Environmental Regulation of Experimental Leaflet Abscission[1]

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Leaf abscission is a terminal developmental sequence initiated by the onset of senescence (excision and deblading in experiments with explants) and terminated by separation of the leaf from the main axis of the stem (3, 4, 8, 9, 13). Studies of abscission are greatly facilitated

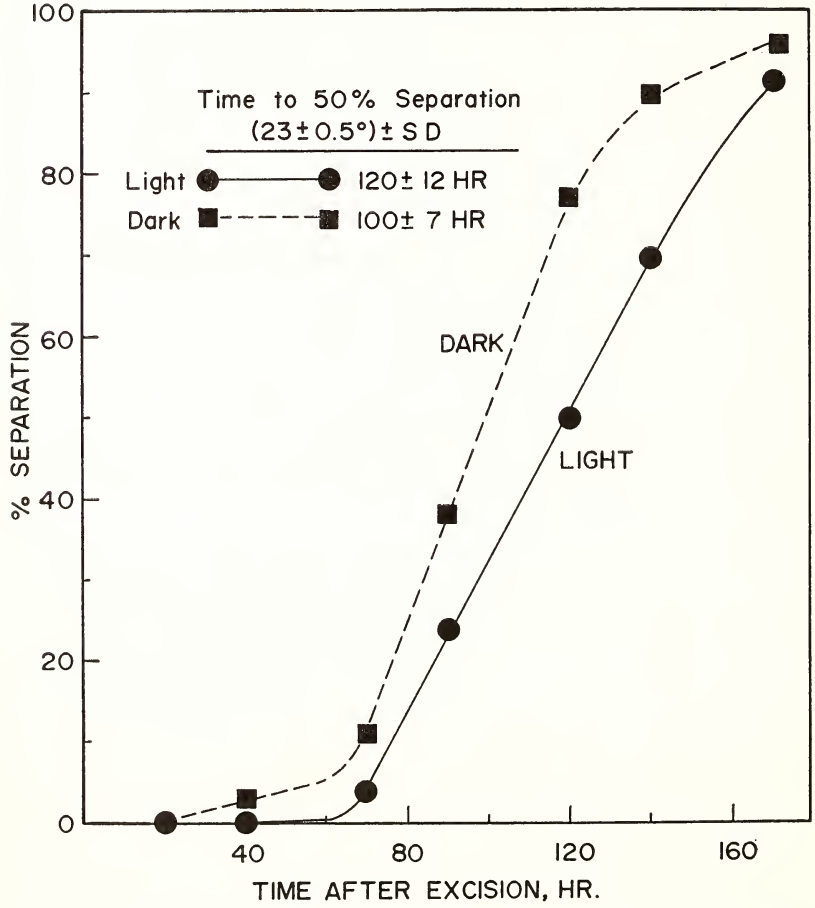

Figure 1. Transition profiles of separation layer formation at 23° comparing explants in light and darkness. Explants were prepared from plants grown in the greenhouse at high light and long photoperiod.

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by use of explants (excised abscission zones and adjacent tissues) which respond uniformly and rapidly but exhibit widely varying rates of abscission depending upon treatment conditions.

The behavior of an explant population is described by a transition profile, a simple sigmoid defined by two parameters: the position of the midpoint (time to 50% separation) and the steepness of the slope at this point (or by an equivalent parameter, the transition width). The time at which abscission reaches the midpoint of the transition is a measure of the rapidity at which separation layers are formed. The transition width is a measure of the heterogeneity of the population. A very broad transition is indicative of a heterogeneous population whereas a sharp transition is indicative of a synchronous population.

With bean, natural abscission of primary unifoliate leaves occurs between 30 and 40 days after planting with plants grown under greenhouse conditions (10). Explants of primary leaves reach 50% separation at about 100 hours following the procedures of Leopold and coworkers (5, 10, 13). It is desirable to utilize explants with very sharp transition profiles (synchronous response). For many types of abscission studies, it would be equally desirable to utilize explants with 50% separation within a day after cutting rather than the usual 100 hours. The rapid abscission rate would permit initiation and termination of kinetic studies during a single working day instead of over a period of several days or weeks.

TABLE 1. *Abscission of Bean Explants Harvested From Plants Grown Under Varying Conditions of Temperature, Light Intensity and Photoperiod. Abscission of explants was determined at three temperatures under continuous, low-intensity light (100 ft-c) or darkness.*

Conditions for Growing Plants Prior to Harvesting Explants			Time to 50% Separation of Explants (Hr ± SD) at Conditions Indicated			
Temperature	Light Intensity	Photoperiod	29° Light	24° Light	22° Light	22° Dark
22.5°	4,500 to 4,800 ft-c	8 Hr	26±4	58±8	73±10	110±20
		12 Hr	30±1	69±11	104±12	90±12
		16 Hr	32±4	69±11	105±6	100±10
	700 to 1,000 ft-c	8 Hr	21±1	40±6	55±10	101±12
		12 Hr	23±3	52±8	60±9	150±10
		16 Hr	28±5	64±10	73±20	133±20
		20 Hr	29±2	50±13	73±20	127±11
30°	700 to 1,000 ft-c	8 Hr	24±3	40±7	42±1	154±19
		12 Hr	27±3	34±6	44±5	118±6
		16 Hr	26±3	40±1	60±20	155±13
		20 Hr	27±3	35±6	68±20	103±20

Materials and Methods

Bean plants (*Phaseolus vulgaris* L., var Red Kidney) were grown under greenhouse or controlled environmental conditions. Unless specified otherwise, explants were cut from unifoliate leaves of 10 to 20 day old plants grown in metal flats containing potting soil overlayed with sand. The flats were filled to a depth of 5 cm with the soil followed by a layer of seeds which were then covered to a depth of about 1 cm with sand. Greenhouse-grown plants were supplemented with low intensity fluorescent and incandescent light to provide a 16 hr. photoperiod.

The 1 to 2 cm long debladed explants were cut to include at least 5 mm of tissue on either side of the distal abscission zone at the pulvinus-

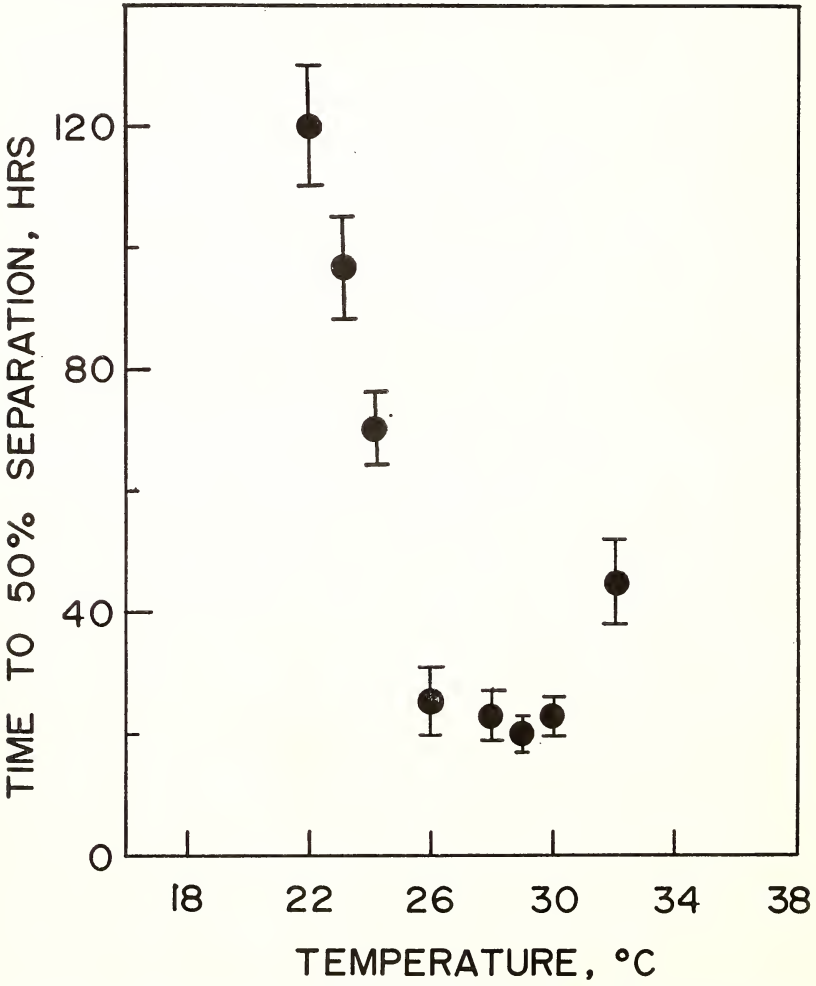

Figure 2. Acceleration of explant abscission in continuous light as a function of temperature. Explants prepared from greenhouse-grown plants. Bars show standard deviations.

petiole juncture (10). Unless specified otherwise, approximately 20 explants were inserted vertically (usually with the petiole end down) to a depth of 4 mm in 1% agar in 1 X 5 cm petri dishes. To facilitate inserting the pulvinus end of the explants into agar, an agar concentration of 0.7% was used. Rate of separation of explants was independent of agar concentration over the range 0.7 to 2%. Results are an average of at least 3 experiments.

The time of separation layer formation was estimated from the time when application of pressure to the pulvinus readily brought about separation. Details of individual experiments are given in the text.

Results

Transition profiles for explants prepared from greenhouse-grown bean plants (Fig. 1) showed a broad transition width when assays were conducted at 23°. In low intensity (100 ft-c) light, explants began to abscise between 60 and 70 hours after cutting but did not reach 100% abscission until after 170 hours (50% separation at 120 hours). With greenhouse-grown plants (high light intensity and long photoperiod), abscission was accelerated when explant assays were conducted in

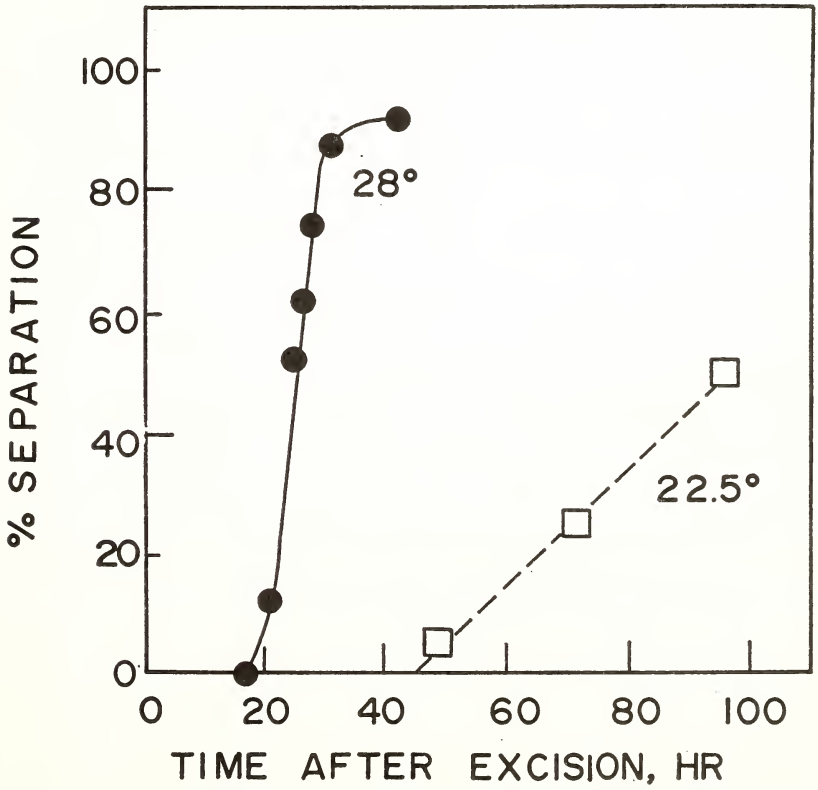

Figure 3. Transition profiles of separation layer formation comparing explant separation in continuous light at 28 and 22.5°.

darkness (50% separation at 100 hours). Except for explants prepared from plants grown under high light intensity and long photoperiod, abscission was generally faster at 22° when explants were incubated in the light (Table 1).

Abscission is extremely sensitive to temperature (Figure 2; Table 1). Abscission was not detected at temperatures below 16° or above 36°. Increasing the temperature from 22° to 26° markedly accelerated abscission (50% separation at 120 hours at 22° vs 50% separation at 24 hours at 26°). The temperature response curve for experimental leaflet abscission exhibited a broad optimum between 26 and 30°, with a decline in rate at 32°. Between 32 and 36°, abscission was further delayed and many of the explants did not abscise. Increasing temperatures decreased the midpoint of the transition profiles (Fig. 2) and at the same time increased the steepness of the slope (decreasing the transition width) as shown in Figure 3 for greenhouse-grown plants (compare 28°, Fig. 3 with 22.5°, Fig. 3 and 23°, Fig. 1). The temperature at which the plants were grown prior to harvesting the explants had much less of an effect on abscission rate than the temperature during the explant bioassay (Table 1).

Effects of light on abscission were less pronounced than the effects of temperature. The most marked effect was the qualitative response to light vs. dark (Fig. 1, Table 1) which varied according to the light intensity and photoperiod at which the plants were grown. With all

TABLE 2. *Abscission of Bean Explants as Influenced by Temperature, Light Intensity and Photoperiod. Plants grown in soil in the greenhouse (16 hr photoperiod).*

Temperature (C°)	Light Intensity (Ft-C)	Photo-period (Hr)	Time to 50% Separation (Hr)
22.5	4,500-4,800	8	88
		12	98
		16	112
	700-1,000	8	68
		12	98
		16	108
		20	112
30	700-1,000	8	44
		12	24
		16	56
		20	24
15	700-1,000	8	>144
		12	>144
		16	>144
		20	>144

plants (except those grown at high light intensity and long photoperiod), incubating explants in low light (100 ft-c) markedly accelerated abscission. Increasing the light intensity from the range 700 to 1,000 ft-c to 4,500 to 4,800 ft-c resulted in no further change in the rate of separation layer formation (Table 2). When explant assays were conducted at 22.5°, separation rate was inversely related to photoperiod with photoperiods of 8, 12, 16 and 20 hours. At 30°, the response to photoperiod was irregular.

TABLE 3. *Acceleration of Abscission in the Light by Inverting the Explants.*

Portion of the Explant Placed in Agar	Time to 50% Separation (Hr ± SD)	
	Dark	Continuous Light
Petiole end	109±9	77±1
Pulvinus end	109±9	46±5

The photoperiod at which the plants were grown affected separation layer formation in explants assayed under continuous light but differences were not marked. Explants prepared from plants grown under low light intensity tended to abscise more rapidly in the light than those from plants grown at high light intensity. This response was independent of photoperiod and assay temperature (Table 1). Most rapid abscission was obtained from bean plants grown at 22.5°, 700 to 1000 ft-c and an 8 hr photoperiod. All differences due to growth conditions of the plants tended to be minimized when explant assays were conducted at a near optimum temperature of 29° as compared with a suboptimal temperature of 22° (Table 1). Explants from plants grown in complete darkness (completely etiolated condition) did not absice when subsequently incubated either in light or darkness.

Time to 50% separation decreased with increasing age of the plant particularly when assays were conducted at 23°. However, at optimum temperature, age of the plant did not appear to be a significant variable (Fig. 4). The most marked response to age was from plants grown at 22.5°, a 12-hour photoperiod and a light intensity of 700 to 1000 ft-c.

TABLE 4. *Influence of Stipules on the Rate of Bean Explant Abscission.*

Number of Stipules Per Explant	Number of Explants Tested	Time to 50% Separation, Hrs
0	200	32
1	100	27
2	80	27

Under these growing conditions, the midpoint of abscission was shortened by about 5 hours comparing 12 day old and 20 day old plants.

With assays conducted in light, acceleration of abscission was achieved by inverting the explants (incubation with the pulvinar end in agar). Inverting the explant was without effect in the dark (Table 3). Ethylene when applied to explants at ambient pressure and temperature provided a further stimulation of abscission rate. The optimum concentration was near 1 ppm (Fig. 5). In some species, the stipules are a natural source of ethylene. With our explants, stipulate and exstipulate explants behaved similarly (Table 4) although abscission rate was reduced slightly by removing both stipules. A shortening of the time to 50% separation equivalent to that with ethylene was obtained by supplying galactose to the agar (Fig. 6). These experiments were con-

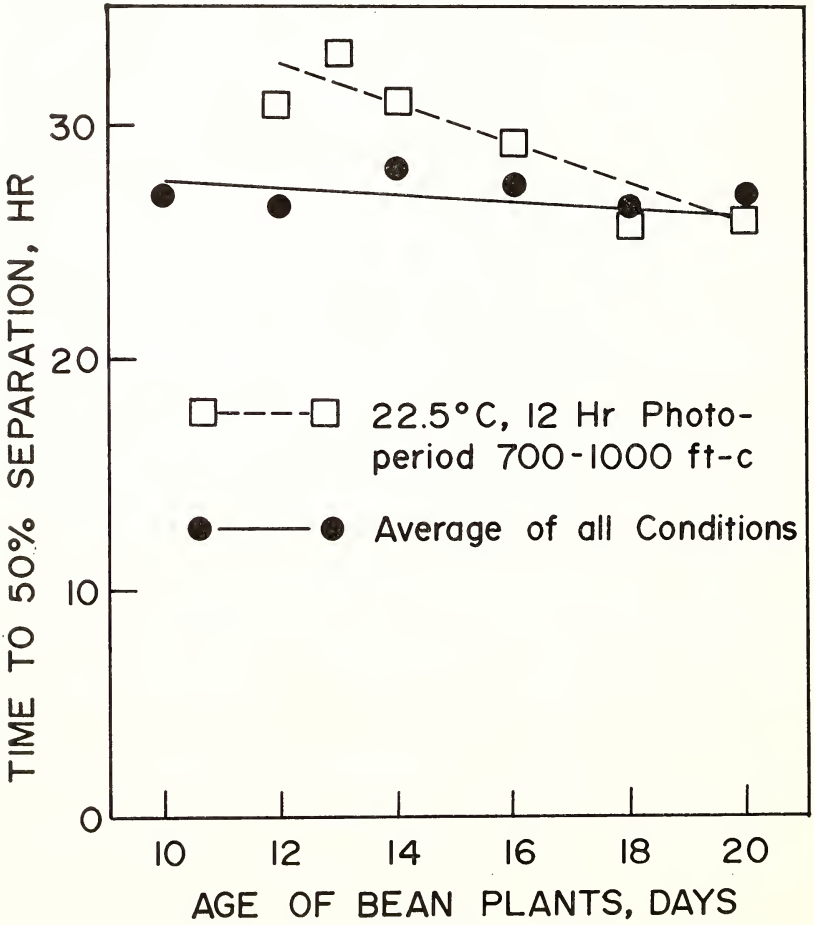

Figure 4. Rate of separation layer formation of explants excised from bean plants of varying ages. All conditions are those listed in Table I. Assays were conducted in continuous light at 28 to 29°.

TABLE 5. *Influence of Concentration and Placement of Sucrose and Glucose on Bean Explant Abscission at 29°. Sugars were supplied in 1% Agar.*

Portion of Explant Placed in Agar	Sugar	Concentration (mM)	Time to 50% Separation (Hr)
Petiole	None	—	42
	Sucrose	3	42
		30	38
	Glucose	3	44
		30	44
Pulvinus	None	—	32
	Sucrose	3	26
		30	24
	Glucose	3	27
		30	29

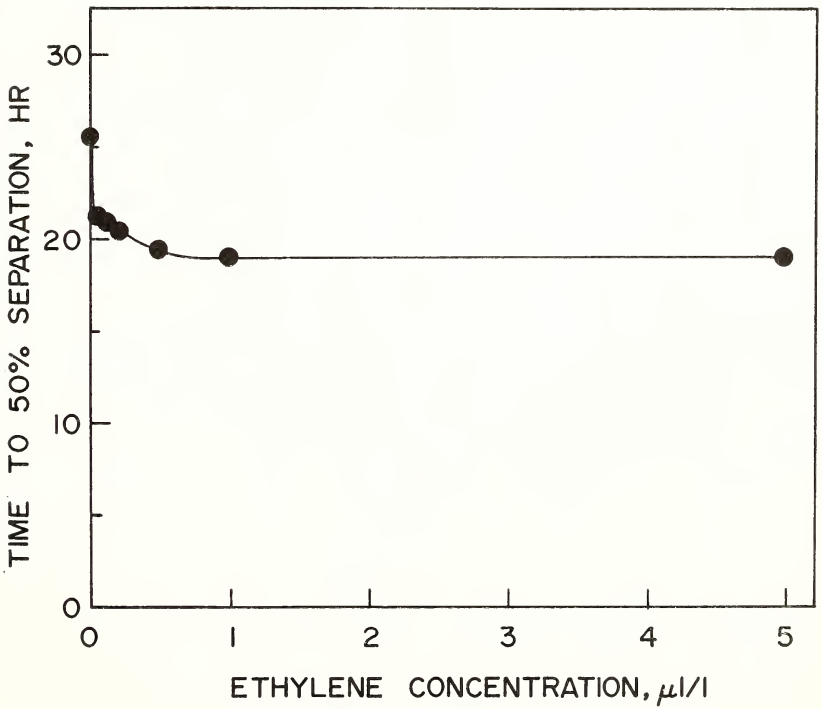

Figure 5. Separation layer formation as a function of ethylene concentration supplied to the gas phase of sealed flasks containing the explants. Explants were obtained from greenhouse-grown plants. Assays were conducted at 28 to 29° in continuous light with the explants inverted (pulvinus end down) in 0.7% agar.

ducted with explants in the inverted position (distal end down). The optimum galactose concentration was 2% and explants treated with 20% galactose did not abscise. Under similar conditions of treatment, sucrose and glucose also stimulated abscission but not to the extent exhibited by galactose (Table 5).

Transition profiles for inverted explants treated with ethylene or galactose (Fig. 7) at 28° show both a steepening of the slope and a marked shortening of the midpoint. When supplied together at optimal concentrations, ethylene and galactose were additive in their effects on rate of separation layer formation (Fig. 7). The studies with ethylene were conducted in sealed flasks with inverted explants (pulvinar end down).

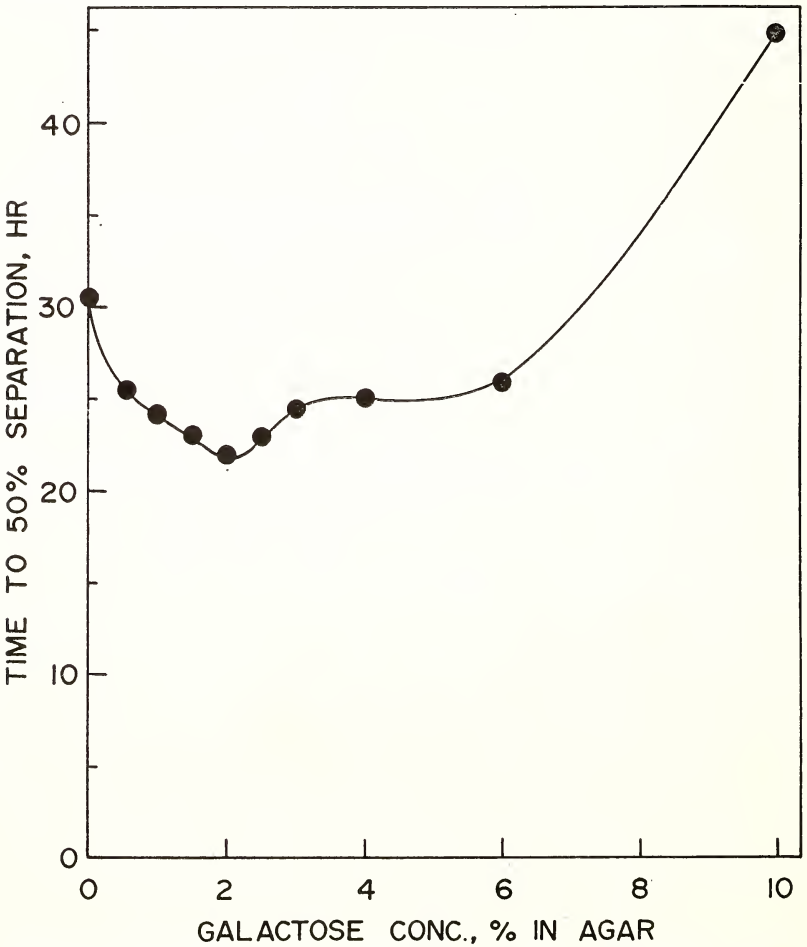

Figure 6. Separation layer formation as a function of galactose concentration in 0.7% agar. Explants were obtained from greenhouse-grown plants and assays were conducted at 28 or 29° in continuous light with the explants inverted (pulvinus end down).

TABLE 6. *Abscission of Bean Explants at Two Temperatures Comparing Plants Grown Under Widely Varying Conditions.*

Growing Conditions for Plants Prior to Harvesting Explants						
					Time to 50% Separation of Explants (Hr)[2]	
Substrate	Light Intensity[1] (Ft-C)	Temperature, °C Day	Night	Chronological Age, Days	22.5°C	30°C
Silicate Soil Conditioner[3]	5,500-5,800	32	24	18	120	60
Soil-Sand	5,500-5,800	32	24	10	96	24
	100	20	7	24	120	48

[1]12 Hour Photoperiod.
[2]12 Hour Photoperiod, 700-1,000 ft-c.
[3]Krum (Silibrico Corporation, Chicago, Illinois).

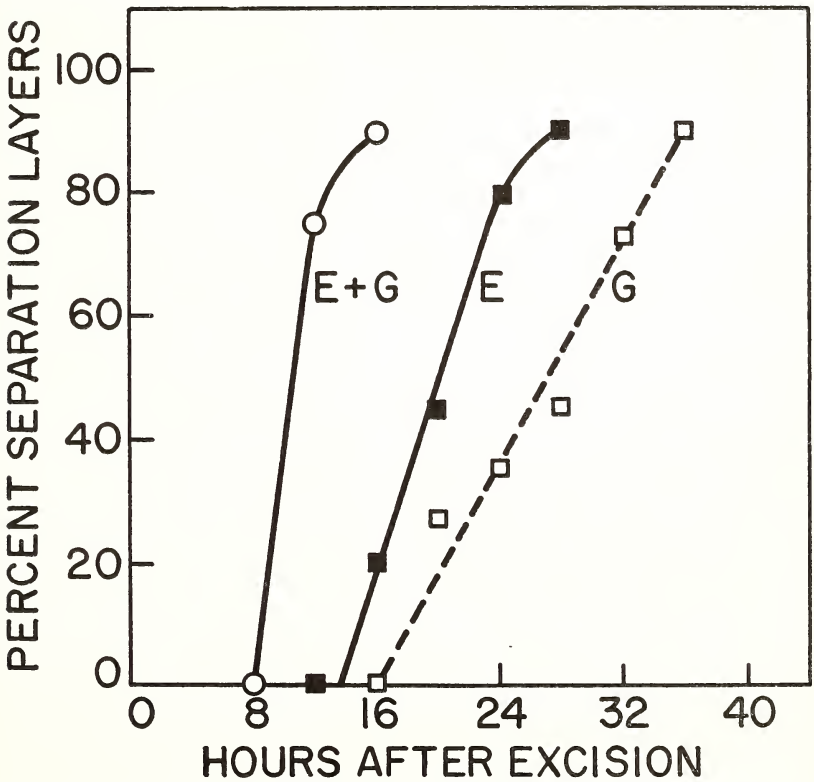

Figure 7. Transition profiles of separation layer formation comparing 1 ppm ethylene in the gas phase (E), 2.5% galacose in the agar phase (G) and 1 ppm ethylene plus 2.5% galactose (E+G). Explants were obtained from greenhouse-grown plants and assays were conducted at 28 to 29° in continuous light with the explants inverted (pulvinus end down).

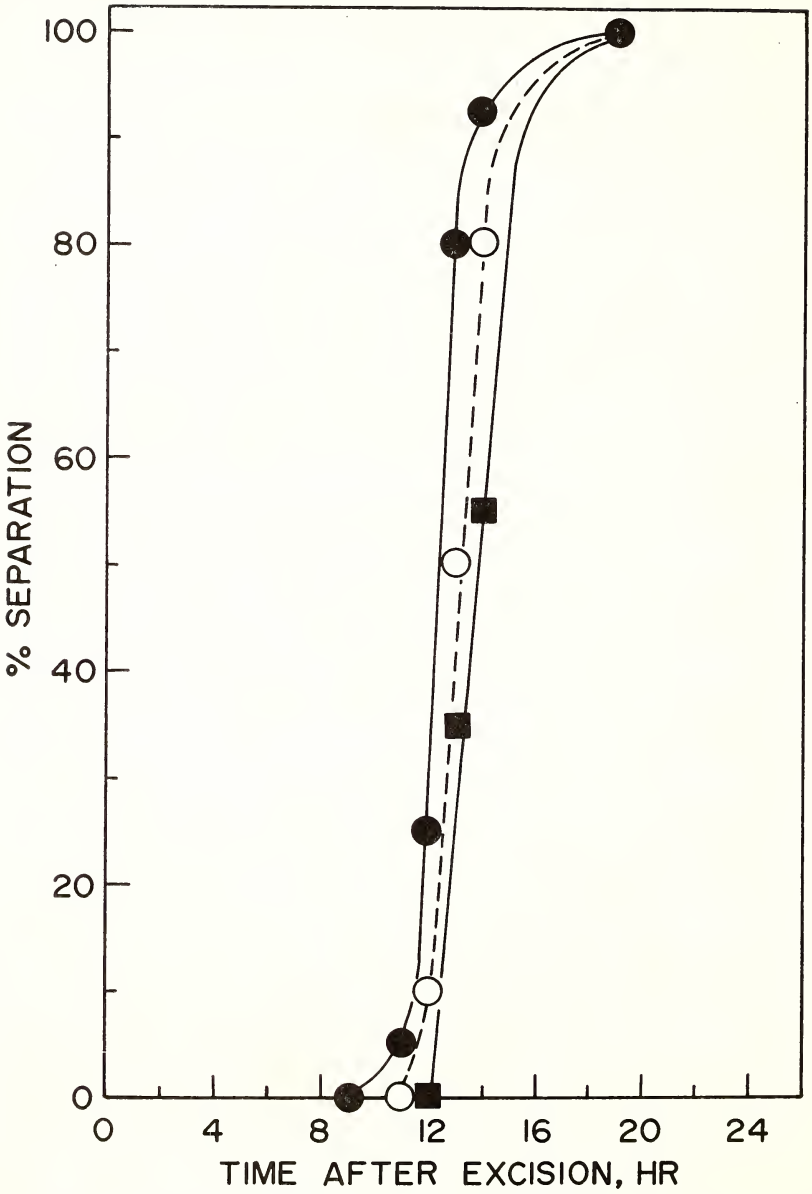

Figure 8. Transition profiles for explants incubated in the presence of 1 ppm ethylene plus 2.5% galactose as described for Figure 7 comparing plants grown under greenhouse (■————■) and controlled environment (●————● — 22.5°, 700 to 1000 ft-c and 8 hr photoperiod; o————o — 30°, 700 to 1000 ft-c and 12 hr photoperiod) conditions.

More detailed transition profiles of rapidly abscising explants (Fig. 8) show a transition width of approximately 2 hours and a midpoint at 12 hours when explants were prepared from plants grown at constant temperature (22.5°), low light intensity (700 to 1000 ft-c) and short (8 hr) photoperiod. With explants from greenhouse-grown plants (variable temperature, long photoperiod, high light intensity), the midpoint was at 14 hours (2 hours later). When compared under less favorable conditions (in the absence of inducers, upright position and 22°), their midpoints differed by as much as 70 hrs (50 hrs to 50% separation from explants from plants grown at 22.5°, low light and 8 hr photoperiod vs. 120 hr for explants from greenhouse-grown plants, Table 1, Fig. 1). The equalizing effect of optimum conditions for the explant assay illustrated in Figure 8 was evident throughout the study. In general, differences due to growing conditions of the plants were minimized as conditions of the explant assay were optimized (Table 1). Also minimized as optimum conditions of explant assay were approached were differences among individual members of each population. The net result was a steepening of the transition profile (Fig. 8).

Discussion

External factors such as climatic conditions have long been known to influence abscission of leaves from intact plants (3, 4). Low carbohydrate levels favor rapid abscission. Moderate nitrogen, low water supply, high light intensity and conditions of carbohydrate accumulation reduce or retard abscission (13). Light also may have an effect through photoperiod. Olmsted (11) observed that constant photoperiods, regardless of length, resulted in delayed abscission and senescence.

The effect of environment on experimental leaflet abscission (studies utilizing explants) has not been studied extensively. Previously, Yamaguchi (reviewed by Addicott (3)) found that the formation of the abscission layer in bean explants exhibited a temperature response curve with a maximum between 25 and 30° and the effects of light and CO_2 on explant abscission were examined by Biggs and Leopold (5).

Being a physiological process, abscission is extremely sensitive to temperature particularly over the range 16 to 26°. Increasing the temperature from 22° to 24°, for example, may shorten the time of 50% separation of explants by nearly 50%. Between 26° and 30°, temperature has little effect on abscission rate with an optimum near 29°. In earlier abscission experiments involving explants at about 23°, it is probable that much of the variation was due to variations in temperature. The temperatures at which the plants were grown prior to harvesting the explants has much less of an effect on abscission rate than the temperature during the explant bioassay.

The response of explants to light is more variable and less pronounced than that due to temperature. With plants grown under low light intensity (conditions that tend to minimize accumulation of carbohydrate reserves), abscission of explants occurs more rapidly in light than in darkness. However, when plants are grown in high light intensity

(conditions favoring accumulation of carbohydrate reserves) just the opposite is true, i.e. explants abscise more rapidly in the dark.

This light-induced delay of abscission with plants grown at high light intensity was investigated by Biggs and Leopold (3). They found that plants in a CO_2-free atmosphere under 300 ft-c of light abscised at the same time as those kept in the dark with or without CO_2, while those kept in ambient air were delayed by about 40 hours. They concluded that the effect of light was due to photosynthesis since in their studies the effect of light was eliminated by added sucrose.

In our studies, conducted with greenhouse-grown plants, sucrose and glucose had slight stimulatory effects on abscission and any regular correlation among light, CO_2, photosynthesis, carbohydrate accumulation and abscission seemed doubtful. A further complication arose from the unexplained observations that explants abscised faster with the pulvinus end in agar (petiole end up) than when the petiole end was in agar (pulvinus end up) but only in light. In darkness, the explant apparently did not sense which end was up.

A possible indirect involvement of photosynthesis in leaf abscission would be through CO_2 removal as it effects the response of the plant to ethylene. Ethylene is a natural promoter of abscission (1, 2, 6, 12) and carbon dioxide (CO_2) is a competitive inhibitor of its action. Thus, a simple explanation of the effects of light and temperature on experimental leaflet abscission might be found in a consideration of the combined rates of photosynthesis and respiration as they affect CO_2 levels. A buildup of CO_2 within the petri plates containing the explants might interfere with the normal acceleration of separation layer formation by endogenous ethylene, particularly at high temperatures. Under conditions where respiratory substrates are non-limiting, photosynthesis would be expected to substantially reduce the CO_2 content of the gas phase of explants incubated in the light with a corresponding accelerated rate of separation layer formation. Both ethylene and galactose appear to accelerate abscission through their specific action in the induction of a pectinase catalyzing the hydrolysis of the cementing substances between adjacent cell layers of the abscission zone (10).

With cotton petioles, Hall and Liverman (7) found that abscission was accelerated in proportion to increased light intensity up to 2,500 ft-c but light intensities from 6,000 to 8,000 ft-c delayed abscission significantly. Acceleration was obtained with red, far-red, fluorescent and ultraviolet light but the effects of light intensity on abscission have not been studied extensively. Similarly, in our studies, plants grown under high light intensity were delayed in their abscission response as compared to plants grown under low light intensity.

Throughout these studies, light and temperature have been most effective in regulating explant abscission during incubation of the explants. Abscission of explants is only little influenced by the conditions under which the plants are grown except for extremes of temperature, nutrition and photoperiod (Table 6). Here, conditions favoring slow

growth of the plants tend to be associated with a reduced rate of separation layer formation.

Summary

Separation layer formation in explants of red kidney bean was markedly accelerated by increasing temperature between 16 and 26° C with an optimum between 28 and 30°. Light effects were largely qualitative. Quantitative changes in light intensity and photoperiod had little effect on the abscission response when explant assays were conducted at optimum temperatures. Light and temperature effects on explant abscission were largely restricted to the explants themselves with minor modulations due to the environmental history of the plants from which the explants were prepared.

By combining near optimal growing conditions of the plants prior to preparation of the explants, optimum temperature and continuous light during the explant assay in conjunction with two inducers of pectinase (galactose and ethylene) supplied under optimal conditions, we shortened the time of experimental leaflet abscission from 100 hours to 12 hours. The development of an experimental system in which the time from excision of the explant to separation layer formation could be confined to a period of 8 hours or less would be of interest not only from the standpoint of practical defoliation but to expedite certain kinetic analyses of abscission events as well.

Literature Cited

1. ABELES, F. B. 1967. Mechanism of action of abscission accelerators. Physiol. Plant. **20**:442-454.
2. ABELES, F. B. and B. RUBINSTEIN. 1964. Regulation of ethylene evolution and leaf abscission by auxin. Plant Physiol. **39**:963-969.
3. ADDICOTT, F. T. 1964. Physiology of abscission. Encyclopedia of Plant Physiol. XV/**2**:1094-1126.
4. ADDICOTT, F. T. and R. S. LYNCH. 1955. Physiology of abscission. Ann. Rev. Plant Physiol. **6**:211-238.
5. BIGGS, R. H. and A. C. LEOPOLD. 1957. Factors influencing abscission. Plant Physiol. **32**:626-632.
6. HALL, W. C. 1958. Physiology and biochemistry of abscission in the cotton plant. Texas Agricultural Experiment Station Miscellaneous Pub. **285**:1-23.
7. HALL, W. C. and J. L. LIVERMAN. 1956. Effect of radiation and growth regulators on leaf abscission in seedling cotton and bean. Plant Physiol. **31**:471-476.
8. HOLM, R. E. and F. B. ABELES. 1967. Abscission: The role of RNA synthesis. Plant Physiol. **42**:1094-1102.
9. MORRE', D. J. 1968. Leaf abscission: The chronology and control of a terminal developmental sequence. Proc. Western Agricultural Experiment Station Collaborator's Conference.
10. MORRE', D. J. 1968. Cell wall dissolution and enzyme secretion during leaf abscission. Plant Physiol. **43**:1545-1559.

11. OLMSTED, C. E. 1951. Experiments on photoperiodism, dormancy and leaf age and abscission in sugar maple. Botan. Gaz. **112**:365-393.

12. RUBINSTEIN, B. and F. B. ABELES. 1961. Relationship between ethylene evolution and leaf abscission. Botan. Gaz. **126**:255-257.

13. RUBINSTEIN, B. and A. C. LEOPOLD. 1964. The nature of leaf abscission. Quart. Rev. Biol. **39**:356-372.