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ABSTRACT

The abscission of petiole explants of *Phaseolus vulgaris* can be correlated with the presence of endogenously produced ethylene. In all cases active promoters of abscission induced the evolution of ethylene prior to observable tissue separation. Substances that had little effect on abscission rates resulted in the production of negligible quantities of ethylene. Stimulatory action on abscission can be reduced by ventilation of the explants. It is concluded that endogenous ethylene participates in the abscission processes in bean-petiole abscission zones.

Introduction

The ability of ethylene to stimulate leaf abscission has been recognized for many years (e.g., CROCKER, HITCHCOCK, and ZIMMERMAN, 1935). Evidence has also been accumulated showing that ethylene is evolved by a wide range of plants and plant parts (DENNY, 1935; BURG, 1962; ABELES and RUBINSTEIN, 1964), and that auxins (MORGAN and HALL, 1962, 1964; ABELES and RUBINSTEIN, 1964) and defoliants (HALL, 1952; JACKSON, 1952) induce ethylene evolution. Since applications of certain auxins and defoliants rapidly accelerate abscission, it is tempting to propose that the increased ethylene evolution induced by these compounds is the causal mechanism of their action.

The above proposition was tested with bean-petiole abscission zones. These explants exhibit a two-stage response when auxin is applied at various times after cutting (RUBINSTEIN and LEOPOLD, 1963). Stage 1, under the conditions to be reported, normally lasts for 48 hr after the explants are prepared and is the period of time in which auxin inhibits abscission. Stage 2 occurs after this 2-day induction period, and applications of auxin during this time accelerate abscission. Ethylene has been found to promote abscission only during the second abscission stage (ABELES and RUBINSTEIN, 1964). In the present experiments active and inactive abscission promoters were investigated in relation to the production of ethylene during stage 2.

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Material and methods

Procedures for the measurement of abscission were similar to those previously described (ABELES and RUBINSTEIN, 1964). Petiole sections 1 cm long were cut from the primary leaves of *Phaseolus vulgaris* L. var. Red Kidney so that the leaf abscission zone was centrally located. The explants were inserted into agar that had been poured to a depth of 3 cm in 43 ± 2 ml bottles. The various compounds were applied by incorporation into the agar. The gas-collection bottles were then sealed by vaccine caps and incubated at 16° C with continuous light of 150 ft-c.

The accumulated gases were measured by withdrawing 2 ml from each bottle with a syringe and injecting the contents into an F&M Model 720 gas chromatograph equipped with a Model 1609 flame-ionization attachment. Oven temperature was 45° C, and helium flowed at 110 ml/min through a ¼-inch O.D. 60-cm activated alumina column; the analysis time was 1 min. By co-chromatography with standards (Olin Mathieson Co.) and the use of mercuric perchlorate and NaCl as specific agents for absorption and release of ethylene (BURCHFIELD and STORRS, 1962), authenticity of the ethylene peak was established.

Areas of peaks were determined with a disc-chart integrator. Sensitivity of the chromatograph permitted the detection of 0.05 mμl ethylene per milliliter and the ethylene content of each sample could be determined with a precision of 5%.

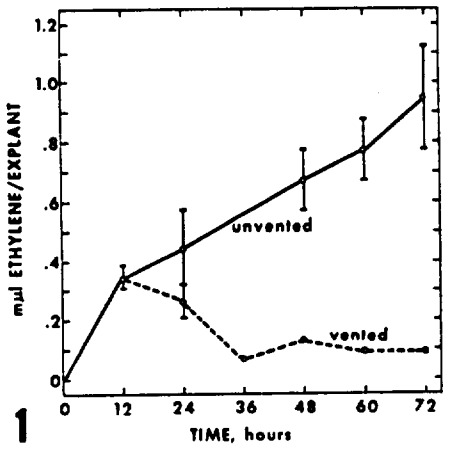
Data are expressed as mμl ethylene evolved per

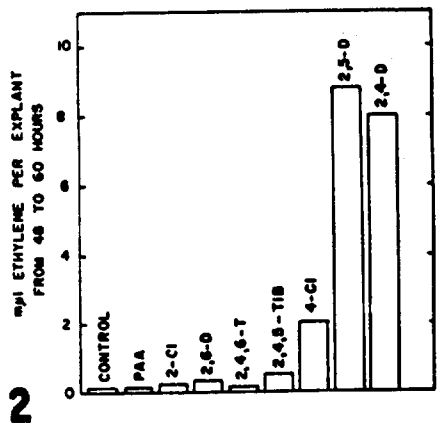
explant but can be converted approximately to parts per million by dividing by 3.4, or expressed as mμl/mg dry weight by dividing by 3.1 and then making the appropriate changes of units.

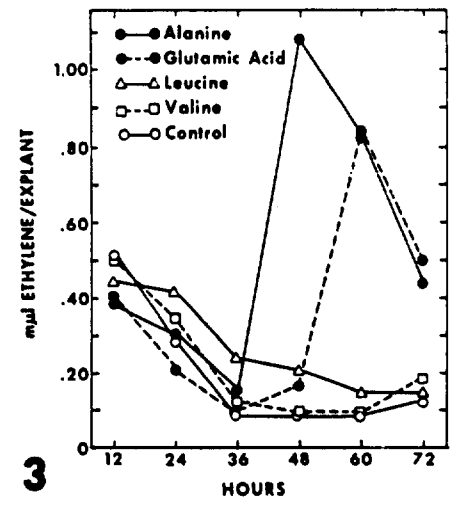
The following phenoxy acids used were a gift from the Amchem Products Inc., Ambler, Pennsylvania: phenoxyacetic acid (PAA), 2-chlorophenoxyacetic acid (2-Cl), 4-chlorophenoxyacetic acid (4-Cl), 2,4-dichlorophenoxyacetic acid (2,4-D), 2,5-dichlorophenoxyacetic acid (2,5-D), 2,6-dichlorophenoxyacetic acid (2,6-D), 2,4,6-trichlorophenoxyacetic acid (2,4,6-T), and 2,4,5-trichlorophenoxy isobutyric acid (2,4,5-TIB). 3,6-endoxohexahydrophthalic acid (Endothal), cis-3,6-endomethylenehexahydrophthalic acid (methylene Endothal), and cis-3,6-endomethylene Δ-4,5-tetrahydrophthalic acid (Δ-methylene Endothal) were obtained from Pennsalt Chemical Corporation, King of Prussia, Pennsylvania.

Results

To determine if detectable quantities of ethylene were evolved from untreated naturally abscising explants, 2-ml gas samples were withdrawn from vented and unvented gas-collection bottles. Accumulated







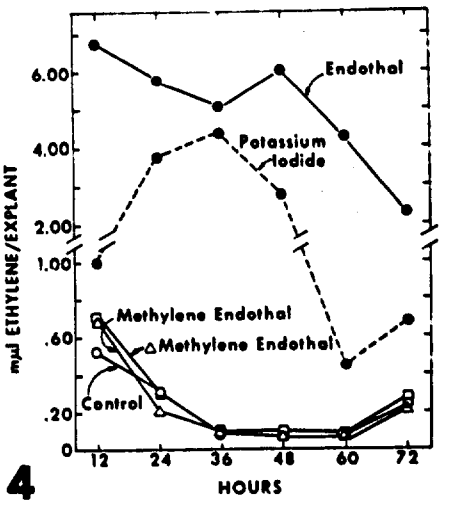


FIG. 1.—Production of ethylene by unvented and vented bean explants. Fifty per cent abscission was reached in unvented explants in 50 hr, by vented explants in 75 hr. Line through each point represents SE.

FIG. 2.—Evolution of ethylene from abscission-zone explants exposed to different phenoxyacetic acids. Explants were placed in plain agar for 48 hr prior to start of experiments.

FIG. 3.—Ethylene evolution by abscission-zone explants after treatment with various L-amino acids at $5 \times 10^{-3} M$. Hours to 50% abscission equal: for alanine, 56; for glutamic acid, 60; for leucine, 73; for valine, 74; and for controls, 75.

FIG. 4.—Effect of various defoliants on ethylene evolution from bean-petiole abscission zones. No abscission was observed for explants placed in Endothal ($10^{-4} M$) or potassium iodide ($5 \times 10^{-4} M$). With methylene Endothal treatments ($10^{-4} M$), 50% had abscised by 74 hr, with Δ-methylene Endothal ($10^{-4} M$) by 76 hr, and controls by 75 hr.

ethylene was removed from "vented" bottles every 12 hr. In the curve labeled "unvented," one bottle from an initial set of six was sampled every 12 hr for abscission rate and ethylene production. Figure 1 shows that ethylene was evolved from both vented and unvented bean explants. The gas production was rapid for 12 hr in the unvented bottles and then continued in a linear fashion until the end of the experiments. Ethylene production by vented explants shows an initial ethylene burst, but after 24 hr, less than 0.15 mμl/explant of the gas could be detected. The initial evolution of ethylene may be similar to the wounding response described by WILLIAMSON (1950) or may represent accumulated tissue ethylene which is released by treatment (BURG and THIMANN, 1959). The unvented treatments, which ultimately contained larger amounts of ethylene, reached 50% abscission at 50 hr and 100% at 72 hr; 50% of the vented explants had abscised by 80 hr.

CHATTERJEE and LEOPOLD (1963) found that the ability of different phenoxyacetic acids to promote abscission during stage 2 was directly related to the growth-promoting activity of the compounds. To examine the relation between ethylene evolution and induction of abscission, explants in stage 2 were transferred to agar containing various substituted phenoxyacetic acids, and ethylene evolved was measured 12 hr later. Data are presented in figure 2 with the compounds arranged by increasing abscission activity from left to right. There appears to be an approximate correlation between stimulatory action on abscission and ethylene production, but it should be noted that the same concentration (10^{-4} *M*) was used throughout this experiment, so that these results are not directly comparable to those of CHATTERJEE and LEOPOLD (1963), who used the minimum concentration that would inhibit abscission during stage 1. In general, however, compounds that are active growth promoters and also potent abscission stimulators (2,5-D; 2,4-D) produced more ethylene than the less active compounds. These data are the results of a representative experiment; since the replications varied widely quantitatively, but were identical qualitatively, the numbers were not pooled.

When investigating the relationship between ethylene evolution and abscission, one must also consider the wide variety of non-auxinic compounds that have a stimulatory effect on leaf abscission. RUBINSTEIN and LEOPOLD (1962) found that amino acids have varying degrees of activity in the bean-petiole abscission test, with L-alanine and L-glutamic acid being among the most stimulatory and L-valine and L-leucine being relatively inactive. These amino acids were applied in agar at 5×10^{-3} *M* to explants in gas-collection bottles, and the evolved ethylene measured over time (fig. 3). The pattern of ethylene evolution is quite different from that following applications of naphthaleneacetic acid when there is an immediate increase in gas production (ABELES and RUBINSTEIN, 1964). No real difference can be seen from the controls until 36 hr after cutting the explants. At that time, ethylene evolution is markedly increased by the two amino acids that stimulate abscission. Abscission occurred 12 hr after the acceleration of ethylene evolution. The amino acids that were weak promoters of abscission, leucine and valine, had little effect on ethylene evolution.

TABLE 1

EFFECT OF AMINO ACIDS AND DEFOLIANTS ON ETHYLENE EVOLUTION AND ABSCISSION OF BEAN PETIOLE EXPLANTS

	Hours elapsed before application of compounds			
	0 hr		48 hr	
	mμl ethylene/explant after 12 hr	Hours to 50% abscission[a]	mμl ethylene/explant after 12 hr	Hours to 50% abscission[a]
Amino acids:				
L-Alanine (5×10^{-3} *M*)	0.57	74	0.75	59
L-Glutamic acid (5×10^{-3} *M*)	0.51	71	0.62	61
L-Leucine (5×10^{-3} *M*)	0.54	70	0.10	74
L-Valine (5×10^{-3} *M*)	0.51	74	0.08	73
Control	0.52	75	0.10	75
Defoliants:				
Endothal (10^{-4} *M*)	6.73	100	8.51	58
Methylene Endothal (10^{-4} *M*)	0.71	70	0.15	68
Δ-Methylene Endothal (10^{-4} *M*)	0.75	73	0.13	73
Potassium iodide (5×10^{-4} *M*)	1.33	98	3.21	56
Control	0.55	75	0.10	75

[a] SE was never greater than ± 4 hr.

All four amino acids were further analyzed for their effects on the two abscission stages. They were supplied for 12 hr to bean explants either immediately after cutting (to observe effects on stage 1) or 48 hr after cutting (to observe effects on stage 2). As can be seen in the first five lines of table 1, none of the amino acids tested had any effect on either ethylene production or abscission rate when present from 0–12 hr after cutting. If applied 48 hr after cutting, however, alanine and glutamic acid markedly increased both ethylene evolution and the rate of abscission. Leucine and valine still had no effect on rate of ethyl-

ene evolution, and explants treated with these amino acids excised at about the same times as the controls.

Similar experiments were performed using compounds that have the ability to defoliate intact bean plants. We have observed that it is possible to defoliate 14-day-old bean plants when they are sprayed until runoff with 10^{-3} *M* Endothal or potassium iodide. Similar results with KI had also been found by HERRETT *et al.* (1962). Two analogs of Endothal, methylene Endothal and Δ methylene Endothal, were found to be inactive as defoliants, however.

KI (5×10^{-4} *M*), Endothal (10^{-4} *M*), and its inactive analogs (10^{-4} *M*) were applied to explants. Figure 4 shows a stimulation of ethylene evolution from explants treated with KI or Endothal, while the Endothal analogs produced only slight differences from the controls. The effects on the two stages of abscission are shown in the second five lines of table 1. Both Endothal and KI stimulated ethylene production during stage 1 (application from 0–12 hr) but abscission was inhibited. When these compounds were applied during stage 2 (after 48 hr had elapsed), ethylene production was again stimulated, but now the abscission rate was accelerated. Methylene Endothal and Δ-methylene Endothal induced a slight stimulation of ethylene evolution during stage 1 but had no effect when applied during stage 2. Abscission rates of explants treated with these two Endothal analogs differed little from that of the control.

TABLE 2

EFFECT OF ALANINE, KI, AND ENDOTHAL ON ABSCISSION OF EXPLANTS IN SEALED AND OPEN BOTTLES[a]

TREATMENT	ABSCISSION 7 HR AFTER TREATMENT[b] (%)	
	Sealed bottles	Open Petri dishes
Control	59 ± 8	43 ± 5
Alanine (10^{-3} *M*)	74 ± 5	49 ± 7
KI (5×10^{-4} *M*)	80 ± 4	52 ± 4
Endothal (10^{-4} *M*)	71 ± 7	40 ± 7

[a] Explants were inserted into plain agar for 48 hr and then placed into sealed gas-collection bottles or open Petri dishes covered with moistened cheesecloth.

[b] Average of nine experiments plus or minus the SE.

The relationship of ethylene to abscission processes may also be tested by removing the ethylene from the environment around the abscission zones; the rate of abscission of these explants can then be compared with similarly treated explants in sealed containers. As shown in table 2, explants placed in the stimulatory substances 48 hr after cutting abscised more rapidly if they were in sealed bottles than if they were in open Petri dishes. The differences were largest at 7 hr after insertion when over 60% of the explants in sealed bottles had abscised while identically treated explants in open dishes showed less than 50% abscission.

Discussion

From the data presented we feel that endogenous ethylene may be implicated in abscission processes. A wide variety of abscission-accelerating substances was applied to bean-petiole explants, and in all cases the evolution of ethylene preceded the stimulation of abscission. For example, certain phenoxyacetic acid compounds that are active as growth regulators have been found to be active promoters of abscission during stage 2 (CHATTERJEE and LEOPOLD, 1963). These compounds also markedly stimulated ethylene evolution. The amino acids alanine and glutamic acid did not immediately stimulate ethylene evolution, but 12 hr before abscission could be observed enough ethylene was produced to account for the subsequent promotion of abscission. It is also interesting that explants treated with these two amino acids 48 hr after cutting evolved greater amounts of ethylene soon after placement.

Both Endothal and potassium iodide rapidly stimulated ethylene evolution, but when these substances were applied immediately after the abscission-zone explants were excised, no abscission occurred. This was also observed by ADDICOTT and co-workers (ADDICOTT and LYNCH, 1955; ADDICOTT, 1964). If the same concentration of the defoliants was applied when explants were in the second stage or 48 hr after cutting, stimulation of ethylene evolution was again observed, and at that time abscission occurred rapidly. It is possible that the defoliants injure the explants or retain them in the first stage; ethylene, as reported earlier (ABELES and RUBINSTEIN, 1964), is ineffective as an abscission stimulant during stage 1. The ethylene evolved by explants to which defoliants were applied 48 hr after cutting was present when the explants were in stage 2, and abscission occurred rapidly thereafter. These results showing that applications of defoliants directly to the abscission zone can stimulate abscission suggest that on intact plants certain defoliants may act directly on the abscission zone, as well as indirectly, by affecting the leaf blade.

Along with the finding that compounds that promote abscission likewise increase ethylene evolution is the fact that other substances that have little effect on explant abscission are unable to stimulate the production of ethylene. Those phenoxyacetic acids that are ineffectual growth promoters also produced little change in time of abscission (CHATTERJEE and LEOPOLD, 1963). These same compounds stimulated only slightly the evolution of ethylene. The amino acids, leucine and valine, were reported

to be relatively poor stimulators of abscission (RUBINSTEIN and LEOPOLD, 1962); they also had no effect on ethylene production during either abscission stage. The two analogs of Endothal, methylene Endothal and Δ-methylene Endothal, which were found to have no activity on whole bean plants, stimulated explant abscission and ethylene evolution only slightly.

The lack of knowledge concerning biosynthetic pathways of ethylene production makes it impossible to employ specific inhibitors to retard ethylene evolution while not affecting other vital metabolic processes. We attempted, therefore, to prevent the accumulation of ethylene at its site of action by placing the explants in open Petri dishes and comparing abscission rates with those of explants in sealed gas-collection bottles. This technique is similar to that of HANSEN (1946), who showed that ventilated bananas ripened later than those in sealed containers. He explained the effect as resulting from accumulated ethylene. Our results indicated that the removal of ethylene tended to delay explant abscission as compared with that of explants in the sealed bottles. This conclusion is further reinforced by experiments in which ethylene evolution from explants in vented and unvented bottles was measured over time (fig. 1). In these cases explants that were vented every 12 hr abscised 25 hr later than unvented explants. Similar results were obtained for auxin-treated explants; it was found that continuous aeration reduced the abscission rates of explants placed in NAA after 48 hr, and control explants flushed with 1.2 ppm ethylene abscised almost as rapidly as explants treated with NAA in sealed bottles (ABELES and RUBINSTEIN, 1964).

We conclude, therefore, that endogenous ethylene is implicated in the processes of bean-leaf abscission. Caution must be exercised, however, in interpreting the data; the method is limited to measuring only the observable separation of the tissues, thus making it impossible to determine if a substance affects the very onset of abscission or just the final dissolution of the cell-wall materials. What is more, the possibility still remains that other substances, both dissolved and volatile, may also participate in initiating and accelerating bean-leaf abscission.

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