CHEMICAL AND PHYSIOLOGICAL CHANGES ASSOCIATED WITH ABSCISSION LAYER FORMATION IN THE BEAN (PHASEOLUS VULGARIS L. CV. CONTENDER)

HARRY PAUL RASMUSSEN

THESIS FOR THE DEGREE OF PHD.
MICHIGAN STATE UNIVERSITY

1965

This is to certify that the

thesis entitled

CHEMICAL AND PHYSIOLOGICAL CHANGES ASSOCIATED WITH ABSCISSION LAYER FORMATION IN THE BEAN (PHASEOLUS VULGARIS L. CV. CONTENDER)

presented by

HARRY PAUL RASMUSSEN

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Horticulture

M. J. Bukovac

M. J. Bukovac
Major professor

Date May 11, 1965

[redacted]

[redacted] K

[redacted] 330

[redacted] K 179

[redacted] K 192

[redacted]

[redacted] K 217

[redacted] E

[redacted] 232

[redacted] K 248

[redacted] 273

[redacted] R 308

[redacted]

ABSTRACT

CHEMICAL AND PHYSIOLOGICAL CHANGES ASSOCIATED WITH ABSCISSION LAYER FORMATION IN THE BEAN (PHASEOLUS VULGARIS L. CV. CONTENDER)

By Harry Paul Rasmussen

The chemical and physiological changes associated with abscission were followed in debladed bean seedlings (Phaseolus vulgaris L. cv. Contender) using histochemical and microchemical procedures. An induction period of 14 hours was found, during which calcium increased in the abscission zone, dry weight increased and pH decreased in both the abscission zone and petiole. Upon completion of induction, abscission layer formation began. Before visual appearance (microscopically) of the abscission layer, calcium decreased in the abscission zone, increased in the petiole, and dry matter decreased in both the abscission zone and petiole. Layer formation did not involve cell division. Swelling of the pectic materials of the cell walls of the potential abscission layer was the first visual change observable in abscission layer formation.

Pectin methylesterase (PME) and polygalacturonase (PG) activity were found in the abscission zone, petiole and stem.

PME activity in the petiole increased rapidly with time after deblading; by contrast, PG decreased rapidly in the petiole during abscission layer formation. The interaction of PME and PG in the petiole established a potential for movement of calcium from the abscission zone to the petiole.

Twenty-four hours after the beginning of pectin swelling the cell wall materials, non-cellulosic polysaccharide and cellulose, began to break down. The cellulose of walls of cells immediately adjacent and distal tothe abscission layer was found to be altered. No lignin was present in the petiole, stem or abscission zone during abscission layer formation.

The alteration of pectic materials coupled with breakdown of cell wall components resulted in the collapse of cells of the abscission layer just prior to separation.

Respiration of the abscission zone tissue increased four days after deblading on a dry weight basis, but not on a section basis. Petiolar respiration steadily decreased over development of the abscission layer both on a section and dry weight basis. Auxin (3-chlorophenoxy-α-propionic acid) increased respiration in both the petiole and the abscission zone at the same time indicating an overall effect on both tissues, not directly on the abscission zone. Auxin also delayed abscission and, correspondingly, delayed the initial increase in calcium, movement of calcium to the petiole, increase in pH and decrease in dry weight.

N-6-benzyl adenine delayed abscission at high

concentrations and induced abscission at low concentrations with the opposite leaf intact, similar to the effect of auxin on abscission. With the opposite leaf removed N-6-benzyl adenine was not effective at any concentration tested.

Microautoradiograms of the abscission zone after application of ^{14}C-naphthalene acetic acid (ring labeled) at both stimulatory (10^{-5} M.) and inhibitory (10^{-3} M.) concentrations indicated that auxin accumulated in the cells immediately adjacent and distal to the abscission layer with no localization in the abscission layer or stem as the abscission layer formed. Thus, the site of action was not directly on the abscission layer.

CHEMICAL AND PHYSIOLOGICAL CHANGES ASSOCIATED WITH ABSCISSION LAYER FORMATION IN THE BEAN (*PHASEOLUS VULGARIS* L. CV. CONTENDER)

By

HARRY PAUL RASMUSSEN

A THESIS

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

DOCTOR OF PHILOSOPHY

Department of Horticulture

1965

ACKNOWLEDGMENTS

To my guidance committee of Drs. D. R. Dilley, H. M. Sell, S. H. Wittwer and R. S. Bandurski, the author wishes to express his sincere appreciation and thanks for their encouragement and suggestions. A special thanks is given to Dr. M. J. Bukovac, chairman of the committee for invaluable counsel and stimulating guidance during the course of my graduate program. I would also like to acknowledge the assistance of Dr. P. Markakis and Dr. A. Kivilaan. My gratitude is especially extended to my wife Mary Jane for her patience, encouragement and support and for her assistance in preparation of this dissertation.

TABLE OF CONTENTS

TABLE OF FIGURES

TABLE OF TABLES

INTRODUCTION

The process of abscission for many years was merely a biological phenomenon which at most was important purely from an academic point of view. The shedding of leaves, branches, flower buds, flowers and fruits has in the past fifty years become of significant economic importance.

The values of being able to control the abscission processes are extremely numerable: (a) fruit thinning by application of growth regulators; (b) sprays of growth regulators at maturity to prevent pre-harvest fruit abscission of apples; (c) delay of petal fall in ornamentals; (d) prevention of ovary abscission prior to and after fertilization; (e) defoliation of economic plants for mechanical harvesting; (f) defoliation of tropical plants for elimination of insect breeding habitats; (g) maintenance of edible portions of harvested vegetables, such as broccoli; (h) dehiscence of fruit and anthers.

The natural formation of the abscission layer can also be hastened by numerous, generally adverse, conditions, i.e. temperature extremes, light intensity and duration, volatile gases, available moisture, fertilization, nutrient status and mechanical injury just to mention a few.

Addicott and Lynch in their review on abscission (5) stated that many of the early investigations of abscission

were primarily of a descriptive nature until recently when a number of physiological studies were undertaken. Unfortunately the majority of most recent studies, although physiological in nature, are still entirely of the descriptive nature. Very little emphasis has been placed on the actual physiological and chemical changes which take place in the abscission zone and its adjacent petiole as the process is initiated and carried through to its termination, that of actual separation.

Since auxin has been shown to be both stimulatory and inhibitory to the formation of the abscission zone it provides a unique system for identifying mechanisms which are operative in controlling the process of abscission.

Perhaps the most exhaustive, most unheralded study into the possible mechanisms and biochemical basis for abscission was reported by Sampson (108). His histochemical approach to abscission is probably the only means by which such a minute and specialized tissue can be adequately studied.

The existence of the many attempts in the literature to explain both the biochemical and physiological mechanisms of both natural abscission and induced abscission (removal of the leaf blade) by descriptive effects of different chemical compounds in either delaying or enhancing the time to abscission led to this study as a means of: (a) identifying changes associated with the abscission process, not only at the time of abscission <u>per se</u>, but the changes which must

occur from the time of deblading until the actual separation; (b) determining the effects of auxin on these processes; and (c) linking together the chain of events beginning with deblading which ultimately leads to abscission.

LITERATURE REVIEW

The study of abscission has intrigued botanists for centuries. There are records of interest in the phenomenon of leaf fall as far back as Theophrastus in 285 B. C. (cited in [27]).

Abscission from a botanical viewpoint may be defined as the separation of an auxillary organ of a plant from its main axis by either a physiological or mechanical action.

In the early 1800's abscission was known to involve two separate and distinct processes (108). The first was separation and the second protection. Although separation and protection are distinct processes, Addicott (2) suggested that both may be induced or controlled by the same mechanism (auxin gradient). The separation process involves cell wall and middle lamella dissolution in one or more layers of cells and in general appears to involve cytolysis to some degree (2). Many early investigators felt that in order for an abscission layer to be formed there must be cell division prior to lyocytosis or cytolysis (5, 22, 72, 74). However since the work of Gawardi and Avery (39) it has been generally agreed that cell division is not a prerequisite to separation, but is likely the early formation of the protective layer (2, 3, 5, 50, 72). Not only does the protective mechanism involve cell division but

also lignosuberization (72). In some species after the primary protective layer has been formed there is the formation of several layers of cells of a secondary protective tissue. Such periderm extensions occur most commonly in woody species (2). In tobacco, cell division occurs readily at the base of the petiole but there is no abscission; however, when the lower leaves are harvested it functions as the protective layer (39).

The process of abscission varies among different species (39). Gawardi and Avery (39) divided plants into three abscission types, namely: Type I - cell division occurred at the base of the petiole, thus forming the "traditional" abscission layer prior to separation, as with poinsetta (Euphorbia pulcherrinia) Willd., cotton (Gossipium herbaceum L.) and pepper (Capsicum frutescens L.), Type II - separation occurred without the formation of an abscission layer, such as in Impatiens (Impatiens Sultani Hook), and Type III - cell division occurred at the base of the petiole but no separation resulted, for example tobacco (Nicotiana tobacum L.).

Morphology and Anatomy

The morphology and anatomy of abscission vary considerably between species.

The abscission layer in cotton bolls occurs as a simple single plate of cells, one cell thick, stretching across the stalk in a definite position marked by a faint

external groove. Each cell of the plate divides once; the two daughter cells separate along the middle lamella and the vascular tissue breaks transversely (50).

Other morphological changes which occur in cotton have been described by Leinweber and Hall (74). They found that morphologically the abscission layer of cotton was not differentiated until very late in leaf maturation. Anatomically the first evidence for abscission layer formation was a thinning of the cell walls in the abscission zone area and poor response to staining. Concomitant with cell wall hydrolysis was a blocking of the tracheary elements by tyloses on the distal side of the abscission layer. The formation of the leaf scar was initiated by the appearance of a periderm layer four or five cells in, toward the stem, from the separation layer. Cork tissue appeared before separation, but following cell division.

Brown and Addicott (22) studied the morphology of abscission in the bean (Phaseolus vulgaris L. cv. Black Valentine) in both debladed petioles on the plant and explants of leaflets. There appeared to be division in one or two cell layers across the abscission zone through which cytolysis and lyocytosis of cells and middle lamella resulted in separation of the petiole from the stem. Following separation a layer of periderm tissue was laid down.

An excellent article on the anatomy of Phaseolus vulgaris was authored by Doutt (35). In describing the

anatomy of the pulvinus she writes: "In a cross section of the pulvinus it is evident that the vascular cylinder consists of a united ring of tissues compressed into a small space in the center with only a very small pith. The cortex is very thick, and consists of eight or more rows of parenchyma cells. When the leaf trace first enters the pulvinus the vascular elements have an amphicribal arrangement, but shortly after some anastomosing, they assume a collateral position with the phloem toward the outside. The endodermis surrounds the united circle of conducting tissue, and delimits it from the cortex."

The anatomical and morphological changes occurring in woody plants appear to be markedly distinct from that of herbaceous plants. Hoshaw and Guard (58) have described the formation of the abscission layer in Quercus polustris and Q. coccinia as having no well defined separation zone at the base of the petioles. All cells of the abscission layer appeared histologically similar to adjoining tissue. Quite unlike herbaceous plants, at the time of high abscission zone activity, the processes leading to separation were found to function within a green area of living tissue at the petiolar base of a marcescent leaf with the remainder of the petiole and leaf tissue dead. A layer of lignified tissue developed at the base of the petiole and separation occurred about two cells below the lignified tissue and involved the next four to five rows of cells. Actual parting involved some separation along the plane of the

middle lamella, while other cells were passively broken through the cell walls at right angles to the middle lamella. During no part of the separation process was cell division evident.

Facey (36) in her work on Fraxinus americana found the separation layer was one or two cells thick. These cells showed considerable softening and were delineated by lignified tissue on both sides. Separation was dependent upon the number of rows of cells involved. When a single row of cells was present, the end walls became lignified and separation occurred by fracture. If two layers of cells were involved the end walls remained soft and separation occurred by intact cell separation or lyocytosis.

An extremely detailed study of different species and their corresponging type of abscission was given by Lee (72).

In Coleus (Coleus blumei), a widely used test plant, the formation of the abscission layer generally occurs in the third pair of leaves from the apex. Cell division begins in two to four layers of cells and proceeds from the adaxial to the abaxial side of the petiole. The formation of the abscission layer appears complete in the first pair of leaves as the sixth pair of leaves emerge. The final thickness of the abscission layer is eight to twelve cells. At the time of separation there is alteration of the cell walls and separation occurs on the distal side of the newly formed layer (108).

Although the morphological and anatomical development of abscission layers appears quite variable, there are several similarities in all cases. The abscission layer is usually found at the base of the auxillary tissue and often appears constricted with a color variation from that of surrounding tissue. The cells of the abscission zone are small, generally round, closely packed with essentially no intercellular spaces. The conductive tissue changes in orientation with respect to that of the stem. During separation formation of tyloses and changes in cell walls appear. Separation generally involves either lyocytosis, cytolysis or both.

Addicott (2) summarized the morphological and anatomical appearance of the abscission zone as a region of cells of arrested development, where processes of cell enlargement and differentiation did not proceed nearly as far as in contiguous regions. He likened the onset of abscission to a resumption of morphological development.

Effect of Other Organs or Tissues on Abscission

The most pronounced effect of other tissues on formation of the abscission layer is that of the leaf blade. Using Valencia orange, Livingston (77) found that the rate of abscission was proportional to the area of leaf blade remaining. Removal of 50 percent of the lamella slightly accelerated abscission and 90 percent removal shortened considerably the time to abscission.

Although the morphological and anatomical development of abscission layers appears quite variable, there are several similarities in all cases. The abscission layer is usually found at the base of the auxillary tissue and often appears constricted with a color variation from that of surrounding tissue. The cells of the abscission zone are small, generally round, closely packed with essentially no intercellular spaces. The conductive tissue changes in orientation with respect to that of the stem. During separation formation of tyloses and changes in cell walls appear. Separation generally involves either lyocytosis, cytolysis or both.

Addicott (2) summarized the morphological and anatomical appearance of the abscission zone as a region of cells of arrested development, where processes of cell enlargement and differentiation did not proceed nearly as far as in contiguous regions. He likened the onset of abscission to a resumption of morphological development.

Effect of Other Organs or Tissues on Abscission

The most pronounced effect of other tissues on formation of the abscission layer is that of the leaf blade. Using Valencia orange, Livingston (77) found that the rate of abscission was proportional to the area of leaf blade remaining. Removal of 50 percent of the lamella slightly accelerated abscission and 90 percent removal shortened considerably the time to abscission.

Swets and Addicott (115) found no effect of leaf blade removal in bean until 90 percent of the blade had been removed. Severing the main vein at the base of the leaf blade retarded the response to defoliants, whereas removal of the mesophyll had no effect.

Using Coleus leaves Myers (87) found that the midvein alone had slight abscission retarding properties. A small amount of lamellar tissue at the apical end of the midvein was less effective than the same amount of tissue at the base of the midvein.

The petiole may also influence the rate of abscission. Long petioles delayed abscission to a greater degree than short petioles (2, 10, 41). The leaf blade contributes the principal controlling effect with some control also being exerted by the petiole.

Rossetter and Jacobs (101) were able to demonstrate that intact nearby leaves accelerated abscission of debladed petioles, and that immature leaves had a stronger stimulating effect than mature leaves. Intact unfolded leaves also stimulated abscission (60).

The presence of petioles of opposite leaves (41), apical buds (60) and removal of roots from Coleus had an accelerating effect on leaf abscission (116).

Fruit and flowers are likewise affected by auxillary organs especially leaves. Yager (126, 127) has shown that removal of upper leaves of tobacco resulted in acceleration of abscission whereas lower leaf removal had no apparent

effect.

Competition for Nutrients

Addicott (8), Chandler (29) and Heinicke (56, 57) have observed that abscission ("June drop") of apples is dependent on the nutrient supply to each young fruit. If a large number of young fruit had been set, "June drop" was heavy whereas if only a small number of fruit had set then drop was light. "June drop" appears to be correlated with leaf size and number again suggesting competition for nutrients.

Environmental Effects on Abscission

Temperature

The rate of formation of the abscission layer in plants, being a physiological phenomenon, is extremely sensitive to temperature. Yamaguchi (review by Addicott) (2) found that the formation of the abscission layer in the leaflet pulvinus of bean exhibited a typical temperature response curve with a maximum between 25 and 30°C. The exposure of plants to extremes in temperature may have an accelerating effect on abscission (26). When extremes of temperature are involved, however, the acceleration is not due directly to temperature but to other factors such as injury or high respiration (5). High temperatures may also act indirectly through dessication (2). Low temperatures have a more direct effect in that it is necessary to have injury to either the leaf, petiole, or both but not death

of the tissue to cause abscission. A killing frost causes the leaves to remain attached (79).

Hall (50) has shown that low temperatures (above freezing) delay abscission by arresting the physiological mechanisms involved.

Light

In 1936 Matzke (83) reported that street lights in New York caused retention of leaves of certain tree species. He concluded that low light intensity (as low as one foot candle) was effective in delaying abscission.

Myers (87) reported one of the few cases where plants placed in the dark showed stimulation of abscission. Young petioles of bean explants abscised faster in the dark than in the light but no difference occurred in older petioles. Healthy explants from any age material had approximately the same abscission rate in the dark (18, 20). Shading by dense foliage tends to accelerate abscission of older leaves on plants (2).

Hall and Liverman (51) found that cotton petiole abscission was accelerated in proportion to increased light intensity up to 2,500 foot candles. Light intensity from 6,000 to 8,000 foot candles delayed abscission significantly. Light quality was also important in abscission. Acceleration was obtained with red, far-red, fluorescent and ultraviolet light.

Light also has an effect through photoperiod. Olmsted (90) observed that constant photoperiods, regardless

of length resulted in delayed senescence and abscission. Change from a long to a short photoperiod (i.e. 20 hours to nine hours) resulted in senescence and abscission at a faster rate than occurred at either photoperiod held constant.

The delay of abscission caused by light was investigated by Biggs and Leopold (20). They found that plants in a CO_2 free atmosphere under 300 foot candles of light abscised at the same time as those kept in the dark with or without CO_2, while those kept in ambient air were delayed about 40 hours. Thus, they concluded that the effect of light was due to photosynthesis. This was confirmed by adding sucrose to all treatments which eliminated the effect of light.

Water

Water appears to play an indirect role in abscission. The rate of development of water deficiency in plants seems to be more important than the actual amount of water available to the plant. If water is continually available through the soil, abscission is minimal, whereas, a rapid water deficiency accelerates abscission (2). Hall (50), however, has shown that if drouth develops slowly over the growing period, in the field, abscission is retarded and defoliation becomes very difficult.

Brown and Addicott (22) and Addicott and Lynch (5) concluded that water influenced or controlled abscission by maintenance of turgor in the cells of the pulvinus. If water deficiency occurred rapidly, cell turgor was lost

and abscission was accelerated. On the other hand, if water deficiency occurred slowly the cells had time to compensate and abscission was not affected.

Carns et. al. (28) found that applying a droplet of distilled water daily to the cut surface of a debladed petiole delayed abscission over that of the control and submersion of the explant completely inhibited abscission (the effect of complete submersion will be discussed later).

Water in the form of rain affects abscission in at least two ways: (a) rain in early afternoon on cotton bolls accelerates abscission by destroying pollen and thus interrupting fertilization (5, 78, 82); (b) rain occurring in late afternoon or evening augments soil moisture and may delay abscission (78). A further function of rain may be in defoliant absorption to induce abscission (50).

High humidity has been shown by Mason (82) to accelerate abscission. It also facilitates activation of certain defoliants (50).

Oxygen and Carbon Dioxide

Reduced oxygen tensions retard abscission (5, 26, 107). Carns (26) has shown that bean explants did not abscise in any medium free of oxygen, however, by bubbling air through water containing completely submerged explants normal abscission occurred. He concluded that oxygen became limiting below 35 percent supplied to the surrounding medium. Water logging of roots has also stimulated abscission which also indicates oxygen deficiency to some extent, although this

may also include an indirect effect upon mineral uptake.

Carbon dioxide in high concentrations will stimulate floral abscission in Nicotiana (2), whereas in combination with oxygen, carbon dioxide concentrations above five percent will delay abscission in bean explants. The apparent contradiction here lies in the tissues involved, stimulation was found in non-photosynthesizing tissue and retardation in tissue which carried on photosynthesis.

Minerals

As long as a plant is supplied with ample mineral elements abscission will be minimal. Deficiency of minerals especially zinc, calcium, boron, magnesium, nitrogen, potassium and sulfur is known to facilitate abscission (2, 5, 23, 50).

Cell Walls in Abscission

Constitutional Materials

Mangin (as reported by Sampson) (108) in 1890 studying membrane composition found that the middle lamella was composed of pectins and did not contain celluloses. Later Lloyd (by Sampson) (108) reported that the middle lamella was rich in calcium thus providing cementing properties. Bonner (21) suggested that differences in appearance of the middle lamella of different plants was due to differences in calcium content. The participation of metal cations in the intercellular cement has been confirmed by Ginzburg (40) by the use of heavy metal chelating agents. He also demonstrated the presence of protein in the intercellular cement

by using proteolytic enzymes and demonstrating that after enzymatic action the pectic materials could no longer be solidified by addition of divalent cations.

Present evidence indicates that the middle lamella is made up of water insoluble pectic substances (69), protein and metal bridges.

Cellulose strands formed by end synthesis are imbedded in a pectic matrix which is bound to cellulose and hemicellulose by calcium and magnesium bridges (98).

Exocellular Changes During Abscission

Many studies have indicated that changes in the middle lamella led to separation of cells during abscission and senescence (2, 3, 5, 18, 22, 36, 39, 58, 72, 108, 113, 118). Most investigators are in general agreement on this. The exception comes in including dissolution of the cell wall material.

Addicott (8) diagrams the three types of lyses involved in abscission of different species of plants as: (a) middle lamella only; (b) middle lamella and primary cell wall; and (c) entire cell.

Sampson (108) studying abscission of coleus histochemically concluded that the changes preceding abscission involved conversion of cellulose to pectose which was then converted to pectin and finally to water soluble pectic acid. He maintained that this conversion provided too much pectin to be solidified by the existing amount of calcium thus the middle lamella was weakened and abscission

resulted.

Facey (36) refuted part of Sampson's work in that she found no conversion of cellulose but merely a hydration which might be interpreted as a loss of cellulose. She felt that there were only two successive cell wall changes: (a) conversion of calcium pectate to pectic acid; and (b) methylation of pectic acid.

All studies on cell wall changes associated with abscission are based on two criteria: (a) solubility of pectic substances; and (b) staining of pectic substances with ruthenium red (18). Kertesz (69) pointed out that ruthenium red is not entirely specific for pectins, and it is not known which portion of the pectin molecule is stained. Further any foreign substance such as protein would alter the solubility of pectin considerably.

Preabscission Changes in Leaf and Petiole

Changes in the biochemistry of senescing tissue have recently been reviewed by Varner (118). However, some of the changes common to most plants will be reviewed briefly.

The most obvious change in leaves occurs in pigments. The loss of chlorophyll being most evident. In some species anthocyanins are increased usually early in the process of abscission (2), only to be lost at the time of separation.

Mason (82) concluded that the proportion of shedding over any given period in cotton fruit was the result of two opposing factors, the rate at which food was synthesized and the rate at which it was utilized in the maturation of

the fruit, and that any check in the former augmented the rate of shedding. Thus, indicating that it must be food material from the leaf or petiole which controls the abscission process. Even so, once the process is initiated certain elements are translocated from the leaf to the stem.

Dry Matter and Nutrients

Oland (89) found the following decreases in presenescent apple leaf tissue during the abscission process: dry matter 16 percent, nitrogen 52 percent, phosphorous 27 percent, and potassium 37 percent. Calcium on the other hand was increased by 18 percent. Similar data were obtained by Chandler (29).

Hall and Lane (53) using defoliants to induce abscission found a rapid hydrolysis of the reserve constituents of the leaf blade. The products were then translocated out of the leaf. Hall et al. (52) also found that the relative concentrations of amino acids in the pulvinoid changed.

Facey (36) concluded that one of the major factors of preabscission changes was the accumulation of organic acids in the abscission layer. The increase in organic acids was sufficient to bring about the change of calcium pectate to pectic acid, but was not sufficient by itself to cause abscission.

Sampson's (108) data indicated that in Coleus nitrates build up in abscission zones as do free reducing sugars and iron. He also found oxidases uniformly distributed in the abscission layer and not in adjacent tissue. Calcium moved

the fruit, and that any check in the former augmented the rate of shedding. Thus, indicating that it must be food material from the leaf or petiole which controls the abscission process. Even so, once the process is initiated certain elements are translocated from the leaf to the stem.

Dry Matter and Nutrients

Oland (89) found the following decreases in presenescent apple leaf tissue during the abscission process: dry matter 16 percent, nitrogen 52 percent, phosphorous 27 percent, and potassium 37 percent. Calcium on the other hand was increased by 18 percent. Similar data were obtained by Chandler (29).

Hall and Lane (53) using defoliants to induce abscission found a rapid hydrolysis of the reserve constituents of the leaf blade. The products were then translocated out of the leaf. Hall et al. (52) also found that the relative concentrations of amino acids in the pulvinoid changed.

Facey (36) concluded that one of the major factors of preabscission changes was the accumulation of organic acids in the abscission layer. The increase in organic acids was sufficient to bring about the change of calcium pectate to pectic acid, but was not sufficient by itself to cause abscission.

Sampson's (108) data indicated that in Coleus nitrates build up in abscission zones as do free reducing sugars and iron. He also found oxidases uniformly distributed in the abscission layer and not in adjacent tissue. Calcium moved

from the abscission layer at the time of abscission.

Respiration

Respiration is known to change either prior to or during the abscission process. Very little work has been done on respiratory changes over the time course of abscission. Carns (26) reported the occurrence of a peak in respiratory activity corresponding to formation of the abscission layer in cotton. With explants in which no abscission layer was formed there was no increase in respiration. These data have been confirmed by several investigators, i.e. Biggs (18) using bean explants and Hall (50) using cotton.

Very few if any reports have appeared in the literature concerning the effect of auxin on respiration of debladed plants. Wedding et. al. (119) demonstrated that 2,4-dichlorophenoxyacetic acid (2,4-D) caused a slight stimulation of respiration when applied to citrus leaves.

Respiration is necessary for abscission to occur as is evidenced by the fact that abscission will not occur if explants are held in any oxygen-free medium. If, however, air is bubbled through the water, abscission occurs normally (26, 28). Carns (18) found that most of the respiratory inhibitors also delayed abscission.

Auxin

The preabscission changes of auxin will be discussed later as will formation of natural abscission inducing substances.

Influence of Auxin on Abscission

The influence of auxin on abscission has been reviewed recently by Addicott (2), Addicott (3), and Addicott and Lynch (5). Consequently, this review will include only the basic effects of auxin on abscission.

Laibach (70) was first to demonstrate that abscission could be delayed by replacing the leaf blade with orchid pollinia (a source of auxin). This was confirmed by LaRue (71) using synthetic indoleacetic acid (IAA). It is now the consensus that the origin of the auxin which controls the abscission process is in the leaf blade and to a lesser degree in the petiole (2, 3, 5, 8, 59, 61, 64, 87, 111, 122, 126, 127).

Many investigators have shown that as the leaf blade senesces the auxin supply drops considerably (2, 3, 5, 111, 126).

Direct evidence that a substance actually moves from the lamella to the abscission zone was provided by Myers (87). By cutting almost completely through a Coleus petiole, leaving only a few cells on the upper side he noted that abscission was delayed. Leaf extracts from young and old leaves have been assayed in the Avena test (121) and it was found that young leaves contained more auxin than older leaves (12, 44, 111).

Sequeira and Steeves (110) have presented indirect evidence by suggesting that the fungus Omphalia flavida, which brings about abscission in coffee, does so by blocking

the flow of auxin from the leaf into the petiole.

Deblading is known to induce abscission, however auxin will completely replace the effect of the leaf blade in Coleus. In general the degree of retardation depends on the experimental material, the growth substance and the site of application (3).

Auxins when applied to either explants or debladed intact plants have two types of influence: (a) delay abscission at high concentrations; or (b) stimulate abscission at low concentrations (2, 3, 4, 5, 12, 13, 15, 19, 23, 38, 60, 61, 63, 64, 71, 87, 104, 116). The acceleration of abscission was first observed following an application of IAA proximal to the abscission layer. The concentration applied seemed unimportant (4). Abscission is auxin concentration dependent only when applied distally.

Leaves and petioles are not the only organs to show an abscission response to auxin. Flowers (2, 3, 5, 13, 60, 61, 126, 127), fruits (2, 3, 5, 13, 23, 50, 60, 61, 81), and buds (5, 49) have also responded to exogenous auxin applications. Most workers have implicated auxin as the primary controlling factor in the plants; however, Van Steveninck (117) feels that two substances are probably involved in the control of abscission in flowers of Lupinus luteus after some of the pods have set.

Transport of Auxin

Mai (as reviewed by Jacobs) (61) reported considerable

changes in the transport properties of ageing Coleus petioles. In young, growing petioles auxin transport was basipetal, in middle aged petioles in both directions and in old petioles there was little if any transport in either direction. These observations have been confirmed by Storey (114).

Not only have differences between tissue ages been shown to affect transport but also the growth regulator used (5). This observation would indicate that some of the differences which were obtained with different experimental materials as well as similar materials could be explained on transport properties from the site of application.

Several investigations have indirectly indicated that transport may play an important role in both accelerated and retarded abscission. Rubenstein and Leopold (104) using carbon labelled naphthaleneacetic acid (NAA), carboxyl labelled, found that abscission responses correlated with the time of application and not with the auxin gradient about the abscission zone, thus indicating transport differences. Differences between proximal and distal applications (3, 4, 5, 19, 50, 104, 114, 116) support the concept that basipetal transport is involved. This has been substantiated by Jacobs (60) in that auxin added above a given debladed petiole had no effect on the rate of abscission of that petiole; however, if applied below, it accelerated abscission. In the latter case there was vascular continuity, but not in the former.

Many times the question has been asked, "Does the abscission layer from a physical barrier to transport or

movement?" No direct proof exists; however, Jacobs (63) believes there is no reason to conclude that the abscission layer is a physical barrier even in older leaves. This area remains to be elucidated.

Does Auxin Act Directly on the Abscission Layer or Indirectly By Maintaining Growth?

Biggs and Leopold (19) concluded that since auxin displays a two-phase action on abscission then the effect must be directly on the abscission zone. The auxin gradient theory of Addicott, Lynch and Carns (7) also implicates a direct effect on the abscission layer.

Other interpretations are that auxin controls abscission indirectly by its effect on growth (63). Shoji and Addicott (112) found essentially no transport of IAA through bean leaf stalks which would indicate indirect control.

Jacobs using Coleus has shown by pattern deblading (60), and petiole elongation measurements with and without auxin (61, 66) that there is a good correlation between petiole elongation and rate of abscission. By deblading, petiole elongation ceased and abscission proceeded; however, if IAA were substituted for the leaf blade, petiole elongation continued and abscission was prevented.

Fertilization and subsequent growth are essential to prevent fruit abscission (13).

Beal (15) demonstrated that stem abscission of Mirabilis jalapa was prevented with IAA by continuation of

growth of the internodes. Similarly, Terpstra (116) concluded that auxin-induced growth proximal to the abscission layer was necessary to render abscission possible. Plants which were low in zinc exhibited stimulated abscission and reduced growth brought about by low auxin (23).

A further substantiation of growth and auxin relationship as given by Avery (12), and Goodwin (48) using Nicotinia and Solidago, respectively, indicated that the auxin content in the leaf, and subsequently the remainder of the plant, corresponded to the rate of growth.

Internal Biochemical and Physiological Effects of Auxin on Abscission

Research of the general effects of auxin on abscission physiology is very extensive; however, the work on its effect on a cellular level is very shallow.

Nelson (88) demonstrated by the use of ^{14}C labelled methionine that there was a build up of methyl groups in the abscission layer causing a solublization of pectin thus facilitating abscission. In senescence and abscission he concluded that the methyl groups came from protein bound methionine in the leaf. Application of IAA with ^{14}C-methionine prevented methylation and delayed abscission.

Rubenstein (102), however, refuted these data finding that the only methyl donor which markedly stimulated abscission was formaldehyde. The effect of methyl donors was not proportional to their methyl donating ability.

Ordin et al. (91) in a continuation of studies on cell wall loosening by auxin (33) found that IAA stimulated incorporation of ^{14}C-methyl from methionine into the pectic material of Avena sections thus loosening the cell walls allowing expansion. The incorporation of methyl groups into pectic materials has been found in a variety of tissues (21, 25, 32, 102, 109, 113, 124).

As Addicott (3) justly points out, however, the suggestion that incorporation of methyl groups facilitates abscission is based on the assumption that abscission requires the conversion of calcium pectate to pectic acid and to water soluble pectin, and on the observation that methyl groups can be incorporated into the pectic substances of the cell wall. Such data should be carefully interpreted.

Two particular studies by Sacher (106, 107) and more recently by Glasziou et al. (46) have shed considerable light on the effect of auxin and membrane permeability.

Sacher demonstrated that in plants treated with auxin, intercellular spaces were filled with air and the membranes maintained their selective permeability. The untreated controls were soft with intercellular spaces filled with water which was accompanied by cellular dissolution. Maintenance of respiratory activity was twice as long in auxin treated tissue as in water treated tissue.

Glasziou (46) using bean endocarp sections treated with auxin found a delay in senescence. This involved maintenance of selective permeability, delay in liquid

logging of air spaces as well as delay in cellular dissolution and exudation. Treatment with auxin caused composition and quantities of sugar to be altered in the tissue, free space and exudate. Tissues without auxin were permeable to sucrose, glucose and fructose after 24 hours. With auxin treatment membrane integrity was maintained for longer periods.

Cleland and Bonner (33) and many others have shown that auxin causes a loosening of cell wall materials which leads to cell enlargement and consequently to growth changes which, as discussed before, may control abscission.

Wilson and Skoog (123) treated tobacco pith with IAA and found cell expansion but no cell division. They found an increase in fresh weight, dry weight, cell wall material, pectic substances, and soluble uronides upon treatment with IAA. The alcohol uronides were found to decrease as a result of incorporation into pectic materials.

Effect of Compounds Other Than IAA on Abscission

Auxin-like Compounds

Naphthaleneacetic acid (NAA) has been used as much or more than IAA in the auxin physiology of abscission.

Milbrath and Hartman (84) in 1940 reported that defoliation could be prevented in holly by spray applications of NAA. The effectiveness of the spray was dependent on concentration, with complete inhibition of abscission at 0.01 percent.

NAA (20ppm) when applied to apple fruit by Luckwill (80) at full bloom caused a reduction in fruit set but did increase the subsequent drop. Post blosson applications of NAA (20 and 50 ppm) reduced the final set of fruit by increasing the early drop of fruitlets. In the former case the reduction was attributed to incompatibility due to excess elongation of the style; the latter was attributed to seed abortion induced by NAA.

Biggs and Leopold (20) and Biggs (18) indicated that NAA reacts similar to IAA in that at high concentrations abscission is delayed and at low concentrations abscission is stimulated. The promotion of abscission was not found in applications to younger leaves however.

The phenoxy acids have also been used extensively in abscission studies. The use of these compounds, however, has been primarily that of chemical evaluation.

Beal (16) studied the reactions of decapitated bean plants to several substituted phenoxy compounds. All compounds tested except 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) caused leaf dwarfing and axillary shoot retardation.

Brown and Addicott (22) using bean explants found that all anatomical changes were delayed after applications of 2,4-D.

Day and Erickson (34) evaluated ring substituted mono-, di-, and trichlorophenoxyacetic acids as to their effectiveness in preventing abscission in lemon leaves. The most active was 4-chlorophenoxyacetic acid. They concluded

that for maximum activity chlorines must be present at the 2 and 4 position and hydrogen at the 6 position. Chatterjee and Leopold (30) and Chatterjee (31) confirmed these findings and simply stated that the promotive effects on abscission were restricted to those compounds which do have auxin activity in growth. All compounds tested were capable of inhibiting abscission, even those which gave no detectable growth activity. Weintraub et al. (120) have tested over 600 compounds for abscission activity.

Osborne (93) evaluated the effect of 2,4,5-T butyl ester in abscission induction in tropical woody species.

Batjer and Thomson (14) found 1-naphthyl N-methyl-carbamate was a very consistent chemical thinner in apples.

Ethylene Effect on Abscission

Addicott (2) concluded that from available evidence ethylene acts on abscission through an effect on auxin. When ethylene is applied in conjunction with auxin-like growth substances they are found to be antagonistic (18, 34, 54, 84) although biochemically non-competitive (2).

Abeles and Rubenstein (1) using gas chromatography found that NAA applied to roots, stems and leaves stimulated ethylene evolution. They concluded that induction of abscission by NAA may be due to stimulation of ethylene evolution. They do not explain why high concentrations delay abscission while at the same time produce the greatest amount of ethylene, even if the gas does not affect abscission until 48 hours after deblading.

Ethylene when applied directly markedly stimulates abscission (1, 54, 77) and is oxygen dependent (100).

Brown and Addicott (22) found that ethylene treatments led to rapid cellular dissolution in the abscission layer and adjoining tissues.

Ethylene is known to exist in leaves (1, 97), fruit (1, 75), roots and stems (1) and may have some influence on the progression of abscission, but does not appear to be a controlling factor.

Amino Acids

Some amino acids have been found to induce abscission. Yager and Muir (128) found methionine to promote abscission of tobacco flowers. Leucine, alanine and glutamic acid were also effective but to a lesser degree. Rubenstein and Leopold (103) found glutamic acid and alanine to be active in bean explants with less stimulation by methionine.

Recent work by Lieberman (75) indicated that when methionine was infiltrated into apple tissue ethylene was produced. Further, the ability of the tissue to transform methionine was dependent on the tissue age. If such a system were operative in the leaf and petiole during senescence and abscission, then acceleration by amino acids may be explained.

Sucrose

The first indication of the effect of sucrose in delaying abscission was by Brown and Addicott (22) where sucrose, when applied to explants, delayed cell division and vascular changes, thus suggesting sucrose inhibited abscission.

Biggs and Leopold (20) and Biggs (18) demonstrated that sucrose inhibited abscission at high concentrations. When applied to plants low in carbohydrates they found that sucrose stimulated abscission. Recent studies by Gorter (41) have confirmed the abscission retarding effect of sucrose; however, when applied to young nodes it may accelerate abscission in Coleus.

Kinins and Gibberellins

Osborne and Moss (94) in 1963 found that when kinins were added concomitantly to the proximal and distal side of the abscission layer accelerated abscission resulted, whereas kinetin applied directly to the abscission layer retarded abscission.

Contrary to Osborne's work, Gorter (41) observed that kinetin retarded abscission and was dependent on concentration.

Chatterjee (31) using bean explants found that kinetin showed a two phase curve similar to auxin with promotion at lower concentrations and inhibition at higher concentrations.

The gibberellins like the kinins have received very little attention. Carns et al. (27) in 1961 demonstrated that gibberellins accelerated abscission in cotton explants. Chatterjee (31) and Rosen and Siegel (100) have also shown that gibberellin accelerates abscission. The degree of acceleration found by these workers was small, however, compared to auxins and ethylene.

Abscission Factors

The first evidence for an abscission factor was presented by Osborne (92) in 1955. She discovered that as leaves matured and reached senescence they produced a chemical substance or substances which had the property of accelerating the abscission of intact abscission zones. Diffusates from both leaf and petiole were active. Since this original work several other laboratories have reported such a factor. Biggs (18) found a similar substance in beans. The factor was increased by conditions favorable to abscission and they also concluded that it was neither an auxin or a substrate. Hall et al. (52) likewise demonstrated the presence of an abscission promoting factor(s) in the pulvinoids of debladed cotton leaves. This factor(s) was heat stable, soluble in ether and ethanol but not in water, slightly inhibitory to elongation of Avena coleoptile sections and gave a negative Salkowski reaction for indole. They concluded that the factor was probably a growth inhibitor and definitely not an amino acid since none of the amino acids evaluated had any effect on abscission of cotton. Rubenstein and Leopold (103) on the other hand, found that the abscission factor was extractable in the amino acid fractions.

Jacobs et al. (62) provided evidence against the hypothesis that the previously reported steady decline in auxin activity was not due to an increase in senescence factor activity.

"Abscisin"

In 1961 Liu and Carns (76) isolated and crystallized an abscission promoting substance ("Abscisin") from cotton burrs. It was found to contain 67 percent carbon and 6 percent hydrogen. The compound was acidic in nature and soluble in chloroform and dilute NaOH. Addicott (2) reported that "abscisin" had been isolated from young cotton fruits and the compound reached a maximum concentration at time of spontaneous abscission of young cotton fruit. He also suggested that "abscisin" was a competitive inhibitor of auxin action. Most of the evidence now available indicates that "abscisin" is an abscission accelerating factor but probably is not the controlling mechanism.

Defoliants

Studies with defoliants have added very little information to the knowledge of natural abscission processes. Most defoliation studies have been performed on cotton which differs from plants such as Coleus and bean in layer formation and response to natural growth substances. In general, defoliants induce abscission by leaf and petiole injury (10, 18, 50, 53, 73, 74, 115). Natural abscission in cotton involves cell division (50); some defoliants such as sodium chlorate-metaborate stimulate hydrolysis of cell walls without cell division (74), thus again placing doubt on the role of cell division in abscission.

Enzymatic Activity in Abscission

The earliest suggestion of the function of enzymes in abscission was by Bonner (21). He hypothesized that the abscission of leaves was possibly due to a middle lamella protopectinase. He postulated also that the enzyme polygalacturonase could act in abscission by breaking down pectin chains.

Not until 1958, when Osborne (95) reported changes in pectin methylesterase (PME) across the abscission zone of bean, was any work done on enzymes of the abscission process. Osborne found high PME activity in young leaf tissue which decreased as the leaf grew older. She observed the greatest fall in activity in the pulvinus concluding that a PME gradient from the pulvinus to either the stem or the petiole was necessary to prevent abscission. Acceleration of abscission decreased PME activity and retardation maintained or increased PME activity. Yager (125) substantiated Osborne's work with tobacco pedicels where auxin increased and methionine decreased PME activity. Further, pectic enzymes caused cell dissolution in vitro (as would be expected).

The majority of work on intercellular enzymes has been done with respect to explanations of auxin induced cell enlargement. Byerrum and Sato (25), Sato et al. (109) using radish tissue found a transmethylation from methionine to pectin. Ordin et al. (91) found greater incorporation of methyl groups from methionine in Avena coleoptile sections after treatment with IAA than before. Wu and Byerrum (124)

found incorporation of methyl groups from formaldehyde, glycine and serine in that order. Cleland (32) observed that auxin induced methylation in cold and hot water soluble pectins of monocotyledons but found no methylation in either the water soluble or acid soluble fractions in dicotyledons.

Sterling and Kalb (113) reported a continual decrease in methyl ester content of pectic substances in ripening peach fruits and consequently an increase in water soluble pectin.

Direct studies of the effect of auxin on cell wall enzymes have centered around pectin methylesterase. Kertesz (69) and Bryan and Newcomb (24) reported that 2,4-D increased the pectin methylesterase content of Red Kidney beans and tobacco pith respectively.

Glasziou and co-workers in Australia have shown that auxins and auxin-like materials, i.e., IAA, 2,4-D and NAA stimulate binding of PME to the cell walls of several types of tissue (Jerusalem artichoke, tobacco pith, bean endocarp and Avena coleoptiles) (42, 43, 44, 46, 47, 107). Each auxin had its own optimum concentration. Other factors such as the presence or absence of calcium ions are also involved in binding of PME. The presence of calcium ions markedly enhanced the activity of PME (45, 69). The effectiveness of calcium would indicate that the PME bond is probably a salt linkage. The enzyme appears to be localized in the free space and 90 percent of the PME is adsorbed to the cell walls <u>in vivo</u> (45). Glasziou concluded that the cell wall

may contain a group of enzymes functional in cell wall metabolism.

Enzymes other than PME have received only limited attention. Sampson (108) demonstrated the presence of oxidases in the abscission layer. Similarly, Hall and Morgan (55) described a large change in IAA oxidase activity in colytedonary abscission zones of cotton. Sequeira and Steeves (110) concluded that an oxidase enzyme was responsible for induced foliar abscission in coffee and Coleus brought about by the attack of the fungus Omphalia flavida.

Shoji and Addicott (112) working with bean leaf stalks and Storey (114) working with cotton have demonstrated the presence of an auxin inactivator which could possibly be an oxidase system.

Many enzymes are known to be inactivated in plants. Bell et al. (17) found 29 species of plants which were capable of inhibiting pectinase and 14 species which would inhibit cellulase activity. Such mechanisms of inhibition by plants very strongly favor the participation of enzymes of different specificities in controlling the abscission process.

As mentioned earlier Bonner (as reviewed by Addicott) (3) has suggested the function of two enzymes in cellular dissolution. The first, PME, has already been discussed. The second is polygalacturonase (PG). This enzyme has been reported infrequently in higher plants (tomato, avocado, pear and bean endocarp) (107). Kertesz (69) concludes that

the velocity of pectin hydrolysis increases roughly in proportion to the decrease in extent of esterification. Again relating PME and PG in pectin hydrolysis. The data presented by Wilson and Skoog (123) on the increase in the uronide in tobacco pith tissue also suggest the possible participation of PG.

Abscission Concepts

The number of concepts presented on control mechanisms is almost as numerous as the number of investigators in the field.

Growth

The theory which has recently been advanced by Jacobs et al. (66) seems to be supported to the greatest extent, either directly or indirectly, by data in the literature (12, 13, 15, 23, 48, 60, 62, 63, 66, 116). The theory states that auxin prevents abscission indirectly by causing growth. The author feels this theory would be acceptable if some other criterion than extension were used to measure growth. This stems from the fact that gibberellin causes very marked extension but has a slight stimulating effect on abscission of explants.

Auxin Gradient

Addicott et al. (6, 7) presented the concept that as long as there was an auxin gradient from the distal side of the abscission zone to the stem, abscission would not take place. If the gradient were lost or reversed abscission would proceed normally or be accelerated respectively. This

hypothesis has been confirmed by Shoji et al. (111), Storey (114) and many others.

Auxin-Ethylene Balance

Hall (54) proposed this hypothesis with work on cotton suggesting that through this concept natural abscission as well as accelerated or retarded abscission could be explained. Abeles and Rubenstein (1) are the most recent workers to substantiate a possible balance between auxin and ethylene. Addicott et al. (9) were unable to find any relationship between these two compounds in bean explants.

Methionine-Auxin

Yager and Muir (128, 129) propose that methionine, as a methyl donor, and indoleacetic acid interact to control abscission. In close correlation with this Rubenstein and Leopold (103) suggested that the decrease of auxin and accumulation of amino acids act to promote abscission.

Auxin Concentration

Since high concentrations of auxins inhibit abscission and low concentrations accelerate abscission, Gaur and Leopold (38) felt the quantitative effect was more significant than the qualitative effect. This concept has been supported by Sacher (105).

Auxin-Auxin Balance

Using debladed patterns in Coleus and synthetic IAA at different petioles relative to the one being tested, Jacobs (60) concluded that an auxin-auxin balance was operative in all types of abscission.

Auxin-Senescence Factor Balance

Osborne (92) states this hypothesis as follows: "Leaf abscission may be controlled not only by endogenous auxins but also by some substance or substances which are produced as the leaf matures and which reach a maximum at senescence." A later report by Jacobs et al. (62) provided evidence against this hypothesis, at least in the above stated form, although they still find an abscission accelerating factor in senescent tissue.

Auxin, Gibberellin and "Abscisin"

Carns et al. (27) and Carns (26) propose the interaction of auxin, gibberellin and the abscission accelerating hormone provides the common mechanism which regulates the process of abscission.

MATERIALS AND METHODS

General Methods

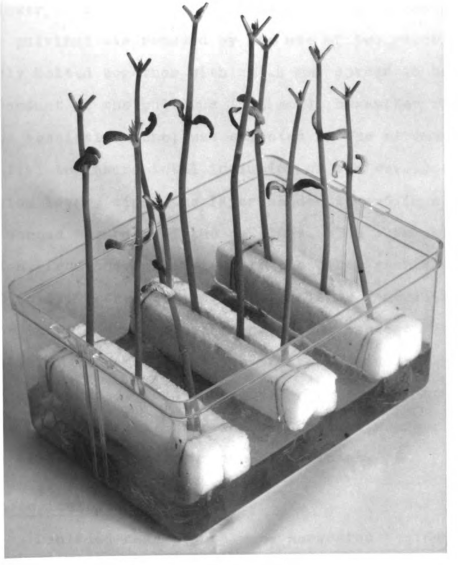
Phaseolus vulgaris L., cv. Contender (Joseph Harris Co. Inc. Rochester, N. Y.) was selected as the test plant because of its uniform growth characteristics and its relatively large diameter petioles and stem.

Seeds were germinated in the greenhouse under the natural day length and minimum day and night temperatures were maintained at approximately 17° C. The plants were grown in coarse, white quartz sand until the petiole length of the primary leaves was approximately 1.5 cm. or the lamina was 25 percent expanded. The seedlings were then carefully lifted to avoid excessive root injury and positioned between two 13.5 X 1.5 X 2.5 cm. styrofoam strips held together with rubber bands. The mounted plants were placed in clear plastic boxes 14 X 19.5 X 9.5 cm. containing approximately 750 ml. of tap water (Figure 1).

Induction of abscission layer formation was achieved by deblading the primary leaves to a petiole length of 1.0 cm. After deblading the plants were transferred to a controlled environment growth room under continuous light at 400 foot candles and a temperature of 21° C., unless otherwise stated.

Throughout many of the experiments the debladed bean plants were decapitated immediately prior to the beginning

Figure 1.--Equipment design and method used in mounting debladed bean plants for experimentation.



of a given assay or determination. The stem was cut 1.5 cm. below the attachment of the primary petioles. The stem with its adjoining petioles (explant) was then cut in half longitudinally. This made it possible to completely remove the growing tip and axillary buds with essentially no injury to the lower pulvinar area or to the petiole. A section of the lower pulvinus was removed by the use of two razor blades solidly bolted together with a 1.4 mm. spacer in between. This amount of the pulvinus (1.4 mm.), hereafter referred to as the abscission zone, was selected as the minimum width essential to insure total inclusion of the developing abscission layer, since the layer seldon formed in a straight line across the base of the pulvinus. The abscission zone section, from one cut surface to the other consisted of 25 to 30 cells, whereas the abscission layer generally involved two or three layers of cells and seldom exceeded five rows of cells. Since the formation of the abscission layer occurred so rapidly and the visual changes were so evident, it was felt that the error induced by the incorporation of these extra cells would be non-significant.

Morphological Studies

Debladed bean plants were harvested beginning 24 hours after blade removal, the explant was cut longitudinally and immediately killed and fixed in FAA.[1] The tissue was placed

[1] A fixing solution containing five milliliters of 40 percent formaldehyde, five milliliters of glacial acetic acid and 90 milliliters of 70 percent ethyl alcohol.

under vacuum for four to six hours and held in the FAA solution for 24 hours. The tissue was then dehydrated in a gradient series of tertiary butyl alcohol as described by Johansen (68) and embedded in Fisher Tissuemat (melting range 56-58° C.). The embedded tissue was mounted and cut longitudinally at 13 microns (u) with a rotary microtome.

The sections were affixed to glass slides with Haupts adhesive (68), stained with safranin-fast green, transferred to xylene and permanently mounted in Piccolyte (General Biological Supply House).

Ten plants were harvested and sectioned daily for five consecutive days. Progressive development of the abscission layer was followed, the number of plants showing the presence of an abscission layer was recorded.

Cell Division in the Abscission Zone (Histones-Basic Amino Acids)

Chemically fixed (FAA) tissue, representing plants taken daily over the five day period (ten plants per day) following deblading, was sectioned at 12 u and mounted on slides with Haupts adhesive. Nucleic acids, the only substances known to interfere with this otherwise specific reaction, were removed by extraction with 15 percent trichloroacetic acid for 30 minutes on a boiling water bath. The mounted specimens were rinsed three times in 70 percent ethanol. The sections were stained in 0.1 percent fast green (aqueous solution) at pH 8.0 (67), washed in water,

placed in 95 percent ethanol, dehydrated, and mounted in Piccolyte. Fast green at pH 8.0 is specific for basic amino acids and since these amino acids are normally associated with genetic material, a positive test was indicative of tissues actively engaged in cell division. Aloe flower buds were embedded, sectioned and used as controls since cells are known to be actively dividing in bud tissue.

Chemical Changes During Development of the Abscission Layer

Pectic Material

Localization and physical changes of pectins were studied during the formation of the abscission layer. Ten seedlings were harvested at 0, 2, 3, 4 and 5 days after deblading, fixed in FAA and embedded in Tissuemat. After sectioning at 12 u and removal of paraffin, the sections were stained with ruthenium red for 20 minutes and mounted in glycerine jelly (General Biological Supply House). The sections were observed microscopically for physical changes and localization of pectins.

Calcium Content of Abscission Zones and Petioles

Ten plants per day were harvested for the zero through five days after deblading and embedded in paraffin. After sectioning the paraffin was removed and calcium was identified in the tissue by the silver method as given by Jensen (67). Sections were treated with $AgNO_3$, the excess removed by washing with water, developed in a solution of 0.5 percent amidol (2,4-diaminophenol dihydrochloride) for two minutes, washed

in water and developed in two percent thiosulfate. The sections were then dehydrated, stained lightly with fast green and mounted. The presence of calcium in the tissue was apparent from the localization of silver grains as observed microscopically. The experiment was performed three times using ten single plant replications for each of the six consecutive days following deblading. This test was found to be less specific than described, since in addition to replacing the insoluble calcium in the pectic chain it also readily formed the silver salt of pectic acid (reaction with free carboxyl groups).

Quantitative Determination of Calcium in the Abscission Zone and Petiole

The histochemical determination of calcium in the abscission zone ($AgNO_3$ method) indicated an accumulation of calcium in the abscission zone during abscission layer formation. Since the histochemical determination was only qualitative at best and lacked in specificity, the calcium content of the abscission zone and petiole on a quantitative basis was next determined.

Tyrner's method as modified by Jensen (67) was employed, where calcium is precipitated by chloranillic acid (2,5-dichloro-3,6-dihydroxyquinone). Optical density measurements were made at 500 mu on a Bausch and Lomb Colorimeter.

Bean plants were debladed and mounted as described under general methods. To one half of the test population

10^{-3} M. 3-chlorophenoxy-3-propionic acid (3-CP) (99) was applied in a lanolin paste to the cut surface of the petiolar stub. The other half of the test population was retained for a control comparison. Ten seedlings representing ten single plant replications were sampled each day, for six days for controls and nine days for 3-CP treated plants, during the formation of the abscission layer. At each harvest time the two abscission zones (from a single plant, from each of ten plants) and their corresponding petioles (1.4 mm. abscission zone, 1.0 cm. petiole section) were placed in glass beakers, dried at 60° C. and dry weight determined. Both abscission zones and a one mg. aliquot of ground petiole tissue were placed in 6 X 50 mm. Pyrex test tubes (Kimble Laboratory Glassware) and set in an aluminum block 15.1 X 15.1 X 5 cm. with holes 6.3 mm. deep. The block was then placed in a muffle furnace and plant tissue ashed at 450° C. for two hours and then cooled in the furnace.

If the ash were not white, the residue was heated again until all the carbon was oxidized. Upon removal from the furnace one small drop of triple distilled water was added to each tube and the ash was dissolved in 1:3 HCl. After evaporating to dryness the residue was resuspended in 50 ul. of 0.1 N. acetic acid, warmed for 30 minutes at 60° C., and then 200 ul. of triple distilled water and 100 ul. of 0.1 percent chloranillic acid were added and the sample agitated and permitted to stand for 12 hours. Percent transmittance was obtained at 500 mu. on a Bausch and Lomb

Spectronic 20 with a microcell adapter using a 0.4 ml. volume cell and a 1.0 cm. light path (Micro Chemical Specialties Company). Solutions were transferred to the cell with a 1.0 cc. B-D Yale Tuberculin syringe with a B-D-20, 7 cm. needle. The cell was rinsed with triple distilled water after each reading. The amount of calcium per sample was determined by running a standard calcium curve for each experiment. The data were calculated and expressed as ug. of calcium per section. Quantitative calcium determinations were repeated five times for non-treated sections and twice for auxin treated tissue.

Cell Wall Changes

Abscission is known to involve the breakdown or at least a weakening of the cell walls in the abscission zone (36). If the formation of the layer involved breakdown of the cell walls, it would be important to know which of the cell wall materials were being affected and at what stage of the abscission process initial breakdown products appeared. Ten single plant replicates were harvested at each sample date during the formation of the abscission layer (0, 2, 3, 4 and 5 days after deblading) and sections (10 u) prepared as previously described.

Four slides each containing approximately ten sections were prepared for each plant at each harvest date. The paraffin was removed with xylene and the slides were coated with paraloidin to prevent loss of sections during the

extraction procedure. One slide was designated to represent the total cell wall materials, the remaining three slides were placed in a Coplin jar containing 0.5 percent ammonium oxalate. The Coplin jar in turn was placed in a water bath at 90° C. for 12 hours. One of these slides was selected and used to designate cell wall constituents minus pectic materials. The remaining two slides were placed in a Petri dish containing four percent NaOH. The sections were extracted for 12 hours at room temperature. The NaOH was then carefully removed with a drawn-out, throw-away pipet and small rubber bulb. The slides were allowed to air dry before removal from the Petri dishes to prevent loss and/or movement of the sections (four percent NaOH removes the paraloidin as well as the hemicelluloses). One slide was removed to represent cell walls with hemicellulose removed. Tissue on the final slide was extracted in a Petri dish with 17.5 percent NaOH for 12 hours at room temperature. The NaOH was again removed as before and the slides allowed to air dry. This final slide represented the cellulose content of the sections with all pectins, hemicelluloses and non-cellulosic polysaccharides removed.

The mounted sections after undergoing various extractions were passed through a gradient alcohol series including: absolute, 95, 70 and 50 percent, respectively. The NaOH crystals which formed on the last two slides were dissolved in the 50 percent alcohol (alcohol series also added and removed with a pipet). The slides were then passed

through the reverse sequence (50, 70, 95 percent and absolute) and again coated with paraloidin. The carbohydrate materials were stained with periodic acid-Schiff reagent with all sections receiving identical times. Sections were examined microscopically over the time course of abscission layer formation and rated as to amount of specific cell wall materials (pectins, hemicellulose, non-cellulosic polysaccharides, and cellulose) and to visual changes which occurred in these specific cell wall constituents. For a better comparison of cell wall materials, photomicrographs were taken under identical light conditions (15 u. amps) and shutter speeds (1/25 sec.).

This experiment was performed twice and the data presented are representative of both.

Site of Action of ^{14}C-NAA[2] and Its Effect on Abscission Layer Development

Debladed bean plants were mounted in the styrofoam strips and placed in the plastic boxes as described. ^{14}C-NAA at 10^{-3} M. or 10^{-5} M. in a lanolin paste was applied to the cut petioles. These concentrations provided a means of delaying (10^{-3} M.) and accelerating (10^{-5} M.) onset of the abscission process (19).

Two plants per day at each concentration were harvested and two mm. of the distal end of the petioles containing the

---

[2]Specific activity of ^{14}C-NAA was 1.25 mc/mm.

^{14}C-NAA were removed and discarded. To prevent a displacement of the NAA present in the tissue the explants were quickly frozen (one minute) in the Freon flow freezing block of an International Equipment Company Cryostat (Model CTD). The explants were mounted and completely immersed in H_2O which after freezing served as a cutting medium to prevent tearing of the tissue upon cutting. When an undue amount of cleaning of the plastic anti-roll plate was necessary, the sections had a tendency to be attracted to the plastic plate. Under these conditions a one percent solution of sodium carboxymethylcellulose (Hercules Powder Company), anionic form, when used as a freezing medium eliminated most of the static electricity problems.

The sections were cut at 58 u. and mounted on glass slides coated with autoradiographic adhesive[3] (67) and allowed to air dry. Under a Wratten series II safelight (Eastman Kodak Company) liquid emulsion, type NTB2 (Eastman Kodak Company) was spread evenly over the sections with a glass rod. The emulsion was then dried and exposed in a dark box containing $CaCl_2$ as a desicant for seven days. The microautoradiograms were developed in a solution consisting of: 1.125 grams amidol, 4.5 grams anhydrous sodium sulfite and two ml. of ten percent potassium bromide. The emulsion was developed for 45 minutes at 14° C. and fixed in sodium thiosulfate at one-third saturation for one hour. After washing

---

[3]Five grams gelatin in 1000 ml. warm distilled water plus 0.5 grams of chromium potassium sulfate.

in cold (10° C.) water for two hours the slides were stained with Azure B, dehydrated, and mounted in Piccolyte. Microscopically, the appearance of reduced silver indicated the localization of radioactive NAA.

Physiological Changes Associated with Abscission Layer Development

Changes in Dry Weight of Abscission Zones and Petioles During the Progress of Layer Formation

Dry weights were determined on the two abscission zones and petioles of the same plant daily from deblading through separation. Dry weights were also determined for similar sections from plants which had been treated with 3-CP at 10^{-3} M. which delayed abscission four to five days in our test system.

Basipetal Movement of ^{45}Ca, ^{32}P, and ^{86}Rb Through The Abscission Zone[4]

A lanolin emulsion of ^{45}Ca, ^{32}P and ^{86}Rb was prepared by adding the appropriate isotope in water to warm lanolin 1:1 (v/v) ratio and mixing with a glass rod until uniform white emulsion was formed. A quantity of lanolin containing the desired isotope (approximately 1000 cpm.) was applied to the cut surface of one petiole of each debladed plant. A fresh cut was made just before the addition of the lanolin emulsion. The movement over a 24 hour period for each element

---

[4]Specific activity of ^{45}Ca 11780 mc/g, ^{32}P carrier free, ^{86}Rb 11050 mc/g.

was established after 0, 1, 2, 3, 4, 5, 6 and 7 days following deblading. Twenty-four hours after treatment the plants were harvested and the distal end of the petiole with the lanolin paste was removed and discarded. The plants were then divided into two samples, the treated petioles (including the abscission zone) and the remainder of the plant (opposite petiole, stem and roots). The samples were placed in 50 ml. glass beakers and dried at 60° C. for 24 hours. The samples were cut into small pieces, placed in a nickel plated planchet, and counted with a Tracerlab Versa/matic II scaler and an end window Geiger-Müller tube for ten minutes. The results are expressed as the percent of radioactive material absorbed which was translocated through the abscission zone into the plant (basipetal movement). Ten single plant replications were employed for each harvest time and each isotope, with each experiment being repeated four times.

Effect of N-6-Benzyl Adenine on Abscission

N-6-Benzyl adenine (N-6-BA) is known to cause amino acids(86) and other compounds to move toward and accumulate at the point of application. If N-6-BA can induce directed transport then an application of N-6-BA to a debladed petiole should either: (a) decrease the time for abscission layer formation by removal of metabolites from the abscission zone or (b) delay abscission layer formation by directing metabolites from other parts of the plant to the abscission layer of the petiole to which N-6-BA has been substituted

for the leaf blade.

Ten plants were mounted, five of which had both primary leaves removed to a 1.0 cm. petiole length, the remaining five plants had one leaf removed, the other left intact. N-6-BA was added at concentrations of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} M. in lanolin to the cut surface of one petiole. The effect of N-6-BA was determined by noting the time required for 100 percent abscission. Petioles were subjected to a 5 g. stress with an abscission petiole tester as described by Mitchell, et al. (85). Comparable plants treated with lanolin alone served as controls.

pH Changes of the Abscission Zones and Petioles Associated with Formation of the Abscission Layer

Two treatments were designated in a group of uniform debladed plants; namely, (a) control (non-treated) and (b) auxin-treated (10^{-3} M. 3-CP in lanolin applied to one of the cut surfaces of the petiolar stub). Ten plants were harvested daily for six days after deblading in the non-treated group and for 11 days after deblading in the auxin-treated group. At each harvest the plants were separated into: (a) a 1.4 mm. section containing the abscission layer and (b) the 1.0 cm. petiolar stump. The 20 sections of comparable tissue were pooled and homogenized (glass homogenizer) with 10 ml. of triple distilled water. The hydrogen ion concentration of the resulting solution was determined on a Beckman Research pH meter, Model 1019, which is accurate to three decimal places.

This experiment was performed three times and each run was considered a replication.

Time Course Study of Respiration of Abscission Zones and Petioles During Abscission Layer Formation

Bean plants were debladed and half were treated by applying 10^{-3} M. 3-CP to the cut petiole and held under environmental conditions of 400 foot candles and 21° C. The time course for non-treated plants was 0, 1, 2, 3, 4, 5 and 6 days, and for 3-CP treated plants 0, 1, 2, 3, 4, 5, 6, 7, 9 and 11 days following deblading. Fifteen plants were harvested at each date and divided into abscission zone and petiole sections (1.4 mm. and 1.0 cm. respectively). The lanolin paste containing the auxin was carefully removed with tissue paper. The sections were then placed in Warburg vessels in 1.0 ml. of distilled water. Three-tenths ml. of ten percent KOH were added to the center well along with a small piece of filter paper. Two separate runs were made, each run containing two replications.

Respiration measurements were taken every 30 minutes for three hours. Oxygen uptake was then examined on the basis of dry weight, section and percent of control.

To determine whether or not there was a change in the respiration rate during the first 24 hours after debladding of non-auxin-treated plants, which might correspond to early changes associated with induction of the abscission process, 15 plants were harvested every six hours for 24 hours and

respiration rates determined as oxygen uptake per section per hour over a period of three hours.

Determination of Pectin Methylesterase Activity

Changes in pectin methylesterase activity during the abscission process were followed. Forty (1.4 mm.) abscission zone sections (representing 20 plants) were homogenized in 20 ml. of distilled water in an ice bath, and dialyzed (24 hours, 3.5° C.) against three liters of M/15 phosphate buffer at pH 6.0 to remove soluble salts and sugars. Similarly, 40 petiole (1.0 cm.) sections and 40 stem (1.4 mm.) sections, taken 1.0 cm. below the primary petioles, were homogenized and dialyzed. The determination of PME activity was carried out as described by Osborne (95) modified as follows: after dialyzing for 24 hours the crude extract was added to 2.0 ml. of a one percent solution of purified citrus pectin (Nutritional Biochemicals Corporation) made up in 0.1 M. NaCl and containing 0.00175 percent methyl red adjusted to pH 6.0 before using. This mixture was incubated at 24° C. for two hours. The reaction mixture was then centrifuged at 7000 R.P.M. for ten minutes. The clear supernatant was drawn off with a pipet, transferred to colorimeter tubes, and optical density determined at 550 mu. Two determinations were made for each plant part. The experiment was repeated three times and standard deviations determined.

Determination of Polygalacturonase Activity

The following procedure was developed for determination of polygalacturonase activity in the abscission zone, petiole

and stem of debladed bean plants during the abscission process. Twenty plants were selected daily and sectioned as described for PME. Sections of comparable tissue were pooled and homogenized in a glass homogenizer in ten ml. of distilled water. All steps of the procedure were carried out in the cold at 3-4° C. The homogenate was dialyzed against three liters of M/15 phosphate buffer at pH 6.0 for 24 hours. This step was shown to remove most of the reducing sugars in the homogenate. The dialysate was adjusted to a volume of ten ml. and added to one gram of G-25 Sephadex (100-300 u particle size, water regain 2.5± 0.2 g/g, Pharmacia, Upsala. Sweden). The Sephadex dialysate slurry was shaken on a Rotary Evapomix (Buchler Instruments) for ten minutes. The Sephadex and crude wall material were centrifuged out at 4000 R.P.M. for ten minutes in a refrigerated centrifuge. The supernatant was removed with a pipet and thoroughly mixed to eliminate any protein gradient which may have developed during centrifugation. A 1.0 ml. aliquot of the clear supernate was added to test tubes containing two ml. of 0.01 percent polygalacturonic acid or two ml. of water, used as controls. The tubes were incubated at 23° C. for four hours at pH 6.0 (pH remained constant throughout the experiment). At the termination of the incubation period the samples were placed in a boiling water bath for ten minutes. The hot reaction mixture was then filtered through Whatman no.2 filter paper and allowed to cool. Two ml. of ethyl ether were added to the filtrate. The extraction with ether removed lipids and perhaps other

materials which interfered with the final color complex formation, and chlorophyll, which absorbs at the same wave length as Prussian Blue. The water and ether phases were separated and t he ether phase discarded. Any residual ether in the water phase was removed with an air stream. The resulting water phase was assayed for reducing sugars. The method used was that of Parke and Johnson (96) modified by adding methyl alcohol just before the ferric ammonium sulfate solution. One ml. each of ferricyanide and carbonate-cyanide was added to each tube, the mixture was then boiled on a water bath for 15 minutes, allowed to cool and ten ml. of absolute methyl alcohol added. Five ml. of ferric ammonium sulfate solution were added and 15 minutes were allowed for color development. To insure uniform particle size in the resulting emulsion, each sample was homogenized. Optical density was determined at 650 mu. The enzyme activity reported is based on the difference in optical density between extract and substrate and extract and water. Duplicates were run in each determination and the experiment repeated three times.

Determination of the Induction Period of Abscission

Abscission and many biochemical and physiological changes associated with abscission layer formation can be delayed with exogenous auxin. In the bean, the leaf blade can only be partially replaced by 10^{-3} M. 3-CP, therefore, one may assume that there must be a time after which application of auxin no longer effectively delays the formation of

the abscission layer.

Plants were debladed and 3-CP at 10^{-3} M. was applied to the cut petiole in lanolin every hour for the first eight hours, thereafter at 12, 16, 20, 24, 48 and 72 hours after debladed. For control comparisons two treatments were established; (a) lanolin alone and (b) lanolin containing 3-CP applied at time of debladed. A fresh cut was made on each petiole immediately before auxin application. The petioles were tested for separation with five grams of stress and the data expressed as time to 50 percent abscission.

Temperatures of 21° C. and 25° C. (under continuous light of 400 foot candles) were used to determine if the induction time, or the time in which auxin could delay abscission layer formation, varied with different temperatures. Each time period was represented by ten plants and time to abscission was based on the first five petioles to separate. Simple regression equations were calculated and the best fit lines were drawn.

RESULTS

Morphological Development of the Abscission Layer

The development of the abscission layer was followed in longitudinal sections of explants taken at 2, 3, 4 and 5 days after deblading (Figure 2). No evidence of abscission layer formation was apparent for the first two days (Figure 2A), however, three days after deblading the abscission layer began to form at a point just below the axillary bud on the adaxial side of the petiole and proceeded toward the abaxial side (Figure 3). Walls of cells on the distal side of the abscission layer stained darker with fast green than those on the proximal side, indicative of marked changes in cell wall material (Figure 2B). Four days after deblading (Figure 2C) the cells of the abscission layer began to collapse and the layer as such was well defined. The cells of the abscission layer were completely collapsed after five days and the petiole was about to abscise (Figure 2D). No abscission layer formation was apparent through the conductive tissue (Figure 3).

The percent of a given population in which an abscission layer was apparent at 0, 1, 2, 3, 4 and 5 days after deblading is illustrated in Figure 4. The abscission layer was apparent in 20 percent of the test population after two days and 90 percent after three days. By four

Figure 2.--Photomicrographs illustrating the development of the abscission layer in Contender bean at two days (A), three days (B), four days (C), and five days (D) after deblading. All at 100 X magnification.

Arrows indicate abscission layer or potential abscission layer. P-Petiole, S-Stem.

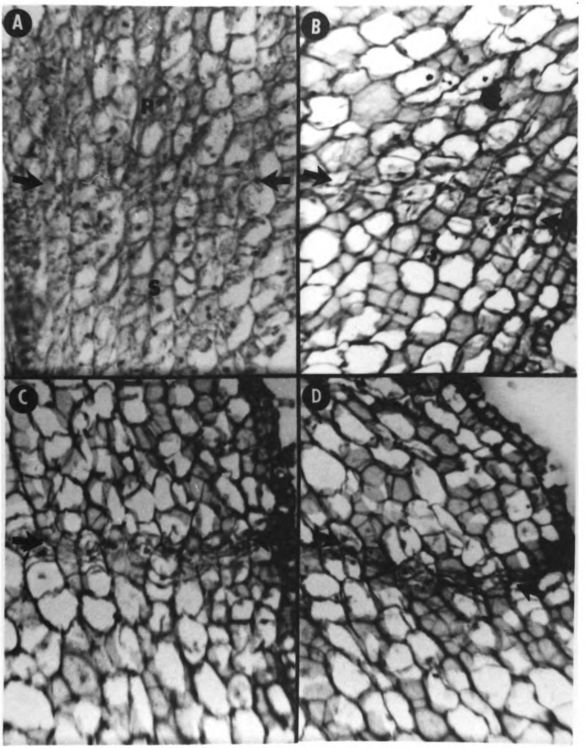

A
B
C
D

Figure 3.--Photomicrograph illustrating the appearance of the abscission zone in the lower pulvinus of Phaseolus vulgaris L. Cv. Contender. Fifty X magnification.

Arrows indicate abscission layer. P-Petiole, S-Stem.

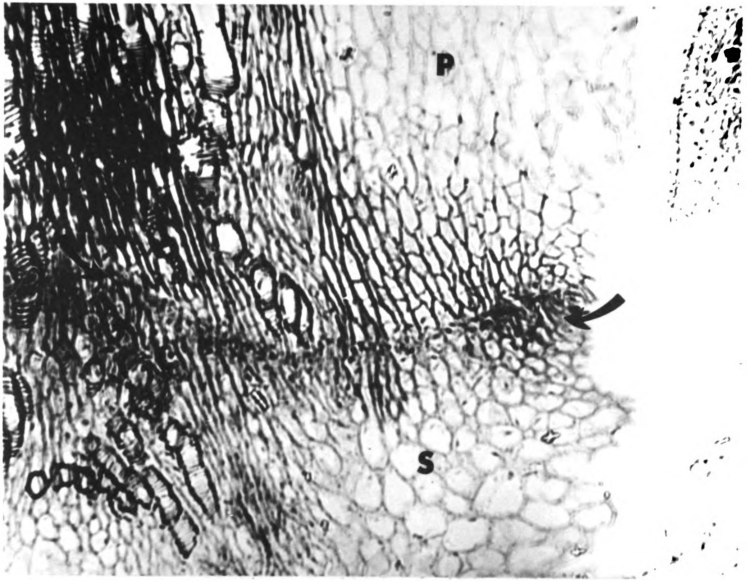
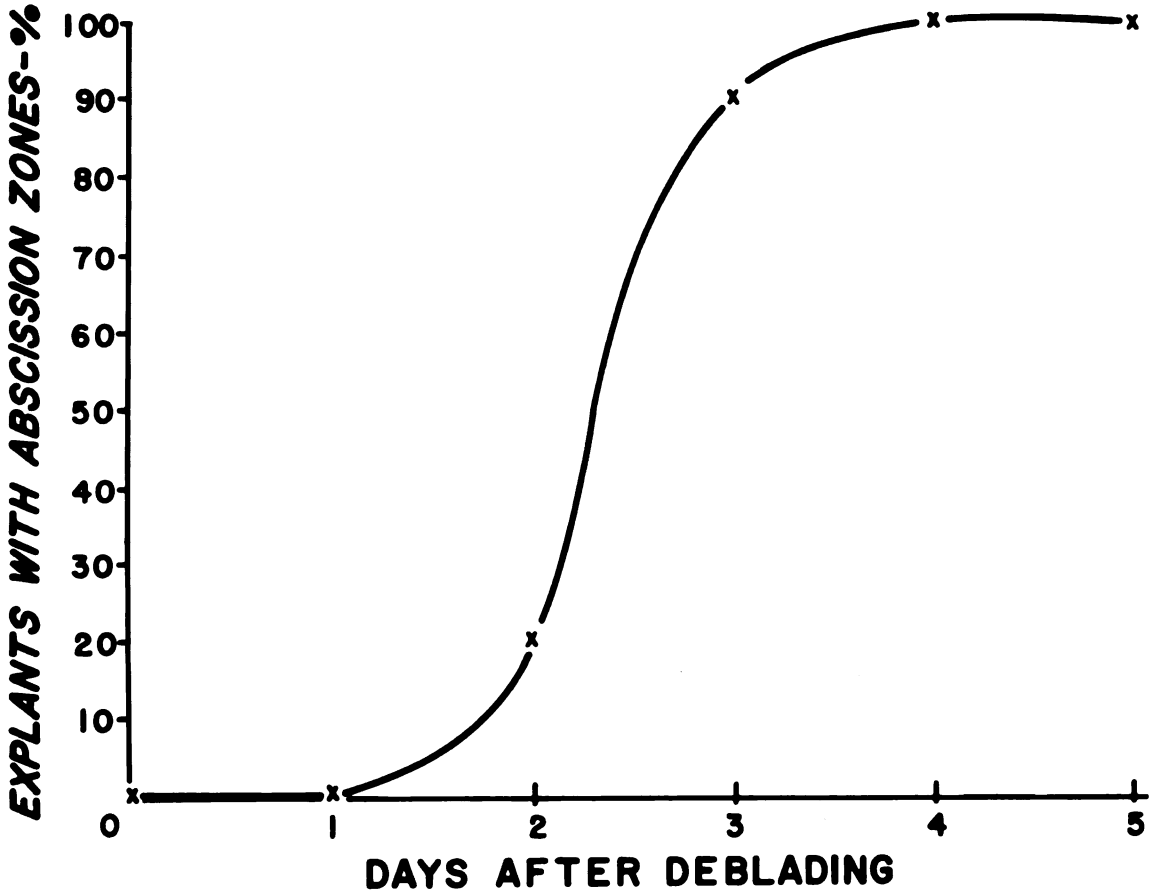

P
S

Figure 4.--Relationship of days after deblading to the appearance (microscopically) of the abscission layer in a ten explant sample.


EXPLANTS WITH ABSCISSION ZONES-%
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
DAYS AFTER DEBLADING

days, the abscission layer was present in the entire test population (100 percent). The debladed bean seedling, under the described environment, thus provided an excellent test system in which the rapid formation of the abscission layer in a major portion of the population was assured.

Cell Division

From microscopic inspection, no cell division was apparent in the abscission zone during the course of abscission layer formation. Further, no histones (basic amino acids) were detected over the entire five day period of abscission layer development confirming the absence of cell division. Over the entire time period, sections of Aloe flower bud were used as controls and stained positive in each experiment confirming the validity of this test.

Chemical Changes During Abscission Layer Formation

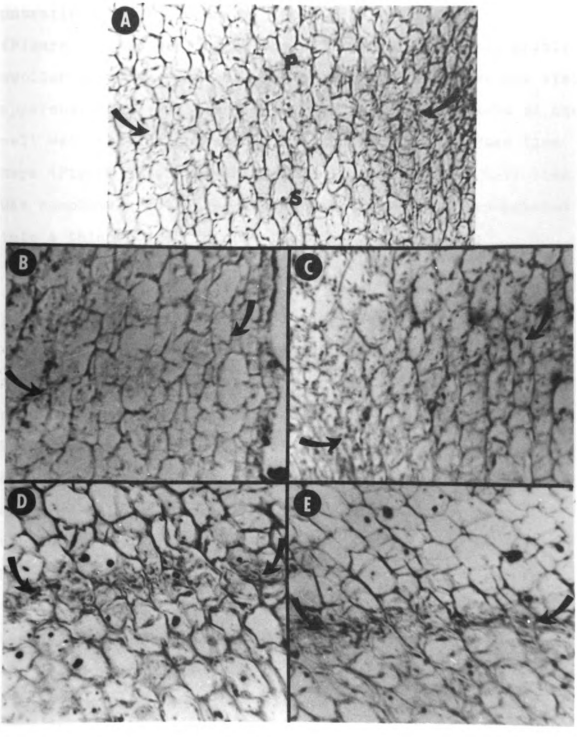
Pectins

The greater degree of staining (safrenin-fast-green) of cell walls with time after deblading was indicative of gross changes in the cell wall and middle lamella.

Using ruthenium red as a specific stain for pectins, the progression of abscission layer formation was followed for 0, 2, 3, 4 and 5 days after deblading (Figure 5). Immediately after deblading (0 days) the cell walls of the potential abscission layer appeared as sharp, well defined cell outlines (Figure 5A). Two and three days after deblading (Figures 5B and C) there was slight swelling of pectins and

Figure 5.--Photomicrographs of sections stained with ruthenium red illustrating the changes in pectin materials during formation of the abscission layer. (A) zero days, (B) two days, (C) three days, (D) four days and (E) five days after deblading. A at 50 X magnification, B, C, D and E at 100 X magnification.

Arrows indicate abscission layer or potential abscission layer. P-Petiole, S-Stem.


A
B
C
D
E

less well defined cell outlines were apparent through the potential abscission layer. Four days after deblading (Figure 5D) the pectins of the cell walls were considerably swollen and discontinuous. Some cell wall collapse was also apparent. Cell wall continuity was lost and remnants of the cell wall pectins appeared considerably swollen after five days (Figure 5E). The abscission layer tissue at this time was completely collapsed and appeared crushed or compressed into a thinner area than at four days.

Calcium

Changes in pectin, which were evident during the development of the abscission layer, led to an investigation of changes in calcium which may explain the swelling and probable loss of pectin materials from the abscission zone. The localization of calcium in a potential abscission zone of a non-induced plant is illustrated in Figure 6A. Calcium appeared only in the cell walls and was uniformly distributed throughout the abscission zone, petiole and stem tissues. By contrast, calcium was found to build up in and adjacent to the abscission layer in debladed plants (Figure 6B). A band of calcium appeared across the entire pulvinus, with the greatest concentration on the adaxial side of the petiole.

The build up of calcium in the abscission zone of plants at 2, 3, 4 and 5 days after deblading is illustrated in Figure 7. Microscopic examinations of sections from 15 plants per day indicated that the calcium content reached a maximum one day after deblading (Figure 7A) then apparently

Figure 6.--Photomicrographs illustrating the localization of calcium in the abscission zone of bean by the silver nitrate method. (A) non-induced, (B) induced by deblading. 100 X magnification.

Arrows indicate abscission layer or potential abscission layer. P-Petiole, S-Stem.

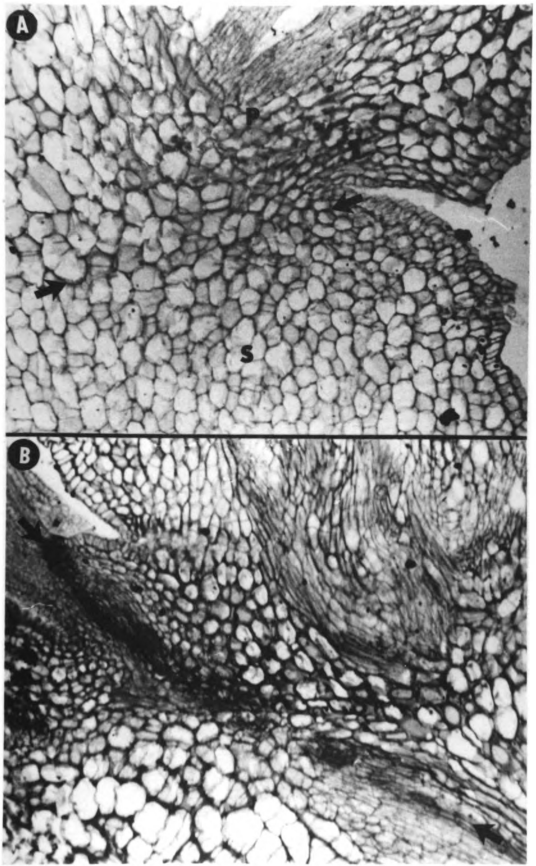
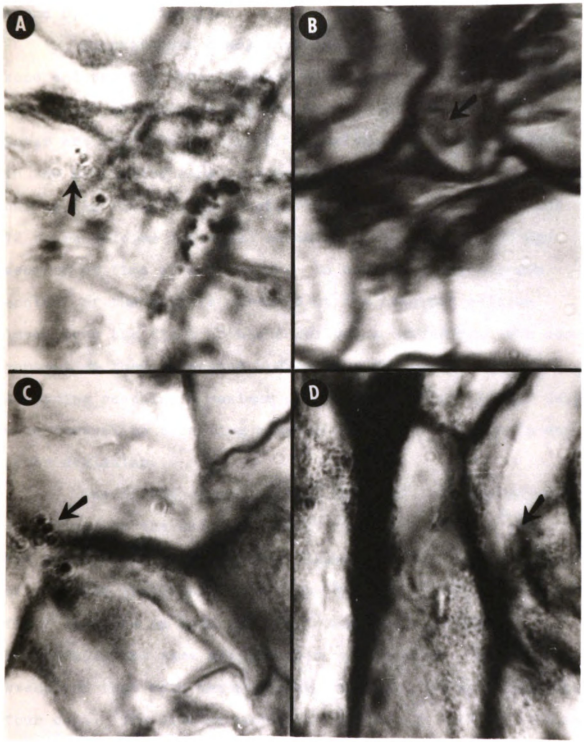

A
S
B

Figure 7.--Photomicrographs illustrating the localization of calcium in the abscission zone of the bean during development of the abscission layer. (A) two days, (B) three days, (C) four days and (D) five days after deblading. All at 1000 X magnification.

Arrows indicating silver grains which represent calcium.


A
B
C
D

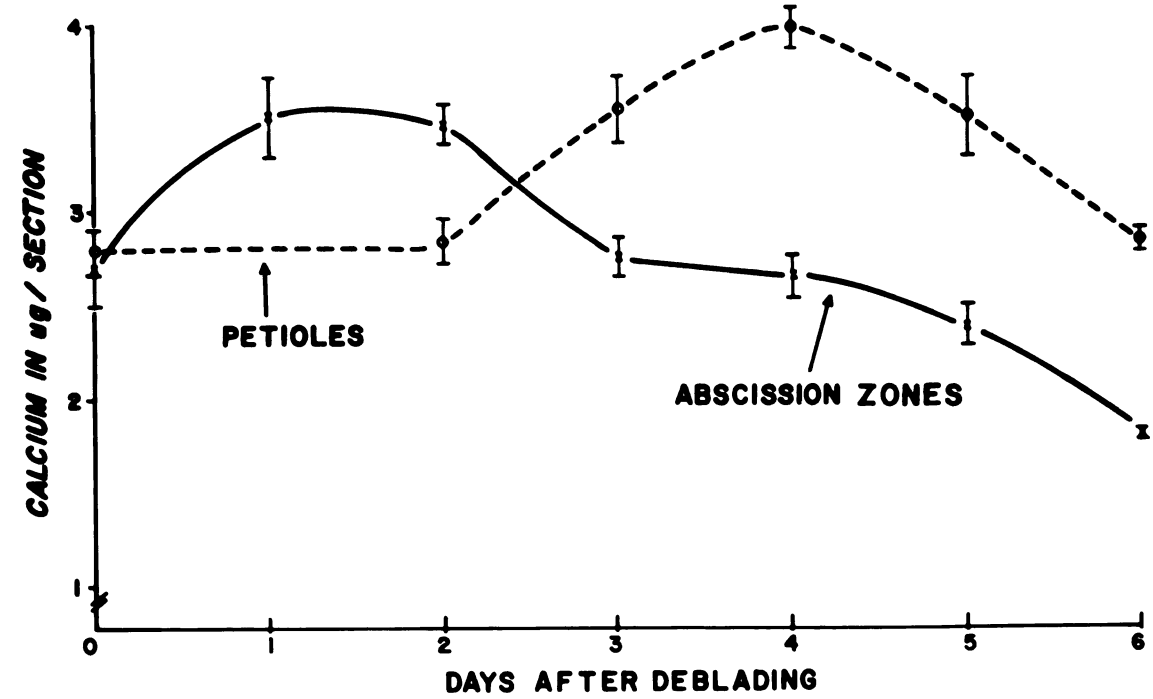
maintained that maximum until separation occurred (Figure 7B, C and D, respectively).

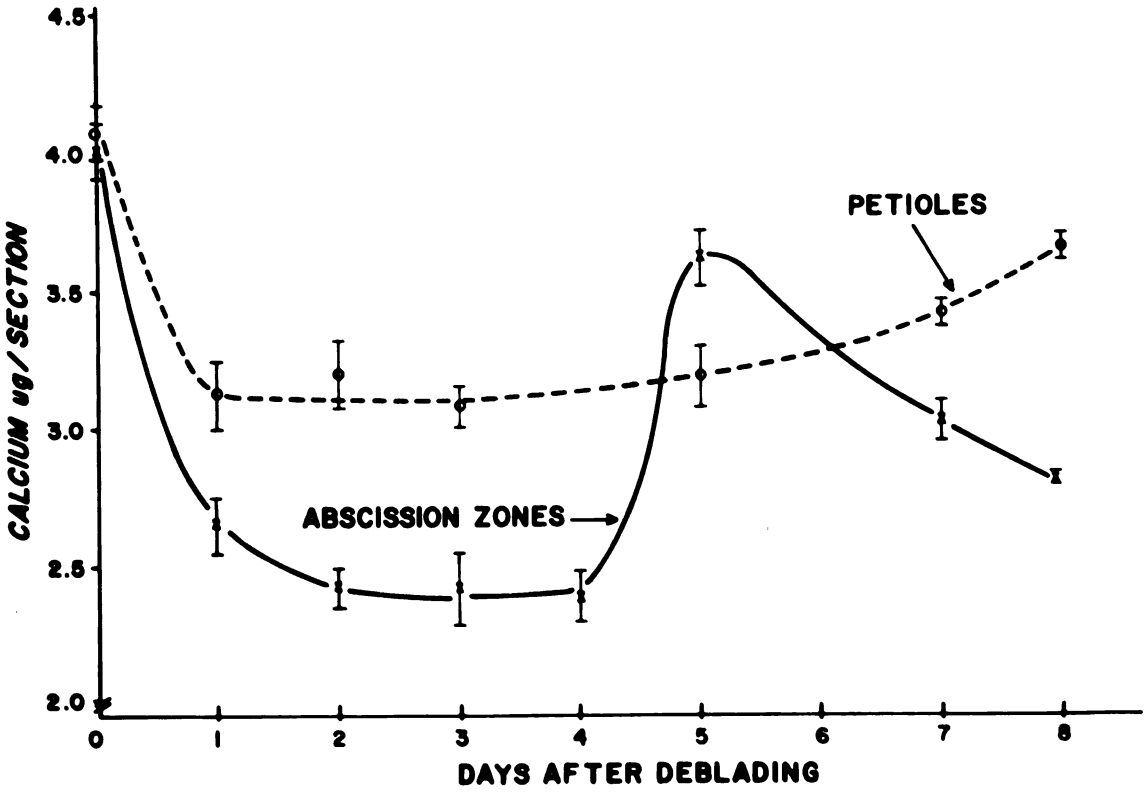
Quantitative changes of calcium in the abscission zone and petiole under induced abscission (deblading) conditions with relation to the development of the abscission layer are depicted in Figure 8. Calcium increased rapidly in the abscission zone to a maximum of 3.55 ug/section during the first 24 hours after deblading. Between 48 and 72 hours calcium decreased reaching a minimum of 1.80 ug/section at time of separation. In contrast, the calcium content of the petiole remained at the original calcium level over the first 48 hours following deblading. The concentration then increased between two and three days after deblading reaching a maximum of 3.80 ug/section at four days with separation occurring between the sixth and seventh day after deblading.

The effect of auxin (10^{-3} M. 3-CP), immediately after deblading, on the calcium content of the abscission zone and petiole is illustrated in Figure 9. With the exception of a marked simultaneous decrease in calcium during the first 24 hours in both the abscission zone and petiole, essentially the same relationship was obtained with the exception that both maximum peaks were delayed by approximately four days. The calcium concentration in the abscission zone with auxin decreased from 4.0 to 2.5 ug/section during the first 48 hours following deblading. Calcium in the abscission zone then increased to a maximum of 3.75 ug/section

Figure 8.--Calcium content of debladed Contender bean abscission zones and petioles during abscission layer formation.

Figure 9.--Calcium content of debladed, auxin treated (10^{-3} M. 3-chlorophenoxy-α-propionic acid) Contender bean abscission zones and petioles during abscission layer formation.


CALCIUM IN ug/SECTION
4
3
2
1
PETIOLES
ABSCISSION ZONES
0
1
2
3
4
5
6
DAYS AFTER DEBLADING


CALCIUM ug/SECTION
4.5
4.0
3.5
3.0
2.5
2.0
PETIOLES
ABSCISSION ZONES
0
1
2
3
4
5
6
7
8
DAYS AFTER DEBLADING

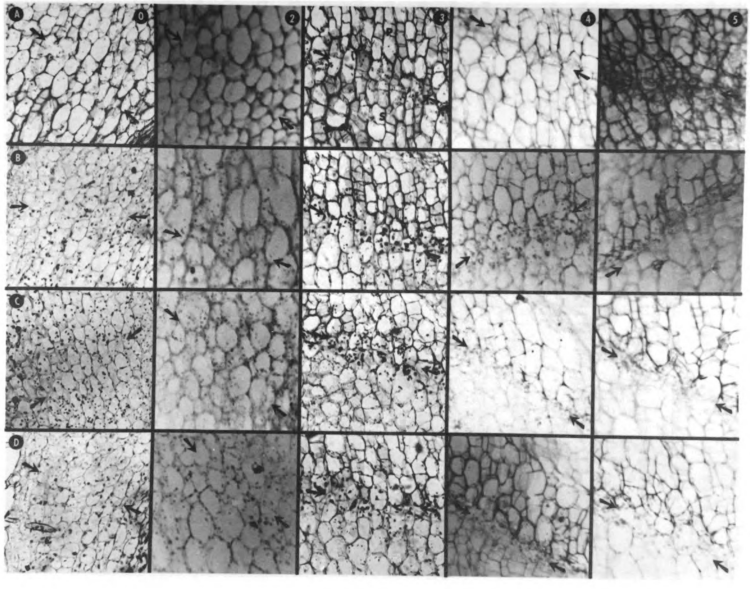
after six days and decreased to a minimum of 2.80 ug/section at eight days or approximately two days before separation. The petiole calcium decreased from 4.1 to 3.2 ug/sections during the first 24 hours after deblading, remained relatively constant (3.3 ug/section) for five days after deblading, and then increased reaching a probable maximum eight days after deblading or two days before separation.

Cell Wall Changes

Abscission zone sections from which pectins, hemicelluloses, and non-cellulosic polysaccharides were sequentially extracted 0, 2, 3, 4 and 5 days after deblading are illustrated in Figure 10. At zero days after deblading no abscission layer was present and no changes in the pectins, hemicelluloses or non-cellulosic polysaccharides (Figure 10-B0, C0, D0, respectively) in the potential abscission layer (illustrated by arrows) were apparent. Again at two days after deblading there were no apparent differences in these cell wall constituents of the potential abscission layer. (The random black spots which are especially evident in Figure 10B0 and C0 are primarily hemicellulose and non-cellulosic polysaccharides. Since the spots are not evident in the non-extracted sections of Figure 10-A0 and A2 they are apparently artifacts of the extraction technique.) Three days after deblading the abscission layer was well formed (Figure 10-A3). In the control tissue (Figure 10-A3) small localized dark areas appeared in the abscission layer indicating breakdown of one or more of the cell wall constituents

Figure 10.--Photomicrographs illustrating abscission zone tissue subsequent to cell wall extraction in the bean during formation of the abscission layer at zero, two, three, four, and five days after deblading. (A) Control (no extraction), (B) pectins extracted, (C) pectins and hemicelluloses extracted, (D) pectins, hemicelluloses and non-cellulosic polysaccharides extracted. Zero and three at 200 X magnification, two, four, and five at 400 X magnification.

Arrows indicating abscission layer or potential abscission layer.
P-Petiole, S-Stem.


A
0
2
3
4
5
B
C
D

perhaps by enzymes. Extraction of the pectins with ammonium oxalate (Figure 10-B3) resulted in no marked change in the number of localized spots indicating that pectins were not broken down and giving rise to the dark staining particles. After extraction of both the pectins and hemicelluloses, the tissue still stained similarly to the control eliminating the hemicelluloses as being the altered cell wall material (Figure 10-C3).

The dark staining material of tissue sections after removal of pectins, hemicelluloses and non-cellulosic polysaccharides was lower than for control tissue although still evident (Figure 10-D3). This suggested the non-cellulosic polysaccharides and cellulose were, at least in part, the wall components which were beginning to break down upon formation of the abscission layer. Adjacent to the abscission layer on the distal side, the cells (two to five layers) stained darker than corresponding cells on the proximal side or other cells of the petiole. In the same extraction series (Figure 10-3A through D), the cell wall material which is staining dark is probably cellulose. Lignins may possibly interfere, however, a phloroglucinol test (67) for lignins over the five day period was negative, thus confirming that cellulose was altered in these cells. The absence of lignin coupled with darker staining suggest the presence of more reactive sites for dye binding.

Examination of numerous sections of 25 plants indicated further development of the abscission layer with more

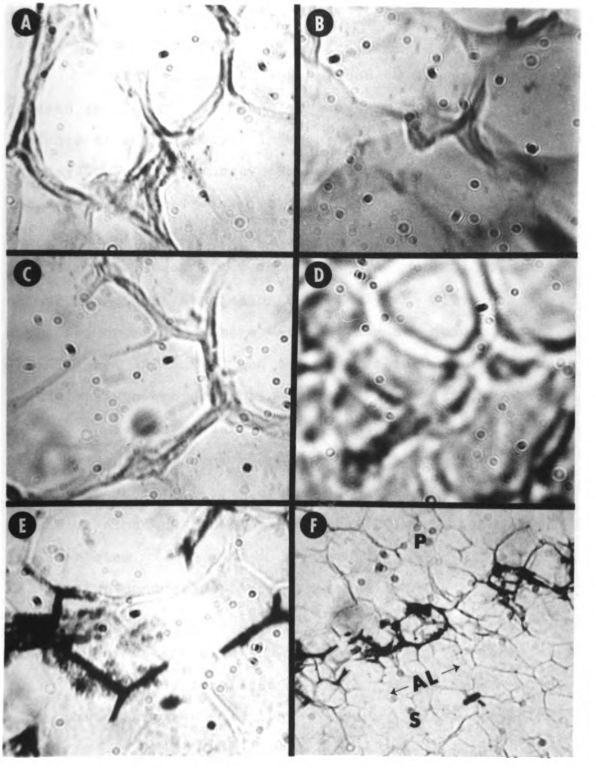
cell wall materials (non-cellulosic polysaccharides and cellulose) being broken down as was evident from smaller sized, more numerous, dark staining particles four days after deblading (Figures 10-4A through D). Cellular integrity was probably lost as indicated by the poorly defined cell walls in the abscission layer. The cells distal to the abscission layer again stained darker (Schiff stain) indicating further change in the composition of the cellulose in this area. Five days after deblading (Figure 10-5A through D) the abscission layer was fully developed. An increased number of dark staining particles after sequential extraction indicated that the non-cellulosic polysaccharides and celluloses were almost completely broken down in the abscission layer (Figure 10-5B through D). The density of staining in the cells adjacent and distal to the abscission layer was at a maximum at five days after deblading.

Site of Action of ^{14}C-NAA and Its Effect on Abscission Layer Development

The application of ring labeled, ^{14}C-NAA at 10^{-5} M. accelerated the formation of the abscission layer. Two days after deblading the abscission layer was well defined with only traces of the ring labeled NAA appearing uniformly distributed in the petiole, stem and abscission zone tissue (Figure 11A). Not until six days after deblading did ^{14}C-NAA accumulate in the tissue adjacent to the abscission layer (distal) (Figures 11B, C, D and E). Localization of ^{14}C-NAA

Figure 11.--Microautoradiograms of the lower pulvinus after treatment with 10^{-5} molar ^{14}C-naphthaleneacetic acid (ring labeled) at two days (A), three days (B), four days (C), five days (D), and six days (E) after deblading. All at 400 X magnification. (F) six days after deblading at 100 X magnification.

P-Petiole, S-Stem, AL-Abscission layer.


A
B
C
D
E
F
P
AL
S

occurred in tissue immediately distal to the abscission layer (AL), with none appearing in either the abscission layer, per se, or stem tissue (Figure 11F).

^{14}C-NAA at 10^{-3} M. when placed on the cut petiole delayed the formation of the abscission layer. There was no evidence of an abscission layer or accumulation of NAA after two days. However, three days after deblading an initial indication of abscission layer formation appeared with a slight localization of NAA (Figure 12B). NAA localization became more evident after four days (Figure 12C) as did the appearance of the abscission layer. By the fifth and sixth day after deblading, there was considerable accumulation of NAA (Figures 12D and E) and it was localized in the tissue just distal to the abscission layer (Figure 12F). As with 10^{-5} M. there was no localization in the abscission layer. It is evident that some block to movement of materials through the cortex tissue of the abscission layer was operative since no ^{14}C-NAA was detected in the abscission layer or stem.

The conductive tissue appeared to be a pathway for ^{14}C-NAA movement. At 10^{-5} M. NAA (Figure 13A) no radioactivity was detected in the xylem elements. By contrast at 10^{-3} M. NAA was localized in individual xylem elements. (Both photomicrographs of tissue two days after deblading.)

Figure 12.--Microautoradiograms of the lower pulvinus after treatment with 10^{-3} molar ^{14}C-naphthaleneacetic acid (ring labeled) at two days (A), three days (B), four days (C), five days (D), and six days (E) after deblading. All at 400 X magnification. (F) six days after deblading at 100 X magnification.

P-Petiole, S-Stem, AL-Abscission Layer.

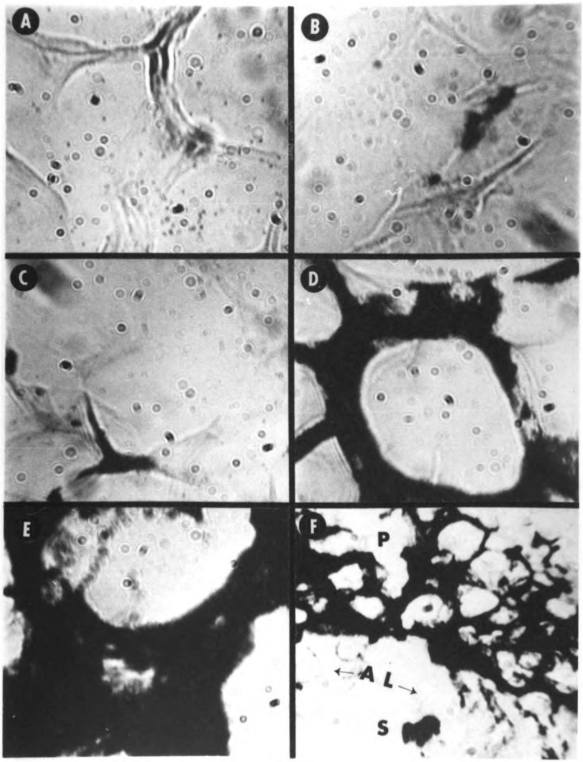
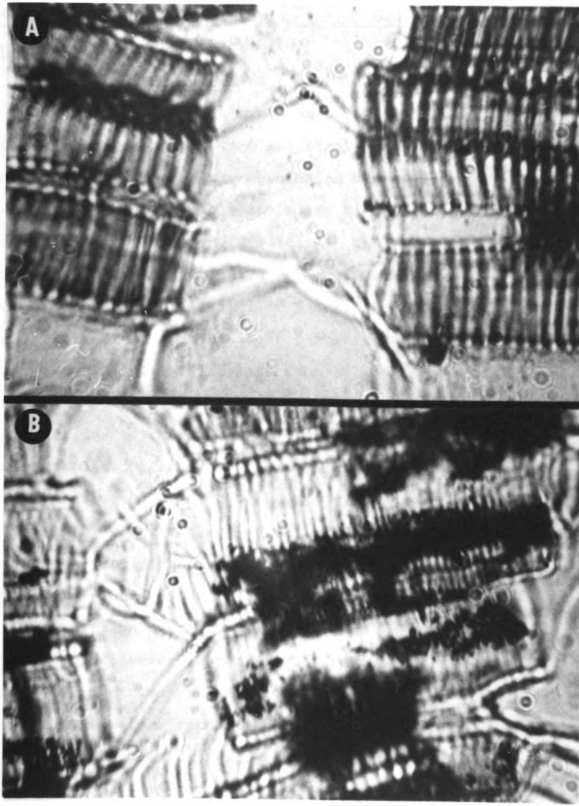

A
B
C
D
E
F
P
A L
S

Figure 13.--Microautoradiograms of the conductive tissue after deblading and treatment with (A) 10^{-5} molar and (B) 10^{-3} molar ^{14}C-naphthaleneacetic acid (ring labeled). Both at 400 X magnification.


A
B

Physiological Changes Occurring During Development of the Abscission Layer

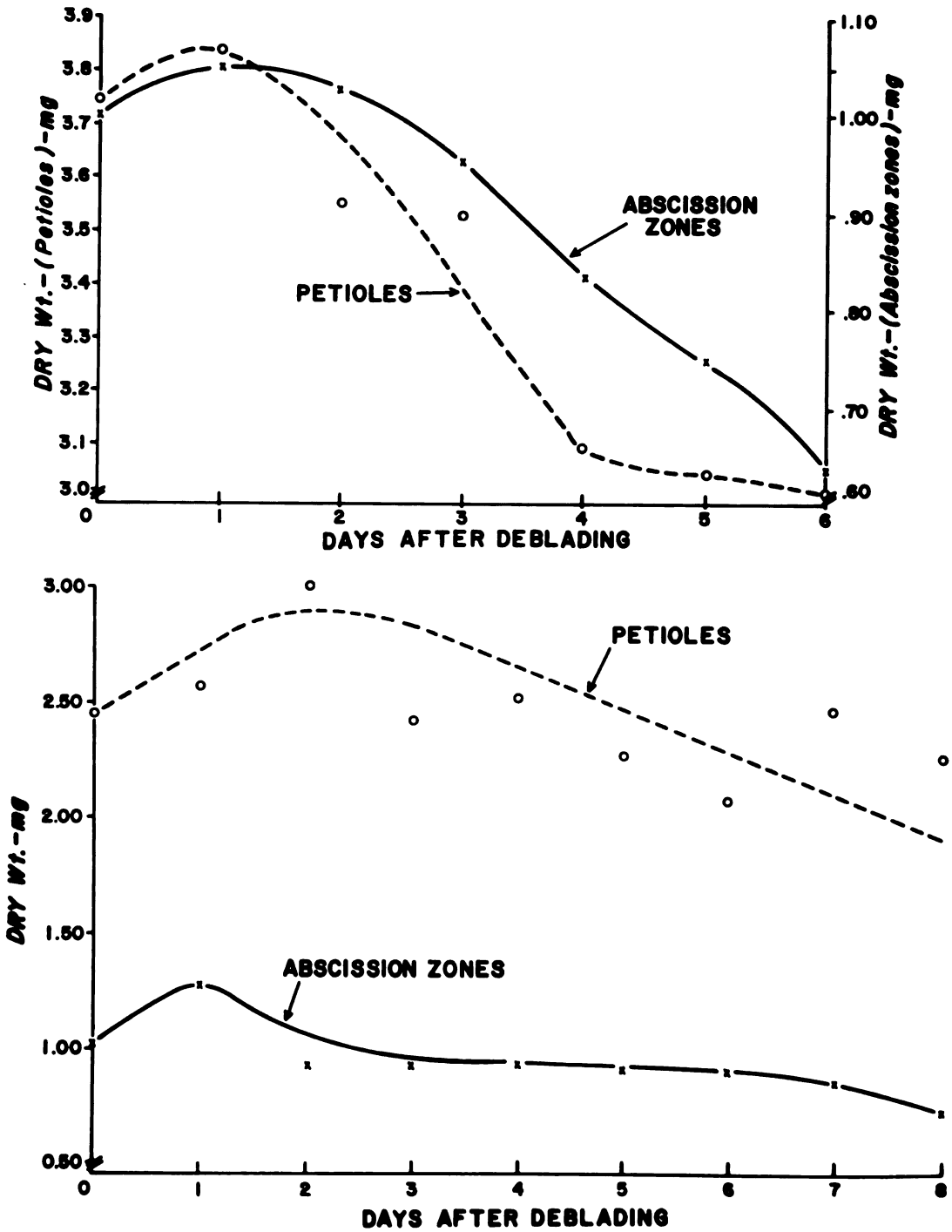
Dry Weight Change

Changes in dry weight observed during the development of the abscission layer are illustrated in Figure 14. An increase in weight of the abscission zones from 1.00 mg/section immediately after deblading to 1.05 mg/section 24 hours after deblading was noted. The dry weight then decreased reaching a minimum value of 0.63 mg/section at time of separation. A similar change in dry weight during the course of abscission layer formation was observed for petioles; an initial increase from 3.74 mg/section to 3.85 mg/section during the first 24 hours, decreased more rapidly than the dry weight of the abscission zones until four days after deblading, then decreased slightly until separation (3.0 mg/section).

The change in dry weight after deblading was markedly altered by applying auxin (10^{-3} M. 3-CP) to the debladed petioles. The abscission zones increased in dry weight from 1.01 mg/section over the first 24 hours after which there was a slow decrease in dry weight over the remaining seven days with a minimum value of .75 mg/section after eight days, or two days before separation. Petioles treated with auxin likewise gained in dry weight over the first 48 hours from a value of 2.48 mg/section to 2.75 mg/section, followed by a slow, steady decline to a minimum value of 2.00 mg/section eight days after deblading. Again, with the auxin-treated

Figure 14.--Change in dry weight of the abscission zones and petioles of bean plants during the development of the abscission layer.

Figure 15.--Change in dry weight of abscission zones and petioles after treatment with 10^{-3} molar 3-chlorophenoxy-α-propionic acid (auxin), and during the formation of the abscission layer.


DRY Wt.-(Petioles)-mg
DRY Wt.-(Abscission zones)-mg
ABSCISSION ZONES
PETIOLES
DAYS AFTER DEBLADING
DRY Wt.-mg
PETIOLES
ABSCISSION ZONES
DAYS AFTER DEBLADING

tissue as with the non-treated tissue the rate of dry weight loss in the petiole was greater than the rate of loss in the abscission zone. A corresponding loss in dry matter from auxin treated petioles and abscission zones was extended over an eight day period compared to six for non-treated tissue. For comparative purposes the dry weight data expressed as percent of control are listed in Table 1.

Movement of ^{32}P, ^{86}Rb and ^{45}Ca Basipetally Through The Abscission Zone

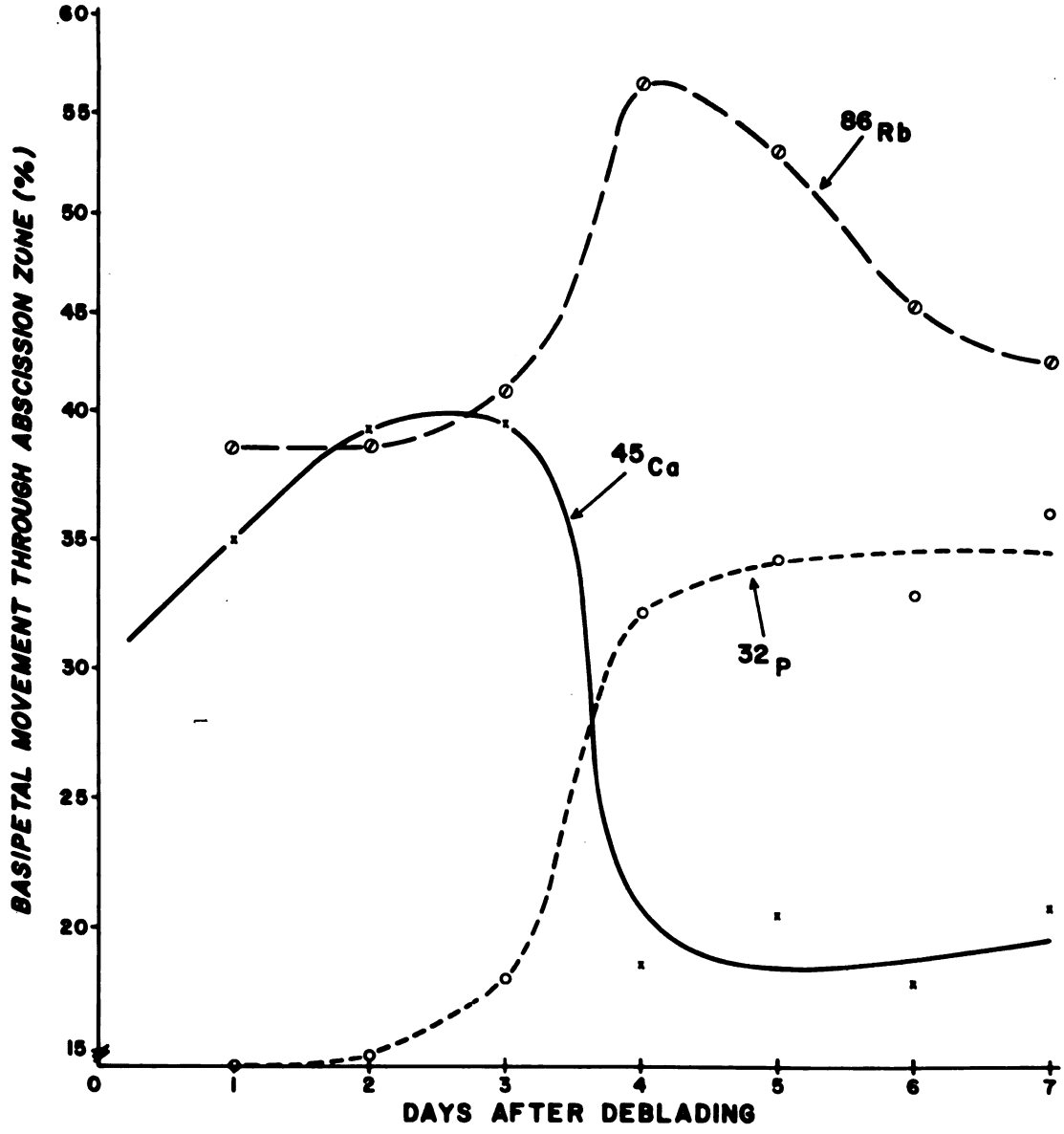
The loss in dry weight of the petioles may have resulted, in part, from an export of materials. The amount of ^{32}P, ^{86}Rb and ^{45}Ca which moved through the abscission layer as a percentage of that absorbed was determined at various stages in the development of the abscission layer and is reported in Figure 16. Little movement of ^{32}P was detected until three days after deblading, then 18 percent of the absorbed phosphorus moved through the abscission zone in a 24 hour period. Four days after deblading 33 percent moved through in a 24 hour period and remained at this level until separation. Rubidium, was utilized to confirm the altered movement of phosphorous from the petiole as the abscission layer formed. The curve obtained for ^{86}Rb was essentially the same as that for ^{32}P with the exception that the amount which moved from the petiole to the stem was higher.

When ^{45}Ca was applied, the reverse of the curves for ^{32}P and ^{86}Rb was obtained in that a higher percentage of

Table 1.--Dry weight changes of abscission zones and petioles treated with 10^{-3} M. 3-chlorophenoxy-α-propionic acid.

Days after deblading	Percent of nontreated	
	abscission zone	petioles
0	112	106
1	128	162
2	130	108
3	125	113
4	147	137
5	133	133
6	126	152
7	85	133
8	83	129

Figure 16.--Basipetal movement of ^{32}P, ^{86}Rb and ^{45}Ca through the abscission zone of Contender bean following daily application to debladed petioles. Curves are averages of four experiments.


BASIPETAL MOVEMENT THROUGH ABSCISSION ZONE (%)
60
55
50
45
40
35
30
25
20
15
0
1
2
3
4
5
6
7
DAYS AFTER DEBLADING
86Rb
45Ca
32P

absorbed calcium moved through the abscission zone during the first three days. The amount then dropped from 40 percent to 18 percent between the third and fourth day after deblading. This lower value (18 percent) was then maintained throughout the remaining three days. Although the percentage was rather high the actual count was low indicating that very little actual calcium moved into the petiole. With all three isotopes, there was a significant change in movement between the third and fourth day after deblading; a time when the abscission layer is evident and cellular integrity is lost.

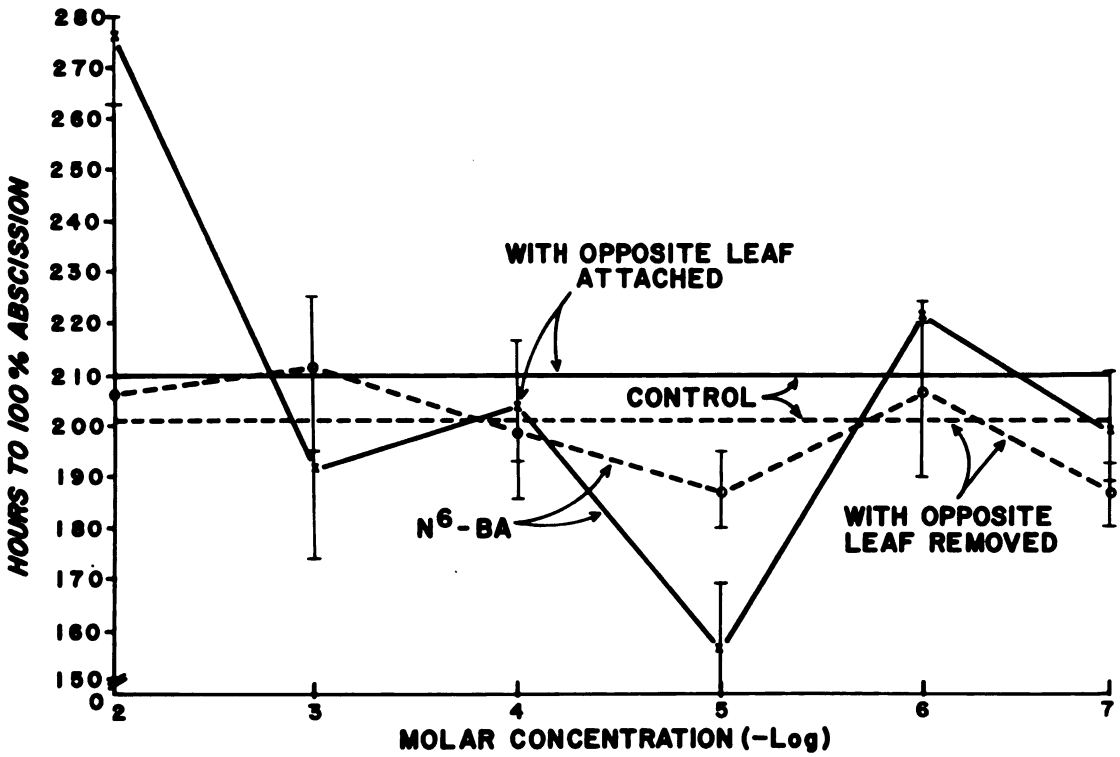
The Effect of N-6-Benzyl Adenine on Abscission

The effect of N-6-BA at concentrations of 10^{-7} through 10^{-2} M. on time to abscission is recorded in Figure 17. No significant difference was noted with N-6-BA, irrespective of concentration, to time of petiole separation when the opposite primary leaf was removed. By contrast, when the opposite leaf was left in tact, a significant delay (65 hours) at 10^{-2} M. over the comparable control was apparent. Similarly, at 10^{-5} M. N-6-BA, with the opposite leaf attached, a significant acceleration (about 50 hours) of abscission occurred (Figure 17). These results are similar to those obtained for auxin at 10^{-3} and 10^{-5} M.

pH Determinations of Abscission Zones and Petioles During Abscission Layer Formation

With deblading and no auxin application to the debladed petiole, the pH of the abscission zones dropped from

Figure 17.--The effect of N-6-Benzyl adenine (N-6-BA) on abscission of bean petioles, with the opposite leaf removed and with the opposite leaf intact.

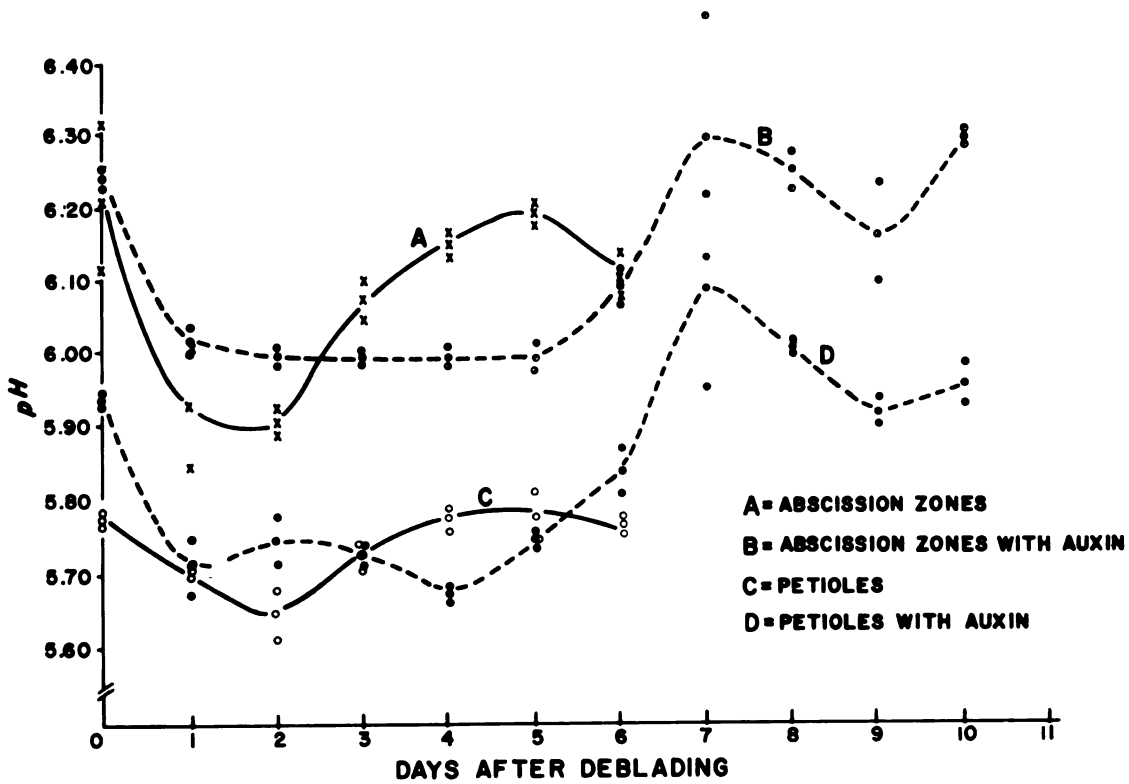

HOURS TO 100% ABSCISSION
280
270
260
250
240
230
220
210
200
190
180
170
160
150
0
2
3
4
5
6
7
MOLAR CONCENTRATION (-Log)
WITH OPPOSITE LEAF ATTACHED
CONTROL
N6-BA
WITH OPPOSITE LEAF REMOVED

6.20 to 5.90 after 48 hours. Similarly the pH of the petioles decreased from 5.80 to 5.65 after 48 hours. Two days after deblading the pH of the abscission zones began to increase reaching a maximum pH of 6.22 five days after deblading (Figure 18A). Similarly, the petiolar pH began to increase two days after deblading reaching a maximum of 5.80 after five days (Figure 18C). Auxin (3-CP, 10^{-3} M.) applied to the cut petioles had no apparent influence on the initial drop in pH of either petioles or abscission zones. The pH in the abscission zones and petioles decreased from 6.22 to 6.02 and 5.93 to 5.71, respectively, after 48 hours. Auxin delayed the subsequent increase in pH of both the abscission zones and petioles by approximately three days. The pH of the abscission zone began to increase five days after deblading reaching a maximum of 6.30 after seven days (Figure 18B). Similarly the pH of the petiole began to increase four days after deblading reaching a maximum of 6.10 after seven days (Figure 18D). It is significant that with auxin the pH peaks occur at the same time (seven days).

Respiration of Abscission Zones and Petioles During Abscission Layer Formation

The overall respiration pattern from the time of deblading through separation was followed with and without auxin treatment (10^{-3} M. 3-CP). Respiration of the non-treated abscission zones (expressed on a section basis) remained relatively constant from deblading through

Figure 18.--Change in pH of debladed bean petioles and abscission zones with and without treatment with auxin during abscission layer formation. (A) abscission zones without auxin, (B) abscission zone with auxin (10^{-3} M. 3-chlorophenoxy-α-propionic acid), (C) petioles without auxin, (D) petioles with auxin. Curves drawn as averages of two experiments.


6.40
6.30
6.20
6.10
6.00
5.90
5.80
5.70
5.60
pH
A
B
C
D
A= ABSCISSION ZONES
B= ABSCISSION ZONES WITH AUXIN
C= PETIOLES
D= PETIOLES WITH AUXIN
0
1
2
3
4
5
6
7
8
9
10
11
DAYS AFTER DEBLADING

separation (Figure 19A). There was a steady decline in the respiration rate of the petiole tissue with the minimum occurring at time of separation (six days) (Figure 19C). When auxin was added to the cut petiole and the rate of oxygen uptake measured, there was an apparent stimulation in respiration of the abscission zones from the second through the fifth day after deblading. Thereafter, the respiration rate decreased slightly below that of the control through the eleventh day (Figure 19B). The auxin treated petioles, (Figure 19D) on the other hand, displayed an immediate rise in respiration followed by a dip two days after deblading, after which the rate again increased and steadily declined through the eleventh day. The respiration rate of the treated petioles remained significantly higher than that of the non-treated petioles throughout the period of abscission layer formation.

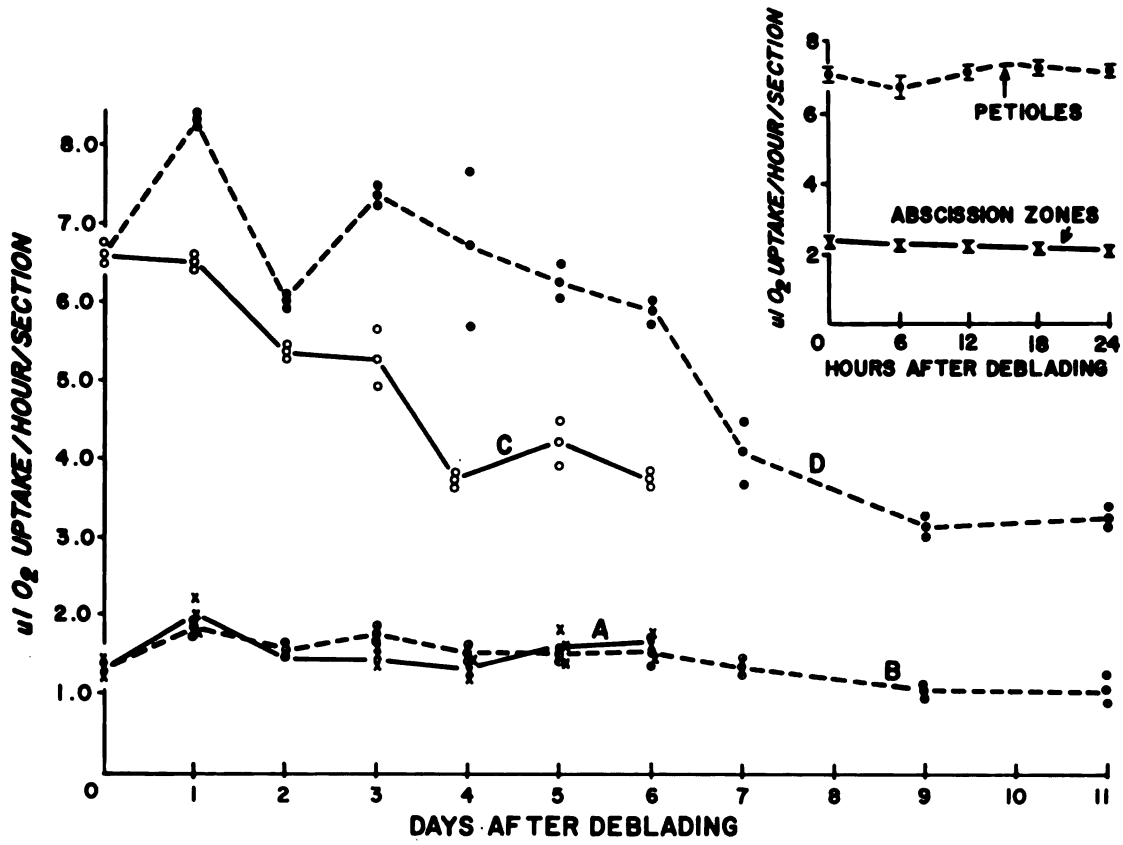
If the respiration data of Figure 19 are replotted as percent of control (Figure 20) the petioles undergo an immediate but slight tissue response to 3-CP, which is not apparent in the abscission zone. Both the petiole and abscission zone tissue reached peak respiration rates four days after deblading indicating that 3-CP affected both tissues at the same time and stimulated respiration in a like manner.

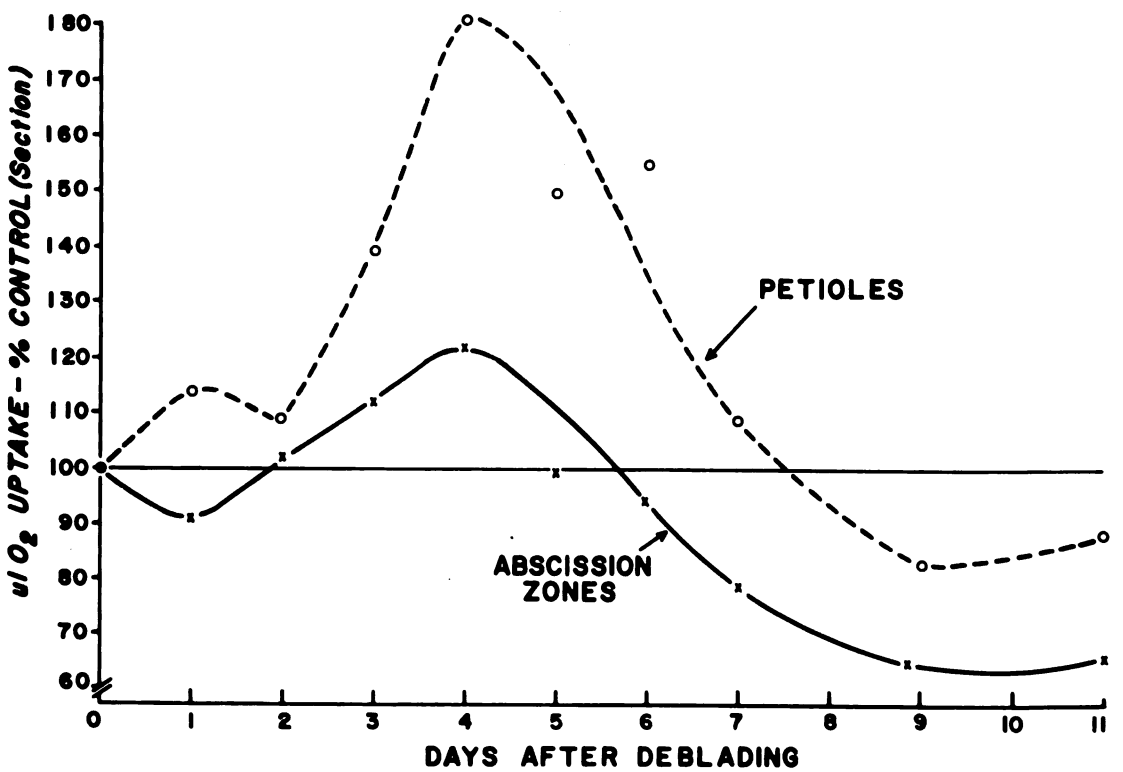
The induction of abscission presumably takes place during the first 24 hours after deblading. Following this assumption and determining respiration for non-auxin treated

Figure 19.--Respiration rate per section (1.4mm abscission zone, 1cm petiole) of abscission zones and petioles of bean following deblading, with and without auxin treatment. (A) abscission zones without auxin, (B) abscission zones with auxin (10^{-3} M. 3-chlorophenoxy-α-propionic acid), (C) petioles without auxin, (D) petioles with auxin. Curves drawn through the sample average.

Insert: Respiration rate of non-treated petiole and abscission zone sections during the first 24 hours after deblading.

Figure 20.--Oxygen uptake of auxin treated, debladed bean abscission zones and petioles expressed as percent of control on a section basis.


µl O2 UPTAKE/HOUR/SECTION
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
1
2
3
4
5
6
7
8
9
10
11
DAYS AFTER DEBLADING
A
B
C
D
µl O2 UPTAKE/HOUR/SECTION
8
6
4
2
PETIOLES
ABSCISSION ZONES
0
6
12
18
24
HOURS AFTER DEBLADING

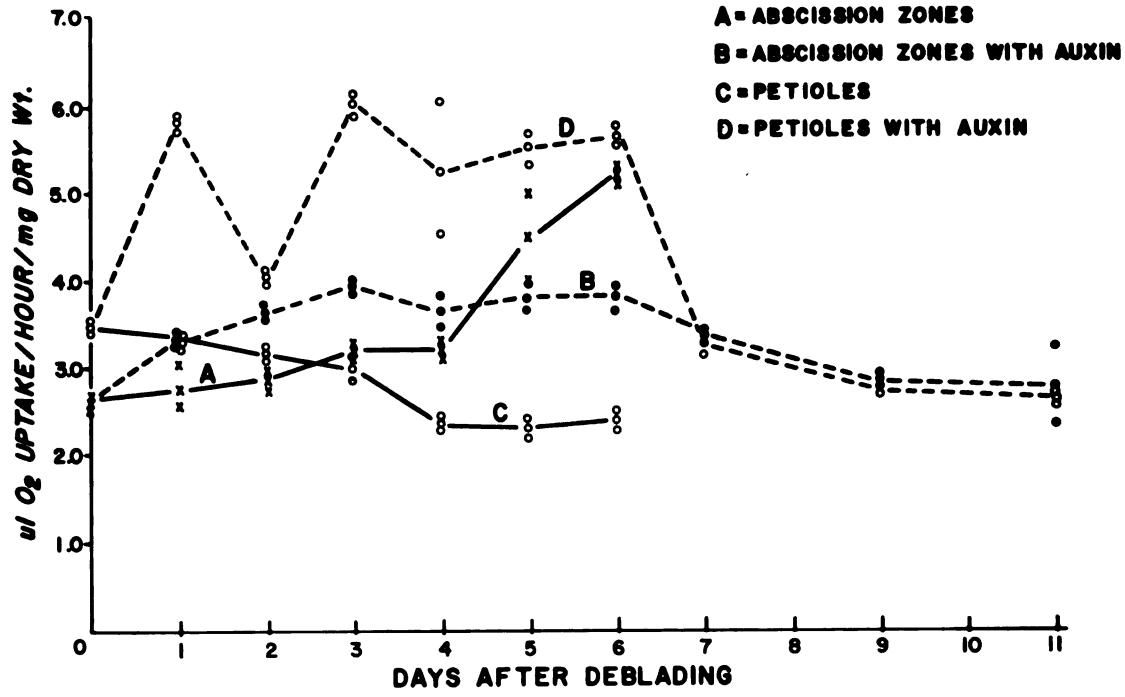

µl O2 UPTAKE - % CONTROL (Section)
180
170
160
150
140
130
120
110
100
90
80
70
60
0
1
2
3
4
5
6
7
8
9
10
11
DAYS AFTER DEBLADING
PETIOLES
ABSCISSION ZONES

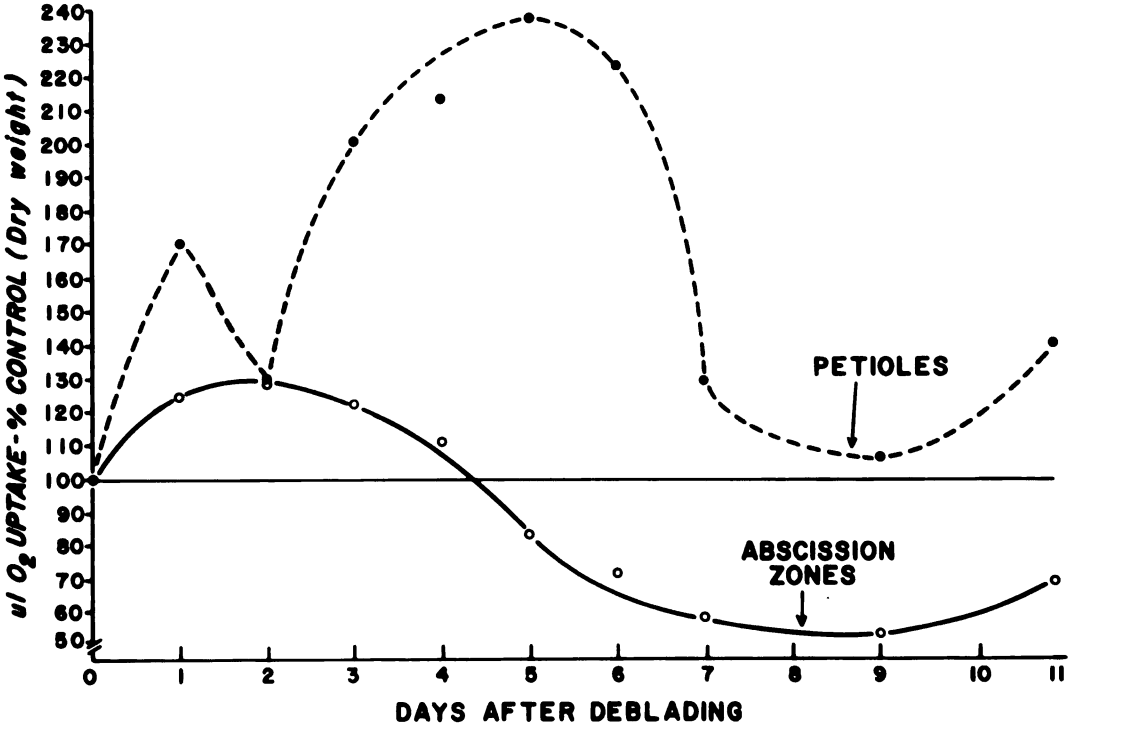
tissue, it was felt that possibly a change in respiratory rate could occur during the first 24 hours and which may not have been detected by respiration studies on 24 hour intervals. Analysis of oxygen uptake every six hours during the first 24 hour period after deblading indicated no change in respiration of the abscission zones or the petioles (Figure 19 Insert).

Since the dry weight changes varied markedly during formation of the abscission layer, the respiration data were corrected for dry weight and reported in Figures 21 and 22. On a dry weight basis the abscission zone respiration rate gradually increased for the first four days with a marked stimulation beginning four days after deblading (Figure 21A). The respiration rate of the petioles declined with increasing time after deblading, similar as when expressed on a per section basis but to a lesser degree (Figure 21C). Abscission zones from auxin treated plants maintained a higher rate of respiration than non-treated tissue until four days after deblading. From six days after deblading through separation the respiratory rate gradually decreased (Figures 21B). By contrast, the auxin-treated petioles exhibited an immediate increase after 24 hours, followed by a sharp decrease at two days. The rate again increased sharply at three days and remained high through six days following deblading at which time respiration dropped sharply at seven days with a slight decline continuing through separation (Figure 21D).

Figure 21.--Respiration rate in terms of dry weight of abscission layer and petiole sections of Contender bean following deblading, with and without auxin treatment. (A) abscission zones without auxin, (B) abscission zones with auxin (10^{-3} M. 3-chlorophenoxy-α-propionic acid), (C) petioles without auxin, (D) petioles with auxin. Curves drawn through the sample average.

Figure 22.--Oxygen uptake of auxin treated, debladed bean abscission zones and petioles expressed as percent of control on a dry weight basis.


A = ABSCISSION ZONES
B = ABSCISSION ZONES WITH AUXIN
C = PETIOLES
D = PETIOLES WITH AUXIN
µl O2 UPTAKE/HOUR/mg DRY Wt.
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
1
2
3
4
5
6
7
8
9
10
11
A
B
C
D
DAYS AFTER DEBLADING


µl O2 UPTAKE-% CONTROL (Dry weight)
240
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
0
1
2
3
4
5
6
7
8
9
10
11
PETIOLES
ABSCISSION ZONES
DAYS AFTER DEBLADING

Expressing these respiration data as percent of control, resulted in an entirely different relationship of auxin affect on petioles compared to abscission zones. The peak rate of respiration in the abscission zones occurs at two days after deblading, whereas, the peak in the petioles occurred five days after deblading (Figure 22). The discrepancy which occurs between the two methods of expressing the data will be discussed later.

Enzyme Activity

Pectin Methylesterase

The enzyme pectin methylesterase (PME) was found to be active in the abscission zone, petiole and stem of the bean. PME activity was highest in the abscission zone, followed by the petiole and lowest in the stem (Figure 23). Abscission zone PME exhibited essentially no change during abscission layer formation with a slight decline at separation. By contrast, petiole PME activity increased sharply approximately 24 hours after deblading and continued increasing through separation. Stem PME activity increased 24 hours following deblading with a gradual decrease through separation.

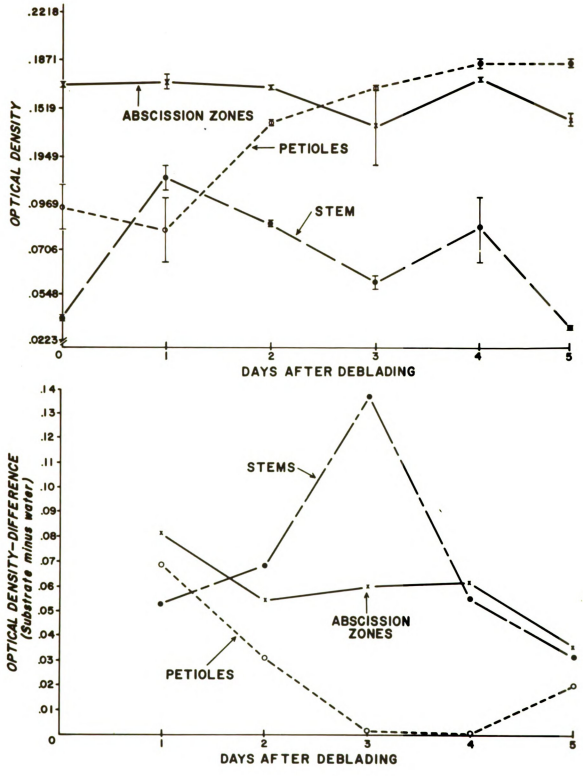
Polygalacturonase

The changes and solubilization of pectin materials during abscission suggested the possibility of enzymatic action other than PME.

The enzyme polygalacturonase was found to be active in the abscission zone, petiole, and stem of the bean during

Figure 23.--Pectin methylesterase activity in the abscission zone, petiole and stem of Contender bean during formation of the abscission layer induced by deblading.

Figure 24.--Polygalacturonase activity in the abscission zone, petiole and stem of Contender bean during formation of the abscission layer induced by deblading.

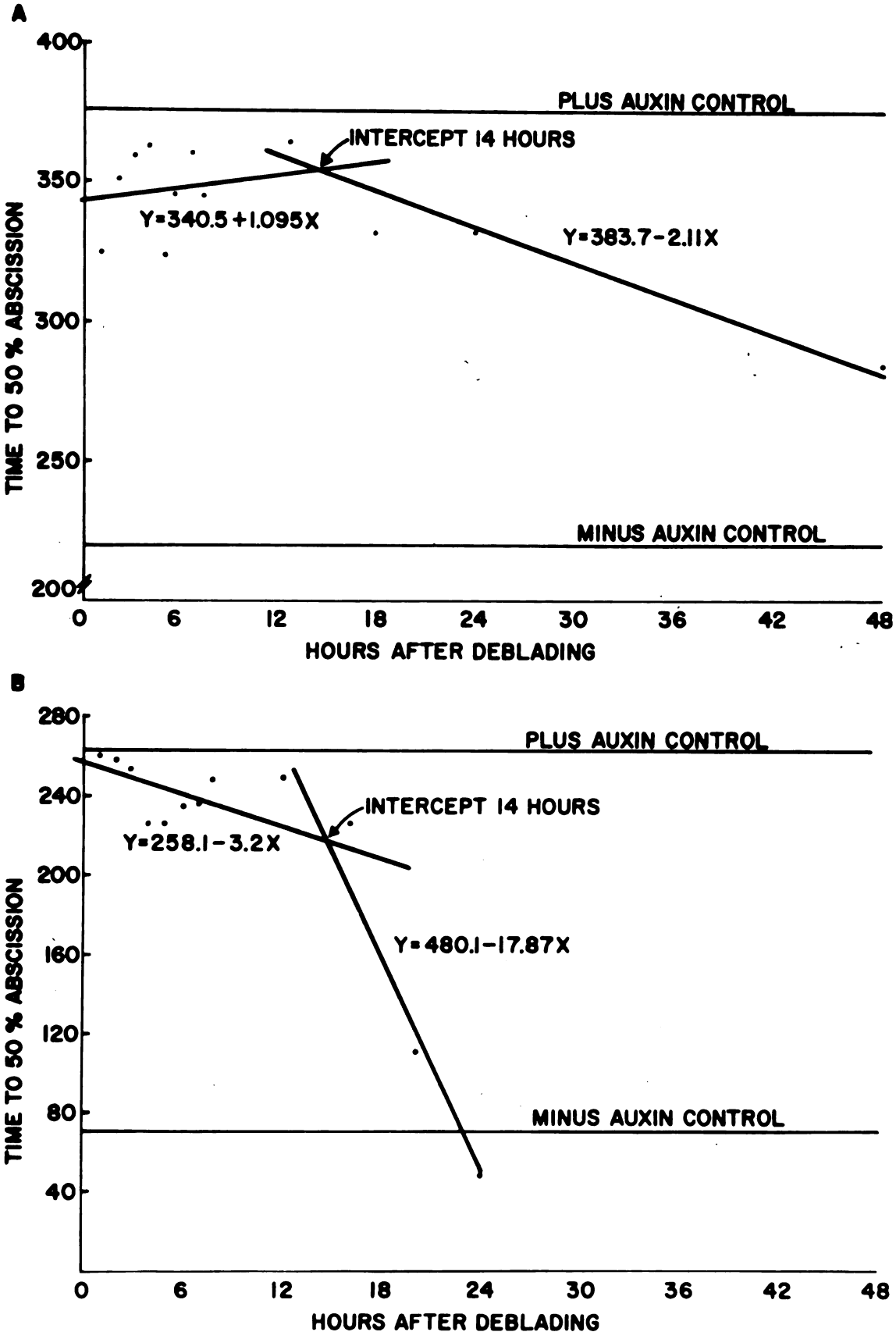

OPTICAL DENSITY
.2218
.1871
.1519
.1949
.0969
.0706
.0548
.0223
ABSCISSION ZONES
PETIOLES
STEM
0
1
2
3
4
5
DAYS AFTER DEBLADING
OPTICAL DENSITY-DIFFERENCE
(Substrate minus water)
.14
.13
.12
.11
.10
.09
.08
.07
.06
.05
.04
.03
.02
.01
0
STEMS
ABSCISSION ZONES
PETIOLES
1
2
3
4
5
DAYS AFTER DEBLADING

abscission layer formation (Figure 24). The abscission zones had moderate enzymatic activity which remained essentially the same for the first four days after deblading and then dropped slightly just prior to petiole separation. In the petioles, the activity declined rapidly for the first three days after deblading with no activity apparent at three or four days. On the fifth day some activity was detectable. Polygalacturonase activity in the stem, quite unlike that of the petiole and abscission zone, increased rapidly to a maximum at three days after deblading then dropped sharply to a minimum at five days.

Effective Time of Auxin Application in Delaying Abscission

Auxin (3-CP) applied up to approximately 14 hours after deblading delayed petiole abscission over the non-auxin treated control and was essentially equally as effective as when applied immediately after deblading. Comparable results were obtained at 21° C. (Figure 25A) and 25° C. (Figure 25B). The application of auxin to debladed petioles after 14 hours was significantly less effective than during the first 14 hours, and at 25° C. when applied 24 hours after deblading accelerated abscission as compared to either the auxin-treated or non-treated control. Calculation of best fit lines through the two apparent slopes at each temperature resulted in an intercept for both temperatures at about 14 hours. These data can be interpreted as demonstrating the presence of an

Figure 25.--Effect of auxin (3-CP) on abscission of debladed petioles when applied at various times after deblading.
A-400 foot candles continuous light and 21°C.
B-400 foot candles continuous light and 25°C.


A
400
350
300
250
200
PLUS AUXIN CONTROL
INTERCEPT 14 HOURS
Y=340.5+1.095X
Y=383.7-2.11X
MINUS AUXIN CONTROL
TIME TO 50 % ABSCISSION
0
6
12
18
24
30
36
42
48
HOURS AFTER DEBLADING
B
280
240
200
160
120
80
40
PLUS AUXIN CONTROL
INTERCEPT 14 HOURS
Y=258.1-3.2X
Y=480.1-17.87X
MINUS AUXIN CONTROL
TIME TO 50 % ABSCISSION
0
6
12
18
24
30
36
42
48
HOURS AFTER DEBLADING

abscission induction period of approximately 14 hours, during which auxin can reverse or delay the effect of defoliating. After induction auxin is less effective, and at higher temperatures may actually accelerate abscission.

DISCUSSION

The abscission process can be described as a sequence of events which is initiated by the onset of senescence in nature, and by deblading in experimental material; the entire process being terminated by separation of the auxillary organ from the main axis or stem of the plant.

Abscission layer formation in the lower pulvinus of bean plants with roots attached is typical of most higher plants. Terpstra (116) found that removal of roots signigicantly accelerated abscission. Proximal applications of auxin retarded abscission in intact plants and accelerated abscission in explants (2). The presence of roots, therefore, is an important factor in abscission and strongly supports the desirability of the debladed bean seedling test system used in these studies.

Induction, Abscission Layer Development and Cell Wall Changes

Upon deblading, the petiole, abscission zone or both undoubtedly involve an induction mechanism. Using debladed plants at 21° C. and hourly applications of 10^{-3} M. 3-CP the induction period in Contender bean was found to be completed approximately 14 hours after deblading. At a higher temperature (25° C.) the induction time was the same, but the formation of the abscission layer was accelerated, and separation occurred earlier.

Rubenstein and Leopold (104) using bean explants (as described by Addicott et al. [11]) treated with 5×10^{-4} M. NAA found an induction period of six or more hours. Their shorter induction period may have resulted from: (a) explants versus intact plants; (b) auxin concentration. Nevertheless, by using two separate test systems two separate laboratories have come to the same conclusion: auxin delays abscission by inhibition of the inductive mechanism. Auxin application following completion of induction was less effective in delaying abscission and may accelerate separation.

Respiration of both the abscission zone and petiole tissue during the first 24 hours following deblading (during induction) did not change appreciably.

The abscission layer begins to form upon completion of induction. Appearance of the abscission layer microscopically, in a population of debladed plants, was not apparent until two to three days after deblading. The abscission layer in the bean begins to form at the junction of the axillary bud and the petiole and proceeds across the adaxial cortical tissue to the conductive tissue. In the bean, as in many other plants, no layer is formed through the conductive tissue. The layer then develops across the abaxial cortical tissue completing the abscission layer.

Cell division has been reported as a necessary prerequisite to abscission in Black Valentine bean (22) and cotton (50). Histones (basic amino acids), which generally occur in the genetic material of the cell just prior to

division, were not present in the abscission zone during abscission layer formation and separation. This indicates that abscission in Contender bean plants does not involve cell division. Further, no increase in respiration was evident during layer formation in the abscission zone, which would be expected if cell division were taking place.

Development of the abscission layer appeared to involve a separation and dissolution of cells, implicating changes in the pectic materials of both the middle lamella and the cell walls. The pectic materials (identified as stained areas with ruthenium red) began swelling two days after deblading and continued to swell through separation. The change in pectic materials suggested possible changes in the calcium content of the abscission zone. Quantitative determinations of calcium revealed a decrease in calcium of the abscission zone beginning two days after deblading. This loss of calcium corresponded to the first appearance of swelling of the pectic materials. As the calcium content continued to decrease there was a concomitant increase in swelling or configurational change in the pectic substances. If alteration in pectic materials is a prerequisite for abscission layer formation as suggested by several investigators (2, 36, 108), the delay induced in abscission by exogenous auxin may be reflected in a delay in loss of calcium and subsequent swelling of the pectic materials. The conversion of calcium pectate to pectic acid is perhaps the chemical change responsible for pectin swelling (2, 108).

If this assumption is correct, then a quantitative delay in loss of calcium should be indicative of the effect of auxin on conversion of calcium pectate to pectic acid. Auxin (10^{-3} M. 3-CP) delayed abscission of debladed petioles by approximately four days (Figures 8 and 9), and caused a corresponding four day delay in the minimum inflection point of calcium in the abscission zone and thus conversion of calcium pectate to pectic acid. The degree of pectin swelling, therefore, appears to be correlated with the loss of calcium.

Configurational changes observed in pectic materials led to an investigation of the pectic enzymes. Demethylation of pectins by pectin methylesterase (PME) has been reported to occur in the Coleus abscission zone (95). In these studies marked PME activity was found to occur in the abscission zone with correspondingly lower amounts in the petiole and stem. It is significant that PME activity of the abscission zones remained relatively constant during abscission layer formation dropping only slightly at separation. In contrast, petiolar PME increased sharply 24 hours after deblading and continued to increase through separation. In stem tissue PME activity increased sharply during the first 24 hours followed by a slow gradual decline in activity through separation. The separation of cells (abscission) cannot be explained on the basis of PME activity, per se.

Significant, however, is the observation that considerable activity of the pectin hydrolyzing enzyme, polygalacturonase (PG), which has not been reported prior to this

time, was found in the abscission zone, petiole and stem during abscission layer formation. PG activity in the abscission zone, like PME, remained relatively uniform throughout abscission layer development. In the petiole PG activity began to fall rapidly 24 hours after deblading with essentially no activity detectable after three days. In stem tissue PG activity was maximum three days after deblading then dropped to a minimum during the later stages of abscission.

Naturally occurring cell wall pectins have been reported to be highly methylated (18 percent) (69). Other carboxyl groups participate in formation of calcium bridges between pectic acid chains thus solidifying the pectins. The PME which was found in the abscission zone was probably activated by the initial increase in calcium (69), thus demethylating the pectic chains. The decrease in calcium, after 48 hours, coupled with the demethylation by PME continued to provide free carboxyl groups in the cell walls of the abscission layer. Thus, providing the necessary substrate for PG hydrolysis of the pectin chain. Two adjacent free carboxyl groups are essential if PG is to cleave the pectic acid chain. This is probably the first step in cellular dissolution of the abscission layer.

The presence of calcium ions is known to inhibit PG hydrolysis of pectic acid (33a). The movement of calcium from the abscission zone therefore removes this enzyme inhibitor leaving PG in the active form in the abscission

layer, and resulting in pectic acid hydrolysis. By contrast, PME activity in the petiole begins to increase and this increase corresponds to the movement of calcium from the abscission zone to the petiole. PG activity, although relatively high in the petiole originally, rapidly decreased and this also occurred at the same time that calcium increased, thus suggesting that as calcium moves to the petiole it may cause inhibition of PG activity. The increase in PME activity coupled with the decrease in PG activity of the petiole provide numerous free carboxyl groups, thus establishing an excellent physical system for movement of calcium from the abscission layer to the petiole. The pH of both the abscission zone and petiole during abscission layer formation is within the range of PME and PG activity, although not at their optimums.

Auxin is known to bind PME to cell walls (43) and in this form PME is inactive. Hence, as long as auxin supply to the petiole and abscission layer is adequate it will delay abscission by maintaining PME in the bound form, thereby, delaying the alteration of pectin. If the leaf is debladed, the auxin source is removed, PME is released from the cell walls, demethylates pectin, and provides a potential for calcium movement from the abscission zone. These studies have established that all of these events can be delayed to some degree by replacing the leaf blade with auxin.

The interaction of PME and PG in the stem coupled with physical stress correlate closely with the conversion

of the cellular stem to the hollow stem further supporting the activity of these enzymes in cellular dissolution.

The collapsing of cells in the abscission layer indicates that cell wall components other than pectin are altered or broken down. Facey (36) concluded that cellulose is altered by hydration but not broken down.

Sequential extraction of cell wall components two days after deblading indicated no cell wall changes in the potential abscission layer. Concomitant with the appearance, microscopically, of the abscission layer was the appearance of small dark staining particles indicative of breakdown of one or more cell wall materials, probably by enzymatic action. The cells (two to four layers) distal and immediately adjacent to the abscission layer stained much darker in contrast to the corresponding cells immediately adjacent on the stem side of the abscission layer. Ammonium oxalate extraction (pectins) produced no change in the dark staining fragments, nor the dark staining cells thus eliminating the pectins and pectic enzymes as the cause and source, respectively, of the dark staining fragments. Four percent NaOH, which extracted the hemicellulose did not alter the staining characteristics, thus eliminating the hemicelluloses. Extraction of the non-cellulosic polysaccharides (17 percent NaOH) resulted in a partial reduction of the dark staining fragments in the abscission layer, suggesting that the non-cellulosic polysaccharides and cellulose were those cell wall materials probably broken down by enzymatic action.

The dark staining cells immediately adjacent to the abscission layer were not altered by extraction suggesting that the differences in staining properties of these cells may be due to changes in cellulose content or cellulose configuration. Lignin, the only known substance which interferes with the above reaction, was not present in the abscission zone as confirmed by a negative phloroglucinol test. Thus, the dark staining reaction of the cells adjacent to the abscission layer can be attributed to cellulose.

Preston (98), in studying the structure of cell walls, concluded that molecular chains of pectic acid are linked both to hemicullulose and cellulose by calcium bridges and phosphate groups. Therefore, removal of calcium, coupled with enzymatic breakdown of cellulose and non-cellulosic polysaccharides would account for abscission layer formation and organ separation. In the adjacent, distal cells calcium is also decreasing, solubilizing the pectin and allowing for expansion and hydration of the cellulose chain, thus opening more binding sites for dye reaction. The cells on the proximal side do not lose calcium, the pectins are not altered, thus no hydration and as a result, no dark staining.

The breakdown of cell wall materials does not begin until after the pectins have lost considerable calcium and become markedly swollen. The calcium change, pectin swelling, and cell wall breakdown follow a logical sequence of events leading to separation.

Respiration

The participation of enzymes in the abscission process may explain why abscission is oxygen dependent. The dependence of abscission on respiration has been amply demonstrated (2, 3, 26, 28, 73, 100). The increase in respiration of non-auxin-treated abscission zones (Figure 20) on a dry weight basis, corresponded closely to the most rapid swelling of pectic materials and to the breakdown to cell wall constituents. The respiratory rise in the abscission zones was not evident on a section basis. Respiration per section gives a much better evaluation of normal abscising tissue since loss in dry matter is characteristic of senescence (Figure 14). Thus, correcting for the loss may lead to false assumptions. Petiolar respiration (non-treated) decreased continuously during abscission layer formation, indicative of senescing tissue. Auxin (10^{-3} M. 3-CP) applied to the cut petiole stimulated respiratory activity above that of the control. Similarly, the rate of respiration of the auxin-treated petiole remained well above that of the non-treated petiole indicating that auxin was maintaining the metabolic activity of both the abscission zone and the petiole. It is significant that maximum oxygen uptake of both the auxin-treated petioles and abscission zones occurred at the same time. This simultaneous occurrence of peaks in both tissues indicates that auxin may be delaying abscission by maintaining metabolic activity in the petiole and abscission zone concomitantly, and not by direct action on the

abscission layer as has been suggested (18, 19).

Dry Weight Changes, Metabolite Movement and pH

The application of auxin to plant tissues affects two important processes: (a) growth and (b) membrane permeability (105, 106, 107). By maintaining membrane permeability and growth, the loss in dry weight of auxin-treated abscission zones and petioles should be significantly reduced. This in fact did occur in the bean. Auxin-treated abscission zones increased in dry weight during the first 24 hours as did the non-treated controls; however, the loss of dry matter in the auxin treated, following the initial increase, was slower and dry matter levels were higher over a longer period of time. The auxin-treated petioles increased in dry weight for 24 hours longer than non-treated petioles and, again, the rate of loss in the auxin-treated petioles was slower than in the control.

The initial loss in dry weight of non-treated abscission zones corresponded to the beginning of swelling in the pectic materials. The greatest loss in dry matter occurred at the time the cell walls were beginning to break down and at this time membrane permeability would be expected to be low and as a result low retention of metabolites would be expected.

The loss in dry weight of non-treated petioles through the fourth day can likewise be attributed to loss in membrane permeability. At this point (four days), however, the abscission zone is well formed and imparts a

block of further movement of metabolites through the cortical tissue of the petiole.

By contrasting the change in dry weight of auxin-treated and non-treated tissue, the data confirm the concept that auxin is maintaining cellular integrity and stimulating growth resulting in maintenance of dry matter by delaying senescence.

Changes in permeability were further established with ^{86}Rb, ^{32}P and ^{45}Ca. Early in the development of the abscission layer phosphorus export was very low as was rubidium export. In contrast, calcium export was greatest during the same time period. Between the third and fourth day the export rate was altered considerably in all three cases, Rb and P increasing and Ca decreasing. This suggests that membrane integrity was lost at this time markedly altering the movement of metabolites from the petiole to the stem. Once membrane integrity was lost movement of Rb and P rapidly reached a maximum indicating the absence of a physiological barrier. With the increase in membrane permeability movement of exogenous Rb and P would be primarily through the xylem.

Calcium was probably moving from the petiole in complex with organic materials. Hence, as long as cellular integrity was maintained and metabolites were available for complexing with calcium, movement from the petiole was possible. Once cellular integrity was lost the metabolites were utilized in respiration and calcium no longer moved

from the petiole. Although there was basipetal movement of calcium the actual amount moving into the stem was very low.

The buffering capacity of the cells of the abscission zone and petiole would be expected to be lost as membrane permeability increased and cellular dissolution occurred. The pH of abscission zones and petioles, both in treated and non-treated seedlings, after deblading dropped initially. The increase in hydrogen ions occurred during the induction period, although the significance of this increase is not understood.

In the non-auxin-treated abscission zones and petioles the rise in pH began at the same time the pectins began to swell and calcium was decreasing. The maximum pH peaks corresponded to the breakdown of cell wall materials, more rapid dry weight loss and movement of isotopes (except calcium). By contrast the pH peak for auxin-treated abscission zones and petioles occurred at the same time in reference to the physiological changes, but all occurred three days later than in non-auxin-treated tissue, further substantiating that auxin was maintaining cellular integrity.

Mothes (86) demonstrated that metabolites would move toward and accumulate at the site of application of kinetin. If the rate of movement of nutrients and metabolites could be stopped or reversed in the petiole then abscission should be delayed.

High concentrations (10^{-2} M.) of N-6-BA significantly

delayed abscission when the opposite leaf was left intact, whereas with the opposite leaf removed there was essentially no effect on abscission indicating that the stem was not an adequate source of metabolites. Thus, at high concentrations N-6-BA directed movement from a source of metabolites (opposite intact leaf) to the cut petiole maintaining growth and cellular integrity for an extended length of time. At low concentrations (10^{-5} M.) N-6-BA accelerated abscission in both plants with an opposite leaf and plants with the opposite leaf removed (to a lesser degree in the latter). Transport again appeared to be directed to the site of application but the concentration was not high enough to cause movement of metabolites from the opposite leaf. Hence, there was a depletion of metabolites from the lower petiole and abscission zone resulting in accelerated abscission layer formation. The biphasic curve for N-6-BA resembles closely that of auxin in that high concentrations delay abscission and low concentrations stimulate abscission. These data are supported by the findings of Osborne (94) where kinetin applied to both ends of a single abscission zone explant accelerated abscission, and when applied directly to the abscission layer delayed abscission. These findings suggest that in the first case transport was directed away from the abscission layer and in the second case to the abscission layer.

Site of Auxin Action

The effects of high and low auxin levels on abscission are well documented (2, 3, 4, 5, 6, 7, 8, 15, 18, 19, 20, 30,

31, 34, 36, 37, 38, 41, 49, 50, 51, 54, 55, 58, 59, 60, 62, 63, 65, 70, 71, 77, 79, 84, 87, 88, 93, 99, 102, 104, 108, 110, 111, 114, 116, 122, 126, 127, 129). Auxin will completely replace the leaf blade in Coleus; however, with many plants auxin can at most only delay the process.

Both 10^{-3} M. and 10^{-5} M. ^{14}C-NAA (ring labeled) when applied to the cut petiole accumulated in the cell walls immediately adjacent and distal to the abscission layer. No localization, at either concentration, occurred in the abscission zone per se or in any tissue proximal to the abscission layer. The accumulation of NAA (10^{-3} M.) in this tissue suggests that the formation of the abscission layer and the resulting dissolution of cells formed a physical barrier to movement, through the cortex, of materials both in and out of the petiole.

The addition of 10^{-3} M. NAA to the cut petiole delayed abscission by three days, whereas treatment with 10^{-5} M. NAA accelerated abscission by approximately two days over control plants. Although the end result of high and low concentrations of NAA produced inhibition and acceleration, respectively, the localization and accumulation were similar, that is, accumulation occurred only in the cell walls distal and immediately adjacent to the abscission layer. This accumulation at both 10^{-3} and 10^{-5} M. increased the auxin gradient from petiole to abscission layer (auxin distal/auxin proximal) but did not prevent abscission. In fact, 10^{-5} M. NAA not only accelerated abscission but also increased the auxin gradient

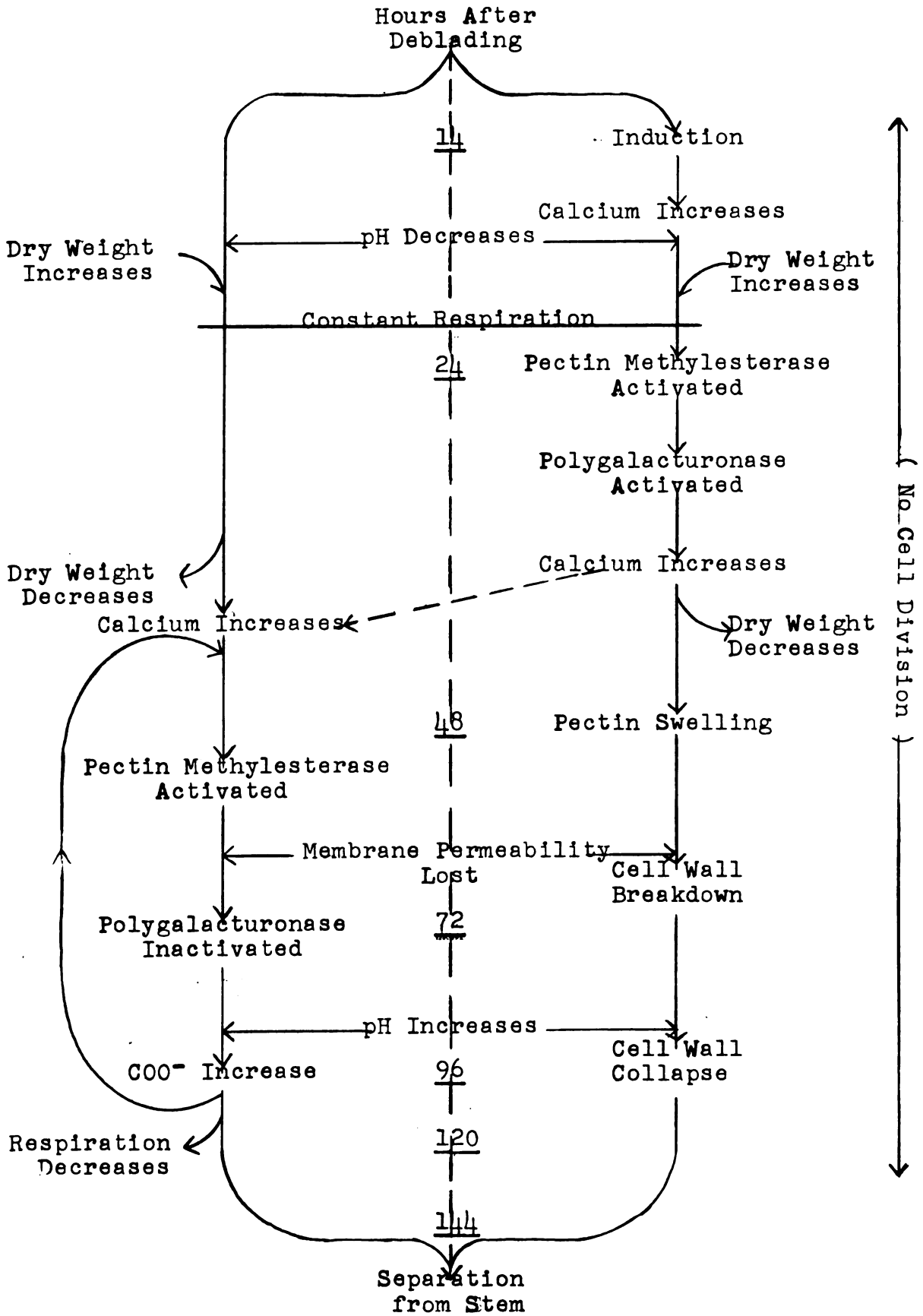
(Figure 11E), thereby providing direct evidence contrary to both the "Auxin Gradient Theory" and the "Auxin Concentration Theory" as control mechanisms in abscission.

NAA (10^{-3} M.) applied after deblading moved at higher concentrations in the xylem (Figure 13B) than in the cortex of the same petiole.

Pathway of abscission

This study has indicated that there is a sequence of events, beginning with deblading, which occur during the development of the abscission layer. Figure 26 illustrates these changes and is presented as a purposed pathway for abscission in the bean.

Figure 26.--Sequence of changes in the petiole and abscission zone during abscission layer formation.


Hours After Deblading
14
Induction
Calcium Increases
pH Decreases
Dry Weight Increases
Dry Weight Increases
Constant Respiration
24
Pectin Methylesterase Activated
Polygalacturonase Activated
Calcium Increases
Dry Weight Decreases
Calcium Increases
Dry Weight Decreases
48
Pectin Swelling
Pectin Methylesterase Activated
Membrane Permeability Lost
Cell Wall Breakdown
Polygalacturonase Inactivated
72
pH Increases
Cell Wall Collapse
COO⁻ Increase
96
120
Respiration Decreases
144
Separation from Stem
No Cell Division

SUMMARY

Abscission layer formation in the bean (Phaseolus vulgaris L. cv. Contender) was studied with respect to its morphological, chemical, and physiological changes utilizing microchemical and histochemical techniques.

1. Morphological Changes
   a. An induction period of 14 hours was established in the bean. The length of induction was found not to be affected by altering the environmental conditions. No increase in respiration was detectable during the induction period.
   b. The appearance of the abscission layer, microscopically, occurred two to three days following deblading; by four days all plants sampled from a debladed population contained an abscission zone.
   c. Cell division does not occur in abscission layer formation as indicated by a negative test for histones.
2. Chemical Changes in the Abscission Zone and Petioles
   a. The pectin materials of the abscission layer began to swell two days after deblading. The degree of swelling increased until five days after deblading (layer formation complete).

b. Calcium content of the abscission layer increased 24 hours after deblading then decreased gradually to a minimum at separation.

c. Calcium in the petiole increased as the calcium in the abscission zone decreased. The decrease in calcium of the abscission zone can be accounted for by the increase in calcium of the petiole.

d. Auxin treatment (10^{-3} M. 3-CP) of debladed petioles delayed the build up of calcium in both the abscission zone and petioles by four days.

e. The non-cellulosic polysaccharides and celluloses were broken down in abscission layer formation beginning three days after deblading and continuing until separation occurred.

f. The cellulose was altered in the cells immediately adjacent to the abscission layer on the distal side beginning three days after deblading with no alteration of the cells on the proximal side of the abscission layer.

g. There was no lignin accumulation in the abscission zone until five days after deblading, then a slight amount appeared on both sides of the abscission layer.

h. Pectin methylesterase and polygalacturonase were operative in abscission layer formation, structurally weakening the cell walls leading to separation.

i. Pectin methylesterase activity of the petiole

coupled with the decrease in polygalacturonase activity provide a physical mechanism for calcium movement from the abscission zone to the petiole.

3. Physiological Changes in the Abscission Zone and Petiole During Abscission Layer Formation

   a. The dry weight of the abscission zone increased during the first 24 hours after deblading, then decreased rapidly to a value one half that of its maximum by the time of separation. Dry weight of the abscission zone after auxin treatment also increased during the first 24 hours but then decreased at a much slower rate.

   b. The dry weight of non-auxin treated petioles increased during the first 24 hours following deblading. The dry weight then fell at a faster rate than the abscission zones for four days after deblading. The loss was then checked by the complete formation of the abscission layer which acted as a physical barrier to movement through the cortical tissue. Auxin treated petioles gained dry weight for 48 hours after deblading then decreased at a much slower rate than the non-treated petioles.

   c. ^{32}P and ^{86}Rb when applied to the debladed petiole did not move out of the petiole into the stem until membrane integrity was lost (three days after deblading) in the cells of the petiole after which movement was by simple diffusion. ^{45}Ca moved only

when there were metabolites to complex with it and carry it out. Once cellular integrity was lost ^{45}Ca did not move from the petiole.

d. The pH of the abscission zone and petiole of non-treated tissue dropped initially then increased to a peak five days after deblading. With auxin treated tissue there was an initial decrease, however, the rise was delayed until seven days after deblading. The initial drop in pH may possibly be associated with initial abscission layer formation although the rise in pH both with and without auxin was a result of abscission layer formation and was not responsible for its initial formation.

e. N-6-Benzyladenine delayed abscission at 10^{-2} M. when the opposite leaf was intact; when the opposite leaf was removed abscission time was not affected by any concentration.

f. The time course study of respiration of auxin treated and non-treated tissues indicated that auxin controls abscission indirectly by stimulating growth of both the petiole and the abscission zone.

4. Site of Action of Auxin on Abscission

a. When ^{14}C-NAA (10^{-5} M.) was applied to the cut petiole abscission was accelerated. No NAA was localized in the abscission layer. Six days after deblading NAA was localized in the cell walls immediately adjacent to the abscission layer on

the petiole side.

b. Application of ^{14}C-NAA (10^{-3} M.) to cut petioles delayed abscission. Concomitant with the first visual evidence of abscission layer formation was a localization of NAA in the cell walls immediately adjacent to the abscission layer on the petiole side. As abscission layer formation progressed, NAA continued to accumulate just above the abscission layer. No localization occurred in the abscission layer per se.

c. After deblading ^{14}C-NAA (10^{-3} M.) was found to move through the xylem elements as well as between the cells of the cortex.

d. By direct proof the "Auxin Gradient Theory" and the "Auxin Concentration Theory" were found to be invalid as control mechanisms in abscission.

LITERATURE CITED

1. Abeles, F. B. and B. Rubenstein. 1964. Regulation of ethylene evolution and leaf abscission by auxin. Plant Physiol. 39: 963-969.

2. Addicott, F. T. 1964. Physiology of abscission. Encycl. of Plant Physiol. XV/2: 1094-1126.

3. _____. 1961. Auxin in the regulation of abscission. Encycl. of Plant Physiol. 14: 829-838.

4. _____, and R. S. Lynch. 1951. Acceleration and retardation of abscission by indole-acetic acid. Science 114: 688-689.

5. _____, and R. S. Lynch. 1955. Physiology of abscission. Annual Review of Plant Physiol. 6: 211-238.

6. _____,J. B. Storey and D. S. Louie. 1955. Auxin, auxin gradient, and leaf abscission in cotton. 10th Ann. Cotton Def. Conf., Memphis, Tenn.

7. _____,R. S. Lynch and H. R. Carns. 1955. Auxin gradient theory of abscission regulation. Science 121: 644-645.

8. _____.1954. Abscission and plant regulators. In: Plant Growth Regulators in Agriculture. Edited by H. B. Tukey. Wiley and Sons, New York. pp. 99-116.

9. _____,G. Sekera, R. S. Lynch and H. K. Pratt. 1954. Auxin and ethylene in the regulation of abscission. Proc. Cotton Def. Conf., Memphis, Tenn. 8: 42-44.

10. _____,and V. T. Walhood. 1954. Leaf abscission as affected by blade, petiole, and defoliant. Proc. of 8th Annual Beltwide Cotton Def. Conf. 38-41.

11. _____,R. S. Lynch, G. A. Livingston and J. K. Hunter. 1949. A method for the study of foliar abscission in vitro. Plant Physiol. 24: 537-539.

12. Avery, G. S.,Jr. 1935. Differential distribution of a phytohormone in the developing leaf of Nicotiana, and its relation to polarized growth. Torrey Botanical Club Bulletin 62: 313-330.

13. 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.

14. _____,and B. J. Thomson. 1961. Effect of 1-Napthyl N-Methyl-carbamate (Sevin) on thinning apples. Amer. Soc. Hort. Sci. 77: 1-8.

15. Beal, J. M. and A. G. Whiting. 1945. Effect of indoleacetic acid in inhibiting stem abscission in Mirabilis jalapa. Botanical Gazette 106: 420-431.

16. _____. 1946. Reactions of decapitated bean plants to certain of the substituted phenoxy compounds. Botanical Gazette 108: 166-186.

17. Bell, T. A., J. L. Etchells, C. F. Williams and W. L. Porter. 1962. Inhibition of pectinase and cellulase by certain plants. Botanical Gazette 123: 220-223.

18. Biggs, R. H. 1958. Physiological basis of abscission in plants. Ph.D. Thesis, Purdue University.

19. _____,and A. C. Leopold. 1958. The two-phase action of auxin on abscission. Amer. Jour. Bot. 45: 547-551.

20. _____,and A. C. Leopold. 1957. Factors influencing abscission. Plant Physiol. 32: 626-632.

21. Bonner, J. 1936. The chemistry and physiology of the pectins. Botanical Review 2: 475-497.

22. Brown, H. S. and F. T. addicott. 1950. The anatomy of experimental leaflet abscission in Phaseolus vulgaris L. Amer. Jour. Bot. 37: 650-656.

23. Brown, L. C. and C. C. Wilson. 1952. Some effects of zinc on several species of Gossypium L. Plant Physiol. 27: 812-817.

24. Bryan, W. H. and E. H. Newcomb. 1954. Stimulation of pectin methylesterase activity of cultured tobacco pith by indoleacetic acid. Physiologia Plantarum 7: 290-297.

25. Byerrum, R. U. and C. S. Sato. 1956. The biosynthesis of pectin methyl ester groups. Proc. Plant Physiol. Meetings 31:37.

26. Carns, H. R. 1951. Oxygen, respiration and other critical factors in abscission. Ph.D. Thesis, University of Calif. at Los Angeles.

27. _____,F. T. Addicott, K. C. Baker and R. K. Wilson. 1961. Acceleration and retardation of abscission by Gibberellic acid. In: Plant Growth Regulation. Iowa State University Press, Ames. pp. 559-565.

28. _____,F. T. Addicott and R. S. Lynch. 1951. Some effects of water and oxygen on abscission in vitro. Plant Physiol. 26: 629-630.

29. Chandler, W. H. 1951. Deciduous Orchards. Lea and Febiger, Philadelphia. pp. 436.

30. Chatterjee, S. K. and A. C. Leopold. 1963. Auxin structure and abscission activity. Plant Physiol. 38: 268-273.

31. _____. 1963. Regulatory systems controlling leaf abscission. Ph.D. Thesis, Purdue University.

32. Cleland, R. 1963. The occurrence of auxin-induced pectin methylation in plant tissues. Plant Physiol. 38: 738-740.

33. _____,and J. Bonner. 1956. The residual effect of auxin on the cell wall. Plant Physiol. 31: 350-354.

33a. Corden, M. E., J. E. Elson and D. L. Bell. 1964. Inhibition of polygalacturonase by cations. Phytopath. 54: 1432.

34. Day, B. E. and L. C. Erickson. 1954. Relative effectiveness of the mono-, di-, and trichlorophenoxyacetic acids in preventing abscission of lemon leaves. Botanical Gazette 115: 285-288.

35. Doutt, M. T. 1932. Anatomy of Phaseolus vulgaris L. var. black valentine. Mich. Tech. Bull. 128: 1-31.

36. Facey, V. 1950. Abscission of leaves in Fraxinus americana L. New Phytologist 49: 103-116.

37. Gardner, F. E. and W. C. Cooper. 1943. Effectiveness of growth substances in delaying abscission of Coleus petioles. Botanical Gazette 105: 80-89.

38. Gaur, B. K. and A. C. Leopold. 1955. The promotion of abscission by auxin. Plant Physiol. 30: 487-490.

39. Gawadi, A. G. and G. S. Avery, Jr. 1950. Leaf abscission and the so-called "abscission layer." Amer. Jour. Bot. 37: 172-180.

40. Ginzburg, B. Z. 1961. Evidence for a protein gel structure cross-linked by metal cations in the intercellular cement of plant tissue. Jour. Expt. Bot. 12: 85-107.

41. Gorter, C. J. 1964. Studies on abscission in explants of Coleus. Physiologia Plantarum 17: 331-345.

42. Glasziou, K. T. 1957. The effect of 3-indolylacetic acid on the binding of pectin methylesterase to the cell walls of tobacco pith. Australian Jour. Bio. Sci. 10: 337-341.

43. _____. 1957. The effect of auxins on the binding of pectin methylesterase to cell wall preparations. Australian Jour. Bio. Sci. 10: 426-434.

44. _____,and S. D. Inglis. 1958. The effect of auxins on the binding of pectin methylesterase to cell walls. Australian Jour. Bio. Sci. 11: 127-141.

45. _____. 1959. The localization and properties of pectin methylesterase of avena coleoptiles. Physiologia Plantarum 12: 670-680.

46. _____,J. A. Sacher and D. R. McCalla. 1960. On the effects of auxins on membrane permeability and pectic substances in bean endocarp. Australian Jour. Bio. Sci. 47: 743-752.

47. _____. 1958. Effect of 2,4-dichlorophenoxyacetic acid on pectin methylesterase in tobacco pith sections. Nature 181: 428-429.

48. Goodwin, R. H. 1937. The role of auxin in leaf development in Solidago species. Amer. Jour. Bot. 24: 43-51.

49. Gur, A. 1962. The role of growth substances in the abscission of Avocado buds. Phyton 19: 179-184.

50. Hall, W. C. 1958. Physiology and biochemistry of abscission in the cotton plant. Texas Agriculture Experiment Station Bulletin. No. 138.

51. _____,and J. L. Liverman. 1956. Effect of radiation and growth regulators on leaf abscission in seedling cotton and bean. Plant Physiol. 31: 471-476.

52. _____,F. A. Herrero and F. R. H. Katterman. 1961. Leaf abscission in cotton. IV Effects of a natural promoter and amino acids on abscission in colytedonary node explants. Botanical Gazette 123: 29-34.

53. _____,and H. C. Lane. 1952. Compositional and physiological changes associated with the chemical defoliation of cotton. Plant Physiol. 27: 754-768.

54. _____. 1952. Evidence on the auxin-ethylene balance hypothesis of foliar abscission. Botanical Gazette 113: 310-322.

55. _____,and P. W. Morgan. 1964. Auxin-ethylene interrelationships. In: Regulateurs Naturels De La Croissance Vegetale. pp. 727-745

56. Heinicke, A. J. 1919. Concerning the shedding of flowers and fruits and other abscission phenomena in apples and pears. Amer. Soc. Hort. Sci. 16: 76-83.

57. _____. 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.

58. Hoshaw, R. W. and A. T. Guard. 1949. Abscission of marcescent leaves of Quercus palustris and Q. coccinea. Botanical Gazette 110: 587-593.

59. Jacobs, W. P. 1955. What makes leaves fall? Sci. Amer. 193 (5): 82-89.

60. _____. 1955. Studies on abscission: The physiological basis of the abscission-speeding effect of intact leaves. Amer. Jour. Bot. 42: 594-604.

61. _____. 1962. Longevity of plant organs: Internal factors controlling abscission. Ann. Rev. Plant Physiol. 13: 403-433.

62. _____,J. A. Shield, Jr. and D. J. Osborne. 1962. Senescence factor and abscission of Coleus leaves. Plant Physiol. 37: 104-106.

63. _____. 1962. The role of native growth substances in controlling the shedding of organs and abscission. Proceedings of the 16th International Horticultural Congress 5: 619-626.

64. _____. 1958. Further studies of the relation between auxin and abscission of Coleus leaves. Amer. Jour. Bot. 45: 673-675.

65. _____. 1959. What substance normally controls a given biological process? Developmental Biology 1: 527-533.

66. _____,M. P. Kaushik and P. G. Rochmis. 1964. Does auxin inhibit the abscission of Coleus leaves by acting as a growth hormone? Amer. Jour. Bot. 51 (8): 893-897.

67. Jensen, W. A. 1962. Botanical Histochemistry. W. H. Freeman and Company, San Francisco.

68. Johansen, D. A. 1950. Plant Embryology. Chronica Botanica Co. Waltham, Mass.

69. Kertesz, Z. I. 1951. The Pectic Substances. Interscience Publishers. New York, New York.

70. Laibach, F. 1933. Versuche mit wuchsstoffpaste. Ber. Deutsch Bot. Ges. 51: 386-392.

71. LaRue, C. D. 1936. The effect of auxin on the abscission of petioles. Proc. Nat. Acad. Sci. 22: 254-259.

72. Lee, E. 1911. The morphology of leaf fall. Annals of Botany 25: 52-106.

73. 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. Botanical Gazette 120: 144-151.

74. _____,and W. C. Hall. 1959. Foliar abscission in cotton. III. Macroscopic and microscopic changes associated with natural and chemically induced leaf-fall. Botanical Gazette 121: 9-16.

75. Lieberman, M. 1964. Ethylene biosynthesis in fruits. Proceedings of the First Eastern Experiment Station Collaborators' Conference on Post-Harvest Physiology (in press).

76. Liu, W. and H. R. Carns. 1961. Isolation of abscisin, an abscission accelerating substance. Science 134: 384-385.

77. Livingston, G. A. 1950. In vitro tests of abscission agents. Plant Physiol. 25: 711-721.

78. Lloyd, F. E. 1920. Environmental changes and their effect upon boll-shedding in cotton. Annals of New York Academy of Sciences 29: 1-131.

79. Louie, D. S. 1960. Physiology of leaf abscission in cotton. I. Leaf moisture and defoliations. II. Acceleration of abscission by IAA. Ph.D. Thesis, University of California, Los Angeles.

80. Luckwill, L. C. 1953. Studies of fruit development in relation to plant hormones. II the effect of naphthalene acetic acid on fruit set and fruit development in apples. Jour. Hort. Sci. 28: 25-40.

81. _____. 1957. Hormonal aspects of fruit development in higher plants. Symposia of the Society of Experimental Biology 11: 63-85.

82. Mason, T. G. 1922. Growth and abscission in sea island cotton. Annals of Botany 36: 457-484.

83. Matzke, E. B. 1936. The effect of street lights in delaying leaf-fall in certain trees. Amer. Jour. Bot. 23: 446-452.

84. Milbrath, J. A. and H. Hartman. 1940. Holly defoliation prevented by a-NAA. Science 92: 401.

85. Mitchell, J. W., G. A. Livingston and P. C. Marth. 1958. Test methods with plant regulating chemicals. USDA Agri. Handbook No. 126. pp. 2-3.

86. Mothes, K. and L. Englebrecht. 1961. Kinetin-induced directed transport of substances in excised leaves in the dark. Phytochemistry 1: 58-62.

87. Myers, R. M. 1940. Effect of growth substances on the abscission layer in leaves of Coleus. Botanical Gazette 102: 323-338.

88. Nelson, R. F. 1960. An investigation of a chemical mechanism of foliar abscission. Ph.D. Thesis, Iowa State University.

89. Oland, K. 1963. Changes in the content of dry matter and major nutrient elements of apple foliage during senescence and abscission. Physiologia Plantarum 16: 682-693.

90. Olmsted, C. E. 1951. Experiments on photoperiodism, dormancy, and leaf age and abscission in sugar maple. Botanical Gazette 112: 365-393.

91. Ordin, L., R. Cleland and J. Bonner. 1957. Methyl esterification of cell wall constituents under the influence of auxin. Plant Physiol. 32: 216-220.

92. Osborne, D. J. 1955. Acceleration of abscission by a factor produced in senescent leaves. Nature 176: 1161-1163.

93. _____. 1958. The role of 2,4,5-T butyl ester in the control of leaf abscission in some tropical woody species. Tropical Agriculture 35: 145-158.

94. _____,and S. E. Moss. 1963. Effect of kinetin on senescence and abscission in explants of Phaseolus vulgaris L. Nature 200: 1299-1301.

95. _____. 1958. Changes in the distribution of pectin methylesterase across leaf abscission zones of Phaseolus vulgaris. Jour. Exp. Bot. 9: 446-457.

96. Park, J. T. and M. J. Johnson. 1949. A submicro determination of glucose. Journal of Biological Chemistry 181: 149-151.

97. Pratt, H. K. 1954. Direct chemical proof of ethylene production by detached leaves. Plant Physiol. 29: 16-18.

98. Preston, R. D. 1964. Structural Plant Polysaccharides. Endeavour 90: 153-159.

99. Rasmussen, H. P. and M. J. Bukovac. 1963. Effects of 3-chlorophenoxy-α-propionic acid on abscission of peach fruits and leaves. Abstract of Amer. Soc. Hort. Sci. Meetings. pp. 64-65.

100. 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.

101. Rossetter, F. N. and W. P. Jacobs. 1953. Studies on abscission: The stimulating role of nearby leaves. Amer. Jour. Bot. 40: 276-280.

102. Rubinstein, B. 1962. Biochemical systems controlling bean leaf abscission. M. S. Thesis. Purdue University.

103. _____,and A. C. Leopold. 1962. Effects of amino acids on bean leaf abscission. Plant Physiol. 37: 398-401.

104. _____,and A. C. Leopold. 1963. Analysis of the auxin control of bean leaf abscission. Plant Physiol. 38: 262-267.

105. Sacher, J. A. 1957. Relationship between auxin and membrane-integrity in tissue senescence and abscission. Science 125: 1199-1200.

106. _____. 1959. Studies on auxin-membrane permeability relations in fruit and leaf tissues. Plant Physiol. 34: 365-372.

107. _____,and K. T. Glasziou. 1959. Effects of auxins on membrane permeability and pectic substances in bean endocarp. Nature 183: 757-758.

108. Sampson, H. C. 1918. Chemical changes accompanying abscission in Coleus blumei. Botanical Gazette 66: 32-53.

109. Sato, C. S., R. U. Byerrum and C. D. Ball. 1957. The biosynthesis of pectinic acid methyl esters through transmethylation from methionine. Journal of Biological Chemistry 224: 717-723.

110. Sequeira, L. and T. A. Steeves. 1954. Auxin inactivation and its relation to leaf drop caused by the fungus Omphalia flavida. Plant Physiol. 29: 11-16.

111. Shoji, K., F. T. Addicott and W. A. Swets. 1951. Auxin in relation to leaf blade abscission. Plant Physiol. 26: 189-191.

112. _____,and F. T. Addicott. 1954. Auxin physiology in bean leaf stalks. Plant Physiol. 29: 377-382.

113. Sterling, C. and A. J. Kalb. 1959. Pectic changes in peach during ripening. Botanical Gazette 121: 111-113.

114. Storey, J. B. 1957. Auxin physiology of cotton in relation to leaf abscission. Ph.D. Thesis, University of California, Los Angeles.

115. Swets, W. A. and F. T. Addicott. 1955. Experiments on the physiology of defoliation. Amer. Soc. Hort. Sci. 65: 291-295.

116. Terpstra, W. 1956. Some factors influencing the abscission of debladed leaf petioles. Acta Botanica Neerlandica 5: 157-170.

117. Van Steveninck, R. F. M. 1959. Abscission-accelerators in lupins (Lupinus luteus L.). Nature 183: 1246-1248.

118. Varner, J. E. 1961. Biochemistry of senescence. Ann. Rev. Plant. Physiol. 12: 245-264.

119. Wedding, R. T., L. C. Erickson and B. L. Brannaman. 1954. Effect of 2,4-dichlorophenoxyacetic acid on photosynthesis and respiration. Plant Physiol. 29: 64-69.

120. 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. Botanical Gazette 113: 348-362.

121. Went, F. W. and K. V. Thimann. 1937. Phytohormones. Macmillan Co. New York.

122. Wetmore, R. H. and W. P. Jacobs. 1953. Studies on abscission: the inhibiting effect of auxin. Amer. Jour. Bot. 40: 272-276.

123. Wilson, C. M. and F. Skoog. 1954. IAA induced changes in uronide fractions and growth of excised tobacco pith tissue. Physiologia Plantarum 7: 204-211.

124. Wu, P. H. L. and R. U. Byerrum. 1958. Studies on the biosynthesis of pectinic acid methyl esters. Plant Physiol. 33: 230-231.

125. Yager, R. E. 1960. Possible role of pectic enzymes in abscission. Plant Physiol. 35: 157-162.

126. _____. 1959. Effect of removal of leaves at various developmental stages upon floral abscission in tobacco. Phyton 13: 125-131.

127. _____. 1960. Some effects of leaves and indoleacetic acid upon floral abscission in Nicotiana tabacum. Botanical Gazette 121: 244-249.

128. _____,and R. M. Muir. 1958. Amino acid factor in control of abscission. Science 127: 82-83.

129. _____,and R. M. Muir. 1958. Interaction of methionine and indoleacetic acid in the control of abscission in Nicotiana. Society for Experimental Biology and Medicine 99: 321-323.