

THESIS



3 1293 10446 3900



ABSTRACT

MORPHOLOGICAL AND PHYSIOLOGICAL ASPECTS OF CHERRY FRUIT ABSCISSION WITH REFERENCE TO 2-CHLOROETHYLPHOSPHONIC ACID

By

Vernon Arie Wittenbach

Morphological and histochemical changes, occurring in the transition zone between the pedicel and fruit of sour (Prunus cerasus L. cv. Montmorency) and sweet (Prunus avium L. cv. Windsor) cherry during maturity, were followed in the absence and presence of 2-chloroethylphosphonic acid (CEPA). A natural weakening of the tissue at this zone was observed to begin near the start of the third growth phase. However, no visible signs of abscission were observed until 2-3 weeks prior to maturity. Changes in pectins, cellulose, and polysaccharides in the walls of cells of the abscission layer preceded separation in the sour cherry. Comparable changes and a well-defined abscission layer were not observed in sweet cherry. CEPA accelerated abscission by apparently increasing the rate of development of the naturally occurring processes. An increase in ethylene evolution from cherry fruits near the onset of the third growth phase was correlated with the simultaneous decline in fruit

removal force, however, the level of ethylene was extremely low. Physiological aspects of cherry fruit abscission were established using an excised fruit technique. Stage of fruit development had a pronounced effect on the sensitivity of fruit explants to CEPA. 3-Indoleacetic acid, CEPA, gibberellin A₃ and abscisic acid hastened abscission in sour cherry. Only CEPA had a similar accelerating effect on sweet cherry. Cycloheximide applied to cherry fruit explants inhibited abscission and also negated the effect of CEPA. This could indicate that protein synthesis is necessary for cherry fruit abscission to occur.

MORPHOLOGICAL AND PHYSIOLOGICAL ASPECTS OF
CHERRY FRUIT ABSCISSION WITH REFERENCE
TO 2-CHLOROETHYLPHOSPHONIC ACID

By

Vernon Arie Wittenbach

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Horticulture

1970

ACKNOWLEDGMENTS

The author wishes to express his sincere appreciation to Dr. M. J. Bukovac for his assistance and encouragement during the course of this program of study.

Appreciation is also extended to Dr. H. P. Rasmussen and Dr. C. E. Cress, members of my guidance committee, for their suggestions and for reading the manuscript. I would like to thank Dr. D. R. Dilley for his assistance and the use of his laboratory equipment and Mr. V. Shull for the use of the electron microprobe. The suggestions and help of fellow laboratory workers were also appreciated.

A special thanks is extended to my wife Pam for her continuous encouragement, patience and assistance in the preparation of this dissertation.

TABLE OF CONTENTS

	Page
ACKNOWLEDGMENTS	ii
LIST OF TABLES	v
LIST OF FIGURES.	vi
LIST OF APPENDICES.	viii
INTRODUCTION	1
LITERATURE REVIEW	4
Leaf Abscission	4
Fruit Abscission.	6
Cell Wall Changes and Enzymes in Abscission.	9
Protein Synthesis in Abscission.	12
Effect of Growth Regulators on Abscission	13
Auxins	13
Ethylene.	16
Gibberellins	19
Kinins	19
Absciscic Acid	20
Other Factors Influencing Abscission	21
CEPA.	22
MATERIALS AND METHODS.	25
Characterization of the Natural Fruit	
Abscission Process	25
General Methods	25
Cherry Fruit Development	26
CEPA Modification of FRF at Maturity	26
Sour Cherry	26
Sweet Cherry.	27
Upper vs. Lower Zone	27
Morphology of Abscission	28
Histochemical Changes in the Abscission	
Zone During Fruit Separation	29
Ethylene Evolution	31
Characterization of the Abscission Process in	
Detached Fruit Explants.	32

	Page
General Methods	32
Effect of Age on Fruit Abscission.	33
Response of Mature Fruit Explants to Growth Regulators	33
Role of Protein Synthesis	36
RESULTS	37
Characterization of the Natural Fruit Abscission Process	37
Change in FRF with Fruit Development	37
Sour Cherry	37
Sweet Cherry.	38
CEPA Promotion of Fruit Abscission	41
Sour Cherry	41
Sweet Cherry.	41
CEPA Effect on Upper vs. Lower Zone.	41
Morphology of Abscission	45
Sour Cherry	45
Sweet Cherry.	48
Histochemical Changes During Abscission	58
Ethylene Evolution from Cherry Fruits During Development.	68
Characterization of Fruit Abscission in Detached Fruits	74
FRF as Effected by CEPA and Stage of Fruit Development	74
Response of Mature Fruit Explants to Growth Regulators	77
Effect of a Protein Synthesis Inhibitor on Abscission	80
DISCUSSION	83
Abscission Process	83
Comparative Response of Sour and Sweet Cherries to Plant Growth Substances Active in Leaf Abscission	86
Role of CEPA in Natural Fruit Abscission.	91
SUMMARY	95
LITERATURE CITED	98
APPENDIX	111

LIST OF TABLES

TABLE	Page
1. Effect of CEPA on FRF at the upper and lower abscission zones of sweet cherry	44
2. An estimate of changes in cell-wall constituents of the abscission zone immediately prior to cell separation	64
3. Effect of CH and CEPA on abscission of sour cherry fruit explants	81
4. Effect of CH and CEPA on abscission of sweet cherry fruit explants	82

LIST OF FIGURES

FIGURE	Page
1. Bioassay used to study abscission in detached fruits.	34
2. Sour cherry fruit growth and FRF of the upper and lower abscission zones	39
3. Sweet cherry fruit growth and FRF of the upper and lower abscission zones	39
4. Effect of CEPA on fruit removal force of sour cherry	42
5. Effect of CEPA on fruit removal force of sweet cherry.	42
6. The two potential abscission zones of cherry fruits at the time of CEPA application	46
7. Development of the separation layer at the lower abscission zone of sour cherry.	49
8. Detailed development of the separation layer in the cortical tissue at the lower abscission zone of sour cherry.	51
9. Changes occurring in the lower abscission zone of sweet cherry	53
10. Detailed development of abscission in the cortex at the lower abscission zone of sweet cherry.	55
11. Sections from the lower abscission zone stained for polysaccharides.	60
12. Sections from the lower abscission zone stained for pectins	62
13. Localization of calcium in the lower abscission zone.	66
14. Ethylene evolution from sour cherry fruits	69

FIGURE	Page
15. Ethylene evolution from sweet cherry fruits . .	71
16. Change in FRF of CEPA-treated sour cherry fruit explants as effected by stage of fruit development	75
17. Change in FRF of CEPA-treated sweet cherry fruit explants as effected by stage of fruit development	75
18. Effect of growth regulators on FRF of sweet and sour cherry fruit explants. . . .	78

LIST OF APPENDICES

APPENDIX	Page
A1. Number of replications and fruit per replication from which the data on ethylene evolution was obtained	112
A2. Standard deviations of the FRF values presented in Figure 2 for 'Montmor- ency' sour cherry	113
A3. Standard deviations of the FRF values presented in Figure 3 for 'Windsor' sweet cherry.	114

INTRODUCTION

Mechanical harvesters are revolutionizing the fruit industry. Blueberries and sour cherries are two crops where mechanical harvesting has become a standard practice. In addition, attempts are being made to mechanically harvest grapes, apples, pears, plums, oranges, sweet cherries, and olives, as well as several other fruit crops.

One of the most serious problems to be overcome in harvesting these crops mechanically is the high retention force with which the fruit is held to the pedicel (Lamouria and Hartmann, 1959; Hendershott, 1965; Cooper et al., 1968; Bukovac, 1969). Cain (1967) has shown a high correlation between the fruit removal force and percentage of sour cherries harvested mechanically. In addition to lowering the per cent of fruit removal, high retention forces result in greater fruit injury (Cooper et al., 1968).

Therefore, recent work has been directed toward the use of chemicals to lower the fruit removal force. Chemicals have the advantage of being easily manipulated and of inducing the desired response in a short period of time. Earlier studies have shown that a wide variety of compounds were capable of causing abscission of leaves (Weintraub et al., 1952) and immature fruit (Batjer, 1954). Unfortunately, few of these chemicals have shown promise in hastening

abscission of mature fruits, since they either cause excessive defoliation, injury to fruit and/or tree, or exhibit too wide a variation in response.

New and different compounds are continually being tested. 2-Chloroethylphosphonic acid (CEPA, Ethrel), which was recently introduced, has been shown to promote abscission of peaches, apples, plums, and cherries (Edgerton and Greenhalgh, 1969; Bukovac et al., 1969). However, much more research needs to be carried out with this chemical before it is released for general use.

Control of abscission has several important practical advantages (Bukovac, 1969). First, a higher percentage of fruit can be removed by the harvester without damaging the tree. Second, a lower fruit removal force would result in less bruising and tearing of the fruit, thereby providing a higher quality product. Third, the availability of efficient chemicals would give growers the opportunity to program their harvests.

Despite the need to control fruit abscission, few studies have been carried out to further our understanding of the chemical and physiological aspects of this process. Most work in this area has been directed toward the phenomenon of leaf fall. Although fruits may have evolved from leaves there is little doubt that the fruit represents a much more complicated system (Jacobs, 1962). For instance, the fruit encloses one or more seeds with a very complex development

of their own. In addition, the fruit is capable of very limited photosynthesis, and therefore, most of the organic material required for growth must come from other plant organs.

Due to these differences in subtending organs, there exists a need to further identify the fruit abscission process. The present study was undertaken with three main objectives: 1) to compare the natural abscission process in maturing sweet and sour cherry fruits, 2) to study the changes associated with the promotion of cherry fruit abscission by CEPA, and 3) to define the physiological parameters of fruit abscission through the use of fruit explants.

LITERATURE REVIEW

Leaf Abscission

The phenomenon of leaf fall has intrigued man for thousands of years. Records from as far back as 285 B.C. include reference to leaf abscission (cited by Addicott, 1965).

Several detailed anatomical studies of leaf fall were conducted prior to the beginning of this century. Lee (1911) summarized some of these earlier works and presented an excellent description of the morphological changes accompanying leaf abscission in 45 dicot species.

Shortly after this extensive study, Sampson (1918), concentrating on Coleus blumei Benth., investigated the microchemical and cellular changes occurring during leaf abscission. He concluded that separation resulted from the conversion of cellulose into pectose, which was further transformed to pectin. The accumulation of pectic acid weakened the middle lamella, since there was an insufficient amount of calcium to maintain solidity. However, more recent investigators (Facey, 1950; Valdovinos and Jensen, 1968) have suggested that separation is due to a breakdown of the pectic substances of the middle lamella. These authors indicate no conversion or only secondary changes in cellulose.

Cell division has been shown to be associated with abscission (Lee, 1911; Sampson, 1918; Brown and Addicott, 1950; Facey, 1950). However, these workers could not agree as to whether it was directly involved with separation or only indirectly associated through the formation of a protective layer. Gawadi and Avery (1950) studied secondary cell division in several plant species and concluded that its only role was in the formation of a protective tissue forming the leaf scar.

Brown and Addicott (1950) greatly simplified and advanced the research on abscission by introducing the use of explants. Their work indicated that explants exhibited the normal processes associated with leaf abscission.

Many more studies on leaf abscission have been reported since 1950. Several have dealt with the cellular and chemical changes accompanying abscission (Scott and Leopold, 1966; Osborne, 1968b; Webster, 1968, 1970; Rasmussen and Bukovac, 1969; Bostrack and Daniels, 1969; Choudhuri and Chatterjee, 1970), while others have been concerned more with the ultrastructural changes (Bornman, Spurr, and Addicott, 1967; Morré, 1968; Jensen and Valdovinos, 1967, 1968; Valdovinos and Jensen, 1968; Bednarz, 1970). In addition, the reader is referred to several excellent reviews (Addicott, 1954; Addicott and Lynch, 1955; Addicott, 1961, 1965, 1968; Jacobs, 1962, 1968; Rasmussen, 1965; Carns, 1966; Cooper et al., 1968; Bednarz, 1970).

Fruit Abscission

Heinicke (1917) was one of the first investigators to study fruit abscission. He observed that the fall of flowers and immature apple fruit was preceded by the formation of a layer of cells "similar to that which precedes leaf-fall." Separation occurred at the junction of the fruit stalk and spur, which appeared to be a naturally weak zone. In addition to describing the anatomy of the abscission zone, Heinicke (1917, 1919) determined histochemically the changes occurring during the formation of the abscission layer. The abscission zone had a lower affinity for cell wall stains, and in most cases a lignified layer was formed in the abscission zone just prior to separation.

The fall of immature apples has since been studied in greater detail (MacDaniels, 1936; McCown, 1938). Secondary cell division was found to precede the formation of a well-defined abscission layer 6-8 cells in width. Separation resulted from the breakdown of the pectic compounds of the middle lamella and occurred within the limits of or distal to the abscission layer (McCown, 1938). However, apples which survived the June drop showed no indication of secondary cell division or the formation of an abscission layer (McCown, 1938). In mature fruits separation resulted from the disintegration of walls of pre-existing cells, and even the abscission zone, 20-30 cells in width, did not determine the path of separation (MacDaniels, 1936; McCown, 1938).

Therefore, even in apples, where separation of mature and immature fruit occurs at the same abscission zone, the two processes appear to be quite different.

McCown (1943) in further studies concluded that depending on variety abscission was initiated independently in the cortex and pith. In the pith, separation was preceded by swelling and extension of walls which was accompanied by a physical change in the cellulose. Next the secondary cellulose wall gradually disintegrated with concomitant changes in the middle lamella. The dissolution of the pectic compounds of the middle lamella then allowed separation of cells. Final separation of the fruit was due to the mechanical tearing of the outer cortex and epidermal tissue and rupturing of the vessels and fibers across the path of separation. Soon after separation a protective layer formed leaving a fruit scar.

Another early study of abscission in mature fruits was carried out by Barnell (1939) on avocado and mango fruits. In both there are two potential "abscission zones". The upper zone, at the junction of the pedicel and spur, is activated prior to the fall of immature fruits and after the separation of mature fruits. The lower zone at the transition region between the fruit and pedicel, is involved in the separation of fruit at maturity.

Barnell followed the development of abscission of the lower zone at fruit maturity and of the upper zone after

fruit separation had occurred. Fruit abscission resulted from cleavage occurring along the middle lamellae of the cells. The cell walls and contents remained undamaged after separation. There was no evidence of the formation of an abscission cambium following fruit abscission.

Further studies on fruit abscission have only been conducted within the last few years. Stösser (1967) investigated the anatomical changes associated with the fall of immature sweet cherries. This separation occurred at the upper zone (between the pedicel and spur).

Several recent studies have been concerned with the abscission of mature oranges (Hendershott, 1965; Wilson and Hendershott, 1967, 1968; Rasmussen and Cooper, 1968; Cooper, Rasmussen and Hutchinson, 1969) but only one dealt with the anatomy and histochemistry of this process (Wilson and Hendershott, 1968). These authors described the abscission zone as being made up of slightly smaller cells and compressed tracheary elements. The protoplasm of cells in this zone became granular near fruit maturity, and there occurred a concomitant accumulation of starch. Just prior to separation there appeared a distinct band of cells low in methylated pectins, which traversed the abscission zone. Separation took place through this band of cells and involved a lysigenous dissolution of cell walls. No cell division was observed during or prior to abscission. Also no suberin was deposited during this period, but with the beginning of separation,

lignification occurred distal to the separation layer (in the fruit).

A similar investigation has also been carried out on the abscission of mature sour cherries (Stösser, Rasmussen, and Bukovac, 1969a, 1969b). Here too the abscission zone represented an area of structural weakness. Prior to separation the walls of cells in the abscission zone showed a partial degradation of cellulose, pectins, and non-cellulosic polysaccharides. Calcium and magnesium were lost from these cell walls prior to and/or during cell separation. The separation of cells occurred without rupturing of cell walls. Final separation of the fruit was brought about by mechanical breaking of the vascular strands.

Fruit "explants" have been used in several laboratories to study the abscission process and to evaluate chemicals for their effect on abscission (Wilson and Hendershott, 1967, 1968; Rasmussen and Cooper, 1968; Stösser et al., 1969a; Zucconi, Stösser, and Bukovac, 1969). They have become an important tool for studying fruit abscission, since the abscission process is apparently identical to that observed in separation of mature fruit (Wilson and Hendershott, 1968; Stösser et al., 1969a).

Cell Wall Changes and Enzymes in Abscission

Cells of abscission zones, whether in leaves or fruits, are generally characterized by their smaller size and thinner walls (Addicott, 1965; Stösser et al., 1969a).

However, despite these anatomical differences the cells appear to have the same structural components and chemistry as other cells (Valdovinos and Jensen, 1968; Jensen and Valdovinos, 1968; Bednarz, 1970).

The primary cell wall is composed of densely packed cellulose fibrils in an essentially crystalline array. These microfibrils are embedded in a matrix of usually amorphous and highly swollen material, consisting of hemicelluloses, pectic substances, and proteins (Setterfield and Bayley, 1961; Preston, 1964; Ro  lofsen, 1965; White, Handler, and Smith, 1968).

The composition of the primary cell wall and middle lamellae has been well established except for the presence of proteins. The major uncertainty is the arrangement of this material.

The timing of abscission, the highly localized region to which separation is confined, and the specificity of breakdown material, suggested the functioning of enzymes in abscission. Hence, Bonner (1936) hypothesized that leaf abscission was due to a middle lamellae protopectinase and the enzyme polygalacturonase. Protopectinase was a logical choice, since pectic substances had been known to comprise the middle lamellae (Sampson, 1918).

Pectin methylesterase (PME) was the first enzyme to be studied in detail. Osborne (1958) found PME activity to be higher in the pulvini of non-senescent bean leaves. There

appeared to be a decreasing gradient of activity from the pulvinus to the petiole. During senescence of the leaf this gradient fell and was generally reversed at the time of abscission. These results have since been confirmed by LaMotte et al. (1969).

Yager (1960a) added more evidence to the possible role of pectic enzymes. He found that it was possible to duplicate the separation of cells which normally occurs in abscission by incubating tissue from the abscission zone with various pectic enzymes. It also was noted that factors which normally influence abscission, such as auxin, had a similar effect on these enzyme preparations.

More recently evidence has been presented for the involvement of polygalacturonase (PG) in abscission (Rasmussen, 1965). This enzyme was found to be active in the abscission zone, petiole, and stem of the bean during abscission layer development. Both PME and PG are believed to function in the breakdown of pectins.

Cellulase has also been indicated as possibly having a role in the formation of the abscission layer. Abeles (1969) found an increase in cellulase activity during abscission in explants of bean, cotton and Coleus. Cellulase activity was localized in the cell separation layer and the increase in activity preceded the loss of break strength in the abscission layer (Craker and Abeles, 1969).

Ethylene, which hastens the abscission process, enhanced the activity of cellulase in bean explant abscission zones (Horton and Osborne, 1967). 2, 4, 5-Trichlorophenoxyacetic acid, which delays abscission, was demonstrated to have the opposite effect. In addition, cellulase activity has been shown to increase during tomato fruit ripening (Dickinson and McCollum, 1964) thereby being present at the time of fruit abscission.

Indoleacetic acid oxidase has also been suggested as having a role in abscission (Hall and Morgan, 1963). Work with the IAA oxidase system in intact cotton plants revealed that certain phenols were cofactors while others were inhibitors of the system in vitro (Morgan, 1964). Therefore, using these phenols, further research was conducted to establish their effect on abscission of cotyledonary explants of cotton (Schwertner and Morgan, 1966). Results demonstrated that the cofactor phenols accelerated abscission while the inhibitory phenols delayed abscission, thereby suggesting a possible function for this enzyme in abscission.

Protein Synthesis in Abscission

Several authors have recently suggested that protein synthesis was essential for the development of the abscission layer (Lewis and Bakhshi, 1968; Abeles, 1968; Morré, 1968). Abeles and Holm (1966, 1967) showed RNA and protein synthesis inhibitors could greatly delay abscission in explants of bean, Coleus, and cotton depending on the concentration

used and the time of application. They also demonstrated that ethylene which accelerated abscission, caused an enhanced synthesis of RNA and protein in the separation layer. Abeles (1968) later hypothesized that RNA and protein synthesis were required for the formation of enzymes involved in the separation of cells during abscission.

Other evidence, however, indicated the enzymes in abscission may already be present in the zone prior to the induction of abscission (Valdovinos and Ernest, 1967). The enzymes may be attached to the walls of cells in the potential separation layer or be held in various unknown structures observed in these cells (Jensen and Valdovinos, 1967). Thus, the initiation of abscission would simply involve the activation or release of these enzymes.

Secondary cell division has often been proposed as being necessary for organ detachment (Addicott and Lynch, 1955; Webster, 1970). If cell division was necessary, it would explain the need for RNA and protein synthesis in abscission. However, Bednarz (1970) has obtained evidence indicating that cell division is not necessary for abscission to occur.

Effect of Growth Regulators on Abscission

Auxins

Laibach (1933) was the first to demonstrate that abscission, induced by deblading, could be delayed by placing

orchid pollinia (a source of auxin) on the cut surface. Soon afterwards LaRue (1936) using synthetic IAA found it to be more effective in delaying abscission in debladed Coleus than a variety of auxin-containing substances.

Although applied auxin was shown to have an influence on abscission, there was no evidence that auxin present in the leaf blade had a similar effect. This was, however, demonstrated by a later study in which the auxin content of bean leaves was followed during development (Shoji, Addicott, and Swets, 1951). Results of this work showed a normally high auxin content in the leaf, but as the leaflet yellowed and approached abscission, there occurred a rapid fall in auxin level.

Several investigators have since shown that auxin applied distal to the abscission zone delayed abscission in direct relation to the concentration applied (Addicott and Lynch, 1951; Gaur and Leopold, 1955; Biggs and Leopold, 1958). However, proximal applications hastened abscission. In response to these opposite effects of auxin, Addicott, Lynch, and Carns (1955) proposed the auxin gradient theory to explain the control of abscission. According to this theory abscission is initiated by a fall in the ratio of distal to proximal auxin levels.

Further work designed to test this hypothesis through the use of labeled auxin has failed to show the presence of such a gradient across the abscission zone

(Rubinstein and Leopold, 1963; Rasmussen and Bukovac, 1966). Other interpretations, suggesting a quantitative rather than a qualitative response to auxin, have been proposed (Gaur and Leopold, 1955; Biggs and Leopold, 1957). However, evidence obtained from the study by Rasmussen and Bukovac (1966) using autoradiographic techniques also argues against these explanations.

Rubinstein and Leopold (1963) demonstrated a two-stage response of abscission to auxin--an induction stage during which auxin delayed abscission and a later stage where abscission was stimulated by auxin. In addition, they observed a two-phase concentration effect of auxin on abscission. Low concentrations (10^{-6} M) exhibited a slight acceleration of abscission while high concentrations (10^{-3} M) delayed abscission. The authors explained these effects as the consequence of actions on the two separate stages. Low auxin levels and proximal application, due to their lower activity and slower transport, would actually be acting on the second stage and thereby exhibiting the proper, accelerating response.

Both the gradient theory and the two phase action of auxin imply a direct effect of auxin on the abscission zone. However, other investigators have suggested an indirect role for auxin (Sacher, 1957; Jacobs, Kaushik, and Rochmis, 1964). Sacher (1957) concluded that auxin delayed abscission by maintaining membrane-integrity of tissue in the abscission

zone. Further support for this role of auxin was provided in a later study (Sacher, 1959). Jacobs et al. (1964) put forth the hypothesis that auxin delayed abscission by maintaining petiole elongation.

Although almost all the work designed to discover the effect of auxin on abscission has been carried out on leaf tissue, there is evidence to indicate that auxin may also play an important role in flower and fruit abscission (Gardner, Marth, and Batjer, 1939; Luckwill, 1948; Batjer, 1954; Yager, 1960b). Luckwill (1948) found a correlation between low levels of auxin in apple seeds and the fall of immature and mature fruits. Yager (1960b) observed that abscission of unpollinated tobacco flowers could be hastened by removing the younger leaves (source of auxin) and greatly delayed by application of IAA to the cut petioles of the removed leaves.

Ethylene

Ethylene has long been known to hasten abscission (Sampson, 1918). Gawadi and Avery (1950) and Hall (1952) suggested that abscission may be controlled by the ratio of auxin to ethylene. More studies implicate ethylene as a primary endogenous regulator of abscission and senescence processes (Abeles, 1966, 1967; Abeles and Holm, 1966; Burg, 1968). Because it is a gas and, hence, is freely diffusable in plant tissue, it hastens abscission regardless of the site of application.

Several investigations have demonstrated that auxin applications of greater than 10^{-6} M will induce ethylene formation in plant tissue (Morgan and Hall, 1964; Abeles and Rubinstein, 1964; Burg and Burg, 1966; Abeles, 1968; Hallaway and Osborne, 1969). Osborne (1968a) concluded that defoliation brought about by auxin was due to an accelerated senescence induced by ethylene, which was produced in the area of auxin application.

Therefore, Abeles (1967) has proposed the difference in response observed for distal and proximal applications of auxin as being due to differences in transport. Distally applied auxin inhibits abscission regardless of the ethylene produced, since it is rapidly transported to the abscission zone. Conversely, auxin applied proximally stimulates abscission because movement is slow and the ethylene produced dominates the response.

Although this explanation is attractive it fails to account for the acceleration of abscission obtained from distally applied auxin at low concentration. However, this acceleration is small and might be a response to stress induced by the application of the low level of auxin, since stress resulting from numerous sources has been shown to cause ethylene production in plant tissue (Nichols, 1966; Vines, Grierson, and Edwards, 1968; Pratt and Goeschl, 1969).

Other works have explained the acceleration of abscission by ethylene as resulting from its ability to

reduce the amount of diffusable auxin in the plant (Valdovinos, Ernest, and Henry, 1967; Burg, 1968; Goldsmith, 1968; Beyer and Morgan, 1969). Beyer and Morgan (1969) and Goldsmith (1968) found that prolonged treatment with ethylene resulted in an inhibition of basipetal auxin transport in peas and cotton. Other studies using ethylene indicated an increased destruction of auxin brought about by enhanced IAA oxidase activity and a decrease in the rate of auxin synthesis (Hall and Morgan, 1963; Valdovinos et al., 1967).

There is also evidence suggesting that ethylene may function by increasing membrane permeability (Von Abrams and Pratt, 1967). Such changes in membrane-integrity could result in the release of enzymes involved in abscission or the release of phenols and other substances which enhance IAA oxidase activity. However, recently Sacher and Salminen (1969) concluded that ethylene had no effect on membrane permeability of tissue sections from several plant species.

Abeles and Holm (1969) also found evidence for the stimulation of RNA and protein synthesis by ethylene in bean abscission zones. Abeles (1968) later suggested that ethylene hastened abscission by enhancing the synthesis of cell wall degrading enzymes in the abscission zone. But dela Fuente and Leopold (1969) argued against this concept on the basis of the short persistence of the ethylene stimulus (approximately one hour) after removing the gas.

Gibberellins

Distal applications of gibberellin (GA) have been shown to slightly hasten abscission in bean and cotton explants (Chatterjee and Leopold, 1964; Devlin and McIntyre, 1966; Bornman et al., 1967). Chatterjee and Leopold (1964) demonstrated that concentrations of GA from $10^{-3}M$ to $10^{-7}M$ all promoted abscission. They also suggested that this effect was principally on the first stage of the abscission process.

However, Berman (1969) applied GA proximally to cotton and observed a delay in abscission. Muir and Valdovinos (1970) have also shown that GA applied to debladed Coleus plants had no effect on the abscission process. But, applying GA to the stem apex resulted in a hastening of abscission in direct proportion to the concentration applied. In addition, such treatments of GA caused an increase in the level of endogenous auxin. Therefore, the authors concluded that GA accelerated abscission by increasing the level of auxin proximal to the abscission zone.

Kinins

Kinins, like gibberellins, produce variable responses on abscission. Osborne and Moss (1963) observed distal applications of kinetin to accelerate abscission of bean explants, while applications directly to the abscission zone delayed abscission.

Other studies using bean explants and debladed bean plants with the opposite leaf intact have indicated a two-phase response to kinetin (Chatterjee and Leopold, 1964; Rasmussen, 1965). Low concentrations applied distally showed some promotion while higher concentrations delayed abscission. Rasmussen (1965) explained these findings on the basis of directed transport induced by the applied kinetin. High levels caused a mobilization of materials from the opposite leaf to the abscission zone, while low levels were only capable of mobilizing substances from nearby to the point of application, thereby depleting the abscission zone of metabolites and hastening abscission.

Recently, proximally applied kinins were shown to hasten abscission of cotton explants (Berman, 1969). This too might be due to a mobilization of materials out of the abscission zone.

Abscisic Acid

The role of abscisic acid (ABA) in abscission and other plant processes has been thoroughly covered in a recent review (Addicott and Lyon, 1969). Levels of ABA in cotton fruits have been correlated with natural fruit abscission (Davis, 1968). ABA has further been shown to hasten abscission of attached cherry fruits and cherry fruit explants (Zucconi et al., 1969). In the latter study abscission layer development in the treated fruits was found to be quantitatively identical to the controls.

In addition, ABA has been demonstrated to be a potent accelerator of abscission in cotton explants (Bornman et al., 1967). Craker and Abeles (1969) observed a two-fold effect of ABA on cotton and bean explants. It caused an increased production of ethylene and also increased the activity of cellulase.

However, Weaver and Pool (1969) found that ABA was relatively ineffective compared to other compounds in promoting the abscission of grape berries and flowers. Other studies have suggested that some plants may rapidly inactivate ABA and thereby seriously limit its potential usefulness (Addicott and Lyon, 1969).

Other Factors Influencing Abscission

Environmental factors such as light, temperature, water, minerals, oxygen, and other gases can greatly alter the time and rate of abscission (Carns, Addicott, and Lynch, 1951; Addicott, 1954, 1965, 1968; Addicott and Lynch, 1955; Rosen and Siegel, 1963; Simons, 1963). Sucrose and amino acids have also been demonstrated to influence abscission (Brown and Addicott, 1950; Biggs and Leopold, 1957; Hall, Herrero, and Katterman, 1962; Rubinstein and Leopold, 1962; Addicott, 1965, 1968; Devlin and McIntyre, 1966; Berman, 1969).

Furthermore, work by Osborne (1955) has indicated the presence of an abscission promoting factor produced in senescing leaves. More recent studies have confirmed this

finding (Hall et al., 1962; Jacobs, Shield and Osborne, 1962). In addition, several workers have shown that aging of plants may modify the abscission process in still other important ways (Leinweber and Hall, 1959; Jacobs, McCready, and Osborne, 1966). Therefore, although explants and debladed petioles appear to undergo the normal abscission process, it is still unknown whether identical internal factors are involved.

CEPA

The introduction of 2-chloroethylphosphonic acid (CEPA, Ethrel) as a growth regulating compound has provided a simple means for studying the effect of ethylene. The first reported study of this compound was chemical in nature (Kabachnik and Rosiřskaya, 1946). Later Maynard and Swan (1963) provided important information on the chemical stability of this compound, but they did not realize the important growth regulating properties which it possessed.

The list of growth regulating effects induced by CEPA is rapidly expanding. They include root initiation, auxillary bud stimulation, growth retardation, stimulation of fruit maturity, promotion of flowering, regulation of sex expression in cucumbers, leaf epinasty, defoliation, release of mature fruits, and a number of other responses which closely parallel those obtained with ethylene (Amchem Products Inc., 1967; Warner and Leopold, 1967; Russo, Dostal, and Leopold, 1968; Edgerton and Greenhalgh, 1969; Cooke and

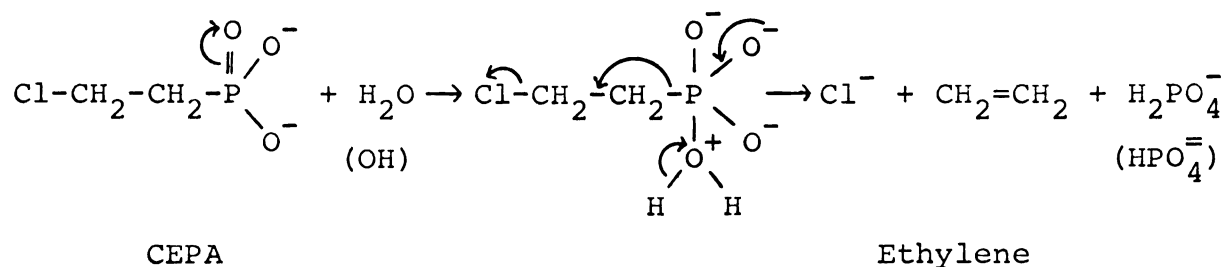
Randall, 1968; McMurray and Miller, 1969; Iwahori, Ben Yehoshua, and Lyons, 1969; Byers, Dostal, and Emerson, 1969).

The induction of pineapple flowering by CEPA was one of the early observations and has since become economically important (Amchem Products Inc., 1967; Cooke and Randall, 1968). Since ethylene was important in fruit ripening (Hansen, 1966), Russo and coworkers (1968) applied CEPA to banana fruits. Russo et al. (1968) observed CEPA to have the same effect on fruit ripening as ethylene gas. These ripening responses were also observed for pears and apples (Edgerton and Blanpied, 1968).

In a more recent study CEPA was shown to have the same growth stimulating effect on fig fruit in growth stage II as ethylene (Crane et al., 1970). This same kind of growth acceleration and ripening brought about by CEPA has also been shown for peaches and tomatoes (Byers et al., 1969; Iwahori et al., 1969; Iwahori and Lyons, 1970). These studies all indicate a potential use of the chemical in regulating fruit ripening in the field.

Warner and Leopold (1967) concluded that CEPA was effective in regulating growth responses through the stimulation of ethylene production in the plant tissue. In a later study they demonstrated that CEPA itself broke down in the plant with the release of ethylene (Warner and Leopold, 1969). They also presented a possible reaction for this breakdown. This mechanism, however, was shown to be

incorrect by Yang (1969). He concluded that the following reaction represented the breakdown of CEPA.



Morgan (1969) found that CEPA hastened the abscission of leaves, debladed petioles, and flower buds of cotton. He also presented evidence to indicate the accelerated abscission was due to ethylene "produced from or by the active ingredients in the formulation".

Bukovac and coworkers (1969) have shown a potential use of CEPA as an aid to mechanical harvesting of sweet and sour cherries and plums. They observed a hastening of abscission within 4 days after application as measured by fruit removal force. At lower concentrations (500 ppm and lower) it was found that the fruit could be loosened significantly without any observable defoliation or other phytotoxic response. Other studies have since confirmed these findings (Anderson, 1969; Bukovac et al. unpublished data).

In addition, CEPA has been observed to promote abscission of mature and immature apples and grapes (Edgerton and Greenhalgh, 1969; Weaver and Pool, 1969). Therefore, data obtained with CEPA further supports the findings that ethylene may play an important role in the control of abscission.

MATERIALS AND METHODS

Characterization of the Natural Fruit Abscission Process

General Methods

The abscission of maturing sour (Prunus cerasus L., 'Montmorency') and sweet cherry (Prunus avium L., 'Windsor') fruits was followed morphologically during the 1968 and 1969 growing seasons. Similar results were obtained for both years, hence only the results obtained in 1969 will be given. A detailed development of abscission was followed for both control and 2-chloroethylphosphonic acid (CEPA) treated fruits. Fruit removal force measurements were collected throughout the growing season so that the progression of abscission could be related to the stage of fruit growth, ethylene evolution from the developing fruit, and anatomical and histochemical changes occurring in the abscission zone.

The terms used in this dissertation to describe cherry fruit abscission are defined below:

Abscission--Separation of the fruit at the fruit: pedicel or pedicel:spur junction brought about by physiological and/or mechanical processes. Synonym--Separation.

Abscission zone--Transition zone between the fruit and pedicel (upper abscission zone) and/or the pedicel and spur (lower abscission zone).

Abscission layer--A well-defined layer of cells in the abscission zone which undergo physiological changes leading to abscission. Synonym--Separation layer.

Cherry Fruit Development

Fruit growth and FRF measurements were made throughout the growing season. Fresh weight was determined twice a week from random samples of 20 or more fruit. All samples were taken in the morning at approximately the same time.

FRF measurements at the upper and lower abscission zones were taken once a week with a Hunter Mechanical Force Gauge (Hunter Springs, Hatfield, Pa.) fitted with a claw. The procedure used to determine FRF at the lower abscission zone was similar to that described by Cain (1967). The fruit was pulled from its pedicel in line with the long axis immediately after picking the fruit with stems attached. FRF values at the upper zone were obtained by holding the spur in the claw at its point of attachment to the branch and pulling the pedicel from the spur, again in line with the long axis. A random sample of 20 fruits was used for FRF measurements at each zone for each date.

CEPA Modification of FRF at Maturity

Sour Cherry,--Three large branches on each of two uniform trees were selected and treated with CEPA at 0, 500, or 1000 ppm approximately 10 days prior to maturity. CEPA, acid-anhydride formulation (Amchem 66-329), was applied as a

foliar spray using 0.1% Tween 20 (polyoxyethylene-20-sorbitan monolaurate) as a surfactant. FRF was determined on a random sample of 20 fruits from each branch at 0, 4, 8, and 12 days after application.

Sweet Cherry.--Nine 7-year-old trees were selected for uniform vigor and fruit load. CEPA, acid formulation (Amchem 68-240), at 0, 500, and 1000 ppm was applied 5-7 days prior to optimum brining maturity (12-14 days before fresh market maturity) to single trees assigned to a randomized block design with three trees in each block. Application was made in the evening with a high pressure sprayer using a single nozzle gun, and trees were sprayed to the drip point. No wetting agent was used. FRF measurements were taken on a random sample of 25 fruits from each tree at time of treatment and thereafter every other day for 14 days.

Upper vs. Lower Zone.--To determine if CEPA influenced the pedicel:spur (upper) and fruit:pedicel (lower) abscission zones similarly, six pairs of branches with uniform fruit load were selected for treatment. One branch of each pair was assigned as the control and received only 0.1% Tween 20. The other branch was sprayed with CEPA at 1000 ppm and 0.1% Tween 20. The spray was applied to the drip point two weeks prior to optimum maturity.

FRF measurements were made on 10 randomly selected fruits from each branch 12 days after treatment. The pedicel

was cut in two with a pair of scissors, thereby making it possible to record the pull force on both abscission zones from a single fruit.

Morphology of Abscission

Samples of fruit for the morphology study were obtained from the same CEPA treatments as the fruit retention force measurements. A random sample of 15 fruit was collected from each of the 0, 500, and 1000 ppm treated branches of sour cherry. These collections were made at time of treatment and thereafter every other day for 14 days.

For sweet cherries, 10 fruits representative of that date were selected from each tree. Samples were collected daily for the first 10 days and every other day thereafter for the next 6 days. Therefore, a total of 30 fruits for each treatment was gathered for both sweet and sour cherry at each date.

Fruits were detached from the tree by cutting the pedicel above the upper abscission zone with a razor blade. The detached fruits were immediately killed and fixed in FAA (Formalin-acetic acid-alcohol, formulation from Jensen, 1962). Later, blocks of tissue containing the upper or lower abscission zone were cut from the spur and pedicel or fruit and pedicel respectively, dehydrated in a gradient series of t-butyl alcohol (Sass, 1958), embedded in Fisher Tissuemat (melting range 56-58°C) and mounted on wooden blocks. The tissue was cut at 12 μ m and affixed to glass slides with

Haupts adhesive, using 4% formalin to flatten and expand the sections (Jensen, 1962). The sections were passed through an alcohol series to water, stained with iron hematoxylin, dehydrated to xylene, and permanently mounted in Lipshaw mounting medium (Lipshaw Manufacturing Company, Detroit).

Fifteen fruits were sectioned for each treatment and collection date. Since 500 ppm CEPA was nearly as effective as 1000 ppm, only photomicrographs of sections from fruit treated with the lower concentration are presented.

Histochemical Changes in the Abscission Zone During Fruit Separation

Changes in pectin, lignin, cellulose, polysaccharides, and starch were followed histochemically in the abscission zone during fruit maturation in both the sour and sweet cherry. FAA fixed tissue, representative of the various stages of abscission, was embedded in paraffin and sectioned at 15 μ m. All experiments were performed twice with similar results.

The following staining reactions, as outlined by Jensen (1962), were used to localize the various constituents:

<u>Constituents</u>	<u>Test Used</u>
Pectin	Ruthenium Red
Pectin	Hydroxylamine-Ferric Chloride
Lignin	Phloroglucinol-HCl
Polysaccharides	Periodic Acid-Schiff's Reagent
Starch	Iodine Potassium Iodide

Changes occurring in cellulose orientation of cell walls in the abscission zone were studied using plane-polarized light. Paraffin was removed from the sections, and they were mounted directly in Lipshaw mounting medium prior to observation.

The distribution of water insoluble calcium and magnesium across the abscission zone was followed using an electron microprobe (Applied Research Laboratories Model EMX - SM). Paraffin sections cut at 15 μm were mounted directly on quartz slides using only 4% formalin to flatten the sections on a warming plate. The sections were then incinerated by increasing the temperature from 21 $^{\circ}\text{C}$ to 350 $^{\circ}\text{C}$ over a period of 5 hours then held at 350 $^{\circ}\text{C}$ for 6 hours in a muffle furnace. The gradual increase in temperature prevented the paraffin from spattering (Bednarz, 1970).

After incineration, the remaining stable white ash was coated with a conducting layer of carbon (approximately 200 $\text{\AA}$ thick) and the specimen was examined with the electron microprobe. Operating conditions were 25 kilovolts electron accelerating potential and a sample current of 0.05 microamperes.

The tissue scanned was the abscission zone just below the epidermal indentation. The distribution of Ca and Mg was obtained using two procedures. First, a scanning x-ray micrograph was obtained for the entire area. Then a 50 μm wide beam was centered on the X axis and the section was moved under the beam, thereby providing a qualitative

line scan of Ca and Mg. Similar patterns of distribution for both Ca and Mg were obtained, and since the level of Mg was far below that of Ca, only the results for Ca are presented.

Ethylene Evolution

Ethylene evolution from sweet and sour cherry fruit was measured twice a week from early development to past maturity. The fruit was carefully detached from the tree at the upper abscission zone, immediately transferred to the laboratory and the pedicel was cut with a razor blade 3 to 4 mm from the fruit.

Fruits during growth stages I and II (Tukey, 1934) were weighed, placed in 30 ml flasks, and sealed with a rubber vaccine cap. Larger fruits, later in the season were sealed in 264 ml glass jars with vaccine caps fitted in the covers (see Appendix, Table A1 for number of fruits per container and number of replications). Filter paper wicks saturated with 10% NaOH were sealed in the containers above the fruit to remove carbon dioxide.

The sealed containers, including appropriate controls (lacking only the fruit), were then transferred to a circulating water bath at $25^{\circ}\text{C} \pm 1^{\circ}$. Oxygen was added with a syringe every hour to maintain the level near normal. The level of oxygen and carbon dioxide was checked after 4 and 8 hours using a Vapor Fractometer Model 154 B.

Ethylene determinations were made after 0, 1, 2, 4, and 8 hr. The data obtained from the 8 hr determinations are presented in the results. Samples of 100 μ l were taken from the 30 ml flasks and 1.0 ml samples from the large containers. These collections were made with gas-tight Hamilton syringes using the technique described by Lyons, McGlasson, and Pratt (1962). The samples were injected into a Varian Aerograph Series 1200 gas chromatograph system, using a 30 inch column containing Poropak Type R. Amounts of ethylene were determined by comparing peak heights to that of a known standard.

Values of ethylene were converted to $\text{nl fruit}^{-1} \text{ hr}^{-1}$ and $\mu\text{l kg fresh weight}^{-1} \text{ hr}^{-1}$. In these determinations correction was made for the volume displaced by the fruit.

Characterization of the Abscission Process in Detached Fruit Explants

General Methods

Explants made up of the fruit and pedicel of Prunus cerasus L. cv. Montmorency (sour cherry) and Prunus avium L. cv. Windsor (sweet cherry) were used to study the physiology of fruit abscission. Uniform fruits (based on size and color) were selected, detached from the tree at the upper abscission zone, and transferred immediately to the laboratory. The pedicels were then trimmed under water to a uniform length of about 2.5 cm and the explants were positioned in 4 ml test tubes containing 3.7 ml of the designated

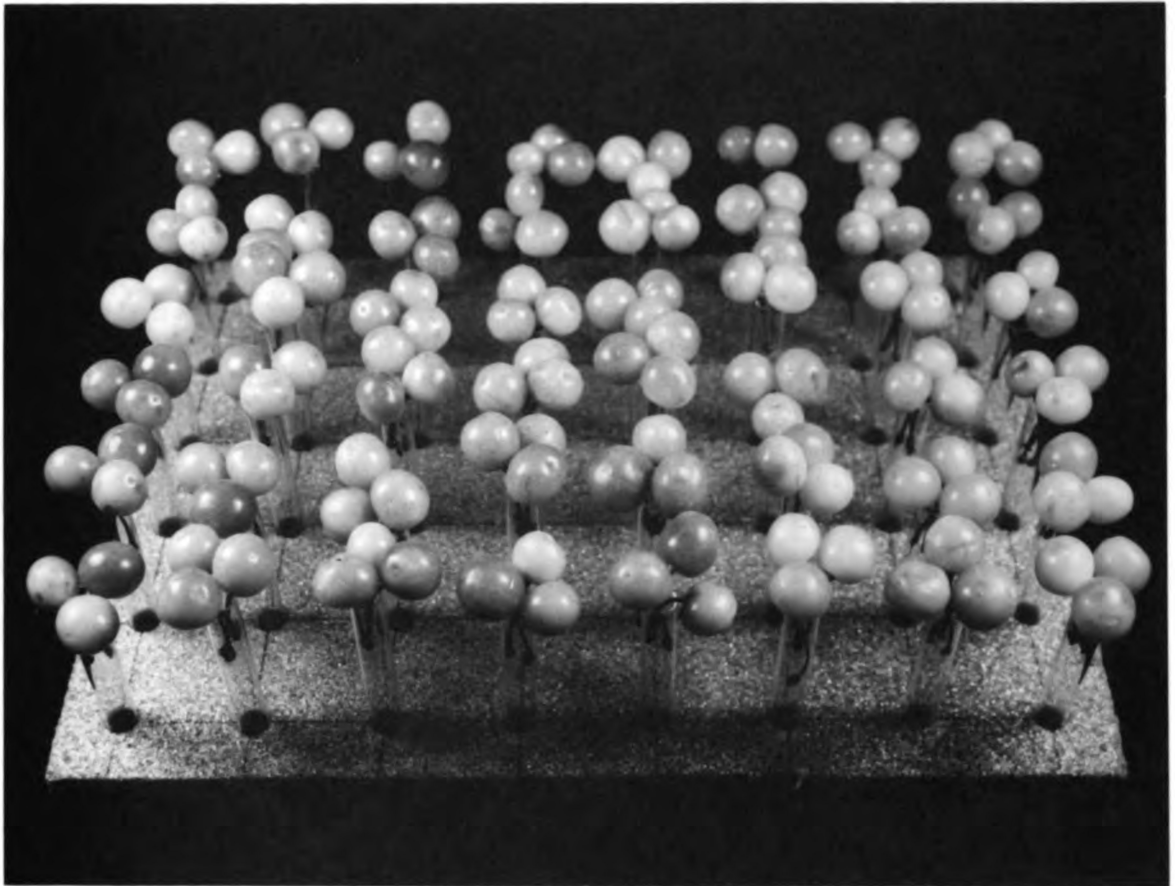
treating solution (Figure 1). Two to four fruits were used per tube, depending on the stage of development, and each tube represented one replication of a treatment. The tubes were arranged in a randomized block design with 8-12 replications. Explants were held in the dark for 80 hours at 25°C. The volume of test solution was maintained by addition of distilled water as needed. At the end of 80 hours the FRF was measured.

Effect of Age on Fruit Abscission.--This experiment was conducted to establish the effect of stage of fruit development on abscission and sensitivity to ethylene (CEPA). Fruit explants, collected weekly from the end of growth stage I to maturity, were assayed as described above using CEPA at 0, 10^{-5} , 10^{-4} , and 10^{-3} M.

Response of Mature Fruit Explants to Growth Regulators.--Sweet and sour cherry fruit explants were obtained approximately 2-4 days prior to the first histochemical signs of cell separation in the abscission zone (10-12 days prior to maturity). The following growth regulators and concentrations were used to establish the abscission process:

<u>Compound</u>	<u>Concentrations Used (M)</u>
3-Indoleacetic acid (IAA)	0, 10^{-7} , 10^{-6} , 10^{-5} , 10^{-4} , 10^{-3}
2-Chloroethylphosphonic acid (CEPA)	0, 10^{-6} , 10^{-5} , 10^{-4} , 10^{-3}
(RS)-Abscissic acid (ABA)	0, 10^{-7} , 10^{-6} , 10^{-5} , 10^{-4} , 10^{-3}
Gibberellin A ₃ (GA)	0, 10^{-6} , 10^{-5} , 10^{-4} , 10^{-3}

Figure 1. Photograph illustrating the bioassay used to study abscission in detached fruits.



The effect of these compounds on abscission was determined by measuring the FRF after 80 hr and comparing the values with those of the controls.

Role of Protein Synthesis.--Cycloheximide (CH) was used to indicate if protein synthesis was involved in fruit abscission. Cycloheximide resembles transfer RNA, therefore, the peptide chain being formed readily attaches to it. But unlike transfer RNA, cycloheximide remains bound to messenger RNA, and hence, terminates the formation of new protein molecules.

Cycloheximide was either applied directly to the abscission zone (0.25 μ g) or used as the treating solution for the fruit explants (10 ppm). In addition, it was used in combination with CEPA to establish if it would inhibit the CEPA-enhanced abscission.

RESULTS

Characterization of the Natural Fruit Abscission Process

Change in FRF with Fruit Development

Sour Cherry.--During the first few weeks of fruit development, the pedicel represented the weakest connection between the fruit and branch (Figure 2). Hence, when a force was applied to the fruit, separation occurred near the middle of the pedicel generally leaving a section of exposed conducting tissue, which did not separate along the same plane. Toward the end of stage I of fruit growth the spur region (between the pedicel and spur and the spur and branch) became the weakest point of attachment, resulting initially in a sharp decline in FRF (standard deviations for the FRF values are given in Table A2-Appendix).

Another decrease in FRF at the spur occurred just prior to the start of the third growth phase. After this reduction there was a strengthening of the tissue and by fruit maturity the FRF at this zone had reached a maximum.

The lower abscission zone showed a very different pattern of development, in that, this zone continued to strengthen after anthesis until the start of the second growth phase, at which time it had reached a maximum value.

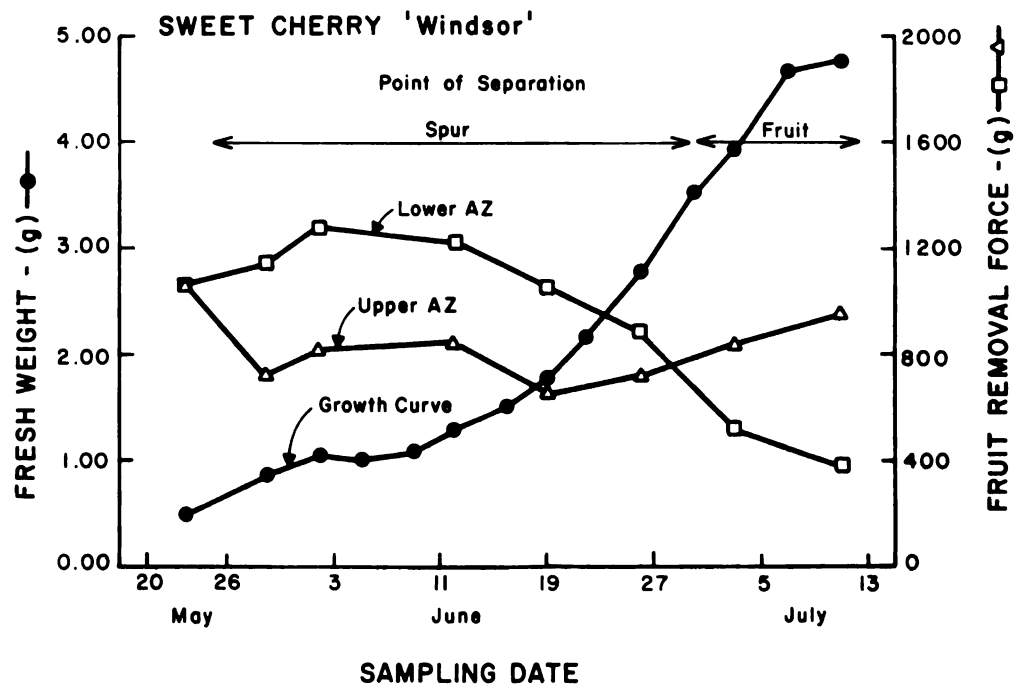
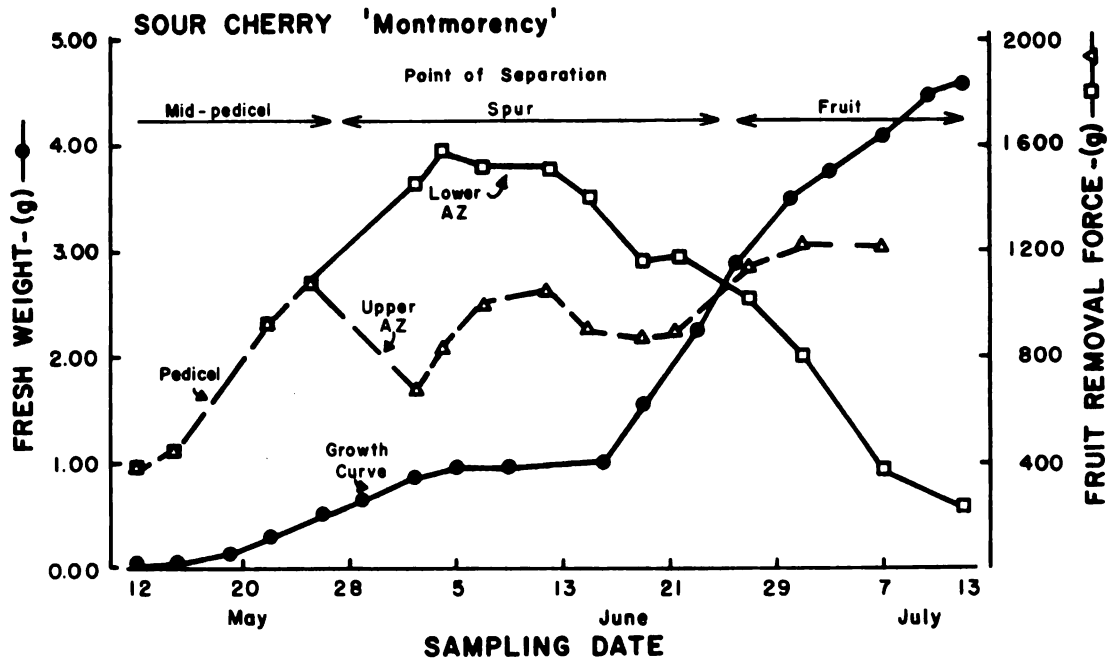
With the beginning of growth stage II there was a gradual decline in FRF, which became more pronounced just prior to the start of the third growth phase. This rapid decline in FRF continued through to maturity resulting in the lower zone becoming the weakest point of fruit attachment.

Sweet Cherry.--In sweet cherries the FRF of the upper and lower abscission zones never reached as high a value in mid-season as was observed for sour cherries (Figure 3). During the first few weeks of fruit development the pedicel represented the weakest region. Then just prior to the start of growth stage II, there was a weakening of tissue at the upper zone (standard deviations for the FRF values are given in Table A3-Appendix). Another decrease in FRF at the upper zone appeared to be associated with early stage III of fruit growth and then, as the fruit approached maturity there was an apparent strengthening of the tissue resulting in a rise in FRF.

The FRF at the lower abscission zone was observed to increase steadily during stage I of fruit growth, reaching a maximum value with the start of stage II. During the second growth phase there was only a slight decline in FRF, but as the fruit entered stage III this reduction became pronounced. About two weeks prior to maturity the lower zone represented the weakest region of fruit attachment. However, even at maturity there was only about half as great a difference in FRF between the upper and lower zone as observed for the sour cherry.

Figure 2. A comparison of sour cherry fruit growth and FRF of the upper and lower abscission zones from full bloom to maturity.

Figure 3. A comparison of sweet cherry fruit growth and FRF of the upper and lower abscission zones from early development to maturity.



CEPA Promotion of Fruit Abscission

Sour Cherry.--CEPA at 500 and 1000 ppm, applied 10 days prior to maturity, caused a 50% reduction in FRF (Figure 4). No significant difference was observed between concentrations, however, both caused a significant reduction from the control. Maximum response to the chemical was observed between 4 and 8 days after treatment.

At 1000 ppm some leaf yellowing and abscission was evident (about 5%), but at 500 ppm only a few yellow leaves were observed (1%). No other injury was apparent on the tree, and the fruit showed no phytotoxicity.

Sweet Cherry.--A similar response to CEPA was observed for sweet cherries (Figure 5). Both 500 and 1000 ppm caused a significant reduction in the force required to separate the fruit from the pedicel. Maximum reduction over the controls occurred between 6 and 10 days following application.

Again, some leaf abscission and yellowing was observed at 1000 ppm (5%) but this was evident only on the lower, weaker limbs and spurs. Little to no injury was apparent at 500 ppm. Treated fruits appeared to be slightly advanced in maturity as noted by color and firmness.

CEPA Effect on Upper vs. Lower Zone

CEPA did not promote the abscission of sweet cherries at the upper zone at a level (1000 ppm) which markedly reduced the FRF at the lower zone (Table 1).

Figure 4. Effect of CEPA on fruit removal force of 'Montmorency' sour cherry 4, 8 and 12 days after foliar application. Vertical brackets indicate standard deviations for each point.

Figure 5. Effect of CEPA on fruit removal force of 'Wind-sor' sweet cherry 2, 4, 6, 8, 10, 12 and 14 days after foliar application. Vertical brackets indicate standard deviations for each point.

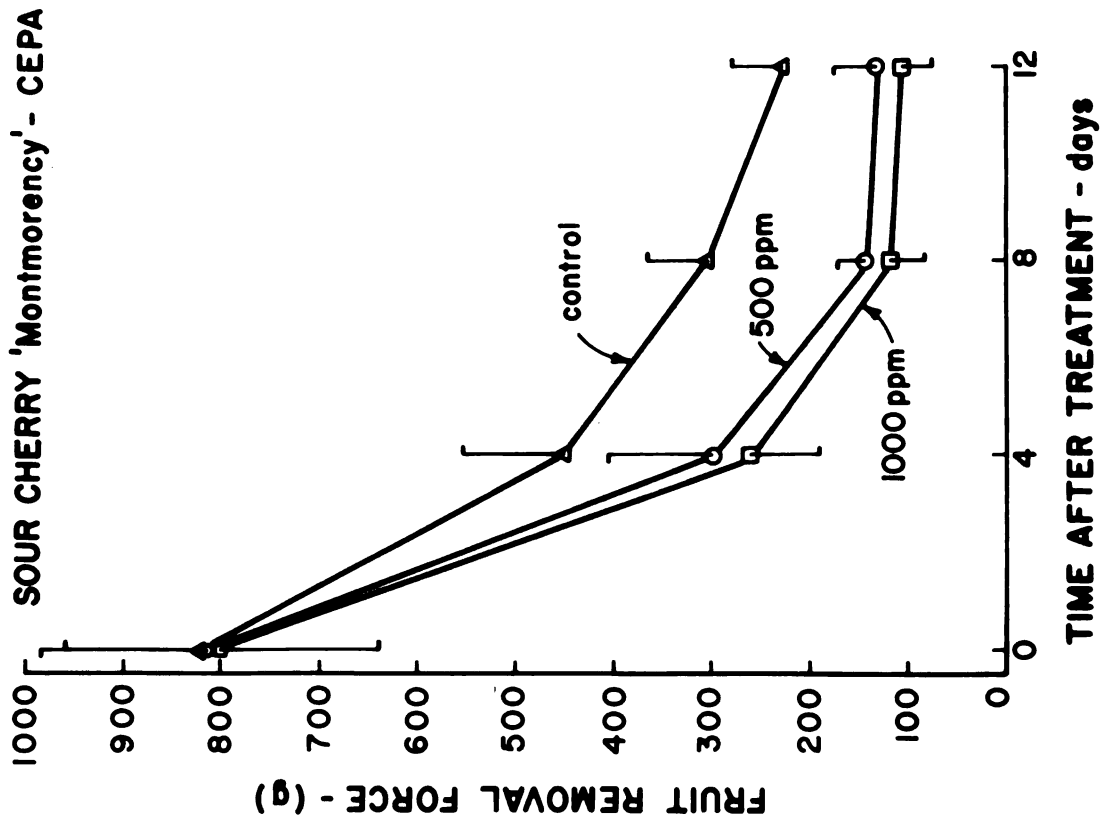
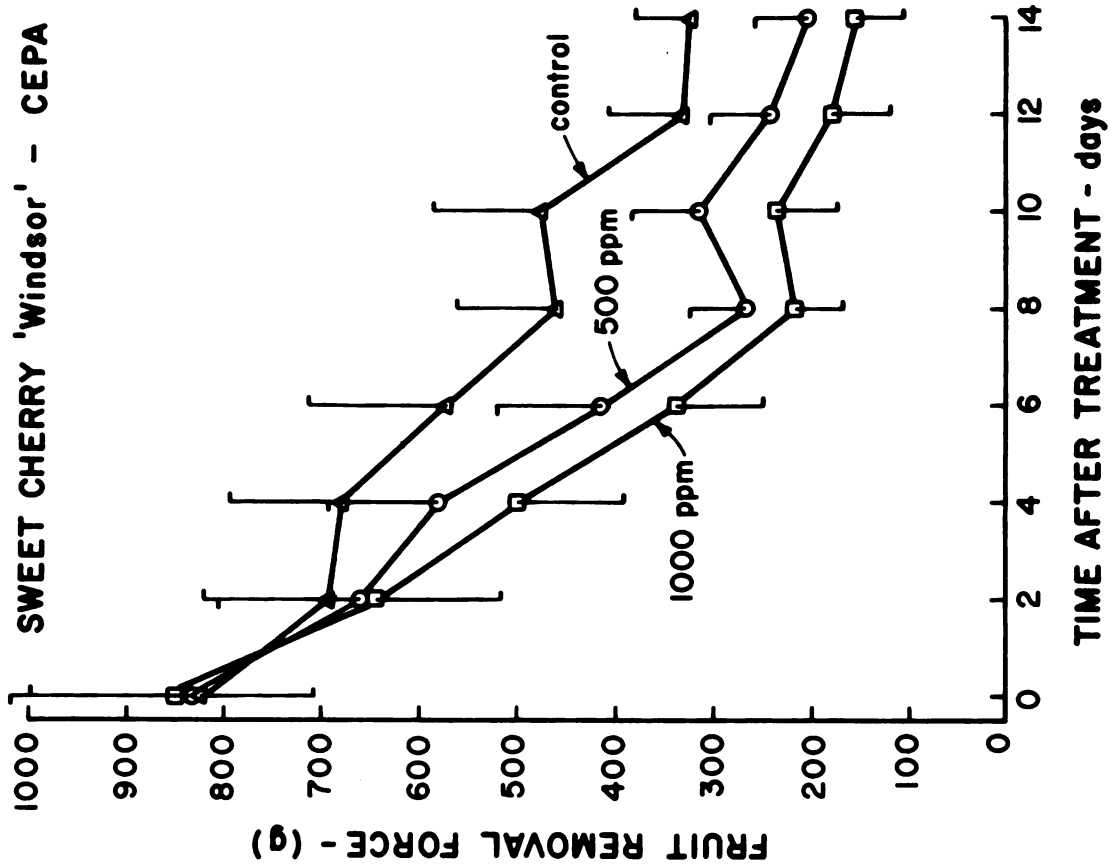


TABLE 1.--Effect of CEPA (1000 ppm) on fruit removal force (FRF) at the upper and lower abscission zones of 'Windsor' sweet cherry 12 days after foliar application.

Concentration	FRF	
	Upper zone	Lower zone
(ppm)	(g)	
0	946 a ¹	391 b
1000	1011 a	204 c

¹Means followed by unlike letters are significantly different at P=0.05 (Tukey's ω test).

Morphology of Abscission

Both sour and sweet cherry fruits exhibit two abscission zones during development (Figure 6). The upper zone is located at the junction of the pedicel and spur and is denoted by the constriction of tissue and the smaller isodiametric cells which comprise the zone (Figure 6A and C). Abscission at this zone occurs at the time of June drop and after detachment of the fruit, either at harvest or earlier in development.

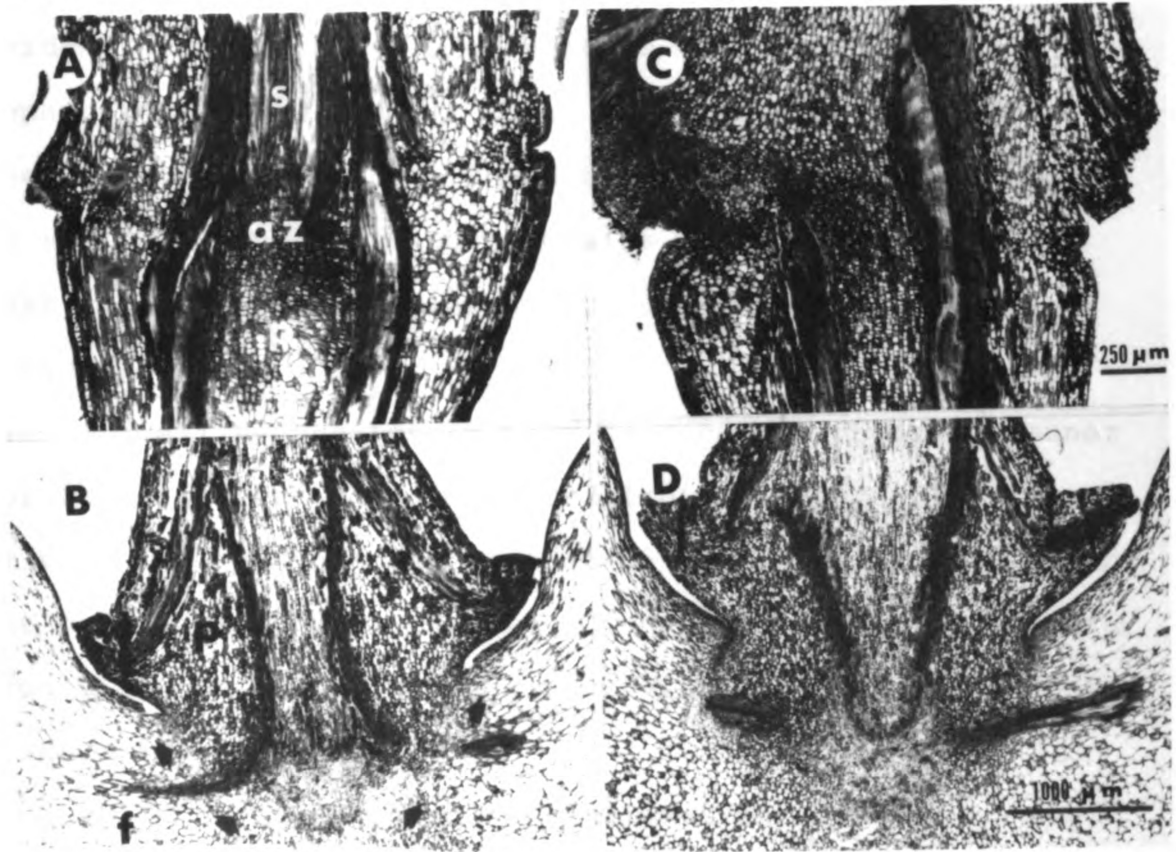
The other transition zone is at the junction of the fruit and pedicel (Figure 6B and D). This lower zone is also characterized by constriction of tissue and the presence of small cells. Abscission is initiated in this region just prior to fruit maturity, although the degree of cell separation is different for 'Montmorency' than for 'Windsor'.

Figure 6 denotes the state of abscission in sweet and sour cherries for the two zones at the time of CEPA application. Since no change was observed in the absence or presence of CEPA in the upper abscission zone from the time of treatment to maturity, the remainder of the data will deal entirely with the lower abscission zone.

Sour Cherry.--Abscission in maturing sour cherries has already been described (Stösser et al., 1969a, 1969b). Separation occurs at the transition zone between the pedicel and fruit. The cells of the abscission zone are small and round compared to the larger cells of the pedicel and the more elongated cells of the fruit.

Figure 6. Photomicrographs illustrating the two abscission zones of cherry fruits at the time of CEPA application. (A) upper zone, sour cherry; (B) lower zone, sour cherry; (C) upper zone, sweet cherry; (D) lower zone, sweet cherry.

az and arrows indicate abscission zone.
p-pedicel, f-fruit, s-spur



The effect of CEPA appears to be a hastening of the normal abscission process (Figures 7 and 8). The separation layer was first identified by a loss in affinity for haematoxylin (Figure 7A and 8B-SL). Then cells all through the crescent shaped abscission layer began to separate (Figure 7B and E). No separation or other change, however, was evident across the vascular bundles (Figure 7D and H). The separation of cells began above the stony pericarp and proceeded up to the natural indentation of the pedicel (Figure 7C and F). Generally there remains a few layers of cells next to the indentation where no cell separation occurs (Figure 7).

Cell separation appears to occur in the same manner for CEPA-treated and non-treated fruits (Figure 8). Generally the cells along the separation layer pull apart leaving the cell walls intact (Figure 8C, D and F), however, separation also occurs through cells leaving cell fragments behind (Figure 8D, G and H).

CEPA, in addition to accelerating abscission, also hastens the degradation of cells in the abscission zone (Figure 8G and H). Cells adjacent to the separation layer in treated fruits exhibit greater distortion of cytoplasm and cell structure than observed in control fruits.

Sweet Cherry.--Separation in the sweet cherry (Figures 9 and 10) is less well-defined than in the sour cherry. Cells in the abscission zone are readily apparent due to

Figure 7. Photomicrographs showing development of the separation layer at the lower abscission zone of 'Montmorency' sour cherry. Control (A-D) and CEPA-treated (500 ppm) fruits (E-H) 2, 4, 6 and 8 days after treatment.

Arrows indicate separation layer.
p-pedicel, f-fruit.

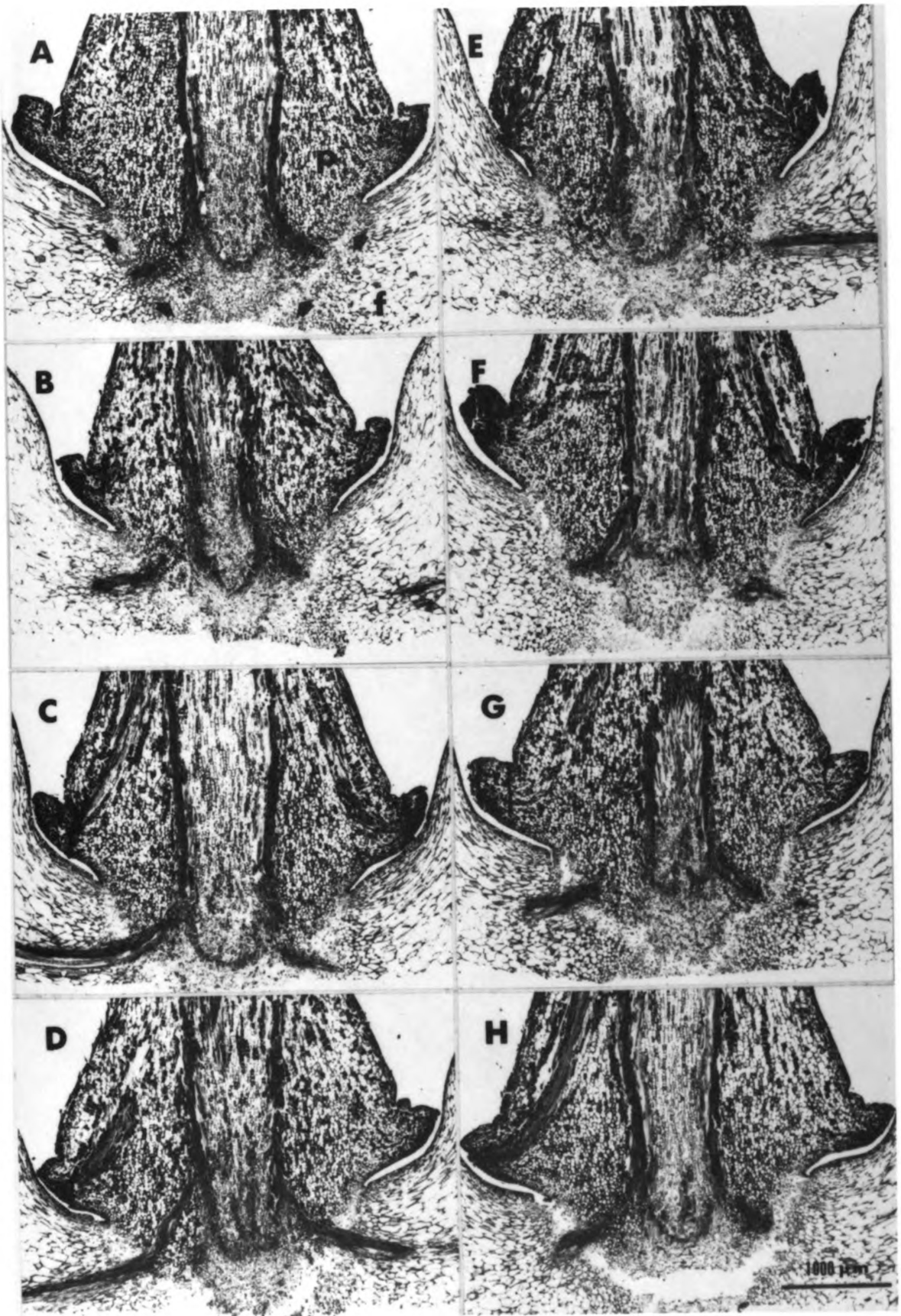


Figure 8. Photomicrographs showing a detailed development of the separation layer in the cortical tissue at the lower abscission zone of 'Montmorency' sour cherry. Control (A-D) and CEPA-treated (500 ppm) fruits (E-H) 0, 2, 4 and 6 days after treatment.

sl-separation layer or potential separation layer, p-pedicel, f-fruit.

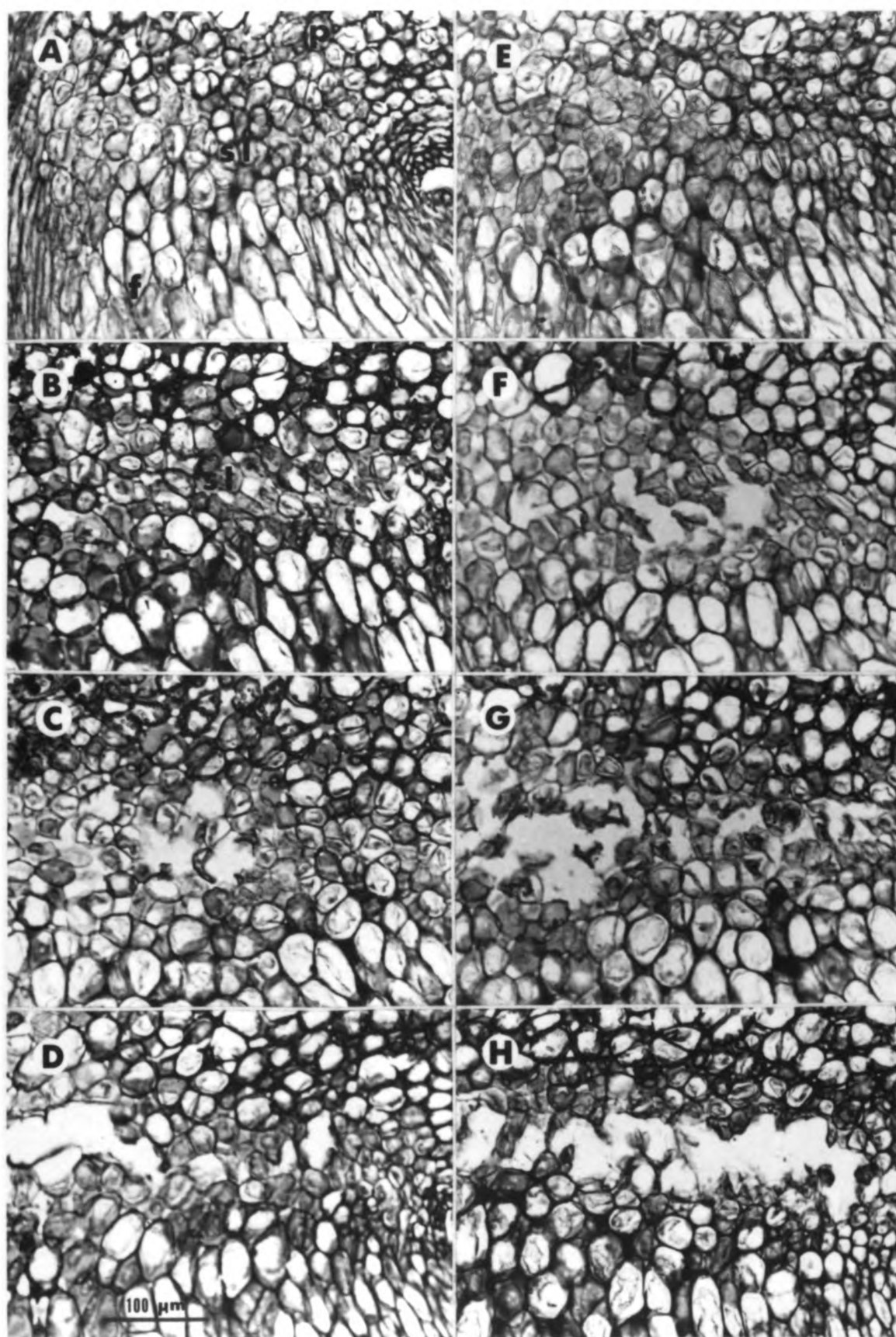


Figure 9. Photomicrographs illustrating changes occurring in the lower abscission zone of 'Windsor' sweet cherry during fruit maturation. Control (A-D) and CEPA-treated (500 ppm) fruits (E-H) 3, 6, 9 and 12 days after treatment.

Arrows indicate abscission. p-pedicel, f-fruit.

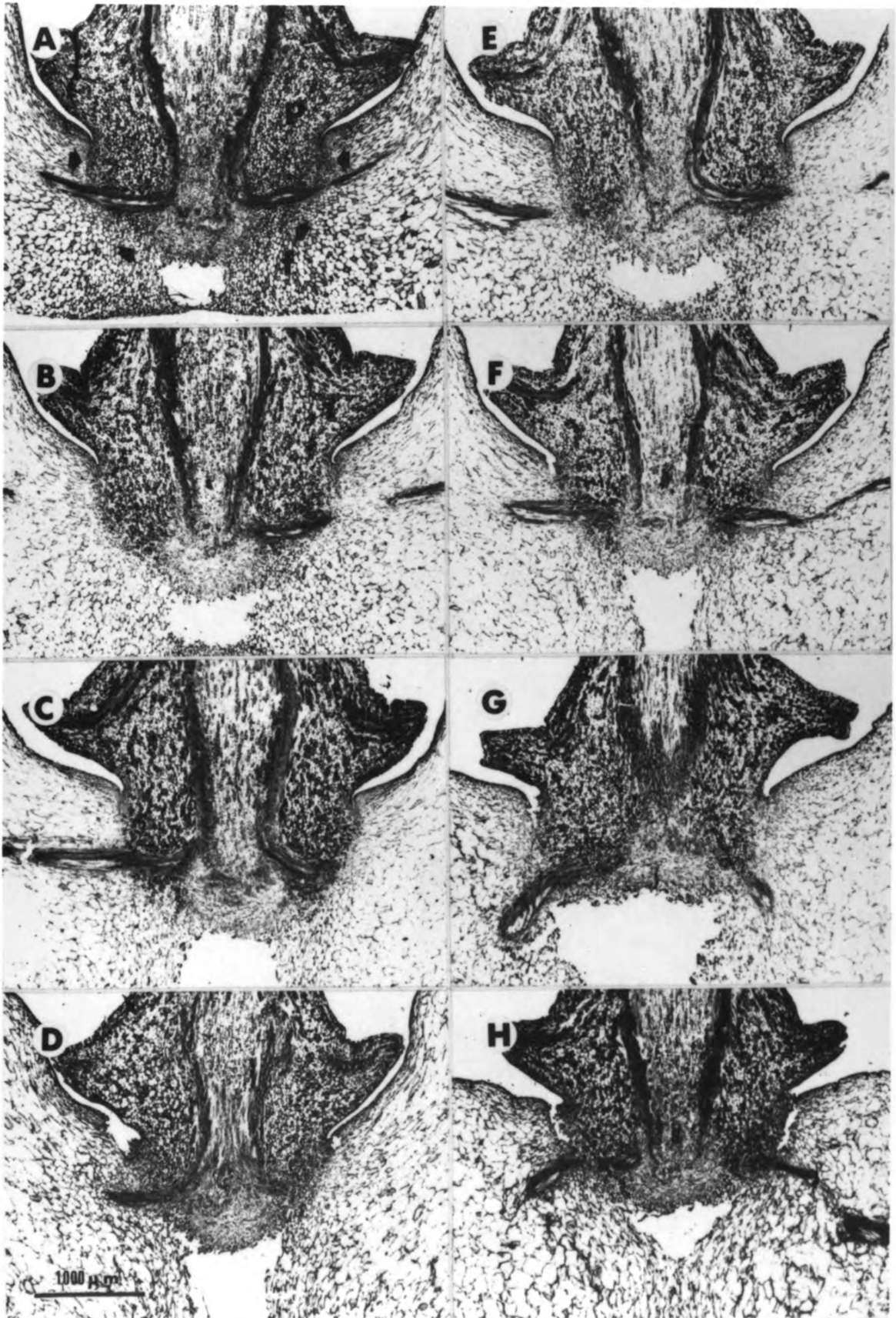
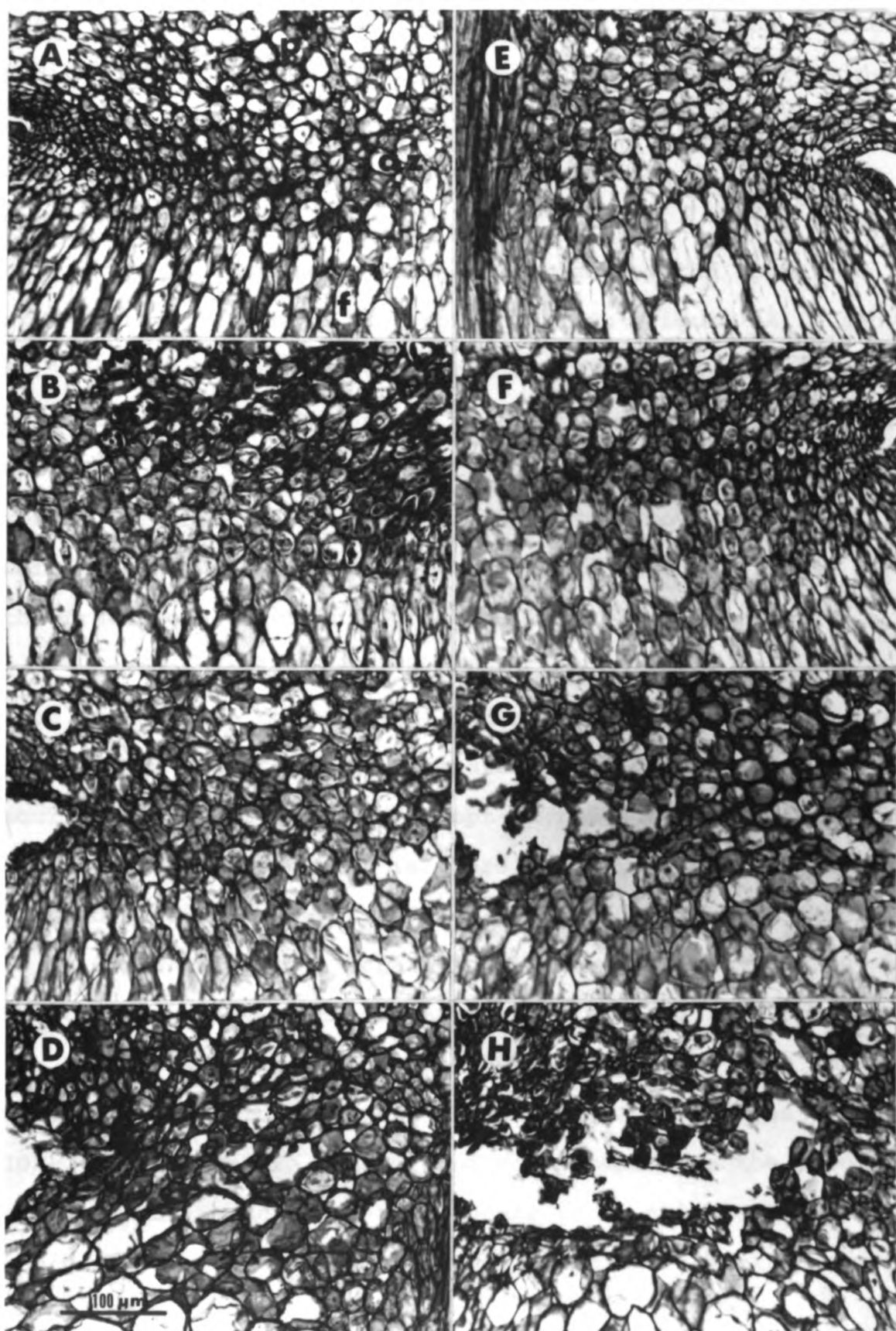


Figure 10. Photomicrographs illustrating a detailed development of abscission in the cortex at the lower abscission zone of 'Windsor' sweet cherry. Control (A-D) and CEPA-treated (500 ppm) fruits (E-H) 0, 4, 8 and 12 days after treatment.

az-abscission zone, p-pedicel, f-fruit.



their small size compared to the larger cells of the adjacent pedicel and fruit tissues. Still these cells are more similar to those in the pedicel due to their thick cell walls in comparison to the thin-walled cells of the fruit (Figure 9A).

Fruit abscission is characterized by the formation of a cavity just above the stony pericarp (Figure 9A, B and E). With the enlargement of this cavity, there appears to be a mechanical stress on the adjacent fruit tissue. Hence, the cells adjacent to the cavity collapse and are pushed in toward the cavity (Figure 9C and F). This movement of cells, which is enhanced by CEPA, later results in the collapse of the fruit lobe which surrounds the pedicel (Figure 9H).

Mechanical stress, prior to the collapse of the lobe, causes the cells at the indentation to pull apart (Figure 9D, F and G). Cell separation as observed in the sour cherry is not evident except in the formation of the cavity above the pit, nor is there any evidence of a well-defined separation layer as seen in the sour cherry. There is no evidence of cell separation in the vascular bundles but they probably break in response to mechanical force.

The structure of cells in the abscission zone appear much different from those in the sour cherry (Figures 8A and 10A). The cells are smaller and more compact in the sweet cherry, and in addition, they appear to be thicker-walled and contain more cementing material than those of the sour

cherry. Separation seldom proceeds in far from the indentation point (Figure 8D and 9D) except in fruits treated with CEPA (Figures 9H and 10G and H) or in fruits left on the tree past maturity.

When cell separation is observed in the cortex, it occurs through cell layers in the distal region of the abscission zone--next to the fruit tissue (Figure 9D and H and Figure 10G and H). If only the latter stages of abscission in CEPA-treated fruits are observed, the cells appear to have been cleanly separated along the middle lamella (Figure 9H and 10H). However, during cell separation in both treated and control fruits, it appears that the cells are generally being torn apart by mechanical force rather than through dissolution along cell walls (Figure 10D and G). It was also found that when control fruits were protected and allowed to become over mature (7-10 days past maturity) that abscission development advanced to the same degree as observed for CEPA-treated fruits 8-10 days after application.

From these observations CEPA appears to function similarly in sweet and sour cherries. In both species CEPA resulted in an apparent hastening of the normal abscission process. No cell enlargement or division was observed in the abscission zone of either the sweet or sour cherry.

Histochemical Changes During Abscission

Prior to cell separation (approximately 4 days), the walls of cells comprising the separation layer in the

abscission zone of sour cherries showed a loss of cell wall constituents. Such changes were not observed in the abscission zone of sweet cherries, indicating differences in the nature of the separation process for the two species.

Therefore, changes in cell wall constituents of the abscission zone and adjacent fruit and pedicel tissue were followed histochemically during abscission. No changes in the cell wall composition of either the fruit or pedicel tissue adjacent to the abscission zone were observed during abscission of either sweet or sour cherry. However, in sour cherries there was a loss of polysaccharides, cellulose, and pectins from the walls of cells of the separation layer in the distal portion of the abscission zone (Table 2). The loss of polysaccharides from the separation layer in sour cherry was readily apparent (Figure 11), whereas the loss of pectins (Figure 12) and cellulose was less pronounced. The 5-8 layers of cells composing the separation layer and undergoing these changes in composition were the cells which later separated.

In sweet cherries similar changes in polysaccharides (Figure 11), pectins (Figure 12), and cellulose did not precede cell separation or even occur later in abscission (Table 2). Instead, separation occurred where the cellulose content per unit area of tissue was naturally lower due to a

Figure 11. Photomicrographs of sections from the lower abscission zone stained for polysaccharides with periodic acid--Schiff's reagent. (A) Sour cherry fruit prior to cell separation. (B) Sweet cherry just prior to the start of cell separation. (C) Sour cherry during cell separation. (D) Sweet cherry during localized cell separation.

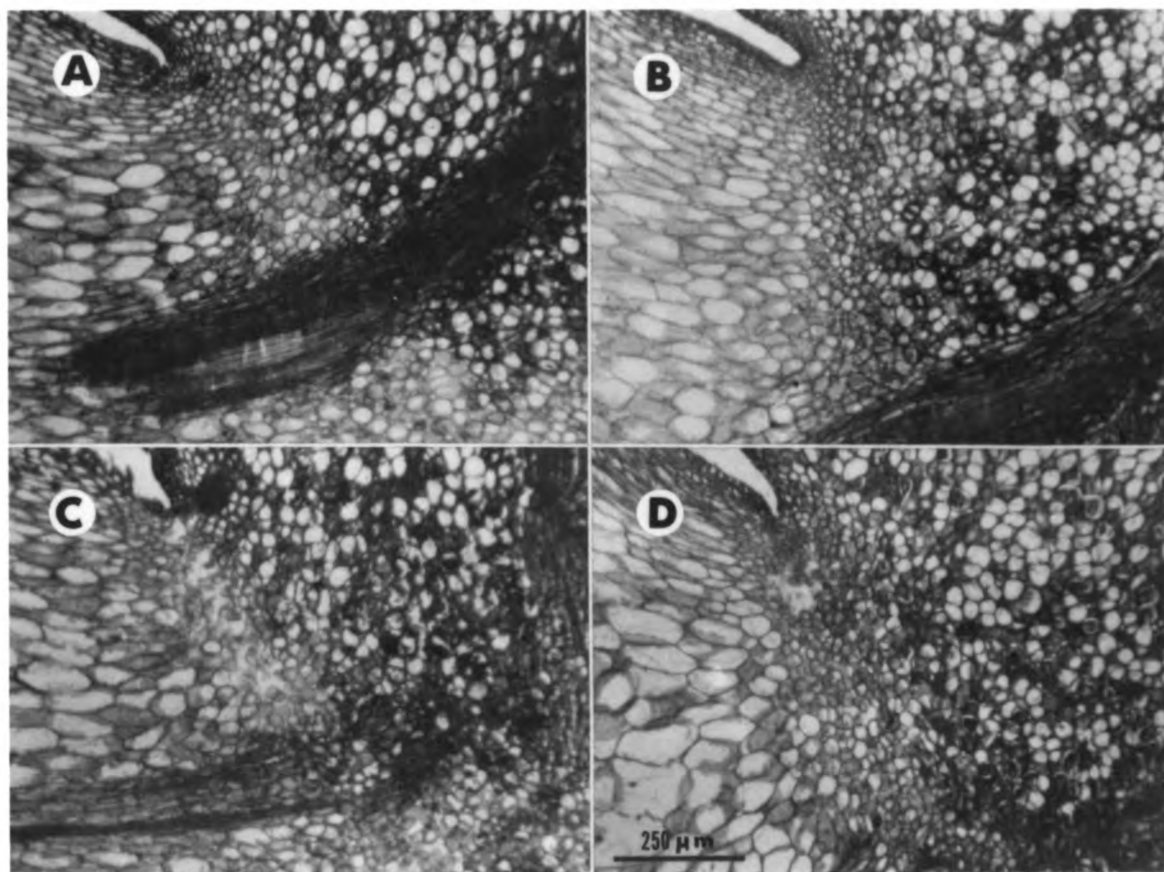


Figure 12. Photomicrographs of sections from the lower abscission zone stained for pectins with ruthenium red (A, B) and hydroxylamine ferric chloride (C, D). Sour cherry just prior to cell separation (A, C). Sweet cherry during localized cell separation (B, D).

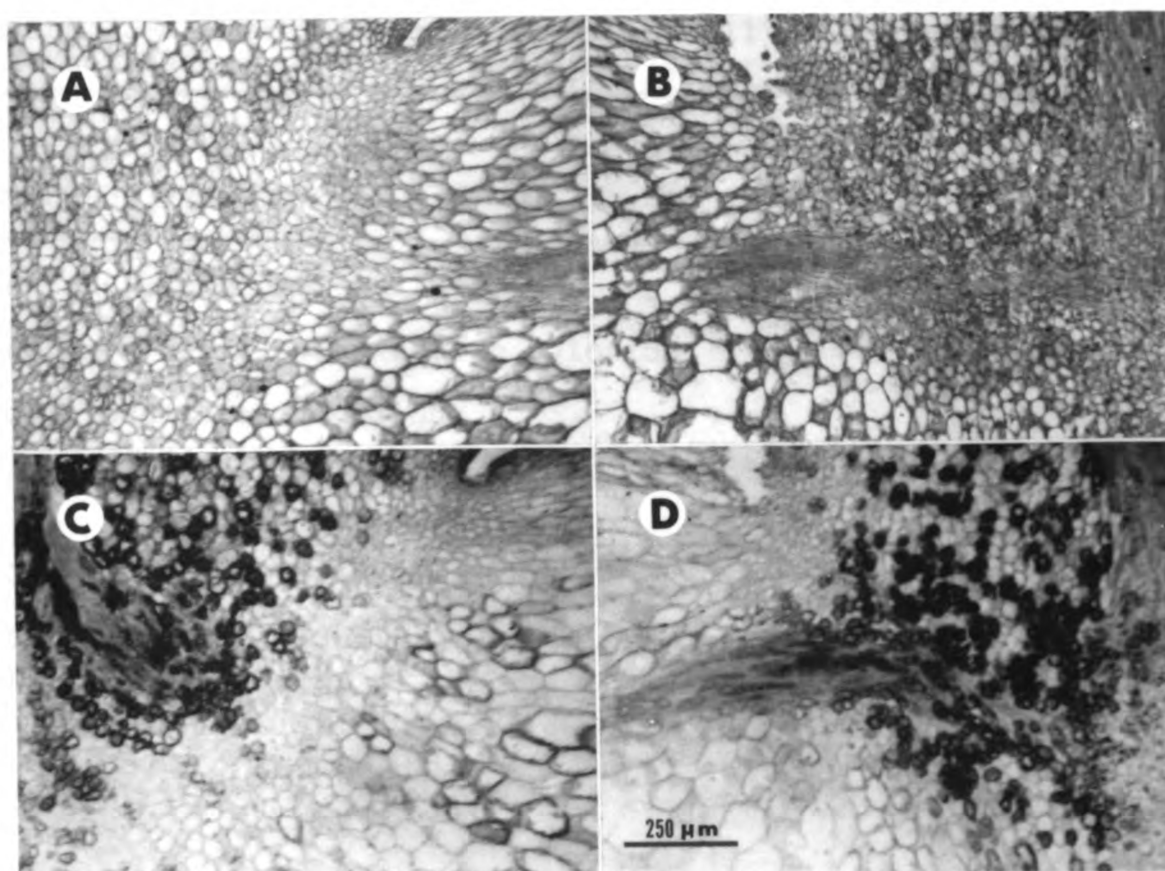


TABLE 2.--An estimate of changes in cell wall constituents of the abscission zone immediately prior to cell separation.

Compound-test	Abscission zone	
	Sour	Sweet
Pectin-Ruthenium red	- ¹	0
Pectin-Hydroxylamine ferric chloride	-	0
Cellulose-Polarized light	-	0
Polysaccharides-PAS ² reagent	-	0

¹0 (no change); - (decrease)

²Periodic Acid - Schiff's Reaction

decrease in cell number per unit area going from the distal region of the abscission zone to the adjacent fruit tissue.

No change was observed in the starch content of the separation layer or abscission zone in either the sweet or sour cherry. However, some build up of starch was observed in a few specialized cells proximal to the abscission zone. Also, no lignification of cells on either side of the separation layer was observed. Only fiber and vascular bundle cells along with cells of the epidermis of the fruit gave a positive reaction. Similar results were found for both the sweet and sour cherry.

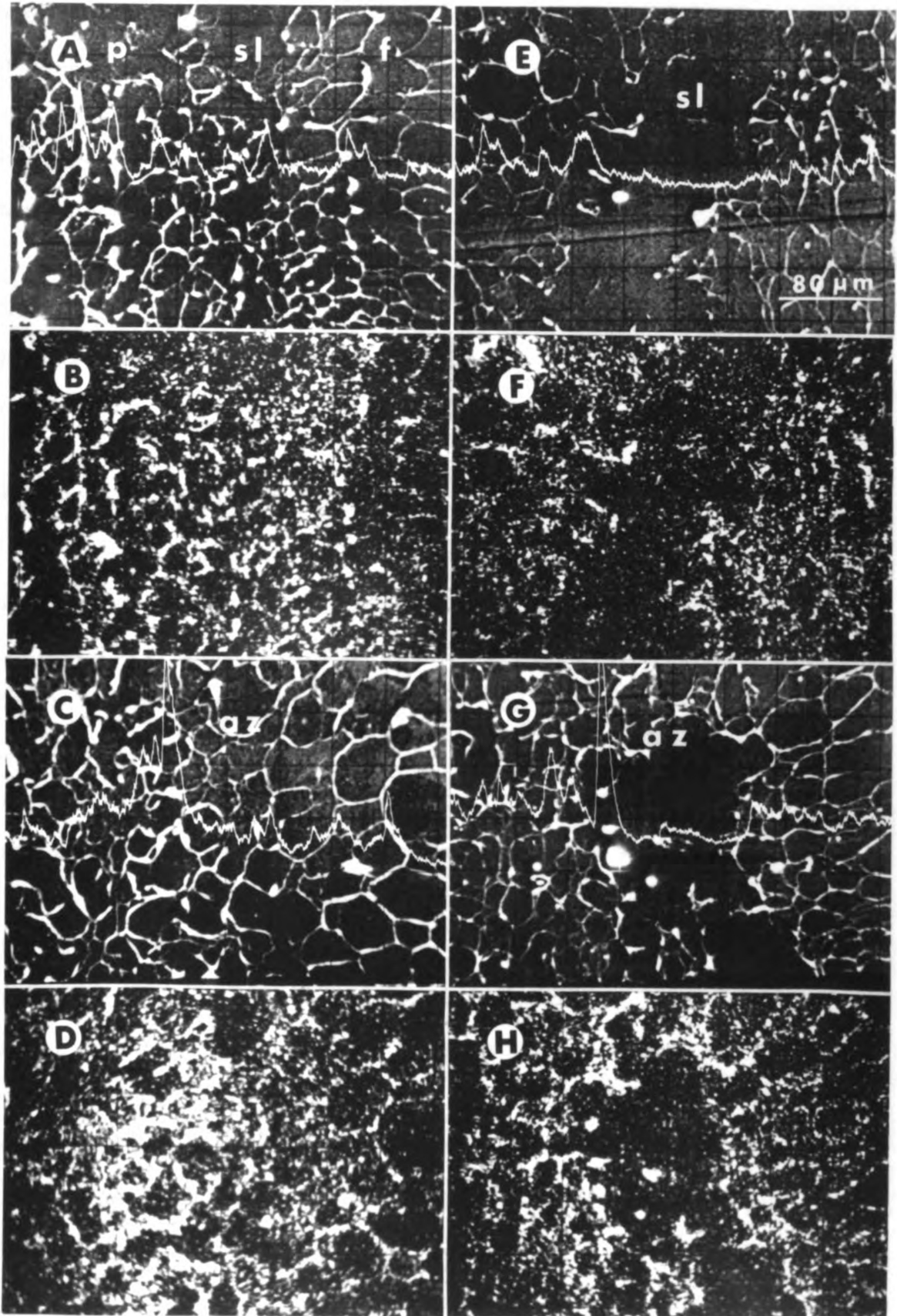
Localization of water insoluble calcium showed that even after the initial histochemical changes in the abscission layer of sour cherry are observed, these cell walls still appear to contain as much Ca as walls of cells in the pedicel region (Figure 13A and B). Although more Ca was observed in the abscission layer than in the walls of cells of the fruit, this was to be expected since the fruit cells are larger and have thinner walls. In treated fruits of the same date, where the cells had already separated there was a loss of Ca from the abscission layer (Figure 13E and F). However, this loss was probably associated with the absence of cell walls in this area (Figure 13E). Therefore, the Ca content appears to be almost completely associated with the cell walls, and as these walls are degraded the Ca is lost.

Similar results were observed for the sweet cherry (Figure 13C, D, G, and H). However, in the sweet cherry a

Figure 13. Localization of calcium in the lower abscission zone of sour and sweet cherry during fruit abscission. (A, C, E, G) Secondary electron micrographs of the abscission zone. The line graph indicates the distribution of calcium for a 50 μm wide band centered on the x-axis. (B, D, F, H) Calcium x-ray micrographs of A, C, E, and G respectively.

- A. Sour cherry at the start of cell separation.
- C. Sweet cherry just prior to cell separation.
- E. Sour cherry treated with CEPA showing complete cell separation in the separation layer.
- G. Sweet cherry treated with CEPA showing localized cell separation.

p-petiole, f-fruit, sl-separation layer,
az-abscission zone.



higher level of Ca is apparently associated with the cells proximal to the abscission layer (Figure 13C and G). This may reflect more cementing material between these cells and represent the thicker-walled cells in this region as was mentioned earlier.

Although a comparison between treated and non-treated cherries is presented, control fruits showing cell separation gave identical results to the treated fruits where the cell walls were completely separated.

Ethylene Evolution from Cherry Fruits During Development

Sweet and sour cherries exhibited a similar pattern of ethylene evolution throughout fruit development (Figures 14 and 15). However, the level of ethylene evolved was low for both species. Sour cherries, on a per fruit basis (Figure 14 - $\text{nl fruit}^{-1} \text{ hr}^{-1}$), showed a decline in the level of ethylene during degradation of the flower parts. Just prior to the start of pit hardening there was a sharp increase in ethylene evolution. The level then fell off during the remainder of growth stage I and stayed relatively constant during most of stage II. However, just before the start of stage III another increase in ethylene evolution was observed. Thereafter, the level of ethylene declined and remained nearly constant until late in development when there was another increase, probably due to tissue degradation.

Figure 14. Ethylene evolution from sour cherry ('Montmorency') fruits during development.

On a per fruit basis ($\mu\text{l fruit}^{-1} \text{ hr}^{-1}$ -●-) and a fresh weight basis ($\mu\text{l kg}^{-1} \text{ hr}^{-1}$ -○-). Vertical brackets indicate standard deviations for each point.

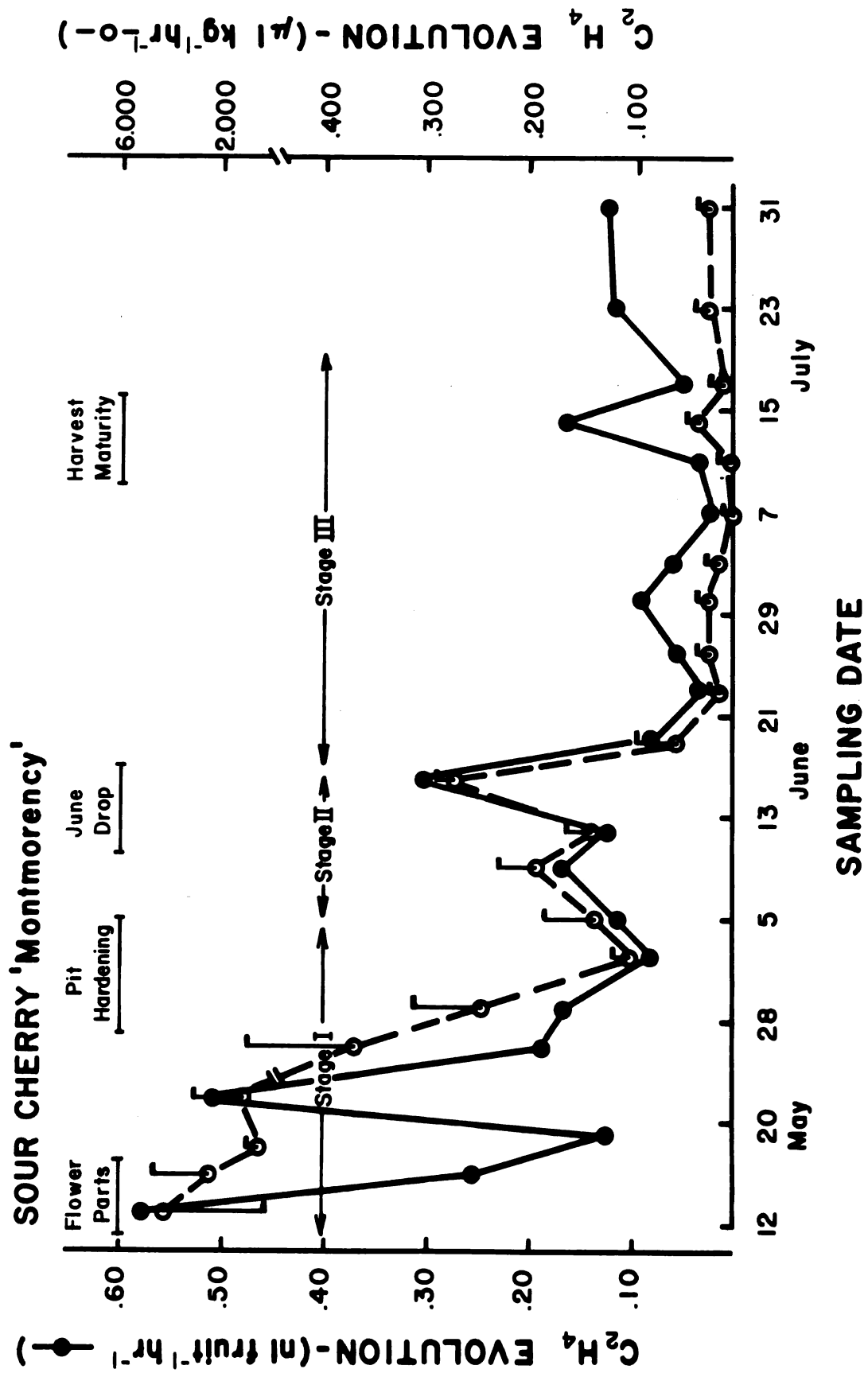
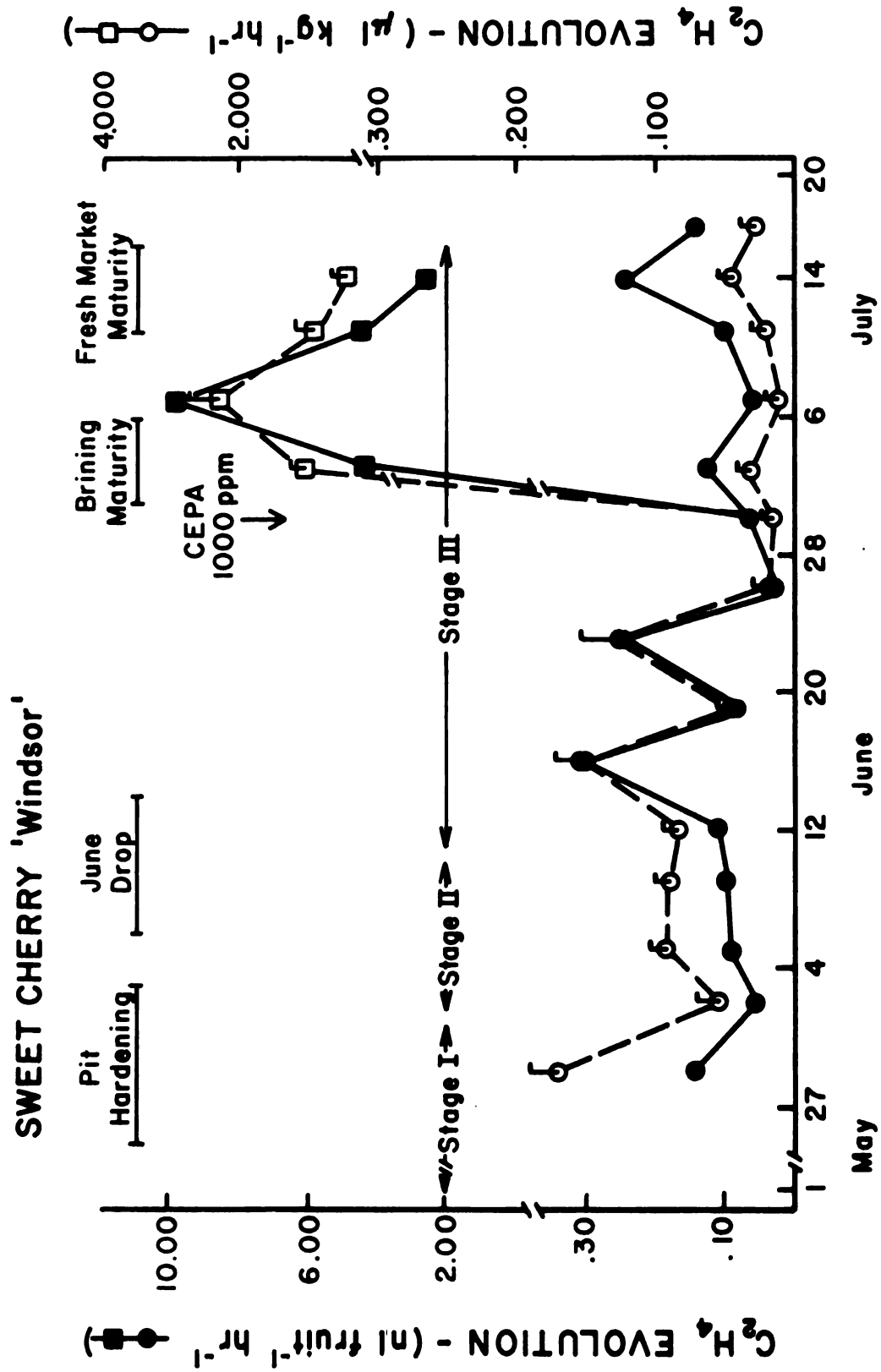


Figure 15. Ethylene evolution from sweet cherry ('Windsor') fruits during development.

On a per fruit basis ($\text{nl fruit}^{-1} \text{ hr}^{-1}$ -●-) and a fresh weight basis ($\mu\text{l kg}^{-1} \text{ hr}^{-1}$ -o-) and each of these as influenced by the application of CEPA at 1000 ppm (-■- and -□-). Vertical brackets indicate standard deviations for each point.



When the results for sour cherries were calculated on a fresh weight basis (Figure 14 - $\mu\text{l kg}^{-1} \text{ hr}^{-1}$), a different relationship was observed. There appeared to be an almost steady decline in ethylene evolution with fruit development. The level of ethylene decreased steadily during stage I, leveled off during stage II, and increased just prior to the beginning of stage III, after which the level again declined and remained low through fruit maturity.

Ethylene evolution from sweet cherries was very similar to that for sour cherries (Figure 15). There was a decline in the level of ethylene at the end of stage I, a leveling off during stage II, and an increase just after the start of stage III. This peak coincided in time with the peak observed for sour cherries which preceded the start of stage III. Shortly after this peak another similar increase in ethylene evolution was observed. Then the level again declined and remained relatively uniform through maturity.

When CEPA was applied to sweet cherries at 1000 ppm the amount of ethylene evolved as well as the development of abscission, was increased markedly over the controls (Figure 15 □ and ■). The level of ethylene from the treated fruits remained high for at least two weeks after treatment (experiment was concluded at this time due to wet weather causing the overripe fruit to crack). However, even though the ethylene level was markedly increased it is theoretically possible that this ethylene was derived solely from the

breakdown of CEPA and not from a stimulation of ethylene production by the fruit.

Characterization of Fruit Abscission
in Detached Fruits

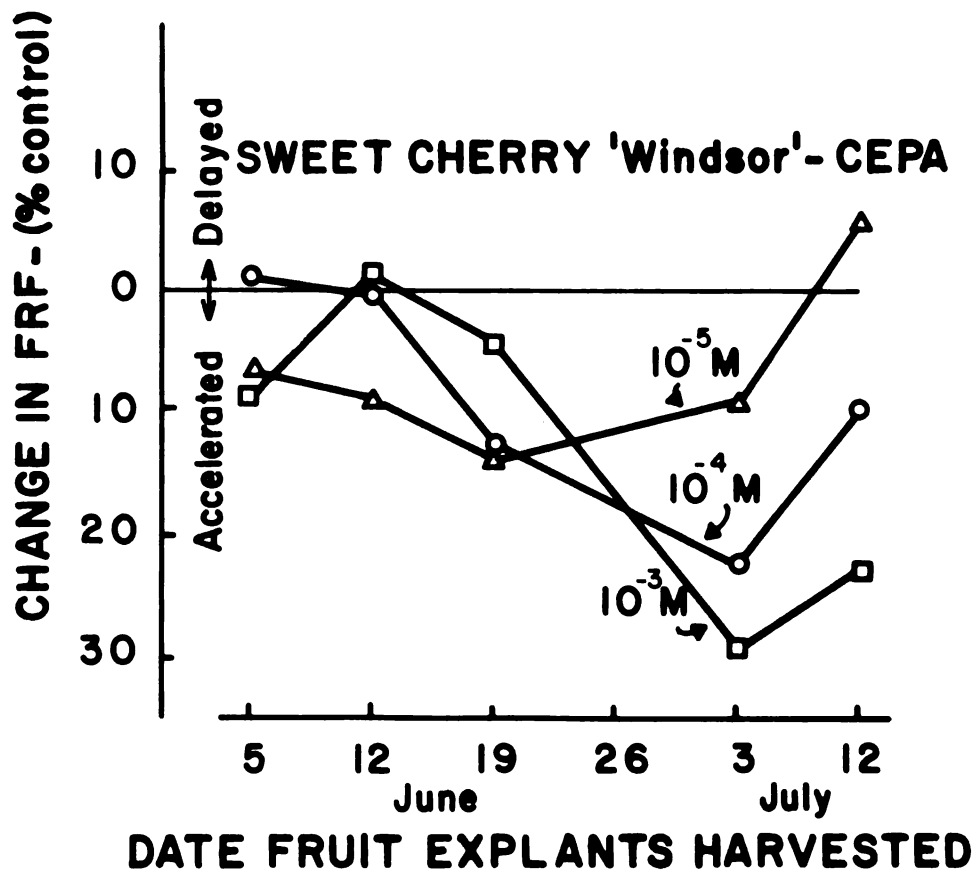
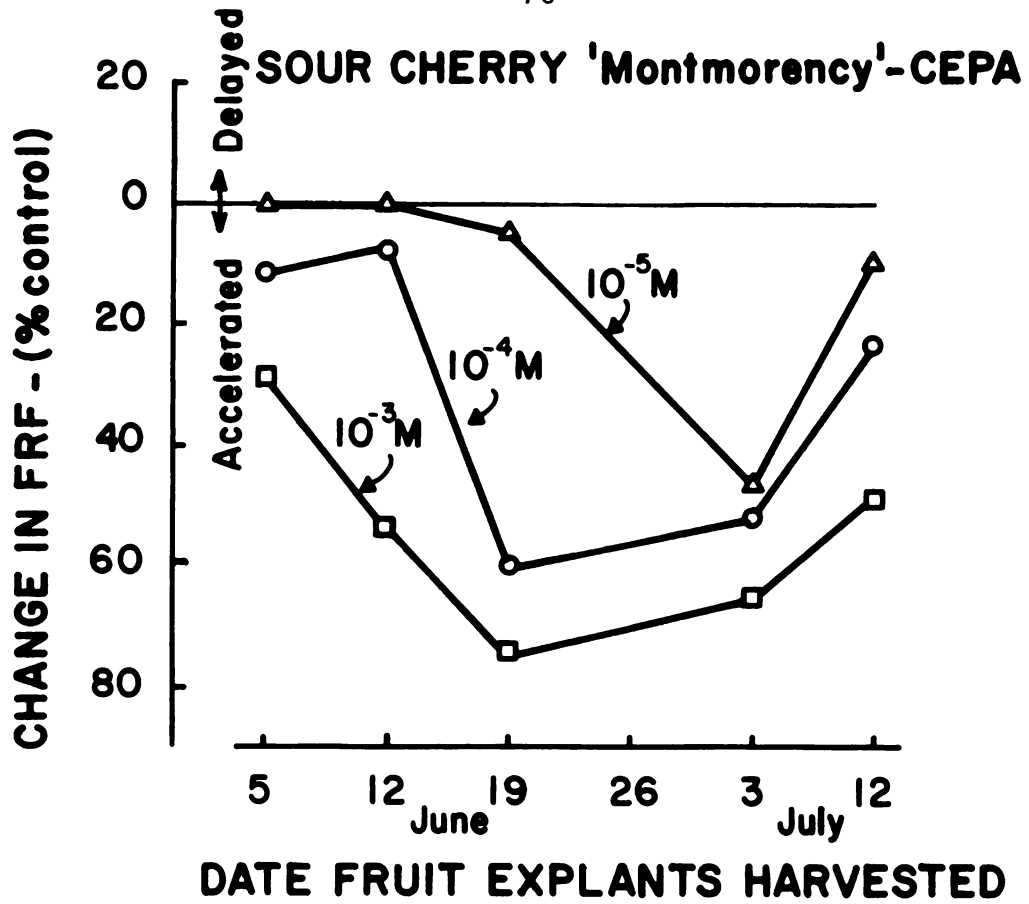
FRF as Effected by CEPA and
Stage of Fruit Development

CEPA at concentrations of 10^{-3} , 10^{-4} , and 10^{-5} M was found to either accelerate or have no influence on abscission of sour cherry fruit explants (Figure 16). Increasing maturity of the fruit enhanced the ability of CEPA to hasten abscission at these concentrations. In addition, the higher the concentration the greater the decrease in FRF over the controls. Explants showed the greatest sensitivity to low concentrations of ethylene around the first of July. This time coincided with the initiation of the separation layer in natural fruit abscission as observed by histochemical changes in these cells.

In sweet cherries different concentrations of CEPA and stage of fruit development had little effect on abscission until near fruit maturity (Figure 17). But even at this late stage of fruit development only the higher concentrations showed an increase in ability to accelerate abscission over the controls, and even this enhancement was far below that observed for sour cherries.

Figure 16. Change in FRF of CEPA-treated sour cherry ('Montmorency') fruit explants as effected by stage of fruit development.

Figure 17. Change in FRF of CEPA-treated sweet cherry ('Windsor') fruit explants as effected by stage of fruit development.



Response of Mature Fruit
Explants to Growth Regulators

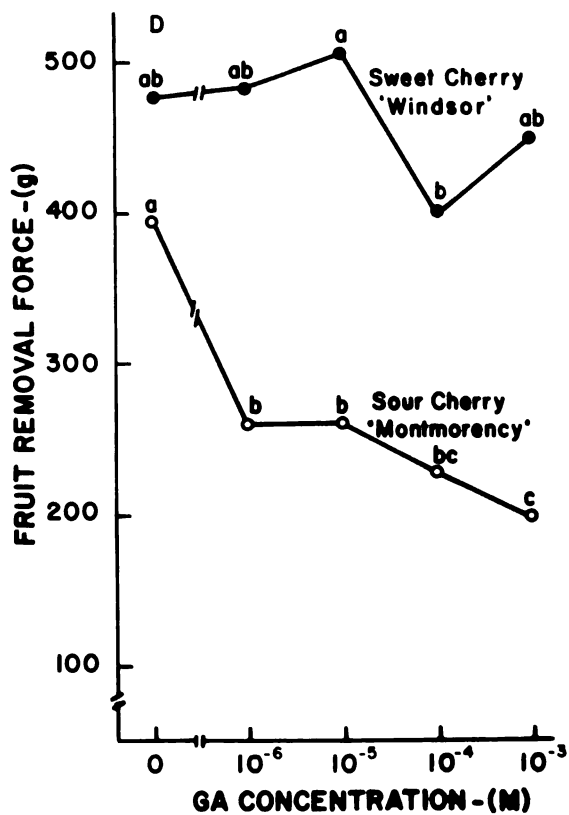
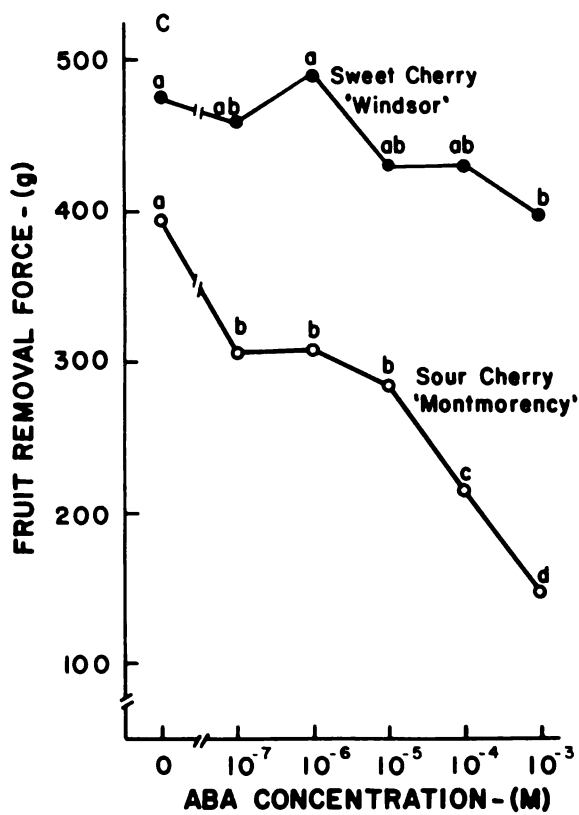
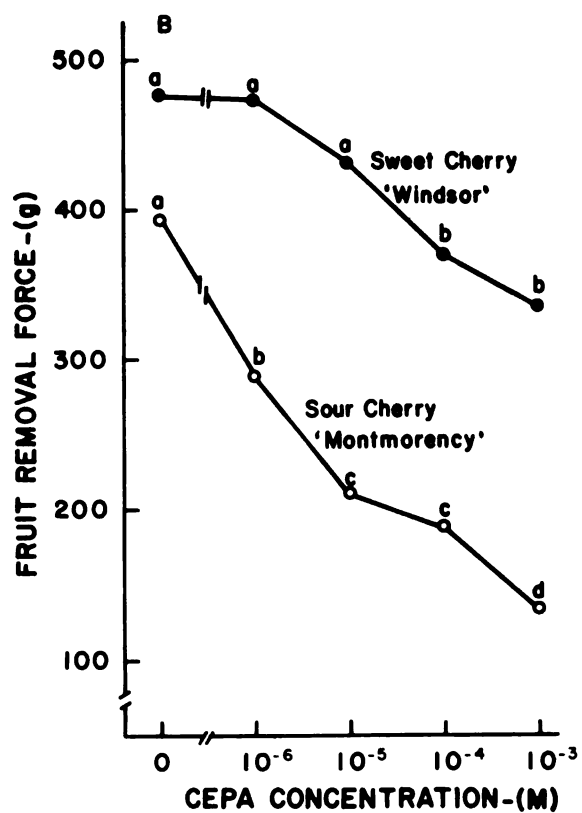
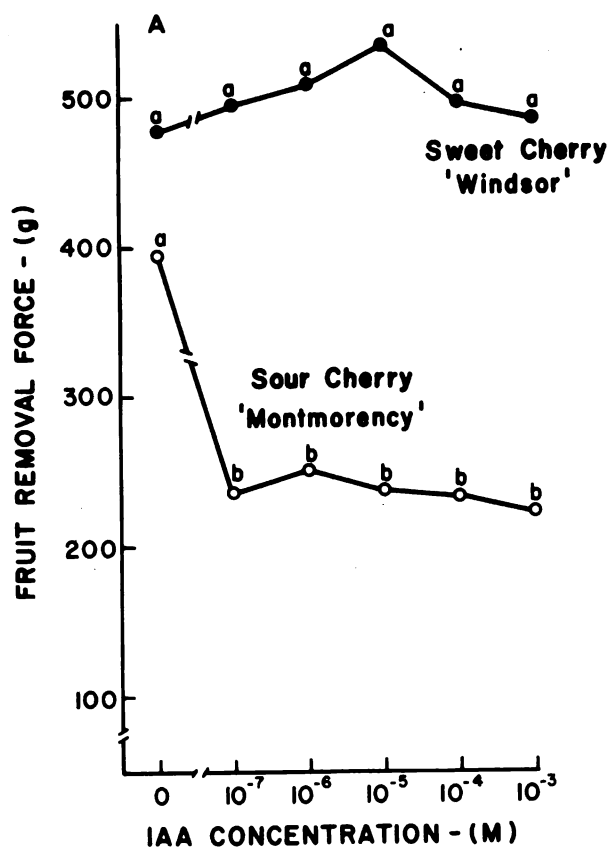
3-Indoleacetic acid when applied to mature sour cherry fruit explants caused an acceleration of abscission over the concentration range of 10^{-7} to 10^{-3} M (Figure 18A). However, the same concentrations brought about no significant change in the abscission rate of sweet cherries, and in fact, at 10^{-5} M showed a slight delay of abscission.

When CEPA was used at concentrations of 10^{-6} to 10^{-3} M, almost identical responses were observed for both sweet and sour cherries (Figure 18B). Increasing concentrations resulted in an increase in abscission. Again though, it is apparent that higher concentrations of CEPA are necessary to obtain an acceleration of abscission in the sweet cherry and the degree of acceleration brought about by these higher levels is less than for the sour cherry.

(RS)-Absciscic acid resulted in a similar hastening of abscission in sour cherries (Figure 18C), but the response was not as linear over the concentration range as observed with CEPA. In addition, ABA only resulted in a significant acceleration of abscission in sweet cherry fruit explants at 10^{-3} M.

Applying gibberellin A₃ to cherry fruit explants caused nearly the same response as observed for IAA (Figure 18D). With sour cherries, concentrations from 10^{-6} to 10^{-3} M resulted in almost a uniform acceleration of abscission. However, with sweet cherries these same concentrations

Figure 18. Effect of 3-indoleacetic acid (IAA), 2-chloroethylphosphonic acid (CEPA), (RS)-abscisic acid (ABA), and gibberellin A₃ (GA) on FRF of sweet ('Windsor') and sour ('Montmorency') cherry fruit explants.



had little or no influence on fruit separation, except at 10^{-4} M which hastened abscission.

Effect of a Protein Synthesis
Inhibitor on Abscission

The addition of cycloheximide to cherry fruit explants completely inhibited the development of fruit abscission as indexed by force necessary to separate the fruit from the pedicel (Tables 3 and 4). It also completely counteracted the accelerating effect of CEPA when both were applied as a mixture to explants. When cycloheximide was applied directly to the abscission zone it resulted in an almost complete inhibition of abscission in sweet cherries and was able to prevent CEPA enhancement of abscission in the sour cherry.

TABLE 3.--Effect of cycloheximide (CH) and 2-chloroethylphosphonic acid (CEPA) on abscission of sour cherry ('Montmorency') fruit explants.

Treatment	Fruit removal force	
	(g)	(%)
Control	234 a ¹	100
CEPA (10^{-3} M)	121 b	52
CH (3.57×10^{-5} M)	323 c	138
CEPA (10^{-3} M)+CH ² (3.57×10^{-5} M)	329 c	141
CEPA (10^{-3} M)+CH (0.25 μ g at AZ ³)	244 a	104

¹Means followed by unlike letters are significantly different at P=0.05 (Tukey's ω test).

²Applied as a mixture.

³Abscission zone.

TABLE 4.--Effect of cycloheximide (CH) and 2-chloroethylphosphonic acid (CEPA) on abscission of sweet cherry ('Windsor') fruit explants.

Treatment	Fruit removal force	
	(g)	(%)
Control	313 a ¹	100
CEPA (10^{-3} M)	241 b	77
CH (3.57×10^{-5} M)	370 c	118
CEPA (10^{-3} M)+CH ² (3.57×10^{-5} M)	396 c	127
CEPA (10^{-3} M)+CH (0.25 μ g at AZ ³)	412 c	132

¹Means followed by unlike letters are significantly different at P=0.05 (Tukey's ω test).

²Applied as a mixture.

³Abscission zone.

DISCUSSION

Abscission Process

Cherry fruits exhibit two potential sites for abscission (Figure 6). Immature fruits abscise with the pedicel attached, indicating abscission development between the pedicel and spur. There appears to be a natural weakening of the tissue at this zone near the end of the first growth phase (Figures 2 and 3). Hence, the weakening of this tissue may be related to the development of June drops. However, as the fruit matures there is a restrengthening of the tissue at the upper abscission zone and the abscission zone between the fruit and pedicel begins to weaken (Figures 2 and 3). Thus mature fruits generally separate at the lower abscission zone.

Although abscission of mature fruit occurs at the lower zone for both 'Montmorency' sour cherry and 'Windsor' sweet cherry, the process itself appears to be quite different for the two species. In the sour cherry a separation layer is initiated about two weeks prior to maturity. The formation of this layer is characterized by the loss of polysaccharides, pectins, and the degradation of cellulose in the walls of cells comprising the separation layer. These cells later separate leaving the fruit attached to the pedicel by only the vascular bundles. No comparable well-defined

layer is formed in the sweet cherry. Instead, separation appears to be more of a result of physical stress on distal cells of the abscission zone brought about by the weight and enlargement of the fruit. Hence, there are chemical changes accompanying abscission which are apparently distinct for the sour cherry.

In addition, at maturity the separation of cells in the abscission zone of sour cherries is much more extensive and occurs throughout the defined separation layer, whereas only localized cell separation is evident in the abscission zone of sweet cherries. This is reflected by the marked difference in FRF values of mature fruits for the two species. At optimum maturity the FRF of sour cherries was approximately 250 g compared to 350-400 g for sweet cherries.

Results from this study suggest two variations between the sweet and sour cherry which may in part explain the formation of a separation layer in the sour cherry but not in the sweet cherry. First, definite morphological differences are evident in the cells making up the abscission zone for the two species. In the sweet cherry (Figure 10) smaller cells with thicker cell walls and possibly more cementing material was observed than was found in the sour cherry (Figure 8). Also, in the sweet cherry a higher calcium content appeared to be present proximal to the abscission zone than in the sour cherry (Figure 13). This may be indicative of more cementing material and stronger bonding between these cells.

Histochemical evidence suggests the possible role of enzymes in the formation of the separation layer in sour cherry and the absence or presence of only low levels of these same enzymes in sweet cherry. Sour cherry exhibits the formation of a separation layer due to the loss of cell wall constituents from the 5-8 layers of cells comprising the layer. Similar losses of cell wall constituents have also been observed during leaf abscission in bean and Coleus (Lee, 1911; Sampson, 1918; Rasmussen, 1965; Valdovinos and Jensen, 1968; Webster, 1970). Several investigators have demonstrated the loss of these cell wall constituents to be associated with an increase in the level of enzyme activity responsible for their breakdown (Osborne, 1958; Rasmussen, 1965; Abeles, 1969; LaMotte et al., 1969). The loss of pectins has been correlated with an increase in pectin methyl-esterase and polygalacturonase (Osborne, 1958; Rasmussen, 1965; LaMotte et al., 1969). Osborne (1968b) and Abeles (1969) have also shown an increase in cellulase activity during abscission.

Although sweet cherries may not have sufficient specific enzymes involved in abscission as is suggested for sour cherries, the results obtained using cycloheximide indicate that protein synthesis in general is important (Tables 3 and 4). Cycloheximide completely inhibited abscission of both sweet and sour cherry fruit explants. In sweet cherry perhaps cycloheximide, by inhibiting protein synthesis, inhibited the overall ripening process causing a

complete inhibition of fruit enlargement and, hence, the resulting increase in mechanical stress at the abscission zone. Cycloheximide would also stop the formation of any degradative enzymes which may be formed prior to or at maturity and involved in the general breakdown of fruit tissue.

Comparative Response of Sour and Sweet
Cherries to Plant Growth Substances
Active in Leaf Abscission

Treatment of cherry fruit explants with growth regulating compounds also established a differential response of sweet and sour cherries. IAA, CEPA, ABA and GA all caused a hastening of separation in sour cherries (Figure 18). Sweet cherries, however, responded only to CEPA and to higher concentrations of ABA, both of which accelerated abscission. GA and IAA did not influence abscission significantly over the controls.

One explanation to account for the differential response of sour and sweet cherries is that sour cherries are more sensitive to injury, and hence, the acceleration of abscission was caused by ethylene produced as a result of injury induced by the applied compounds. However, this apparently was not the case, since preliminary studies on ethylene evolution brought about by mechanically injuring the flesh and pedicel of cherries showed no higher sensitivity for sour cherries than sweet cherries. In fact, neither one showed a large increase in evolved ethylene due

to injury. Therefore, it would appear that the acceleration of abscission was due solely to the effect of the growth regulators on the tissue of the fruit, pedicel, and abscission zone.

Auxin and gibberellin were observed to have a similar influence on abscission of cherry fruit explants (Figure 18A and D). Both have been shown to stimulate plant growth (Went, 1942; Galston and Hand, 1949; Brian and Hemming, 1955; Wittwer and Bukovac, 1957). And their presence has been correlated with the growth of fruit (Nitsch, 1950; Wright, 1956; Coombe, 1960). Since the cells of the abscission zone appear active--at least during the early stages of abscission--it might be expected that growth regulators would effect separation through an influence on the cells of the abscission zone. These cells in sour cherries may be induced later in fruit development to produce the enzymes which are involved in the breakdown and subsequent loss of cell wall constituents from the separation layer. Hence, IAA and GA might cause these cells to remain active for a longer period and continue synthesizing enzymes involved in abscission. The result would be a hastening of fruit separation. However, in sweet cherries no evidence was found to suggest the presence of cell wall degrading enzymes in the abscission zone. Therefore, IAA and GA would only maintain these cells in an active state longer and thereby possibly delay abscission.

Conversely, the addition of IAA and GA may influence cell separation indirectly through the mobilization of material away from the separation layer. In sour cherry this enhanced mobilization in combination with cell wall degrading enzymes in the separation layer would result in an increase in cell separation and degeneration. However, in sweet cherry, which apparently lacks sufficient cell wall degrading enzymes, the cells of the abscission zone would remain in an active state and, thereby, inhibit the ability of IAA and GA to mobilize material away from the abscission zone. Therefore, neither IAA or GA would have a significant influence on abscission.

The results obtained with IAA are similar to those observed for bean, where auxin was applied to the petiole after abscission had already been initiated (Rubinstein and Leopold, 1963). This may indicate that induction of abscission had already occurred prior to the harvest of the fruit explants.

(RS)-abscisic acid was observed to promote abscission of sour cherry fruit explants at all concentrations applied (Figure 18C). Lower concentrations (10^{-7} - 10^{-5} M) had nearly the same accelerating effect, while higher concentrations (10^{-4} - 10^{-3} M) caused a further enhancement of separation. Craker and Abeles (1969) suggested a two fold effect for ABA: 1) increased ethylene production, and 2) increased cellulase activity. Cellulose was one of the constituents of

cell walls of the separation layer found to be altered or lost during abscission. Thus, ABA at low concentrations may be increasing the synthesis of cellulase. Furthermore, the effect may be completely saturated in our bioassay at a concentration of 10^{-7} M. The further enhancement of separation brought about by high levels of ABA might be caused by ethylene being produced in response to ABA toxicity of the tissue. In sweet cherries only ABA at 10^{-3} M caused a significant acceleration of abscission. Therefore, this response may also be due to ethylene being produced as a result of the toxic level of ABA.

Treatment of cherry fruit explants with CEPA resulted in a hastening of abscission for both species (Figure 18B). However, sour cherries appeared to be more sensitive and showed a greater response than sweet cherries. Furthermore, it was observed that with fruit maturation sour cherry fruit explants became increasingly more sensitive to the enhancement of abscission by CEPA (Figure 16). Even at 10^{-5} M, CEPA resulted in a significant reduction in FRF when applied to fruit explants harvested 1-2 weeks prior to maturity. Sweet cherries failed to demonstrate such an increase in sensitivity with fruit development (Figure 17). Instead only higher concentrations of CEPA (10^{-3} and 10^{-4} M) were capable of significantly reducing the FRF and this was evident only for fruit explants harvested about 2 weeks prior to maturity.

CEPA breaks down readily to yield ethylene and the responses induced by CEPA are identical to those for ethylene.

Consequently it seems that the effect of this compound on abscission may be indirect via ethylene. Abeles and Holm (1966) found evidence for the stimulation of RNA and protein synthesis by ethylene. Other results have indicated that ethylene may function by increasing membrane permeability (Von Abrams and Pratt, 1967).

The application of cycloheximide to cherry fruit explants indicated the importance of continued protein synthesis for the progression of abscission. Therefore, ethylene may hasten the abscission of both sweet and sour cherries by stimulating protein synthesis. The more pronounced response and greater sensitivity of sour cherries might be related to the presence of sufficient enzymes which are directly involved in abscission. Enhanced protein synthesis induced by ethylene would increase the synthesis of these enzymes and thereby hasten the formation of the separation layer.

Moreover, ethylene may influence abscission by increasing membrane permeability of cells of the abscission zone. This alone could accelerate abscission by enhancing the mobilization of materials out of the abscission zone. The cells comprising the zone would, therefore, become weaker and separate more readily. The ability of ethylene to have a greater influence on the abscission of sour cherries might again be related to the possible presence of enzymes in the separation layer. Perhaps these enzymes are bound to the

cell membranes and addition of ethylene, by increasing membrane permeability, results in the release of these enzymes.

Role of CEPA in Natural Fruit Abscission

CEPA when applied as a foliar spray substantially reduced the FRF of sweet and sour cherries (Figures 4 and 5). The practical implications include the ability to economically harvest cherries mechanically, extension of the harvest period for both sweet and sour cherries, greater fruit recovery and shorter shaking time made possible by the lower FRF at optimum brining and/or fresh market maturity. Also the short interval between application and optimum response (4-8 days for 'Montmorency' and 6-10 days for 'Windsor') provides the grower with a valuable tool to program harvest.

What then is the mode of action of CEPA in fruit abscission? Cell separation in CEPA-treated and control fruits appears identical both morphologically and histochemically except that in treated fruits separation proceeds faster. Therefore, it appears that CEPA functions by accelerating the normal abscission process and perhaps fruit ripening in general. CEPA has been demonstrated to accelerate maturation of apples, pears, bananas, peaches, tomatoes and figs (Edgerton and Blanpied, 1968; Russo et al., 1968; Byers et al., 1969; Iwahori et al., 1969; Crane et al., 1970). In addition ethylene, which is the active component and breakdown product of CEPA, has been termed the 'ripening hormone' by post harvest physiologists due to its importance as a natural regulator of ripening.

From the results obtained on ethylene evolution from sour and sweet cherry fruits (Figures 14 and 15) it appears that a correlation exists between the increase in evolved ethylene near the start of the third growth phase and the weakening of tissue at the lower abscission zone (Figures 2 and 3). However, since the increase in evolved ethylene occurred on the same date for sour and sweet cherry, the enhanced evolution may merely have been the result of some environmental stress. Also it is not known whether the level of ethylene at this time is sufficient to initiate the abscission mechanism, or is in fact even representative of that in the fruit tissue. Assuming that it is, then there is a possibility that natural abscission of cherry fruits is under the control of ethylene. But even if ethylene is not the natural trigger for abscission in cherries, this would not preclude it from hastening the process once it was triggered in the fruit.

The decline in FRF of the lower abscission zone occurs far earlier than any visible changes in the anatomy or histochemistry of the zone (Figures 2 and 3). Only after the FRF has dropped well below 1000 g for sour cherries is there any sign of a separation layer or loss of cell wall constituents, and for sweet cherries a well-defined separation layer was never observed. Therefore, the process of fruit abscission is not restricted to the last week or two of development, but would appear to be initiated a month or

more in advance. Anything applied later than this would only be accelerating or delaying the development of abscission, which has already been set in motion.

Stösser (1970) reported on abscission in four sweet cherry varieties (grown in Germany) which had been treated with a foliar application of CEPA at 2000 and 4000 ppm. He concluded that CEPA resulted in an induction of abscission. From our observations on abscission of 'Windsor' sweet cherries, CEPA appears to only hasten the normal abscission process. The term induction would indicate that CEPA actually set in motion the entire process leading to cell separation. From our results on FRF changes during fruit development (Figures 2 and 3), it would appear that induction may actually occur a month or more in advance of fruit maturity. If these same changes in FRF apply for varieties used by Stösser, then it seems improbable that CEPA applied 8-10 days prior to full maturity could in fact result in the induction of abscission. Perhaps the high concentrations used (2000 and 4000 ppm) hastened cell separation to the extent that it was mistaken for induction of abscission. If this was the case, then control fruits should also undergo a similar type of cellular separation if left on the tree past full maturity. However, Stösser does not mention whether, in fact, this did occur, or how cell separation proceeded in the control fruits.

Despite the high concentrations of CEPA used by Stösser, the observance of such an extensive degree of cell

separation in the abscission zone may indicate that some varieties of sweet cherry are capable of forming a separation layer similar to that of 'Montmorency' sour cherry. Since the ability to abscise has not been a factor in selection of sweet cherry varieties and, in fact, a large market requires that the stem remain attached, varieties over the years have probably been selected against the ease of pedicel removal.

Therefore, although the degree of cell separation which accompanies abscission may vary due to histological and physiological differences in the abscission zone of cherry fruits, it is apparent that certain compounds such as ethylene (CEPA) are able to enhance abscission once it has been induced.

SUMMARY

A comparative study of fruit abscission in the sour (Prunus cerasus L. cv. Montmorency) and sweet (Prunus avium L. cv. Windsor) cherry during ripening was followed in the absence and presence of 2-chloroethylphosphonic acid (CEPA). Although two abscission zones are present, mature fruits of both species abscise at the junction of the fruit and pedicel. A natural decline in fruit removal force at this zone was observed at the start of the third growth phase. CEPA applied 10-14 days prior to optimum maturity significantly hastened abscission.

Despite these similarities in response, there were distinct morphological and histochemical differences in the fruit abscission of sour and sweet cherries. In sour cherry a well-defined separation layer was formed approximately two weeks prior to maturity. The formation of this layer was characterized by the loss of polysaccharides, pectins, and cellulose from the walls of cells comprising the layer. Separation later proceeded through this layer generally leaving the cell walls intact, although frequently cells were ruptured. No comparable layer was observed in the sweet cherry. Instead, abscission appeared to occur where the cells of the fruit joined those of the abscission zone. The actual separation of cells was not as well-defined as in the

sour cherry but rather was localized and appeared to be due more to physical stress than a weakening of the cell walls. Physical differences in the cells comprising the abscission zone were also observed. The cells in the abscission zone of 'Windsor' were smaller and thicker-walled in comparison to those of the sour cherry.

Cell separation in CEPA-treated cherries appeared identical to that in the control fruits. CEPA accelerated abscission by apparently increasing the rate of development of the naturally occurring processes. The ability of this compound to hasten separation might be interpreted to mean that ethylene was the natural promoter of fruit abscission. A study of ethylene evolved from cherry fruits during development did show an increase in evolved ethylene which was correlated with the weakening of tissue at the lower abscission zone, however, the level of ethylene was extremely low.

Physiological aspects of cherry fruit abscission were established using an excised fruit technique. With fruit development sour cherries exhibited an increasing sensitivity to CEPA enhancement of abscission. Such a change in sensitivity was not observed for the sweet cherry, which only responded to CEPA late in fruit development.

3-Indoleacetic acid, CEPA, gibberellin A₃, and abscisic acid hastened abscission in sour cherry fruit explants. Only CEPA had a similar accelerating effect on sweet cherries. The other compounds failed to influence

abscission in the sweet cherry except for abscisic acid at 10^{-3} M which promoted abscission.

Cycloheximide, when applied to cherry fruit explants, completely inhibited the development of abscission. It also negated the effect of CEPA. This would suggest that protein synthesis is necessary for the development of cherry fruit abscission.

LITERATURE CITED

LITERATURE CITED

- Abeles, F. B. 1966. Auxin stimulation of ethylene evolution. *Plant Physiol.* 41:585-588.
- _____. 1967. Mechanism of action of abscission accelerators. *Physiol. Plantarum* 20:442-454.
- _____. 1968. Role of RNA and protein synthesis in abscission. *Plant Physiol.* 43:1577-1586.
- _____. 1969. Abscission: Role of cellulase. *Plant Physiol.* 44:447-452.
- _____, and R. E. Holm. 1966. Enhancement of RNA synthesis, protein synthesis and abscission by ethylene. *Plant Physiol.* 41:1337-1342.
- _____. 1967. Abscission: Role of protein synthesis. *Ann. N. Y. Acad. Sci.* 144:367-373.
- Abeles, F. B., and B. Rubinstein. 1964. Regulation of ethylene evolution and leaf abscission by auxin. *Plant Physiol.* 39:963-969.
- Addicott, F. T. 1954. Abscission and plant regulators. In: Plant Growth Regulators in Agriculture. Edited by H. B. Tukey. Wiley and Sons, New York. PP. 99-116.
- _____. 1961. Auxin in the regulation of abscission. *Encycl. Plant Physiol.* 14:829-838.
- _____. 1965. Physiology of abscission. *Encycl. Plant Physiol.* 15/2:1094-1126.
- _____. 1968. Environmental factors in the physiology of abscission. *Plant Physiol.* 43:1471-1479.
- _____, and R. S. Lynch. 1951. Acceleration and retardation of abscission by indoleacetic acid. *Science.* 114:688-689.
- _____. 1955. Physiology of abscission. *Ann. Rev. Plant Physiol.* 6:211-238.

- Addicott, F. T., R. S. Lynch, and H. R. Carns. 1955. Auxin gradient theory of abscission regulation. *Science*. 121:644.
- Addicott, F. T., and J. L. Lyon. 1969. Physiology of abscisic acid and related substances. *Ann. Rev. Plant Physiol.* 20:139-164.
- Amchem Products, Inc. 1967. Amchem 66-329, a new plant growth regulator. Information sheet 37.
- Anderson, J. L. 1969. The effect of ethrel on the ripening of Montmorency sour cherries. *HortScience* 4:92-93.
- Barnell, E. 1939. Studies in tropical fruits. V. Some anatomical aspects of fruit-fall in two tropical arboreal plants. *Ann. Bot.* 3:77-89.
- Batjer, L. P. 1954. Plant regulators to prevent preharvest fruit drop, delay foliation and blossoming, and thin blossoms and young fruit. In: Plant Regulators in Agriculture. Edited by H. B. Tukey. Wiley and Sons, New York. PP. 117-131.
- Bednarz, R. M. 1970. The changes in anatomy and fine structure as related to the physiology of abscission in the lower pulvinus of bean (Phaseolus vulgaris L.) Ph.D. Thesis. Mich. State Univ.
- Berman, T. 1969. The effect of amino acids and growth regulators on abscission in cotton explants. *Israel J. of Agr. Res.* 19:129-130.
- Beyer, E. M., and P. W. Morgan. 1969. Ethylene modification of an auxin pulse in cotton stem sections. *Plant Physiol.* 44:1690-1694.
- Biggs, R. H., and A. C. Leopold. 1957. Factors influencing abscission. *Plant Physiol.* 32:626-632.
- . 1958. The two-phase action of auxin on abscission. *Amer. J. Bot.* 45:547-551.
- Bonner, J. 1936. The chemistry and physiology of the pectins. *Bot. Rev.* 2:475-497.
- Bornman, C. H., A. R. Spurr, and F. T. Addicott. 1967. Abscisin, auxin, and gibberellin effects on the developmental aspects of abscission in cotton (Gossypium hirsutum). *Amer. J. Bot.* 54:125-135.

- Bostrack, J. M., and R. R. Daniels. 1969. Leaf retention: A seasonal study of the abscission region of juvenile oak trees. *Phytomorphology*. 19:17-21.
- Brian, P. W., and H. G. Hemming. 1955. The effect of gibberellic acid on shoot growth of pea seedlings. *Physiol. Plantarum* 8:669-681.
- Brown, H. S., and F. T. Addicott. 1950. The anatomy of experimental leaflet abscission in Phaseolus vulgaris. *Amer. J. Bot.* 37:650-656.
- Bukovac, M. J. 1969. Chemical promotion of cherry fruit abscission. *Mich. Agr. Exp. Sta. Res. Report* 101:15-16.
- _____, F. Zucconi, R. P. Larsen, and C. D. Kesner. 1969. Chemical promotion of fruit abscission in cherries and plums with special reference to 2-chloroethylphosphonic acid. *J. Amer. Soc. Hort. Sci.* 94:226-230.
- Burg, S. P. 1968. Ethylene, plant senescence and abscission. *Plant Physiol.* 43:1503-1511.
- _____, and E. A. Burg. 1966. The interaction between auxin and ethylene and its role in plant growth. *Proc. Nat. Aca. Sci.* 55:262-266.
- Byers, R. E., H. C. Dostal, and F. H. Emerson. 1969. Regulation of fruit growth with 2-chloroethanephosphonic acid. *BioScience* 19:903-904.
- Cain, J. C. 1967. The relation of fruit retention force to the mechanical harvesting efficiency of Montmorency cherries. *HortScience* 2:53-55.
- Carns, H. R. 1966. Abscission and its control. *Ann. Rev. Plant Physiol.* 17:295-314.
- _____, F. T. Addicott, and R. S. Lynch. 1951. Some effects of water and oxygen on abscission in vitro. *Plant Physiol.* 26:629-630.
- Chatterjee, S. K., and A. C. Leopold. 1964. Kinetin and gibberellin actions on abscission processes. *Plant Physiol.* 39:334-337.
- Chaudhri, S. A. 1957. Some anatomical aspects of fruit drop in citrus. Ph.D. Thesis. Univ. Fla., Gainesville.

- Choudhuri, M. A., and S. K. Chatterjee. 1970. Seasonal changes in the levels of some cellular components in the abscission zone of Coleus leaves of different ages. *Ann. Bot.* 34:275-287.
- Cooke, A. R., and D. I. Randall. 1968. 2-Haloethanephosphonic acids as ethylene releasing agents for the induction of flowering in pineapples. *Nature* 218:974-975.
- Coombe, B. G. 1960. Relationship of growth and development to changes in sugars, auxins, and gibberellins in fruit of seeded and seedless varieties of Vitis. *Plant Physiol.* 35:241-250.
- Cooper, W. C., G. K. Rasmussen, and D. J. Hutchison. 1969. Promotion of abscission of orange fruits by cycloheximide as related to site of treatment. *Bio-Science* 19:443-444.
- _____, G. K. Rasmussen, B. J. Rogers, P. C. Reece, and W. H. Henry. 1968. Control of abscission in agricultural crops and its physiological basis. *Plant Physiol.* 43:1560-1576.
- Craker, L. E., and F. B. Abeles. 1969. Abscission: Quantitative measurement with a recording abscissor. *Plant Physiol.* 44:1139-1143.
- _____. 1969. Abscission: Role of abscisic acid. *Plant Physiol.* 44:1144-1149.
- Crane, J. C., N. Marei, and M. M. Nelson. 1970. Growth and maturation of fig fruits stimulated by 2-chloroethylphosphonic acid. *J. Amer. Soc. Hort. Sci.* 95:367-370.
- Davis, L. A. 1968. Gas chromatographic identification and measurement of abscisic acid and other plant hormones in the developing cotton fruit. Ph.D. Thesis. Univ. Calif., Davis.
- dela Fuente, R. K., and A. C. Leopold. 1969. Kinetics of abscission in the bean petiole explant. *Plant Physiol.* 44:251-254.
- Devlin, R. M., and O. M. McIntyre. 1966. Stimulation of abscission of petioles of Phaseolus vulgaris by sucrose and gibberellic acid. *Physiol. Plantarum* 19:741-747.

- Dickinson, D. B., and J. P. McCollum. 1964. Cellulase in tomato fruits. *Nature* 203:525-526.
- Edgerton, L. J., and G. D. Blanpied. 1968. Regulation of growth and fruit maturation with 2-chloroethanephosphonic acid. *Nature* 219:1064-1065.
- _____, and W. J. Greenhalgh. 1969. Regulation of growth, flowering and fruit abscission with 2-chloroethanephosphonic acid. *J. Amer. Soc. Hort. Sci.* 94:11-13.
- Facey, V. 1950. Abscission of leaves in Fraxinus americana L. *New Phytol.* 49:103-116.
- Galston, A. W., and M. E. Hand. 1949. Studies on the physiology of light action. I. Auxin and the light inhibition of growth. *Amer. J. Bot.* 36:85-94.
- Gardner, F. E., P. C. Marth, and L. P. Batjer. 1939. Spraying with plant growth substances to prevent apple fruit dropping. *Science* 90:208-209.
- Gaur, B. K., and A. C. Leopold. 1955. The promotion of abscission by auxin. *Plant Physiol.* 30:487-490.
- Gawadi, A. G., and G. S. Avery. 1950. Leaf abscission and the so-called "abscission layer." *Amer. J. Bot.* 37:172-180.
- Ginzburg, B. Z. 1961. Evidence for a protein gel structure cross-linked by metal cations in the intercellular cement of plant cells. *J. Exp. Bot.* 34:85-107.
- Goldsmith, M. H. M. 1968. The transport of auxin. *Ann. Rev. Plant Physiol.* 19:347-360.
- Hall, W. C. 1952. Evidence on the auxin-ethylene balance hypothesis of foliar abscission. *Bot. Gaz.* 113:310-322.
- _____, F. A. Herrero, and F. R. H. Katterman. 1962. Leaf abscission in cotton. IV. Effects of a natural promoter and amino acids on abscission in cotyledonary node explants. *Bot. Gaz.* 123:29-34.
- Hall, W. C., and P. W. Morgan. 1963. Auxin-ethylene interrelationships. In: Regulateurs Naturels De La Croissance Vegetale. PP. 727-745.
- Hallaway, M. and D. J. Osborne. 1969. Ethylene: A factor in defoliation induced by auxins. *Science* 163:1067-1068.

- Hansen, E. 1966. Postharvest physiology of fruits. *Ann. Rev. Plant Physiol.* 17:459-480.
- Heinicke, A. J. 1917. Factors influencing the abscission of flowers and partially developed fruits of the apple Pyrus Malus L. *Cornell Univ. Agr. Exp. Sta. Bull.* 393:43-114.
- _____. 1919. Concerning the shedding of flowers and fruits and other phenomena in apples and pear. *Proc. Amer. Soc. Hort. Sci.* 16:76-83.
- Hendershott, C. H. 1965. The effect of iodoacetic acid on citrus fruit abscission. *Proc. Florida State Hort. Soc.* 36-41.
- Horton, R. F., and D. J. Osborne. 1967. Senescence, abscission and cellulase activity in Phaseolus vulgaris. *Nature* 214:1086-1088.
- Iwahori, S., S. Ben Yehoshua, and J. M. Lyons. 1969. Effect of 2-chloroethanephosphonic acid on tomato fruit development and maturation. *BioScience* 19:49-50.
- _____, and J. M. Lyons. 1970. Maturation and quality of tomatoes with preharvest treatments of 2-chloroethylphosphonic acid. *J. Amer. Soc. Hort. Sci.* 95:88-91.
- Jacobs, W. P. 1962. Longevity of plant organs: Internal factors controlling abscission. *Ann. Rev. Plant Physiol.* 13:403-436.
- _____. 1968. Hormonal regulation of leaf abscission. *Plant Physiol.* 43:1480-1495.
- _____, M. P. Kaushik, and P. G. Rochmis. 1964. Does auxin inhibit the abscission of Coleus leaves by acting as a growth hormone? *Plant Physiol.* 51:893-897.
- Jacobs, W. P., C. C. McCready, and D. J. Osborne. 1966. Transport of the auxin 2, 4-dichlorophenoxyacetic acid through abscission zones, pulvini, and petioles of Phaseolus vulgaris. *Plant Physiol.* 41:725-730.
- Jacobs, W. P., J. A. Shield, and D. J. Osborne. 1962. Senescence factor and abscission of Coleus leaves. *Plant Physiol.* 37:104-106.
- Jensen, T. E., and J. G. Valdovinos. 1967. Fine structure of abscission zones I. Abscission zones of the pedicels of tobacco and tomato flowers at anthesis. *Planta* 77:298-318.

- Jensen, T. E., and J. G. Valdovinos. 1968. Fine structure of abscission zones. III. Cytoplasmic changes in abscising pedicels of tobacco and tomato flowers. *Planta* 83:303-313.
- Jensen, W. A. 1962. Botanical Histochemistry. W. H. Freeman & Co., San Francisco.
- Kabachnik, M. I., and P. A. Rosiĭskaya. 1946. Organophosphorus compounds. I. Reaction of ethylene oxide with phosphorus trichloride. In: *Chemical Abstracts* 42:7241-7242. 1948.
- Laibach, F. 1933. Versuche mit wuchsstoffpaste. *Ber. Deutsch Bot. Ges.* 51:386-392.
- LaMotte, C. E., C. Grochnauer, L. R. LaMotte, J. RajMathur, and L. R. Davies. 1969. Pectin esterase in relation to leaf abscission in Coleus and Phaseolus. *Plant Physiol.* 44:21-26.
- Lamouria, L. H., and H. T. Hartmann. 1959. Studies of methods of harvesting olives mechanically. *Proc. Amer. Soc. Hort. Sci.* 73:203-212.
- LaRue, C. D. 1936. The effect of auxin on the abscission of petioles. *Proc. Nat. Acad. Sci.* 2:254-259.
- Lee, E. 1911. The morphology of leaf-fall. *Ann. Bot.* 25:52-106.
- Leinweber, C. L., and W. C. Hall. 1959. Foliar abscission in cotton. I. Effect of age and defoliants on the respiratory rate of blade, petiole, and tissues of the abscission zone. *Bot. Gaz.* 120:144-151.
- Letham, D. S. 1960. The separation of plant cells with ethylenediaminetetraacetic acid. *Exp. Cell Res.* 21:353-360.
- . 1962. Separation of plant cells with hexameta-phosphate and the nature of intercellular bonding. *Exp. Cell Res.* 27:352-355.
- Lewis, L. N., and J. C. Bakhshi. 1968. Protein synthesis in abscission: The distinctiveness of the abscission zone and its response to gibberellic acid and indoleacetic acid. *Plant Physiol.* 43:359-364.
- Luckwill, L. C. 1948. The hormone content of the seed in relation to endosperm development and fruit drop in the apple. *J. Hort. Sci.* 24:32-44.

- Lyons, J. M., W. B. McGlasson, and H. K. Pratt. 1962. Ethylene production, respiration, and internal gas concentrations in cantaloupe fruits at various stages of maturity. *Plant Physiol.* 37:31-36.
- MacDaniels, L. H. 1936. Some anatomical aspects of apple flower and fruit abscission. *Proc. Amer. Soc. Hort. Sci.* 34:122-129.
- Maynard, J. A., and J. M. Swan. 1963. Organophosphorus compounds I. 2-Chloroalkylphosphonic acid as phosphorylating agents. *Australian J. Chem.* 16:596-608.
- McCown, M. 1938. Abscission of flowers and fruits of the apple. *Proc. Amer. Soc. Hort. Sci.* 36:320.
- . 1943. Anatomical and chemical aspects of abscission of fruits of the apple. *Bot. Gaz.* 105:212-220.
- McMurray, A. L., and C. H. Miller. 1969. The effect of 2-chloroethanephosphonic acid (ethrel) on the sex expression and yields of Cucumis sativus. *J. Amer. Soc. Hort. Sci.* 94:400-402.
- Morgan, P. W. 1964. Distribution of indoleacetic acid oxidase and inhibitors in light-grown cotton. *Plant Physiol.* 39:741-746.
- . 1969. Stimulation of ethylene evolution and abscission in cotton by 2-chloroethanephosphonic acid. *Plant Physiol.* 44:337-341.
- Morgan, P. W., and W. C. Hall. 1964. Accelerated release of ethylene by cotton following application of indolyl-3-acetic acid. *Nature* 201:99.
- Morré, D. J. 1968. Cell wall dissolution and enzyme secretion during leaf abscission. *Plant Physiol.* 43:1545-1559.
- Muir, R. M., and J. G. Valdovinos. 1970. Gibberellin and auxin relationships in abscission. *Amer. J. Bot.* 57:288-291.
- Nichols, R. 1966. Ethylene production during senescence of flowers. *J. Hort. Sci.* 41:279-290.
- Nitsch, J. P. 1950. Growth and morphogenesis of the strawberry as related to auxin. *Amer. J. Bot.* 37:211-215.

- Osborne, D. J. 1955. Acceleration of abscission by a factor produced in senescent leaves. *Nature* 176:1161-1163.
- _____. 1958. Changes in the distribution of pectin methylesterase across leaf abscission zones of Phaseolus vulgaris. *J. Exp. Bot.* 9:446-457.
- _____. 1968a. Ethylene as a plant hormone. In: Plant Growth Regulators. Edited by the Society of Chemical Industry. Staples Printers Limited. PP. 236-250.
- _____. 1968b. Hormonal mechanisms regulating senescence and abscission. In: Biochemistry and Physiology of Plant Growth Substances. Edited by F. Wightman and G. Setterfield. The Runge Press Ltd., Ottawa, Canada. PP. 815-840.
- Osborne, D. J., and S. E. Moss. 1963. Effect of kinetin on senescence and abscission in explants of Phaseolus vulgaris. *Nature* 200:1299-1301.
- Pratt, H. K., and J. D. Goeschl. 1969. Physiological roles of ethylene in plants. *Ann. Rev. Plant Physiol.* 20:541-584.
- Preston, R. D. 1964. Structural plant polysaccharides. *Endeavour* 23:153-159.
- Rasmussen, G. K., and W. C. Cooper. 1968. Abscission of citrus fruits induced by ethylene-producing chemicals. *Proc. Amer. Soc. Hort. Sci.* 93:191-198.
- Rasmussen, H. P. 1965. Chemical and physiological changes associated with abscission layer formation in the bean (Phaseolus vulgaris L. cv. contender). Ph.D. Thesis, Mich. State Univ.
- _____, and M. J. Bukovac. 1966. Naphthaleneacetic acid: Localization in the abscission zone of bean. *Science* 152:217-218.
- _____. 1969. A histochemical study of abscission layer formation in the bean. *Amer. J. Bot.* 56:69-76.
- Roéløfsen, P. A. 1965. Ultrastructure of the wall in growing cells and its relation to the direction of growth. *Advances Bot. Res.* 2:69-149.
- Rosen, L. A., and S. M. Siegel. 1963. Effect of oxygen tension on the course of ethylene- and gibberellin-induced foliar abscission. *Plant Physiol.* 38:189-191.

- Rubinstein, B., and A. C. Leopold. 1962. Effects of amino acids on bean leaf abscission. *Plant Physiol.* 37:398-401.
- _____. 1963. Analysis of the auxin control of bean leaf abscission. *Plant Physiol.* 38:262-267.
- Russo, L., H. C. Dostal, and A. C. Leopold. 1968. Chemical regulation of fruit ripening. *BioScience* 18:109.
- Sacher, J. A. 1957. Relationship between auxin and membrane integrity in tissue senescence and abscission. *Science* 125:1199-1200.
- _____. 1959. Studies on auxin-membrane permeability relations in fruit and leaf tissues. *Plant Physiol.* 34:365-372.
- _____, and S. O. Salminen. 1969. Comparative studies of effect of auxin and ethylene on permeability and synthesis of RNA and protein. *Plant Physiol.* 44:1371-1377.
- Sampson, H. C. 1918. Chemical changes accompanying abscission in Coleus blumei. *Bot. Gaz.* 66:32-53.
- Sass, J. E. 1958. Botanical Microtechnique. The Iowa State Univ. Press, Ames, Iowa.
- Schwertner, H. A., and P. W. Morgan. 1966. Role of IAA-oxidase in abscission control in cotton. *Plant Physiol.* 41:1513-1519.
- Scott, P. C., and A. C. Leopold. 1966. Abscission as a mobilization phenomenon. *Plant Physiol.* 41:826-830.
- Setterfield, G., and S. T. Bayley. 1961. Structure and physiology of cell walls. *Ann. Rev. Plant Physiol.* 12:35-62.
- Shoji, K., F. T. Addicott, and W. A. Swets. 1951. Auxin in relation to leaf blade abscission. *Plant Physiol.* 26:189-191.
- Simons, R. K. 1963. Anatomical studies of apple fruit abscission in relation to irrigation. *Proc. Amer. Soc. Hort. Sci.* 83:77-87.
- Stösser, R. 1967. Über die Ausbildung des Trenngewebes und seine kausale Beziehung zu Fruchtfall und Fruchtentwicklung bei Süpkirschen. *Angew. Bot.* 41:194-209.

- Stösser, R. 1969. Histoautoradiographische Lokalisierung von ^{45}Ca in der Trennzone der Früchte von Sü- und Sauerkirsche. Z. Pflanzenphysiol Bd. 61:314-321.
- _____. 1970. Die Induktion eines Trenngewebes bei Früchten von Prunus avium L. durch 2-chlorathylphosphonsäure. Planta 90:299-302.
- _____, and H. P. Rasmussen, and M. J. Bukovac. 1969a. A histological study of abscission layer formation in cherry fruits during maturation. J. Amer. Soc. Hort. Sci. 94:239-243.
- _____. 1969b. Histochemical changes in the developing abscission layer in fruits of Prunus cerasus L. Planta 86:151-164.
- Tukey, H. B. 1934. Growth of the embryo, seed and pericarp of sour cherry (Prunus cerasus) in relation to season of fruit ripening. Proc. Amer. Soc. Hort. Sci. 31:125-144.
- Valdovinos, J. G., and L. C. Ernest. 1967. Effect of protein synthesis inhibitors, auxin, and gibberellic acid on abscission. Physiol. Plantarum 20:1027-1038.
- _____, and E. W. Henry. 1967. Effect of ethylene and gibberellic acid on auxin synthesis in plant tissue. Plant Physiol. 42:1803-1806.
- Valdovinos, J. G., and T. E. Jensen. 1968. Fine structure of abscission zones II. Cell-wall changes in abscising pedicels of tobacco and tomato flowers. Planta 83:295-302.
- Vines, H. M., W. Grierson, and G. J. Edwards. 1968. Respiration, internal atmosphere, and ethylene evolution of citrus fruits. Proc. Amer. Soc. Hort. Sci. 92:227-234.
- Von Abrams, G. J., and H. K. Pratt. 1967. Effect of ethylene on the permeability of excised cantaloupe fruit tissue. Plant Physiol. 42:299-301.
- Warner, H. L., and A. C. Leopold. 1967. Plant growth regulation by stimulation of ethylene production. Bio-Science 17:722.
- _____. 1969. Ethylene evolution from 2-chloroethylphosphonic acid. Plant Physiol. 44:156-158.

- Weaver, R. J., and R. M. Pool. 1969. Effect of ethrel, abscisic acid, and a morphactin on flower and berry abscission and shoot growth in Vitis vinifera. J. Amer. Soc. Hort. Sci. 94:474-478.
- Webster, B. D. 1968. Anatomical aspects of abscission. Plant Physiol. 43:1512-1544.
- _____. 1970. A morphogenetic study of leaf abscission in Phaseolus. Amer. J. Bot. 57:443-451.
- Weintraub, R. L., J. W. Brown, J. C. Nickerson, and K. N. Taylor. 1952. Studies on the relation between molecular structure and physiological activity of plant growth regulators. I. Abscission-inducing activity. Bot. Gaz. 113:348-362.
- Went, F. W. 1942. Growth, auxin and tropisms in decapitated Avena coleoptiles. Plant Physiol. 17:236:249.
- White, A., P. Handler, and E. L. Smith. 1968. Cell Walls plants and microorganisms. Principles of Biochemistry. McGraw-Hill. New York. PP. 908-916.
- Wilson, W. C., and C. H. Hendershott. 1967. The effects of various chemical treatments on the rate of abscission of 'Hamlin' orange explants. Proc. Amer. Soc. Hort. Sci. 90:123-129.
- _____. 1968. Anatomical and histochemical studies of abscission of oranges. Proc. Amer. Soc. Hort. Sci. 92:203-210.
- Wittwer, S. H., and M. J. Bukovac. 1957. Gibberellins: New chemicals for crop production. Mich. State Univ. Agr. Exp. Sta. Quart. Bull. 39:469-494.
- Wright, S. T. C. 1956. Studies on fruit development in relation to plant hormones III. J. Hort. Sci. 31:196-211.
- Yager, R. E. 1960a. Possible role of pectin enzymes in abscission. Plant Physiol. 35:157-162.
- _____. 1960b. Some effects of leaves and indoleacetic acid upon floral abscission in Nicotiana tabacum. Bot. Gaz. 121:244-249.
- Yang, S. F. 1969. Ethylene evolution from 2-chloroethylphosphonic acid. Plant Physiol. 44:1203-1204.
- Zucconi, F., R. Stösser, and M. J. Bukovac. 1969. Promotion of fruit abscission with abscisic acid. BioScience 19:815-817.

APPENDIX

TABLE A1.--Number of replications and fruit per replication from which the data on ethylene evolution was obtained.

Date	Sour Cherry		Sweet Cherry	
	Reps	Fruit/Rep	Reps	Fruit/Rep
May 13	9	10		
May 16	7	10		
May 19	7	10		
May 22	6	10		
May 26	8	10		
May 29	7	10	4	10
June 2	5	10	3	10
June 5	6	10	4	10
June 9	4	10	3	10
June 12	6	10	2	30
June 16	2	30	2	30
June 19	2	30	2	30
June 23	4	30	4	30
June 26	4	30	4	30
June 30	4	25	4	25
July 3	4	25	2	25
July 7	3	20	2	20
July 11	3	20	2	15
July 14	3	20	2	15
July 17	2	20	2	15
July 23	3	15		
July 31	3	15		

TABLE A2.--Standard deviations of the FRF values presented in Figure 2 for 'Montmorency' sour cherry.

Date	Point of Separation		
	Mid-pedicel	Upper AZ	Lower AZ
May 12	48		
May 15	92		
May 22	170		
May 25	165		
June 2		156	162
June 4		187	72
June 7		202	142
June 12		232	161
June 15		186	182
June 19		194	230
June 22		218	175
June 27		209	182
July 1		248	168
July 7		262	106
July 13			53

TABLE A3.--Standard deviations of the FRF values presented in Figure 3 for 'Windsor' sweet cherry.

Date	Point of Separation		
	Mid-pedice	Upper AZ	Lower AZ
May 23	122		
May 29		124	183
June 2		80	162
June 12		116	128
June 19		150	110
June 26		122	179
July 3		95	141
July 11		156	54