Analysis of the Auxin Control of Bean Leaf Abscission [1, 2]

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The role of auxin in leaf abscission has been recognized ever since Laibach (6) found the abscission of debladed petioles to be influenced by auxin-rich orchid pollinia. Inhibitions of abscission by indoleacetic acid have been reported by LaRue (7) and Myers (8), and promotions of abscission have been described by Addicott and Lynch (1) and Wetmore and Jacobs (13). Later work by Gaur and Leopold (5) and Biggs and Leopold (3) suggested that the promotive and inhibitory auxin effects on leaf abscission were functions of auxin concentration comparable to the classical two-phase scheme of auxin action on growth proposed by Thimann (12).

A further analysis of auxin effects on abscission is reported here using both explant and intact petiole abscission tests, and the existence of two successive steps with opposite auxin sensitivities is suggested. We will attempt to show how these findings lead to a new interpretation of the effects of auxin on leaf abscission.

Materials & Methods

Explant Abscission Test. The test was carried out in the manner described by Rubinstein and Leopold (10). Seeds of *Phaseolus vulgaris* L. var. Red Kidney were grown under controlled environment conditions of 2,000 ft-c, 23 ± 2° and a 16 hour photoperiod for about 14 days. From primary leaves petiole sections were then cut including 5 mm of tissue on each side of the upper abscission zone. Ten explants were used for each treatment.

For proximal applications, substances to be tested were combined with a 1 % agar solution and poured into Petri dishes to a depth of 4 mm. The proximal ends of the petiole explants were then inserted into the agar and the dishes returned to the controlled environment chamber at a light intensity of 400 ft-c.

For distal applications, the proximal ends of the petiole explants were first inserted into plain 1 % agar. Discs of filter paper (5 mm) were then dipped into 50 % ethanol solutions of the substances to be tested (plain 40 % ethanol for the controls) and the wet paper discs placed on the distal ends of the explants.

Intact Petiole Abscission Test. Roots were removed from 11 day old Red Kidney bean plants, the hypocotyls were trimmed to a length of 10 cm, and the plants placed in beakers containing distilled water. The primary leaves were then cut off so that approximately 2.5 cm of the petiole and lower abscission zone were left intact on the plant. Substances investigated with this bioassay were incorporated into lanolin which was then applied to the cut surface of the petioles. In cases where substances were applied at intervals after leaf removal, about 2 mm more of the petiole was removed just prior to application so that each treatment was applied to a freshly cut surface. The plants were kept in the controlled environment chamber during the course of the experiments. The statistical LSD 5 % for both abscission tests described here was 20 hours or less.

Auxin Extraction. α-naphthaleneacetic acid-2-C^{14} (NAA*)[3] (1.3 mc/m M) was fed to bean explants by either the proximal or distal method. At specified time intervals, 15 explants were cut so as to remove one 2 mm section just to the proximal side of the abscission zone and another 2 mm section just distal to the abscission zone. These sections were then homogenized in 95 % ethanol and the mixtures evaporated to dryness in a flash evaporator with temperatures not in excess of 50°. After 10 ml of anhydrous ether and 2 ml of pH 5 potassium phosphate buffer (0.1 M) had been added to the flasks, they were shaken for 3 hours. The ether fraction was then evaporated in a planchet and counted in a windowless flow counter. The numbers presented in the tables are all corrected for background.

Results

It is possible that the abscission process is comprised of more than one physiological stage and that the inhibitory and promotive effects of auxin may be due to the differential action of auxin on these successive stages. To test this hypothesis, bean petiole explants were treated distally or proximally with a high concentration of auxin at various times after leaf removal. Figure 1 shows that when the naphthaleneacetic acid (NAA) was applied immediately after leaf removal (0 hr) no abscission occurred, thus reflecting an inhibition of abscission. If,

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[3] Abbreviations: NAA*, α-naphthaleneacetic acid-2-C^{14}; NAA, α-naphthaleneacetic acid.

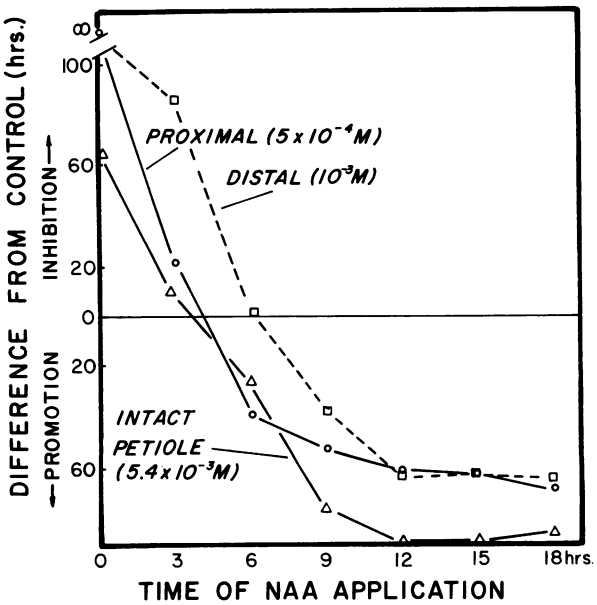

FIG. 1. Abscission responses of bean explants to auxin treatments applied at various intervals after deblading. Concentration of NAA used for each treatment indicated in parentheses. Time of 50 % abscission of controls for each treatment: proximal = 93 hr; distal = 94 hr; intact petiole = 136 hr. Data are plotted as inhibitions or promotions above or below these times.

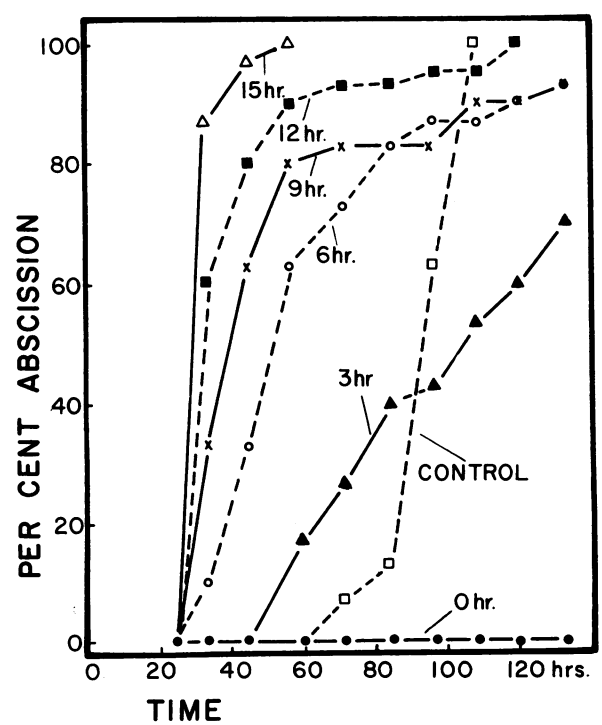

FIG. 2. Time curves showing progression of bean explant abscission. On each line is designated the time elapsed between leaf removal (at time 0 hr) and proximal application of NAA (5×10^{-4} M).

however, the same auxin concentration was applied 3 hours after the explants were cut the inhibitions were much less pronounced, and if applied 9 hours after cutting a very marked stimulation of abscission occurred. It can be seen, then, that the same concentration of NAA applied to the same region of the explant can either inhibit or accelerate abscission depending on the time of auxin application.

Further tests were carried out using the intact petiole abscission test (fig 1). Except for the fact that absolute inhibitions were never achieved with NAA, the results were essentially the same as those with explants. The longer the time between deblading and applying the auxin, the less effective was the inhibition. If as much as 9 hours elapsed between leaf removal and auxin application, the same concentration of auxin which had inhibited earlier now caused striking stimulations of abscission.

The progress of abscission by explants with various times of auxin application can be seen for proximal treatments in figure 2. The per cent abscission of the untreated controls rose sharply after 85 hours and complete abscission occurred shortly thereafter. The curve for explants treated with auxin 15 hours after cutting showed a much earlier rise, abscission being markedly stimulated by the auxin application. Curves for auxin applications earlier than 9 hours after leaf removal show some initial abscission followed by a markedly slower rate, and when auxin was applied 3 hours after deblading 30 % of the explants still showed no abscission even after 130 hours. These data seem to demonstrate a progressive change in auxin response.

The results suggested the existence of two steps during leaf abscission: a first stage or induction period which is inhibited by auxin and a later stage which is promoted by the same auxin concentration. To further determine the sensitivity of the two stages to auxin, two groups of explants were used. Both groups were treated proximally with 5×10^{-4} M NAA for various lengths of time from one minute to two hours, but one group was placed in the auxin immediately after cutting while the other was treated with auxin 18 hours later. The results of this experiment are presented in figure 3. If the explants were placed in NAA for only 1 minute immediately after cutting, abscission was inhibited by more than 50 hours over the control. This presumably represents an auxin inhibition of the induction period. If, however, 18 hours elapsed before the explants were placed in NAA for 1 minute, there was a promotion of almost 60 hours. This latter effect demonstrates the auxin stimulation of the second stage of abscission.

Experiments were then set up to determine whether the two stages of abscission could be retarded by low temperature. This was done by exposing the explants to low temperatures (4°) during the first or second stage and applying high concentrations of auxin so as to observe the progress of the separate periods. The change from inhibitory to

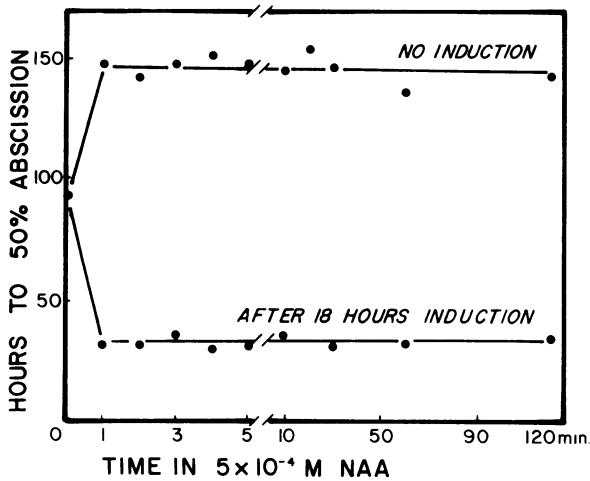

FIG. 3. Bean explant abscission as affected by various durations of proximal NAA (5×10^{-4} M) treatment immediately after deblading or 18 hours after deblading.

promotive responses to the auxin would indicate completion of the induction stage. In table I it can be seen that low temperature retarded abscission to about the same extent whether given during the first or second stage (#1, 2, 3). For example, controls at 22° abscised at 98 hours, and 18 hours of cold only slightly delayed abscission to 111 or 112 hours. The abscission of explants which were held at 22° was greatly accelerated by auxin applied after 18 hours (#5) as had been shown in previous experiments. However, the abscission of explants which were first placed in the cold for 18 hours was completely inhibited when treated with the same auxin concentration at 22° (#6). These results suggest that the changes occurring during the induction period have been arrested by low temperatures. The cold temperatures also retard the second stage since inhibitions rather than promotions were obtained with explants which were simultaneously exposed to cold and placed in auxin during the 18 to 36 hour period (#7 vs 5).

The effects of a range of NAA concentrations on abscission are known to exhibit a two-phase curve (3) and representative data are shown in figure 4. The explants treated distally without an induction period show only an inhibition of abscission with increasing concentrations of NAA under the conditions of these tests. The proximally treated explants, however, show a marked promotion at concentrations of 10^{-5} M to 10^{-4} M, with inhibitions at higher concentrations. If leaf abscission is composed of two stages of which the first is inhibited by auxin, how then is it possible for NAA at a concentration of 5×10^{-5} M to stimulate abscission when it is applied proximally to the explant immediately after deblading?

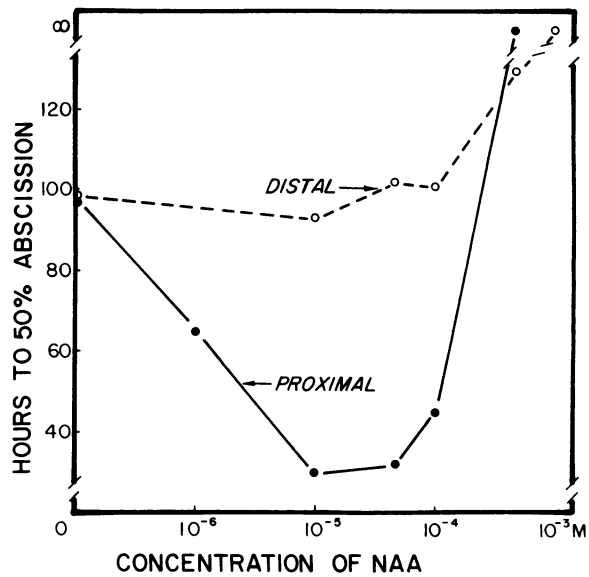

FIG. 4. Abscission of bean explants in response to proximal or distal applications of various concentrations of NAA.

An explanation for the abscission stimulation occurring with dilute auxin concentrations can be deduced from the data plotted in figure 5. If explants are placed immediately in the promotive concentration of NAA (5×10^{-5} M) for various lengths of time, they must remain in the NAA for over 16 hours before an acceleration of abscission becomes evident as shown by the curve for no induction in

Table I

Effects of Periods of Low Temperatures on Abscission of Bean Petiole Explants
NAA applied proximally at 5×10^{-4} M only in time periods indicated.

Time Period:	0 to 18 hr		18 to 36 hr		After 36 hr		Hours to
Treatment	NAA	Temp °	NAA	Temp °	NAA	Temp °	50 % abscission
#1		22		22		22	98
2		4		22		22	111
3		22		4		22	112
4	NAA	22	NAA	22	NAA	22	No abscission
5		22	NAA	22	NAA	22	34
6		4	NAA	22	NAA	22	No abscission
7		22	NAA	4	NAA	22	125

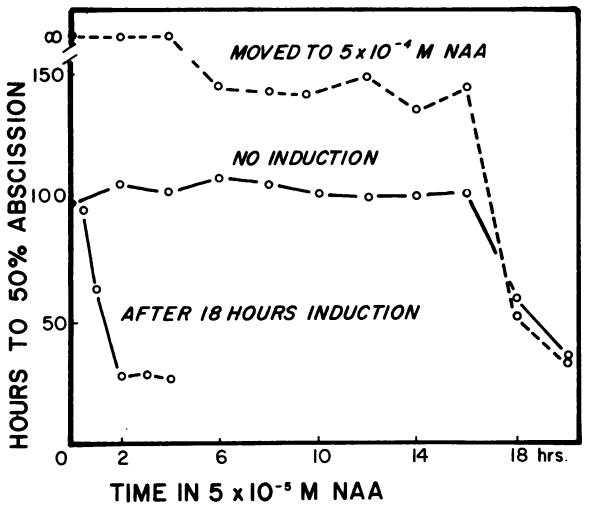

FIG. 5. Bean explant abscission as affected by proximal applications of 5×10^{-5} M NAA for various lengths of time. Explants were exposed to the NAA at once after deblading (no induction), or after an 18 hour induction period in plain agar; following the times in NAA, explants were moved to plain agar or to agar containing 5×10^{-4} M NAA.

figure 5. But when the explants are held for 18 hours in plain agar before being placed in the NAA, an exposure of only slightly more than 1 hour is needed to produce a stimulation. This indicates that even when the explant is placed immediately in NAA, the promotion caused by the auxin is due only to its action on the second step. The action of 5×10^{-5} M NAA on the first step was further investigated by moving the explants from the promotive concentration after various lengths of time to 5×10^{-4} M NAA (fig 5). Explants which were in 5×10^{-5} M NAA for 15 hours or less and then transferred to 5×10^{-4} M NAA were inhibited almost 40 hours over the control, but it is already known that when the explants are kept in plain agar for 15 hours before the transfer to 5×10^{-4} M NAA, there is a marked stimulation of abscission (see fig 1). From these data, therefore, it is evident that even the promotive concentration of auxin somewhat inhibits the first stage of abscission.

It is also possible to test abscission promoting substances other than auxins to determine whether their action is due to a stimulation of the second step or a shortening of the induction period. For example, the amino acid alanine has been reported to accelerate abscission (10), and its relative effectiveness in altering the two stages of abscission is illustrated in figure 6. The explant must remain in alanine over 16 hours in order for a promotion to occur (as shown by the curve for no induction) while 5 hours of alanine exposure is sufficient for an acceleration if applied 18 hours after cutting. Thus it is implied that, like auxin, the alanine acts by stimulating the second stage of abscission. If explants are transferred to 5×10^{-4} M NAA after various times in alanine, the stimulatory action of the auxin remains like that in figure 1 where the explants were first placed in plain agar; so these data do not indicate any action of alanine on the induction period.

Attempts to explain the effects of auxin on leaf abscission have been based on a gradient of auxin across the abscission zone (2) or on the total auxin concentration in the abscission zone (3, 5). It would be helpful, therefore, to establish the relative quantities of auxin which may occur on either side of the abscission zone after promotive or inhibitory auxin treatments. This has been done by applying NAA* to bean petiole explants either at once or after the induction period, and extracting 2 mm sections just above and just below the abscission zone. In this way it is possible to compare the rate of abscission with the amount and location of the added auxin. Data for the distribution of radioactivity after distal applications of NAA* are presented in table II. In each case, the petiole explants were exposed to NAA* for just 4 hours. As in the earlier experiments, when 10^{-3} M NAA* was applied immediately (#2) an *inhibition* of abscission was obtained, but when the same concentration was applied 18 hours later (#3) a *stimulation* of abscission occurred (see also fig 1); however, the measurements of radioactivity indicate that the gradient of auxin about the abscission zone was in the same direction for both treatments. Similar gradients were found for applications of 10^{-4} M NAA* which had no measurable effect on the abscission rate (#4, 5). Neither the radioactivity in the proximal or distal sections nor the total radioactivity for each treatment appears to

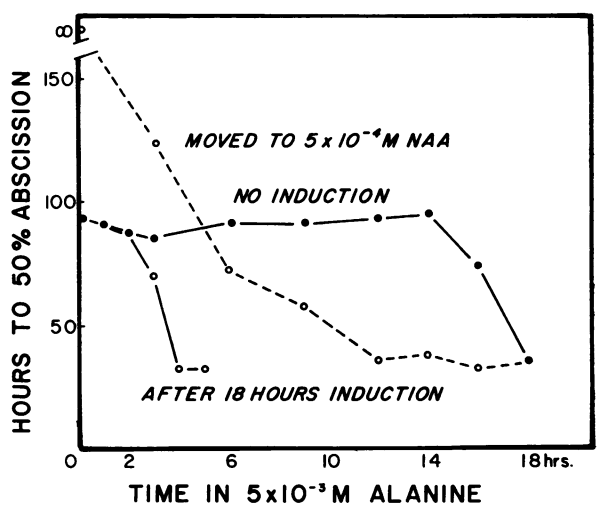

FIG. 6. The effects of alanine on bean explant abscission. Proximal applications of 5×10^{-3} M alanine were made for various lengths of time as in figure 5. Explants were exposed to alanine at once or after an 18 hour induction period; they were then moved to plain agar, or to agar containing 5×10^{-4} M NAA.

be correlated with the rate of explant abscission. The smaller amount of NAA* present in the explants which were treated with auxin after 18 hours is probably due to a decreased uptake after 18 hours on agar.

Proximal applications of NAA* (table III) likewise showed no apparent correlation between abscission rate and either the auxin gradient or auxin concentration. It can be seen that more radioactivity was found in the proximal sections of all explants regardless of whether an inhibition or promotion of abscission occurred. For example, when explants were placed in NAA* for 10 minutes after 18 hours in plain agar (#3), 50 % abscission occurred in only 27 hours and there was slightly more radioactivity in the proximal side. When explants were placed in NAA* for 10 minutes immediately after debladding (#2), abscission was inhibited by 44 hours over the control but the gradient shows even more radioactivity in the proximal side. Only small concentration differences were apparent between the two treatments. From figure 5 it is known that 5×10^{-5} M NAA* applied immediately promotes abscission only if the explants remain in the auxin for over 18 hours; if applied after an 18 hour induction period, promotions were obtained with only a 2 hour exposure. Such experiments with NAA* (#4, 5) exhibited as wide a difference in auxin gradient between these two promotive treatments as between the promotive and inhibitory treatments. Comparisons of the concentration differences between table II and III are not valid because of the different nature of the test methods.

Discussion

From the experiments in which auxin was applied to bean explants and intact petioles at various times after leaf removal, it appears that leaf abscission can be divided into two stages. The first of these stages is primarily retarded by auxin while the second stage is markedly accelerated by auxin applications. In contrast, the amino acid alanine which stimulates abscission, has no apparent effect on the initial stage but does accelerate the second. Thus promotions of the second stage of abscission are shared by auxins and other natural substances.

A simple method employed by Czapek (4) for separating the stages of geotropism was used here to further separate the stages of abscission. The procedure consisted of exposing the petiole explants to low temperatures during each abscission stage. It was found that the first stage is clearly retarded by cold since abscission was subsequently inhibited by NAA exposure, while abscission of explants induced for the same period at 22° was subsequently stimulated by NAA. The events during the second stage are also slowed by cold as evidenced by the inhibitions obtained when explants are exposed simultaneously to NAA and low temperatures for 18 hours after an 18 hour induction period at 22°. There is good reason, then, to suspect that both stages consist of events which are retarded by cold.

The existence of two abscission stages also has been found using indoleacetic acid (IAA) but since the presence of an IAA inactivating system has been reported for bean petioles (11), the data for IAA were not considered as reliable as those for NAA.

The fact that auxin inhibits during a first stage and promotes during a second suggests new interpretations of some existing theories of leaf abscission. Addicott et al. (2) have suggested that abscission is controlled by the gradient of auxin across the abscission zone; if there is more auxin on the distal side (as would develop following distal applications) an inhibition of abscission should be evident, and when there is more auxin on the proximal side a stimulation of abscission should take place. This theory is difficult to defend in view of the fact that either promotions or inhibitions can be obtained by auxin treatment to any side of the abscission zone (3, 5, 6, 9, 13). The results in figure 1 also conflict with the gradient theory, for either promotions or inhibitions can be obtained with applications to the same side of the abscission zone, depending only on the time of application. Measurements of the radioactivity on either side of the abscission zone after applying labeled NAA do not support the involvement of a gradient of the applied auxin, for distal applications apparently produced gradients in the same direction whether they inhibited or promoted abscission (table II), and also proximal applications produced the opposite gradients whether they inhibited or promoted abscission (table III). The differences in effects between proximal and distal auxin treatments may be a consequence of a readier movement of the auxin to the abscission zone following a distal treatment, hence the greater tendency for inhibition effects (3).

A concentration theory has been proposed by Gaur and Leopold (5) and Biggs and Leopold (3), in which the promotion of abscission by low auxin

Table II

Distribution of Radioactivity in the Distal & Proximal Sides of the Abscission Zone After Distal Applications of NAA to Bean Explants*

	Treatments	Counts/minute		Hours to 50 % abscission
		Distal 2 mm	Proximal 2 mm	
1.	Control	...	...	99
2.	10^{-3} M NAA* 4 hr	1550	680	150
3.	Untreated 18 hr, then 10^{-3} M NAA* 4 hr	940	680	35
4.	10^{-4} M NAA* 4 hr	141	86	104
5.	Untreated 18 hr, then 10^{-4} M NAA* 4 hr	110	88	101

Table III

Distribution of Radioactivity after Proximal Applications of NAA to Bean Explants*

After each auxin treatment, explants were placed in plain agar for 4 hours prior to extraction.

Treatments	Counts/minute		Hours to 50 % abscission
	Distal 2 mm	Proximal 2 mm	
1. Control	...	...	95
2. 5×10^{-4} M NAA* 10 min	230	450	139
3. Plain agar 18 hr, then 5×10^{-4} M NAA* 10 min	250	280	27
4. 5×10^{-5} M NAA* 20 hr	62	188	26
5. Plain agar 18 hr, then 5×10^{-5} M NAA* 2 hr	66	90	30

concentrations and the inhibition by high concentrations are considered to be reflections of a two-phase action of auxin similar to the actions on growth (12). As originally proposed, this concept of abscission effects implied that the promotion and the inhibition effects are reflections of the same auxin action in the plant. The assignment of the promotion and inhibition auxin effects to separate steps in the abscission process calls for a reinterpretation of the concentration theory. It is clear that the same concentration of auxin can either markedly inhibit or promote abscission depending on when the auxin is applied (fig 1), and the apparent amount of auxin reaching the abscission zone is not itself correlated with the effects obtained (table II and III). A reinterpretation of the concentration effects is suggested from these data: the promotive effects of dilute concentrations of auxin applied immediately to petiole explants can be considered to be a consequence of an amount of auxin just low enough to allow the induction stage to proceed to completion and yet high enough to stimulate the second stage of abscission (see fig 5). Thus the apparent two-phase concentration curve can be an expression of two separate auxin actions on the abscission processes.

Summary

An analysis of the control of leaf abscission by auxin was carried out using petiole explants and debladed intact petioles of *Phaseolus vulgaris* L. var. Red Kidney.

I. By adding α-naphthaleneacetic acid (NAA) at various intervals following leaf removal, the presence of two abscission stages was revealed—an induction period of about six hours or more which is inhibited by auxin, and a later stage which is stimulated by auxin.

II. Low temperatures were shown to separately retard each of the abscission stages, permitting a further distinction between them.

III. The amino acid alanine which stimulates abscission was found to act on the second stage and to have no apparent effect on the induction period.

IV. The two-phase concentration effects of auxin on abscission are described as the consequence of actions on two stages with separate and roughly opposite auxin sensitivities.

V. Experiments with radioactive NAA indicate that under the conditions of the bean tests, the abscission response is correlated with the time of auxin application and not with the gradient of auxin about the abscission zone nor with the total concentration extractable from that region.

Literature Cited

1. Addicott, F. T. & R. S. Lynch. 1951. Acceleration & retardation of abscission by indoleacetic acid. Science: 114: 688–89.
2. Addicott, F. T., R. S. Lynch, & H. R. Carns. 1955. Auxin gradient theory of abscission regulation. Science 121: 644–45.
3. Biggs, R. H. & A. C. Leopold. 1958. Two-phase action of auxin on abscission. Am. J. Botany 45: 547–51.
4. Czapek, F. 1895. Untersuchungen uber Geotropismus. Jb. Wiss. Botan. 27: 243–339.
5. Gaur, B. K. & A. C. Leopold. 1955. The promotion of abscission by auxin. Plant Physiol. 30: 487–90.
6. Laibach, F. 1933. Wuchsstoffversuche mit lebenden Orchideenpollinien. Ber. Deut. Botan. Ges. 51: 336–40.
7. LaRue, C. D. 1936. The effect of auxin on the abscission of petioles. Proc. Nat. Acad. Sci. U.S. 22: 254–59.
8. Myers, R. M. 1940. Effect of growth substances on the absciss layer in leaves of Coleus. Botan. Gaz. 102: 323–38.
9. Portheim, L. 1941. Further studies on the action of heteroauxin on *Phaseolus vulgaris*. Ann. Botany 5: 35–46.
10. Rubinstein, B. & A. C. Leopold. 1962. Effects of amino acids on bean leaf abscission. Plant Physiol. 37: 398–401.
11. Shoji, K. & F. T. Addicott. 1954. Auxin physiology in bean leaf stalks. Plant Physiol. 29: 377–82.
12. Thimann, K. V. 1937. On the nature of inhibitions caused by auxins. Am. J. Botany 24: 407–12.
13. Wetmore, R. H. & W. P. Jacobs. 1953. Studies on abscission: the inhibiting effect of auxin. Am. J. Botany: 40: 272–76.