

# Auxin Structure & Abscission Activity<sup>1, 2</sup>

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Since the early work of Laibach (7) abscission has been considered to be controlled by auxin, but comparative effects have been reported for only a few auxins (4, 5, 13) and it is not clear to what extent abscission activity is really related to auxin activity. Moreover, when comparative studies have been made only the inhibitory effects of auxins have been compared (4).

The aim of the present study is to compare quantitatively the abscission effects of a selection of chemicals having various degrees of auxin activity. Both inhibitory and promotive aspects have been considered in an effort to clarify the extent to which abscission effects are related to auxin effects on growth.

## Materials & Methods

The bioassay for abscission was used as described by Rubinstein and Leopold (10). Seeds of *Phaseolus vulgaris* L. var Red Kidney were sown in vermiculite in controlled environment chambers and the plants were maintained under uniform conditions (16 hr photoperiod, 2000 ft-c and  $24 \pm 2^\circ$ ). Plants were approximately 15 days old when the primary leaves were excised and the blades trimmed off. The petiole pieces were then cut by a 1 cm cutter so that the abscission zone was in the center of the explant.

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

For distal treatments, the petiole explants were inserted into 1% agar in the usual manner, and the chemicals being tested were added to the distal end. Discs 2.5 mm of Whatman No. 2 filter paper were dipped into 40% ethanol solutions of the substances to be tested (controls having plain 40% ethanol-dipped discs) and placed on the distal ends of the explants. The dishes were returned to the controlled environment chamber. The paper discs were wetted

once after 24 hours with the solution being tested.

The following compounds were included in the study: Indoleacetic acid (IAA), Naphthaleneacetic acid (NAA), Phenoxyacetic acid (PAA), 2-chlorophenoxyacetic acid (2-Cl), 4-chlorophenoxyacetic acid (4-Cl), 2,4-dichlorophenoxyacetic acid (2,4-D), 2,5-dichlorophenoxyacetic acid (2,5-D), 2,6-dichlorophenoxyacetic acid (2,6-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 2,4,6-trichlorophenoxyacetic acid (2,4,6-T), 2,4,5-trichlorophenoxy isobutyric acid (2,4,5-T-IBA), *d*-2,4-dichlorophenoxy-2-propionic acid (*d*-2, 4-DP), *l*-2,4-dichlorophenoxy-2-propionic acid (*l*-2,4-DP). The phenoxy acids were obtained from the Amchem Company, Ambler, Pennsylvania.

In experiments following changes in auxin effects with induction times, explants were inserted in agar for specified times, after which they were treated either proximally or distally with relatively high concentrations of the auxin material. The treatments were all carried out in light of 400 ft-c, 16 hour photoperiod, and  $24^\circ \pm 2^\circ$ .

The time of abscission was determined visually. As the explants approached the final stages of abscission, the abscission zone was seen as a whitish line, while adjacent tissue was still green or yellow and opaque. At this stage, a slight pressure was applied to the explants which readily brought about separation. Readings were taken every 12 hours and the time required for abscission of 50% of the 10 explants was taken as the experimental result. The least significant difference (LSD 5%) for the abscission tests described here was between 18 and 21 hours. All experiments reported were repeated at least three times with consistent results.

For determination of growth promoting activity of the compounds, the Avena coleoptile straight growth test was used (9). Coleoptile sections (4 mm) from 3 mm below the tip were floated for 3 hours on glass distilled water containing 1 mg/liter of  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ . Ten sections were put in 1.0 ml of solution containing 2% sucrose plus a buffer at pH 5.0 ( $\text{K}_2\text{HPO}_4$  1.794 g/liter + citric acid monohydrate 1.019 g/liter) and the growth substance being tested. Sections were incubated about 20 hours in the dark at  $25^\circ$  in a rotator and the final lengths were measured under a microscope with an ocular micrometer. Growth is recorded as percentage increase over controls.

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## Results

In order to compare the abscission activities in relation to changes in the molecular structure, the relative effects of a series of auxins and auxin analogues were measured with the bean abscission test. The effects on abscission were compared by both proximal and distal methods of application to petiole explants over a concentration range of  $10^{-8}$  to  $10^{-3}$  M (fig 1 & 2).

Both IAA and NAA showed the well-known two-phase action on abscission, weaker concentrations promoting abscission when applied proximally and stronger concentration inhibiting it with either position of application. Promotion of abscission was maximal with proximal applications of  $10^{-5}$  M in both cases (fig 1a). Lack of promotion with distal application agrees with Biggs and Leopold (3) who obtained promotions only with bigger explants of primary leaves.

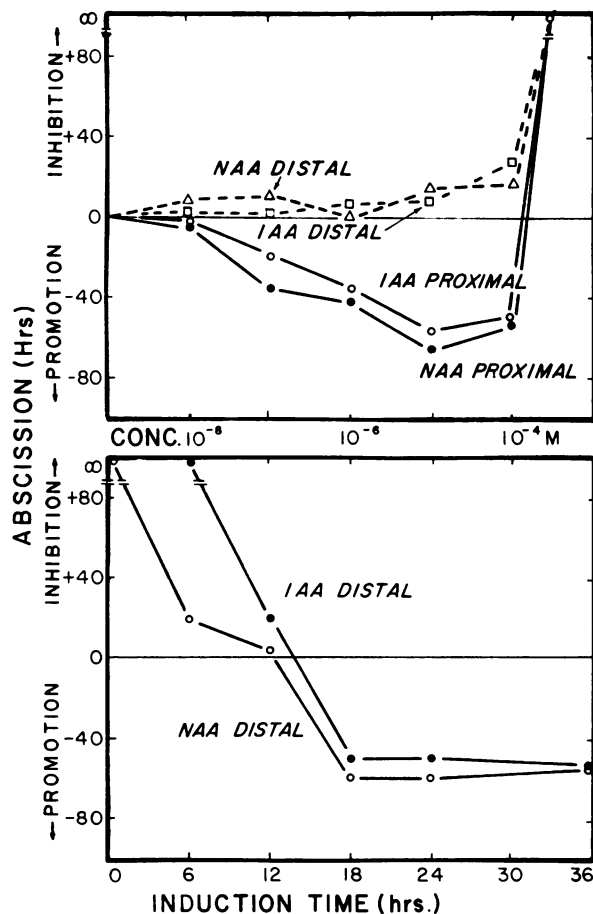


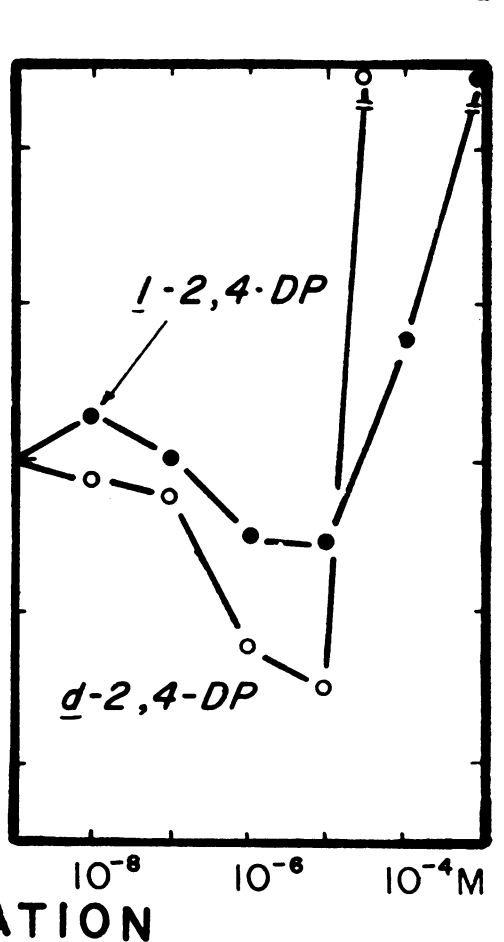
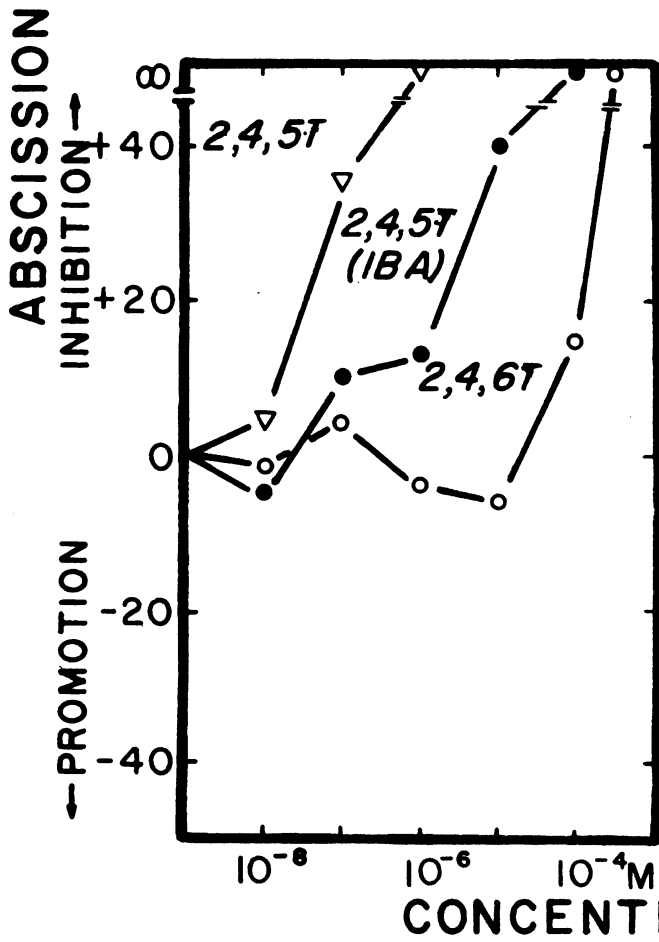
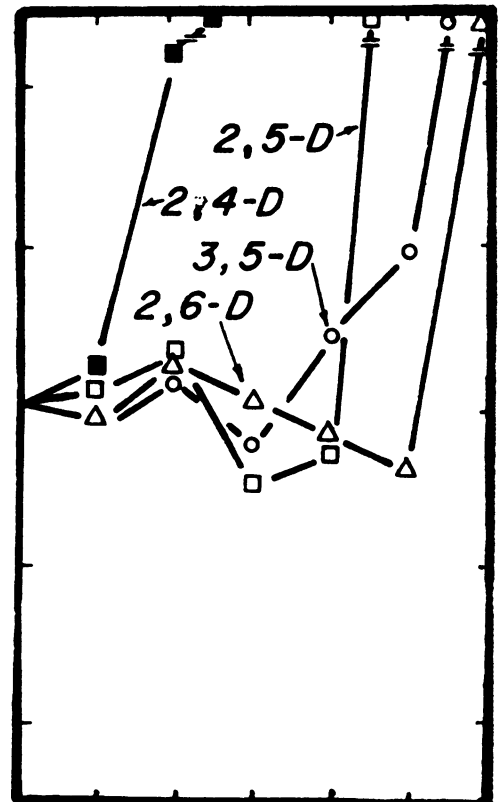
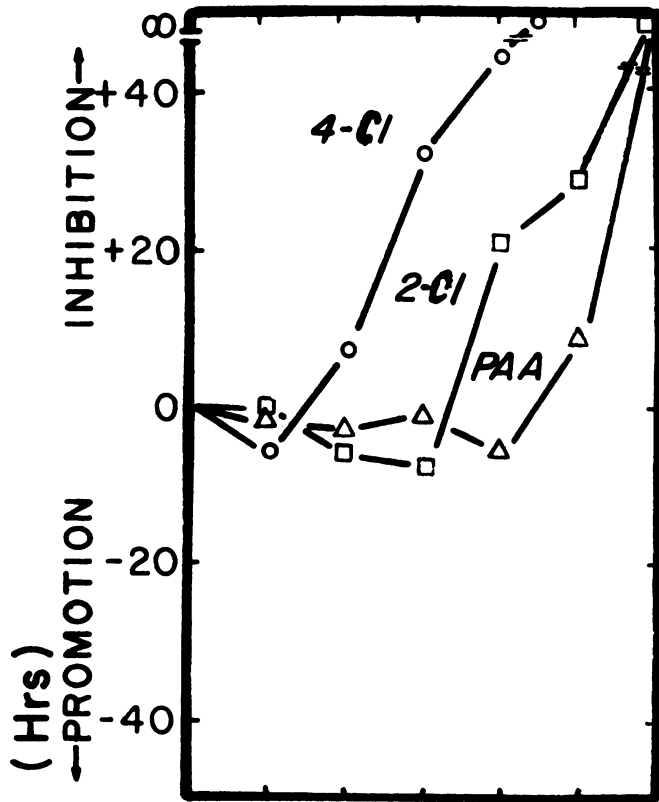
FIG. 1. Abscission responses of bean explants to indoleacetic and naphthaleneacetic acids. a (upper), with proximal or distal applications of various concentrations. b (lower), distal applications of  $5 \times 10^{-4}$  M after various intervals of induction. Time of 50% abscission of controls: for a, 90 hours proximal and 92 hours distal; for b, 91 hours.

In contrast with IAA and NAA, mono-chloro phenoxyacetic acids (fig 2a) did not produce any detectable promotion of abscission. Inhibition effects set in at relatively low concentrations with 4-Cl as compared with 2-Cl or the unsubstituted phenoxyacetic acid. Dichlorinated substitutions (fig 2b) likewise produced no detectable promotion. Inhibition was very strong with 2,4-D, intermediate with 2,5-D and only occurring with concentrations above  $10^{-4}$  M with 2,6-D and 3,5-D. In the trichlorinated series (fig 2c) 2,4,5-T was as inhibitory as 2,4-D, 2,4,5-T-IBA was much less and 2,4,6-T was as weak as phenoxyacetic acid. Among the optical isomers tested, significant promotion of abscission was clear with *d*-2,4-DP whereas the *l*-2,4-DP had no significant promotive effects (fig 2d).

The above data reveal the general effectiveness of the phenoxy compounds on inhibition of abscission, with those compounds possessing greatest auxin activity (like 2,4-D & 2,4,5-T) producing the strongest inhibitions. It is noteworthy that compounds with little or no auxin activity (2,6-D, 2,4,6-D, 3,5-D) produced some inhibition. Promotions of abscission were not detected with any of the phenoxy compounds except dextro-2,4-DP.

To permit a comparison of the abscission inhibiting activities of these phenoxy compounds with their growth activities, straight growth tests were carried out. The maximum promotion of coleoptile section growth obtained with each compound has been entered in table I. Growth promotions were of course greatest for 2,4-D and 2,4,5-T with intermediate promotions by 2,5-D and *d*-2,4-DP and little or no promotions with the diortho substituted acids, the isobutyric acid, the laevo acid or 3,5-D. Comparison of the growth promoting activity and the abscission inhibiting activity in table I indicates a close agreement, substances causing the greatest stimulation of growth being most inhibitory of bean explant abscission. Minimum concentrations required for complete inhibition of abscission were lowest with the strongest growth promoters. For example, minimum concentrations for complete inhibition of abscission by 2,4-D and 2,4,5-T were  $5 \times 10^{-7}$  M and  $10^{-6}$  M respectively. These two compounds showed high growth activity (53% & 33% respectively). Substances producing a very small stimulation of growth (e.g. 2,6-D & 3,5-D) required 1,000 to 2,000 fold higher concentrations to inhibit abscission completely.

In a previous publication the abscission process has been shown to be comprised of more than one physiological stage, with the auxin NAA inhibiting a first stage and promoting a second one (Rubinstein & Leopold, 10). Experiments were undertaken to establish whether the same molecular requirements would hold for the two separate effects of auxin. Explants were given various lengths of induction time after which they were given high concentrations of IAA and NAA and six phenoxy compounds selected to include strong auxins (2,4-D & 2,4,5-T), weak auxins (2,5-D & *d*-2,5-DP), and nonauxins



**Table I**  
Comparative Activities on Growth & Abscission of a Series of  
Phenoxyacetic Acid Derivatives

Growth as maximal % increase of coleoptiles over controls within the concentration range  $10^{-3}$  to  $10^{-7}$  M. Abscission as minimum concentration for complete inhibition of bean explants, or hours promotion after 36 hours induction; compounds applied distally.

Compounds	Growth activity	Abscission activity	
		Minimum conc. for complete inhib. of abscission	Abscission promotion in second stage (hr)
PAA	+ 2%	$10^{-3}$ M	
2-Cl	+ 4%	$10^{-3}$ M	
4-Cl	+ 14%	$5 \times 10^{-5}$ M	
2,6-D	+ 4%	$10^{-3}$ M	6
3,5-D	+ 8%	$5 \times 10^{-4}$ M	8
2,5-D	+ 22%	$5 \times 10^{-5}$ M	28
2,4-D	+ 53%	$5 \times 10^{-7}$ M	55
2,4,6-T	+ 4%	$5 \times 10^{-4}$ M	
2,4,5-T-IBA	+ 8%	$10^{-4}$ M	
2,4,5-T	+ 33%	$10^{-6}$ M	48
<i>d</i> -2,4-DP	+ 20%	$5 \times 10^{-5}$ M	31
<i>l</i> -2,4-DP	+ 10%	$10^{-3}$ M	

**Table II**  
Induction Effects on Abscission Responses to a Series of Auxins & Auxin Analogues  
Applied Proximally in Initially Inhibitory Concentrations

Difference from controls for 50% abscission of the bean explants after varying induction period. (++) = complete inhibition; + = inhibition in hours over control; - = promotion in hours over controls).

Compound	Conc	Abscission after hrs of induction					
		0	6	12	18	24	36 (hr)
IAA	$5 \times 10^{-4}$ M	++	++	+ 8	-52	-55	-55
NAA	$5 \times 10^{-4}$ M	++	+25	- 6	-55	-60	-62
2,4-D	$5 \times 10^{-7}$ M	++	++	+45	-15	-50	-55
2,4,5-T	$10^{-6}$ M	++	++	+10	-38	-42	-45
2,5-D	$5 \times 10^{-5}$ M	++	+12	-25	-30	-26	-29
<i>d</i> -2,4-DP	$5 \times 10^{-5}$ M	++	+15	-28	-30	-32	-36
<i>l</i> -2,4-DP	$10^{-3}$ M	++	+15	+10	- 8	-10	- 8
3,5-D	$5 \times 10^{-4}$ M	++	+ 8	+ 2	- 8	0	- 6
2,6-D	$10^{-3}$ M	++	+ 5	- 6	- 8	- 9	- 6

(3,5-D & 2,6-D). A high concentration of each of the compounds was used to insure that any effect on the first stage would be only inhibitory. Both proximal and distal applications were separately tested with comparable results.

The data for abscission effects of nine compounds applied proximally are reported in table II. When the auxins were applied immediately after leaf removal (0 hr) inhibition of abscission occurred in every instance. If the same concentrations were applied after increasing lengths of time, inhibitions became less pronounced and in the cases of the most

active auxins a very marked stimulation of abscission ultimately occurred. Thus high concentrations of IAA and NAA produced 55 or 62-hour promotions when applied 36 hours after cutting. Similarly, the strongest phenoxyacetic auxins, 2,4-D and 2,4,5-T produced 55 and 45-hour promotions of abscission. Moderate promotions occurred with 2,5-D and *d*-2,4-DP (29 & 36 hr) whereas the *l*-2,4-DP, 2,6-D and 3,5-D did not give significant stimulations of abscission even after a 36-hour induction period.

Similar experiments were carried out with distal applications, and the responses are presented in fig

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FIG. 2. Abscission responses of bean explants to distal applications of phenoxyacetic acid derivatives at various concentrations. Time for 50% abscission of controls for each treatment: 92 hours. a (upper left). Phenoxyacetic acid and its monochloro substitutions. b (upper right). Dichloro substitutions. c (lower left). Trichloro substitutions. d (lower right). Optical isomers of 2,4-DP.

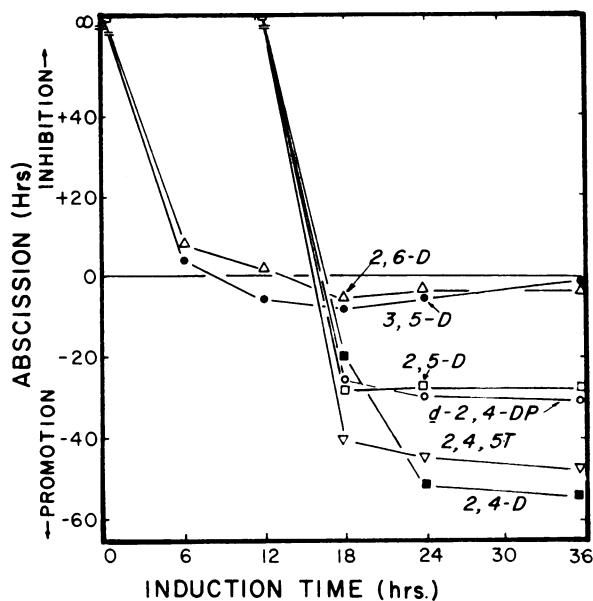


FIG. 3. Changes in abscission responses of bean explants with increasing time before application of various phenoxyacetic acids. Distal applications of initially inhibitory concentrations: 2,6-D:  $10^{-3}$  M, 3,5-D:  $5 \times 10^{-4}$  M, 2,5-D:  $5 \times 10^{-5}$  M, *d*-2,4-DP:  $5 \times 10^{-5}$  M, 2,4,5-T:  $10^{-6}$  M, 2,4-D:  $5 \times 10^{-7}$  M. Time for 50% abscission of controls: 92 hours.

1b and 3. The time curves for IAA and NAA are again closely alike, both stimulating abscission 50 to 60 hours and after similar induction periods (fig 1b). With the phenoxyacetic acids the induction periods led to a deterioration of the inhibitory effects, and as in the proximal treatments the largest promotions were obtained with 2,4-D and 2,4,5-T (55 & 44 hr), intermediate promotions with *d*-2,4-DP and 2,5-D (32 & 28 hr) and no significant promotions with 2,6-D and 3,5-D (fig 3).

Thus it can be seen that the inhibitory effects of high concentrations of these auxins are converted to accelerative effects with the interpolation of an induction period. But among the compounds tested, accelerative effects are found only for the compounds which are effective auxins, in the stimulation of growth. Numerical comparisons of the promotion effects on growth and on abscission are given in table I.

### Discussion

While the control of abscission has been considered to be a function of auxin, it has not been made clear whether the molecular requirements for auxin effects on abscission were similar to those for growth. The experiments reported here indicate that inhibitions of abscission can be obtained with compounds having very little growth-stimulating auxin activity, but that the concentrations required for these inhibitions reflect similarities to the relative activities of

the compounds in the stimulation of growth. On the other hand, promotions of abscission were obtained only with those compounds tested which do satisfy the structural requirements for growth activity.

The results obtained with the phenoxyacetic acids indicate such a correspondence between abscission activity and growth activity. Among the compounds tested, those having the highest growth activity (2,4-D, 2,4,5-T) appeared to be the most strongly inhibitory of bean explant abscission, and among the less active auxins the inhibitory effects on abscission become less pronounced with decrease in growth activity. However, even the compounds with the lowest auxin activity (e.g. phenoxyacetic acid) showed some inhibition of abscission at relatively high concentrations.

With respect to the promotive effects of auxins on abscission, Rubinstein and Leopold (10) have established that such effects are restricted to a second stage of abscission; for that reason, the promotive effects have been measured here after various durations of induction periods in which no auxin was applied. This method permits the unmasking of promotive effects of the phenoxyacetic acids which were not detectable through the use of the usual concentration series (cf fig 2 & 3). Promotions were found only for compounds with good growth-stimulating activity (2,4-D, 2,5-D, 2,4,5-T, & *d*-2,4-DP, as well as NAA & IAA).

Some departures from the parallelism between auxin activity and abscission inhibition have been reported by Day and Erickson (4) using lemon cuttings as experimental material. They have shown that some of the auxin homologues claimed to have no auxin activity (e.g. the diortho substituted phenoxyacetic acids) are quite active in preventing abscission. The data from the present study are in agreement; while the diortho or the isobutyric compounds do inhibit abscission in bean petioles, they do so only at concentrations markedly higher than the compounds having higher auxin activity in growth.

Experiments on the auxins present in developing currant fruits led Wright (14) to conclude that the fruits contained an auxin which was not effective in stimulating growth but was effective in the inhibition of the fruit abscission. Our experiments suggest that an auxin with quite low growth stimulating activity could show up in the abscission bioassay as an abscission inhibitor, in the same manner as the currant fruit auxin.

One of the most dramatic cases of structural modification of auxin activity is the case of the optical isomers of  $\alpha$ -propionic acid auxins. In the stimulation of growth, the dextro form is consistently active and the laevo form is weak or inactive (12,1). In the abscission test, the *d*-2,4-DP is found to be quite effective in both the inhibitory and in the promotive actions. By contrast, the *l*-2,4-DP is as weak in inhibiting abscission as is phenoxyacetic acid, and

yielded no apparent promotion effect. The striking difference in abscission activity with such subtle change in structure is no less surprising for its similarity to the growth activity of the two isomers.

In the past, abscission studies with bean have commonly been done with NAA, since Shoji and Addicott (11) showed that IAA is readily destroyed in the bean petiole. The relevance of these NAA experiments has been criticized by Addicott (2) in the context that synthetic NAA may have antiauxin activity, thus serving to lower the effectiveness of endogenous auxin rather than acting as an auxin. Jacobs (6) too has rejected the NAA effects on abscission as not being identical with IAA or endogenous auxin effects. The experiments reported here permit a precise comparison of the actions of NAA and IAA on abscission and from these results it appears that each of the NAA influences is precisely comparable to those of IAA. Not only is the concentration curve for IAA proved to be similar to that of NAA for both proximal and distal modes of application, but each auxin can be similarly altered from an inhibitor to a promoter of abscission by insertion of an induction period. It is to be noted that both IAA and NAA move in the same polar transport system inside the plant (8) and both are active in the *Avena* curvature test. It would seem unlikely that these two compounds do not act in the same auxin manner on abscission.

### Summary

The effects of various auxins on leaf abscission of *Phaseolus vulgaris* L. have been compared by studying the abilities of indoleacetic acid, naphthaleneacetic acid and various phenoxyacetic acids to inhibit or to promote abscission. The compounds were selected to represent a range of auxin activity associated with substitutions of the aromatic ring, of the acidic side chain, and with optical isomerism.

I. Using the bean abscission test, it was found that all of the compounds tested were capable of inhibiting abscission. Some inhibitory activity was obtainable even from compounds with no detectable growth activity. But the relative inhibitory effects were increased with structural changes which increased the growth activity of the auxin.

II. The promotive effects on abscission were restricted to those compounds which do have auxin activity in growth. This was especially evident when the promotive effects were released by including an

induction period before the application of the auxins or auxin analogues.

III. All of the auxins tested share in a quantitative way the ability to inhibit abscission and to promote abscission.

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