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ABSTRACT

THE CHANGES IN ANATOMY AND FINE-STRUCTURE AS RELATED TO THE PHYSIOLOGY OF ABSCISSION IN THE LOWER PULVINUS OF BEAN (*PHASEOLUS VULGARIS* L.)

by

Richard Michael Bednarz

Abscission in the lower pulvinus of debladed primary leaf petioles of bean plants (*Phaseolus vulgaris* L.) was examined structurally at the cellular and subcellular levels by means of light and electron microscopy. Certain structural aterations which occurred during abscission were correlated with physiological changes. In addition, the effect of the application of abscission delaying quantities of the auxin, 2,4-D, upon these changes was studied.

The first indication of abscission occurred the second day after deblading; a layer of cells, a few cells distal to the eventual line of separation, stained with phloroglucinol-HCl. The third day after deblading, cell division in the abscission zone resulted in the formation of a protective layer. Due to cell division, mother cells

formed nests of daughter cells; the protective layer was composed of two tiers of these nest type cells. The protective layer was located 3 to 7 cells distal to the junction of the petiole and stem and proximal to the phloroglucinol-HCl stained layer. Separation occurred on the distal side of the protective layer approximately 6 days after deblading. The protective layer was left as the leaf scar and the phloroglucinol-HCl stained layer remained with the separated petiole. Commensurate with protective layer formation, separation began along the middle lamellae of older cell walls adjacent to the protective layer. Pectin content of the cell walls decreased as wall separation progressed. Separation was almost exclusively confined to the middle lamella.

The application of 2,4-D (7 x 10^{-5}M) delayed the formation of the protective layer approximately six days. Separation, which was delayed approximately seven days, again occurred distal to the protective layer.

Ethylene (5 ppm) treatment hastened separation. Cell division did not occur prior to separation in ethylene promoted abscission but separation occurred through the normal plane.

The cytoplasm of abscission zone cells changed only slightly for the first 3 days after deblading. Although lysigenous cavities were apparent in older cell walls of the separation layer at this time, the only indication of cytoplasmic permutation was a slight swelling of chloroplast thylakoids. Five days after deblading, at the advanced stages of separation, the cytoplasm was deteriorated and the chloroplasts appeared senescent. The mitochondria and plasmalemma appeared unaltered at even the most advanced stages of cell wall separation.

Accordingly, respiration remained relatively constant throughout abscission. A decline in the chlorophyll content of the abscission zone occurred after deblading, yet carbon fixing capacity was maintained. The abscission zone increased in dry weight the first day after deblading and lost weight thereafter.

At the ultrastructural level, 2,4-D had a deleterious effect upon cellular membranes. The chloroplasts were especially affected by 2,4-D exhibiting a nearly complete loss of thylakoid structure after five days of treatment with 2,4-D (7 x 10^{-5}M). Cell wall integrity was maintained following 2,4-D application.

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Richard Michael Bednarz

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INTRODUCTION

As early as the middle of the eighteenth century the morphological aspects of leaf fall had attracted the serious attention of science (DuHamel, 1758). Schact (1859) and Von Mohl (1860) indicated that leaf drop involved two phenomena, separation and protection. At the turn of the century Tison (1900) examined abscission in over eighty species and grouped them into eleven categories according to their manner of leaf fall. The categories included the mode of separation, the time of inception of cell division, and the method of cell proliferation during foliar abscission. A reevaluation of these categories by Lee (1911) reduced this unwieldy number to three general classes for the 45 deciduous dicots he examined. Gawadi and Avery (1950) extended these three general types of abscission to non-woody species.

Facey (1950) critically studied cell wall changes during abscission in *Fraxinus americana* L. with the light microscope. Rasmussen (1965) and Bornman *et al*. (1967) conducted further light microscopic examinations of the cell wall during abscission in bean and cotton.

With the increasing use of the electron microscope one could expect that a study of abscission, utilizing the high resolving power of this instrument, would have been undertaken. At the inception of this research in 1967, no such study had appeared in the literature. Since that time three investigations on the ultrastructure of the abscission layer have been published. Jensen and Valdovinos, in a series of three papers (1967; 1968; Valdovinos and Jensen, 1968), examined the fine structure of abscission zones of the pedicels of tomato and tobacco. Similar studies of abscission in explants of *Coleus Blumei* Benth. and *Gossypium hirsutum* L. were reported by Bornman (1967). Finally, abscission of explants and intact plants in the upper pulvinus of unifoliate bean petioles was published by Morré (1968).

The great variability (as will become evident in the literature review which follows) in both the physiology and anatomy of abscission of different plants demonstrates that separation does not occur in the same manner between species. In the case of the bean, a widely used test plant, abscission at the upper pulvinus of the primary leaf petiole does not parallel separation at the lower pulvinus of that petiole. Consequently, an ultrastructural examination of abscission in the lower pulvinus of bean may be equally rewarding as have previous studies of the fine structure of abscission zones of other tissues.

While the primary efforts of this study involved an electron microscopic examination of abscission in the lower pulvinus of the petiole of intact bean plants (Phaseolus vulgaris L. cv. Contender), the scope of the study was not restricted to use of the electron microscope alone. Correlative physiological studies, light microscopy, and histochemical techniques were included to substantiate the fine structural observations or to further our knowledge concerning the process of abscission. The plant used in this study was selected to attempt to correlate ultrastructural changes with investigations by Rasmussen (1965) and Rasmussen and Bukovac (1967; 1969) on the physiological and anatomical changes associated with abscission in this same tissue.

LITERATURE REVIEW

Abscission refers to the detachment of leaves, flowers, fruits or other parts from the plant. Typically, abscission is a physiological process. At the base of an abscising organ a layer of cells remains alive and the physiological processes which lead to separation occur in this cell layer regardless of the state of the abscising organ. For example, the leaves of deciduous species are usually dead when they are shed in autumn, whereas in certain species, flowers may still be capable of setting fruit when they abscise.

The abscission process is of interest not only academically but also agriculturally. A noteworthy example of successful employment of abscission retardation is the prevention of preharvest fruit drop of apples (Gardner and Batjer, 1939) and pears (Allen and Davey, 1945). Induced abscission has been extensively employed to defoliate several crops prior to harvesting, the leaves of which would hinder the mechanical pickers. Several million acres of cotton are defoliated annually for this purpose in the United States alone (Carns, 1966). Also, chemical methods of thinning of young fruit, for the purpose of increasing the size of those remaining has been successfully practiced (Murneek, 1950).

Anatomy

Abscission of most organs involves a layer of cells located at the base of the organ. These cells are characterized by being smaller in size and with a denser protoplasm than surrounding cells (Facey, 1950). Their cell walls are thin and nearly void of lignin and suberin (Scott *et al*., 1948). Secondary cell division usually occurs in the abscission zone in many species and this phenomenon has been proposed to cause organ detachment (Addicott and Lynch, 1955). However, Lee (1911), in a study of 45 dicot species, determined that cell division may either precede or follow leaf detachment depending on species. Similarly, McCown (1939) reported that young apple fruits abscised while the abscission zone was meristematically active but that mature fruits did not require the meristematic process to abscise. In herbaceous species, foliar abscission involved secondary cell division at the base of the petiole in *Euphorbia pulcherrima* Willd., *Gossypium herbaceum* L., *Capsicum frutescens* L., and *C. annuum* L. On the other hand, cell division did not occur in *Impatiens Sultani* Hook. until after leaf detachment (Gawadi and Avery, 1950). Irrespective of the time at which cell division occurred, once the organ abscises the new cell layers become lignified and suberized thereby affording the plant a barrier to pathogen invasion (Lee, 1911).

Actual separation of cells in the abscission zone which allows the leaf to drop may result from the dissolution of the walls of the cells which make up this zone. Cell walls, which have no secondary thickenings, are composed of two basic layers: the primary wall, and an intercellular middle lamella (Anderson, 1935). The primary wall is optically active (anisotropic) and contains cellulose, hemicellulose, pectic compounds, and other polysaccharrides (Bailey, 1940). The middle lamella is isotropic and is composed of cellulose-free colloidal matter, mainly calcium pectate (Dippel, 1898). The divalent calcium ion cross-links adjacent pectin molecules thereby promoting adhesion between neighboring cells (Facey, 1950). Ginzburg (1961) demonstrated that magnesium, iron, or copper could also function as cementing ions. Methylation of these metal ion binding sites of the pectins in the abscission zone would produce water soluble pectins in the middle lamella resulting in cellular separation (Facey, 1950).

Separation of cells in the abscission zone has been observed to occur in one of three ways. First, only the middle lamella between cells dissolved and the primary wall remained intact (Lee, 1911). Second, the middle

lamella and the primary walls of adjacent cells dissolved leaving only a thin layer of cellulose covering the cells (Tison, 1900). Third, complete dissolution of several layers of cells occurred (Hannig, 1913). Lloyd (1916) was unable to find dissolution of entire layers of cells and claimed that what Hannig described as layers of cells in a state of solubilization was, in reality, a few layers of cells which had extremely thin cell walls. Mechanical injury during fixing or sectioning could easily rupture the thin walls thereby giving the appearance of cellular dissolution. Increased fragility of abscission zone cells could be due to the wall swelling which has been observed to precede separation (Lee, 1911; Facey, 1950; Rasmussen, 1965; Bornman _et al_., 1967).

Structural weakness of the abscission zone is further indicated by its smaller diameter than that of adjacent tissues (Addicott, 1954). In addition, the abscission zone is often delineated by an external indentation (Addicott, 1954). A fine structural study of tomato floral abscission (Jensen and Valdovinos, 1967) indicated that this indentation extended into the pedicel having branches which followed along the middle lamellae between cells. Further indication of weakness is evident from the pattern of vascularization in the abscission zone. Doutt (1932) reported that a single vascular bundle traversed the pulvinus of bean whereas several traces entered the pulvinus from the stem and eleven bundles exited into the petiole. Thus the support of several bundles in neighboring cells is reduced to just a single strand in the pulvinus.

Reviewing the morphological aspects of abscission, organ detachment occurs in a well defined abscission zone located at the base of the organ. This zone is often characterized by meristematic activity. Swelling of the walls of cells in the region of separation precedes detachment. The vascularization and diameter of the abscission zone differs from adjacent portions of the plant. Thus, an area develops which is structurally weaker than other cells of the petiole and through this weakened area separation occurs.

Injury

Injury to healthy plant parts which normally would remain attached until maturity or senescence results in premature abscission. Livingston (1950) indicated that in Citrus, leaves fall in proportion to the area of leaf removed. Leaf injury due to infection by microorganisms, feeding by various insects, or chemical injury are often followed by abscission (Addicott and Lynch, 1955). These and other environmental factors which either retard or accelerate abscission act indirectly through their effects on the organ involved rather than directly upon the abscission zone.

Water

Water affects abscission since it must be available for the cells of the abscission zone to function. In addition, a loss of turgor of cells distal to the abscission zone occurred after deblading trifoliate bean leaf petioles (Brown and Addicott, 1950). They suggested that the difference in turgor between cells distal and proximal to the abscission zone created a physical stress sufficient to cause separation.

An excess of water around explants of Citrus was reported to retard or prevent abscission (Livingston, 1950). Carns et al. (1951) suggested that the excess water probably interfered with the oxygen supply.

Oxygen, Carbon Dioxide and Respiration

Coleus plants retained leaves under conditions of low oxygen pressure (Molisch, 1886; Sampson, 1918). Bean explants in oxygen free or oxygen reduced environments did not abscise and abscission increased with increasing oxygen concentrations up to a maximum of 50% (Carns et al., 1951).

Ten percent carbon dioxide accelerated floral abscission in Nicotiana tabacum L. from 34 to 17 hours. Conversely, retardation of abscission by 5 to 22.5% carbon dioxide in oxygen was observed with bean explants (Addicott and Lynch, 1955).

Respiration was maximum in cotton explant abscission zones coincident with abscission (Leinweber and Hall, 1959). Respiratory enzyme inhibitors (arsenate, azide, cyanide, fluoride, iodoacetate, malonate, and zinc chloride) applied to bean explants retarded abscission (Carns, 1951). It is evident, therefore, that abscission is a physiological process requiring respiration.

Temperature

The effect of temperature on abscission is similar to its effect on other physiological processes. For example, rates of abscission rise to a maximum between 25°C and 30°C, and decline at higher temperatures (Addicott and Lynch, 1955). Temperature probably has an indirect effect upon abscission by altering respiration, food translocation or producing injury (Meyer and Anderson, 1952).

The effect of freezing temperatures on abscission of cotton leaves is dependent on intensity. If frost is light, abscission is accelerated, if heavy abscission is prevented. Light frost only injures the leaf blade, not the petiolar abscission zone; thus, abscission proceeds as with other leaf injury. With a heavy frost, the abscission zone tissue is injured or killed and becomes incapable of the metabolic processes necessary for abscission (Addicott and Lynch, 1955).

Photoperiod

Wiesner (1904) followed leaf drop of various trees through the summer and autumn and decided that shortening of the light period was responsible for leaf fall. Some trees when grown under constant daylight conditions retained their leaves throughout winter (Kramer, 1936; Olmstead, 1951). Matzke (1936) reported that the leaves of some trees growing near street lamps retained their leaves longer. Wareing (1956) indicated that not all species reacted this way, and in those that did, frequent anomolies were observed.

Olmstead (1951) demonstrated increased leaf fall in sugar maples at light intensities too low for photosynthesis. The increased separation was due to decreased hormone level rather than carbohydrate deficiency. Likewise, La Rue (1936) reported that leaving a small segment of the leaf blade on the petiole retarded abscission. Since this leaf segment was too small to carry on a substantial level of photosynthesis, La Rue concluded that some substance manufactured in the leaf influenced abscission when present in low concentrations.

Auxin

In 1933 Laibach demonstrated that abscission was retarded when orchid pollen gains, high in auxin content, were applied to debladed Coleus petioles. La Rue (1936) confirmed these results with purified synthetic IAA. Myers (1940) applied 1% IAA in lanolin to debladed Coleus petioles which were opposite intact petioles. The IAA treated petioles stayed on as long as the intact ones. Earlier Küster (1916) surmised that a substance produced in the leaf blade kept the petiole from abscising. He demonstrated that even one square centimeter of leaf left on the petiole delayed abscission considerably. Myers (1940) found more diffusible auxin could be obtained from young leaves than old leaves and Wetmore and Jacobs (1953) determined that a similar correlation existed between leaf longevity and the amount of diffusible auxin that could be obtained from cut leaves. They concluded that after a leaf reached a specific stage of development, diffusible auxin from the leaf blade was the abscission controlling factor.

Jacobs (1955) proposed that abscission was due to the balance of auxin on either side of the abscission zone. Thus the "auxin-auxin balance" theory of leaf abscission in Coleus was formulated. Accordingly, when the flow of auxin from the leaf blade decreased, the abscission speeding effect of auxin from the apex or from younger leaves then took effect and abscission commenced. The results of abscission studies in Coleus using several deblading patterns produced the evidence for the theory. Jacobs demonstrated that two sided deblading, where the intact leaves were not vascularly connected with the debladed ones, resulted in slower abscission than if a spiral deblading pattern was employed and the intact leaves were directly connected with the debladed petioles. Four sided deblading caused strikingly longer retention of the petioles. The presence of intact leaves and the proximity of these leaves to the debladed petioles, as in the case of spiral deblading, resulted in accelerated abscission (Rossetter and Jacobs, 1953). Yager (1960b) found that removal of nearby leaves speeded abscission of unfertilized tobacco pedicels, an opposite physiological effect to that observed by Rossetter and Jacobs (1953). The apical bud has also been shown to exert an influence on abscission of debladed petioles. When the apical bud was removed, abscission of the debladed petioles was retarded. One percent IAA in lanolin substituted for the apical bud in returning the time course pattern of abscission of debladed petioles to that of control (Jacobs, 1955).

Addicott and several co-workers have, since 1951, proposed that auxin initiated abscission due to the auxin gradient across the abscission zone. The "auxin gradient" theory as proposed by Addicott stated that abscission was initiated following a decrease in the ratio of auxin present distally to auxin present proximally with respect to the abscission zone. Conversely, abscission did not occur if the distal supply of auxin remained high (Addicott et al., 1955). Initial work by this group (Shoji et al., 1951) demonstrated that in bean, the distal region to the separation layer contained a higher concentration of auxin than the proximal region. Addicott and Lynch (1951) pointed out that depending upon the concentration and the position of application with respect to the abscission zone, IAA and 2,4-dichlorophenoxyacetic acid (2,4-D) either accelerated or retarded abscission in petiole explants and intact plants of cotton or bean. Approximately 25% retardation occurred with distal application of IAA, and 25% acceleration with proximal IAA treatment in bean. Kinetin also had an effect similar to IAA but was less active (Osborne and Moss, 1963; Chatterjee and Leopold, 1964).

Leopold and co-workers have opposed the auxin gradient theory of abscission and have proposed other mechanisms for the action of auxin (Gaur and Leopold, 1955; Rubinstein and Leopold, 1963). By imposing artificial gradients across the abscission zone of primary leaf explants of bean, Gaur and Leopold (1955) found no correlation between the direction of the gradient and abscission but that a high concentration of auxin inhibited abscission and a low concentration accelerated it. Thus, they concluded that the concentration of auxin controlled abscission rather than the gradient of auxin across the abscission zone. A similar two phase action of auxin activity in root, bud and stem growth was demonstrated by Thimann (1937). Auxin exhanced growth at certain concentrations but inhibited it at higher concentrations.

Rubinstein and Leopold (1963) determined a second action of auxin in abscission. Abscission, they proposed, is a two stage process; the first stage, or induction period, is inhibited by auxin and the second stage is promoted by the same auxin concentration. These conclusions have been substantiated by others (Chatterjee and Leopold, 1963; Abeles and Rubinstein, 1964; Rasmussen and Bukovac, 1969). An induction period of 6 or more hours occurred in the upper pulvinus of bean with NAA (Rubinstein and Leopold, 1963). In the same system IAA gave similar results as NAA but stage II began between 12 and 18 hours after deblading (Chatterjee and Leopold, 1963). The lower pulvinus abscission zone of intact debladed bean plants had an induction period of 14 hours using 3-chlorophenoxy-alpha-propionic acid as an auxin (Rasmussen and Bukovac, 1969).

Chatterjee and Leopold (1963) applied radioactive NAA to either the proximal or distal side of upper pulvinus of bean explants immediately or after an 18 hour induction period. The results indicated that abscission was correlated with time of application and not with the gradient or total auxin concentration. Regardless of the direction of the gradient, abscission could be induced by altering the time of application of auxin following induction. A one minute application of NAA increased the rate of abscission the same as a 120 minute application.

Direct evidence against the "auxin gradient" and total "concentration of auxin" theories was presented by Rasmussen and Bukovac (1966) using autoadiographic techniques. These authors demonstrated that although high and low concentrations of NAA retarded or accelerated abscission respectively, a similar accumulation of radiolabelled NAA occurred on the distal side of the abscission zone in both cases.

The theories outlined above, namely the "auxin gradient," "auxin balance," and the "concentration of auxin" have, for the most part, been descriptive. Jacobs *et al*. (1964) advanced a "growth theory" which provided a physiological basis for regulation of abscission by auxin. Jacobs indicated that abscission was delayed as long as the petiole maintained its growth. The role of auxin, in the theory, was to mediate growth of the petiole. Growth, however, must be defined by parameters other than, for example, extension; for although giberrellic acid induces elongation it has little effect upon abscission.

IAA Oxidase and Inhibitor System

An endogenous IAA oxidase and inhibitor system was demonstrated in cotton by Morgan (1964). Schwertner and Morgan (1966) determined that the addition of an IAA oxidase cofactor, 2,4-dichlorophenol increased IAA-1-^{14}C decarboxylation above that of control tissue. Likewise, less decarboxylation of IAA-1-^{14}C was seen with the addition of an IAA-oxidase inhibitor, catechol, than with IAA alone. The monophenol, 2,4-dichlorophenol hastened abscission whereas the dihydroxyphenol, catechol, delayed abscission. Tomazewski and Thimann (1966) suggested that the phenols may control auxin balance since the simple addition of a second hydroxyl group changes the molecule's function from auxin destruction to auxin protection. Valdovinos and Ernest (1967) reported, however, that IAA destruction in Coleus did not increase under conditions of accelerated abscission.

Pectin Metabolism

Facey (1950) reported a shift from calcium pectate to pectic acid in Fraxinus americana and during the abscission process the pectic acid fraction of the middle lamella became methyl esterified into water soluble pectins. Two enzymes are believed to function in promoting pectin dissolution in the middle lamella (Bonner, 1936). Modern terminology refers to these as pectin methylesterase (PME), a specific catalyst for the hydrolysis of methyl ester bonds, and polygalacturonase (PG), which catalyzes the hydrolysis of polygalacturonic acid.

Using bean explants treated with 2,4-D, Osborne (1958) found that PME activity was maintained with auxin applications which inhibited abscission. Just prior to abscission, PME activity was high in the pulvinus. During senescence, however, PME activity gave a low content of soluble methyl ester pectins providing additional sites for metal ion linkages and thereby preserving intercellular integrity.

Yager (1960a) reported increased PME activity in tobacco pedicel tissue treated with IAA at concentrations which retarded abscission. He further demonstrated that the amino acid, methionine, which promoted abscission, decreased PME activity. Yager's data suggested that methionine influenced PME activity and abscission by acting as a donor of methyl groups to the pectic substances of cells comprising the abscission zone. Ordin _et al_. (1955) demonstrated that methionine functioned as a methyl donor in plant tissues, i.e., in _Avena_ coleoptiles. Valdovinos and Muir (1965) reported preferential incorporation of methionine into the pectic substances of abscission zones of cotton and _Coleus_. Using methionine labelled at different carbon positions they demonstrated that the radioactivity in the abscission zone was attributed to the methyl group of methionine. Under their experimental conditions, abscission was promoted. Addition of IAA with radiolabelled methionine suppressed the incorporation of methyl groups into the pectic substances of the abscission zone and abscission was retarded.

Rasmussen (1965) observed polygalacturonase activity in the petiole and abscission zone of bean plants after deblading. Although PG activity remained relatively constant in the abscission zone from deblading until separation six days thereafter, in the petiole PG activity diminished and was undetectable after 3 days. Rasmussen suggested that movement of calcium from the abscission zone to the petiole accounted for the changes in enzymatic activity. Calcium ions are known to inhibit the hydrolysis of pectic acid (Corden *et al.*, 1964). Thus, the movement of calcium from the abscission zone removed the inhibition of PG resulting in pectin hydrolysis, whereas the increase in calcium levels in the petiole inhibited PG. The loss of calcium from the abscission zone, coupled with demethylation of pectin carboxyl groups by PME, increased the number of free carboxyl groups in the abscission zone. This provided the necessary substrate for PG which lead to pectin hydrolysis.

Senescence and Mobilization

There is evidence to suggest that abscission and senescence are related. Osborne (1955) reported that agar diffusates from the leaves of woody species contained some metabolic product which was capable of promoting abscission. This accelerator was in higher concentrations in old leaves than in young leaves. Senescent Coleus petioles contained a senescent factor which accelerated abscission by about twenty hours (Jacobs et al., 1962). Both IAA (Osborne and Hallaway, 1960) and kinetin (Osborne and Moss, 1963) which retarded abscission retarded senescence also.

Osborne and Moss (1963) found that kinetin applied directly to the abscission zone delayed abscission, but promoted it when applied to either side. Osborne and Moss suggested that the abscission zone was sensitive to the withdrawal of metabolites with such withdrawal leading to localized senescence of the abscission zone. Scott and Leopold (1966) determined that abscission was preceded by a mobilization of several metabolites to regions proximal to the abscission zone. Similarly, Rasmussen (1965) determined that calcium moved from the abscission zone into the petiole in debladed bean plants.

Increased auxin levels in tissues bordering the separation layer may promote metabolite movement from the abscission zone. Addicott and Lynch (1951) determined that IAA, like kinetin, caused mobilization of metabolites to its point of application. Rasmussen and Bukovac (1966) reported NAA localized distal to the lower pulvinal separation layer following application to the debladed petiole. Jacobs et al. (1966) determined that the pulvinus obstructs basipetal 2,4-D flow through bean petioles.

The amino acids leucine, methionine, tryptophan, valine, and phenylalanine have been reported to increase during senescence (Hall *et al.*, 1962). High concentrations of amino acids in the abscission zone promoted abscission (Rubinstein and Leopold, 1962; Hall *et al.*, 1962; Valdovinos and Muir, 1965; Abeles, 1967). Valdovinos and Muir (1965) proposed that the effect of amino acids in abnormal amounts was that they act as antimetabolites in the abscission zone altering the amino acid sequences in normal protein systhesis.

De la Fuente and Leopold (1968) stated that it was unlikely that abscission was due to localized senescence in the separation layer. They argued that senescence leads to a responsiveness to the action of ethylene. Moreover, Burg (1968) proposed that ethylene may even regulate the onset of senescence.

Protein Synthesis and Ethylene

In 1950, Gawadi and Avery hypothesized that two forces acted upon foliar abscission: hormonal balance and ethylene production. Ethylene is known to be produced in a number of plant tissues (Biale and Young, 1954; Pratt, 1957; Burg and Burg, 1962; Lyons *et al.*, 1962) and auxin has been shown to influence the production of ethylene (Abeles and Rubinstein, 1964; Hall and Morgan, 1964; Morgan and Hall, 1964; Burg and Burg, 1966). Abeles (1966) indicated that the mechanism of auxin on the enhancement of ethylene production is through the formation of enzymes used in ethylene biosynthesis. Up to 84% of auxin stimulated ethylene production was overcome with protein synthesis inhibitors such as actinomycin-D (Abeles and Holm, 1966). The senescent bean explants used in these experiments had increased leucine-^{14}C and orthophosphate-^{32}P incorporation after ethylene administration, thereby indicating ethylene enhanced protein and RNA synthesis. However, ethylene did not promote protein synthesis in young tissue. Rubinstein and Abeles (1965) proposed that known promoters of abscission act through their effect on ethylene production.

Certain physiological effects produced by ethylene could implicate the gas in abscission. For example, concentrations of ethylene which promoted abscission when supplied to bean tissue decreased PME activity (Osborne, 1958). Also, Lyons and Pratt (1964) suggested that the permeability of mitochondrial membranes from cauliflower buds increased after ethylene treatment. Such ethylene mediated permeability changes occur regularly in the ripening processes of climacteric fruits (Hansen, 1966). Other changes in permeability associated with abscission have also been observed (Rasmussen, 1965). Thus a role of ethylene in abscission may be in the regulation of one of these processes.

Abscisic Acid

Liu and Carns (1961) isolated an abscission inducing substance in crystalline form from cotton burs and called the compound abscisin. A more active substance, abscisin II (redesignated abscisic acid by Addicott *et al*., 1968), was isolated from cotton fruits by Okhuma *et al*. in 1963. The chemical structure of abscisin II, which accelerated cotton explant abscission, was determined by Okhuma *et al*. (1965). Subsequently, Cornforth *et al*. (1965) confirmed the structure by synthesis.

Bornman *et al*. (1967) found abscisin II to be an "extremely potent accelerant of abscission" in cotton explants. Whole cell dissolution led to separation after abscisin II treatment rather than normal separation along middle lamellae. Due to restricted cell division, a separation layer did not form following abscisin II treatment (Bornman *et al*., 1967). Abscisin II accelerated abscission and senescence in a variety of species other than cotton and the compound counteracted auxin and gibberellin effects in several test systems although inactive itself (Addicott *et al*., 1965). In *Perilla ocymoides* L. explants, abscisin II accelerated but could not overcome

IAA retardation (El-Antably et al., 1967). Further, abscisin II was not effective in causing abscission of intact leaves of a wide variety of both woody and herbaceous species although it had other marked physiological responses.

Gibberellin Effects on Abscission

Gibberellic acid (GA_3) had little or no effect on abscission of certain deciduous trees, retarded abscission in others (Brian et al., 1959), and accelerated abscission in bean explants (Chatterjee and Leopold, 1964). Cotton explant abscission was accelerated following proximal GA_3 application (Carns et al., 1961; Bornman et al., 1967), but was retarded at low concentrations (Carns et al., 1961). Distal GA_3 application accelerated cotton explant abscission (Carns et al., 1961). In Coleus, Muir and Valdovinos (1965) found that GA_3 applied to the apex accelerated abscission. Bornman et al. (1967) concluded that GA_3 accelerated both the processes of cell division in the abscission zone and the schizogenous breakdown of the middle lamella of separation layer cells.

Concluding Remarks on the Physiology of Abscission

In general, healthy plant parts remain attached until maturity or senescence. The close association between senescence and abscission is evident from the several changes which occur prior to or during the abscission process; e.g., cell wall hydrolysis, susceptibility to ethylene treatment, changes in membrane permeability, senescent factor diffusate accumulation, and mobilization of nutrients from the abscission zone of the abscising organs.

Auxin has been shown to be an effective abscission regulating substance. Much work to date has been descriptive concerning the role of auxin in abscission; studies of the sites of auxin action, the gradient, balance, and total concentration of the hormone in the abscission zone are examples of this type of work. The regulation of pectin metabolism, the movement of metabolites, delay of senescence, and other effects of auxin upon growth and development provide a more meaningful role for the action of auxin or abscission.

Recently, two new areas of abscission control have developed. The effect of ethylene in the abscission process has been actively pursued but the role of ethylene as an endogenous abscission promoter has not been clearly established. The identification of abscisic acid by Okhuma _et al_. (1963) and its effects upon the abscission and senescence processes is still difficult to assess from the reports available.

MATERIALS AND METHODS

Definition of Terms

The terms used in this dissertation with reference to abscission in the lower pulvinus of bean are defined below:

Abscission zone - The region at the base of the petiole in which the morphological and physiological changes associated with abscission occur.

Protective layer - The layer of cells which develops as a result of cell division in the abscission zone. Once petiole detachment occurs, these cells remain on the plant as the leaf scar.

Separation layer - The bi-layer of cells in the abscission zone directly involved in the separation of the petiole from the plant.

Distal; Proximal - With respect to a reference point (e.g., the protective layer) distal refers to areas on the leaf blade side of that reference point. Proximal refers to regions to the stem side of that point.

Adaxis; Abaxis - Adaxis refers to the upper surface of the petiole; i.e., the side nearest the axis of petiole and stem. Abaxis refers to the under side of the petiole, the side furthest from the stem-petiole axis.

General

The general methods used in this study closely followed those previously described by Rasmussen (1965). Bean plants, Phaseolus vulgaris L. cv. Contender, were grown in the greenhouse under natural daylight conditions in a white quartz sand and Turface (calcined clay) 1:1 mixture. Minimum greenhouse temperature was 20°C. After 8 to 11 days, or when the primary leaf blades were approximately 25% expanded, the plants were removed from the germinating box and mounted between styrofoam stips held together with rubber bands. Ten to twelve plants mounted in this manner were placed in plastic boxes containing 750 ml of Hoagland's nutrient solution (Hoagland and Arnon, 1950) with continuous aeration. The plants were then transferred to a controlled environment growth chamber under 400 foot candles of continuous fluorescent lighting with a temperature of 21°C.

Abscission of the primary leaf petiole at the lower pulvinus was induced by deblading. Lanolin paste (appx. 15 mg) was applied to the cut surface of the 1.0 cm petiole. Chemical treating solutions were dissolved in the lanolin when these were utilized. Abscission was tested by applying a 5 gram pressure to the adaxial surface of the petiole (Mitchell and Livingston, 1968).

In the physiological studies, a transverse section of 1.4 mm width was cut from the abscission zone of the lower pulvinus unless otherwise specified. The disc was cut by means of two razor blades bolted together with a 1.4 mm spacer.

Light Microscopy

A portion of the petiole and adjacent stem, less than 0.5 mm in length, containing the abscission zone, was excised from the plant. The tissue was prepared using a modification of the procedures outlined by Feder and O'Brien (1968). The tissue was fixed immediately in a 10% aqueous solution of acrolien (Shell Oil Company) or a 4% gluteraldehyde solution (Fisher) in 0.1M phosphate buffer at pH 7.0. Fixation was carried out at 0°C for 24 hours.

The tissue was dehydrated in 100% methyl cellosolve (3 changes in 24 hours) followed by absolute ethanol (3 changes). The tissue was infiltrated in propylene oxide using successively higher concentrations of Epon (25%, 50%, 75%) and finally two changes of 100% Epon. The tissue was then cast into aluminum boats containing fresh Epon mixture with polymerizing catalyst dimethylaminomethylphenol (DMP-30) at 2% V/V. The resin was cured at 60°C for 3 days.

The tissue was cut from the blocks and, with proper orientation, mounted on cured Epon-filled gelatin capsules by means of epoxy cement. Sections (1μ) were cut using dry glass knives with an LKB Ultratome. The individual sections were expanded by floating on a drop of distilled water on a glass slide. Gentle warming over a bunsen burner evaporated the water, flattened the sections, and affixed the sections to the slide. The sections were stained 15 to 45 minutes in a Coplin jar containing a 0.05% toluidine blue solution in benzoate buffer at pH 4.4. The slides were rinsed briefly in running water and allowed to dry. Coverslips were mounted in a permanent mounting medium (Lipshaw Manufacturing Company, Detroit).

Photographs of the specimens were taken using Kodak High Contrast Copy Film. The film was developed in D-76 developer for 7 minutes at 70°C followed by 5 minutes in Kodak fixer.

Anatomical Effects of Ethylene

Ethylene Promoted Petiole Separation

Ethylene was supplied with a continuous flow system to debladed bean plants set in Hoagland's nutrient solution as described in "General Methods." Eight plants were placed in a sealed 9 liter glass dessicator at 20°C.

Ethylene was supplied through one port of a two-holed rubber stopper in the lid of the dessicator. The ethylene was administered at 5 ppm in air for 17 hours with a flow rate of 3 liters per hour. Fluorescent lighting (300 foot-candles) was supplied continuously during the treatment. Fixation and subsequent handling of the tissue followed the procedure outlined under "Light Microscopy."

Protective Layer Formation After Ethylene Promoted Abscission

Ethylene was administered as described above for 30 hours by which time the periole had separated. The plants were then transferred to the growth chamber for four days and the abscission zones were prepared for light microscopy using the Epon embedding procedure and toluidine blue staining previously described.

Histochemical Methods in Light Microscopy

Fresh tissue was used for histochemical studies. At various dates after deblading, and also after petiole separation, free-hand longitudinal sections were cut with a razor blade through the petiole and adjacent stem. Staining procedures used followed the methods given by Jensen (1962):

Compound	Test Used
Lignin	Phloroglucinol - HCl
Lignin	Chlorine Sulfite
Aldehydes	Periodic Acid - Schiff's Reagent
Protein	Ninhydrin
Starch	Iodine Potassium Iodide
Callose	Analine Blue (visual method)

Pectin Localization

Abscission zones from 12 day old greenhouse grown plants which were either not debladed or had been debladed five days previously were fixed in aqueous 10% acrolien at 0°C. The tissue was placed in 60% ethanol before staining according to the following schedule:

Hydroxylamine-Ferric Chloride Staining (Schmid and Machado, 1968)

14% Hydroxylamine; 14% NaOH 1:1	1 hour
N/10 HCl	20 minutes
2.5% $FeCl_3$ in N/10 HCl	30 minutes
N/10 HCl	20 minutes

All solutions were prepared in 60% ethanol.

Specimens were dehydrated through the standard alcohol series and into n-butanol prior to paraffin infiltration. Tissue blocks were sectioned with a rotary microtome at 10 microns and, depending upon subsequent treatment, were affixed to either glass or quartz slides.

Sections mounted on quartz slides were incinerated by bringing the temperature from 21°C to 550°C over a 5 hour period. This prevented the paraffin from spattering.

A stable white mineral ash remained after incineration. The slides with affixed tissue or mineral ash were coated with a conducting layer of carbon prior to examination under an electron microprobe (Applied Research Laboratories Model EMX-SM). Operating conditions were 25 kilovolt electron accelerating potential and a sample current of 0.05 or 0.1 microamperes. The samples were analyzed for either iron or calcium.

Electron Microscopy

Sections of the petiole and adjacent stem containing the abscission zone were fixed for electron microscopy in 4% gluteraldehyde in 0.1M phosphate buffer at pH 7.0 - 7.2. Fixing, dehydrating, and embedding followed this schedule:

Fixation-0°C

Gluteraldehyde fixative	1 to 4 hours
Buffer rinse (three changes)	15 minutes each
1.0% osmic acid in buffer (pH 7.0-7.2)	1 hour

Dehydration and Embedding-21°C

50% ethanol	15 minutes
80% ethanol	15 minutes
95% ethanol	15 minutes
100% ethanol	15 minutes
Propylene oxide (PO) (three changes)	15 minutes
3PO :1Epon 812	15 minutes
1PO :1Epon 812	30 minutes
1PO :3Epon 812	30 to 60 minutes
Pure Epon 812 mixture	overnight

Epon 812 Preparation (Luft, 1961)

I Mixture A
62ml Epon 812 resin
100ml DDSA (Dodecanyl succinic anhydride)

II Mixture B
100ml Epon 812 resin
89ml NMA (Nadic methyl anhydride)

III Mix 7 parts A with 3 parts B

IV Add 0.2ml of DMP-30 per 10ml of Epon mixture

Polymerization

35°C	8 to 24 hours
45°C	8 to 24 hours
60°C	3 to 5 days

Styrene: Butyl Methacrylate Embedding

Mohr and Cocking (1968) suggested that an embeddant of styrene and butyl methacrylate (7:2 v/v) resulted in good infiltration of highly vacuolated cells. Some tissue was embedded in this mixture but polymerization difficulties led to only limited use of this embeddant. Tissue was dehydrated normally except that it was stained overnight in a saturated uranyl nitrate solution in 70% ethanol. Polymerization with this embeddant was accomplished at 60°C in covered aluminum boats for 3 days.

Mounting

Tissue blocks were cut out of the Epon and affixed on top of cured Epon filled size 00 gelatin capsules with epoxy cement. The specimens were oriented so that longitudinal sections could be cut.

Sectioning and Staining

Sections were cut on an LKB Ultratome utilizing glass knives broken free-hand. Sections of silver to gold interference colors (ca. 90 nanometers) were floated on water and picked up on naked athene-type 200 mesh copper grids.

The grids, with affixed sections, were immersed in an aqueous 2% uranyl acetate solution (Watson, 1958) for 15 to 20 minutes. The styrene-butyl methacrylate embedded sections were not stained in this manner since the uranium salt had been incorporated during dehydration. After staining, the grids were floated section-side down in a second stain of lead citrate (Reynolds, 1963). Lead staining was carried out on stain droplets on dental wax in a closed petri dish. Several sodium hydroxide pellets were added to the petri dish to minimize lead carbonate precipitation. The grids were then dipped in a weak (0.1N) sodium hydroxide solution prior to two rinses in distilled water. The grids were air dried on Whatman #2 filter paper and stored in closed petri dishes.

Electron Microscopy

A Hitachi HU-11 electron microscope operating at 75 kilovolts accelerating voltage was used for specimen examination. Visual records were made with Kodak Electron Image Plates.

Physiological Changes in the Abscission Zone During Abscission

Effect of Varying Concentrations of 2,4-D on Time to Separation

Lanolin paste containing concentrations of 10^{-9}M to 10^{-4}M, 2,4-D were applied to debladed petioles. A fresh application of lanolin was made every three days until separation. Abscission was tested daily by applying a 5 gram force to the adaxial side of the petiole (Mitchell and Livingston, 1968). After 50% of the petioles in a given treatment had abscised the testing was terminated. Ten plants (20 petioles) were used per treatment and the experiment was repeated four times.

Chlorophyll Content of the Abscission Zone

Greenhouse grown plants were transferred to the growth chamber 48 hours before deblading. Thirty abscission zones, 10 per replication, were cut from the petioles daily through separation and placed in 3 ml of 80% acetone. The experiment was repeated once.

Chlorophyll was extracted (Arnon, 1949) in the dark for 24 hours. Analysis for chlorophyll was done with a Beckman Model DU spectrophotometer utilizing Arnon's (1949) equation for chlorophyll present in a 1-cm cell:

$$\text{Total Chlorophyll}_{\text{mg/l}} = 20.2\ \text{OD}_{645} + 8.02\ \text{OD}_{663}.$$

Carbon Dioxide Fixation by Abscission Zone Sections

Twelve day old greenhouse grown seedlings were transferred to the growth chamber 48 hours prior to treatment. Treatment involved deblading the petiole to 1 cm and applying 2,4-D (7 x 10^{-5}M) in lanolin or plain lanolin paste to the cut petiole. Sequential sowing dates allowed plants with different deblading dates and with varying lengths of 2,4-D pretreatment to be harvested simultaneously. A treatment consisted of 24 excised 1.4 mm abscission zone discs segregated into three aliquots. Excised discs were laid flat on a piece of moist filter paper with each group of 8 abscission zones arranged randomly on the paper. The filter paper and sections were then placed in a clear "Plexiglas" assimilation chamber (10.5 x 6.0 x 2.5 cm). A porcelain boat containing 22.5μc $NaH^{14}CO_3$ was placed in the chamber. The chamber was sealed, after which excess 0.1N HCl was injected via a rubber sealed port into the boat releasing the radioactive carbon dioxide. Assimilation continued for 10 minutes under 1600 foot-candles of incandescent light filtered through 8 cm of cold running water. Room temperature during the experiment was 20.5°C.

Immediately upon removal from the assimilation chamber the tissue was frozen on dry ice. The frozen tissue was exposed to formaldehyde vapors for 30 minutes. The tissue was homogenized using a minimal amount of 2% gluteraldehyde. The homogenate was evaporated in 5 cm aluminum planchets and activity measured on a Beckman Lowbeta II counter with a thin window gas-flow detector.

Respiratory Changes in the Abscission Zone

Debladed bean plants had either 2,4-D (7 x 10^{-5}M) in lanolin or lanolin alone applied to the cut petiole. The time course for respiratory studies of the non-treated control plants was 0,1,2,3,4, and 5 days after deblading. For the auxin treated abscission zones respiration was measured at 1,2,3,4,5,6,7,9, and 11 days after deblading. Fifteen plants of each treatment were harvested daily and the 30 abscission zones segregated into 3 aliquots each representing a replicate. The experiment was performed twice.

The discs were placed in 1.0 ml of distilled water in Warburg vessels. KOH (10%) was added to the center well of the vessels to absorb CO_2. Oxygen uptake measurements were taken every 45 minutes over a 3 hour period.

A hydrobarometer was used to correct for barometric fluctuations.

Abscission zones 1.4 mm in thickness were cut from intact Impatiens Sultani Hooke petioles and placed in the side arm of Warburg vessels (1.0 ml of water and the KOH solution were added to the vessels as before). The abscission zones were placed in the side arm since it was thought that respiration would be affected if the sections were floated on water for several hours (Ohmura and Howell, 1960). Oxygen uptake was measured at half-hour intervals the first 6 hours and at 45 minute intervals from 6 to 15 hours. Abscission zones from Impatiens which had been debladed for 12 hours were also cut and placed in Warburg vessels. Oxygen uptake was measured for 13 hours every 45 minutes. This represented the twelfth through the twenty-fifth hours after deblading the petiole. Either stem or petiole sections (1.4 mm thick) from the same plants were analyzed for respiration concurrent with the abscission zones.

Twenty-six to twenty-eight Impatiens abscission zones comprised each of 3 replicates. The average dry weight of the replicates was 3.8 mg. The petiole and stem replicates had an average dry weight of 3.7 and 3.5 mg respectively.

Dry Weight Changes

The dry weight of the abscission zones used in the above respiration studies was determined. After respiration was measured, the tissue was dried for 24 hours at 60°C and the dry weight of each replicate recorded.

RESULTS

Light Microscopic Examination of Abscission

Development of a Protective Layer

The first and second days after deblading, the abscission zones in the lower pulvinus were anatomically indistinguishable from abscission zones of non-debladed plants. The adaxial side of the petiole was characterized by having thick walled collenchyma cells at the junction of the stem and petiole. These thick walled cells extended a few cell layers into the petiole from the point of juncture of the stem and petiole. Distal to these thick walled cells, the petiole was composed of thinner walled, large, parenchyma cells. On the abaxial side of the petiole, below the anastomosed vascular tissue, there was no differentiation between the cells of the pulvinus and stem. The cells were all parenchymatous.

Commencing on the adaxial side, about three days after deblading, cell division occurred in one or two layers of cells (Figure 1B). These layers of newly divided cells, hereafter referred to as the protective layer, were generally located from three to seven cells distal to the point of stem-petiole juncture. Within 24 hours, the protective

Figure 1. Anatomy of abscission in the lower pulvinus of bean. All at X500.

A. Abscission zone of a petiole 2 days after deblading. The stem-petiole junction is in the upper left-hand corner with the petiole extending to the right.

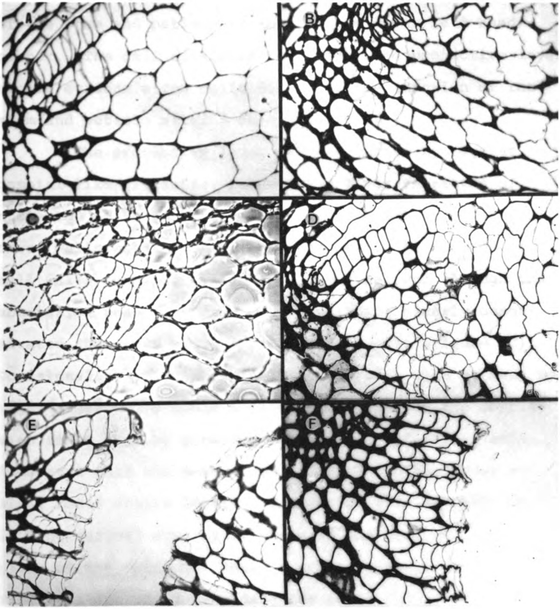
B. Abscission zone of a petiole at 3 days.

C. At 4 days. Phase contrast optics.

D. At 5 days. Separation commencing.

E. At 5 days. Separating petiole.

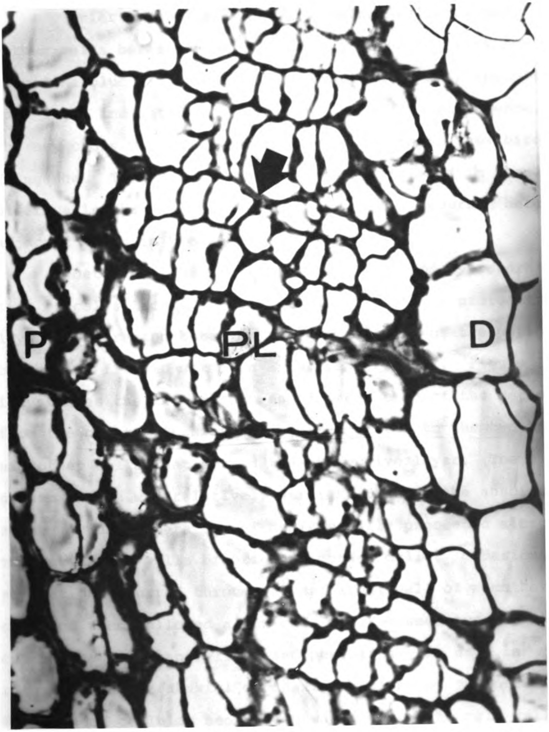
F. At 6 days. The leaf scar as it appears after separation.


A
B
C
D
E
F

layer extended through the pulvinus to the abaxial side forming a plate of cells between the petiole and stem. Figure 1A is a photomicrograph of an abscission zone from a plant two days after deblading. The point of juncture of the stem and petiole is seen in the upper left-hand corner. The cell divisions which form the protective layer became evident a few cells distal to the junction of the stem and petiole after 3 days (Figure 1B).

The protective layer was not formed by regular cambial-like divisions; rather this layer consisted of nucleated cells which divided by the formation of several cross walls within a single mother cell. All of the new cell walls remained thin. A longitudinal cut through the abscission zone four days after deblading clearly demonstrated the cross walls in the mother cells (Figure 1C). Occasionally, in this plane, a mother cell with as many as eight cross walls could be seen. Sections of the abscission zone, made by rotating the petiole 90° on its axis, indicated that the mother cells formed another cross wall along their entire length. This cross wall was not visible in longitudinal cuts since the wall was parallel to the plane of the section. Thus, a single mother cell gives rise to a "nest" of many daughter cells. A cell containing 19 such daughter cells is visible in Figure 2 (arrow).

Figure 2. The abscission zone sectioned parallel with the adaxis. P = proximal; PL = protective layer; D = distal. X 1,500.


P
PL
D

The Separation Process

Prior to separation, the original walls of the mother cells became swollen and had a lower affinity for toluidine blue strain. Sections examined under plane polarized light indicated a concomitant loss of birefringence. The new cross walls did not swell nor did these show birefringence. The swelling was often so pronounced in the older walls that large intercellular spaces occurred between adjacent cells in the separation layer.

Detachment of the petiole was effected by separation of cells located on the distal side of the protective layer. Separation began on the adaxial side of the petiole commencing five to six days after leaf removal. Figure 1D illustrates the initial stages of separation of the petiole from the plant. Separation occurred distal to the two tiers of mother cells that formed the protective layer. The plane of separation was relatively straight through the abscission zone. Actual separation of cells usually proceeded along the anticlinal walls of original mother cells. Occasionally, separation occurred through a periclinal wall of a mother cell and then followed a new cell wall between two daughter cells (the terms anticlinal and periclinal are used in reference to the abaxial or adaxial surface of the petiole). The mode of cellular separation was between the cell walls. However, an occasional cell was ruptured. This may have been due to mishandling these fragile cells during sample preparation. Separation, which began at the adaxis, progressed sequentially, one cell after another, down through the petiole to the vascular tissue producing an ever widening gap between the petiole and stem. At this advanced stage of separation, it was not possible to excise the abscission zone for anatomical study without the petiole detaching completely.

The Leaf Scar After Abscission

Figure 1F depicts the leaf scar as it appears immediately after separation of the petiole. For the most part, the original mother cells were left on the stem side following abscission. Considering the number of daughter cells within the two tiers of mother cells left on the stem, the layer of cells which affords the plant protection following leaf drop is several cells thick. Those cells with surfaces exposed to the air become somewhat collapsed following separation.

Two days after separation, the cells exposed to the air, as well as three to four cell layers internal to these, stained red with phloroglucinol-HCl. This indicated a lignification of cells of the protective layer. No staining reaction was observed the first day after separation.

Following separation, if plain lanolin paste was applied to the leaf scar, the cells of the protective layer did not become lignified. No lignification was evident even at six days after separation when stained with phlorglucinol-HCl.

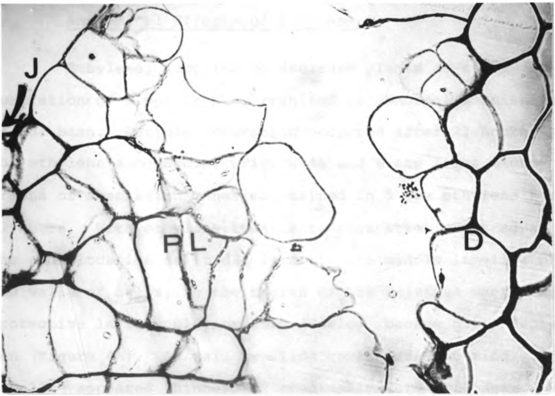
The Effect of Auxin on Development of the Protective Layer

Auxin (2,4-D) supplied to the petiole at a concentration of 7 X 10^{-5}M in lanolin paste, caused a 7-day delay in the formation of the protective layer. With auxin treatment, the protective layer did not become evident until the ninth day after deblading. At that date a few cell divisions were apparent in the abscission zone. By the eleventh day, two tiers of mother cells produced nests of daughter cells. Except for the enlarged nature of the cells, due to 2,4-D treatment, the protective layer which formed was anatomically similar to that formed in non-auxin treated abscission zones.

Separation Following Auxin Treatment

The separation process was anatomically the same in the auxin treated plants as in control plants. Again, separation proceeded from the adaxial side of the petiole along a line distal to the two tiers of mother cells (Figure 3). Separation proceeded along the anticlinal walls of the original mother cells and occasionally along cell outlines of new cell walls. The leaf scar was, therefore,

Figure 3. Separating petiole treated with 7 x 10^{-5}M 2,4-D for 13 days. J = stem petiole junction; PL = protective layer; D = distal. X 1,250.


J
PL
D

left with a protective covering of one to two layers of mother cells.

When treated with 2,4-D (7×10^{-5}M), fifty percent of the petioles had abscised four days after anatomical development of the protective layer. This was only a one day delay from that of control plants.

Anatomical Effects of Ethylene

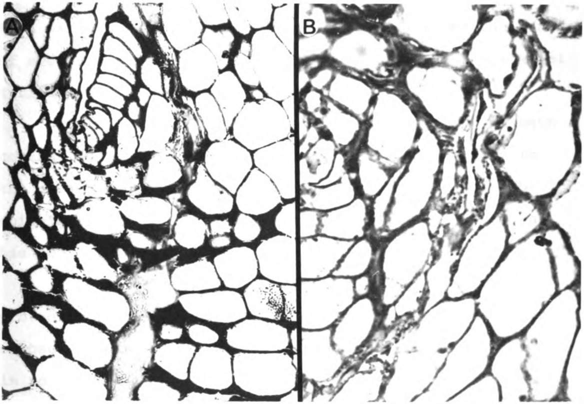
Ethylene, supplied to debladed plants at a concentration of 5 ppm in air, resulted in abnormal abscission in the bean. Petiole separation occurred after 22 hours in the ethylene atmosphere. Figure 4A and B are light micrographs of abscission zones maintained in 5 ppm ethylene for 22 hours. With ethylene treatment, separation occurred at the same location as it did in air. The middle lamellae of the walls of cells, in the region of the pulvinus where the protective layer would normally develop, became quite swollen (Figure 4A). As wall swelling continued, the middle lamellae appeared thinner and eventually were no longer evident. Thus, intercellular spaces became increasingly more evident and much larger prior to separation. Most of the intercellular spaces were located along a single plane through the abscission zone. Separation occurred through this plane.

The separatory process was not as orderly in ethylene treated tissue as in control or auxin treated

Figure 4. Separation following ethylene treatment.

A. Separation just distal to the stem-petiole junction. X 925.

B. Magnified cell walls displaying ethylene induced separation. X 1,800.

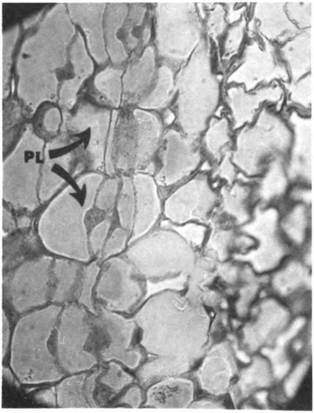

A
B

tissue. Normal separation commenced on the adaxial side of the petiole. A gap developed between the petiole and the stem. As separation progressed towards the abaxial side of the petiole, the gap was like a wedge, gradually working its way between the petiole and stem. After ethylene treatment, separation occurred between adjacent cells or groups of cells along the separatory plane. This separation was observed to occur at random places along the line of eventual separation rather than beginning at the adaxial surface and proceeding to the abaxial surface.

Although cell division preceded separation in both control and auxin treated petioles, this was not true in the presence of ethylene, however, ethylene promoted abscission occurred through the normal separatory plane as has been previously indicated.

After ethylene promoted defoliation, a narrow protective layer formed over the surface of the stem when exposed to air. Lanolin applied to the surface resulted in no protective layer formation. Instead the cells in immediate contact with the lanolin became extremely distended. The protective layer which formed following ethylene treatment was morphologically dissimilar to that which formed under normal conditions (Figure 5). Only three to four layers of cells formed the protective layer after ethylene promoted abscission as opposed to about five to seven layers under normal circumstances. These layers of

Figure 5. The protective layer formed after ethylene promoted abscission. Distal to the protective layer (PL) are collapsed cells which are sloughing-off. X 600.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cells seemed to originate from cambial-like activity of a typical wound response. The cells of the protective layer were aligned in tiers the same as cork tissue aligns. This type of protective layer was in contrast to the nest type which normally developed as described earlier. Furthermore, the nest type divisions occurred prior to organ detachment; after separation, the stem was left with a protective covering of one or two layers of these nests of cells.

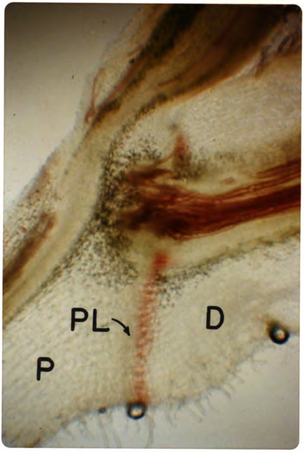
Histochemical Observations of a Layer of Cells Distal to the Separation Layer

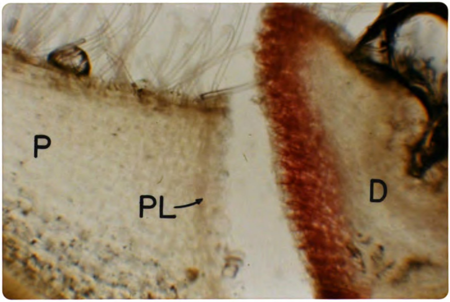
Two days after deblading the cell walls of a band of cells, approximately three cell layers wide, stained positively to phloroglucinol-HCl. This phloroglucinol positive layer was located approximately two cells distal to the separation layer. The stained layer became evident two days after deblading which was one day prior to cell division which formed the protective layer. By the second day after leaf removal, 7 of 8 petioles stained positive to phloroglucionol-HCl. Initially, those cells on the adaxial side of the petiole were the only ones that stained. By three days, the cells of this layer stained more deeply and the band was continuous across the abscission zone (Figure 6A). Staining of these cells intensified up to the time of separation, which occurred on the blade side of the protective

Figure 6. Phloroglucinol-HCl staining on the distal side of the separation layer. D = distal; P = proximal; PL = protective layer.

A. Three days after deblading. X 175.

B. Six days after deblading indicating separation at the abaxis. The stained cells separate with the petiole. X 440.


PL
D
P


P
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D

layer. The stained cells, and one or two layers adjacent to the separation layer separated with the petiole (Figure 6B).

The results of a series of staining reactions to determine the cell wall constituents which reacted positively to phloroglucinol-HCl appear in Table 1.

Table 1. Histochemical Tests on a Layer of Cells Distal to the Separation Layer.

Test	Testing For	Reaction
Phloroglucinol-HCl	Lignin (non-specific)	+
Chlorine-Sulfite	Lignin	-
PAS Reagent	Aldehydes	+
Ninhydrin	Protein; Amino Acids	-
IKI	Starch	-
Aniline Blue (visual)	Callose	-

Although the results of the histochemical tests were somewhat inconclusive, it may be surmised that the compound which stained with phloroglucinol-HCl was lignin, since the Chlorin-Sulfite test for lignins failed to cause a color reaction in the lignified xylem elements of bean petioles. The positive PAS reaction for aldehydes can be explained since lignin interferes with this reaction (Jensen, 1962).

Pectin Localization

The distribution of pectin in the abscission zone was examined in non-debladed plants and in plants which had been debladed for 5 days. A staining reaction was used wherein aklaline hydroxylamine hydrochloride reacted with methyl esters of pectin to produce pectin hydroxamic acid. The pectin hydroxamic acid subsequently complexed with ferric ions. Thus the distribution of iron in the abscission zone paralleled that of pectin.

The distribution of pectin in the abscission zone (Figures 7 and 8) was indicated by the localization of iron using the electron microprobe. The left-hand column in both figures is a secondary electron image of the tissue. In the right-hand column are the corresponding x-ray oscillograms. The iron distribution in the abscission zone of a non-debladed petiole is depicted in Figure 7A and B. The protective layer (pl) should form where indicated in Figure 7A. Tissue distal to the protective layer or on the leaf blade side is labelled "d." As is evident from the iron oscillogram (Figure 7B), pectin was distributed uniformly throughout the abscission zone prior to development of the protective layer. Figure 7C and D indicate the distribution of pectin in the abscission zone of a petiole five days after deblading. The protective layer, which was very evident, was defined by having cell walls that were less intense and appeared thinner than the cell walls of tissue either distal (d) or proximal (p) to it. The protective layer had the nests of cells referred to previously. The iron x-ray oxcillogram

Figure 7. Localization of pectin and calcium in the abscission zone. d = distal; pl = protective layer; p = proximal. All at X 230.

A. Secondary electron image. Non-incinerated control.

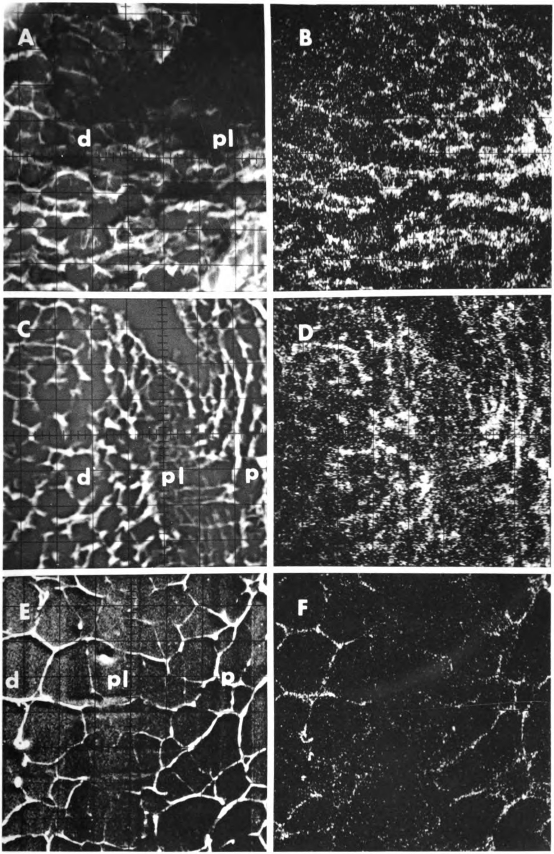
B. Iron x-ray oscillogram of A.

C. Secondary electron image of an abscission zone 4 days after deblading; non-incinerated.

D. Iron x-ray oscillogram of C.

E. Secondary electron image of an abscission zone 5 days after deblading; incinerated.

F. Calcium x-ray oscillogram of E.


A
d
pl
B
C
d
pl
p
D
E
d
pl
p
F

(Figure 7D) shows less iron in a band which coincides with the protective layer. Thus, the pectin content of protective layer cells was lower than that of the surrounding tissue.

The distribution of calcium was examined with the microprobe five days after deblading in sections which had not been stained with hydroxylamine-ferric chloride. Pectin distribution should coincide with the distribution of calcium since the tissue was given no chemical treatment that would remove the calcium from the middle lamella (the dehydration series used in sample preparation would have removed water and alcohol soluble calcium in the cells). The protective layer (pl) was clearly evident in this tissue exhibiting its characteristic fragile appearance (Figure 7E) in the secondary electron image. The distribution of calcium (Figure 7F) was similar to that of iron in tissue which had been stained with hydroxylamine-ferric chloride. The protective layer again contained less metal than the surrounding tissue.

Microincineration of the hydroxylamine-ferric chloride sections prior to microprobe analysis resulted in sharper definition of the distribution of pectin. The protective layer is evident in Figure 8A and again the iron x-ray oscillogram (Figure 8B) revealed less pectin in the protective layer than in adjacent tissue.

Figure 8. Localization of pectin in the abscission zone; 4 days after deblading. Labelled as in Figure 7.

A. Secondary electron image. The white line indicates the path of the electron beam during the line scan in Figure 9. X 235.

B. Iron x-ray oscillogram of A. X 235.

C. Secondary electron image of distal cells. X 870.

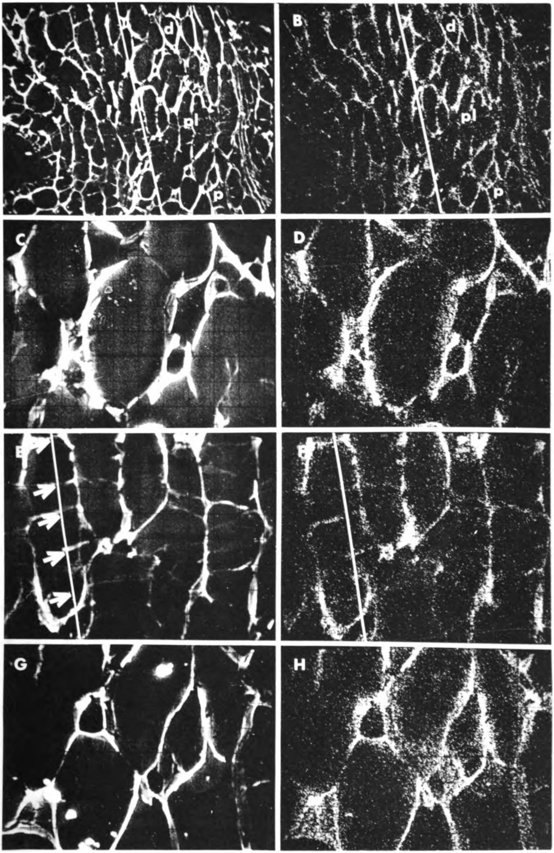
D. Iron x-ray oscillogram of C. X 870.

E. Secondary electron image of protective layer cells. The white line indicates the path of the electron beam during the line scan in Figure 9. Arrows indicate new cross walls. X 870.

F. Iron x-ray oscillogram of E. X 870.

G. Secondary electron image of proximal cells. X 870.

H. Iron x-ray oscillogram of G. X 870.


A
d
pl
p
B
d
pl
p
C
D
E
F
G
H

The fragile cross walls are visible in an original mother cell of the protective layer (Figure 8E). The periclinal walls of the mother cell, to which the cross walls are attached, also appear quite delicate. The iron (viz. pectin) distribution of these cells is shown in Figure 8F. The cross walls and the periclinal walls of the original mother cell exhibit less iron than the cells on either the distal (Figure 8C and D) or proximal side (Figure 8G and H) of the protective layer. In the latter tissue the walls are thicker and contain normal amounts of pectin.

A semi-quantitative estimate of the amount of pectin in the various regions of the abscission zone was made with a line scan. The focused electron beam which generates the x-rays was held stationary while the tissue sample was driven under the beam. In Figure 8A and B, the white line crossing perpendicular to the protective layer indicates a portion of the line scan. The scan was made through the protective layer cells in Figure 8E and F. The resulting scan is represented in Figure 9. A distance of 240 microns was scanned which included the protective layer. The ordinate indicates the intensity of x-ray emission. The high intensity peaks which occur along the distance axis correspond to cell walls. The protective layer is delineated by two open arrows in the center of the graph. The peaks in iron intensity at the open arrows are the end walls of one of the original mother cells which formed the protective layer. Cells on the distal side of the protective layer are on the left with proximal cells on the right.

The solid black arrows (Figure 9) indicate the analysis of the cross walls of the nest of cells. These arrows correspond to those in Figure 8E. As is evident, the cross walls in the protective layer contain less iron (pectin) than the walls of cells in adjacent tissue. Similarly, the anticlinal end walls (indicated by the open arrows) of the original mother cell contain more iron (pectin) than the new cross walls.

The above data indicate that the distribution of pectin in the abscission zone was uniform prior to deblading. Four days after deblading, when the protective layer was well developed, there was less pectin in the protective layer than in adjacent tissue. Histochemical procedures which utilized hydroxylamine-ferric chloride stain and the distribution of calcium in unstained abscission zones support these conclusions.

Electron Microscopic Observations During the Abscission Process

Changes in the Cell Wall During Abscission. I. Cell Separation

The cell walls of several adjacent cells of the abscission zone of non-debladed plants are shown in Figure 10. Treatment of sections with heavy metal salts (uranium

Figure 9. Line scan indicating pectin content across the abscission zone. Open arrows indicate the anticlinal end walls of a protective layer mother cell shown in Figure 8E. The closed arrows along the abscissa indicate the new crosswalls in the nest of cells in Figure 8E. Tissue distal to the protective layer is on the left and proximal tissue on the right of the line scan.

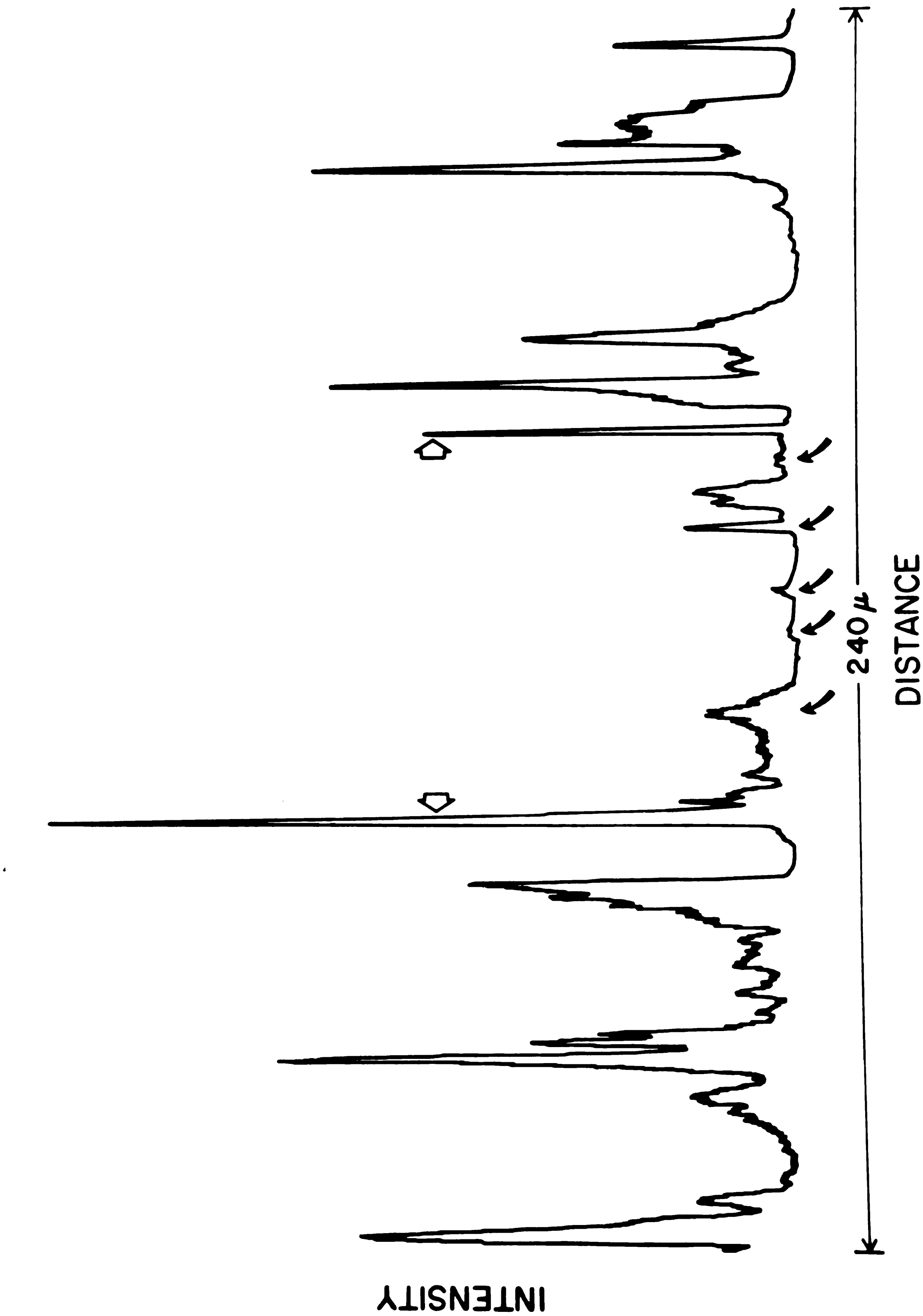
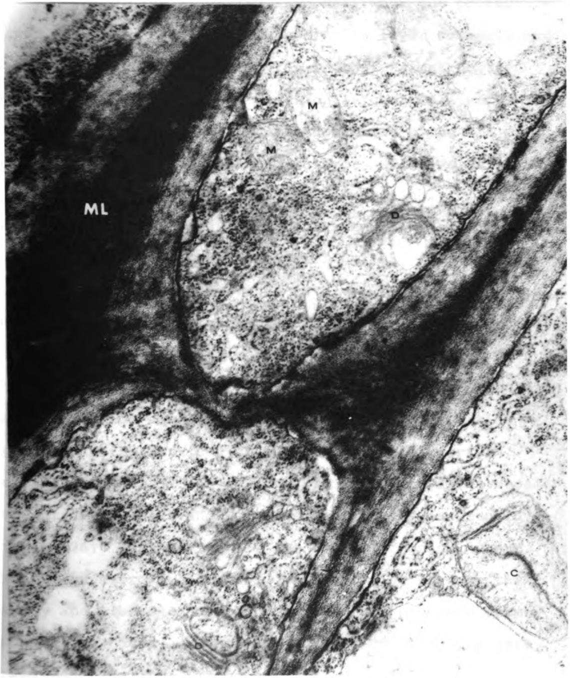

240µ
DISTANCE
INTENSITY

Figure 10. Cells from non-debladed tissue. ML = middle lamella; C = chloroplast; M = mitochondria; D = dictyosome. X 25,000.


M
M
ML
D
C
D

and lead), to increase contrast, caused increased electron density in the middle lamella.

Three days after leaf removal, when the protective layer was visibly evident, cells in the layer commonly had a lack of dark staining material in the central portion of the cell walls (Figure 11). The largest cell wall, labelled "cw" in Figure 11, was separating along its middle lamella. Note that the thinner walls, which extend from the separating wall, were not separating. Correspondingly, a line of dark staining material was evident along the center of these thin cell walls.

The sequence of events involved in the separation of adjacent cells of the separation layer is illustrated in Figure 12A to D. At the time when the amount of dark staining material of the middle lamella decreased (approximately three days after leal removal), discontinuities appeared in the matrices of the cell walls of certain cells in the separation layer. Lysigenous cavities became evident (Figure 12A and B). One such cavity is seen in Figure 12A. To the right of the photomicrograph, where the cavity narrows, electron dense material borders the cavity and also extends along the line of probable extension of the cavity. Numerous cavities of this type were observed in cell walls by the third day after deblading. The cell wall in Figure 12B had less heavy metal staining in the middle lamella than control tissue (Figure 10).

Figure 11. Separation layer cells at early stage of separation; 3 days after deblading. CW = cell wall; V = vacuole; C = chloroplast; S = starch grain; N = nucleus; D = dictyosome. X 18,000

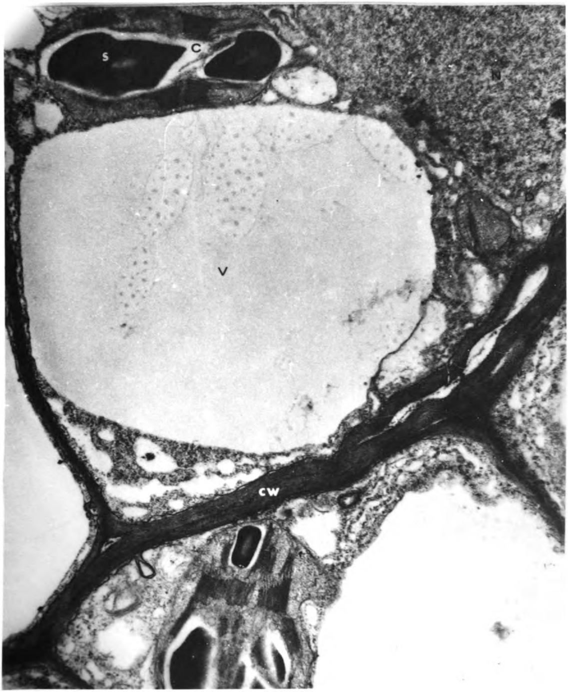

S
C
N
D
V
CW

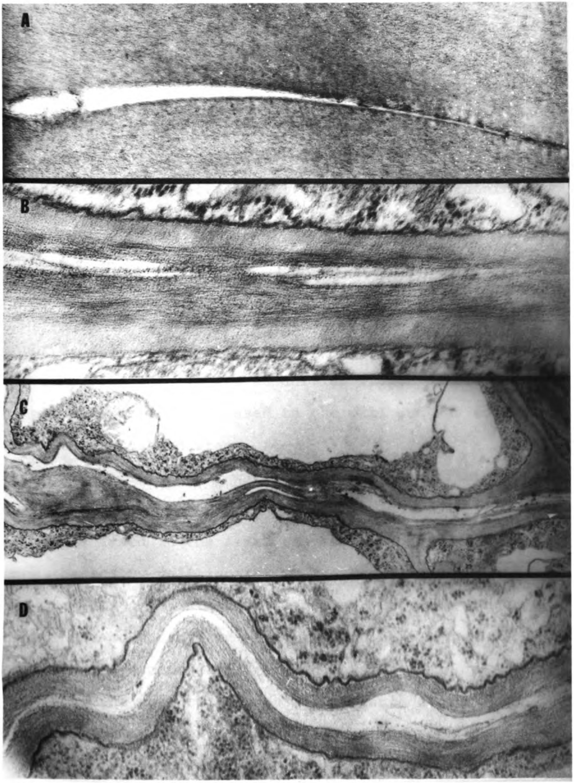
Figure 12. The mode of cell wall separation.

A. Initial stage of separation; 3 days after deblading. X 90,000.

B. Lysigenous cavities in the wall; 3 days after deblading. X 74,000.

C. Extensive separation along the middle lamella; 5 days after deblading. X 20,000.

D. Separation following wall convolutions; 5 days after deblading. X 35,000.


A
B
C
D

It was in the middle lamellar region that the lysigenous cavities developed (Figure 12B). The cavities enlarged by coalescing, which resulted in extensive separation of the cell walls between adjacent cells (Figure 12C). Again, note that the thinner walls, extending from the separating wall in Figure 12C, had no lysigenous cavities within them and their middle lamellae still contained dark staining pectinaceous material.

The cell walls in an advanced stage of separation are seen in Figure 12D. The line of separation followed the convolutions of the cell walls and remained in the center of the walls. The fibrous matrix of the wall was less compacted than it was at earlier stages of separation. The plasmalemma was appressed to the wall and was continuous at this advanced stage.

The swollen cell wall in Figure 13, indicated that regions of the cell wall, in addition to the middle lamella, may become lysed in the final stages of separation. The space, caused by wall separation and swelling, is filled with numerous short strands of fibrous material. These strands are comparable in appearance to that material which normally composes primary cell wall.

In the advanced stages of cell wall separation, extreme deformation of certain walls in the separation layer was observed (Figure 14). The walls appeared swollen and the matrix of the walls was not compact. Lysigenous

Figure 13. Degradation of the primary wall; 5 days after deblading. CW = cell wall. X 21,600.

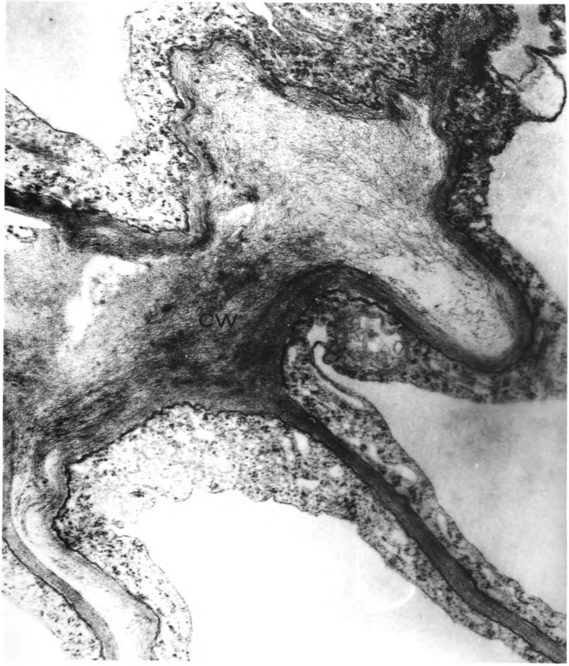
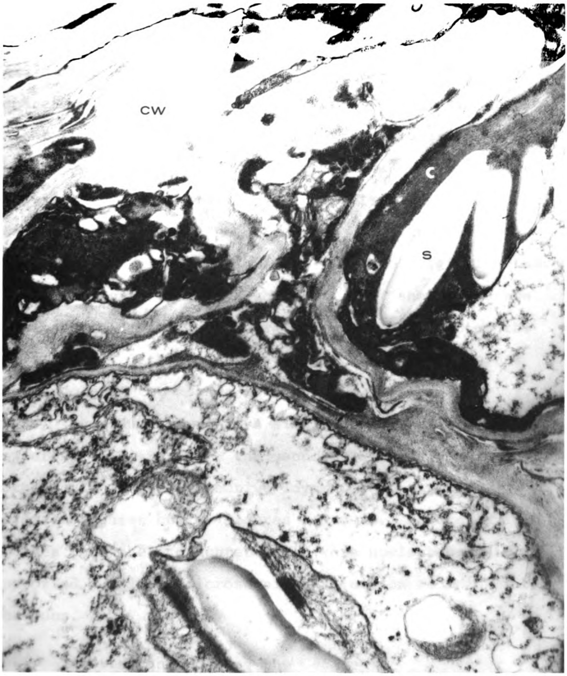

CW

Figure 14. Cell wall collapse; 5 days after deblading. CW = cell wall; C = chloroplast; S = starch. X 23,300.


CW
C
S

cavities were apparent and the walls might be quite convoluted and collapsed upon themselves.

Changes in the Cell Wall During Abscission. II. Cell Division in the Abscission Zone

The third day after leaf removal, when slight separation of cell walls was apparent, cytokinesis occurred in the abscission zone. The vast intracellular vacuole at this stage was traversed by a thin bridge of cytoplasm (Figure 15). Within this narrow cytoplasmic strand a cell plate was laid down. The reticular cell plate was connected at each end to the cell wall of the original mother cell. In Figure 15 this mother cell wall shows evidence of separation (arrow). The reticulate appearance of the new cell wall later changed to a continuous wall across the vacuole of the mother cell.

Eventually, within a single mother cell, several new cross wall traversed the vacuole (Figure 16). Each mother cell in the protective layer formed a nest of several daughter cells (Figures 1 and 2). Figure 16 indicates that these daughter cells were nucleated; this is supported by light microscopic examination of thicker sections.

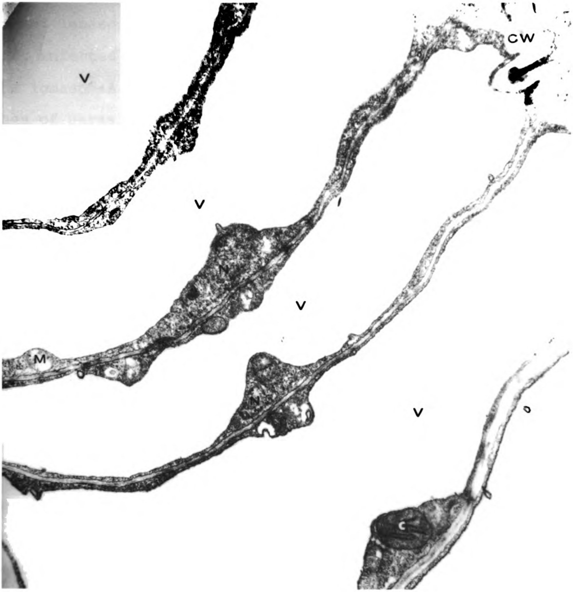
Protuberances Along the Cell Wall: Lomasomes

The term lomasome was initially used by Moore and McAlear (1961) to indicate a spongy structure that was

Figure 15. Cell division during wall separation; 3 days after deblading. CW = cell wall; CP = cell plate; L = lomasome body; V = vacuole; N = nucleus; C = chloroplast. X 16,300.


V
L
CP
C
V

Figure 16. Daughter cells in a mother cell of the protective layer; 4 days after deblading. CW = cell wall; V = vacuole; M = mitochondria; N = nucleus; C = chloroplast. X 6,300.


V
CW
V
V
M
N
N
V
C

continuous with the cell wall and limited by the plasma membrane. While these structures have been described as lomasomes in the fungi, such formations between the wall and the plasmalemma, although observed, have not been termed lomasomes in higher plants, except in the case of rust infected wheat leaf cells (Ehrlich, et al., 1967). The lomasomes in wheat leaf cells were of host, rather than of parasite origin. The plasmalemma invaginations into cells of the protective layer produced structurally similar formations to wheat leaf lomasomes and the inclusions within both invaginations were similar. The term lomasome will, therefore, be used to designate such wall features in the abscission zone cells of bean.

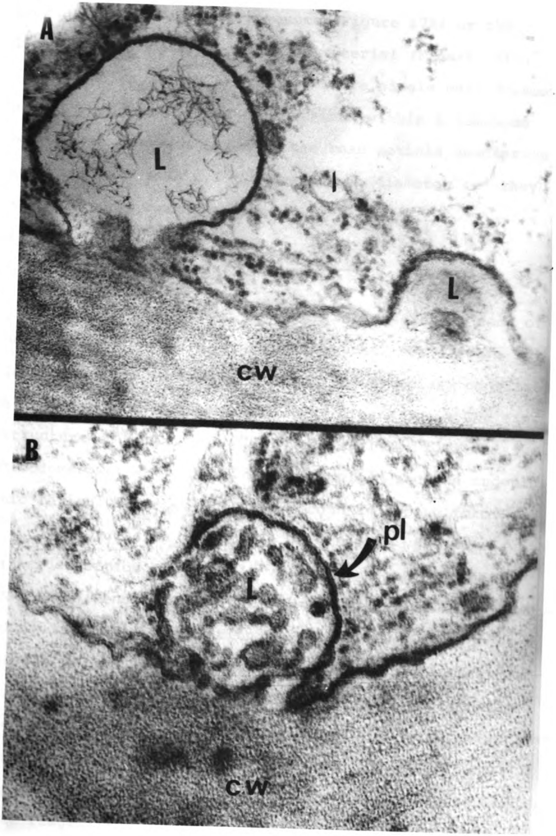
The lomasomes were visible throughout the development of the protective layer. The number of lomasomes was not related to time after leaf removal. Neither could the point of origin of cell wall lysis be associated with a lomasome (Figure 17). Not all cell walls of cells in the protective layer had lomasomes. However, lomasomes were evident often enough to insure that they were neither artifacts nor infrequent cell wall anomolies. Thus, a descriptive characterization of these cell wall protuberances follows.

Structurally, the lomasome is an invagination of the plasmalemma into the cytoplasm. Cell wall material does not fill this invagination. Rather, fibrillar

Figure 17. The structure of lomasome bodies (L) on the cell wall. CW = cell wall; pl = plasmalemma.

A. Lomasome containing fibrillar material. X 98,000.

B. Lomasome containing vescicular material. X 157,000.


A
L
l
L
cw
B
pl
L
cw

inclusions may fill the lomasome (Figure 17A) or the lomasome may contain vescicular material (Figure 17B). The vescicles within the lomasome are single unit membrane bound. Many vescicles are contained within a lomasome body. The lomasomes seen in the bean petiole abscission zone are less than 0.5 micrometers in diameter and they are generally round in shape.

Cytoplasmic Changes During the Abscission Process

The cytoplasm of cells in the abscission zone of non-debladed plants was normal in appearance (Figure 10). In addition to being quite dense, the cytoplasm contained a full complement of intracellular organelles. The fine structure of these organelles was usual for plant tissue. Ribosomes were abundant in the cytoplasm and the endoplasmic reticulum was of both the smooth and rough form.

Abscission zone cells were highly vacuolated with a large portion of a cell's volume occupied by a single vacuole. Hence, the cytoplasm was restricted to the pariphery of the cell appearing as a marginal band appressed to the cell wall. The membrane of the vacuole (the tonoplast) generally was the most difficult to preserve as compared to other cellular membranes (e.g., the plasmalemma, and all of the boundary membranes of the various intracellular organelles). At late stages of separation, the fragility of the tonoplast was even more pronounced.

At the time the protective layer began to form and both cell division and cell wall separation had commenced (*viz*. 3 days after deblading), little change in the cytoplasm or its constituents was observable. Only the chloroplasts showed noticeable structural changes (chloroplast changes will be treated separately under a subsequent heading). The large vacuole increased in size, such that only a strip of cytoplasm, generally thinner than the cell wall, surrounded the cell.

As has been discussed earlier, cytoplasmic bridges traversed the vacuole in dividing cells. No increase in the number of dictyosome bodies or of detached dictyosome vescicles were evident in the cytoplasmic bridges as the new cell plate formed (Figure 15) although dictyosomes have been implicated in cell plate formation (Whaley and Mollenhauer, 1963).

At the lattermost stages of separation, the appearance of the cytoplasm changed considerably. Figure 18 shows a portion of three cells from a petiole five days after leaf removal. The cell wall (CW) is separating. The loose appearance of the cytoplasm in two of the cells is in contrast to the more compact nature of the cytoplasm in the third cell. This senescent appearing cytoplasm is present in cells on both sides of the separating cell wall. In addition to its non-compact appearance, the cytoplasm of these cells contains numerous membraneous

Figure 18. Senescent appearing cytoplasm of abscission zone cells at late stage of abscission; 5 days after deblading. CW = cell wall; C = chloroplast; M = mitochondria. X 25,000.


C
CW
M

fragments. These may be fragments of endoplasmic reticulum. Attachment of ribosomes along the endoplasmic reticulum occurs regularly. Senescent appearing cytoplasm of this type is common in cells at this stage of development of the protective layer.

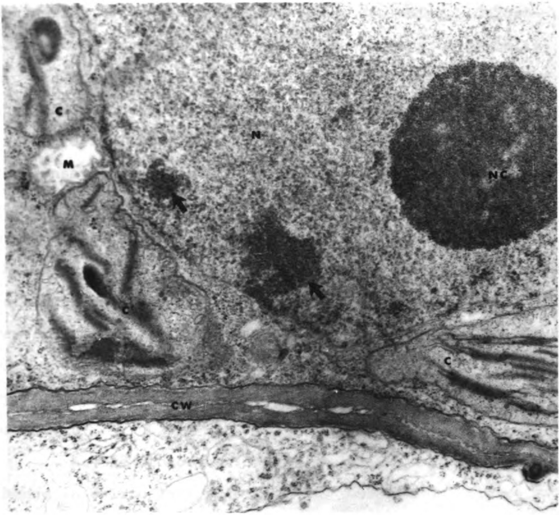
The fine structure of the nucleus of a protective layer cell is given in Figure 19. Patches of perinucleolar chromatin material, similar in electron density to the ribonucleic acids (RNA) of the nucleolus, are dispersed throughout the nuclei of most protective layer cells. Indeed, sections through the nucleolus support the conclusion that these patches are composed of material similar to nucleolar RNA. The cell in Figure 20 shows proribosome bodies in both the nucleolus and diffused patches of perinuclear chromatin. Nuclei with similar fine structural characteristics were also observed in non-abscising abscission zone cells (nuclei of other species also have similar characteristics, O'Brien and McCully, 1969).

The mitochondria remained similar to the control tissue even at the late stages of separation. There was no evidence of the crystalline cored microbodies as found in the abscission zones of tomato and tobacco pedicels by Jensen and Valdovinos (1967; 1968).

Figure 19. Fine structure of the nucleus in a separation layer cell; 5 days after deblading. N = nucleus; CW = cell wall; V = vacuole; M = mitochondria. X 25,800.


M
N
CW
V
D
M

Figure 20. The nucleolus and diffused chromatin material in the nucleus of a separation layer cell; 5 days after deblading. NC = nucleolus; N = nucleus; CW = cell wall; M = mitochondria; C = chloroplast. Arrows indicate the patches of chromatin. X 35,000.


C
M
N
NC
C
C
CW

Chloroplast Permutations During the Abscission Process

The chloroplasts, in cells of the abscission zone, responded to leaf removal by undergoing fine structural changes. Only the chloroplasts, of all intracellular organelles, showed permutations after deblading. Chloroplast structural changes became evident prior to cytoplasmic senescence. The fine structure of the chloroplasts of abscission zone cells from non-debladed plants are given in Figure 21A. The organelle has a double, unit membrane. The dense stroma contains ribosomes, osmiophilic globules, as well as a well developed thylakoid system. The grana are oriented so that they all are aligned parallel with the long axis of the chloroplast. Starch grains were often interspersed among the grana stacks. The chloroplasts were themselves oriented with their long axes parallel with the cell wall to which, owing to the thin band of cytoplasm surrounding the cell, they were in close proximity.

As the protective layer developed, only a slight change in chloroplast structure was perceptible (Figure 21B). The stroma remained dense and the osmiophilic bodies and starch grains were still present. The stacks of grana did, however, become slightly less compact. Less membrane to membrane contact occurred in the accordion-like pleating of membranes which composed the grana stacks

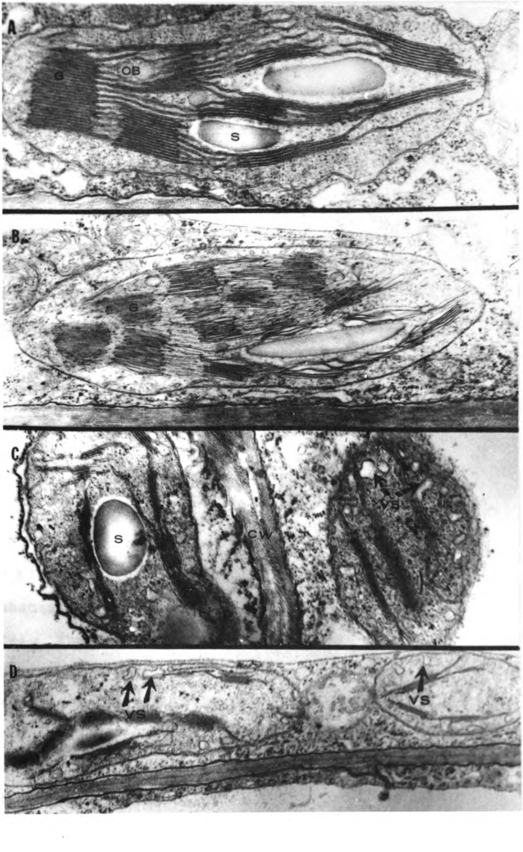
Figure 21. Chloroplast permutations of abscission zone cells during abscission.

A. Control tissue with normal chloroplast. G = grana; OB = osmiophilic bodies; S = starch grain. X 37,500.

B. Swelling of the thylakoids; 3 days after deblading. X 19,200.

C. Disoriented thylakoids; 5 days after deblading. Arrows indicate vescicles (VS). The cell wall (CW) is separating. X 33,000.

D. Confluency of the vescicles with the internal membrane of the plastids; 5 days after deblading. X 31,700.


A
G
OB
S
B
G
C
S
CW
VS
D
VS
VS

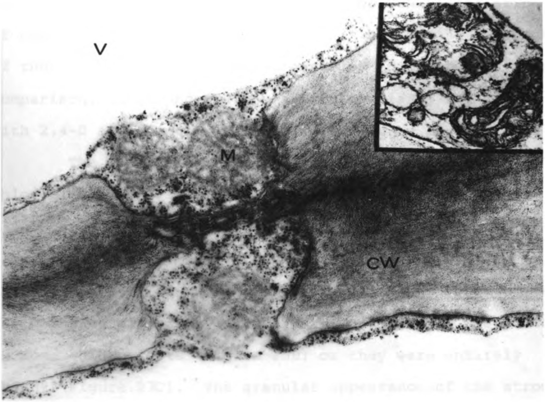
and, therefore, spaces were apparent. The general appearances of the thylakoids indicated that swelling had occurred.

By the time cell separation was in an advanced stage, most of the chloroplasts of the protective layer showed dramatic structural alterations. The thylakoid system was sparse (Figure 21C). The arrangement of the thylakoids within the chloroplasts was not orderly, rather groups of grana stacks and frets might lie perpendicular to others (Figure 21C). Numerous vescicles appeared in the stroma and these were generally located around the periphery of the organelle. These vescicles were often confluent with the inner of the two limiting membranes of the chloroplasts (Figure 21D).

The Effect of Abscission Delaying Concentrations of Auxin, 2,4-Dichlorophenoxyacetic Acid, on the Ultrastructure of the Abscission Zone

Five days after continuous application of 2,4-D (7×10^{-5}M) to the cut petiole, the walls of cells in the abscission zone were unchanged from the control (Figure 22). The dark staining of the middle lamella is evident and extends through the pit pair. The middle lamella stained as intensely in the 2,4-D treated tissue as in non-debladed controls. No separation of cell wall was evident although extensive separation had occurred by five days in non-auxin treated plants. Furthermore, no

Figure 22. The effect of 2,4-D on the cell wall and mitochondria of abscission zone cells. The cell wall (CW) has a dark staining middle lamella. Mitochondria (M) lie in the pit cavity. The petiole has been debladed and treated with 7 X 10^{-5}M 2,4-D for 5 days. V = vacuole. X 27,500. The insert shows mitochondria from non-auxin treated control tissue. X 37,500.


V
M
CW

cell division was evident in the 2,4-D treated abscission zones.

The plasmalemma was visible as a single, unit, membrane bordering the cell wall. This limiting membrane appeared intact. However, the limiting membranes and the cristae of the mitochondria were drastically altered by the 2,4-D treatment. The hard dark line, characteristic of osmium fixed membranes, was not visible in the membranes of the mitochondria in the pit cavities in Figure 22. For comparison, mitochondria from abscising tissue not treated with 2,4-D are shown in the insert of Figure 22.

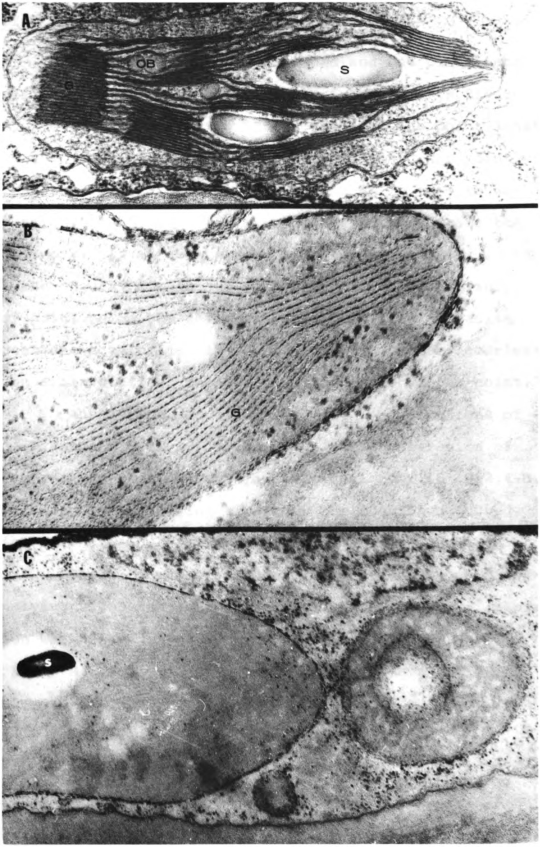
The effects of 2,4-D on the membranes of the chloroplasts are likewise quite striking. Figure 23A is a chloroplast from the abscission zone of a non-debladed plant. After treatment with 2,4-D for five days, the thylakoid system of the chloroplasts was almost obliterated. The membrane of the thylakoids were only faintly discernible in some chloroplasts (Figure 23B) or they were entirely absent (Figure 23C). The granular appearance of the stroma was lost. Starch grains and ribosomes appeared to be present in the stroma. The double limiting membranes of the chloroplasts were also effected by the 2,4-D. The membranes might be discontinuous (Figure 23C) or not apparently visible, as in the smaller of the two chloroplasts in Figure 23 C.

Figure 23. The effect of 7 X 10-5M 2,4-D on the chloroplasts of abscission zone cells.

A. Control chloroplast. G = grana; OB = osmiophilic body; S = starch grain. X 39,000.

B. Disruption of the grana (G) and limiting membrane of the chloroplast five days after 2,4-D application. X 79,000.

C. Complete obliteration of the grana five days after 2,4-D application. X 40,800.


A
OB
S
G
B
G
C
S

Effect of 2,4-D on the Time to Abscission

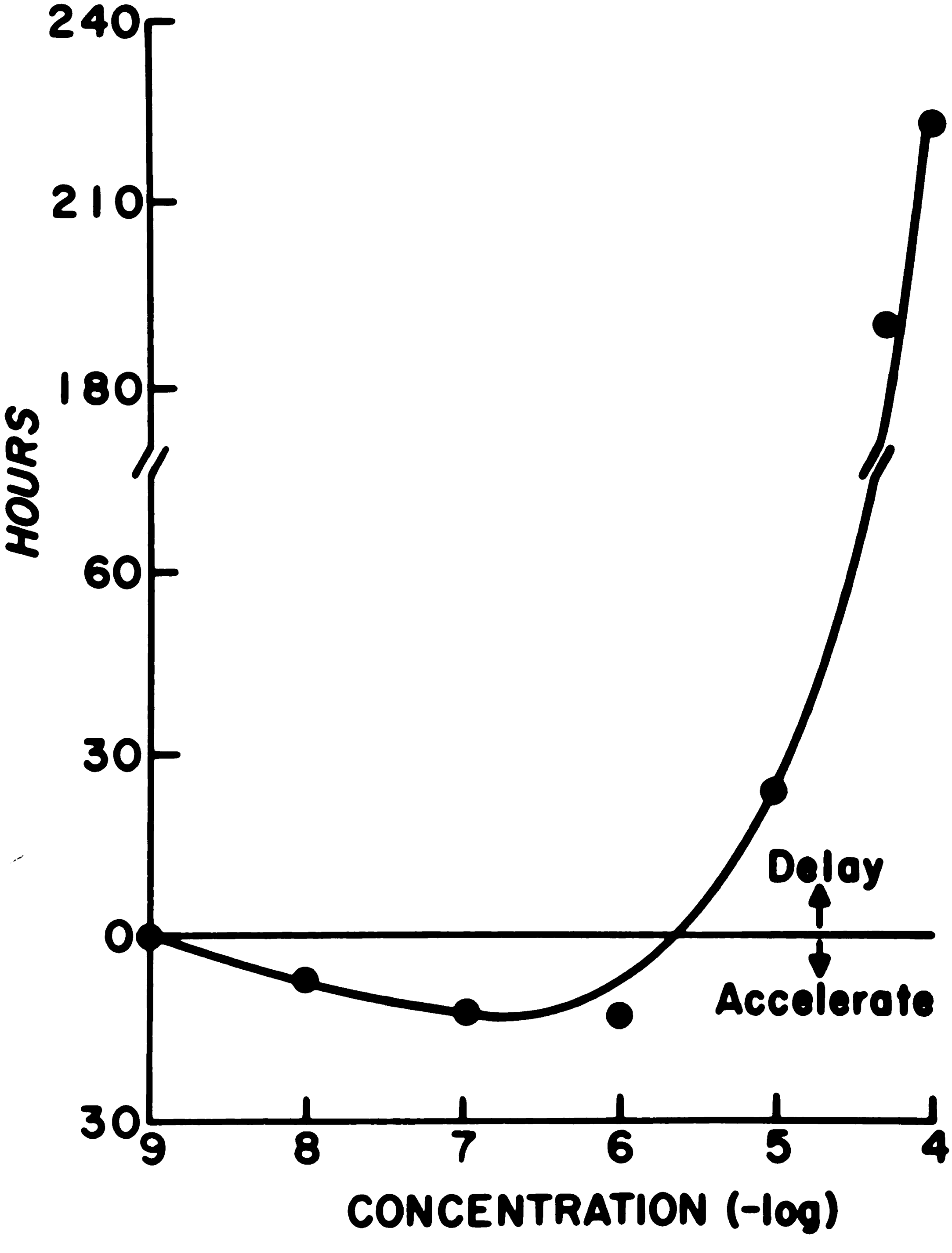
Petioles which were debladed and had plain lanolin applied to the petiole abscised in 6.2 days. In Figure 24, the line intersecting the ordinate at the point labelled zero represents this control condition. Any point along this zero line indicates no change in time to abscission. Points above the zero base line indicate the treatment delayed abscission. The amount of delay would be the "Hours" coordinate on the ordinate. Conversely, any point below the zero base line indicates the auxin treatment accelerated abscission. The amount of acceleration again would be the "Hours" coordinate of the point.

Abscission was accelerated at concentrations of 2,4-D ranging from 10^{-6}M to 10^{-8}M. An acceleration of 17 hours was observed with an application of 10^{-7}M 2,4-D. No change in abscission time over the control occurred with 10^{-9}M 2,4-D.

Separation was retarded for one day or longer by applying 2,4-D concentrations of 10^{-5}M or greater. Separation was delayed one day with 10^{-5}M, 2,4-D, whereas 7×10^{-5}M 2,4-D delayed separation for 8 days. A 10 day delay in time to separation resulted from treatment with 10^{-4}M 2,4-D.

If 2,4-D was applied at concentrations of 10^{-3}M or greater, toxicity occurred. Marked curvature, yellowing, swelling, and necrosis of the petiole was evident

Figure 24. The effect of varying concentrations of 2,4-D on the number of hours retardation or acceleration of abscission of treated petioles from the control. Each point on the curve represents 4 replicates of 20 abscission zones. Time to 50% abscission of control petioles was 149 hours.


240
210
180
60
30
0
30
HOURS
Delay
Accelerate
9
8
7
6
5
4
CONCENTRATION (-log)

within a few days. Even at lower concentrations some of these symptoms were evident at the point of application. However, these maladies were confined to the distal portion of the petiole at 2,4-D concentrations of 10^{-4}M or less.

Chlorophyll Changes

There was a decrease in chlorophyll in the abscission zone after deblading (Figure 25). In addition to a marked constriction of the petiole at the abscission zone a few days after leaf removal, there was also a noticeable loss in coloration in that region. From the original green color of a non-debladed petiole, the abscission zone appeared slightly yellow to white at the time of petiole separation. The chlorophyll content of abscission zone sections, plotted as a percent of non-debladed petioles versus successive dates after deblading, gives a decreasing log function. The rate at which chlorophyll content decreased in the abscission zone was thus greater with time. The fifth day after deblading, the chlorophyll content of the abscission zone was only 41% of its initial value.

Treatment of the petiole with 2,4-D(7×10^{-5}M) altered the chlorophyll content of the abscission zone with time (Figure 26). During the twelve day time course of the experiment, the chlorophyll content generally

Figure 25. The change in chlorophyll content with time after deblading; plotted as a percent of day zero. Each point on the curve represents an average of 3, ten abscission zone, replicates.

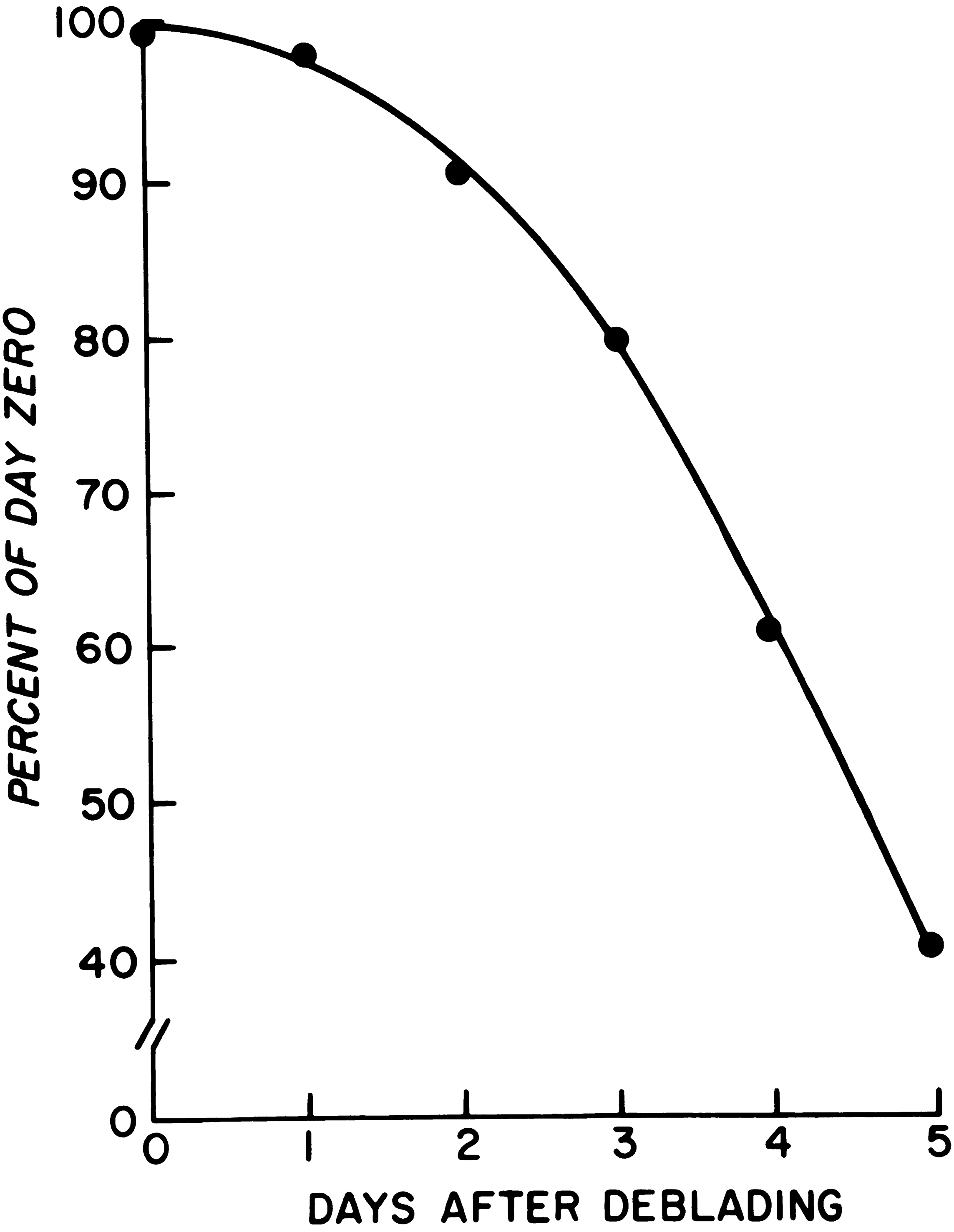
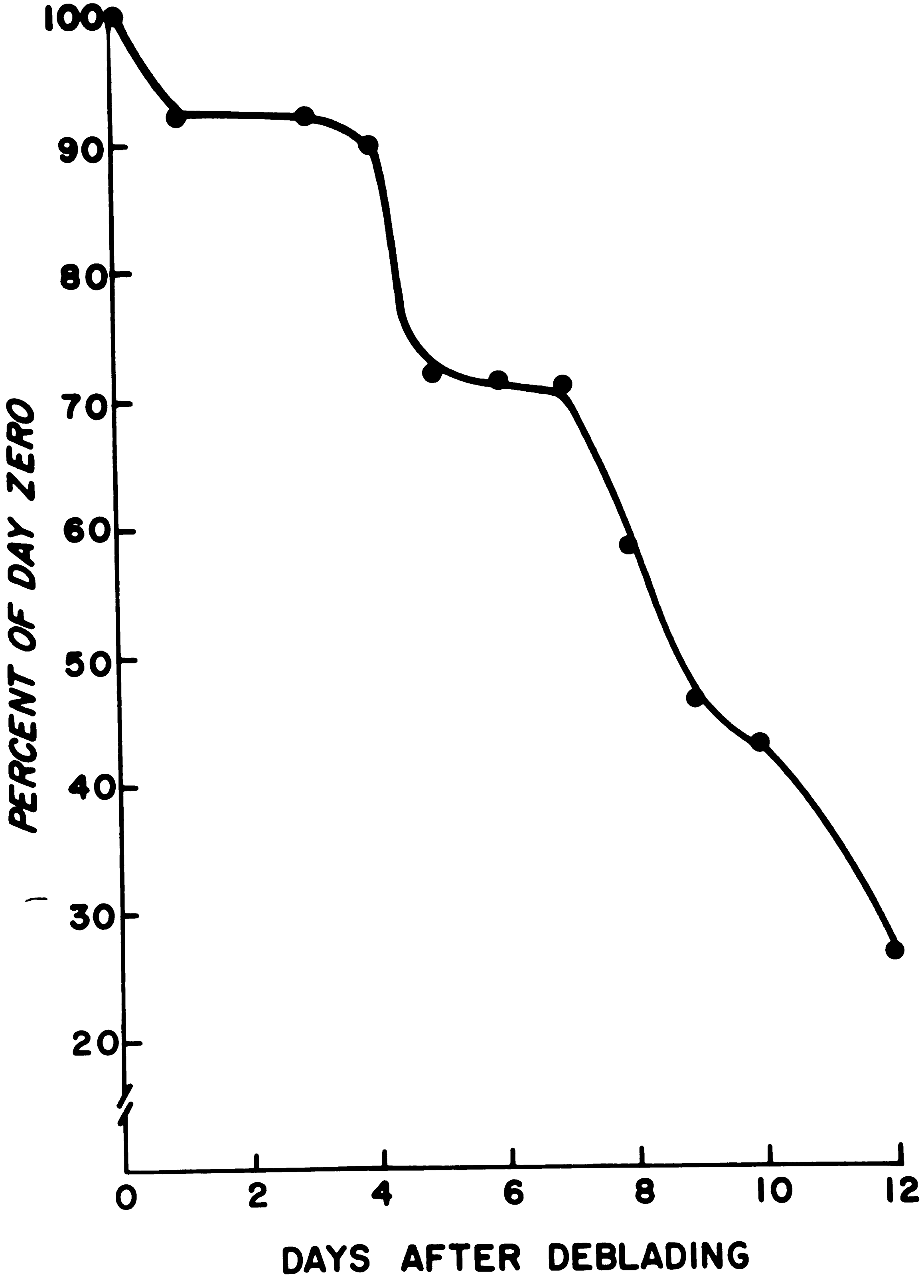

100
90
80
70
60
50
40
0
PERCENT OF DAY ZERO
0
1
2
3
4
5
DAYS AFTER DEBLADING

Figure 26. The change in chlorophyll content with time after deblading as effected by application of 7 X 10^{-5}M 2,4-D to the cut petiole. Chlorophyll content is expressed as a percent of the amount present in the abscission zones of non-debladed petioles.


100
90
80
70
60
50
40
30
20
PERCENT OF DAY ZERO
0
2
4
6
8
10
12
DAYS AFTER DEBLADING

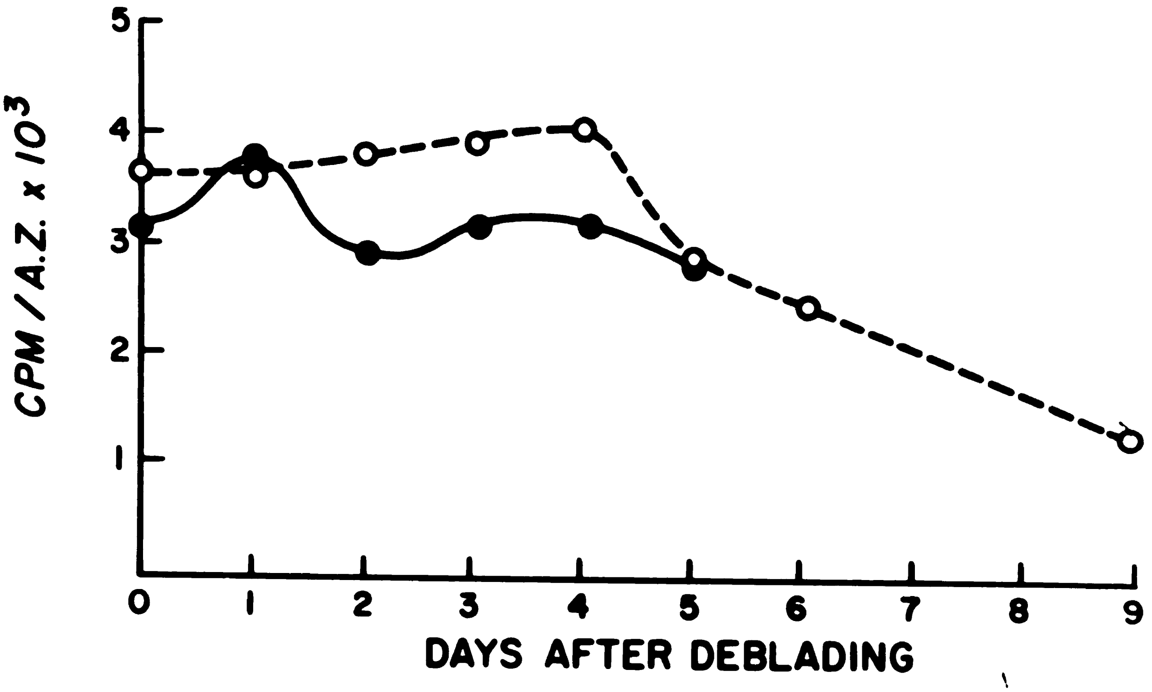
decreased. However, two plateau areas are evident on the graph. The chlorophyll content of the abscission zone remained constant at about 90% for the first four days after deblading. A sudden drop in chlorophyll content occurred at day five to 72% of day zero. This level was maintained through the seventh day after deblading. After the seventh day, the rate of chlorophyll loss again decreased sharply and then remained constant. Twelve days after deblading, the chlorophyll content was only 27% of day zero. Separation occurred thirteen days after deblading.

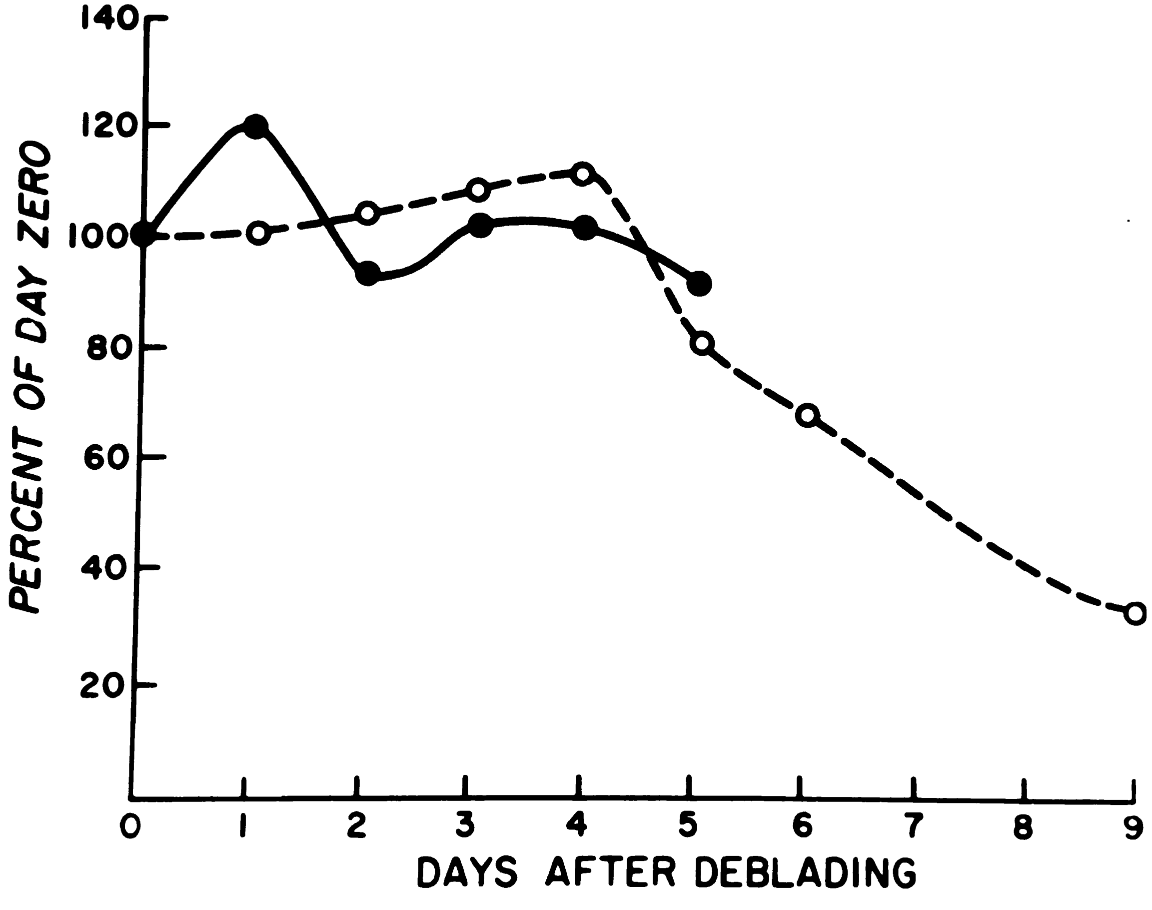
Carbon Dioxide Fixation

The ability of excised, non-treated, abscission zones to fix carbon dioxide in the light was observed throughout the development of the protective layer. Results were similar when expressed as radio-activity (counts per minute) per abscission zone section or as a percent of the initial activity of non-debladed petioles. Carbon dioxide fixation by abscission zones increased 20% the first day after leaf removal (Figures 27 and 28). Fixation on subsequent days varied only slightly from the non-debladed sections at day zero. Values for carbon fixing capacity remained within 90% of day zero for days 2,3,4, and 5.

Figure 27. Carbon dioxide fixation of control and 2,4-D treated abscission zones (per section). CO_2 fixation, as counts per minute per abscission zone section were recorded on successive dates after deblading. Control tissue is the solid line; tissue treated with 7 X 10^{-5}M 2,4-D is the broken line.

Figure 28. Carbon dioxide fixation of control and 2,4-D treated abscission zones (percent control). Solid line is control tissue; broken line is tissue treated with 7 X 10^{-5}M 2,4-D.


CPM / A.Z. x 10^3
5
4
3
2
1
0
1
2
3
4
5
6
7
8
9
DAYS AFTER DEBLADING


PERCENT OF DAY ZERO
140
120
100
80
60
40
20
0
1
2
3
4
5
6
7
8
9
DAYS AFTER DEBLADING

With a 2,4-D (7 X 10^{-5}M) treatment, there was a slight rise in carbon dioxide fixing capacity for the first four days after deblading (Figure 27 and 28). A sharp decrease in the rate of fixation (80% of day zero) occurred at day five. Thereafter, the ability of the 2,4-D treated sections to fix carbon continually decreased and radioactivity per section was less than that of non-auxin treated abscission zones. By the ninth day, the sections fixed only 34% of the carbon dioxide they had fixed at day zero.

Dry Weight Changes

Changes in the dry weight of auxin treated (7 X 10^{-5}M 2,4-D) and control abscission zones were measured from the time of deblading through separation (Figure 29). Immediately after deblading a weight of 0.50 mg per abscission zone section (1.4mm) was obtained. The first day after deblading an increase to 0.58 mg per section was attained in the non-auxin treated plants. Thereafter, the sections lost weight at a constant rate reaching the lowest value of 0.38 mg per section five days after deblading. These results agree with the weight changes of bean abscission zones obtained by Rasmussen and Bukovac (1969). Visually, the petiole appeared constricted in the region of the abscission zone by the fourth day after deblading. Fifty percent abscission of the petioles occurred six days after deblading.

As in control the dry weight of 2,4-D treated abscission zones increased to 0.58 mg per section one day after deblading. The dry weight then remained constant until day 7. From 7 days through separation, the abscission zones lost weight reaching a minimum of 0.44 mg per section at separation.

Respiratory Changes in the Abscission Zone

Respiration in Bean

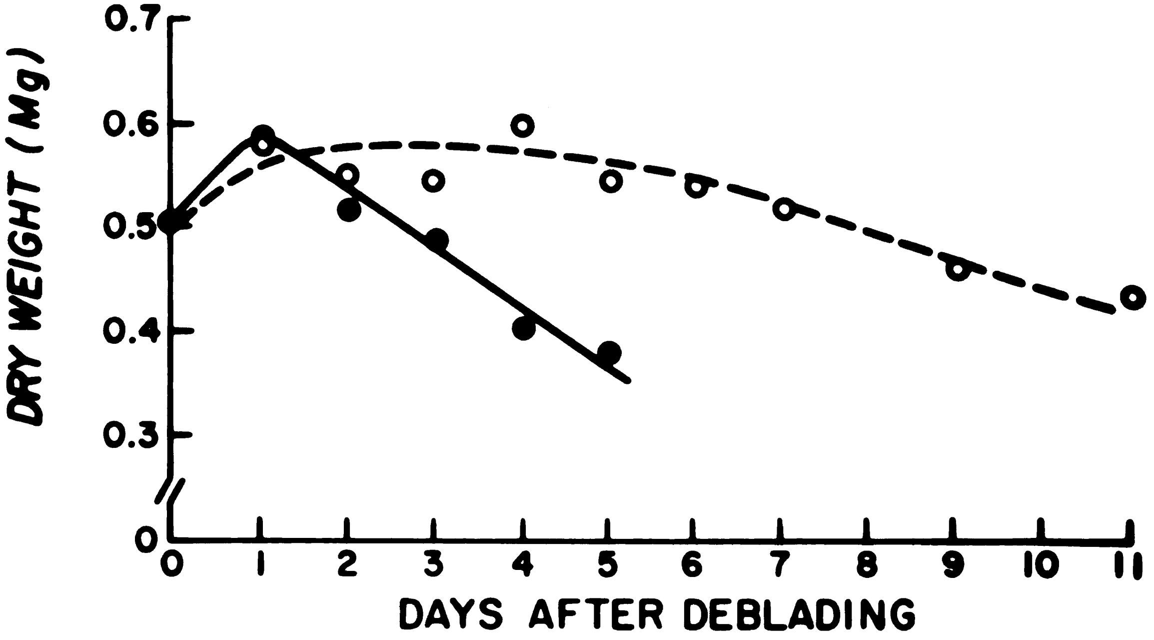
Respiration in the lower pulvinal abscission zones of bean plants was measured by oxygen consumption. Respiratory data were collected throughout the time course of protective layer development from plants with and without auxin treatment (7 X 10^{-5}M 2,4-D).

The oxygen consumption per abscission zone section (Figure 30) indicated little change in respiration in non-auxin treated abscission zones for three days following leaf removal. Respiration began to decrease the fourth day after deblading. This decreased rate became more pronounced; the day preceding separation the rate was less than half of the original rate.

Application of an abscission delaying concentration of 2,4-D (7 X 10^{-5}M) altered the pattern of respiration. Recall that the protective layer became evident in the lower pulvinus following treatment with 7 X 10^{-5}M 2,4-D about the ninth day after deblading. For four

Figure 29. Dry weight changes of control (solid line) and 7 X 10^{-5}M 2,4-D treated (broken line) abscission zones from deblading to separation.

Figure 30. Respiration of control (solid line) and 7 X 10^{-5}M 2,4-D treated (broken line) abscission zones from deblading to separation.


DRY WEIGHT (Mg)
0.7
0.6
0.5
0.4
0.3
0
0
1
2
3
4
5
6
7
8
9
10
11
DAYS AFTER DEBLADING

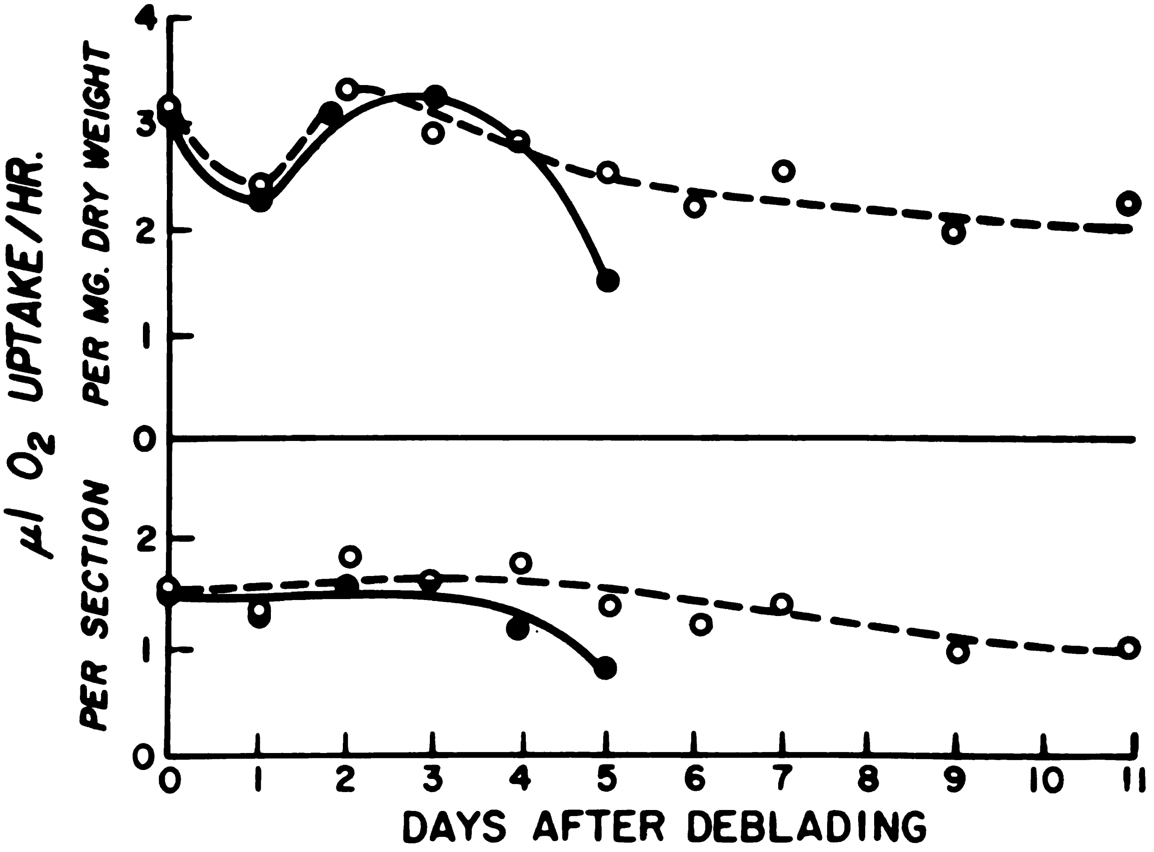
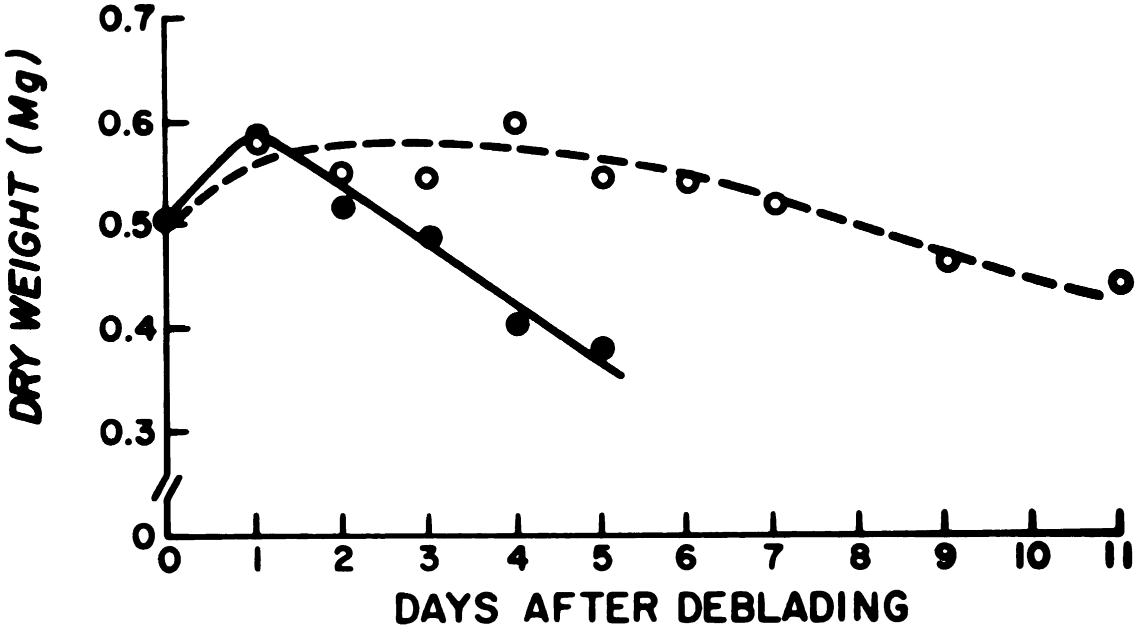
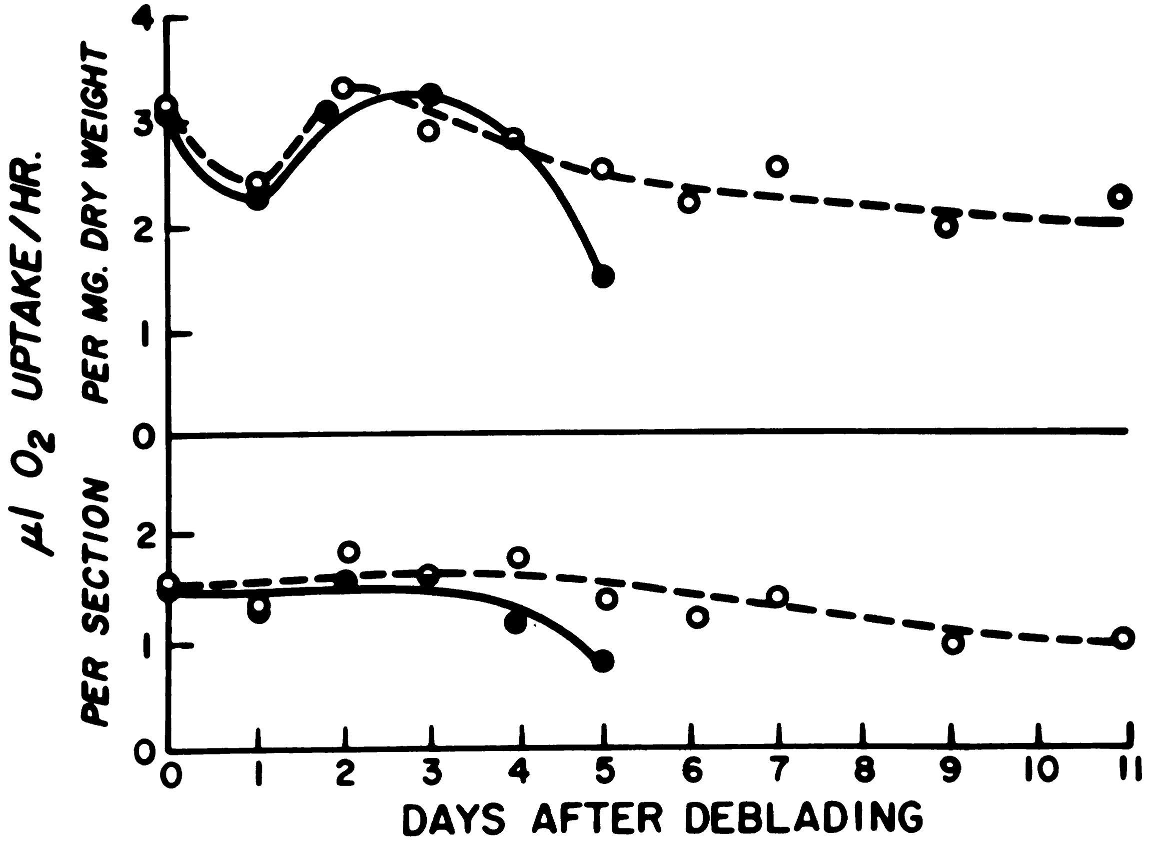

µl O$_2$ UPTAKE/HR.
PER MG. DRY WEIGHT
PER SECTION
4
3
2
1
0
2
1
0
0
1
2
3
4
5
6
7
8
9
10
11
DAYS AFTER DEBLADING

Figure 29. Dry weight changes of control (solid line) and 7 X 10^{-5}M 2,4-D treated (broken line) abscission zones from deblading to separation.

Figure 30. Respiration of control (solid line) and 7 X 10^{-5}M 2,4-D treated (broken line) abscission zones from deblading to separation.


DRY WEIGHT (Mg)
0.7
0.6
0.5
0.4
0.3
0
0
1
2
3
4
5
6
7
8
9
10
11
DAYS AFTER DEBLADING


µl O2 UPTAKE/HR.
PER MG. DRY WEIGHT
PER SECTION
4
3
2
1
0
2
1
0
0
1
2
3
4
5
6
7
8
9
10
11
DAYS AFTER DEBLADING

days following deblading, respiration, on a section basis, continued at the same rate. From the fifth through the eleventh days there was a gradual decrease in respiration. Just prior to separation, the abscission zones respired at approximately 60% of the initial rate. Separation commenced twelve days after deblading.

Since the dry weight of abscission zone sections fluctuated with time after deblading, the respiration data was recalculated on a dry weight basis (Figure 30). Due to the initial increase in dry weight the first day after leaf removal, respiration of both auxin treated and control abscission zones declined the first day after deblading. From the second to the fourth day, respiration in the control abscission zones was approximately the same as occurred immediately following leaf removal. The fifth day, the rate of respiration decreased to half of its initial value.

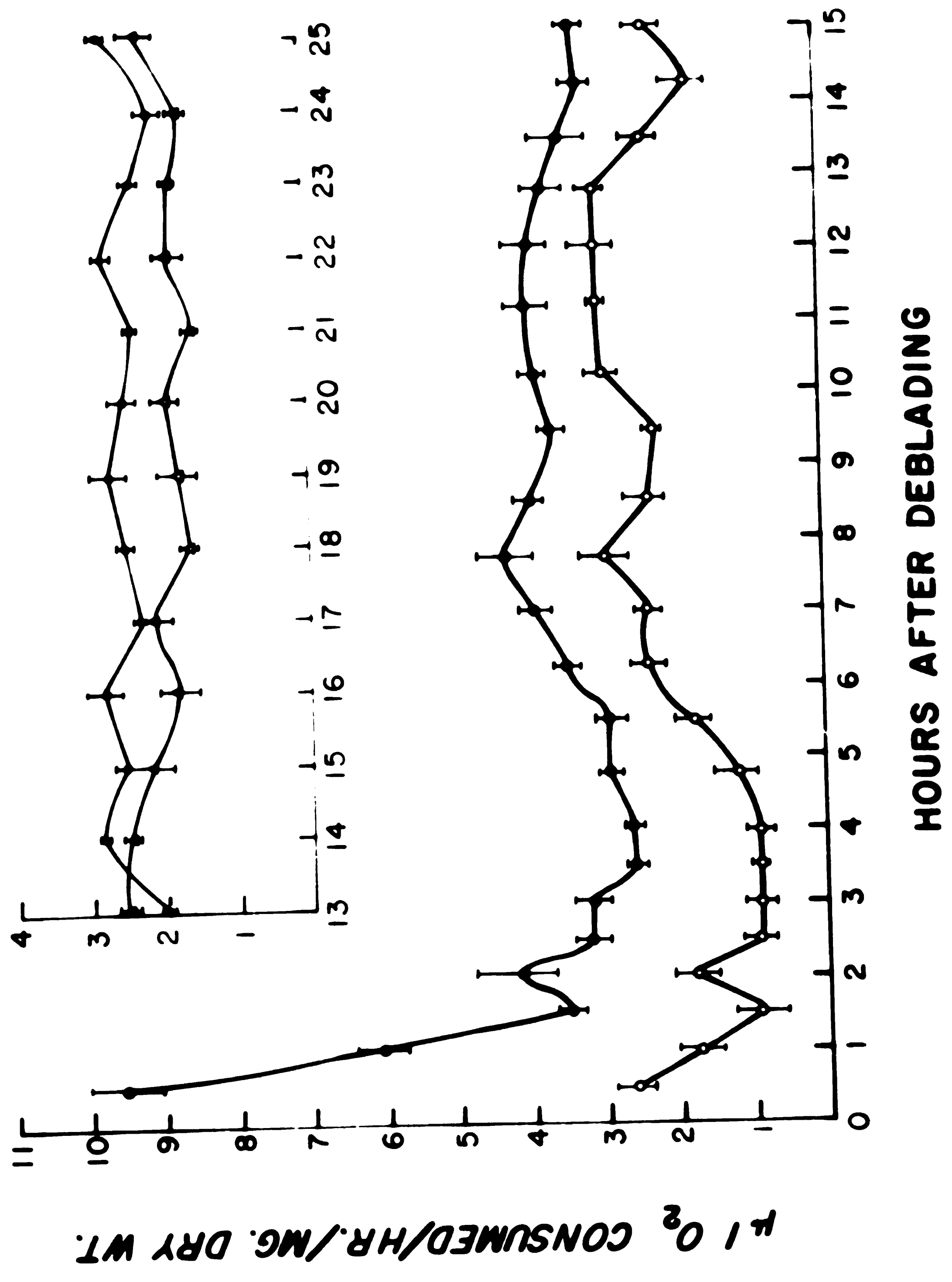
As with control tissue, if oxygen consumption is plotted on a per unit dry weight basis, there is an initial drop in respiration the first day after leaf removal in auxin treated abscission zones. The second day, the respiratory rate returned to its initial value. Thereafter, there was a steady decrease in respiratory rate until separation.

Respiration in Impatiens Sultani

One would expect that during the course of abscission, the events leading to separation might require increased energy. By recording respiratory data on a daily basis perhaps a peak in respiratory activity was missed. Rasmussen (1965) investigated respiration in lower pulvinal abscission zones of primary bean petioles during the first 24 hours after leaf removal which included the induction stage. He noted no change in respiration during the induction stage of abscission.

A more critical examination of respiration during abscission could be done using *Impatiens Sultani* abscission zone sections. Excised *I. Sultani* abscission zones (1.4mm) abscised in a moist chamber at the same time they would if they were not removed from the plant. Cell division did not precede abscission in *I. Sultani*. Thus, this species was well suited for investigating respiratory activity during the abscission process since one could follow respiration in a Warburg apparatus, from the time of abscission zone excision until well after that same abscission zone separated. Respiratory data of abscission zones, petiole, and stem sections of *I. Sultani* appear in Figure 31. Fifty percent of the abscission zones separated at 17 hours in this system. The experiment was terminated at 25 hours with separation complete. The high rate of respiration which occurred immediately after excision

Figure 31. Respiration in *Impatiens Sultani* abscission zone, petiole, and stem sections. Respiration was measured in abscission zone (solid circles) and petiole (open circles) sections through the first fifteen hours after excision of the tissue from the plant. Respiration of abscission zone sections (solid circles) from petioles which had been debladed for 13 hours prior to the respiration studies is indicated in the insert. Respiration of stem sections (open triangles) from the same plants was measured concurrently.


µl O2 CONSUMED/HR./MG. DRY WT.
HOURS AFTER DEBLADING
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
1
2
3
4
5
6
7
8
9
10
11
13
14
15
16
17
18
19
20
21
22
23
24
25
1
2
3
4

might be attributed to tissue response to injury caused by cutting. Other than this initial high rate, the pattern of respiration of the abscission zone sections closely resembled that of the non-abscising tissues of the petiole or stem. No apparent respiratory rise was evident either before, during, or after abscission that could be attributed to the separation process.

DISCUSSION

Ultrastructural Aspects of Abscission in the Lower Pulvinus of Bean

Three previous studies of the ultrastructure of abscission have appeared in the literature. Abscission in the upper pulvinus of unifoliate leave of explants and intact bean plants was investigated by Morre' (1968); explants of cotton and Coleus were examined by Bornman (1967); and the pedicels of tomato and tobacco flowers were studied by Jensen and Valdovinos (1967; 1968; Valdovinos and Jensen, 1968). Many similarities in fine structure exist between abscission in the above tissues and the lower pulvinus of bean. However, the results of the present study indicate that in addition to the similarities, differences also exist.

The Cell Wall

Cell wall separation in the lower pulvinus of bean occurred along the middle lamella between adjacent cells. Cell walls of Coleus, cotton, tomato and tobacco pedicels, and upper pulvinal bean explants also separated along their middle lamellae. Prior to separation, a change in the middle lamella was observed. Bornman (1967) stained for pectin ester groups and found that pectin was concentrated in the middle lamella in control tissue. As abscission progressed, the pectic content of the middle lamella apparently decreased (Figures 7, 8, and 9). Results of the present study agree with Bornman in that there was an apparent loss or change in the interwall substances with time after deblading. The lysigenous cavities observed in bean lower pulvinal abscission zones were also evident in the cell walls of cotton explants when the esterified pectins decreased (Bornman, 1967). Similar cavities developed in *Coleus* explants (Bornman, 1967) and in pedicels of tomato and tobacco flowers (Valdovinos and Jensen, 1968).

Wall changes in the lower pulvinus indicated that components of the primary wall might become hydrolyzed during the abscission process. This observation substantiates results of other ultrastructural studies (Bornman, 1967; Valdovinos and Jensen, 1968) and agrees with the concept of an involvement of cellulose degradation in abscission (Horton and Osborne, 1967).

Crystal Containing Microbodies and Abscission

Jensen and Valdovinos (1968) proposed that the structural changes of crystal containing microbodies (CCBs) which they observed during tomato and tobacco floral abscission indicated the activation of cell wall hydrolytic enzymes within the microbodies. However CCBs were not present in the lower pulvinal abscission zones of bean nor were they evident during abscission of petioles of cotton, Coleus (Bornman, 1967), or bean upper pulvini (Morré, 1968). CCBs have been observed over a wide range of other plant tissues (Gerola and Bassi, 1964; Thornton and Thimann, 1964; Bouck, 1965; Marinos, 1965; Walles, 1965; Price, 1966; Arnott and Smith, 1967; Petzold, 1967; Villiers, 1967; Frederick, et al., 1968; Frederick and Newcomb, 1969), and their function is apparantly not directly involved with the abscission process (Tolbert, et al., 1969). Thus, the absence of these microbodies in the abscission zone is not surprising and presents additional evidence against the hypothesis of Jensen and Valdovinos that the CCBs are involved in cell wall hydrolysis and abscission.

Membrane Integrity and Abscission

Membrane breakdown does not occur before cell wall separation in the lower pulvinus of bean. Jensen and Valdovinos (1968) reported only the limiting membranes of the CCBs became disrupted before separation, the other membrane systems remained intact. On the other hand, Bornman (1967) suggested that the progressive degeneration of cellular membranes led to cell wall dissolution. The degeneration of the plasmalemma supposedly allowed cell wall hydrolytic enzymes to come into contact with the wall. However, even at the most advanced stages of cell wall hydrolysis, the integrity of the plasmalemma was maintained in the lower pulvinus of bean (Figure 12 and 13).

Abscission delaying quantities of auxin (IAA) maintained both the healthy appearance of the cytoplasm and the continuity of cellular membranes in cotton (Bornman, 1967). The application of an abscission delaying concentration of auxin (2,4-D) to the lower pulvinus of bean caused pronounced damage to cellular membranes. The thylakoid systems in the chloroplasts and other membrane systems were completely obliterated by the auxin treatment. Thus, if cell wall hydrolytic enzymes were kept from contact with the cell wall by the plasmalemma, the disruptive effect of 2,4-D upon cellular membranes should have hastened abscission. This was not the case. Although massive membrane disruption occurred following 2,4-D treatment, abscission was delayed. The middle lamellar region of the cell wall appeared the same as control tissue after five days of 2,4-D application and apparently none of the cell wall components were being hydrolyzed.

It would, therefore, seem more likely that auxin, rather than delaying abscission by maintaining the integrity of membranes, effects its delaying influence on other systems. Auxin could retard abscission by directly inhibiting the enzymes involved in cell wall hydrolysis, by inhibiting the production of these enzymes, or through its effects upon PME activity, metabolite movement, and growth in general as previously discussed.

The Role of the Protective Layer in the Abscission Process

Rasmussen (1965) reported that cell division did not occur in the lower pulvinal abscission zone of bean prior to petiole detachment. He based his conclusion on microscopic observation and the apparent lack of histones in the abscission zone over the period of development of what he termed the abscission layer. The results of the present study, utilizing recently developed microtechnical procedures, demonstrated that the protective layer forms by cell division. Separation occurs on the distal side of the protective layer with only a few of the newly formed cell walls of the protective layer becoming involved in the separation process. Extensive wall separation occurs principally in older cell walls. Indeed, separation of the old walls of a mother cell was noticed simultaneous with the new wall synthesis which would form daughter cells within the mother. Thus the two processes, separation and cell division can occur concurrently.

Separation is not, however, dependent upon cell division. In addition to the non-involvement of the new cell walls in the separation process (Figure 15), separation could be induced without cell division (Figure 4). Ethylene treatment caused the petiole to abscise without prior cell division. When the petiole was forced to abscise before formation of a protective layer, cell division at the exposed surface occurred after the petiole had fallen (Figure 5). It appeared that these divisions occurred for protection of the plant as indicated by the rapid lignification of the new cell walls.

Thus several lines of evidence indicate that separation and cell division are distinct processes. First, under normal abscission, induced by leaf removal, separation occurs on the distal side of the cell division layer rather than through the layer (Figure 1). Second, new cell walls form during development of the protective layer and these are not the walls involved in separation (Figure 15). Third, separation can be induced to occur without prior cell division (Figure 4). Finally, if separation is induced before cell division, a protective layer will form after the petiole falls (Figure 5).

Additional support of the idea that abscission involves two separate processes is found in the literature. Abscission of leaves of woody species may or may not be preceded by the formation of a protective layer (Schact, 1859; Von Mohl, 1860; Tison, 1900; Lee, 1911). _Impatiens_ _Sultani_ normally abscises without cell division, forming its protective layer after petiole detachment

(Gawadi and Avery, 1950). These examples support the conclusion that separation need not require cell division in the lower pulvinus of bean.

Abscission and Senescence

The association between abscission and senescence can be seen in the changes in the gross appearance of the petiole prior to separation. From a healthy green appearance before deblading, the abscission zone takes on a light yellow color, characteristic of senescence, at separation. In addition, the lower pulvinus becomes markedly constricted and collapsed just before separation instead of being thicker than the petiole at leaf removal.

Jacobs (1962) consolidated the viewpoint that abscission may be, at least indirectly, a consequence of senescence. Jacobs' "Growth Theory" suggested that as long as the petiole continued to grow, abscission was inhibited. Evidence favoring Jacobs' theory has been presented by Osborne and Moss (1963) who have shown that the application of kinetin directly to the abscission zone delayed abscission whereas distal or proximal applications accelerated abscission. These studies implied that abscission was regulated by the metabolite level in the abscission zone. Scott and Leopold (1966) supported the observations of Osborne and Moss by showing abscission in the bean was a result of movement of metabolites out of the abscission zone.

The active cell divisions which form the separation layer (Tison, 1900; Lee, 1911) have been used as arguments against the idea that abscission is a result of senescence (Carns, 1966). Bornman (1967) supported this concept by demonstrating that in cotton, separation occurred through a newly formed layer of cells. However, results of the present study indicate that separation in the bean is not a consequence of new cell division (Figure 1 and 15). This conclusion is substantiated by Webster (1968) who observed little cell division during abscission in the upper pulvinus of bean. It has also been shown that cell division does not precede separation in certain species (Gawadi and Avery, 1950), and separation can even be induced to occur without cell division by chemical treatment in poinsettia, Euphorbia pulcherrima (Gawadi and Avery, 1950), or with ehtylene treatment (Figure 4) as was done with the bean (both species normally precede separation with cell division). Although arguments have been raised that the idea of senescence in the abscission zone cannot be reconciled with active cell division, ample evidence suggests that cell division is not a requirement for abscission and that division and separation are two distinct processes in many plants.

The fine structural changes in the abscission zone indicate that senescence and abscission are closely related (Figure 14 and 18). In addition to the degenerative state of the cytoplasm at the late stages of abscission, the swelling of the chloroplast thylakoids, which appears the third day after leaf removal (Figure 21), is characteristic of the early stages of senescence. With osmium fixation, similar alterations in chloroplast structure have been observed in senescing wheat leaves (Shaw and Manocha, 1965) and in cucumber cotyledons (Butler, 1967). The invaginations which occur on the inner membrane of the chloroplasts (Figure 21C and 21D) have been observed in the abscission zones of pedicels (Jensen and Valdovinos, 1967) and in petioles of senescing leaves of _Impatiens Sultani_ (Bednarz, unpublished observations). Similar invaginations have been reported in non-mature chloroplasts which are developing thylakoid systems (Lemoine, 1968; Spurr and Harris, 1968). If these invaginations are a response to thylakoid synthesis as Spurr and Harris suggest, then they may also be a response to thylakoid degradation in that translocation of lipid materials from the plastids to the ground cytoplasm may be occurring.

The decrease in chlorophyll content after deblading can be accounted for by the observed changes in chloroplast structure of both control (Figure 21) and 2,4-D treated (Figure 23) abscission zones. However, the maintenance of carbon dioxide fixing capacity by non-auxin treated abscission zones cannot be reconciled with the obvious senescence of the plastids unless one argues that the chloroplasts seldom reach their operating capacity.

The constancy of respiration during abscission is also difficult to assess. The fine structural characteristics of the mitochondria did not vary with time after deblading. Butler (1967) noted only a slight change in cristae size in mitochondria of senescing cotyledons of cucumber. He indicated that senescence of the cotyledons involved no decrease in respiration.

Eilam (1965) determined that the increase in permeability which occurred early in senescence was independent of respiration. Rasmussen (1965) noted increased permeability in the bean during abscission. Thus, it seems that the respiratory system remains active although dramatic alterations occur in the cytoplasm and cytoplasmic membranes during senescence and separation.

The maintenance of the fine structure of the mitochondria coupled with the relative constancy of respiration throughout the abscission process indicates that metabolic activity must be maintained for senescence or abscission. The rough endoplasmic reticulum seen in cells having extremely degenerated cytoplasm has also been observed in abscissing pedicels of tobacco and tomato (Jensen and Valdovinos, 1968). The increased protein levels preceding abscission of bean petioles (Abeles and Holm, 1966) may imply that the rough endoplasmic reticulum is active in the synthesis of cell wall hydrolytic enzymes or in the formation of specific degradative enzymes causing cellular senescence. The diffused chromatin material in the nuclei of abscission zone cells may contribute to the protein synthesizing machinery of the cells during senescence. Proribosome particles of nucleoli and chromatin reportedly are "discharged into the ground substance of the cytoplasm" and "are converted there into ribosomes that can act in protein synthesis" (O'Brien and McCully, 1969). Varner (1965) has suggested that cellular senescence might result from production of m-RNA which would direct the synthesis of degradative enzymes.

Senescence does occur during abscission. Whether or not the structural and physiological changes that are evident during abscission are related to the wall separation process or to senescence of the abscission zone has not been determined. It would seem more likely that those physiological changes which become evident late in the abscission process are involved with cellular senescence. Cell wall separation occurs prior to any alterations of the cytoplasm (Figure 11) save the slight swelling of the chloroplast thylakoids. Butler (1967) suggested that thylakoid swelling is a sign of the early stages of senescence. Likewise, Eilam (1965) indicated that permeability changes are also an early sign of senescence.

The thylakoid swelling noted in this thesis and the increased permeability observed by Rasmussen (1965) became evident the third day after leaf removal. Apparently these are the first detectable signs of senescence in bean abscission.

Observations in the lower pulvinus after deblading suggest that metabolic changes occur well before the third day after deblading. Results of physiological studies in this thesis and by Rasmussen (1965) indicate that the first 24 hours after deblading is a time of increased cellular activity. The increase in dry weight which occurs during the first 24 hours (Figure 29) may reflect increased protein levels in the abscission zone. Thus, the synthetic machinery for producing degradative enzymes may be formed quite early in the abscission process; a conclusion supported by RNA and protein synthesis inhibitor studies during the induction period (Abeles and Holm, 1966, 1967; Holm and Abeles, 1967). This could be a direct result of decreased auxin levels in the tissue due to removal of the organ responsible for the flow of auxin past the abscission zone, the leaf. However, it still cannot be ascertained if the increased cellular activity is necessary for the production of degradative enzymes used in the wall separation or senescence processes or both. Thus, the association between abscission and senescence remains undefined.

The Separation Process

The detachment of the petiole from the stem occurs several cell layers distal to the stem-petiole juncture (Figure 1 and 4). There are structural reasons for separation to occur through this plane.

Anatomically, the pulvinar region of the bean is weaker than the rest of the petiole. Doutt (1932) has shown that the leaf traces have an amphicribal arrangement before they enter the pulvinus. In the pulvinus, the vascular bundles anastomose such that a single collateral element, surrounded by endodermis, is centered in the cortex. Upon leaving the pulvinus, the vascular tissue is arranged as a ring of separate collateral bundles, thus affording the petiole greater support than the pulvinus.

The abscission zone is composed of cells that have less secondary wall support than cells on either side of it. Immediately distal to the line of separation, two to five layers of cells become lignified (Figure 6). The widespread occurrence of a lignified layer in plant species (Tison, 1900; Lee, 1911) may indicate that lignification distal to the line of separation is characteristic of abscising tissue. Lignification of this layer of cells strengthens the area of the petiole distal to the line of separation. Thick walled collenchyma cells are found at the junction of the petiole and stem (Figure 1). These collenchyma cells extend a few cell layers into the stem but they are restricted to the adaxial side of the petiole. Thus, the area of the petiole where separation begins is sandwiched between two structurally stronger tissues. Proximal to the separation layer are thick walled collenchyma cells and distal to it are the lignified layers of cells.

The structural changes of the walls that occur after deblading favor separation in the protective layer. The layer forms by a series of cell divisions within mother cells (Figure 1). Most of the new cell walls which form are anticlinal with respect to the adaxis of the petiole. These new walls lie parallel with the eventual plane of separation. By the use of staining reactions for electron microscopy (Figures 10 and 11), histochemical procedures with the microprobe (Figures 7, 8 and 9) and corroborative histochemical tests by Rasmussen and Bukovac (1969), it is indicated that the new cell walls, and the protective layer in general, contain less pectic substances than adjacent tissues. A decrease in the calcium content in the protective layer, concomitant with separation, has been shown by both microprobe analysis (Figure 7) and by chemical techniques (Rasmussen, 1965). This evidence indicates that a loss or change in pectic substances occurs in the separation layer as abscission progresses. The new cell walls inherently contain little wall cementing pectin when they are formed and the walls of the original mother cells lose their wall cementing pectins with time after deblading.

Results of this study suggest that cell wall components other than pectins are altered prior to separation (Figure 13). Facey (1950) postulated a hydration but no degradation of cellulose in abscission of *Fraxinus americana*. Rasmussen and Bukovac (1969) sequentially extracted cell wall components and determined that both cellulose and non-cellulosic polysaccharides are degraded during development of the abscission layer. Horton and Osborne (1967) indicated that the degradation of cellulose is controlled enzymatically. The alteration in the structure of pectin, cellulose, and other wall polysaccharides further weakens the walls of cells in the abscission layer.

The actual plane of separation through the abscission zone is delineated quite early by the appearance of lignified tissue on the distal side of the separation layer (Figure 6). Cells of the separation layer separate along their middle lamellae leaving intact cells on both sides of the line of separation (Figure 1). Generally, the anticlinal walls of the most distal of the two tiers of mother cells separate, but since the layers of mother cells are not aligned perfectly with the plane of separation, occasional separation occurs through a periclinal wall of a mother cell and thence along the middle lamella of the cross wall of a daughter cell (Figure 1). Thus, it seems apparent that separation is not predetermined to occur between any particular two layers of cells; rather the general weakness of the walls of cells in the separation layer results in separation along the path of least resistance through the petiole. Anatomical differences of tissues bordering the separation layer likewise indicate that the path of separation is through a weak area of the abscission zone.

The Contribution of this Study to the Problem of Leaf Abscission

A controversy concerning the participation of cell division in the formation of the separation layer has appeared in the literature (Carns, 1966). The results of the current study indicate unequivocally that separation and cell division are two distinct processes in abscission at the lower pulvinus of unifoliate bean leaves (Figure 1 and 4). This conclusion provides an answer to another question posed by Carns (1966) concerning the role of active cell division in the separation process. Carns justly questions the association between senescence and abscission if the separation layer is formed through new cell proliferation. The results of the study of abscission in bean show that the new wall synthesis and cell divisions which form the protective layer simultaneous with the degradative processes leading to wall hydrolysis and cellular senescence (Figure 15).

Thus the association between senescence and separation remains an unresolved issue in the process of abscission.

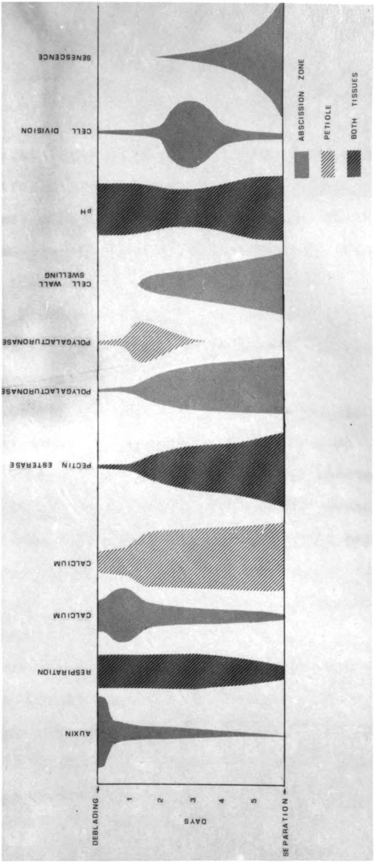
The results of the present study also provide evidence establishing a mode of wall separation in foliar abscission of herbaceous species. Although some hydrolysis of primary wall material was evident during separation (Figure 13), hydrolysis primarily occurred in the region of the middle lamella between cells (Figure 12) and this hydrolysis was presumably linked to changes in pectic compounds. Thus, added emphasis upon the role of pectolytic rather than cellulolytic enzymes in the separation process is suggested.

Finally, the semi-quantitative analysis of pectin distribution in the abscission zone using the microprobe established a new micro-technical procedure for the localization of compounds using specific staining reactions.

The Sequence of Events Leading to Abscission

The chronology of events that lead to abscission in the lower pulvinus of bean is summarized in Figure 32. The results of this study and that of Rasmussen (1965) were used in compiling this summary. The figure indicates both the qualitative and relative quantitative changes in the abscission zone and petiole that occur from the time of deblading until separation of the petiole from the stem. The dimensions of the individual patterns in the figure are not intended to be compared with each other, except in the case of calcium where it is believed that calcium from the abscission zone is translocated into the petiole. The modulation of pectin hydrolysis by calcium has been discussed in the Review of Literature section and more thoroughly by Rasmussen (1965).

Figure 32. A chronology of events during abscission.


SENESCENCE
CELL DIVISION
pH
CELL WALL SWELLING
POLYGALACTURONASE
POLYGALACTURONASE
PECTIN ESTERASE
CALCIUM
CALCIUM
RESPIRATION
AUXIN
DEBLADING
1
2
3
4
5
SEPARATION
DAYS
ABSCISSION ZONE
PETIOLE
BOTH TISSUES

SUMMARY

Anatomical and physiological changes during abscission of unifoliate bean leaves (Phaseolus vulgaris L. cv. Contender) were examined in this study. The first indication of abscission occurred the second day after leaf removal. The walls of a layer of cells, just distal to the eventual line of separation, stained red with phloroglucinol-HCl. These walls stained more intensely as abscission progressed.

Approximately the third day after deblading, a protective layer resulted by means of cell division in the abscission zone. The protective layer was located adjacent and proximal to the phloroglucinol-HCl stained layer. Due to cell division, mother cells formed nests of cells; the protective layer was composed of two tiers of these nest-type cells. Cell division did not occur in ethylene promoted abscission.

Commensurate with the formation of the protective layer, separation began along the middle lamellae of older cell walls in the protective layer. Progressively less pectin was found in the middle lamella as separation proceeded. The cytoplasm did not undergo changes (except

for the slight swelling of the chloroplast thylakoids) until after the first appearance of cell wall separation.

The fifth day after deblading, wall separation was well advanced. Separation was generally confined to the middle lamella although there were indications of hydrolysis of other wall polysaccharides in addition to pectin. The cytoplasm was in a deteriorated condition and the chloroplasts appeared senescent at this time; however, the plasmalemma was still intact.

Fifty percent petiole abscission occurred by the sixth day after deblading. Separation occurred on the blade side of the most distal of the two tiers of mother cells.

Respiration remained relatively constant throughout abscission. Following an initial increase in dry weight the first day after leaf removal, the abscission zone lost weight. Chlorophyll content declined after deblading, however, the abscission zone sections maintained most of their carbon fixing capacity.

The application of 2,4-D (7 x 10^{-5}M) delayed both the formation of the protective layer and separation for approximately seven days but did not interfere with the mode of separation. At the ultrastructural level, 2,4-D was observed to have a deleterious effect upon cellular membranes. The chloroplasts especially were affected by 2,4-D exhibiting a nearly complete loss of internal thylakoid structure after five days of treatment. On the other hand, cell wall integrity was maintained following 2,4-D application.

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