PHYSIOLOGY OF CHERRY FRUIT ABSCISSION

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ABSTRACT

PHYSIOLOGY OF CHERRY FRUIT ABSCISSION

By

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SECTION I. EVIDENCE FOR A TWO-PHASE PROCESS AND THE INVOLVEMENT OF ETHYLENE

Abscission in the sour cherry (Prunus cerasus L., cv. Montmorency) can be separated into two distinct phases. Phase I, a pre-separation phase, is characterized by increasing break-force, and explants prepared from fruits during this phase exhibit no potential for abscission. Ethylene and 2-(chloroethyl)-phosphonic acid (ethephon) have no effect on abscission when applied early in Phase I, whereas later applications shorten the phase. The separation phase, Phase II, is characterized by a declining break-force, and explants have a potential for abscission. Exogenous ethylene hastens abscission during this phase, while lowering the level of endogenous ethylene delays abscission. Neither rates of ethylene evolution nor levels of endogenous ethylene in the fruit were correlated with abscission. Cycloheximide inhibited abscission while promoting ethylene production. The possible roles of ethylene and protein synthesis in abscission are discussed.

SECTION II. A ROLE FOR ETHYLENE IN MECHANICALLY-INDUCED ABSCISSION OF IMMATURE FRUITS

Injury-induced abscission of sour cherry fruit (Prunus cerasus L., cv. Montmorency) was correlated with a marked increase in ethylene evolution from the seed following treatment. Ethylene evolution subsequent to injury of the seed at various stages of development suggested that the induced ethylene production was associated with the nucellar tissue. Ethephon also induced immature fruit abscission; however, abscission followed seed abortion. Although exogenous ethylene appeared to directly influence abscission at the peduncle:pedicel zone, injury-induced ethylene did not appear to act directly at this zone. The mechanisms involved in abscission of immature and mature fruit, although associated with different zones, appear to be similar. The possible role of ethylene in immature cherry fruit abscission is discussed.

SECTION III. PEROXIDASE ACTIVITY IN THE ABSCISSION ZONE IN RELATION TO SEPARATION

Peroxidase activity was demonstrated in the abscission zone and adjacent tissues of sour cherry fruit (Prunus cerasus L., cv. Montmorency) from Stage I of fruit growth to maturity. Activity was greatest in the receptacle and abscission zone tissues, with only a low level in the fruit. A histochemical difference was observed in the peroxidase of the abscission layer from that of the adjacent tissues. Moreover, peroxidase activity in the abscission zone increased to a maximum at a stage of development coinciding with the initiation of the separation phase. This

increase in activity was accompanied by an increase in two of the major isoenzymes and the appearance of a third. The relationship between changes in peroxidase and abscission in fruit explants was less clear. Ethylene and ethephon had no significant effect on total peroxidase activity; however, ethylene appeared to increase the activity of a basic isoenzyme. Cycloheximide treatment decreased total and isoenzyme activity. Lowering the endogenous ethylene level did not reduce total activity, although the activity of a basic isoenzyme was decreased.

PHYSIOLOGY OF CHERRY FRUIT ABSCISSION

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Guidance Committee:

The Paper-Format was adopted for this dissertation in accordance with departmental and university regulations. The dissertation body was separated into three sections. The first section was prepared for publication in Plant Physiology. The second and third sections were styled for publication in the Journal of the American Society for Horticultural Science.

GENERAL INTRODUCTION

The process of fruit abscission has not been extensively studied. Auxin sprays promote abscission of immature fruit and delay abscission of mature fruit, while ethylene and ethylene producing chemicals promote abscission of both immature and mature fruit.

Work in this laboratory has been concerned with sour cherry fruit abscission. Immature and mature fruit abscission can be studied independently in the cherry, as separation of immature fruit occurs at the peduncle:pedicel abscission zone, while mature fruit abscise at the pedicel:fruit zone. Special emphasis has been directed toward the process of abscission in mature fruit, since control of this process would provide a basis for improved hand and machine harvest as well as programed harvesting.

Maturation of sour cherry fruit is accompanied by a decline in break-force at the pedicel:fruit zone. This decline has been associated histochemically with the loss of pectic substances and polysaccharides and a change in cellulose orientation in the walls of cells of the abscission layer. These results suggest the involvement of cell-wall degrading enzymes in cherry fruit abscission. In addition, enzymes not involved in cell wall degradation have also been localized in the abscission layer. Furthermore, the rate of abscission can be altered by exogenous application of chemicals. Cycloheximide will inhibit abscission,

while ethephon, an ethylene releasing chemical, will promote separation.

These studies have provided us with the means to investigate the process of cherry fruit abscission and to answer some of the physiological questions relating to abscission of immature and mature fruit. The present investigation was undertaken with three main objectives: 1) to examine the influence of ethylene on abscission of mature fruit, 2) to establish the role of the seed and ethylene in mechanically induced abscission of immature fruit, and 3) to study the relationship between peroxidase and mature fruit abscission.

SECTION I

EVIDENCE FOR A TWO-PHASE PROCESS AND THE INVOLVEMENT OF ETHYLENE

INTRODUCTION

Auxin and ethylene have both been implicated as natural regulators of leaf abscission (5, 20). Early workers in this field suggested that abscission might be controlled by a balance between these two hormones (15, 17). Evidence favoring such a balance was provided indirectly by the separation of the leaf abscission process into two distinct phases based on sensitivity to auxins and ethylene (1, 28), i.e., an induction phase during which auxin delayed abscission and ethylene had no effect, and a later phase when both auxin and ethylene stimulated abscission. Recently, the length of phase I in bean leaf explants has been altered by supplying or removing ethylene (3, 21). The latter study (21) demonstrated the importance of endogenous ethylene, since removing ethylene from the system significantly delayed abscission.

The process of fruit abscission has not been extensively studied. Applications of ethylene and ethephon, an ethylene releasing chemical, will enhance fruit abscission in a wide range of plant species (10, 11, 13, 14, 16, 18, 19, 23). Moreover, ethylene treatment increases the level of cell wall degrading enzymes in fruit abscission zones (19, 21, 26, 27) and regulates enzyme secretion into the cell wall (26). Endogenous ethylene also appears to have an important role, since reducing the internal level markedly delays abscission in young cotton fruit (23).

Earlier, we presented evidence which suggested the presence

of two phases in cherry fruit abscission (33). We not describe a series of experiments designed to more critically define these phases in relation to fruit development, to determine the effects of both exogenous and endogenous ethylene on abscission, and to further elucidate the mechanism involved in abscission.

MATERIALS AND METHODS

General Methods. Sour cherry fruit (Prunus cerasus L., cv. Montmorency) in various stages of development, were collected as needed just prior to initiating an experiment. Separation was followed at the abscission zone between the pedicel and fruit, the point of natural separation at maturity. Procedures used to quantitatively determine the fruit removal force (FRF) at this zone and in conducting the fruit explant bioassay have been previously described (11, 33).

Change in Abscission Potential with Development. Changes in FRF were determined on a uniform sample of 20 fruit at weekly intervals from the end of Stage I of fruit growth through maturity. Abscission potential, the capacity of explants to exhibit abscission, was determined in explants prepared from comparable fruit at the same time. FRF was measured after 80 hr with explants held in distilled water at 23$\pm$ 2 C (33). Stage of fruit development was monitored by obtaining fresh weight measurements twice weekly utilizing a representative sample of 20 fruits.

Effect of Exogenous Ethylene. Ethephon [2-(chloroethyl)-phosphonic acid (Amchem Products, Inc)] and ethylene gas were used to determine the effect of exogenous ethylene on the two

phases of abscission. Ethephon was supplied in the explant treating solution at 10^{-3} M. The effects of cycloheximide (10^{-4} M) on ethylene evolution and abscission of explants prepared from fruits in different stages of development was also established. Control explants were held in distilled water.

Explants were treated with 10 μl/l ethylene gas in 10 liter desiccators. A flow-through system was employed in which ethylene was premixed with compressed air and allowed to bleed into the desiccators through a vacuum regulator (Matheson model 49) at a rate of 8.5 liters/hr controlled by a needle valve to the vacuum line. Control explants were held under the same conditions in ethylene-free air.

To make certain that the influence of cycloheximide and ethylene on abscission was not simply an indirect effect due to altered fruit enlargement, fresh weight of the fruit was recorded before and after treatment.

Effect of Endogenous Ethylene. If ethylene is involved in abscission, then reducing the endogenous level early in the separation phase should delay abscission. Endogenous levels of ethylene were reduced to one-fifth by subjecting explants in desiccators to 0.2 atm pressure. The same flow-through system was used as described earlier except pure oxygen was allowed to bleed into the desiccators, thereby maintaining its partial pressure at atmospheric level. As a control for the reduced pressure, an additional treatment containing 50 μl/l ethylene in pure oxygen was supplied to explants at 0.2 atm and compared to 10 μl/l ethylene in compressed air at one atm.

Ethylene Evolution and Internal Concentrations. Ethylene evolution was determined twice a week from full bloom until after maturity. Fruits were detached from the tree and the pedicel was cut 3 to 4 mm above the fruit. Ten fruits were sealed in 25 ml flasks or 10 to 30 fruits in 265 ml glass containers, depending on the stage of fruit development. A filter paper wick saturated with 10% KOH was sealed in each container to absorb CO_2. Two to 4 replications were used. Sealed containers, including appropriate controls (lacking only fruit) were held in a water bath at 25$\pm$ 1 C for 8 hr. CO_2 and O_2 levels were monitored using a Perkin-Elmer Vapor Fractometer, Model 154B, and O_2 was supplied to maintain the atmosphere at 21$\pm$ 3%. Ethylene evolution was determined by assaying 1.0 ml of the gas phase by gas chromatography (29).

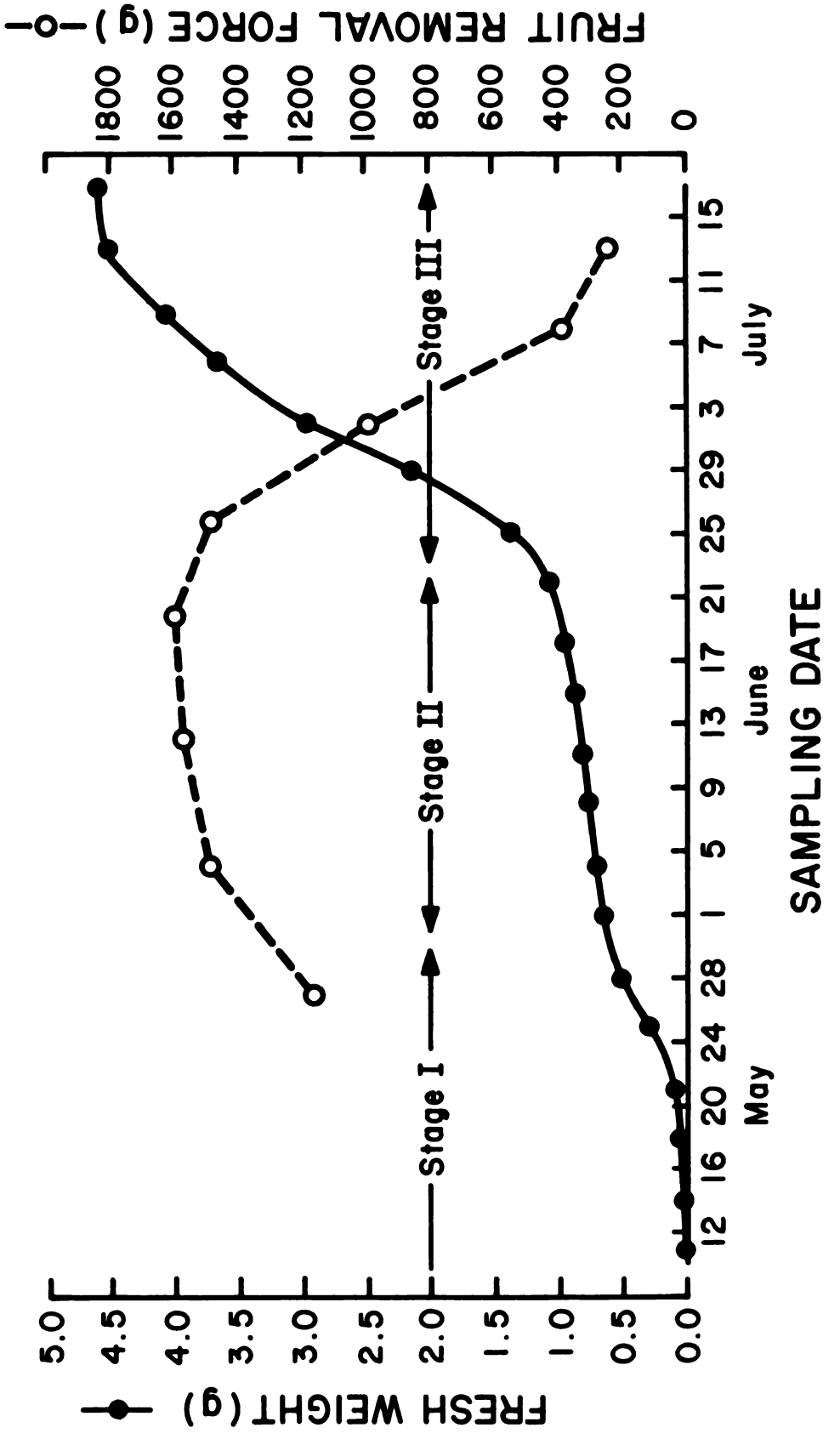
The concentration of endogenous ethylene was determined using a modification of the procedure of Beyer and Morgan (8). Twenty five to 50 fruit were subjected to a vacuum of 200 mm for 30 sec. The extracted gas was then assayed for ethylene by gas chromatography.

RESULTS

Change in Abscission Potential with Development. FRF increased early in fruit development (Figure 1) indicating a strengthening of the abscission zone tissue. Then at the beginning of Stage III a perceptible decline in FRF began, which increased in rate and reached its lowest value at fruit maturity.

The abscission potential of explants prepared from fruits

Figure 1.--Fruit removal force (FRF) at the pedicel:fruit abscission zone in relation to stage of fruit development. FRF and fresh weight measurements were made on a sample of 20 fruit.


FRESH WEIGHT (g)
FRUIT REMOVAL FORCE (g)
SAMPLING DATE
Stage I
Stage II
Stage III
May
June
July
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
0
200
400
600
800
1000
1200
1400
1600
1800
12 16 20 24 28 1 5 9 13 17 21 25 29 3 7 11 15

in different stages of development is shown in Table 1. Abscission potential becomes apparent for the first time in explants harvested June 26, i.e., the beginning of Stage II of fruit growth, which corresponds to the first evidence of a decrease in FRF in attached fruits (Figure 1).

Influence of Exogenous Ethylene. Ethephon did not promote abscission in explants prepared from fruits in mid-Stage II of development (Table 2). However, it markedly enhanced abscission in explants from fruits collected June 18 (late Stage II)--one week before control (non-ethylene treated) explants demonstrated a potential to abscise--and in explants from fruits in Stage III of development.

Cycloheximide inhibited abscission in explants obtained from fruit in late Stage III of development; however, it significantly reduced the FRF of explants from fruits (late Stage II) harvested June 18 (Table 2). Cycloheximide caused a rapid and marked enhancement of ethylene production in explants from fruits harvested June 22 (Table 3).

Explants prepared at different stages of fruit development responded similarly to both ethylene gas at 10 μl/l and to ethephon (compare Table 2 and Table 4).

Because of the close correlation between fruit enlargement in Stage III and the onset of the decline in FRF, the effects of ethephon and cycloheximide on these two processes were assessed at an early stage of fruit development. There was no significant effect of either ethephon or cycloheximide on fresh weight; however, both significantly reduced the FRF (Table 5). Similar

TABLE 1.--Abscission Potential of Fruit Explants.

Explants were prepared at weekly intervals from the end of Stage I of fruit growth to maturity. The explants were positioned with the cut end of the pedicel in distilled water and held in the dark at 23‡ 2 C. At the end of 80 hr the fruit removal force (FRF) was recorded and used as an index of the abscission potential. Each treatment included 20 single fruit replications.

Date explants prepared	FRF		
	Initial (g)	After 80 hr (g)	% of Initial
May 27	1167 a[1]	1269 a	108.7
June 4	1485 a	1428 a	96.2
June 11	1530 a	1509 a	98.6
June 18	1550 a	1542 a	99.5
June 25	1512 a	1260 b	83.3
July 2	998 a	408 b	40.9
July 8	398 a	321 b	80.7

[1]Mean separation (in rows) by Tukey's ω test, P = 0.01.

TABLE 2.--Effect of Ethephon and Cycloheximide on Fruit Removal Force of Explants Prepared from Fruit at Different Stages of Development.

Sour cherry fruit explants were prepared at weekly intervals from mid-Stage II to mid-Stage III of fruit growth. Explants were positioned with the cut end of the pedicel in test tubes containing distilled water, ethephon (10^{-3} M), or cycloheximide (10^{-4} M) and held in the dark at 23± 2 C. After 80 hr the fruit removal force (FRF) was recorded and used as a measure of abscission. Each treatment included 20 single fruit replications.

	FRF (g)		
Date explants prepared	Control	Ethephon	Cycloheximide
June 11	1502 a[1]	1452 a	1456 a
June 18	1545 a	936 c	1440 b
June 25	1281 a	334 b	1262 a
July 2	408 b	218 c	771 a

[1]Mean separation (in rows) by Tukey's ω test, P = 0.05.

TABLE 3.--Effect of Cycloheximide on Ethylene Production by Fruit Explants.

Explants prepared from fruits harvested June 22 were positioned with the cut end of the pedicel in small beakers of distilled water or cycloheximide (10^{-4} M) and sealed in 264 ml glass containers. The gas phase was assayed after 1 and 4 hr for ethylene by gas chromatography.

Time of sampling	Ethylene ($\mu l\ kg^{-1}\ hr^{-1}$)	
	Control	Cycloheximide
1 hr	0.021 b[1]	1.179 a
4 hr	0.016 b	11.099 a

[1]Mean separation (in rows) by Tukey's ω test, P = 0.01.

TABLE 4.--Effect of Exogenous and Endogenous Ethylene on Abscission of Explants Prepared from Fruits at Different Stages of Development.

Fruit explants were subjected to the indicated treatments in desiccators using a flow-through system. Explants were positioned with the cut end of the pedicel in distilled water in the dark at 23± 2 C. Fruit removal force (FRF) was measured after 72 hr.

Date explants prepared	FRF (g)			
	Air, at 760 mm	Air + 10 µl/l C_2H_4, at 760 mm	O_2, at 150 mm	O_2 + 50 µl/l C_2H_4, at 150 mm
May 27	1292 a[1]	1118 b	1395 a	1185 ab
June 4	1433 b	1373 b	1537 a	1396 b
June 11	1531 a	1358 b	1514 a	1386 b
June 18	1543 a	1291 b	1548 a	1302 b
June 25	1278 b	383 c	1384 a	362 c
July 2	492 b	320 c	673 a	302 c

[1] Mean separation (in rows) by Tukey's ω test, P=0.01.

TABLE 5.--Influence of Ethephon and Cycloheximide on Fruit Enlargement and Abscission.

Explants were prepared June 18 and positioned with the cut end of the pedicel in test tubes containing distilled water, ethephon (10^{-3} M) or cycloheximide (10^{-4} M) and held in the dark at 23± 2 C. After 80 hr the fresh weight and fruit removal force (FRF) were recorded.

Measurement	Control	Ethephon	Cycloheximide
Fresh wt (g)	0.90 a[1]	0.89 a	0.86 a
FRF (g)	1545 a	936 c	1440 b

[1]Mean separation (in rows) by Tukey's ω test, P = 0.05.

observations have been made with ethephon on intact fruit in the field and with ethylene on fruit explants in the laboratory (unpublished data).

Influence of Endogenous Ethylene. Reducing the endogenous level of ethylene to one-fifth by reduced pressure significantly inhibited abscission of explants prepared from fruits in Stage III of development, June 25 and July 2 (Table 4). Adding ethylene (50 μl/l) at 0.2 atm to provide a level equivalent to 10 μl/l ethylene at one atm (column 3 vs 5, Table 4) resulted in an identical response and confirmed that reduced pressure had no significant effect on abscission other than indirectly by reducing the endogenous ethylene level.

Ethylene Evolution and Concentrations in the Internal Atmosphere. The rate of ethylene evolution with cherry fruit development (Figure 2) was similar whether determined on a per fruit (nl fruit^{-1} hr^{-1}) or fresh weight (μl kg^{-1} hr^{-1}) basis. There was almost a steady decline in evolution with fruit development. The level of ethylene decreased rapidly during Stage I, except for an increase just prior to pit hardening. The level then declined during the remainder of Stage I and remained relatively constant during most of Stage II. Just before the start of Stage III another increase was observed. Thereafter, the level declined and remained nearly constant until near maturity when another small peak was observed.

Ethylene concentrations in the internal atmosphere of cherry fruit were also low for the period monitored (from mid-Stage II to 1 week prior to maturity--Table 6). The concentration was

Figure 2.--Ethylene evolution from sour cherry fruits during development. Fruits were sealed in containers with KOH (10%) wicks to absorb CO_2. Ethylene evolution was determined by assaying 1.0 ml of the gas phase by gas chromatography. These values were obtained for a different season than the rest of the studies, and therefore, the dates cannot be compared. However, the values are related to stage of fruit development, which should be comparable between years.

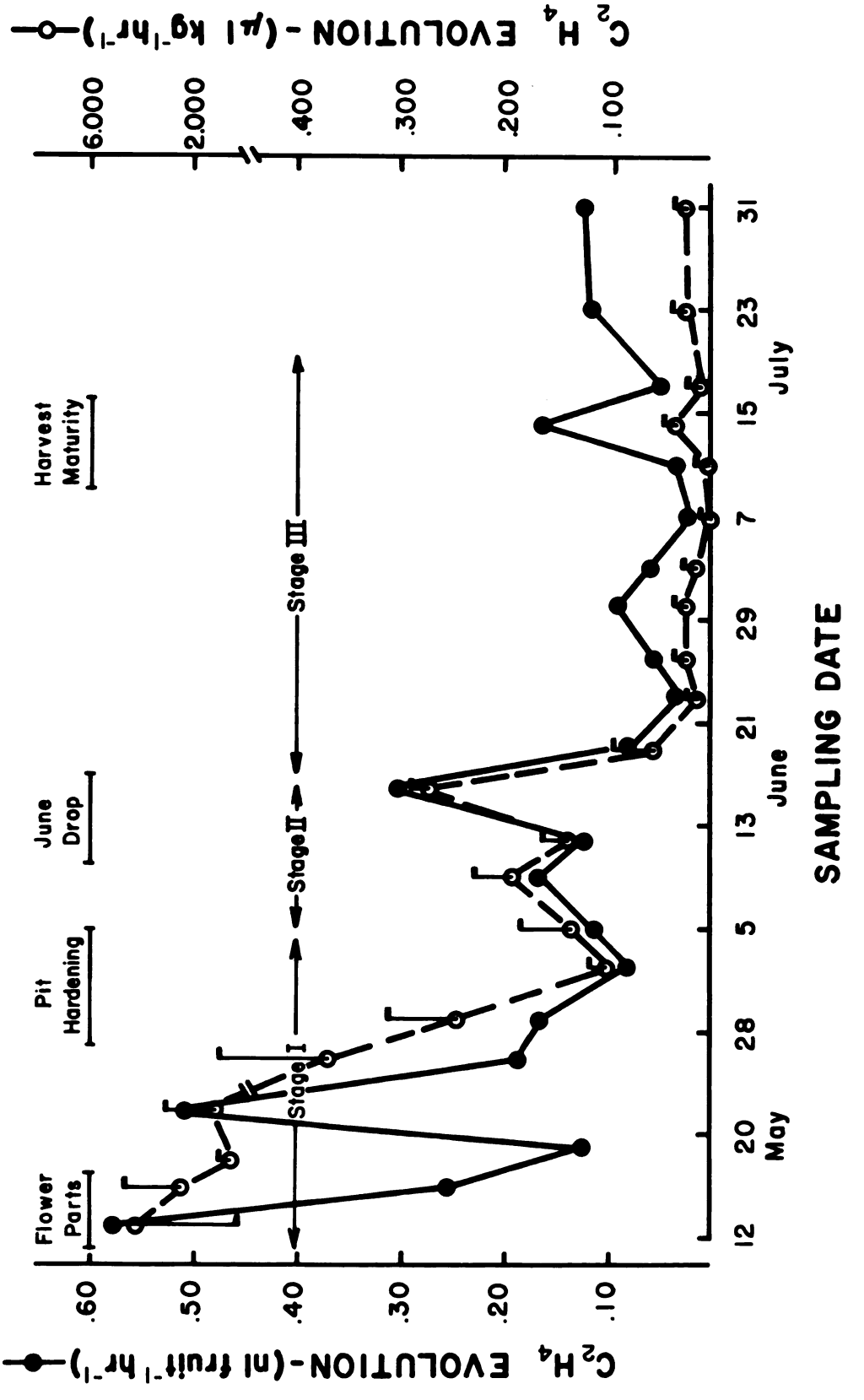

C2 H4 EVOLUTION - (nl fruit-1 hr-1)
.60
.50
.40
.30
.20
.10
C2 H4 EVOLUTION - (µl kg-1hr-1)
6.000
2.000
.400
.300
.200
.100
Flower Parts
Pit Hardening
June Drop
Harvest Maturity
Stage I
Stage II
Stage III
12
20
28
5
13
21
29
7
15
23
31
May
June
July
SAMPLING DATE

TABLE 6.--Ethylene Concentration in the Internal Atmosphere of Cherry Fruit at Different Stages of Development.

A vacuum of 200 mm was applied for 30 sec to a sample of 25 to 50 fruit. The extracted gas was then assayed for ethylene by gas chromatography. Each value represents the average of three replications and is followed by its standard deviation.

Date	Stage of development	Ethylene (nl/l)
June 15	Mid-Stage II	50.2 ± 5.2
June 21	Late Stage II	34.3 ± 4.8
June 29	Early Stage III	86.9 ± 18.2
July 5	Mid-Stage III	84.7 ± 16.1

lowest at a time corresponding to the end of Stage II (Figure 1) and increased to a level 2.5 times greater during Stage III.

DISCUSSION

Based on changes in FRF and the abscission potential of explants, the cherry fruit abscission process can be separated into two distinct phases. We have demonstrated with attached fruits that the pedicel:fruit abscission zone passes through two phases as indexed by force required to separate the fruit from its pedicel. During the early stages of fruit growth increasingly greater force is required to effect separation. However, at the end of Stage II or beginning of Stage III, there is a precipitous decline in FRF leading to separation at maturity (Figure 1). Undoubtedly, the increase in FRF associated with Phase I, the pre-separation phase, is related to the development of the pedicel and fruit tissues, particularly of the vascular cylinder. During Phase II, the separation phase, the decline in FRF is related to the formation of the abscission layer (31).

Furthermore, these two phases can be demonstrated based on a marked change in abscission potential of explants from fruits in varying stages of development (Table 1). Explants from fruits in Stage I or II of development exhibit no potential to abscise, while an abscission potential can be demonstrated in explants from fruits in Stage III.

Therefore, Phase I can be characterized by an increasing break-force and by an absence of a potential for abscission in explants. This phase is characteristic of fruits during Stage I and most

of Stage II of fruit development. Phase I can more accurately be labeled as the pre-separation phase rather than the induction phase which has been associated with leaf explant studies, since there is no potential for abscission in explants during this phase. Phase II, the separation phase, is characteristic of fruit near the end of Stage II and all of Stage III. It is denoted by a declining break-force and a potential for abscission in explants. Furthermore, auxin, ethylene (supplied as ethephon), abscisic acid, and gibberellin promote abscission in explants during this phase (33).

Additional evidence supporting the two-phase nature of the abscission process in sour cherry derives from the differential response to ethylene of explants from fruits in various stages of development (Tables 2 and 4). Ethylene had no effect on abscission of explants from fruits in Stage I or early Stage II, but consistently promoted abscission of explants from fruits in late Stage II and Stage III. The significantly lower FRF associated with ethylene treatment during Stage I is apparently due to an inhibition of the increase in FRF (Table 1 *vs* 4) rather than an effect on abscission *per se*. Similar data were obtained with ethephon applied to trees under field conditions (unpublished data). These findings parallel those reported by Abeles (1) for abscission of cotton explants. He later suggested (2) that juvenility factors, e.g., auxins present at high levels during the non-responsive phase may overcome the action of ethylene. Once separation has been initiated, ethylene may promote abscission by increasing the synthesis of enzymes required (21, 22, 25, 26, 28)

as well as their secretion into the cell wall (4, 26).

The ability of ethylene to shorten Phase I (Table 4, June 11 and 18; Table 2, June 18) supports the work on leaf abscission (3, 21), which demonstrated that ethylene could influence the induction or aging phase. This influence may result from the capacity of ethylene to reduce auxin transport (7) and/or auxin destruction (24) and thereby lower the level of auxin in the abscission zone.

Evolution rates (Figure 2) and concentrations (Table 6) of ethylene in the internal atmosphere indicate relatively low levels of production by cherry fruit during separation, and confirm the observation of Blanpied (9). The high levels of ethylene evolved at bloom and shortly thereafter may play a role in the abscission of immature cherry fruit, as was demonstrated by Lipe and Morgan (22) for immature cotton fruit. Interestingly, there was a significant peak at the transition between Stage II and III of fruit growth, but the levels during Stage III were much lower, when FRF was rapidly decreasing and separation was underway. Even though the endogenous level of ethylene remained low throughout separation, the endogenous ethylene present appeared to play a role. Reducing the endogenous level by 80% shortly after the start of the separation phase and midway through separation significantly inhibited abscission (Table 4). This indicates the importance of a continuous supply of ethylene during the separation phase. These results support those of Jackson and Osborne (21) on leaf abscission and Lipe and Morgan (23) for young fruit abscission.

Although reduced pressure failed to completely block abscission early in the separation phase (Table I, June 25 initial FRF *vs* Table 4, June 25), we believe this is due to the inability to select a sample of fruit sufficiently synchronous in abscission development. Our data suggest that once the separation phase is initiated and underway, reducing the endogenous level by 80% fails to completely block abscission.

It should not be overlooked that due to the morphology and low permeability of the cuticle (6), the tissue containing the abscission zone may represent the path of least resistance for ethylene diffusion from the fruit. If this is so, then the level of ethylene in the abscission zone might be several fold higher than that in the fruit. Another consideration is that only ethylene produced in or near the abscission zone may be important in abscission. Hence, our measurements on whole fruit may have masked localized changes in or near the abscission zone. Ethylene production in the abscission zone was not determined because of the latter's shape and proximity to the fruit tissue (32); removal from the fruit would have resulted in excessive injury.

Finally it is possible that the level of ethylene in the abscission zone remains at a low but potentially active level. As the level of auxin, or other juvenility factors, declines below a critical threshold, the cells in the abscission layer may become more sensitive to ethylene, thereby initiating separation. This was the basis of the aging-ethylene hypothesis proposed by Abeles (2).

The influence of cycloheximide on abscission (Table 2) would appear to be two-fold. Firstly, cycloheximide induced a dramatic increase in ethylene evolution within a very short time after treatment (Table 3) and hastened abscission of explants from fruits in Stage II. This increase in ethylene may have resulted from injury as has been reported for other fruits (13, 18). Secondly, near maturity cycloheximide inhibited abscission of explants (Table 2) and intact fruits (12), as well as blocked ethylene-enhanced abscission (33). This suppression of abscission may have resulted from inhibition of protein synthesis necessary for abscission (30).

Since inhibition of protein synthesis by cycloheximide negates the influence of ethylene evolution, protein synthesis appears to be involved in ethylene-enhanced cherry fruit abscission. Based on the enzymatic studies cited, the proteins required may be enzymes involved in abscission. The fact that cycloheximide continues to inhibit abscission until separation is nearly complete (Table 2) would indicate the necessity for continuous synthesis of these enzymes. However, this study also showed the requirement for a continual presence of ethylene which further suggests a role for ethylene in the synthesis of enzymes necessary for cherry fruit abscission.

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SECTION II

A ROLE FOR ETHYLENE IN MECHANICALLY INDUCED ABSCISSION OF IMMATURE FRUITS

INTRODUCTION

The role of the seed in the abscission of immature fruit is unclear. Tukey (18) showed that mechanical destruction of the seed in developing sour cherry and peach fruit during Stage II of growth resulted in an abrupt cessation of fruit growth and subsequent abscission. A similar effect was demonstrated by maleic hydrazide-induced seed abortion in apricot fruit prior to pit hardening (5). However, as seed destruction was successively delayed, higher percentages of fruits persisted and developed normally (5, 18). These studies suggested that the seed played a dominant role during the early stages of fruit development.

Previously we observed that application of ethephon, an ethylene-releasing chemical, to sweet cherry fruit in Stage II of growth caused seed abortion and abscission of the immature fruit at the peduncle:pedicel zone (3). By contrast, treatment of fruit early in Stage III resulted in an acceleration of maturity and hastening of abscission at the pedicel:fruit zone. Ethephon also causes abscission of immature peach fruit (2, 6, 15). In peach, however, immature fruit abscission was enhanced primarily at the fruit:receptacle juncture, the same site of mature fruit abscission (6).

Questions remain as to the role of the seed and ethylene in immature fruit abscission. Does seed abortion induce abscission by shutting off the supply of auxin or some other juvenility

factor to the zone (10, 17), or does abortion of the seed result in the release of ethylene which then promotes abscission at the zone (7, 16)? We outline in this paper a series of experiments designed to further our understanding of the role of the seed and of ethylene in mechanically-induced abscission of immature sour cherry fruit.

MATERIALS AND METHODS

General methods. Sour cherry fruit (Prunus cerasus L., cv. Montmorency) were selected as a test system because of the marked dependency of fruit growth on the seed and the well-defined stages of fruit and seed development (12, 19). Furthermore, separation of immature fruit occurs at the peduncle:pedicel zone while mature fruit abscise at the pedicel:fruit zone, thereby allowing abscission at the two zones to be studied independently.

Fruit development was monitored by determining fresh weight twice weekly on a sample of 20 fruit. Seed development was recorded by photography of fruits cut in half longitudinally through the suture.

Abscission at the peduncle:pedicel zone was quantitatively determined by measuring the fruit removal force (FRF) with a Hunter Mechanical Force Gauge (Hunter Spring, Hatfield, Pa.) fitted with a claw. FRF was obtained by holding the spur in the claw at its point of attachment to the branch and pulling the pedicel from the spur in line with the long axis.

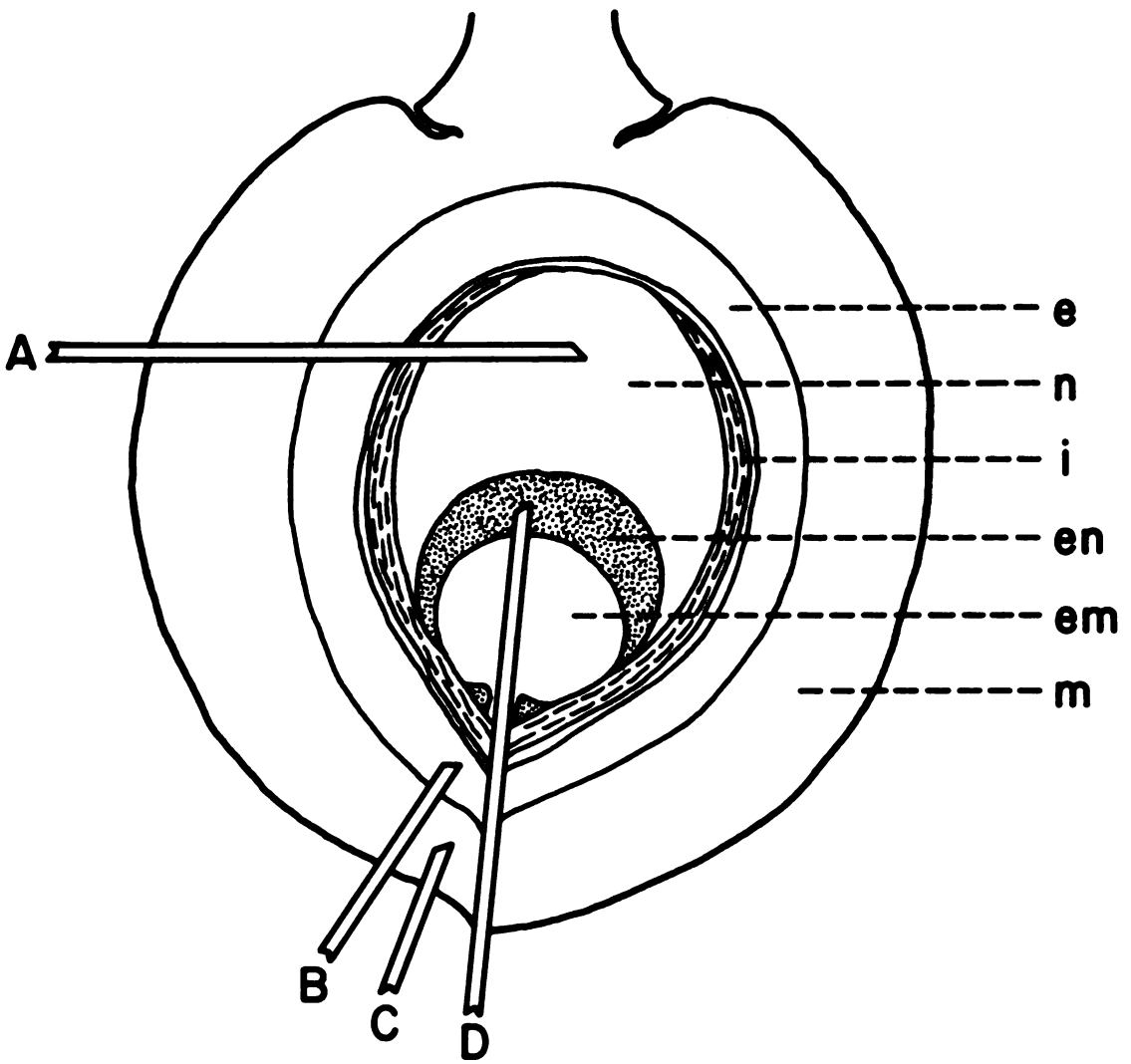
Injury-induced ethylene evolution and abscission. To determine if there was a relationship between tissue injury, ethylene

evolution and abscission, different tissues of the fruit and seed were injured mechanically, and ethylene evolution and abscission were recorded. Injury was induced by drilling (high speed hobby drill, 0.79 mm diam.) into the mesocarp, endocarp, or embryo through the micropylar end and into the nucellus at the chalazal end (Figure 1). Each treatment, consisting of approximately 100 fruits, was replicated three times.

Ethylene evolution was determined by enclosing a sample of 10 fruit collected from each treatment immediately after injury in a 25 or 50 ml flask, depending on stage of fruit development. A filter paper wick satured with 10% KOH was sealed in each flask to absorb CO_2. The flasks, including appropriate controls (lacking only fruit), were held in a water bath at 25± 1° C for 2 hr. Ethylene evolution was determined by assaying 1.0 ml of the gas phase by gas chromatography (14). Abscission was recorded by counting the number of fruits which abscised after 2 weeks.

Injury of the seed at different stages of development. To further elucidate the role of the seed in abscission of fruit at various stages of development, the seed was injured by drilling either into the micropylar or chalazal end (Figure 1). These treatments were repeated weekly from late Stage I until early Stage III of fruit development (corresponding to periods A-E of Figures 3 and 5-I, insert). The effect of the injury on the subsequent development of the seed and on ethylene evolution was determined as outlined above. FRF was obtained as a quantitative measure of abscission at the peduncle:pedicel and pedicel:fruit abscission zones 10 days after treatment.

Figure 1.--Diagram of the sour cherry fruit in Stage II showing four positions of drilling to induce injury of the developing fruit and seed (e, endocarp; n, nucellus; i, integuments; en, endosperm; em, embryo; m, mesocarp). Position A, into the nucellus at the chalazal end of the seed; B, into the endocarp; C, into the mesocarp; D, into the embryo through the micropylar end of the seed.


A
B
C
D
e
n
i
en
em
m

Influence of exogenous ethylene. If ethylene produced as a result of seed abortion is responsible for the abscission of immature fruit, then exogenous ethylene should have a similar effect. Ethephon, as a source of ethylene, was applied as a spray without a surfactant at 500 and 1000 ppm at weekly intervals from late Stage I to early Stage III of fruit development. Seed development was followed and ethylene evolution was recorded within 4 hr after application. The FRF for both abscission zones was measured after 10 days.

The influence of ethylene gas was also determined. Small branches each containing 10 fruits were detached from the tree, the leaves removed, and the bases of the branches were placed in distilled water in 10 l desiccators. The branches were exposed to ethylene (10 μl/l) or compressed air at a flow rate of 8.5 l/hr using the flow-through system previously described (21). The FRF at the peduncle:pedicel zone was determined after 80 hr. In addition, the effect of ethylene gas on ethylene production by the fruit at various stages of development was determined. Fruits were exposed to a flow of 10 μl/l of ethylene for 12 hr and the system was then flushed with ethylene-free air for 2 hr. Ethylene evolution was then determined over a subsequent 2 hr enclosure period as described above.

Influence of injury-induced ethylene. To separate the effect of injury to the seed from the effect of the ethylene evolved as a result of the damage, the endogenous levels of ethylene were reduced to one-fifth normal by subjecting branches with injured fruit to 0.2 atm. Similar branches with injured fruit were exposed

to 1 atm as described earlier. The same flow-through system was used that was previously described (21).

RESULTS

Fruit and seed development. The development of the 'Montmorency' sour cherry fruit occurs in three distinct stages (Figure 2). The FRF at the peduncle:pedicel zone for persisting fruit remains relatively constant from late Stage I through Stage II, thereafter increasing slightly with maturity (Figure 2). No decline in FRF at this zone was observed in healthy fruit prior to or during "June drop".

Seed development is closely related to fruit growth. During Stage I the nucellus and integuments reach their maximum size (Figure 3A). With the onset of Stage II the endosperm and embryo begin their period of rapid enlargement (Figure 3 B-D). As the endosperm and embryo develop, the nucellar tissue is consumed. By early Stage III the embryo is completely developed and occupies almost the entire seed compartment (Figure 3E). Only remnants of the endosperm can be seen at the outer edges of the embryo.

Injury-induced ethylene evolution and abscission. Ethylene evolution as a result of injury was closely related to the fruit tissue damaged (Table 1). There was no significant increase in ethylene evolved when injury was limited to the ovary wall tissue (mesocarp and endocarp). In contrast, there was 22 - 43 times more ethylene evolved following damage to the seed as compared to the control (not damaged) or when only the ovary wall was

Figure 2.--Fruit removal force (FRF) at the peduncle:pedicel abscission zone in relation to stage of fruit development. FRF and fresh weight measurements were determined utilizing a sample of 60 and 20 fruits, respectively. Full bloom occurred on May 2.

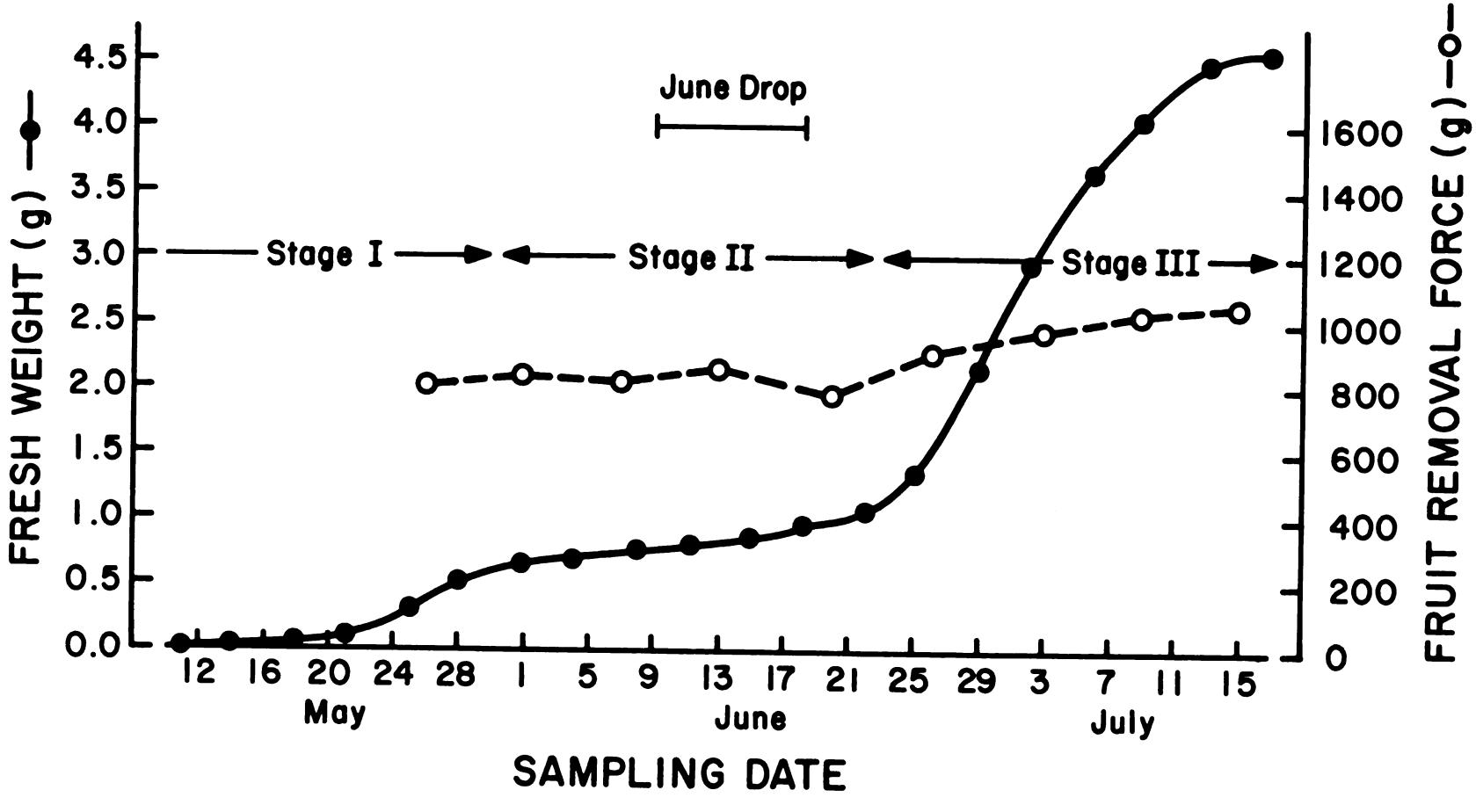

FRESH WEIGHT (g) —●—
FRUIT REMOVAL FORCE (g) —o—
June Drop
Stage I
Stage II
Stage III
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1600
1400
1200
1000
800
600
400
200
0
12 16 20 24 28 1 5 9 13 17 21 25 29 3 7 11 15
May
June
July
SAMPLING DATE

Figure 3.--Photomicrographs of longitudinal sections through the fruit illustrating development of the seed at those times when injury treatments were made. A, late Stage I; B, early Stage II; C, mid-Stage II: D, late Stage II; E, early Stage III.

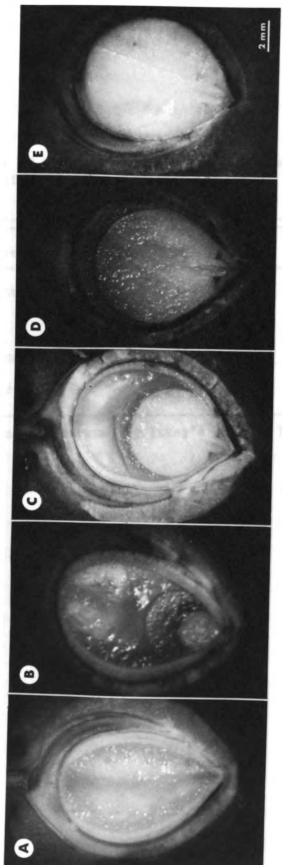

A
B
C
D
E
2 mm

TABLE 1.--Effect of the tissue injured on ethylene evolution and fruit abscission early in Stage II.

Tissue injured	C_2H_4 (μl kg^{-1} hr^{-1})	% Abscission
Control (non injured)	0.24 b[z]	4 b
Mesocarp	0.46 b	5 b
Mesocarp + endocarp	0.36 b	4 b
Seed (micropylar end)	10.33 a	88 a

[z]Mean separation (in columns) by Tukey's ω test, P = 0.01.

injured. Fruit abscission was induced only following injury to the seed, the same treatment that caused a marked increase in ethylene evolution (Table 1).

Injury of the seed at various stages of development. Damaging the seed at either the micropylar or chalazal end during Stage I resulted in degradation of the nucellar tissue and shriveling of the integuments (Figure 4, A and D). In addition, ethylene evolution shortly after injury was markedly increased in comparison with uninjured control fruit (Figure 5-I), and the FRF at the peduncle:pedicel zone was greatly reduced after 10 days. All injured fruits abscised (Figure 5-III) within 2 wk.

Similar results were obtained for the early and mid-Stage II treatments (Figure 5, I and II, B and C). However, neither the endosperm tissue nor embryo appeared to undergo degradation like the nucellar tissue (Figure 4, B and E). The endosperm only appeared to shrivel later, while the embryo showed only the scar of injury from the micropylar end and retarded growth corresponding to the time of treatment. Furthermore, the rate of shriveling of the integuments appeared to be dependent on the degradation of the underlying tissue, i.e. whether it was the endosperm or nucellus.

In late Stage II of development, injury-induced ethylene evolution was greatly reduced (Figure 5-I, D). Correlated with this low rate of ethylene evolution was a slight decrease in FRF at the peduncle:pedicel zone, and the number of fruits which abscised due to injury was considerably less than when injury was induced early in Stage II (Figure 5-II). With the onset of

Figure 4.--Photomicrographs showing the appearance of the seed 4 days after drilling into the seed through the micropylar (A-C) or chalazal (D-F) end. A and D, late Stage I; B and E, mid-Stage II; C and F, early Stage III.

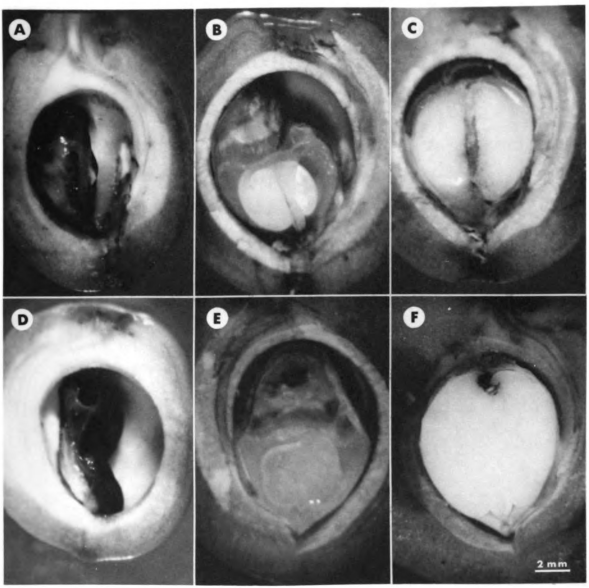
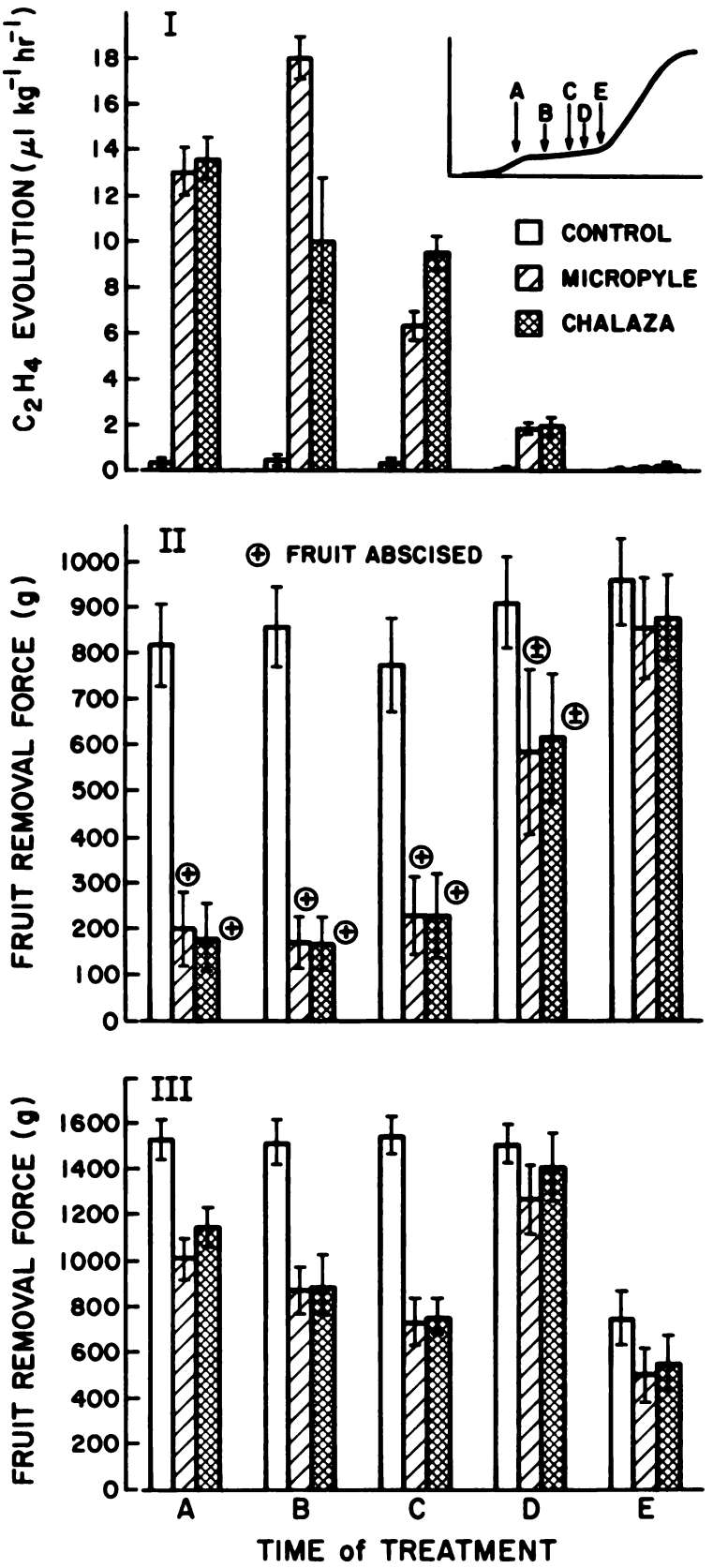

A
B
C
D
E
F
2 mm

Figure 5.--Ethylene evolution (I) and fruit removal force (FRF) at the peduncle:pedicel (II) and pedicel:fruit (III) abscission zones of immature sour cherry fruit. Ethylene determinations were made during the first 4 hr and FRF 10 days after treatment. Control, no injury; micropyle, injury to the seed at the micropylar end; chalaza, injury to the seed at the chalazal end. Insert (I) indicates time of treatment in relation to fruit development (A, May 29; B, June 5: C, June 12; D, June 17; E, June 22). Vertical brackets indicate standard deviations. +, injured fruits abscised; ±, approximately one-half of the injured fruits abscised.


I
C_2H_4 EVOLUTION (μl kg^{-1} hr^{-1})
0
2
4
6
8
10
12
14
16
18
A
B
C
D
E
CONTROL
MICROPYLE
CHALAZA
II
FRUIT REMOVAL FORCE (g)
0
100
200
300
400
500
600
700
800
900
1000
⊕ FRUIT ABSCISED
III
FRUIT REMOVAL FORCE (g)
0
200
400
600
800
1000
1200
1400
1600
A
B
C
D
E
TIME of TREATMENT

Stage III of fruit growth, damage to the seed resulted in no appreciable ethylene evolution (Figure 5-I, E). Moreover, the fruit did not abscise (Figure 5-II) but persisted with no visible effects other than the scar left by the drill (Figure 4, C and F). Injury to the seed at the end of Stage II or start of Stage III resulted in no measurable acceleration of maturity.

Although the abscising fruit separated at the peduncle: pedicel zone, there was a significant reduction in FRF at the pedicel:fruit zone in response to seed injury (Figure 5-III). Furthermore, the magnitude of reduction in FRF increased with subsequent treatments until late in Stage II (Figure 5-III, D) when the injury-induced ethylene was greatly reduced. Thus in the last two treatments (Figure 5-III, D and E) the FRF at the pedicel:fruit zone of the injured fruit was only slightly lower than that of the control fruit.

Influence of exogenous ethylene. Ethephon caused a marked increase in the rate of ethylene evolution shortly after treatment at all stages (Figure 6-I). Fruit treated with either concn at the end of Stage I abscised at the peduncle:pedicel zone (Figure 6-II, A). Furthermore, at this early date both concn caused seed abortion (Figure 7A). However, only the higher concn induced seed abortion and abscission during early and mid-Stage II (Figure 6-II and 7B), even though both concn caused a significant increase in ethylene evolution (Figure 6-I). By the onset of Stage III, neither concn induced seed abortion or abscission (Figure 6-II), although both markedly enhanced ethylene evolution (Figure 6-I). Ethephon in late Stage II slightly accelerated

Figure 6.--Ethylene evolution (I) and fruit removal force (FRF) at the peduncle: pedicel (II) and pedicel:fruit (III) abscission zones of immature sour cherry fruit. Ethylene determinations were made during the first 4 hr and FRF 10 days after treatment. Control (0 ppm) and ethephon (500 and 1000 ppm) treated fruits. See insert Figure 5-I for time of treatment. Vertical brackets indicate standard deviations. +, injured fruits abscised; $\pm$, approximately one-half of the injured fruits abscised.

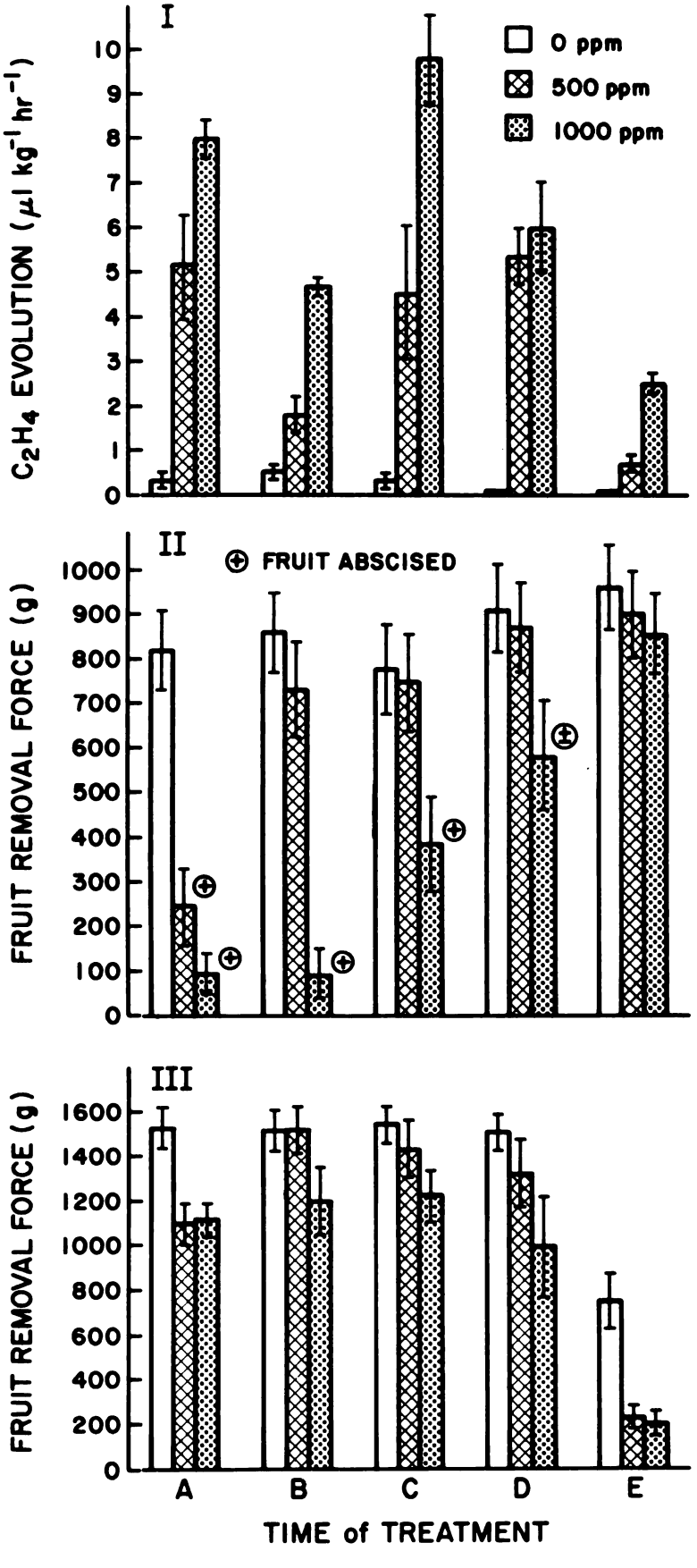
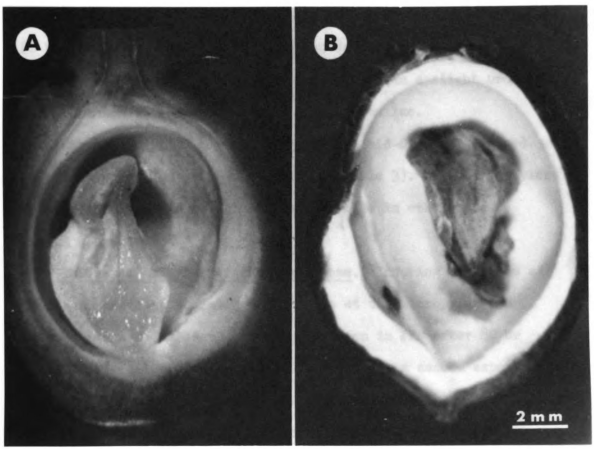

I
0 ppm
500 ppm
1000 ppm
C_2H_4 EVOLUTION (μl kg^{-1} hr^{-1})
0
1
2
3
4
5
6
7
8
9
10
II
⊕ FRUIT ABSCISED
FRUIT REMOVAL FORCE (g)
0
100
200
300
400
500
600
700
800
900
1000
III
FRUIT REMOVAL FORCE (g)
0
200
400
600
800
1000
1200
1400
1600
A
B
C
D
E
TIME of TREATMENT

Figure 7.--Photomicrographs depicting ethephon-induced seed abortion. A, six days after treatment with 500 ppm in late Stage I; B, eight days after treatment with 1000 ppm in early Stage II.


A
B
2 mm

fruit coloring.

Ethephon also caused a reduction in FRF at the pedicel:fruit zone (Figure 6-III). The lower concn was effective during Stages I and III, but only the higher concn caused a significant reduction in FRF during Stage II.

Treatment of detached fruiting branches with 10 μl/l ethylene caused a significant reduction in FRF at the upper zone after 80 hr (Table 2). However, no apparent damage to the seed was observed at the end of the experiment other than a slight brown discoloration at the chalazal end of the nucellus.

Ethylene applied to the fruits during mid-Stage II induced an enhanced rate of ethylene evolution (Table 3). But, if ethylene was applied later in development no increase in ethylene evolution was observed.

Influence of injury-induced ethylene. Injuring the seeds of fruits on detached, defoliated branches at the micropylar end late in Stage I failed to cause a reduction in FRF after 80 hr (Table 2). Similar results were obtained with a second experiment run for 200 hr (unpublished data). Ethylene at either 10 μl/l or 50 μl/l enhanced abscission. Reducing the level of injury-induced ethylene to one-fifth had no significant effect on abscission (Table 2). Injury to the seeds did, however, result in seed abortion.

TABLE 2.--Effect of applied and injury-induced ethylene on fruit removal force (FRF) at the peduncle:pedicel zone and on seed abortion late in Stage I.

Treatment[z]	FRF (g)	Seed abortion
Air, at 760 mm	768 a[y]	-[x]
10 μl/l C_2H_4 in air, at 760 mm	358 b	*
50 μl/l C_2H_4 in O_2, at 150 mm	372 b	*
Injured seed in air, at 760 mm	734 a	+
Injured seed in O_2, at 150 mm	717 a	+

[z]Branches with treated fruit were held in desiccators at the indicated pressure and gas phase conditions for 80 hr.

[y]Mean separation by Tukey's ω test, P = 0.01.

[x]Seed healthy (-), seed aborted (+), seed healthy except for a small brown discoloration at chalazal end of nucellus (*).

TABLE 3.--Effect of ethylene pretreatment on the subsequent evolution of ethylene by detached sour cherry fruit at various stages of development.

Date	Stage of development	Pretreatment[z]	
		Air	10 μl/l C_2H_4
		μl kg^{-1} hr^{-1}	
June 9	Mid-Stage II	0.15 b[y]	1.83 a
June 18	Late Stage II	0.11 a	0.10 a
July 3	Mid-Stage III	0.04 a	0.05 a

[z]12 hr pretreatment followed by 2 hr in ethylene-free air prior to enclosure and sampling.

[y]Mean separation (in rows) by Tukey's ω test, P = 0.01.

DISCUSSION

A correlation appears to exist between ethylene evolution and abscission. Injury to the ovary wall tissue (mesocarp and endocarp) did not significantly increase ethylene evolution or fruit abscission (Table 1). However, damage to the seed markedly enhanced the rate of ethylene evolution and resulted in abscission of immature fruit. Fruit in Stage I to mid-Stage II of growth showed a marked increase in ethylene production and abscised as a result of injury to the seed, regardless of the site of injury (Figure 5, I and II). Damaging the seed in Stage III, however, failed to induce ethylene evolution or abscission. Comparing these results on ethylene evolved with the injury to the seed (Figure 5-I vs. Figure 4), it would appear that the nucellus is the primary tissue responsible for the high rate of ethylene evolved. The integuments are probably not important since little or no ethylene was evolved (Figure 5-I) when the seed was damaged in Stage III (Figure 4). The endosperm and embryo apparently are not involved, since neither begins to enlarge until the onset of Stage II and yet a high rate of ethylene evolution was observed in Stage I. Furthermore, injury to the fully-enlarged embryo did not significantly increase ethylene evolution.

Ethephon caused immature fruits to abscise (Figure 6-II). Ethephon at 500 ppm was effective during Stage I, while 1000 ppm was effective during most of Stage II. Moreover, there appeared to be a correlation between ethephon induced seed abortion and fruit abscission.

On the other hand, fruit abscission was induced on isolated branches at the peduncle:pedicel zone in late Stage I with ethylene without evidence of seed abortion (Table 2). This suggests that ethylene can induce abscission at this zone provided it can be supplied to the zone from the fruit or adjacent tissue at an effective level. Perhaps ethylene produced in the nucellus during seed abortion can directly influence abscission of immature fruit, since the vascular system may provide a direct path of low diffusion resistance from the seed to the peduncle:pedicel zone. However, it is also possible that ethylene may influence abscission indirectly by reducing the trasnport of auxin (1) or some other juvenility factor to the zone.

The ability of ethylene treatment to induce ethylene production in fruit during Stage II but not later in development (Table 3), suggests that the ethylene produced is derived from the nucellus. Since little or no injury of the seed was observed following similar treatments (Table 2), the nucellus may produce ethylene autocatalytically rather than simply releasing it upon injury. Therefore, any treatment which will trigger this mechanism, i.e. mechanical, chemical or environmental injury of the seed, may cause a high rate of ethylene production.

The reason for the failure of fruits on detached branches to abscise in response to seed injury is unclear (Table 2). The results suggest, however, that injury-induced ethylene is not the sole factor controlling abscission at the peduncle:pedicel zone. Crane and Nelson (5) showed that apricots with maleic hydrazide-induced aborted seeds persisted as healthy fruit so

long as competition between their growth and vegetative growth was reduced to a minimum. Removal of the leaves from the cherry branches, rather than having the intended effect of enhancing the rate of abscission, may have reduced competition with the fruit.

Crane (4) suggested that growth hormones present in the seed function through the mobilization of substrates to them. Several studies have shown that auxins, gibberellins, and cytokinins are capable of causing the movement of inorganic and organic compounds, as well as other growth hormones to the site of application (8, 9, 13). Moreover, the levels of these hormones are known to be high in the sour cherry seed during the early stages of development[1] (12). On the other hand, ethylene has been shown to have a possible role in auxin destruction (11). Possibly, the ethylene production induced by injury of the seed prior to the completion of growth of the embryo may result in the destruction of the metabolic gradient established by the hormones of the seed. Consequently, the transport of hormones through the peduncle: pedicel zone toward the fruit would be cut off and abscission induced.

Ethylene appears to be involved in the abscission of immature fruit. However, as was shown for mature fruit (21), abscission is apparently controlled by an interaction of factors rather than ethylene alone. The absence of abscission at the peduncle:pedicel zone at maturity (20) may be due to either the presence of juvenility factors or the absence of ethylene at a sufficient level

[1]Hopping, M. E. Isolation, characterization and role of endogenous auxins and cytokinins in sour cherry (Prunus cerasus L., cv. Montmorency) fruit development. Ph.D. Thesis, Michigan State University. 207 P. 1972.

to induce separation. The latter explanation would appear more reasonable, since late in the season the quantity of juvenility hormones is generally at a low level.

Finally, the mechanism of abscission at both zones appears to be similar. Treatments which markedly enhanced ethylene evolution and abscission at the peduncle:pedicel zone, also caused a reduction in FRF at the pedicel:fruit zone (Figures 5 and 6). However, because the FRF was initially much higher at the pedicel:fruit zone the reduction in FRF in response to the treatments, although nearly as great, did not result in separation at this zone.

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SECTION III

PEROXIDASE ACTIVITY IN THE ABSCISSION ZONE IN RELATION TO SEPARATION

INTRODUCTION

Maturation of sour cherry fruit is accompanied by a decline in fruit removal froce (FRF) at the pedicel:fruit abscission zone (21). This decline in FRF has been associated with the loss of pectic substances and polysaccharides and a change in cellulose orientation in the walls of cells comprising the abscission layer (19). These observations suggest the action of cell-wall degrading enzymes in sour cherry fruit abscission as has been demonstrated for other fruit species (5, 10, 14, 16).

Enzymes not involved in cell-wall degradation have also been found in the abscission zone of cherry fruit during separation (12). One of these, peroxidase, has been associated with the break down of 3-indoleacetic acid (IAA) (3), an auxin long implicated in the abscission process. Moreover, peroxidase activity has been linked with the abscission of cotton and bean leaves (9, 11) in which both peroxidase activity and leaf separation were enhanced by ethylene. We have recently demonstrated a role for ethylene in sour cherry fruit abscission (21). Also, IAA is known to be present in sour cherry fruits during the early stages of growth[1]. Thus, peroxidase may play a role in the abscission of cherry fruit by regulating the auxin level.

This study was designed to establish if there was a relationship between peroxidase and cherry fruit abscission. Data are

[1]Hopping, M. E., Isolation, characterization and role of endogenous auxins and cytokinins in sour cherry (Prunus cerasus L., cv. Montmorency) fruit development. Ph.D. Thesis, Michigan State University. 207 P. 1972.

presented on localization, activity, and changes in isoenzyme patterns of peroxidase in the abscission zone and adjacent tissues prior to and during separation.

MATERIALS AND METHODS

General methods. Sour cherry fruit (Prunus cerasus L., cv. Montmorency) were collected at weekly intervals from late Stage I of fruit development through maturity. The samples were frozen immediately and held in dry ice until used. Fruit development was monitored by taking fresh weight measurements twice weekly utilizing a representative sample of 20 fruit. Abscission at the pedicel: fruit zone was followed by measuring the FRF on a sample of 20 fruit at weekly intervals[2].

Abscission bioassay. Fruit explants of the same physiological age were treated with ethephon (10^{-3} M), cycloheximide (10^{-4} M), ethylene (10 μl/l), or reduced pressure (0.2 atm) to produce explants with different degrees of abscission layer development. The preparation of explants and the procedures used in the bioassay have been previously described (20, 21). Explants were treated with ethephon and cycloheximide by placing the cut pedicels of the fruit into the test solutions. Ethylene was administered to explants held in desiccators, and reduced pressure treatments were performed in desiccators as described earlier (21).

Localization of peroxidase activity. Longitudinal sections (24 μm thick) were cut from the abscission zone with a cryostat.

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[2]Wittenbach, V. A., Morphological and physiological aspects of cherry fruit abscission with reference to 2-(chloroethyl)phosphonic acid. M.S. Thesis. Michigan State University. 114 P. 1970.

Peroxidase activity was determined with benzidine using the method of Veech (18). Benzidine in the presence of peroxide and peroxidase yields an unstable blue intermediate, which undergoes autoxidation to give a brown precipitate. However, in the Veech method the blue intermediate is stabilized with nickelous ammonium sulfate. The sections were photographed immediately after staining to record the localization. A second procedure using o-dianisidine (22) was employed to confirm the results obtained with benzidine. Controls consisted of heat-treated sections or substrate-deficient reaction media.

Measurement of peroxidase activity. Peroxidase activity was quantitated for the abscission zone and related tissue at various stages of fruit development. A 4 mm cork borer was used to remove the abscission zone from the frozen fruit. The zone tissue was then excised from most of the receptacle and pericarp tissues. Because of the semispherical shape of the abscission layer, its small size (6-8 cells wide), and its location within the fruit, the excised zone contained in addition to the actual layer a relatively large amount (nearly two-thirds by volume) of fruit (mesocarp) tissue.

Tissue from the abscission zone (~0.1 g from 5 fruits), receptacle, pedicel, or mesocarp (~0.2 g) was mechanically ground in a glass homogenizer with 4 ml of extraction medium containing 0.1% Tween 80 (polyoxyethylene sorbitan monooleate) and 0.5 mM ethylenediaminetetraacetic acid in 0.1 M phosphate buffer, pH 6.0, with 0.25 g insoluble polyvinylpyrrolidine. The extract was centrifuged at 10,000 g for 15 min and the supernatant solution was

decanted. The precipitate was reextracted twice with 2.0 ml of extraction medium as described above. The supernatant solutions were combined and centrifuged at 25,000 g for 30 min. The resulting supernatant solution was dialyzed overnight against deionized distilled water. All of the above procedures were carried out at 0 to 4° C.

Peroxidase activity was determined spectrophotometrically using o-dianisidine (17). Parallel determinations were made using guaiacol (2) to verify the results obtained with o-dianisidine. Enzyme activity was based on the oxidation of o-dianisidine at 460 nm or guaiacol at 470 nm in the presence of H_2O_2 and expressed per mg protein, determined by the Lowry method (6). Controls included heat-inactivated enzyme extract or H_2O_2 deficient medium.

Gel electrophoresis. The enzyme extract, prepared as described above, was lyophilized, and the resulting powder was dissolved in 0.5 ml 0.02 M phosphate buffer, pH 7.0 An aliquot (0.12 ml) of the concentrated enzyme extract, after mixing with a 60% (w/v) sucrose solution (2:1), was applied to the gel just prior to electrophoresis. The amount of protein applied per gel was maintained between 80 and 100 μg. Separation of the acidic proteins was carried out according to the discontinuous gel electrophoretic procedure of Davis (1) using Cyanogum 41 (7), while separation of the basic proteins was performed by the procedures of Reisfeld et al. (15). The gel columns (5 mm diam.) were composed of two sections, the upper 10 mm of a 5.0% (w/v) and the lower 65 mm of a 9.0% Cyanogum 41. The gels were run at 4° C using 1.5 ma per tube for the first 30 min and 3 ma per tube for the remaining

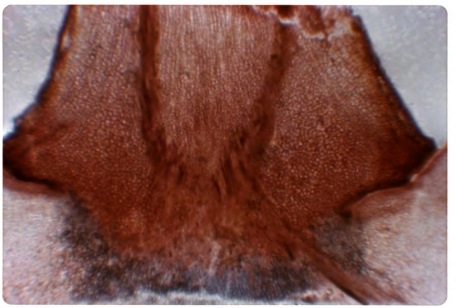
time (approx 90 min for the acidic protein gels and 150 min for the basic protein gels). Peroxidase activity was detected in the acidic protein gels with benzidine (18) or guaiacol (2), while only guaiacol was used for the basic protein gels, due to an interaction between the gel and benzidine. The gels were photographed to record the isoenzyme patterns.

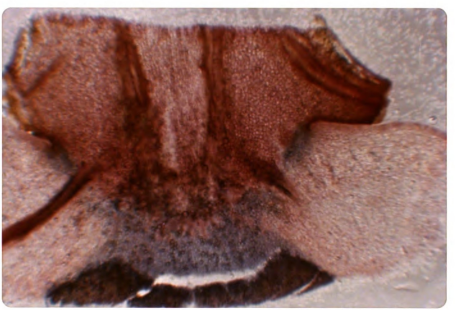
IAA oxidase assay. A relatively large quantity of enzyme extract (150 μg of protein) from the abscission zone was applied to the gels, which were run as described above. After electrophoresis the gels were frozen on dry ice and sliced into 1 mm sections. The sections were then incubated for 24 hr in the dark at 25° C in 0.2 ml of a reaction mixture containing 2 mM IAA, 0.1 mM 2,4- dichlorophenol, and 0.1 mM $MnCl_2$ in 0.1 M phosphate buffer, pH 6.0 (4). At the end of the incubation period 0.2 ml of 0.5% p-N,N- dimethylaminocinnamaldehyde in 1 N HCl was added to each tube (4, 8). After 1 hr the solutions were removed from the tubes and the optical density of the IAA oxidation products (8) was determined at 562 nM.

RESULTS

Localization of peroxidase. Peroxidase activity, as denoted by the blue reaction product, was localized principally in those cells through which the abscission layer forms (Figure 1, upper). However, a brown reaction product, indicating autoxidation of the blue intermediate, was observed in the receptacle tissue and to a much lesser extent in the fruit tissue. Autoxidation occurred irrespective of how soon after treating with benzidine the nickelous

Figure 1.--Localization of peroxidase in the abscission layer and associated tissues at mid-Stage III (upper) and at the end of Stage I (lower). The abscission layer is denoted by the blue reaction product.





ammonium sulfate solution was added. Furthermore, both reaction products, denoting peroxidase activity, were observed in the abscission layer and the adjacent tissues from late Stage I (Figure 1, lower) to maturity. The endocarp also gave a positive reaction for peroxidase in the early stages of development (Figure 1, lower). A similar differential staining for peroxidase between the abscission layer and the receptacle and fruit tissue was observed using o-dianisidine. In this case a green reaction product was formed in the abscission layer and a black reaction product in the adjacent fruit and receptacle tissues.

Peroxidase activity. Peroxidase activity in the fruit (mesocarp), abscission zone, receptacle, and pedicel tissues determined at various stages of fruit development is recorded in Table 1. Enzyme activity was highest in the receptacle, intermediate in the abscission zone and pedicel and lowest in the fruit at all times.

The activity of peroxidase in the abscission zone increased with fruit development until early Stage III of fruit growth (Table 2). In the latter weeks of maturation the activity declined. The initial decline in FRF which signals the start of separation was observed June 26, coinciding with the peak of peroxidase activity. However, the marked drop in FRF which followed was accompanied by a decline in peroxidase activity.

Although ethephon treatment resulted in a 78% reduction in FRF after 80 hr compared to a 59% reduction for the control, it had no significant effect on peroxidase activity (Table 3). Cycloheximide caused a significant reduction in enzyme activity after 10 hr compared with the control, and this decreased enzyme activity was

TABLE 1.--Peroxidase activity in the abscission zone and associated tissues as related to stage of fruit development.

	Activity per mg protein[z]		
Tissue	June 7	June 21	July 5
Fruit (mesocarp)	4.6 c[y]	6.2 d	3.5 c
Abscission zone	22.1 b	27.2 b	25.8 b
Receptacle	31.0 a	33.4 a	34.9 a
Pedicel	19.6 b	18.0 c	24.6 b

[z]Units of activity, based on ΔA_{460} using H_2O_2 as substrate and o-dianisidine as H^+ donor, per mg protein determined by Lowry's assay.

[y]Mean separation (in columns) by Tukey's ω test, P = 0.01.

TABLE 2.--Peroxidase activity in the abscission zone as related to fruit removal force (FRF) and stage of fruit development.

Date of measurement	Measurement		
	Activity[z]	FRF (g)	Fruit development
May 30	18.4 c[y]	1283	Late I
June 5	23.2 bc	1496	Early II
June 12	25.0 b	1571	Mid II
June 19	27.1 b	1599	Late II
June 26	33.8 a	1480	Early III
July 3	24.4 bc	876	Mid III
July 10	22.0 bc	326	Late III

[z]Units of activity, based on ΔA_{460} using H_2O_2 as substrate and o-dianisidine as H^+ donor, per mg protein determined by Lowry's assay.

[y]Mean separation by Tukey's ω test, P = 0.05.

TABLE 3.--Effect of cycloheximide (CH) and ethephon on peroxidase activity and fruit removal force (FRF) in the abscission zone of fruit explants[z].

Treatment	Activity per mg protein[y] after 0	10	20	40 hr	Reduction in FRF after 80 hr (%)
Control	24.4	27.1 a[x]	28.9 a	27.6 a	59
CH (10^{-4} M)		19.0 b	18.9 b	16.9 b	13
Ethephon (10^{-3} M)		24.3 a	26.7 a	25.3 a	78

[z]Explants held in the dark at 23° C.

[y]Units of activity, based on ΔA_{460} using H_2O_2 as substrate and o-dianisidine at H^+ donor, per mg protein determined by Lowry's assay.

[x]Mean separation (in columns) by Tukey's ω test, P = 0.05.

associated with a significant inhibition of abscission as indexed by the FRF after 80 hr.

Neither treatment of explants with ethylene nor lowering the endogenous ethylene level with reduced pressure affected peroxidase activity (Table 4). However, ethylene caused a 75% reduction in FRF after 72 hr, while lowering the endogenous ethylene to one-fifth resulted in only an 8% reduction in FRF.

Peroxidase isoenzymes. There was a change in the isoenzyme pattern of peroxidase extracted from the abscission zone with fruit development (Figure 2). Four acidic isoenzymes were observed (A-D), A and B representing the major and C and D the minor isoenzyme bands. The two minor bands remained relatively constant during development, whereas isoenzymes A and B increased until mid-Stage II (Figure 2, gel 3) and thereafter decreased slightly or remained constant. Band A did not appear to be present during Stage I (Figure 2, gel 1) and decreased slightly after mid-Stage II. Only 1 basic isoenzyme (E) was observed and it moved only a short distance from the origin. The intensity of band E changed with fruit development, following a pattern similar to the acidic isoenzyme A.

Ethephon and ethylene had little or no effect on the acidic isoenzyme pattern (Figures 3 and 4), however, ethylene appeared to increase the basic isoenzyme (Figure 4). Cycloheximide caused a decrease in isoenzyme A (Figure 3), while lowering the level of endogenous ethylene by reduced pressure had no effect on the acidic isoenzymes (Figure 4).

IAA oxidase activity. The acidic isoenzymes of peroxidase

TABLE 4.--Effect of ethylene and reduced pressure on peroxidase activity and fruit removal force (FRF) in the abscission zone of fruit explants[z].

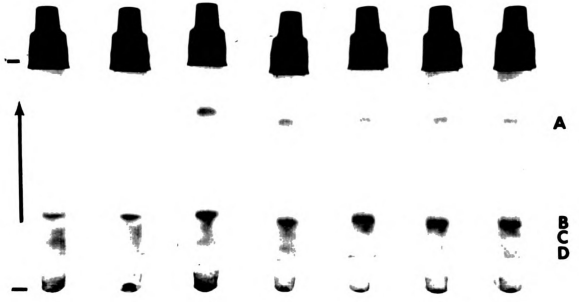
Treatment	Activity per mg protein[y] after 0	10	20	40 hr	Reduction in FRF after 80 hr (%)
Air, at 760 mm	33.8	29.5 a[x]	32.2 a	31.1 a	14
10 µl/l C_2H_2 in air at 760 mm		29.5 a	34.0 a	30.6 a	75
O_2, at 150 mm		30.2 a	32.9 a	33.0 a	8

[z]Explants held in the dark at 23° C.

[y]Units of activity, based on ΔA_{460} using H_2O_2 as substrate and o-dianisidine as H^+ donor, per mg protein determined by Lowry's assay.

[x]Mean separation (in columns) by Tukey's ω test, P = 0.05.

Figure 2.--Zymograms of acidic (upper) and basic (lower) peroxidase isoenzymes extracted from the pedicel: fruit abscission zone at various stages of fruit development. 1, late Stage I; 2, early Stage II; 3, mid-Stage II; 4, late Stage II; 5, early Stage III; 6, mid-Stage III; 7, late Stage III. Arrows denote direction of run and letters denote isoenzymes bands.


A
B
C
D

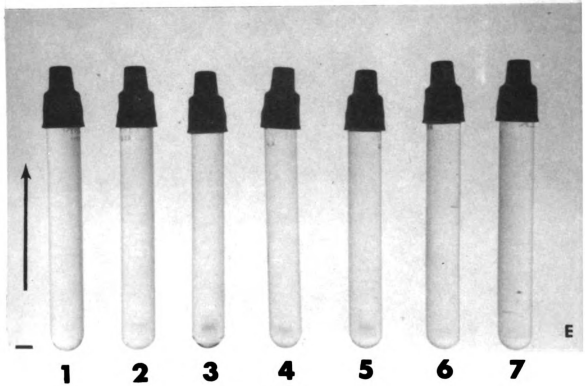

E
1
2
3
4
5
6
7

Figure 3.--Zymograms of acidic peroxidase isoenzymes extracted from the pedicel:fruit abscission zone of fruit explants treated with 10^{-4} M cycloheximide (1, 4, 7), distilled water (2, 5, 8), or 10^{-3} M ethephon (3, 6, 9). Explants were assayed for peroxidase isoenzymes after 10 (1, 2, 3), 20 (4, 5, 6), and 40 (7, 8, 9) hr.

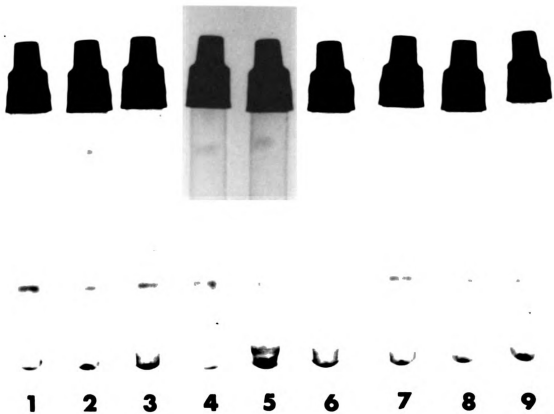

1
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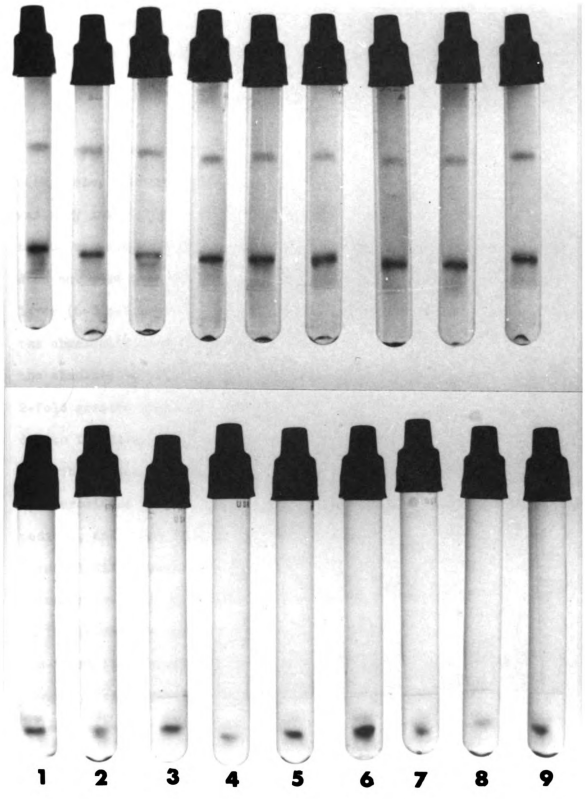
Figure 4.--Zymograms of acidic (upper) and basic (lower) peroxidase isoenzymes extracted from the pedicel: fruit abscission zone of fruit explants subjected to oxygen at 0.2 atm (1, 4, 7), air at 1 atm (2, 5, 8), or 10 μl/l ethylene at 1 atm (3, 6, 9) pressure. Explants were assayed for peroxidase activity after 10 (1, 2, 3), 20 (4, 5, 6), and 40 (7, 8, 9) hr.


1
2
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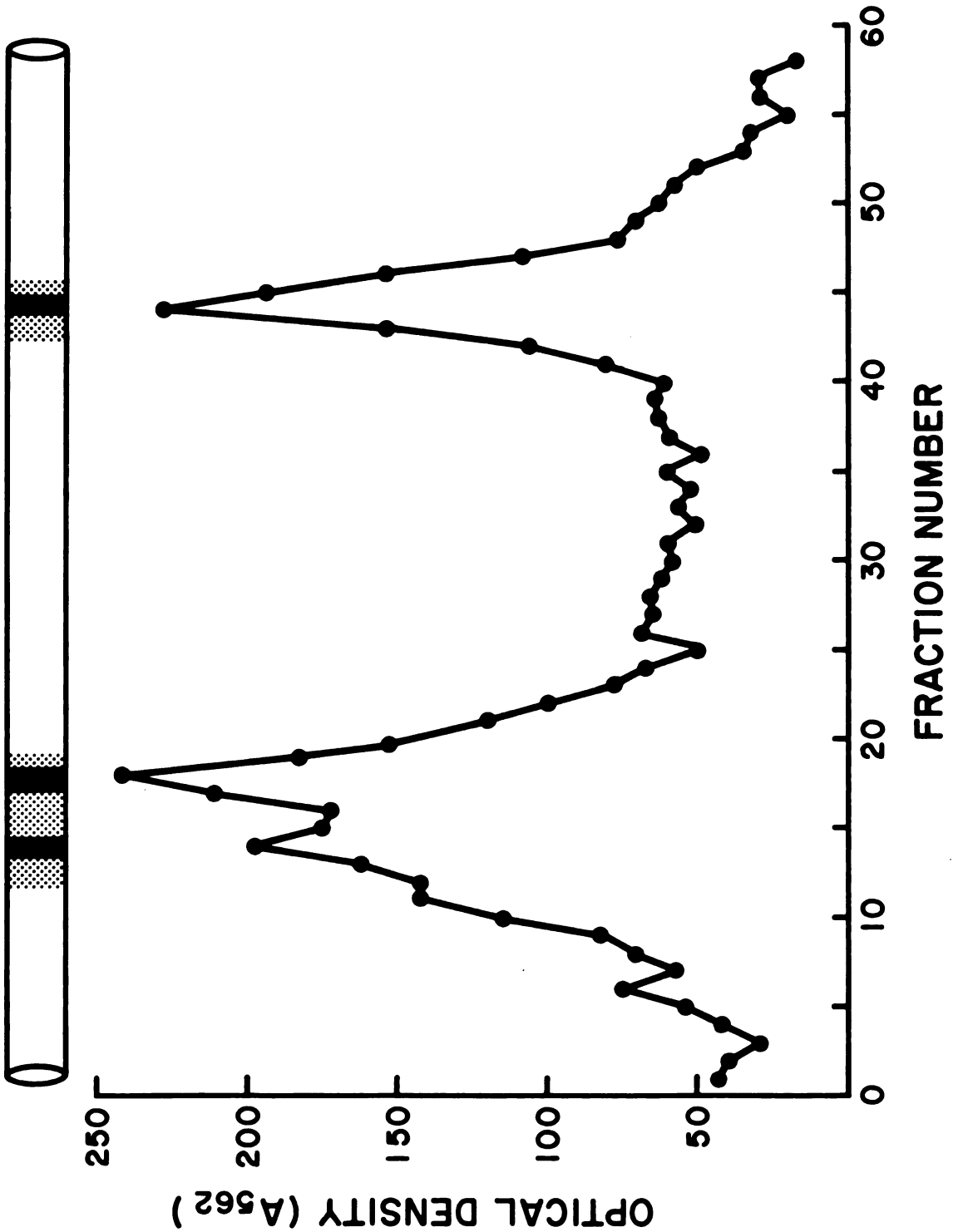
from the abscission zone degraded IAA (Figure 5). There was a close correlation between the intensity of the isoenzyme bands and IAA oxidase activity. The basic isoenzyme could not be analyzed because of an interaction between the reagent and the gel.

DISCUSSION

Peroxidase activity was highest in the abscission zone and receptacle, whereas activity in the fruit (mesocarp), by comparison, was very low (Table 1). Although the activity in the receptacle tissue was slightly higher than that of the abscission zone, this does not take into account the fact that the actual separation layer (6-8 cells wide) comprised only a portion (approx 1/3) of the abscission zone as assayed. Therefore, it is possible that the absolute activity in the abscission layer *per se* was over 2-fold greater than that observed for the excised zone tissue, due to the dilution of activity by the mesocarp tissue (Table 1).

The reason for the difference in reaction products formed by the peroxidase in the layer tissue from that in the receptacle, pedicel, and fruit (mesocarp) tissue is not known. These histochemical differences in activity were observed from late Stage I to maturity. One additional acidic isoenzyme band was observed in the peroxidase extracted from the pedicel tissue, which ran one-third the distance between bands B and A (unpublished data). However, this isoenzyme was not observed in the fruit and was present only as a minor band in the receptacle tissue, which may have resulted from incomplete separation of the pedicel tissue from that of the receptacle. Thus, these histochemical differences

Figure 5.--Degradation of IAA by isoenzymes of peroxidase extracted from the pedicel:fruit abscission zone of sour cherry fruit. The sketch (top) illustrates the isoenzyme pattern in the electrophoretic gel (direction of run - left to right). The graph (bottom) presents optical density of solutions of IAA after 24 hr incubation with segments of gel, followed by addition of p-N,N-dimethylaminocinnamaldehyde. High OD indicates high IAA oxidase activity.


OPTICAL DENSITY (A_{562})
250
200
150
100
50
0
10
20
30
40
50
60
FRACTION NUMBER

are probably not due to differences in isoenzymes. Nevertheless, they may signify a higher capacity for oxidation in the receptacle tissue (18) and/or the result of differences in the location of the enzyme within the cells of the respective tissues (13).

A significant increase in peroxidase activity occurred in the abscission zone with development of the fruit (Table 2), and the maximal activity corresponded with the initiation of the separation phase (Table 2). Furthermore, the enzyme activity did not appear to be associated with lignification, as only a small amount of lignification of vascular bundle cells occurs in the abscission zone[2]. The increase in activity was most likely related to an increase in 2 of the major isoenzymes (B and E) and the appearance of a third (A) major isoenzyme (Figure 2).

Cycloheximide resulted in a significant reduction of peroxidase activity (Table 3), which was reflected by a decline in intensity of isoenzyme A (Figure 3). Moreover, this reduction in peroxidase activity was associated with cycloheximide-induced inhibition of abscission. Ethylene, however, had no effect on total peroxidase activity or on the acidic isoenzyme pattern, even though it markedly enhanced abscission. However, ethylene appeared to enhance the activity of the basic isoenzyme. Lowering the endogenous ethylene level had no effect on peroxidase activity or on the acidic isoenzyme pattern, but but appeared to slightly reduce the basic peroxidase isoenzyme. These results suggest that ethylene does not enhance cherry fruit abscission through an effect on total peroxidase activity, although it may exert some influence on the activity of the basic peroxidase isoenzyme.

Furthermore, it is not clear whether cycloheximide delays abscission through its influence on peroxidase or whether the effect on peroxidase was simply due to a general inhibition of protein synthesis.

The peroxidase isoenzymes from the abscission zone had the capacity to degrade IAA, and therefore, may play a role in abscission through the control of IAA levels. Since activity was localized in the layer early in fruit development, peroxidase may have a general role in the preparation of these cells for separation. Possibly it maintains IAA at a low level and thereby prepares these cells for early senescence and separation. The observed increase in activity to a maximum at the time of induction of the separation phase certainly indicates the potential for a decline in IAA in the abscission zone with the onset of separation.

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