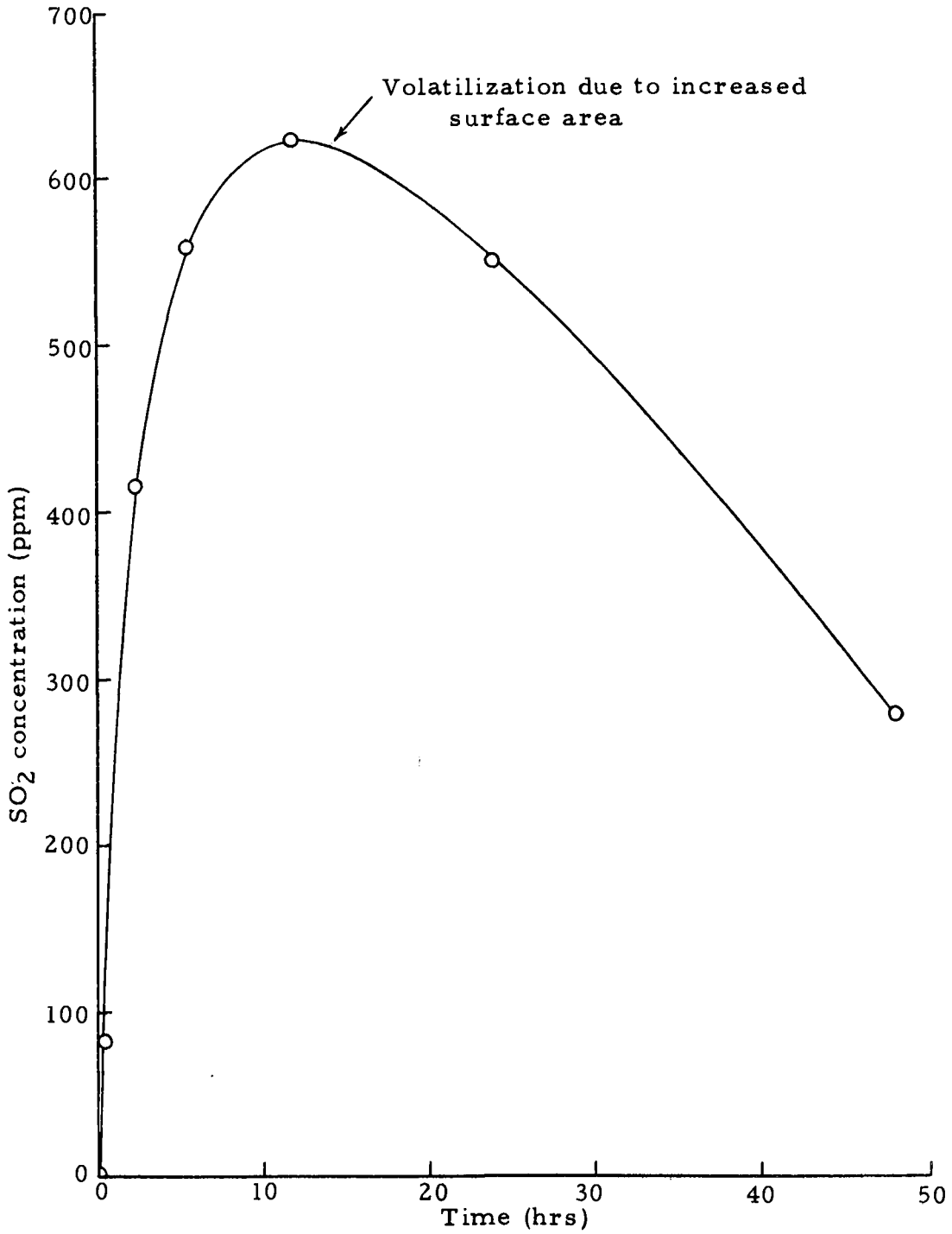

Figure 45. SO_2 (brine) penetration through a standard dialyzing membrane.

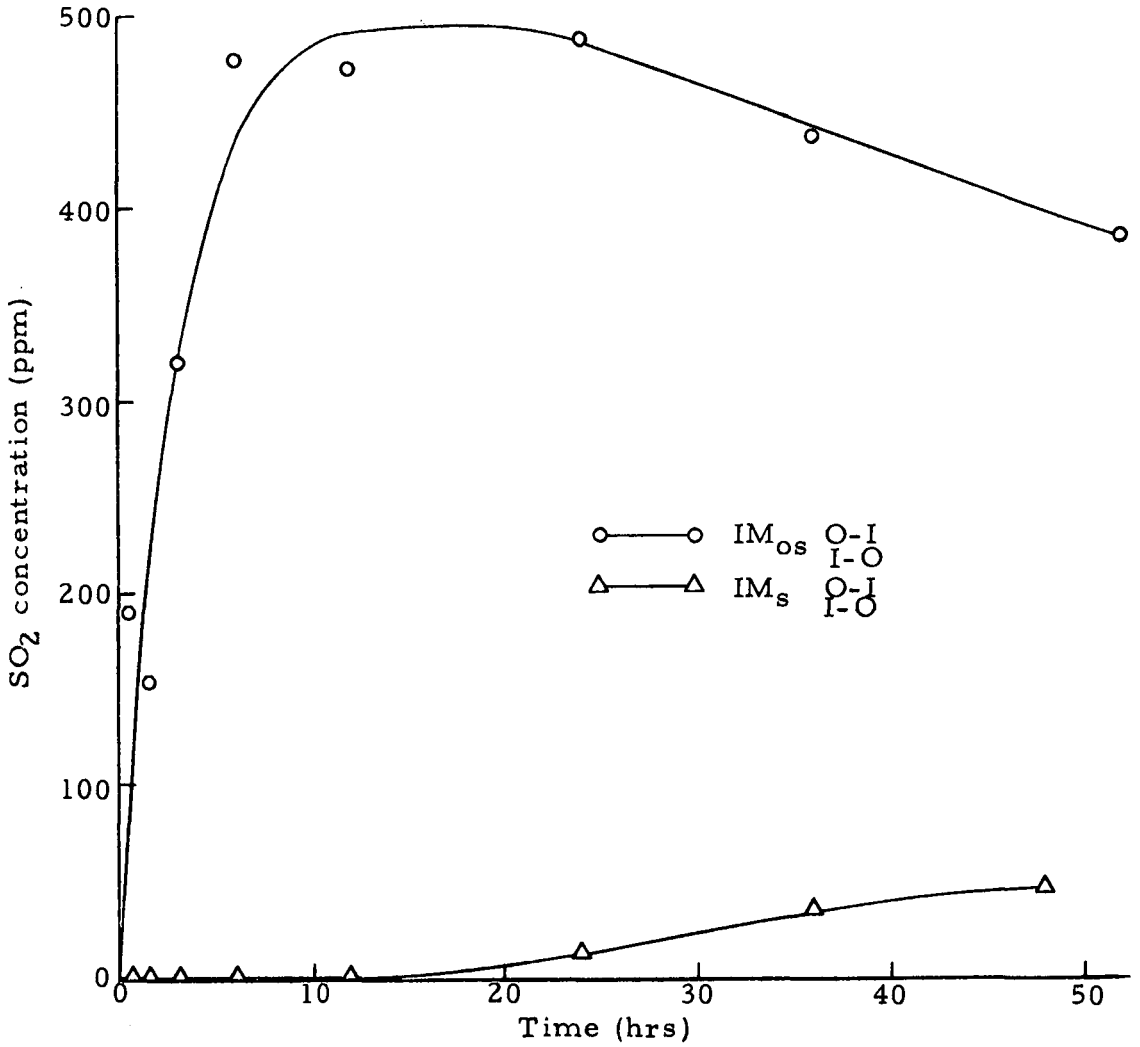

Figure 46. SO_2 (brine) penetration through isolated immature cherry cuticle.

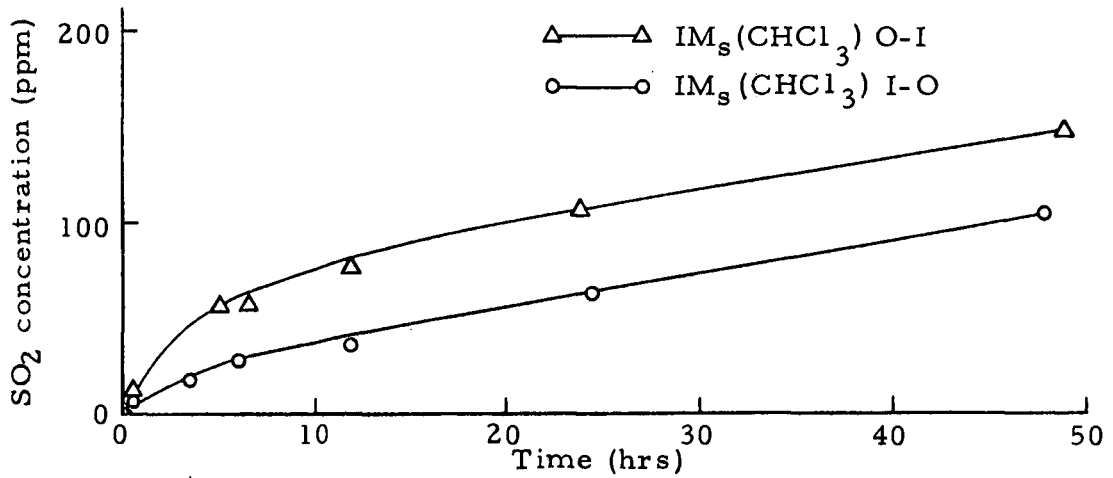

Figure 47. SO_2 (brine) penetration through isolated chloroform dipped immature cherry cuticles.

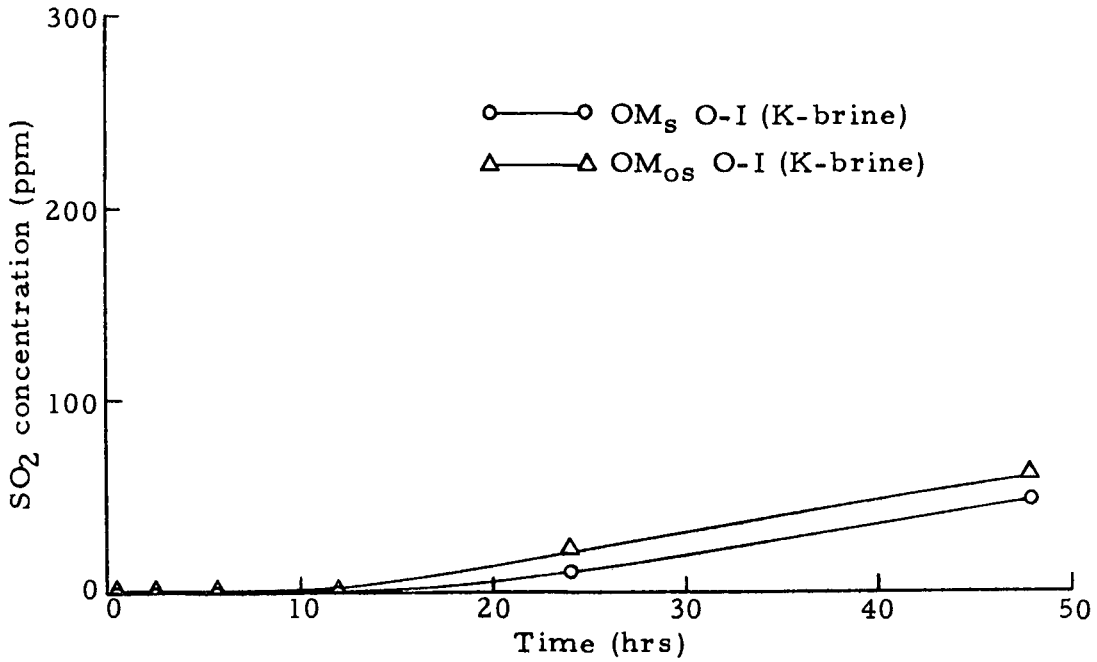

Figure 48. SO_2 (potassium-brine) penetration through isolated overmature cherry cuticles.

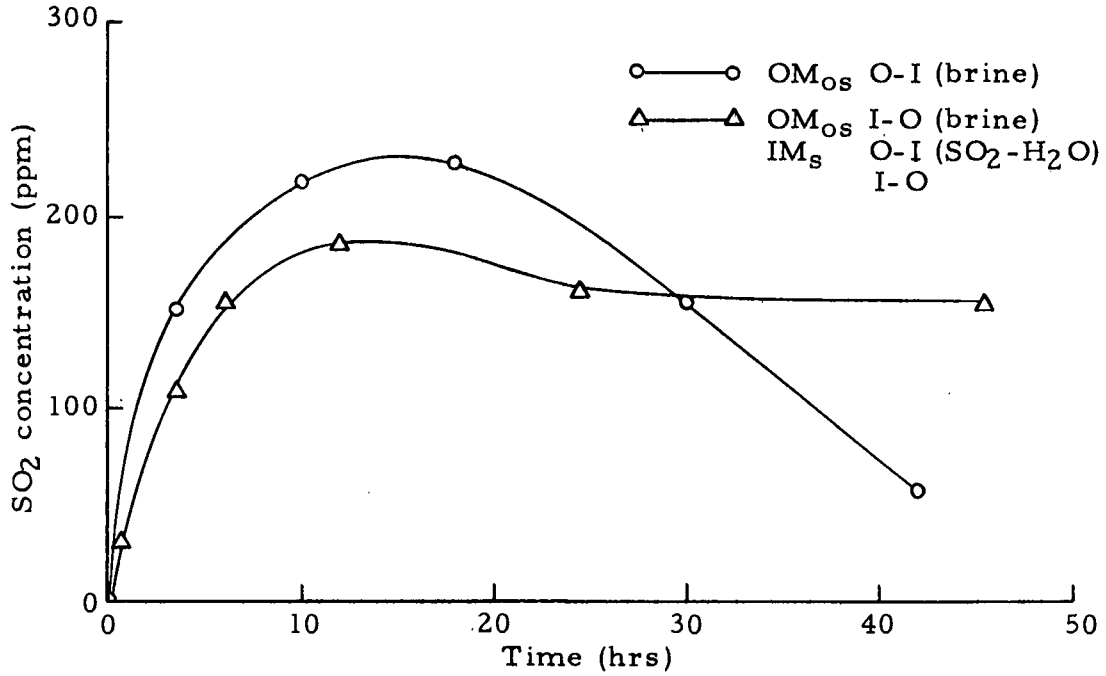

Figure 49. SO_2 (brine and SO_2-H_2O) penetration through isolated overmature and immature cherry cuticles.

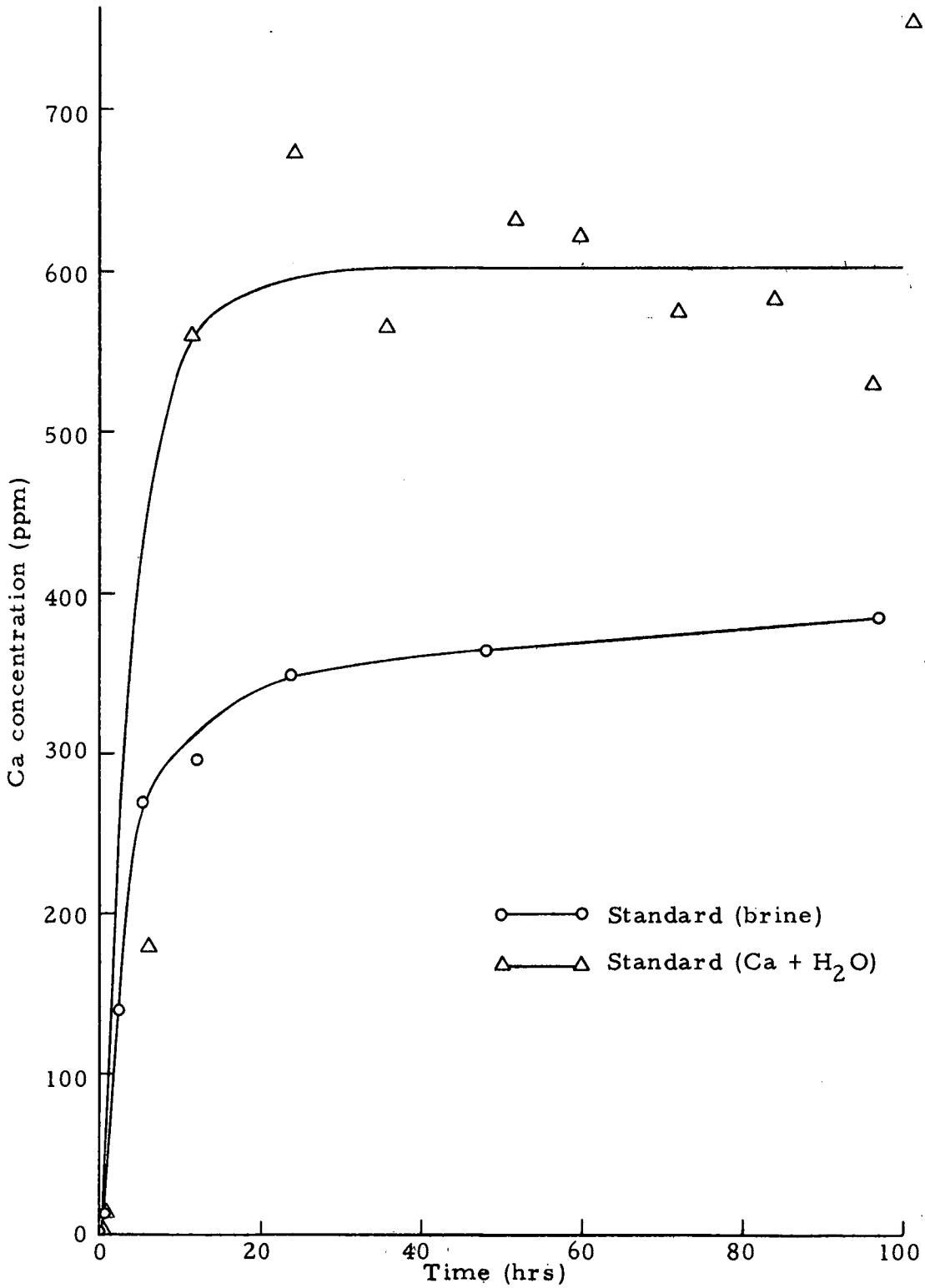

Figure 50. Calcium (Ca-H_2O and brine) penetration through standard dialyzing membranes.

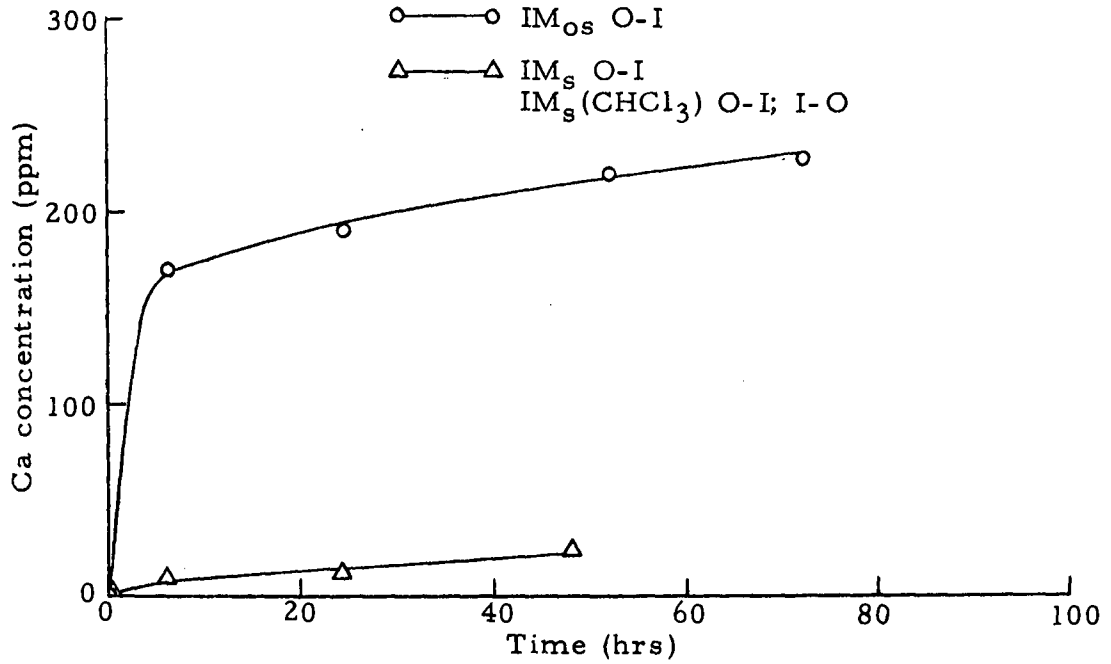

Figure 51. Calcium (brine) penetration through isolated immature and chloroform dipped immature cherry cuticles.

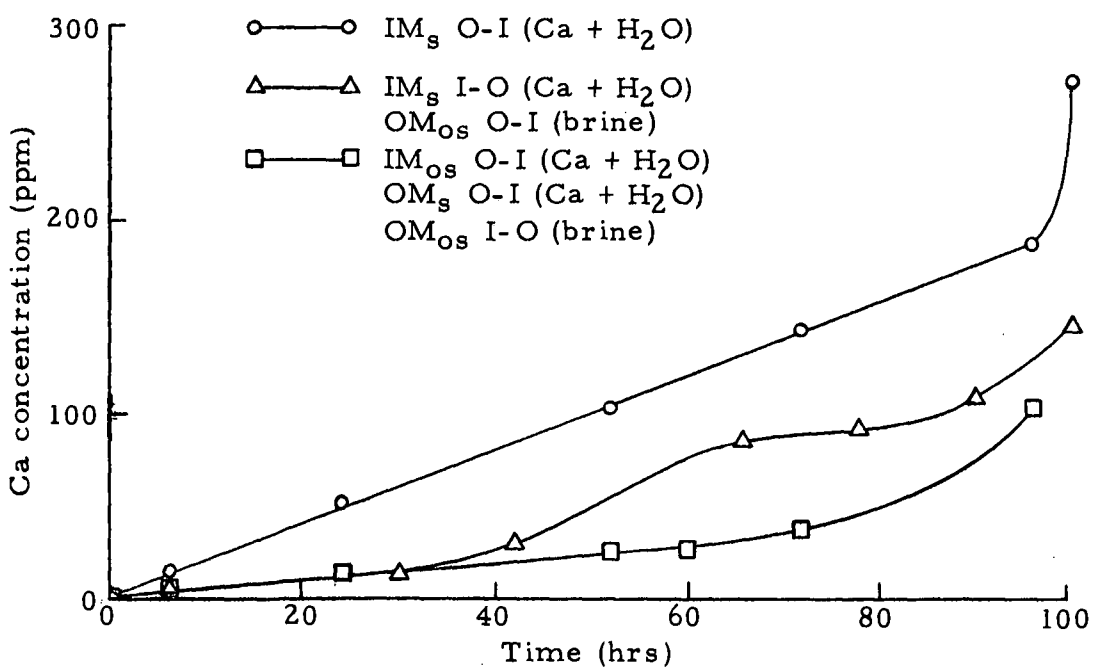

Figure 52. Calcium (Ca-H_2O and brine) penetration through isolated immature and overmature cherry cuticles.

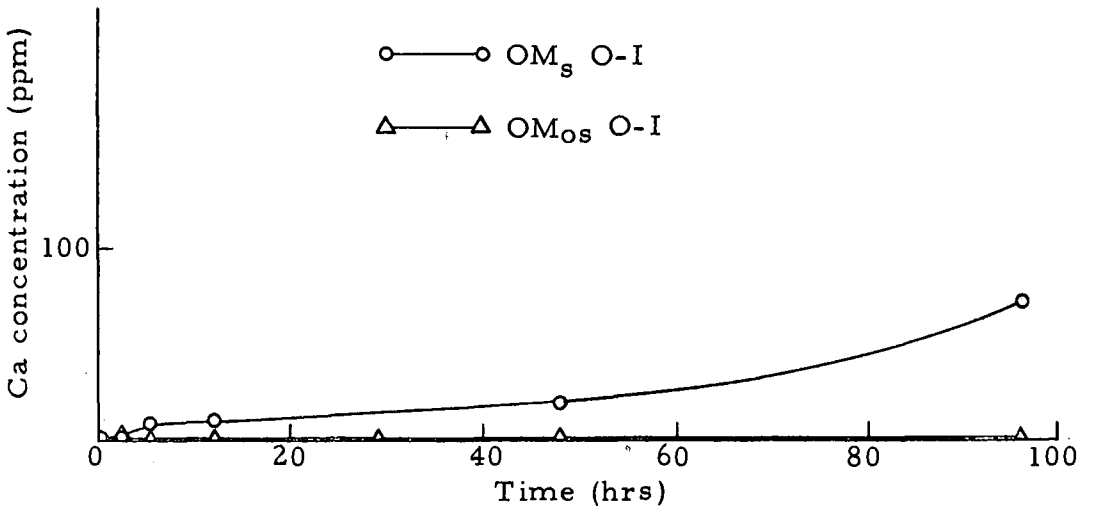

Figure 53. Calcium (potassium-brine) penetration through isolated overmature cherry cuticles.

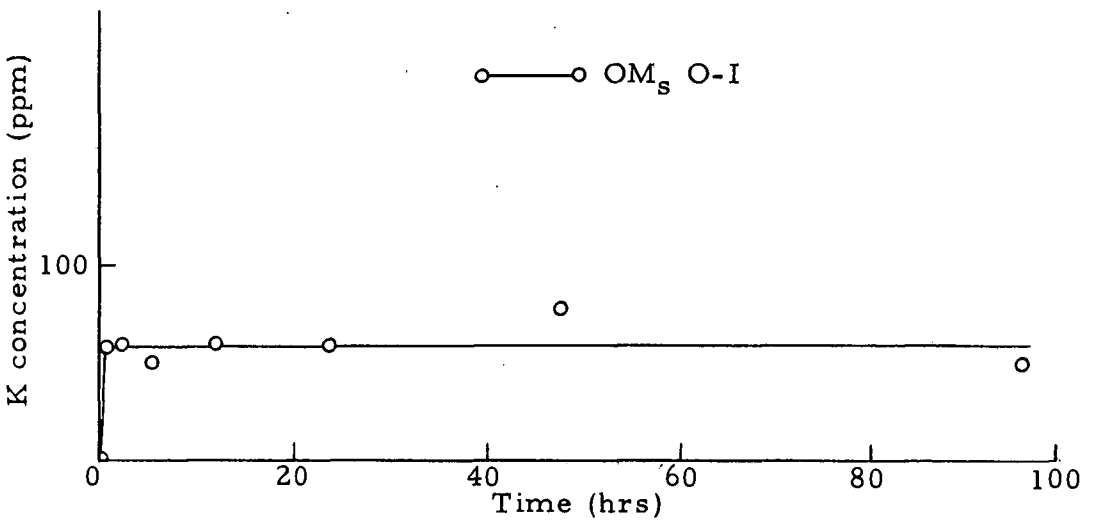

Figure 54. Potassium (potassium-brine) penetration through an isolated overmature cherry cuticle.

along the gradient of polarity in the cuticle.

The slight increase in calcium penetration through the IM_s over the IM_{os} at pH 5.8 (which is reverse at pH 2.8 to 3.0) is probably due to increased ion exchange capacity of the cuticle. As cellulose was found to be more deeply embedded in the cuticle on the suture side in the penetration study, the calcium bound on the surface would penetrate faster along the gradient of polarity than on the opposite side. The cellulose would tend to repel anions after they have penetrated, due to its negative charge.

The greater penetration of SO_2 and H^+ in water could be due to partitioning of molecular SO_2 through the lipophilic areas of the completely undissociated cuticle at pH 1. The penetration of HSO_3^- would also be possible due to the slight positive charge on the cuticle. The sudden drop in pH is caused by the extremely fast dissociation of molecular SO_2 into HSO_3^- and H^+ as it enters the water of the receiver solution.

The lowering of the concentration of SO_2 in the receiving solutions (Figures 45 and 46) after rapid penetration was due to the volatilization of the SO_2. This was found to occur after rapid penetration and equilibrium, due to the large surface area of the receiver solution. Volatilization from the donor tubes and from slow penetrations was found to occur very slowly and not affect the data to a great extent.

Greater penetration of SO_2 was observed to occur from the opposite suture side over the suture side of the isolated cherry cuticles (Figures 41, 46 and 51). This could be accounted for by the greater numbers of ectodesmata on the opposite suture side. This is in contrast to the stomata counting, which showed more stomata on the suture side. However, the overall number is lower than those for leaf cuticles, so that the stomata may not play as great a role in cherries. No other structural explanation was observed for this occurrence. The fact that it happens is illustrated, in that fewer solution pockets occur on the opposite suture side of the cherry. The greater penetration on this side undoubtedly contributes to this occurrence.

The penetration of two brine components (Ca^{++}, H^+) was often greater from the outside surface rather than from the inner surface (Figures 41, 42, 51 and 52). This has also been found true with tomato fruit, onion leaf, and ivy (Hedera helix) cuticles (Schieferstein et al., 1959; Yamada et al., 1964b, 1965b).

The lack of difference in the SO_2 penetration may be due to partitioning through the lipoidal areas of the cuticle. Yamada et al. (1965b) found a similar occurrence with urea penetration.

The penetration of the H^+, was manifested by either an immediate or delayed lowering of pH in the following studies (Figures 39 to 44) shown below:

Brine	Maturity/ side	Orientation	Lowering of pH
Normal	IM_s	O-I	Delayed
Normal	IM_s	I-O	Delayed
Normal	IM_{os}	O-I	Immediate
Normal	IM_{os}	I-O	Immediate
Normal	IM_s $CHCl_3$ dipped	O-I	Immediate
Normal	IM_s $CHCl_3$ dipped	I-O	Immediate
SO_2-H_2O	IM_s	O-I	Immediate
SO_2-H_2O	IM_s	I-O	Immediate
Potassium	OM_s	O-I	Delayed
Potassium	OM_s	I-O	Delayed
Normal	OM_s	I-O	Delayed
Normal	OM_{os}	I-O	Immediate
Normal	Standard dialyzing membrane	O-I	Immediate
Normal	Standard dialyzing membrane	I-O	Immediate

The delay in the lowering of the pH (i. e., initial raising of pH) may be explained by the difference in exchange capacities for the cuticle. The delay in hydrogen ion penetration is probably due to the negative charges on the cuticle, especially on the inner surface. The hydrogen

ions in the receiver solution would tend to migrate to these areas to be bound, thus causing a decrease in pH. The hydrogen ions from the donor solution would be doing the same thing; however, they must compete with other ions and pass the waxy layer to reach these sites. The immediate lowering of pH is most likely due to the penetration of molecular SO_2 with its resulting hydration to $H^+ + HSO_3^-$, as SO_2 was found to be present (figures 46, 47 and 50).

Effect of Wax Removal

The initial penetration of the brine components through the immature cuticle was found to be generally greater through the chloroform stripped cuticle than the control cuticle (Figure 41, 42, 46, 47 and 51). The H^+ ion penetration was increased by a factor of 10, the SO_2 penetration was increased by 7 while the Ca^{++} penetration remained rather constant. The removal of the wax also tended to bring the penetration from the different sides closer together. It was also interesting to note that when cherries that were de-waxed were placed in standard brine along with a control, that the decolorization (bleaching) to a straw yellow of the anthocyanin pigments occurred within a 2 to 20 minute period. This also shows the importance of the wax on the cherry.

The effect of maturity was also found to affect penetration. Generally, the ions penetrated faster through the immature cuticle than the overmature cuticle. SO_2 cuticular penetration was greater for the IM_{os} than the OM_{os} in both directions (Figure 46 and 48).

Calcium penetration remained approximately the same (Figures 51 and 52).

This decrease in penetration with increasing maturity is probably due to the increase in wax. This was also indicated by the increase in penetration exhibited by the wax stripped cuticle.

It was observed that the Red 4 used to check for breaks in the cuticle also gave an indication of the permeability of the cuticle. The dye did not penetrate or stain the intact immature or overmature cherry cuticle after 72 hours. This would indicate that the dye did not penetrate the stomata. It was observed that when pitted whole brined cherries were placed in the dye, the dye was taken up through the pitter holes where the fleshy pericarp was exposed to the dye. The dye then slowly diffused through the cells, by-passing the cuticle. However, secondary bleached cherries were found to pick up the dye fairly uniformly over the entire surface of the cherry. This change in penetration is undoubtedly due to the changes observed in the cuticular structure (Figures 23 to 27) caused by the action of the secondary bleach.

It was also observed that in a number of trials the water from the receiver solution tended to go into the donor solution (as noted by the increased level in the donor tube). This tends to support data from whole cherries soaked in water or brine, as the cherry has soluble solids of from 13.0-25.6 while the brine has a soluble solids

of approximately 3.5. Thus, the cherry has a lower activity (higher osmotic pressure) than the brine (Wagner and Moore, 1954). As the inside of the cherry is isolated from the brine by a semi-permeable membrane, flow of the solvent would be from the brine (region of high activity) into the cherry to dilute the soluble solids of the cherry. Thus, there should be an influx of water and/or ions to equilibrate this gradient. The influx of water into cherry fruit has been observed by many investigators: the increase in weight of cherries the first few days after brining (Beavers et al., 1971), and the increase in weight and diameter of the Bing cherries with a subsequent decrease in soluble solids when soaked in water solutions (Levin et al., 1959; Westwood and Bjornstad, 1970).

The selective rapid penetration of an ion into the cherry would increase the osmotic pressure and thus increase the probability of tissue rupture.

As the cherry absorbs water it increases in size. Since the wax is less elastic than the "spongy" cutin framework (Van Overbeek, 1956) the swelling of the cherry will stretch the cutin which in turn will tend to push any wax deposits further apart. This would then increase the permeability of the cuticle allowing more water and ions to enter, for a saturated cuticle has been found to be more permeable (Middleton and Sanderson, 1965). This is extremely important to cherries that are already turgid when placed in water (rain) or brine.

However, the increase is limited by the elasticity of the skin. Thus, when the pressure becomes too great, the skin splits or cracks, thereby relieving this pressure.

In brining, the same basic process occurs. Except as the water enters so do the SO_2, Ca^{++} and H^+ ions. As the SO_2 enters the cells, they are killed and hardened as noted in the structure studies. As the epidermal cells are the first to be killed they are least susceptible cells to rupture. However, the large thin walled parenchyma cells would still be swelling with water. If the pressure became greater than the elasticity of the parenchyma cell walls, the cells would be ruptured. This would then give the structure defect, known as a solution pocket. Levin et al. (1959) observed that the modulus of elasticity of the skin of the Bing cherry has a lower modulus of elasticity in the longitudinal direction than the transverse direction. Thus, as the moisture is absorbed and swelling increases the skin diameter, it becomes weaker in the longitudinal direction. This may be the cause of solution pockets being parallel to the suture.

Standard Dialyzing Membrane

Movement through the dialyzing membrane was the same in either direction. However, the penetration through the dialyzing membrane (Figures 39, 46 and 50) was greater in all but the following three cases: the penetration of hydrogen in a brine solution through

the chloroform stripped immature cuticle (Figure 42); the second was the penetration of hydrogen ion in a SO_2-H_2O solution through an immature opposite suture cuticle (Figure 44); and finally, the penetration of SO_2 through the immature opposite suture (Figure 49). An equilibrium was reached in the majority of brine penetrations by 50 hours, and in calcium by itself in 100 hours. However, an equilibrium was not reached in 50 hours in the potassium brine study, except for potassium.

Potasssium Brine Penetration

During the 1970-71 cherry season, some cherries were brined in a calcium bisulfite brine that had potassium bitartrate added as a buffer (Gerding, 1971). He found that the number of solution pockets increased from 41 percent at three months to 72 percent after six months storage. The solution pockets were located on the suture (81 percent), parallel with the suture (95 percent) and that most contained gas.

For this reason a similar experiment was set up with isolated cuticles to illuminate the mechanism. The data for this experiment are in Figures 43, 48, 53 and 54. It was found that the SO_2 penetration was delayed for both suture and opposite suture sides, with a resulting lower final concentration (Figure 48). The calcium and hydrogen ion concentration was also retarded, as seen in Figures 43 and 53.

However, the penetration of potassium was immediate, with complete equilibrium being achieved within the first 30 minutes (Figure 54). A possible explanation for this rapid penetration with delay of the other ions would be the penetration of potassium through the selective guard cells. They have been found to be permeable to water and especially permeable to potassium which increases the osmotic pressure within the cells (Zelitch, 1969; Thomas, 1970). They found that this increase in potassium concentration opened the stomatas in light and increased their permeability. Thus, this rapid increase could cause rupture of surrounding cells, which would result in a solution pocket. The smaller ion radius (1.33 Å) of potassium over that of hydrogen (1.38-2.08 Å) could also facilitate faster penetration, as Haile-Mariam and Wittwer (1965) found that the rate of penetration was inversely correlated with the size of the hydrated ion (i.e., larger size, slower rate). The bitartrate ion would be negatively charged and thus it would be attracted to the positive charge of the undissociated cuticle. Once there it could do one of two things: a) penetrate and undergo conversion to succinate or malate, so that it can become involved in the citric acid cycle thus contributing to the CO_2 produced, or if not converted it will inhibit fumarase activity, thus blocking the cycle, or b) it would not penetrate, thus blocking further penetration at that site and others near it due to its size and charge. The enzymes released by cell rupture present within the solution pocket would still be active

as long as the SO_2 penetration was delayed. Thus, the citric acid cycle could continue operating giving off CO_2, ergo a gas pocket.

As the whole cherries brined in this potassium brine would still be initially metabolically active, a metabolic facilitated diffusion process may also occur in addition to the observed penetration. A number of investigators have found metabolic uptake of ions (Jyung and Wittwer, 1964; Franke, 1967; Martin and Juniper, 1970).

Comparison to Whole Cherries

The data penetrations in this study were compared with values obtained using intact cherries (Payne, 1970). This comparison indicated that the general shape of the pH curves were similar (example: IM_{os} O-I; standard membrane) and the penetration of SO_2 through the standard membrane. The calcium penetration by itself was similar while the calcium penetration from brine differed.

Thus, penetration through isolated Royal Anne cherry cuticles would seem to give an estimate of the actual entry of SO_2-calcium bisulfite brine.

Bukovac and Norris (1969) also indicate that while the data obtained from isolated cuticles pertains to isolated cuticles only, it does give an insight into part of the cuticular penetration mechanism. This is particularly true for cherry cuticles used in brining, as the penetration of SO_2-calcium brine and the use of secondary bleach were

observed to alter the structure and penetration of the cherry cuticle. This means that these data and interpretations are restricted to the passage of the ionic constituents of a SO_2-calcium brine through the Royal Anne cherry cuticle.

Kinetic Data

A number of investigators have found that inorganic and organic ions penetrate the cuticular membrane by diffusion (Darlington and Circules, 1963; Jyung and Wittwer, 1965; Franke, 1967; Bayer et al., 1968). The following first order reaction equation was applied to the results of this study:

$$\ln\left(\frac{a}{a-x}\right) = k_1 t$$

a = initial concentration of solute in inner solution

x = concentration in outer solution at time t

$(a-x)$ = solute remaining in the inner solution at time t

k_1 = specific rate constant for a first order reaction

A characteristic criteria for a first order reaction is that a plot of $\ln(a/a-x)$ vs t is linear and has a slope of k. None of the penetration curves in this study were first order. However, after the initial hydration of the dried cuticle and the filling of binding sites, the penetration tended to assume a slower penetration due to saturation of the sites and the rate at which they are removed by the exchange

properties of the cuticle. This slower rate approximated a first order reaction in the following penetrations:

Brine	Ion	Maturity / side	Orientation
Normal	SO_2	IM_{os}	O-I; I-O
Normal	SO_2	OM_{os}	O-I
Normal	SO_2	IM_s $CHCl_3$ dipped	O-I
Normal	Ca	Standard dialyzing membrane	O-I; I-O
Ca-H_2O	Ca	Standard dialyzing membrane	O-I; I-O

Both of the above calcium penetrations approximated two different first order rates: initial hydration-penetration and the slower saturation penetration. The majority of the curves were of the curvilinear type.

Calcium Binding

The location of calcium binding sites was made using alizarin red S dye. With this method, the calcium is covered and surrounded by a birefringent orange-red precipitate, with a faint pink background (McGee-Russel, 1958; Barka and Anderson, 1965). It was found that the calcium binding was so intense that birefringent areas overlapped making the location of individual sites impossible. Likewise comparison between calcium binding and ectodesmata was not possible.

While individual binding sites could not be distinguished, the relative intensity of binding could be made. The binding on the inner surface of the cherry cuticle was much greater than the binding on the surface. This observation is similar to the binding of calcium and $SO_4^{=}$ on isolated astomatous ripe tomato fruit cuticles and stomatous green onion leaf cuticles (Yamada et al., 1964a, 1966).

A possible explanation for this observation would be the gradient of polarity in the cuticular membrane (Crafts and Foy, 1962; Yamada et al., 1964a). The inner surface is more polar with the cellulose and pectin residues left after isolation than the lipoidal surface. Thus, binding of the cation would be expected to be greater on the inner than outer surface.

SUMMARY AND CONCLUSIONS

In this study the structure and the permeability of the Royal Anne cherry were determined in order to ascertain their effects on the penetration of SO_2-calcium bisulfite brine components. The results indicate the following:

Structure

1. The general morphology of the Royal Anne cherry is similar to that of the sour cherry (Prunus cerasus L., var. Montmorency).
2. The intact and isolated cuticles were found to be separated from underlying cells by a layer of pectic substances, which were found to be de-esterfied on storage to pectic acid.
3. The epidermal cells were found to become disorganized and flattened with increased cutinisation on maturity.
4. The epidermis was found to be covered with a continuous cuticle which is randomly interrupted by stomata.
5. The stomata were found to occur more frequently on the suture side of the cherry than the opposite suture side.
6. Scanning electronmicrographs of the surface showed that as the fruit matured and increased in size, the suture and stomata were stretched, flattened and often ruptured in overmature fruit.

7. The surface was found to have a discontinuous birefringent layer of wax which increased from 0 to 0.2 μ with increasing maturity.

8. The amount of surface wax was found to increase from 0.123 $\mu g/mm^2$ to 0.170 $\mu g/mm^2$ (or 13.8 to 18.4 mg/100 g flesh) as the cherries matured.

9. The total lipid decreased from 99.74 to 76.09 mg/100 g flesh with increasing maturity. This in conjunction with the increase in weight and thickness of the surface wax and the thickness of the cuticle indicated that wax and cutin deposition kept slightly ahead of surface expansion and weight increase of the maturing cherry.

10. This amount of wax was found to be less than in most fruits and the same amount or less than in most leaves as reported in the literature. This factor affected the penetration of the cherry as well as the occurrence of splitting and solution pockets.

11. The surface of the cuticle was found to have a smooth to granular sheet or layer of wax which when removed with chloroform revealed a porous sponge-like cuticle. This would allow easy wettability of the surface and allow easier penetration.

12. The surface was found to be altered not only by chloroform but by brining and secondary bleaching.

13. While the preceding procedures remove some of the cuticle they

were found to harden the fleshy parenchyma cells and epidermal cells.

14. Ectodesmata (MP) were found to occur over anticlinal walls and in guard cells on both sides of the cherry fruit. However, they appeared to be more on the opposite suture than the suture side. This reflects areas that are more permeable to polar compounds and undoubtedly played a role in the brine component penetration.

15. The percent weight loss of cherries was increased 2.86 to 4.00 times with chloroform dipped cherries than their respective controls. The loss was found to be linear and each treatment significantly different from its control at the .01 level.

16. Enzymatically isolated cuticles were left with cellulose debris as well as an unidentified birefringent crystalline artifact in the cellulose under the cuticle that has not been previously reported by other investigators. As this was found for both cherry and prune cuticles and not tomato or apple cuticles, both observations are believed to be particular to the two species of Prunus tested.

17. The inside surface of the cuticle could not be described due to this remaining debris.

Penetration

1. Penetration of SO_2 was greater through the opposite suture than the suture side of the cherry cuticle.
2. Penetration of Ca^{++} and H^+ from brine was often greater from the outside than from the inside of the cherry cuticle.
3. Penetration of hydrogen ion was found to be delayed in a number of cases where in others it penetrated immediately.
4. SO_2 was found to penetrate the cuticular membrane faster than calcium of which the concentration difference accounted for part.
5. Individual ions penetrated faster than the combined ions of the brine due to the condition of the ion exchange properties of the cuticle.
6. Initial penetration through chloroform dipped cherries was greater than through the control cuticle; hydrogen ion penetration increased by 10, SO_2 by 7 while calcium stayed approximately the same.
7. Brine bleached the whole chloroformed dipped cherries faster (2-20 minutes) than control cherries.
8. The effect of maturity was found to effect penetration: immature cuticles were penetrated faster than overmature cuticles.
9. Red 4 did not stain or penetrate intact isolated cuticles in 72 hours, which indicated that penetration did not occur through the stomatal pores.

10. It was observed that Red 4 rapidly penetrated whole secondary bleached cherries while it went through the pitter hole in normally brined cherries, which confirmed alteration of the cuticle by the secondary bleach as noted in the structure studies.

11. Potassium was found to penetrate rapidly from a potassium brine with equilibrium being reached in 30 minutes or under.

12. SO_2, hydrogen ion and calcium penetration was delayed in a potassium brine with equilibrium not being reached.

13. Penetration of all constituents studied through a standard dialysis membrane was the same in either direction and in greater amounts as compared to cherry activities in most of the penetration.

14. Comparison of isolated cuticle penetration with whole cherry penetration indicated that this technique gives an insight into the cuticular penetration mechanism.

15. None of the penetrations initially followed a first order reaction rate. However, after initial hydration of the cuticle, a first order reaction rate was approximated.

16. Individual calcium binding sites could not be located with the alizarin red S method. However, the intensity of binding was greater on the inside rather than the outside of the cuticle.

17. Water was found to enter due to the osmotic difference between the brine and the cherry.

18. The rapid penetration of SO_2 and hydrogen from SO_2-H_2O brine but slower penetration from a normal brine; rapid penetration of SO_2 in chloroform dipped cherries with slower penetration in control cherries with the calcium penetration remaining the same for both chloroform dipped and control; the ratio of SO_2 to calcium being greater than 2:1; and the major chemical species of the water-SO_2 being HSO_3^- at the pH range used indicates that:
    a) the calcium is not entering by the same route as the SO_2,
    b) the major penetrating species of SO_2 in water and brine would seem to be either HSO_3^- or molecular SO_2.
19. The greater SO_2 penetration through the cuticle opposite the suture side; the greater number of ectodesmata on this side and occurrence in guard cells rather than the aperture; lack of penetration of Red 4 through intact cuticles and rapid penetration of potassium would indicate that
    a) the stomatal pores are not sites of entry;
    b) the ectodesmata are probably the polar sites used for penetration;
    c) the guard cells are more important to potassium penetration while the ectodesmata in the astomatous region are important for SO_2 penetrations.

Recommendations and Conclusions Concerning Cherry Brining

1. One of the major factors involved in the penetration of the brine components is the waxy layer on the cuticular membrane. Therefore, techniques that alter or remove this wax, such as solvents and surfactants, would allow faster penetration of the brine.

2. It would appear that the defect known as solution pockets occurs due to a combination of factors:

   a) the retarded penetration of SO_2 on the suture side of the fruit;

   b) an inherent structural weakness or low elasticity of the cells in the suture area;

   c) the osmotic gradient between the cherry and the brine allow an influx of water;

   d) the shape, amount and undoubtedly the chemistry of the surface wax, which allows water to more easily wet the surface and penetrate;

   e) the penetration of ions being greater from the outside which allows easier penetration than excretion causing the ion concentration to increase which in turn increases the osmotic pressure, and finally,

   f) the contamination or use of potassium which was found to have immediate penetration while depressing the penetration

of SO_2 thus allowing cell rupture to occur and possibly CO_2 production. The use of the bitartrate salt also contributed to the potassium's effect.

The above factors would indicate that the following procedures should decrease the incidence of solution pockets.

a) Removal of the waxy layer, which would allow greater penetration of SO_2 through the whole cherry killing the tissue before a solution pocket can form. This would have to be done with FDA approved solvents or surfactants.

b) Desiccate the fruit before brining at least 12 hours, as the most rapid weight loss occurs in this period. This is especially important to matured (high soluble solids) full turgid fruit as shriveling causes the cuticle to shrink pulling the wax deposits closer together which would make the waxy layer more compact, thus resisting rapid water penetration. This would allow for better killing and hardening of the tissues, thus decreasing the chance of cell rupture.

c) The use of a more isotonic brine, such as a reused brine (Beavers et al., 1970b), which would alleviate the osmotic pressure differential.

The results and conclusions from this study suggest that the following avenue might be beneficial and applicable to cherry brining:

The use of a lipoidal dip (1 percent oleic acid or 1 percent

ethyl oleate) in conjunction with sodium bisulfite as Ponting (1971) found that in grapes, penetration of SO_2 throughout the fruit occurred in about ten minutes which increased the relative drying rate at 110 F.

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