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Patterns of Ethylene and Carbon Dioxide Evolution during Cotton Explant Abscission

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ABSTRACT

The relationship between abscission and the evolution of ethylene and CO_2 was examined in explants and explant segments of cotton seedlings (*Gossypium hirsutum* L. cv. Acala SJ-1) under both static and flow system conditions, and in the presence and absence of mercuric perchlorate. Explant excision was immediately followed by increased ethylene evolution (wound ethylene); senescence was also accompanied by increased ethylene evolution (senescence ethylene). One or two ethylene peaks were found to interrupt the low background rate of ethylene evolution during the period between excision and senescence. The first intermediate ethylene peak coincided with a rise in CO_2 evolution; however, precedence could not be established. No statistical correlations were discovered between either intermediate ethylene peak and abscission. The best statistical correlation was found between wound ethylene and abscission at 12 hr after excision. No positive correlations were found between senescence ethylene and abscission. Implications of these results for the understanding of the role of ethylene in explant abscission are discussed.

Relationships between a number of different explant treatments and ethylene evolution were also examined. Ethylene production in response to indoleacetic acid applications, abscisic acid applications, and different types of wounding is summarized. It was concluded that the results of the standard abscission bioassay (conducted in Petri dishes) have not been influenced by unnatural ethylene accumulations.

Cotton plants have provided the materials for extensive abscission research, and ETH[2] has been implicated in the regulation of this abscission many times over (1, 8, 9, 19, 21, 24, 26). Among the numerous proposals forwarded to explain the role(s) of ETH in abscission, several have been based on experiments with cotton explants: most notably the hypotheses that abscission may be controlled in part by increasing tissue sensitivity to the ETH already produced (1), and that absicssion may be controlled in part by reduced auxin transport resulting from increased ETH evolution (10). The fact that cotton explants display increased ETH evolution prior to absicssion is not disputed. Cotton explants share this characteristic with bean explants, where correlation between the *in vivo* pattern of ETH production and abscission led Jackson and Osborne (17) to conclude that ETH was responsible for initiating the biochemical events leading to separation, and was hence the natural regulator of abscission.

Despite the attention given to cotton explants as a tool of absicssion research, no detailed account of their endogenous patterns of ETH evolution has yet appeared. This paper is primarily concerned with accomplishing this task for one variety of cotton.

In a flow system specifically designed for this work, the patterns of ETH and CO_2 evolution accompanying abscission in control explants were established and then compared with those patterns in explants treated with IAA and ABA. Abscission of hormone-treated and untreated explants was compared in the presence and absence of MP. Patterns of ETH and CO_2 evolution were also studied in segments of cotton explants and in individual explants.

MATERIALS AND METHODS

General Explant Techniques. All experiments were performed with excised cotyledonary nodes of 14-day-old cotton seedlings (*Gossypium hirsutum* L. cv. Acala SJ-1) or with further subdivisions of this explant. Subdivision of a standard explant consisted of excising the petiole stumps and dividing them each into two segments 2 to 2.5 mm in length. The proximal segments contained the abscission zones with their surrounding tissues and were called abscission zone segments. The distal segments and the axes were referred to, respectively, as petiole stump segments and axial segments. On subdivision, each standard explant yielded two abscission zone segments, two petiole stump segments, and one axial segment. The standard growth regime and cotton explant procedures were followed with minor modifications (7, 12, 28). Briefly, the explants were supported in an upright position by a shallow layer (5 mm) of 1.5% agar covering the base of the explant chamber, approximately 10 g of pressure was applied to the petioles in testing for abscission, and abscised petioles were not removed from the explant chambers until all gas production data had been obtained. Agar droplets were omitted on all stem and petiole stumps unless otherwise indicated.

Flow System, Chromatography, and Sampling. Most experiments were conducted in a flow system. Rates of ETH and CO_2 evolution were determined from chromatographic analysis of gas samples withdrawn from the explant chambers (22, 23). Instantaneous rates of gas evolution were calculated using the appropriate formula (22), and total amounts produced were estimated by cutting and weighing Xerox copies of the individual rate *versus* time graphs.

The atmosphere of the flow system was Purafil-filtered ETH-free air. The flow system was similar to that described by Claypool and Keefer (14) for respiration studies. The air pressure was regulated by a barostat and the flow rate by short lengths of calibrated capillary tubing. The stoppers sealing the explant chambers were provided with glass tubing so that the incoming air stream entered at the base of the chambers and exited at the top. The outflow tubing terminated in a capillary (capacity several hundred liters/min) resting in a test tube containing 8 cm of water. The pressure within the explant chambers (1.01 atm)

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[2] Abbreviations: ETH: ethylene; MP: mercuric perchlorate; N: sample size; *r*: correlation coefficient; PSS: petiole stump segments; AZS: abscission zone segments; AXS: axial segments.

was monitored by manometers connected to the air supply line of each chamber. The volume of the explant chambers ranged from 100 to 250 cm^3. Whenever sampled, the humidity within the chambers was between 50 and 65%, and no special measures were employed to modify the water content of the air. The first 2.5 cm of tubing exiting each chamber was of a construction which could be punctured repeatedly by a 25-gauge needle without introducing a leak into the system, and all atmospheric sampling was done from this point. Care was taken during sampling not to reduce the internal pressure below atmospheric.

Hormone Experiments. Standard explants were cut and placed in opaque explant chambers at 30 C, sealed into the flow system, and aerated (20 liters/min), before initiation of the desired flow rate. Approximately 3 hr elapsed between the cutting of the explants for any one treatment and the incorporation of that flask into the flow system. Twelve hr after excision, the flasks were opened and hormone treatments applied distally in 5-μl droplets of 0.75% agar. Plain agar droplets were applied to the controls. The system was resealed, aerated, and the selected flow rate reestablished. Flasks that were used for abscission data were not used for measurements of gas production because of the occasional release of wound ETH following the test for abscission. Measurements of gas production were obtained from companion flasks in these experiments.

Abscission was also studied as a function of hormone concentration in the absence of accumulated ETH. Vials containing 10 ml of a 0.375 M MP solution were included in the explant chambers, and the ETH levels within the chambers were monitored twice daily. If a rise above 3 nl/l was detected, that chamber was discarded.

Wound ETH Experiments. Wound ETH, the burst of ETH evolution immediately following excision of explants and explant segments, was studied in both static and flow systems. Measurements were begun as soon as possible after excision (often within 8 min).

RESULTS

The endogenous patterns of ETH evolution and abscission in these cotton explants are shown in Figure 1. All data in Figure 1 are from untreated (control) explants. The average peak wound ETH production rate of 4.10 ± 1.95 nl/g·hr is reached 1.26 ± 0.50 hr after excision (flow system data, N = 22). Between 8 and 20 hr following excision, ETH evolution rates have usually reached a low basal level around 0.2 nl/g·hr. Prior to senescence, this low background rate of ETH evolution is interrupted either once or twice by bursts of ETH (intermediate peaks). At least one of these intermediate peaks always occurred (between 12 and 60 hr). In Figure 1, individual intermediate peaks before 24 hr tend to be obscured by the wound ETH. Although there are no points in Figure 1 much below 2 nl/g·hr, this is not inconsistent with basal rates of 0.2 nl ETH/g·hr. It reflects the presence of intermediate ETH peaks in each 12 hr interval.

The division of the data of Figure 1 into three groups is arbitrarily based on the per cent abscission reached at 120 hr from excision. The over-all pattern of abscission (Fig. 1) shows one major peak, during which absicssion is most likely to occur. The greater the final amount of abscission, the more pronounced and earlier is this peak.

CO_2 production remains constant, or nearly so, during wound ETH evolution (see ref. 22). A prominent respiratory rise begins coincident with the first intermediate ETH peak (see Fig. 3). Following excision, CO_2 production rates peak once and then gradually decline to zero.

Senescence in the cotton explant was manifest by a progressive darkening and softening of all tissues, accompanied by a terminal rise in ETH production which subsequently fell to zero with the death of the tissues. Senescence occurred sooner at higher temperatures than at lower ones, but its visual onset and the

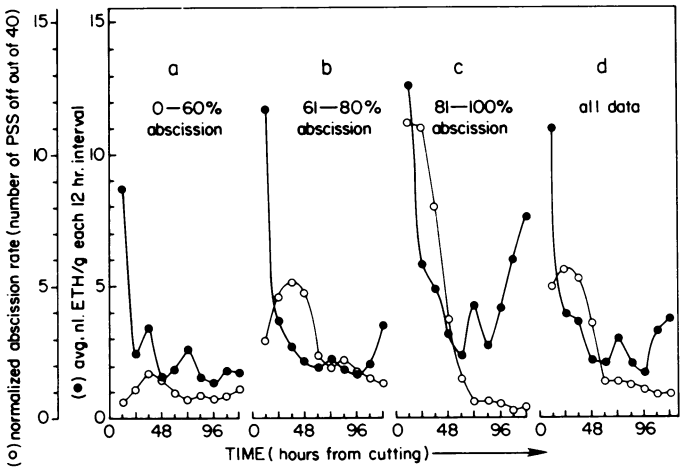

FIG. 1. Patterns of abscission and ETH production in cotton explants. (●): Average rate of ETH production/12-hr interval (based on two to 12 measurements each interval); (○): normalized rate of abscission/12-hr interval. The data were obtained from control explants at 30 C and include some that for unknown reasons displayed abnormally high abscission (c). The data are divided into categories based on the total per cent abscission recorded 120 hr from cutting. The combined abscission data (d) are based on 8,400 abscission zones: 2,440 in a, 1,720 in b, and 4,240 in c. In each figure, the number of abscission events occurring in each 12-hr interval has been summed and normalized on the basis of 20 explants; d is an average of the three previous graphs. Average ETH production rates and their standard deviations in each 12-hr interval are as follows for the combined data (d): 0 to 12 hr: 11.00 ± 5.83 (N = 72); 12 to 24 hr: 3.98 ± 2.93 (N = 64); 24 to 36 hr: 3.64 ± 2.06 (N = 59); 36 to 48 hr: 2.15 ± 1.61 (N = 46); 48 to 60 hr: 2.10 ± 1.06 (N = 41); 60 to 72 hr: 2.97 ± 2.51 (N = 59); 72 to 84 hr: 2.04 ± 2.03 (N = 20); 84 to 96 hr: 1.78 ± 1.71 (N = 25); 96 to 108 hr: 3.37 ± 3.13 (N = 25); 108 to 120 hr: 3.77 ± 4.11 (N = 38). About 75% of the ETH data were obtained simultaneously with abscission data, and the correlation coefficients were calculated from only these paired measurements.

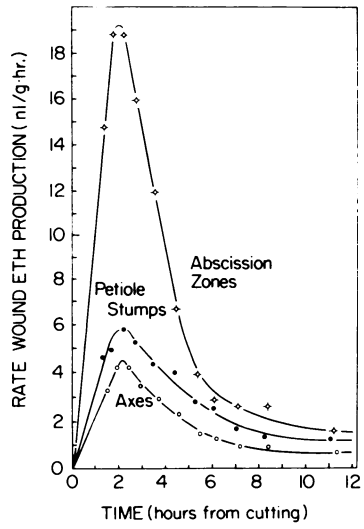

FIG. 2. Wound ETH evolution from explant segments. Wound ETH is characterized by three parameters: T: time at which the maximal rate of wound ETH evolution occurs (hr after cutting); R: maximum rate of wound ETH evolution (nl/g·hr); and Tnls: total amount of ETH produced during the 12 hr immediately following wounding. Essential data for 40 PSS (●): weight: 0.105 g; flow rate: 32.9 ml/hr; free volume: 124 ml; T: 2.25 hr; R: 6.09 nl/g·hr; Tnls: 39.92 nl/g. Essential data for 40 AZS (+): weight: 0.112 g; flow rate: 33.8 ml/hr; free volume: 114 ml; T: 2.10 hr; R: 19.22 nl/g·hr; Tnls: 83.20 nl/g. Essential data for 20 AXS (○): weight: 0.995 g; flow rate: 33.2 ml/hr; free volume: 118 ml; T: 2.19 hr; R: 4.50 nl/g·hr; Tnls: 23.48 nl/g.

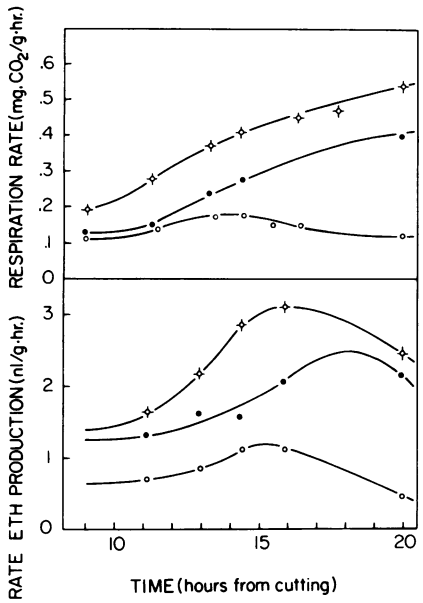

FIG. 3. Simultaneous CO_2 and ETH evolution in cotton explant segments. A representative example from an experiment studying the intermediate peaks in ETH and CO_2 production. All replicates showed a rise in CO_2 evolution accompanying the first intermediate ETH peak. Average time (and standard deviation) of first intermediate ETH peak occurrence was 21.3 ± 3.08 hr. Average peak rate (and standard deviation) of this ETH evolution in AZS was 2.98 ± 0.90 nl/g·hr. These data are continuations of those given in Figure 2, and the tissue weights, explant chamber free volume, and flow rates of the PSS (●), AZS (+), and AXS (○) can be found in the legend to Figure 2.

terminal rise in ETH production were always correlated (23). The 108-hr peak in ETH evolution in Figure 1d represents senescence ETH.

Figures 2 and 3 present a detailed picture of the wound ETH response and the first intermediate ETH peak for explant segments under flow system conditions. Instantaneous rates (22) are given as a function of time. The highest rates (on a weight basis) were always achieved by the abscission zone segments and the lowest rates by the axial segments. The variability in the time of appearance of peaks in ETH evolution from explant segments cut from different seedlings was much greater than that variability seen in segments cut from the same seedlings. Accordingly, representative examples of data are shown to avoid averaging peak rates occurring at different times. (Such an averaging occurs in the data of Fig. 1 resulting in higher values for background ETH evolution and lower values for peak ETH evolution, but the large sample size employed allowed good definition of the over-all ETH evolution pattern.)

The first intermediate peak in ETH evolution is accompanied by simultaneous CO_2 evolution data (Fig. 3). In all six replicates of each segment type, the rise in both ETH and CO_2 evolution occurred within a 2-hr interval, and if one rise regularly preceded the other, it could not be determined from these data. Sixty seven per cent of the time (see Fig. 3), the peak rate of ETH evolution from the abscission zone segments preceded that of the petiole stump segments, and the remainder of the time the peak rates occurred simultaneously; however, the potential triggering effect of this early ETH evolution can not be evaluated at present.

Table I demonstrates the markedly different respiration rates of abscission zone segments, petiole stump segments, and axial segments. Rates of CO_2 evolution were always highest in abscission zone segments and lowest in axial segments. At the same time, it demonstrates how these different rates can combine to match the observations made on intact explants. The CO_2 evolution of standard cotton explants is no more than the sum of the CO_2 evolution of the various regions of the explant; subdivision of the explant did not introduce any "wound CO_2" effects.

IAA, ABA, and GA_3 dose response curves for cotton explant abscission in a flow system are reported in Figure 4. All concentrations of ABA and GA_3 tested provided acceleration. IAA applications of 10^{-3} and 10^{-2} μg/abscission zone provided mild acceleration. Higher concentrations of IAA retarded abscission while lower concentrations were without apparent effect. These dose response curves were obtained in the presence of MP (where external ETH concentrations never exceeded 3 nl/l) and in its absence (where rates of CO_2 and ETH production were determined), and no significant differences in abscission behavior between these two conditions were noted.

Figures 5 and 6 summarize the ETH and CO_2 production data that accompanied the abscission reported in Figure 4. Applications of IAA and ABA 12 hr after excision did not alter the patterns of ETH or CO_2 evolution. Applications of IAA at concentrations of 10^{-2} μg/abscission zone and above were more effective in stimulating increased ETH evolution than applications of ABA at corresponding concentrations. Lower concentrations of both IAA and ABA did not increase the amount of ETH produced; CO_2 production was virtually unchanged at all IAA and ABA concentrations tested. Increased ETH evolution always appeared as an enhancement of the first intermediate ETH peak; however, the CO_2 peak associated with the first intermediate ETH peak was not enhanced by hormone treatment.

DISCUSSION

Ethylene and Abscission Correlations. Both in time of occurrence and in maximum rates observed, the senescence ETH of these cotton explants is remarkably similar to the senescence-related terminal ETH rise reported for bean explants (17, 18). Significant abscission in cotton explants occurs before the onset of senescence ETH (Fig. 1), and the relationship between terminal ETH and abscission reported for bean explants does not hold for cotton explants. The CO_2 evolution patterns of both cotton and bean explants have a single peak (11, 13), but the metabolic functions of cotton explants may decline more rapidly than those of bean explants.

Since wound ETH evolution, the first intermediate ETH peak, and the main peak in abscission activity all occur within 24 hr of each other, Figure 1 suggests the possibility of some correlation between early ETH production and abscission. Whenever the data permitted, correlation coefficients (r) were calculated between rates of ETH production and abscission. For example, average rates of ETH production in each 12-hr interval and the fraction of the total abscission occurring in each 12-hr

Table I. Respiration rates of explant segments and standard explants.

Explant Segment	Rate[1]	Fraction[2] of Total	Net Contribution[3]
PSS	0.376 ± 0.074	0.098	0.037
AZS	0.607 ± 0.186	0.073	0.044
AXS	0.184 ± 0.034	0.829	0.152

Intact Explants[1]: 0.227 ± 0.053; Reconstructed Rate[4]: 0.233 ± 0.049

[1]Rate given as mg CO_2/g·hr (20 hr after excision); averages and SD based on 5 replicates.

[2]Fractions given are averages of 8 replicates (w/w). Average weights of the explant segments were: PSS: 0.12 ± 0.03; AZS: 0.09 ± 0.02; AXS: 1.02 ± 0.09 g.

[3]The product of columns 1 and 2.

[4]The sum of the net contributions of PSS, AZS, and AXS.

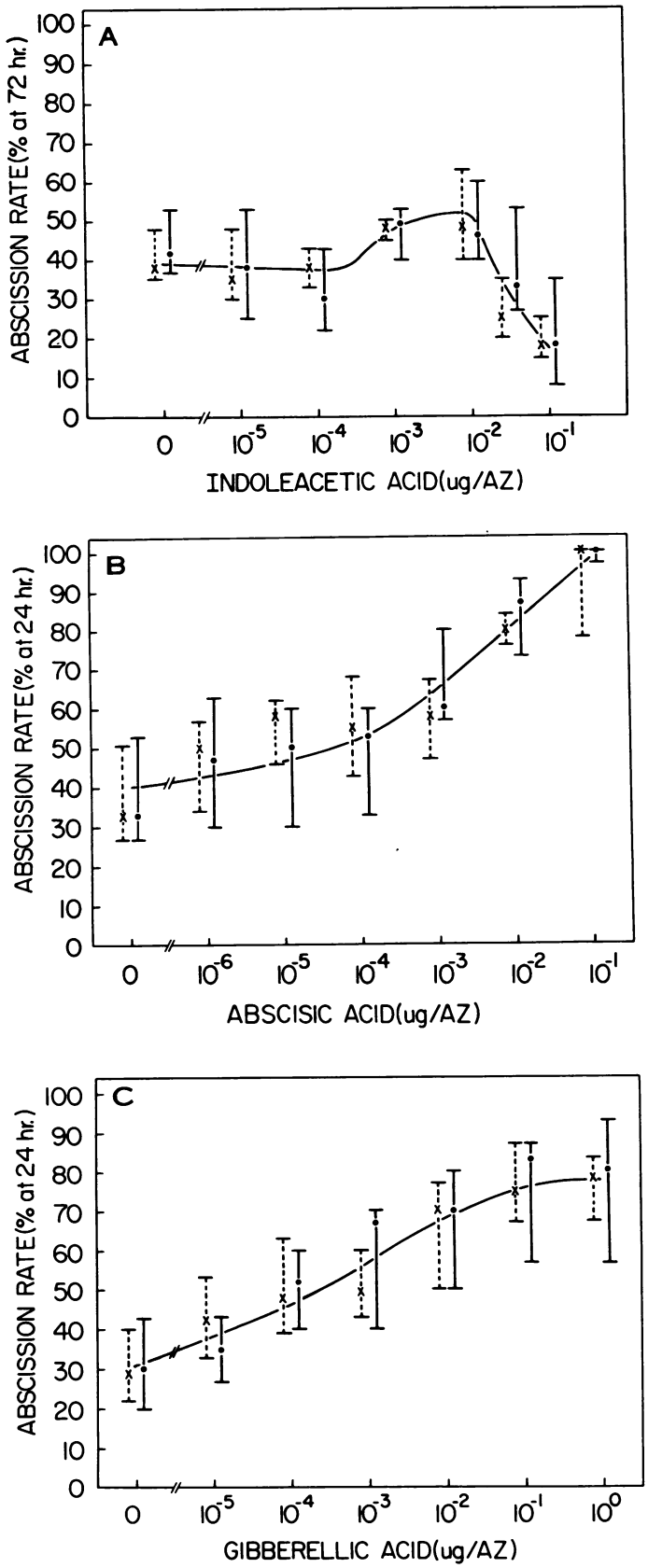

FIG. 4. Abscission responses to distal applications of hormone treatments. Treatments were applied 12 hr after cutting. Experiments were conducted in a flow system both with and without inclusion of MP. (●): median value of abscission in the absence of MP; (——); corresponding 96% confidence intervals; (×): median value of abscission in the presence of MP; (- - -): corresponding 96% confidence intervals. Each data point is based on 12 replicates, and since each value of per cent abscission is calculated from a group of 20 explants, 12 replicates encompass 480 abscission zones.

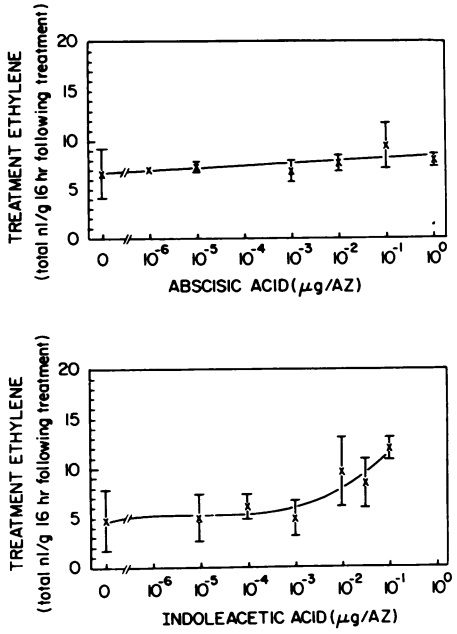

FIG. 5. ETH production (treatment ETH) of cotton explants following distal applications of IAA and ABA. Rates of ETH evolution were monitored in a flow system during the 16 hr immediately following hormone treatment, and often up to 40 hr following treatment; trends over both time intervals were identical. The amounts of ETH produced represent areas under rate *versus* time curves. Number of replicates for any one treatment varied from three to nine (flasks of 20 explants). Averages for each treatment have been graphed with their corresponding 90% confidence limits. Best fit lines connecting the points were provided by linear and cubic regression analyses, respectively.

interval were calculated and tabulated along with the total abscission observed at 120 hr for each individual explant chamber. The data were then grouped on the basis of the total abscission observed at 120 hr after excision (Fig. 1). Correlation coefficients were then calculated between ETH production and abscission within individual 12-hr intervals, between ETH production and abscission 12 hr later, and between ETH production and abscission 24 hr later, for each group of data. No statistically significant correlations between the first intermediate ETH peak and abscission were found.

In addition, individual explants displayed the same pattern of ETH evolution (wound ETH, one or two intermediate ETH peaks, and senescence ETH), but cases in which the first intermediate ETH peak appeared with no accompanying abscission (0% abscission at 120 hr) were common. Axial segments displayed intermediate ETH peaks (Fig. 3), but the petiole stump segments and abscission zone segments were most active in this regard.

The best correlation found was between ETH production and abscission in the 0- to 12-hr interval ($r = 0.83$, N = 41, Fig. 1d). This correlation was just as strong at low rates of abscission ($r = 0.80$, N = 18, Fig. 1a) as at high rates of abscission ($r = 0.79$, N = 19, Fig. 1c), and considering the inherent tissue variability commonly observed (23), this correlation coefficient of 0.83 is remarkably large.

One possibility considered was that an underlying third factor, metabolic activity for example, could be responsible for the

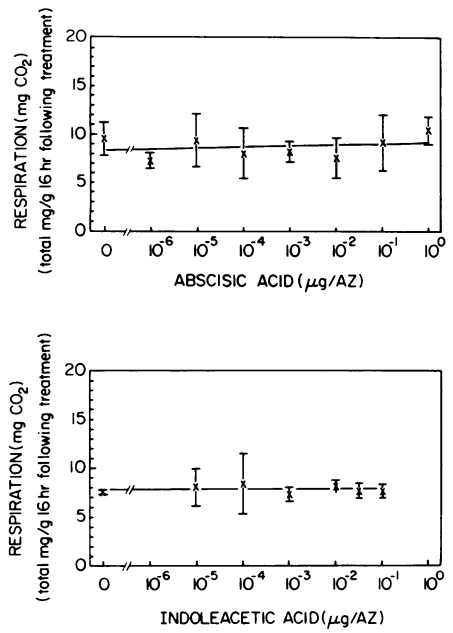

FIG. 6. CO_2 production of cotton explants following distal applications of IAA and ABA. Rates of CO_2 evolution were monitored in a flow system during the 16 hr immediately following hormone treatment, and often up to 40 hr following treatment; trends over both time intervals were identical. Amounts of CO_2 reported represent areas under rate *versus* time curves. Number of replicates for any one treatment varied from three to six (flasks of 20 explants). Averages for each treatment are graphed with their corresponding 90% confidence limits. Best fit lines connnecting the points were provided by linear regression analyses.

above correlation. In this case, good correlations would be expected between total ETH evolution and total abscission (r = 0.32, N = 49) and between wound ETH and total abscission (r = 0.38, N = 73), but these values lend no support to the above suggestion.

A second hypothesis to explain this 0- to 12-hr correlation is one of cause and effect. Prior to excision, cotton abscission zones do not respond to ETH unless their auxin supply has first been diminished (9). Twelve hr after removal of the leaf blade, cotton explant abscission zones are sensitive to ETH (1). During the present experiments, addition of as little as 0.02 μl/1 ETH 12 hr after excision caused statistically significant abscission acceleration (at the 1% level), while addition of 0.01 μl/1 ETH or less had no effect. Excision not only cuts off the supply of IAA to the abscission zone, but it also induces ETH production which may accelerate the inactivation of IAA already present in the explant tissues (16). It is quite possible that on wounding, the onset of tissue sensitivity may occur before 12 hr and before the production of wound ETH has ceased. In this case, the tissue would respond to its own wound ETH.

All correlation coefficients, other than the 0- to 12-hr ones, were small and usually positive (0.04–0.38). After 72 hr, the trend was for small negative numbers to appear, culminating in an r of −0.58 (N = 7) for ETH and abscission in the 108- to 120-hr interval of Figure 1c. The negative correlation coefficients arise from the fact that as senescence approaches, abscission activity is drawing to a close while rates of ETH evolution are increasing.

Ethylene and Hormone Treatments. The basic pattern of ETH evolution did not change with either IAA or ABA treatment. While ETH peaks following ABA treatments were more apt to occur earlier than ETH peaks following IAA treatments, this difference (about 2 hr) was not statistically significant. The slight to nonexistent enhancement of ETH by ABA treatment (Fig. 5) is not a unique observation (20, 27), but it is one which suggests that the role of ABA in this case may be independent of ETH.

In one preliminary experiment, explant segments were treated with ABA at the time of excision. The pattern of ETH evolution in the ABA-treated segments was unaltered, but the peak rates of CO_2 evolution in the abscission zone and the petiole stump segments were reached sooner and sustained longer. The axial segments were similarly affected although the effect was less pronounced. If these preliminary results are typical of ABA treatments at excision, they support the conclusion that ABA regulation of cotton explant abscission is not related to the induction of increased or earlier ETH production.

Wound ETH Evolution. The evolution of wound ETH may not be strictly proportional to the area cut; it may also depend on the type of tissue wounded. The data presented in Figure 2 are consistent with the hypothesis that the region of the abscission zone is more active than other explant regions in producing ETH when wounded. The explant segment data of Figure 2 and similar experiments can be used to calculate predicted values of ETH production for standard explants. Table I gives an example of a similar procedure for CO_2 evolution data of explant segments. If a cut near the abscission zone were not equivalent to the other cuts in terms of wound ETH evolution, then reconstructed rates from explant segment data would not necessarily equal three times the actual measured values (the wounded surface area of a subdivided explant is approximately three times greater than that of an intact explant). In fact, the reconstructed values calculated from explant segments are generally four times the average value of wound ETH produced by intact explants.

Standard Cotton Abscission Bioassay. It seemed possible that the standard cotton abscission bioassay, conducted in loosely covered Petri dishes, could be influenced by accumulated ETH, since it has been demonstrated that ETH concentrations capable of accelerating abscission can accumulate within closed containers (17). Figure 4 duplicates, under conditions where ETH accumulation is not a factor, the earlier IAA, ABA, and GA_3 dose response curves obtained with cotton explants (6). Thus, ETH accumulation within the Petri dishes has evidently not affected the standard cotton abscission bioassay.

While CO_2 was not tested for competitive inhibition with ETH in these experiments, it is unlikely that abscission responses to ETH were obscured by external accumulations of CO_2. Cotton explant abscission was inhibited only 13% in the presence of 1% CO_2 (4), and 0.45% CO_2 was the maximum concentration encountered in these experiments. However, small internal compartments could easily build up inhibitory levels of CO_2, and an internal ETH-CO_2 balance could be a factor in experiments of this kind.

Our results do not exclude the possibility that endogenous ETH may act by influencing the auxin levels in abscising tissues (8, 10, 25). Obviously, if endogenous ETH acts to reduce the auxin transport capacity of the tissue after the auxin source has been removed, abscission in the presence and absence of MP will be identical and endogenous ETH peaks may be unaccompanied by abscission.

However, abscission in these explants can be accelerated by external ETH treatments (5, present paper), and our data indicate that rates of ETH evolution commonly greater than 3 nl/g·hr are characteristic of the abscission zone in explants undergoing the first intermediate peak of ETH evolution. Why this peak in ETH evolution is only occasionally accompanied by abscission is not readily apparent, unless the explants are prevented from responding to this endogenously produced ETH by an internal control mechanism.

The existence of an ETH-CO_2 balance within the tissues is one possibility. The first intermediate ETH peak is invariably accom-

panied by an increase in the rate of CO_2 evolution (Fig. 3), and therefore the internal ETH-CO_2 balance may not be upset. In this case, abscission might not be affected. However, addition of external ETH could accelerate abscission by upsetting the internal balance.

The existence of different sites of ETH action in distinct subcellular compartments is another possibility. Substantial evidence, indicating different concentration dependencies for ETH effects within the same tissue, has recently been discussed (15), and it has been suggested that ETH may have more than one role in abscission (3, 6, 10).

Abscission does occur in many widely divergent species, and in the examination of any such process, the simplifying assumption that one basic biochemical explanation will emerge as the underlying foundation of the process in all species usually must be made as a matter of convenience if not preference. Hydrolytic enzyme activity is undeniably central to the abscission process, but in the search for a single theory to explain abscission, too many species-specific differences leading to the production of the essential hydrolytic enzymes may have been overlooked. It is recognized that different varieties of a single species (including *Phaseolus vulgaris*) may differ in their abscission responses to ETH (2), and the abscission process in different species may possess even more significant differences. Evidence that this is so with cotton and beans may be found not just in suggestions of different patterns of endogenous ETH evolution, but also in the state of morphological development of the abscission zone in the different petioles at the time of explant excision.

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