IN VITRO TESTS OF ABSCISSION AGENTS[1]

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(WITH EIGHT FIGURES)

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Premature fruit fall and leaf fall have for centuries been a source of loss to growers, but in the last ten years methods have been developed which permit the control of these phenomena under many circumstances. It is now possible either to accelerate or to retard the rate of abscission of leaves and fruits by the application of various chemicals.

Detailed analyses of anatomy in relation to abscission have been made since the middle of the eighteenth century but very few investigations of the physiology of abscission have been attempted. In 1936 LA RUE (**4**) demonstrated that the abscission of debladed petioles could be delayed by application of growth substances. Since then a number of experiments have been reported on the regulation of abscission (**1, 2, 3, 4, 5, 7, 10, 11, 12**) but they include relatively little research on the fundamental physiology of abscission. It was the objective of this investigation to study the physiology of abscission with experiments *in vitro*.

Materials and methods

The experiments reported below employed the fully mature leaves of *Citrus sinensis* var. Valencia orange. The leaves of this variety are unifoliolate with two active abscission zones: (1) the laminar abscission zone, between the lamina (blade) and the petiole, and (2) the nodal abscission zone, between the petiole and stem. The laminar abscission zone is characterized externally by an encircling groove.

In all but one of the experiments the laminar abscission zone was excised and was used in this investigation. This zone was contained in a portion of tissue consisting of 6 mm. of the petiole and 10 mm. of the mid rib. The remaining blade tissue and the petiolar wings, if present, were removed. This excised portion of the leaf is subsequently referred to as the "explant." Such explants were mounted on agar in Petri dishes, the relation of the explant to the leaf and the method of mounting being shown in figure 1. The details of this method have already been reported (**1**).

Chemical agents were applied to the explants in either of two ways: (1) by dissolving the agent in the agar upon which the explants rested; or (2) by immersing the explants for known times in solutions of the chemical and then placing them on the agar.

Under field conditions the movement of leaves by air currents and other

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agents places significant stress upon the abscission layer. Since this stress was normally lacking from the *in vitro* experiments of this study it was considered advisable to replace the stress, at least in part. This was done by raising each unabscissed explant at the close of the experimental period, with the tips of a pair of forceps under the petiolar portion. On the graphs the data shown to the left of the broken vertical line are the daily percentages of unaided abscission while the data to the right of the line include that obtained after the application of the stress by lifting on the final day of

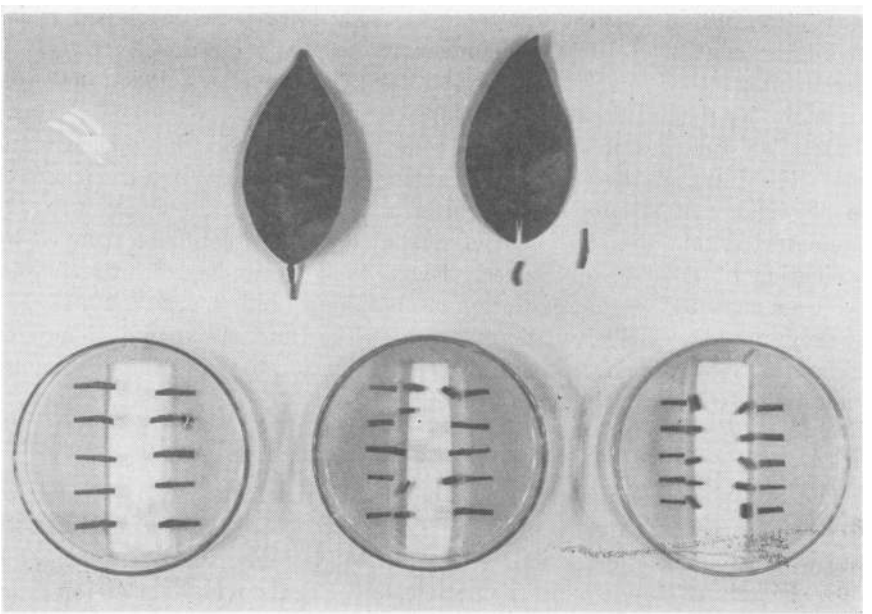

FIG. 1. Photograph showing a normal leaf, a leaf with an explant removed and three agar dishes with explants. The dishes show initial, intermediate and final stages in abscission.

the experiment. The data represented in the graphs are the percentages of abscission obtained from 20 explants per treatment.

Experiments and results

EFFECT OF ETHYLENE

It is common knowledge that ethylene can increase the rate of abscission of leaves from branches. To test the effects of ethylene on explants, Petri dishes with explants were sealed under bell jars of approximately six liters capacity. The atmospheres in the bell jars were modified in the following ways: A. Control (ordinary air). B. One ml. of water saturated with ethylene introduced into the bell jar at the start of the experiment. C. 10 ml. of water saturated with ethylene introduced at the start of the experiment. D. 10 ml. of water saturated with ethylene introduced 48 hours after the start of the experiment. (The ethylene solution employed was

saturated at 25° C.) Figure 2 presents the results. They show that ethylene increased the rate of abscission in excised tissues.

Effect of toxic substances

Pfeiffer (8) reported that a 1% solution of chloral hydrate promoted abscission. Defoliants are available which if applied under the proper conditions will bring about abscission. Chloral hydrate and cyanamid (Experimental Defoliant X-5 of the American Cyanamid Company) were applied separately to explants in order to test their effect on explant abscission. One per cent. chloral hydrate was dissolved in the agar which supported the explants. The cyanamid was dissolved in distilled water in concentrations

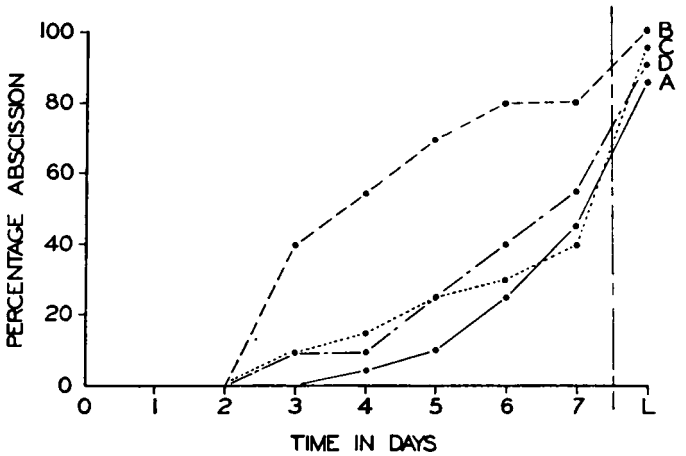

Fig. 2. Effect of ethylene. The explants were placed in bell jars (six liter capacity) and the atmosphere modified in the following ways: A. Control (ordinary air). B. One ml. of water saturated with ethylene added at the start of the experiment. C. 10 ml. of water saturated with ethylene introduced at the start of the experiment. D. 10 ml. of water saturated with ethylene added forty-eight hours after the start of the experiment.

varying from .0001 to 3.% and the explants were dipped momentarily in these solutions. The chloral hydrate and all concentrations of cyanamid inhibited abscission. The rate of inhibition of the various concentrations of cyanamid was directly proportional to the concentration of cyanamid.

Effect of immersion

In the preliminary experiments explants were placed in aqueous solutions. Under these conditions no abscission occured, even after many weeks. After adopting agar as a supporting medium it became of interest to learn more of the effect of immersion on abscission. An experiment was conducted in which explants were immersed in distilled water for the following periods: A. None (control). B. One minute. C. 30 minutes. D. Four hours. E. 24 hours. The explants were then mounted on agar and examined daily. The results are presented in figure 3. They show that immersion inhibits abscission and that the degree of inhibition is proportional

to the duration of the immersion period. These results must be borne in mind where explants have been treated by immersion in aqueous solutions. The immersion tests were repeated a second time in order to determine the reproducibility of results. There was a slight variation between the two tests as could be expected but on the whole the results were comparable.

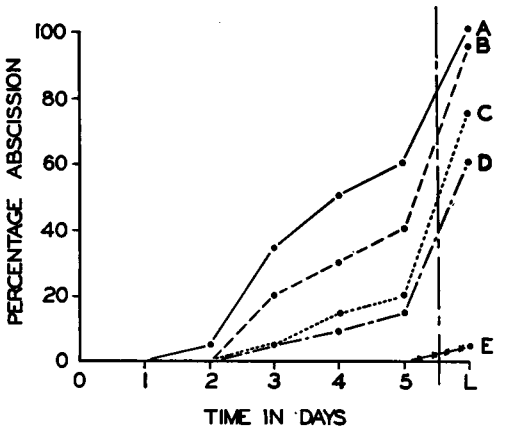

FIG. 3. Effect of immersion in distilled water. Immersion times: A. None (control). B. One minute. C. 30 minutes. D. four hours. E. 24 hours.

Many of the following experiments were conducted before the tests on immersion. However, as can be seen from figure 3 even a short immersion time is effective in reducing the abscission rate. Therefore, whenever the tests entail immersion in aqueous solutions and a water-dipped control is not included Line B of figure 3 should be consulted to determine what fraction of the effect is due to the immersion in water and what is due to the solute. These tests are effect of: Sucrose, 2,4-dichlorophenoxyacetic acid and indoleacetic acid.

EFFECT OF BLADE TISSUE

To determine how much of the blade must be present on the explant to produce an inhibition of abscission, an experiment was performed in which portions of the blade were excised with the explant. Essentially, it was conducted with explants to which part or all of the blade tissue was still attached. The portions of the blade remaining attached in the various treatments were: A. None (usual explant). B. The basal 10% of the blade. C. The basal 25% of the blade. D. The basal 50% of the blade. E. The entire blade. The material was placed on agar in Petri dishes and examined daily. The results are presented in figure 4. They show a high correlation between the percentages of inhibition of abscission and the amount of the blade tissue present.

EFFECT OF SUCROSE

It has been reported that flower abscission in tomatoes can be reduced by the use of sprays containing sucrose (**13**). To determine whether simi-

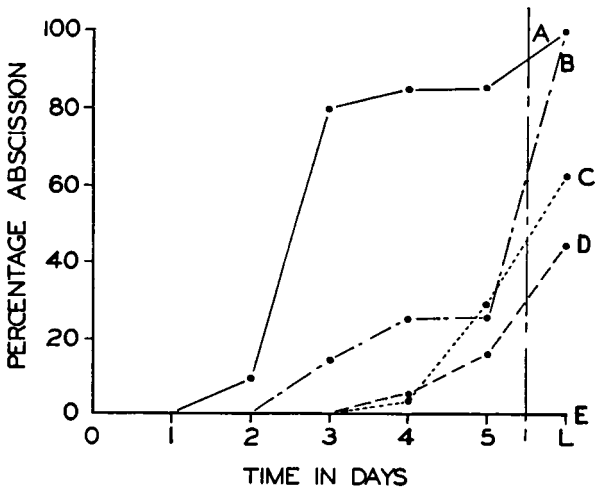

FIG. 4. Effect of blade tissue. Amount of blade present: A. None (usual explant). B. Basal 10% of the blade. C. Basal 25% of the blade. D. Basal 50% of the blade. E. The entire blade.

lar results could be obtained with citrus leaves an experiment was performed in which explants were immersed momentarily in sucrose solutions of the following concentrations: A. None (no immersion control). B. 20%. C. 10%. D. 3%. E. 1%. F. 0.3%. The results are presented in figure 5. They show an inhibition of abscission in explants treated with

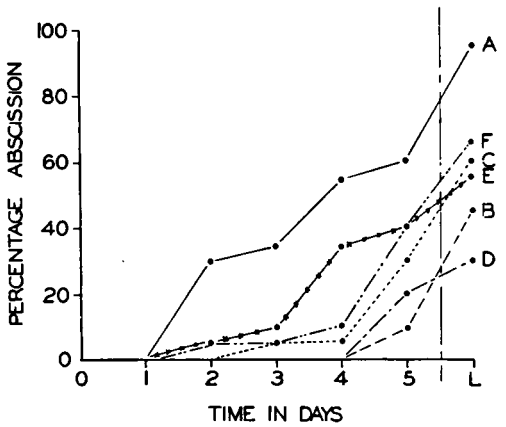

FIG. 5. Effect of dipping in sucrose solutions: A. No dip (control). B. 20% sucrose. C. 10% sucrose. D. 3% sucrose. E. 1% sucrose. F. 0.3% sucrose.

sucrose but there seems to be no particular correlation between the concentration of sucrose employed and the amount of inhibition of abscission.

EFFECT OF 2,4-D

Stewart and his collaborators have worked extensively with 2,4-D (2,4-dichlorophenoxyacetic acid) as an agent for the control of leaf fall and pre-

harvest drop of citrus (**10, 11, 12**). An experiment was performed in which the explants were immersed momentarily in the following concentrations of 2,4-D (the water soluble ammonium salt was used): A. None (no immersion control). B. 1,000 mg./liter. C. 10 mg./liter. D. 0.1 mg./liter. E. 0.001 mg./liter. F. 0.00001 mg./liter. The results are presented in figure 6. A comparison of these data with those obtained following a

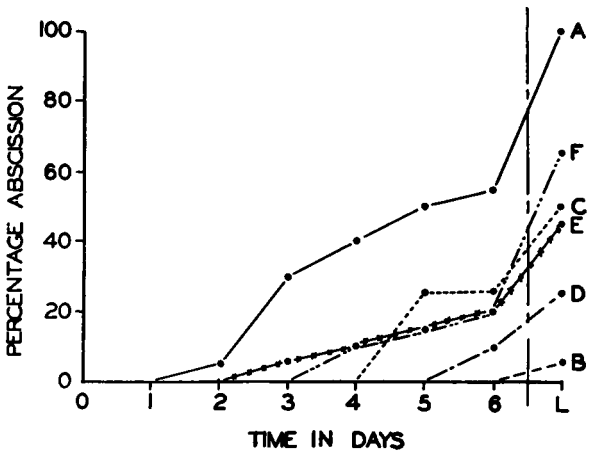

FIG. 6. Effect of 2,4-D. Concentrations of the initial dip: A. No dip (control). B. 1,000 mg./liter. C. 10.0 mg./liter. D. 0.1 mg./liter. E. 0.001 mg./liter. F. 0.00001 mg./liter.

short immersion in distilled water (figure 3, Line B) shows that even the lowest concentration of 2,4-D had some inhibitory effect on abscission.

EFFECT OF IAA

Previous investigators have shown that IAA (indoleacetic acid) inhibits abscission under field and greenhouse conditions (**3, 4, 5, 7**). An experiment similar to the preceding one was performed to determine the effect of IAA on abscission in explants. The concentrations in which the explants were immersed immediately prior to being placed on the agar were: A. None (no immersion control). B. Aqueous solution saturated at room temperature. C. 10 mg./liter. D. 0.1 mg./liter. E. 0.001 mg./liter. F. 0.00001 mg./liter. The results are presented in figure 7. Abscission was inhibited at all concentrations.

ABNORMAL GROWTH RESPONSES

Early in this investigation it was noted that there was an unusual enlargement of the parenchyma proximal to the abscission groove after certain treatments. The enlargement was mainly internal since the epidermis was frequently split in the process. The phenomenon was observed with considerable frequency, and while no quantitative measurements were made, a brief description may be of interest.

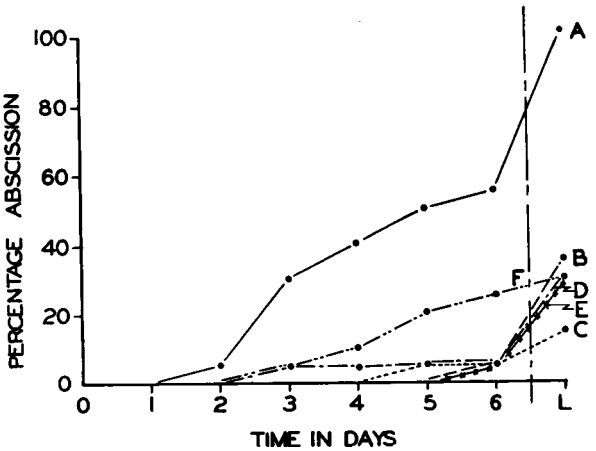

FIG. 7. Effect of IAA. Concentrations of initial dip: A. No dip (control). B. Saturated aqueous solution. C. 10 mg./liter. D. 0.1 mg./liter. E. 0.001 mg./liter. F. 0.00001 mg./liter.

Table I presents a list of conditions which produced this growth response. There is a close correlation between abnormal enlargement and inhibition of abscission. The only exceptions to this correlation were found in the higher concentrations of sucrose, where, it appears very likely, the osmotic factor inhibited cellular activity generally, as well as abscission specifically.

TABLE I

LIST OF EXPLANT TREATMENTS AND PERCENTAGES OF EXPLANTS SHOWING ABNORMAL GROWTH PROXIMAL TO THE ABSCISSION GROOVE

TREATMENT	PERCENTAGE SHOWING GROWTH	TREATMENT	PERCENTAGE SHOWING GROWTH
Immersion in water		Dipped in 2,4-D Solution	
24 hours	65	.00001 mg/L	25
4 hours	25	.001 mg/L	25
½ hour	25	.1 mg/L	25
0 hours	0	10. mg/L	30
		1000. mg/L	25
		Control (No dip)	10
Dipped in Indoleacetic acid solution		Effect of blade	
.00001 mg/L	15	Entire blade	100
.001 mg/L	10	½ blade	55
.1 mg/L	5	¼ blade	?
10. mg/L	0	1/10 blade	10
100. mg/L	10		
1000. mg/L	10	Dipped in sugar solution	
		20% sucrose	0
		10% sucrose	0
		3% sucrose	30
		1% sucrose	15
		.3% sucrose	5

Discussion

It has been observed that application of growth substances, sugars, etc. is followed by localized growth responses in explants. This observation raises the question as to why such overgrowths are so localized. A possible answer, based on the anatomical studies by SCOTT, *et al.* (**9**) is suggested here. These authors have shown the presence of suberized cells in the vicinity of the abscission zones of citrus. Figure 8 is a drawing showing the

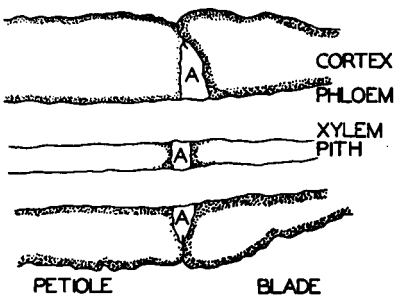

FIG. 8. Diagram of a median longitudinal section thru the Laminar abscission zone. Stipling indicates the extent and degree of tissue suberization. (A) indicates the abscission zone.

location of these suberized cells (stippled area) and their relationship to the abscission zone (A).

The suberized cells are modified parenchyma cells in the pith and in that part of the cortex in and near the bundle sheath, forming a layer two or three cells thick distal to the abscission zone. Proximal to the abscission zone this layer is not quite as thick and the deposit of suberin in the cortical cells adjacent to the abscission zone is light and discontinuous.

This deposition of suberin may be closely related to the restriction of the effects of normal and applied growth substances to the abscission zone. If the applied substances are carried in the normal translocation channel (phloem) there is little chance of their diffusing in large amounts into the surrounding tissues of the blade or petiole, this being prevented by the suberization of the bundle sheath and adjacent tissues in this region. Due to the absence of the bundle sheath at the abscission zone there is no barrier to lateral translocation. Since the suberin deposit of the parenchyma cells proximal, but adjacent, to the abscission zone is discontinuous the diffusing substances can pass into the cortical tissue of the petiole adjacent to the abscission zone. It is obvious that the suberized layer is not completely impermeable to the lateral transport of materials since the deposit is present while the leaf is still in vigorous vegetative condition. It has been suggested by Scott (personal communication) that the transport through the suberized cell walls is through the plasmodesmata. The suberization then would not completely stop the lateral translocation. However, the lateral translocation at the unsuberized abscission zone would be greater than elsewhere.

Many substances, such as sulphur, ammonium thiocyanate, zinc chloride,

chloral hydrate, etc., have been shown to promote abscission in the field. Since these toxic substances differ widely in their chemical properties it is probable that their primary physiological effects are quite different. How does their common abscission-accelerating action come about? It has been reported that the application of an overdose of commercial defoliant to cotton plants results in death of the whole plant, and consequently in no foliar abscission. This indicates that the substances have a toxic action, inactivating the cells of the leaf and thereby producing the same results as artificial deblading. In this investigation the treatment of explants with a commercial defoliant or chloral hydrate inhibited abscission. This inhibition was interpreted as due to the result of death or injury of the cells in the abscission zone. If all of these substances bring about death or injury of the blade tissue, the resultant removal of the inhibitory action of these tissues could account for their effect as promoters of abscission in the field.

The role of ethylene as an accelerator of abscission may be due to its destroying action on auxin. MICHENER (**6**) has suggested that this destruction may possibly occur in intact pea plants. From the present and other investigations, it appears that abscission occurs when a source of growth-promoting substance is not available. Therefore, the possibility that the effect of ethylene in increasing the rate of abscission is one of destruction of growth substances seems reasonable.

It was found in this investigation that abscission is lower than normal in explants immersed in distilled water and then placed on agar plates. Among several possible explanations of this effect are:

First, some substance necessary to abscission may be leached from the explant by the water.

Second, the extra water taken up may release additional abscission inhibitor present in the tissues in a bound state.

Third, the presence of the extra water taken up by the explant during immersion may prevent some metabolic reaction or set of reactions which are necessary for the initiation of the process of abscission.

In the present state of these investigations it is impossible to say which of the above concepts are factors in the delay following immersion. It is hoped that further experiments will clarify this point.

The abscission-inhibiting effect of the growth substances 2,4-D and indoleacetic acid on explants has been exhibited in these tests. However, an analysis of the data (figs. 6 and 7) will show that there is no significant difference between the various concentrations employed.

Summary

1. Abscission in the excised laminar abscission layer of the Valencia orange was studied.

2. Experiments were conducted with explants to determine the effects of: various fractions of blade tissue, immersion in distilled water, sucrose, chloral hydrate, indoleacetic acid, 2,4-dichlorophenoxyacetic acid, ethylene

and cyanamid. Ethylene accelerated abscission, all other treatments inhibited abscission.

3. Many of the treatments brought about enlargement of the tissues proximal to the abscission groove. It is suggested that this may have been the result of interference with free movement of materials by the heavily suberized cells which form a layer surrounding the vascular tissues in the inner cortex and another just distal to the abscission zone in the cortical tissues.

4. This investigation has demonstrated the value of studying abscission in the laboratory using excised tissues of relatively small volume. This method is convenient and the results are comparable to those obtained in the field.

The writer wishes to thank Dr. F. T. Addicott, Dr. F. M. Scott and Dr. R. S. Lynch for their guidance and assistance during this investigation. Thanks are also extended to Dr. S. H. Cameron for the citrus leaves and to E. I. du Pont de Nemours and Company for the 2,4-dichlorophenoxyacetic acid.

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