Plant Physiol. (1967) 42, 1351–1356

Abscission: The Role of Aging

F. B. Abeles, R. E. Holm, and H. E. Gahagan
Department of the Army, Fort Detrick, Frederick, Maryland 21701

Received April 27, 1967.

Summary. Excision of *Phaseolus vulgaris* L. c.v. Red Kidney abscission zone explants results in senescence, mobilization, and abscission. Because these processes take place at about the same time, there has been some question as to whether they are causally related or are occurring in an independent but simultaneous fashion. Data presented here suggest that the latter interpretation is correct. After abscission zone explants are isolated from the leaf an aging process is set into motion and a degradation of metabolites in the pulvinus takes place. During the aging process the explants also become increasingly sensitive to ethylene which in turn promotes cell separation. Indoleacetic acid, cytokinins, and coumarin appear to retard aging since both degradative processes and abscission are inhibited. However, ethylene increased abscission without increasing degradative processes indicating that abscission and senescence are independent processes occurring at the same time.

Aging refers to the changes occurring in cells as a function of time without reference to their ultimate fate. Senescence on the other hand, is a result of aging in which deteriorative processes ultimately lead to the death of the cells (11). The increased sensitivity of abscission zone explants to ethylene (1, 2, 16) is a result of an aging process. The aging process also initiates the senescence of the pulvinus in bean petiole explants and is characterized by a loss of metabolites from the tissue (14) and its eventual death. To some extent the loss of metabolites is influenced by the subtending petiole tissue (14). This directed transport and accumulation is an example of mobilization.

This paper presents evidence that the action of ethylene appears to be independent of senescence and mobilization in the explant. Many processes are initiated as a result of aging, one of which is the increasing capacity to respond to ethylene while other changes such as mobilization occur in a parallel but independent fashion. Since aging appears to be a prerequisite to subsequent sensitivity to ethylene, any compound that inhibits abscission may be acting to block aging. This idea is supported by reports that auxin (14) and 6-furfurylaminopurine (13) which are known to block abscission, also retard senescence. Experiments confirming and extending these reports are presented here. Further support is the discovery that coumarin, a compound that is known to retard degradative processes (5, 9), also retards abscission.

Materials and Methods

Plant Material. Seeds of *Phaseolus vulgaris* L. c.v. Red Kidney sown in 10 cm pots filled with soil were grown for 14 days at 26 ± 2° and 1200 ft-c of fluorescent light (12 hr photoperiod). Abscission zone explants from the primary leaves were incubated in bottles (43 ± 2 ml in volume, 5 cm in diameter, and 2.5 cm high) fitted with a 25 mm diameter rubber vaccine cap at 25° in 400 ft-c of continuous fluorescent light. The 10-mm-long explants (4.5 mm of pulvinal tissue) were placed petiole end down in 3 mm of 1.5 % agar. When required, ethylene was added to the gas phase with a syringe inserted through the vaccine cap.

Analytical Methods

Dry Weight. Changes in the dry weight of pulvinal or petiole tissue were determined after the tissue had dried at 98° for 16 hours.

Chlorophyll. Ten petiole or pulvinal sections were homogenized in 10 ml of methanol for 2 minutes at high speed in a VirTis homogenizer fitted with a 50-ml flask. The homogenate was then filtered through glass wool or Miracloth (Calbiochem Corporation) and centrifuged at 2000 × *g* for 10 minutes. The amount of chlorophyll was determined by measuring the optical density of the solution at 666 mμ in a spectrophotometer.

Ribonucleic Acid. Ten pulvinal or petiole sections were homogenized in 10 ml of 0.01 M tris buffer (pH 7.5) with a VirTis homogenizer for 2 minutes and filtered through Miracloth. A 6-ml aliquot of the filtrate was made 0.2 N with respect to $HClO_4$ and centrifuged at 2000 × *g* for 10 minutes. The pellet was washed at 0 to 4° with 0.2 N $HClO_4$, with 0.05 M formic acid in methanol twice, and at 37° with ether; ethanol:chloroform (2:2:1 v/v/v) for 30 minutes. The washed pellet was then hydrolyzed in 0.3 N KOH for 18 hours at 37°. After cooling,

sufficient 2.4 N $HClO_4$ was added to give a final concentration of 0.2 N $HClO_4$ and the suspension was centrifuged at 4000 × *g* for 10 minutes to yield a clear supernatant. Optical density of the supernatant was measured at 260 and 290 mμ. RNA was calculated by the relationship: mg RNA = (OD 260 mμ-OD 290 mμ) (dilution factor) (0.048).

Protein. After samples of tissue were homogenized and filtered as described above to determine RNA 0.7 ml of 50 % trichloroacetic acid was added to a 7-ml portion to coagulate the protein. The protein was precipitated by centrifugation at 2000 × *g* for 10 minutes and washed at 0 to 4° with 5 % trichloroacetic acid, with ethanol, and then at 60° for 5 minutes with ethanol:ether (3:1 v/v). The washed pellet was then solubilized in 1 ml of 0.1 N NaOH. The protein content of the NaOH solution was determined by the method of Lowry et al. (12).

For convenience, the methodology for each experiment is described with the presentation of results because of the variation in procedures among experiments.

Results

Mobilization in Split and Ethylene Treated Explants. The purpose of the experiment summarized in figure 1 was to determine the role that mobilization played in the loss of metabolites from the pulvinus and to establish the extent to which the cell separation process was autonomous from the loss of metabolites from the pulvinus. If mobilization is a part of the separation process, then any treatment that accelerates abscission should enhance mobilization.

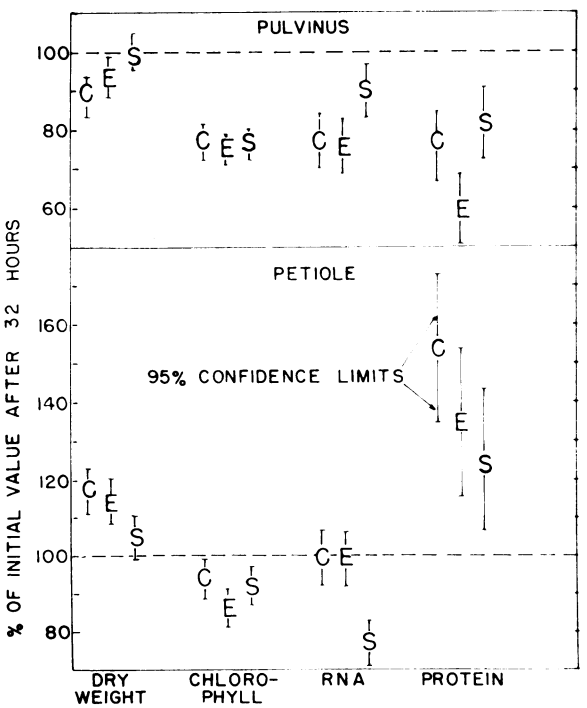

FIG. 1. Mobilization of metabolites in split and ethylene treated explants. C represents controls, E ethylene treated, and S split explants. Each point is the average of 4 replications consisting of 10 explants each. Height of the bar represents 95 % confidence limits. Bottles containing explants were vented and resealed at 8 and 24 hours. Ethylene level was 4 ppm.

Figure 1 shows the levels of dry weight, chlorophyll, RNA, and protein in petiole and pulvinal tissues 32 hours after the explants were excised, expressed as the percent of the initial values. The bottles containing control (C) abscission zone explants were vented and resealed at 8 and 24 hours to reduce the accumulation of ethylene and CO_2. With ethylene treated explants, (E) 4 nl of ethylene were added per ml gas phase after the explants were excised and after each venting. After 32 hours, the abscission of ethylene treated explants was 100 % and that of the controls was 30 to 50 %. The data in figure 1 indicate that, although ethylene accelerated abscission, it had no significant effect on the mobilization of chlorophyll, RNA, and protein or on dry weight changes. Ethylene reduced the protein content of both halves of the explant by about 20 % although the spread of the data makes the significance of the observation questionable.

Mobilization and degradation can be distinguished by comparing changes in metabolites in split versus intact explants, because splitting explants into pulvinal and petiole tissues before placing them into the bottles removes the metabolic sink. The results after 32 hours shown in figure 1 (S) suggest that the best example of mobilization was the changes in RNA levels. In this case, splitting explants maintained RNA levels in the pulvinus and caused a loss in the petiole compared with intact controls.

Chlorophyll changes were not significantly affected by splitting indicating that decreased chlorophyll levels are not caused by mobilization. Intermediate in effect were changes in dry weight and protein content. Examining these parameters shows that splitting explants reduced but did not prevent the loss in pulvinal tissue and the gain in petiole tissue indicating that only part of the observed changes can be attributed to mobilization.

Although ethylene stimulated abscission, our data show that the gas did not stimulate mobilization of any metabolite or result in a decrease in dry weight, chlorophyll, or RNA. Ethylene actually reduced mobilization when expressed as dry weight changes. The ethylene-enhanced explant separation was in effect similar to splitting explants mechanically but because it occurred later, the effect was intermediate between results seen in controls and split explants. However, ethylene treatment resulted in a loss of protein in both petiole and pulvinal tissues. There is no obvious explanation for this effect. Our results suggest that the cell separation process and the loss of metabolites from the pulvinus are independent processes characteristic of aging explant tissue.

Inhibition of Abscission by Indoleacetic Acid (IAA), Cytokinin and Coumarin. IAA, cytokinin

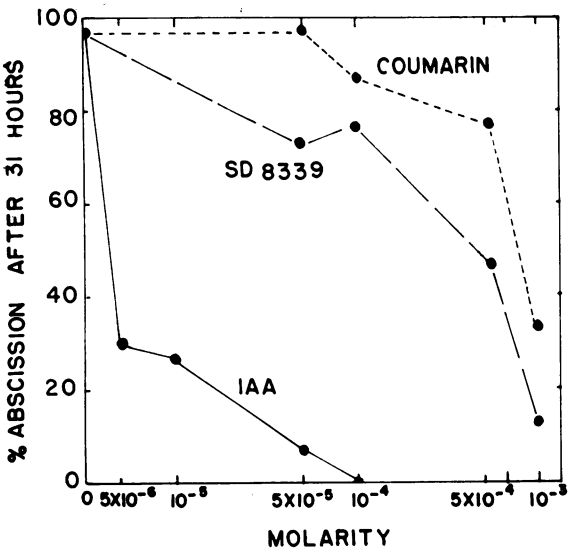

FIG. 2. Inhibition of abscission by indoleacetic acid, cytokinin SD 8339, and coumarin. Explants were placed pulvinal end down in agar containing concentrations of compounds as indicated. Bottles were vented at 8 and 24 hours. Each point is the average of 3 replications of 10 explants each.

SD 8339 [6-benzylamino-9-2(tetrahydropyranyl-9H-purine)], and coumarin retarded abscission of bean petiole explants when applied to pulvinal tissue (fig 2).

Explants were placed pulvinal end down in agar containing various concentrations of these abscission retardants immediately after excision. At 8 and 24 hours the bottles were vented; abscission was measured after 31 hours. This experiment was repeated on 2 other occasions with essentially similar results. Two other cytokinins, 6-furfurylaminopurine and N^6-benzyladenine, also retarded abscission but were less effective on a molar basis. In addition, at higher concentrations these 2 cytokinins doubled the rate of ethylene production from explants as contrasted with cytokinin SD 8339, which did not affect ethylene production. We have also found that cytokinins retarded abscission of explants from coleus (*Coleus blumei* Benth.) and cotton (*Gossypium hirsutum* L. C.V. Acala 4-42) prepared as described earlier (1). Other compounds applied to pulvinal tissue at a concentration of 100 μM or less in agar such as aesculin, caffeic acid, catechol, chlorogenic acid, *p*-coumaric acid, dichlorophenol, ferulic acid, juglone, quercitrin, resorcinol, naringenin, and $MnCl_2$ had no effect on abscission.

Since IAA, cytokinins, and coumarin are normally thought to control different aspects of plant metabolism, i.e., growth, cell division, and growth inhibition, it was of interest to explore the reasons for their ability in common to block abscission.

Although the inhibition of abscission by coumarin has not been described earlier, the result is not completely unexpected as there are a number of examples where auxin and coumarin have similar effects on plants (4, 6, 10, 15) and it is known that coumarin blocks the loss of metabolites from aging plant tissues (5, 9).

Effect of IAA, Cytokinin SD 8339, and Coumarin on Senescence. Earlier workers have reported that naphthaleneacetic acid (14) and 6-furfurylaminopurine (13) retarded aging of bean petiole explants. In figures 3 through 6 these observations have been extended to include the effect of IAA (50 μM), cytokinin SD 8339 (200 μM), and coumarin (200 μM) on levels of dry weight, chlorophyll, RNA, and protein in pulvinal and petiole tissue during abscission.

The retardants had no effect on the weight loss of pulvinal tissue, suggesting that some of the loss represents reserve carbohydrates or other metabolites used to supply energy to the explant during aging. As opposed to the trends shown in figure 1, the petiole tissue did not increase in weight with time. This difference may be because the explants used for the experiments summarized in figure 1 were placed

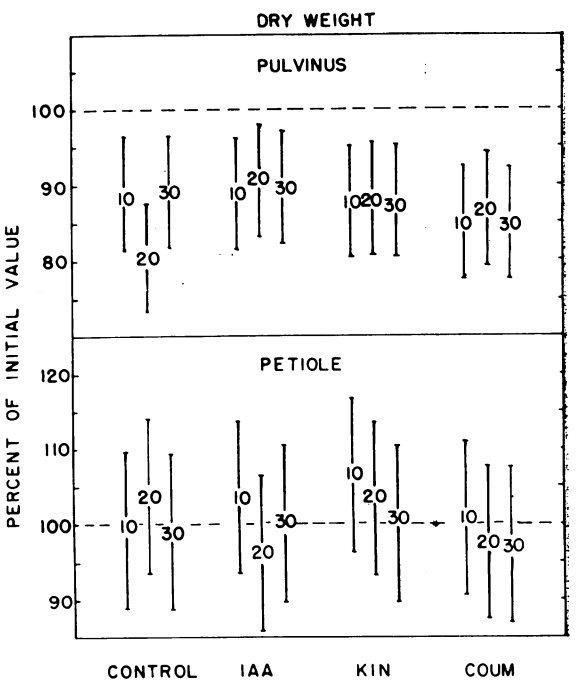

FIG. 3. Effect of abscission retardants on dry weight. The abscission retardants were incorporated into agar and the explants were inserted pulvinal end down. Treated explants abscised 0 to 10 % after 30 hours; controls usually abscised 30 to 50 %. Explants were removed from the bottles after 10, 20, and 30 hours, split into pulvinal and petiole tissue, and frozen. Samples for weight determinations were dried immediately after harvesting. Explants not harvested at 10 and 20 hours were vented at that time. To facilitate comparison, the data are expressed as a percentage of the initial value at the start of the experiment. The mean value of the data at 10, 20, and 30 hours are shown as the numbers 10, 20, and 30 on figures 3 thru 6 and the vertical bars represent 95 % confidence limits determined by an analysis of variance.

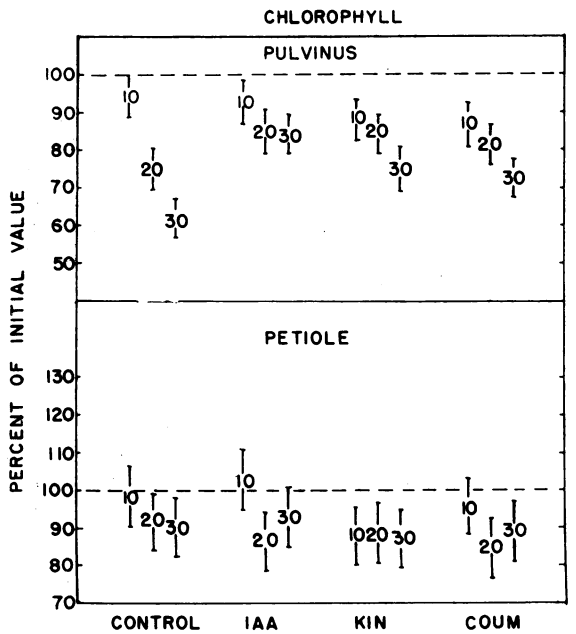

FIG. 4. Effect of abscission retardants on chlorophyll content. For details see figure 3.

petiole end down in the agar and those used in the abscission retardant experiments were placed pulvinal end down.

However, the data in figures 4, 5, and 6 indicated that the retardants did inhibit the breakdown and subsequent loss of chlorophyll, RNA, and protein from the pulvinus. IAA not only prevented the loss of RNA from the pulvinus but caused an increase compared with initial levels.

The retardants had little or no effect on the changes in RNA and chlorophyll content of petiole tissue. Even though retardant-treated explants failed to abscise by 30 hours, the level of chlorophyll and protein in the petiole were similar to those in explants that were rapidly abscising. This supports the idea discussed above that some of the changes of metabolite levels are autonomous events characteristic of aging excised tissue.

The increased protein content normally associated with aging explant petiole tissue was inhibited by the retardants. This inhibition of protein synthesis was most readily observed with explants treated with IAA.

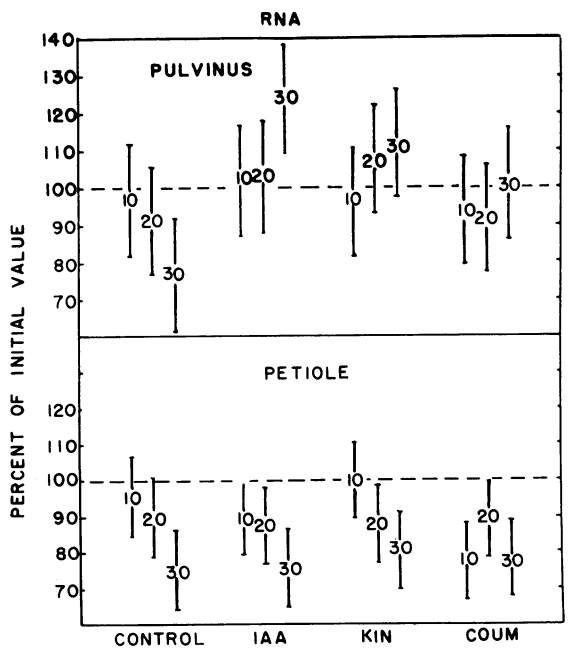

FIG. 5. Effect of abscission retardants on RNA content. For details see figure 3.

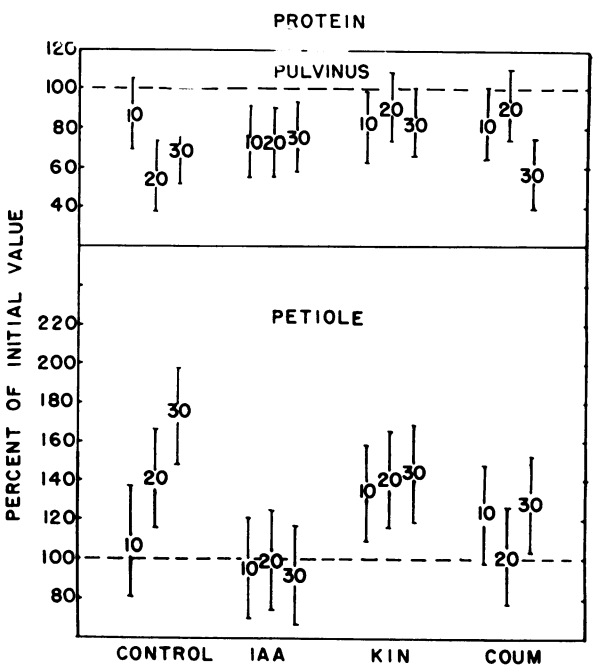

FIG. 6. Effect of abscission retardants on protein content. For details see figure 3.

However, figure 1 suggests that ethylene also decreased protein levels. The possibility remains that part of this auxin effect may be an ethylene effect because auxin has been shown to stimulate ethylene production (3).

Pulvinal aging is characterized by a loss of weight, chlorophyll, RNA, and protein. These are gross changes and as such reflect the total of a large number of reactions consisting in part of the increase in certain components. For example, even though the pulvinus loses protein and RNA, it is still capable of synthesizing new RNA and protein during the abscission process (2) measured as the incorporation of ^{32}P into RNA and ^{14}C-leucine into protein.

To the extent that loss of metabolites is associated with aging and that abscission retardants inhibited

this loss, we can say that the mode of action of auxin, cytokinins, and coumarin in preventing abscission is associated with their ability to retard aging.

The Inhibition of Ethylene Stimulated ^{32}P Incorporation into RNA by IAA and Cytokinin SD 8339. We reported earlier (2) that the stimulation of ^{32}P incorporation into RNA by ethylene in bean explants depended on an aging process. If aging is required for ethylene action, and if auxin and cytokinins retard aging, then these compounds should also inhibit the effect of ethylene on ^{32}P incorporation into RNA. (See table I).

As in the experiments described above, IAA and cytokinin SD 8339 inhibited abscission and RNA breakdown in explant tissue, and ethylene did not significantly alter RNA levels in explants although it stimulated abscission. As reported earlier (2), ethylene stimulated the incorporation of ^{32}P into the RNA of 22-hour-old explants. However, when the ability of ethylene to stimulate abscission was blocked by auxin and cytokinin, its ability to stimulate ^{32}P uptake into RNA was also blocked. The reasons why ethylene caused a reduction of RNA levels as well as the rate of ^{32}P incorporation in auxin and cytokinin treated explants are unknown.

We conclude from this experiment that auxin and cytokinin prevented aging, thereby preventing ethylene from stimulating ^{32}P incorporation into RNA. The rapid incorporation of ^{32}P into RNA and higher RNA content also indicates the relative juvenility of auxin- and cytokinin-treated explants.

Petiole Length Experiments. Gorter found that increasing the proximal tissue of *Coleus rhenaltianus* explants accelerated abscission. Scott and Leopold (14) suggested that the longer stem was a more effective mobilization center thereby accelerating cellular senescence in the abscission zone. However, the acceleration of abscission by larger amounts of proximal tissue may be due to increased availability of carbohydrates or ethylene at the separation zone. Table II presents data showing that decreasing petiole tissue inhibited ethylene evolution and abscission of bean explants but that 1 % sucrose or 2 ppm ethylene alleviated the effect.

In conclusion, the evidence presented here indicates that auxin and cytokinins repress aging and the sensitivity to ethylene in bean petiole abscission zone explants. Excision of the explant probably sets the aging process into motion because the normal supply of auxin, cytokinins, or other as yet unidentified factors are cut off when the leaf blade is removed. After explants are excised, metabolites are lost from the pulvinus, and the rate of loss is influenced in part by a metabolic sink. While mobilization on such a small scale is interesting and deserves further study, it apparently has no direct relation with the cell separation process. Cell separation is stimulated by ethylene and the ability of explants to respond to the

Table I. *Effect of IAA, Cytokinin SD 8339, and Ethylene on ^{32}P Incorporation Into RNA*

Explants with 10 mm petioles were placed pulvinal end down in either plain agar or agar containing 50 μM IAA or 500 μM SD 8339 for 22 hours. The explants were then placed petiole end down in plain agar and 1.5 by 4 mm agar cylinders containing 2 μc ^{32}P were placed on the pulvinus for 8 hours. Where indicated ethylene level was 2 ppm. Data shown are the means plus or minus the standard deviation. The RNA from the explants was extracted and purified by methods described earlier (2).

Treatment	μg RNA 10 explants	CPM μg RNA	% Abscission after 30 hrs
Control	149 ± 3	2864 ± 7	40
Ethylene	156 ± 5	4156 ± 68	100
IAA	173 ± 5	4611 ± 102	0
IAA + Ethylene	151 ± 6	3984 ± 97	10
SD 8339	200 ± 7	4257 ± 81	0
SD 8339 + Ethylene	175 ± 4	2843 ± 26	0

Table II. *Effect of Sucrose and Ethylene on the Abscission of Explants With Different Amounts of Petiole Tissue*

Explants with various lengths of petiole tissue were placed petiole end down in either plain agar or agar containing 1 % sucrose. The bottles were vented 8 and 24 hours after the start of the experiment and 2 ppm ethylene was added at 24 hours to 1 set of explants in plain agar. Ethylene accumulation was measured between 8 and 24 hours after preparing the explants.

	Petiole length			
	2mm	3mm	4mm	5mm
ppm C_2H_4 accumulated	0.12	0.15	0.18	0.22
Treatment		% Abscission after 31 hours		
Control	37	83	87	97
2 ppm C_2H_4	100	100	97	100
1 % Sucrose	100	100	100	100

gas is an integral part of the aging process. We conclude from these results that the action of ethylene in abscission is directed towards hastening the dissolution of cells in the abscission zone and that the gas has no effect on aging of bean petiole explants.

Acknowledgments

We gratefully acknowledge the technical assistance of Mr. L. E. Forrence during these experiments. We also thank Dr. J. van Overbeek of Shell Development Company for cytokinin SD 8339 and Dr. A. C. Leopold for critical advice during the preparation of this manuscript.

Literature Cited

1. Abeles, F. B. 1967. Mechanism of action of abscission accelerators. Physiol. Plantarum 20: 442–54.
2. Abeles, F. B. and R. E. Holm. 1966. Enhancement of RNA synthesis, protein synthesis, and abscission by ethylene. Plant Physiol. 41: 1337–43.
3. Abeles, F. B. and B. Rubinstein. 1964. Regulation of ethylene evolution and leaf abscission by auxin. Plant Physiol. 39: 963–69.
4. Audus, L. J. and J. H. Quastel. 1947. Coumarin as a selective phytocidal agent. Nature 159: 320–24.
5. Basler, E. 1963. Interaction of 2,4-dichlorophenoxyacetic acid, ethylene chlorohydrin and glutathione in the stability of microsomal ribonucleic acid of excised cotton cotyledons. Proc. Oklahoma Acad. Sci. 43: 35–40.
6. Gantzer, E. 1960. Wirkungen von Cumarin auf Wachstum und Entwicklungsvorgange und seine Wanderrungs fahigheit im Pflanzengewebe. Planta 55: 235–53.
7. Gawadi, A. G. and G. S. Avery. 1950. Leaf abscission and the so-called abscission layer. Am. J. Botany 37: 172–80.
8. Gorter, C. J. 1964. Studies on abscission in explants of *Coleus*. Physiol. Plantarum 17: 331–45.
9. Knypl, T. S. 1967. Retardation of chlorophyll degradation in *Zea mays* by coumarin, Phosfon D, and CCC. Naturwissenschaften 54: 146.
10. Leopold, A. C. and K. V. Thimann. 1949. The effect of auxin on flower initiation. Am. J. Botany 36: 342–47.
11. Leopold, A. C. 1964. Plant Growth & Development. McGraw-Hill Book Company, New York. p 194.
12. Lowry, O. H., N. J. Rosenbrough, A. L. Fair, and R. J. Randall. 1951. Protein measurement with Folin phenol reagent. J. Biol. Chem. 193: 265–75.
13. Osborne, D. J. and S. E. Moss. 1963. Effect of kinetin on senescence and abscission in explants of *Phaseolus vulgaris*. Nature 200: 1299–1301.
14. Scott, P. C. and A. C. Leopold. 1966. Abscission as a mobilization phenomenon. Plant Physiol. 41: 826–30.
15. Winter, H. 1954. Der Einfluss von Wirkstoffen, von Röntgen- und Elektronenstrahlen auf die Cambiumtätigkeit von *Beta vulgaris*. Planta 44: 636–68.
16. Yamaguchi, S. 1954. Some interrelations of oxygen, carbon dioxide, sucrose, and ethylene in abscission. Ph.D. Thesis. University of California, Los Angeles.