Kinetics of Abscission in the Bean Petiole Explant[1]

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Received October 11, 1968.

Abstract. **The progress of bean petiole abscission has been followed using quantitative measurements of the mechanical force required to break explants at the separation zone. It is found that the shortest time for a measurable effect of ethylene (1 ppm) in stimulating the development of frangibility is about 1 hr. Removal of the ethylene is followed by a return to the endogenous rate of weakening, the slower rate being established in 1 hr. Application of the inhibitor cycloheximide leads to a cessation of abscission development within one-half hr. As in the stimulations of certain plant processes with auxins, gibberellins, and cytokinins, ethylene stimulation of abscission requires the continuous presence of the regulator.**

Quantitative effects of various chemicals on abscission were first made possible through the use of petiole explants—excised sections containing the abscission zone (6). The conventional explant technique permits measurements of the time required for completion of the abscission processes. We have developed a modification of the explant technique which permits measurements of the time course of abscission development before the visible completion of separation, and employed it in studying the kinetics of ethylene regulation of abscission.

Measurements of the breaking force needed to rupture the abscission zone were first employed by Morre (13), who placed explants in a stress gauge and measured their tensile strength. We have employed a rougher and simpler method (10), using living explants pressed against a pan balance to measure the force needed to bring about breakage at the abscission zone.

In the present study, we are interested in learning about the time required for the onset of ethylene stimulation of abscission processes, and likewise the time over which the stimulus persists after removal of the ethylene.

Materials and Methods

About 250 bean seeds (*Phaseolus vulgaris* L. var. Red Kidney) were planted in Vermiculite in wooden flats (14 × 20 × 4 inches). A fungicide having mercury as the active ingredient was used to control fungus growth. Three to 4 days after planting, 2 teaspoonsful of a 25-10-10 garden fertilizer were spread over the flat. The plants were grown in a growth chamber (2000 ft-c, 16 hr daily), at a temperature of close to 22°. The plants were used at 13 to 17 days after planting, when the primary leaf blade had reached its maximum expansion. About 100 to 150 uniform plants were obtained from a flat.

The leaf blade was cut off with a razor at the juncture of the pulvinus and the blade, and the petiole was cut 6 mm from the abscission zone, to provide approximately a 10 mm explant. These explants were placed basal end down in a Petri dish of 1 % agar, 20 or 30 explants per dish. The open dishes were arranged in transparent plastic boxes in 50 ft-c of light with water-saturated air continually passing through, maintaining a uniform gas medium for all treatments.

Ethylene treatment was effected by placing each dish of explants in a 250 ml beaker, covering with a sheet of parafilm (American Can Company), and injecting ethylene with a plastic syringe to provide 1 ppm concentration. A second sheet of parafilm was then fitted over the first. Ethylene was removed from the explants by placing the dishes in a dessicator, and evacuating twice to 480 mm mercury for 3 min before refilling with air. A comparison of 1, 2, 3, and 5 such evacuations indicated that the rate of abscission was markedly slowed by the first, and not appreciably altered by further evacuations. It was concluded that 2 evacuations were adequate for removal of ethylene from the explants. The evacuation treatment itself did not alter the ability of the explant to proceed with normal abscission; reapplication of ethylene 3 hr after evacuation reinstated the normal ethylene response (that is, the slope of the abscission break force curve such as that in figure 2 returned to the usual ethylene value).

Cycloheximide poisoning was carried out following the method of Abeles and Holm (3), injecting 1 μl of solution directly into the abscission zone by inserting the needle of a 10 μl syringe through the petiole pith to apply 50 mμg per explant.

[1] Journal Paper No. 3493, Purdue University Agricultural Experiment Station, Lafayette, Indiana. Research supported by contract with Department of the Army, Fort Detrick, Frederick, Maryland.

Measurement of the abscission breaking force was done by a method described briefly in a previous paper (10). Each explant is held at the pulvinus end with a pair of blunt forceps. With the adaxial side down, the petiole end is pressed against a platform balance at about a 30 degree angle. The force is applied with increasing weights at 2 to 5 gram intervals. The force at which rupture of the abscission zone occurred was recorded as the abscission breaking force. In most cases 2 groups of 20 explants were measured for each time interval for each treatment. Data are plotted with standard errors.

Our experience has shown that if the explant can withstand forces above 50 grams, it will bend rather than break at the abscission zone. The breaking-force measurement is therefore not effective for explants that are less than 24 hr from cutting or for other conditions in which early stages of abscission are prevented. This test is also ineffective with explants taken from senescing bean leaves where the petiole has become extensively softened. An important factor is the selection of explants of uniform diameter. For any given age of explant the abscission breaking was found to be correlated with the petiole diameter (see also 13).

Results

The progressive decline of the abscission breaking force for untreated explants is shown in figure 1. The first measureable breaking force (below 50 grams) is generally observed about 20 hr after cutting the explant. From 20 to 30 hr the breaking force declines to about 20 grams. From 30 to 90 hr the breaking force falls much more slowly. At about

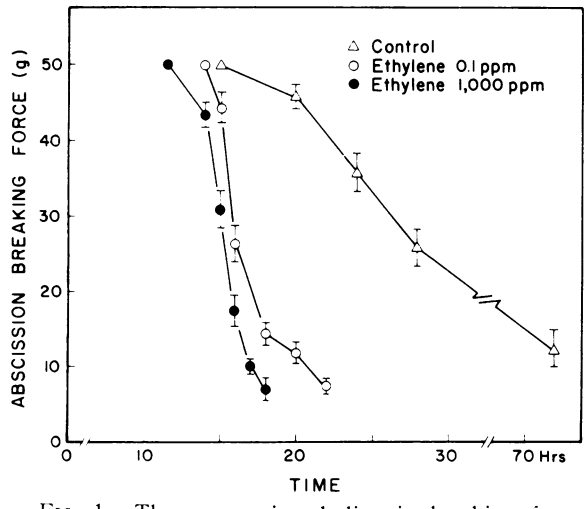

FIG. 1. The progressive decline in breaking force at the abscission zone with time after excision. The curve for control plants is compared with progress curves for bean petiole explants under 0.1 and 1000 ppm ethylene. Vertical lines indicate standard errors.

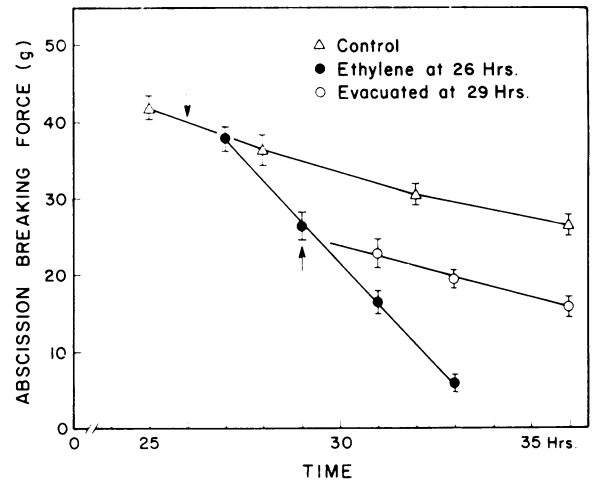

FIG. 2. Changes in decline of the abscission breaking force following the introduction of 1 ppm ethylene (at the twenty-sixth hr), and removal of ethylene by evacuation (at the twenty-ninth hr after excision). Arrows indicate times of ethylene addition or evacuation.

100 hr approximately 50 % of the explants show visible separation of the pulvinus from the petiole.

Application of ethylene results in a much more precipitous decline in breaking force, as shown for 2 concentrations of continually applied ethylene in figure 1. This type of dynamic progress curve allows a study of the kinetics of abscission development.

The effects of ethylene exposure (1 ppm) were examined. Dishes of explants were placed in ethylene atmospheres at 26 hr after cutting, at which time ethylene responsiveness is known to be strong (5, 10). The progress of abscission breaking force is accelerated, as shown in figure 2. When the progress curve for ethylene treated explants is drawn, it appears to depart from the control curve at about 1 hr after the start of the ethylene treatment, indicating that a period of 1 hr is needed for the translation of the ethylene signal into an actual change in abscission breaking force. Evacuation of the ethylene at 29 hr after cutting reinstated the rate of softening to that observed for control explants, as seen in figure 2. By extrapolation, the progress curve for evacuated sections is observed to depart from the ethylene curve at about one-half hr after evacuation, indicating that the ethylene stimulation of softening is lost at about one-half hr after ethylene removal.

In another type of experiment, ethylene was supplied continuously from the time of cutting of the explants until the fourteenth hr, at which time the ethylene was evacuated from a series of dishes. Progress of the abscission breaking force was measured over the next 10 hr (fig 3), during which a lessened rate of softening was observed. In this case the approximate intercept of the curves for evacuated and unevacuated explants was at about 2 hr following evacuation. In another group of dishes the ethylene was evacuated at 16 hr after

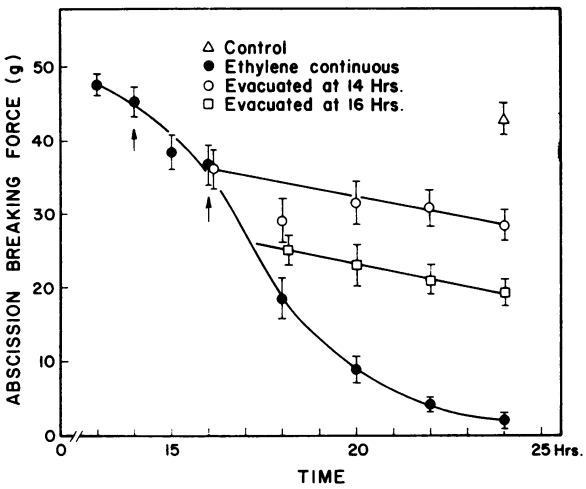

FIG. 3. The decline of abscission breaking force when explants are continuously under ethylene (1 ppm), or when the ethylene is removed by evacuation at the fourteenth or the sixteenth hr after excision.

cutting, and the lessened rate of softening was apparently instated at about 1 hr following evacuation.

The effectiveness of cycloheximide in poisoning the ethylene stimulation of abscission has been reported by Abeles and Holm (3) and by Morre (13). Experiments were carried out to compare the kinetics of cycloheximide poisoning with the effects of removal of the ethylene stimulus. For this purpose ethylene was introduced over a series of dishes of explants at 18 hr after cutting, and the progress of softening was followed as shown in figure 4. A group of dishes was evacuated to remove the ethylene

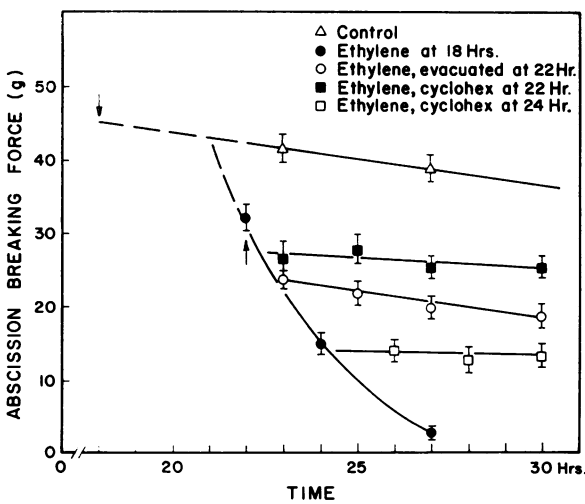

FIG. 4. Inhibition of the progressive decline in abscission breaking force by cycloheximide. Ethylene exposure (1 ppm) began at the eighteenth hr after excision, and cycloheximide injection was performed at the twenty-second or twenty-fourth hr. The poison effect is compared with the effect of ethylene removal through evacuation at the twenty-second hr.

at 22 hr after cutting, and another group was injected with cycloheximide and then returned to the ethylene chamber. The progress curves of the 2 treatments are shown in figure 4. It can be seen that the cycloheximide treatment served to more severely check the progress of abscission development. Judging from the approximate intercepts, the cycloheximide effect was instated in about one-half hr in comparison to 1 hr for the evacuation treatment. A similar cycloheximide treatment was made to another group of explants at 24 hr after cutting, and again the progress of abscission was severely checked, beginning at about one-half hr after injection.

Discussion

Using a method which can follow the progressive development of abscission, we have examined some kinetic features of ethylene stimulations and the persistence of such stimulations after the removal of the ethylene. Measurements of the rate of change of the breaking force after ethylene exposure indicate that 1 to 2 and one-half hrs are needed for the translation of an ethylene signal into a perceptible decline in the abscission breaking force. The experiment was repeated 5 times, and an average time of 1.3 hr for the commencement of ethylene response was obtained. Estimation of the persistence of the ethylene stimulus after removal of the gas by evacuation was repeated 9 times, giving an average time of 1.0 hr for the disappearance of the ethylene acceleration. Four experiments with cycloheximide gave an average of 0.25 hr for the onset of the inhibition of softening.

As mentioned earlier, ethylene treatment brings about a precipitous fall in the abscission breaking force, and evacuation of the ethylene restores the rate to that of the explants not treated with ethylene. From figure 4 it appears that cycloheximide not only inhibited the ethylene action, but also inhibited the endogenous softening.

It has been generally assumed that the endogenous rate of abscission development is determined by the endogenous ethylene production by the explant tissue (1, 2, 5, 8). We were surprised, therefore, to find that evacuation of control explants did not eliminate the endogenous progress of abscission. It is possible that the evacuation treatment did not reduce the internal ethylene concentration over a time period long enough to stop the progress of abscission.

With the development of abscission in bean explants, there is a decline in the protein content of the pulvinus (4, 17). The time curve for the protein degradation is strikingly similar to that of the breaking force as shown in figure 1—that is, there is a relatively rapid decline in protein from 24 to 48 hr, followed by a markedly slower decline (data not shown). Like Abeles *et al.* (4), we have not observed any acceleration of the protein degradation by ethylene treatments.

Evidence has been developed suggesting that the ethylene regulation of abscission takes place through an alteration of the RNA directed protein synthesis, with the consequent production of wall hydrolyzing enzymes in the region of the abscission zone (3, 12, 13, 14). The finding reported here that ethylene causes a new rate of wall softening in about 1 hr after exposure may be compatible with the nucleic acid concept of ethylene control, judging by the timing of some other nucleic acid controlled systems (*cf.* 15). The relatively short persistence of the ethylene stimulus (about 1 hr) after ethylene removal seems difficult to reconcile with the concept of an ethylene alteration of enzyme synthesis. Once the enzymes responsible for cell wall degradation have been formed, it is difficult to see how removal of the ethylene can result in loss of their action within an hr, when the half-life of the most transient of plant enzymes is only rarely less than 20 hr. Rapid termination of the ethylene effect by cycloheximide is likewise difficult to accommodate to the nucleic acid regulation theory.

The relatively brief after-effect of ethylene treatment shows a suggestive similarity to the after-effect of each of the other known types of plant growth regulators. Kinetic experiments on the stimulation of cell elongation by auxin have shown that upon the removal of auxin a new lowered growth rate is established in 40 min (16). Experiments on the stimulation of amylase synthesis by gibberellin have shown that withdrawal of the gibberellin results in a decline in amylase synthesis detectable in 7 hr (9). Studies of the induction of bud formation in moss by cytokinins have shown that removal of the cytokinin results in a complete loss of the bud-forming stimulus in 24 hr (7). These various observations indicate that growth substances generally have effects which are relatively non-persistent. Heslop-Harrison (11) has used the simile of an electric relay in describing the action of a flowering hormone: this type of concept of regulator action may be applicable to plant growth regulators generally, in contrast to the concept of a switching action in which the regulation is a simple turning-on of different genetic signals. In the case of the plant growth substances, once the chemical signal is withdrawn, the immediate alteration which it has produced may be likewise withdrawn.

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