PHYSIOLOGICAL STUDIES OF BRACT KEEPING QUALITY IN POINSETTIA

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ABSTRACT

PHYSIOLOGICAL STUDIES OF BRACT KEEPING QUALITY IN POINSETTIA

By

David Allan Gilbart

A study was made of the factors governing differential bract abscission rates and hence keeping quality among cultivars of poinsettia. Experiments were designed to measure changes in respiration rate, nitrogen pools, carbohydrate pools and ethylene evolution with aging. The effect of exogenous growth regulators such as indoleacetic acid (IAA), gibberellic acid (GA), abscisic acid (ABA), and ethylene on abscission rates of intact plants and explants was determined. A study was made of the extractable and diffusible endogenous growth regulators, especially IAA, together with changes in activity and isoenzymic forms of IAA-oxidase with aging.

The respiration rate declined faster in the bracts of poor keeping cultivars while nitrogenous compounds were mobilized coincident with senescence. No significant correlation was found between any parameter and abscission rate. No ethylene evolution was detected with the method employed.

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Exogenous IAA delayed the abscission of debladed bracts. Other growth regulators were not effective in promoting or delaying abscission. No interaction was found between any growth regulator and cultivar with respect to bract abscission.

Both extractable and diffusible endogenous auxin levels in bracts decreased with time. The rate of decrease was higher in the poor keeping cultivar. The decrease in auxin could be related to an increase in the activity of IAA-oxidase and hydrogen peroxide content with time. The appearance of new isoenzymes was associated with increased enzyme activity.

Differences in keeping quality among cultivars of poinsettia is the result of differential destruction of endogenous auxin through the appearance of new forms of peroxidase and increased levels of hydrogen peroxide together with differential synthesis of auxin through decreased rates of respiration leading to a decrease in the effective concentration of auxin. A threshold level of auxin which is reached earlier in poor keeping cultivars triggers senescence and abscission resulting in mobilization of nitrogenous compounds which possibly further accelerates abscission.

This hypothesis is compatible with inheritance studies of keeping quality in poinsettia.

PHYSIOLOGICAL STUDIES OF BRACT KEEPING QUALITY IN POINSETTIA

By

David Allan Gilbart

A THESIS

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NOTE TO THE COMMITTEE

This thesis has been prepared so that the body of the dissertation is in the form of two journal articles designed for publication in the Journal of the American Society for Horticultural Science. Additional information, by way of a general introduction to the problem, additional data and a general interpretation of the results appear in the appendix.

ACKNOWLEDGMENTS

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ARTICLE I

THE EFFECT OF EXOGENOUS GROWTH REGULATORS ON KEEPING QUALITY AND ABSCISSION IN POINSETTIA

The Effect of Exogenous Growth Regulators on Keeping Quality and Abscission in Poinsettia

Abstract. A study was made of the response of several cultivars of intact and explant poinsettia cultivars to exogenously applied growth regulators. No treatment accelerated the abscission of explants over nontreated controls. Indoleacetic acid (IAA) delayed the abscission of explants relative to its concentration and time of application. Gibberellic acid or abscisic acid alone had no effect but modified the IAA effect in interaction. Ethylene had no effect. There was no interaction between any treatment and cultivar.

INTRODUCTION

Several naturally occurring metabolites and plant growth regulators may influence the rate of foliar and floral abscission when applied exogenously to an explant or intact plant. These include auxins, gibberellic acid, abscisic acid, ethylene, kinin, certain amino acids and protein synthesis inhibitors (11). Varying the experimental conditions may alter the response to any one regulator and 2 or more applied chemicals may interact to produce synergism or compensation. Indoleacetic acid as an abscission retardent and ethylene as an accelerator have the most universal and the greatest quantitative effect.

Most research on abscission has been done using explants of Phaseolus, Gossypium or Coleus. This has been particularly true in the testing of compounds for activity as abscission regulators. The poinsettia has been used in a few abscission studies. Gawadi and Avery (10) showed that applying ethylene chlorohydrin to intact poinsettias induced abscission of young leaves as fast as deblading while a 1% napthaleneacetic acid solution applied to debladed leaf stumps delayed abscission from the normal 5 to 6 days to upward of 20 days. Carpenter (7) found that up to 6 ppm of 2,4,5-trichlorophenoxyacetamide delayed abscission of both leaves and bracts on poinsettia while 2,4,5-trichlorophenoxypropionamide slightly hastened it. However the results were variable, dependent on environmental conditions, and the treatments induced an epinastic response.

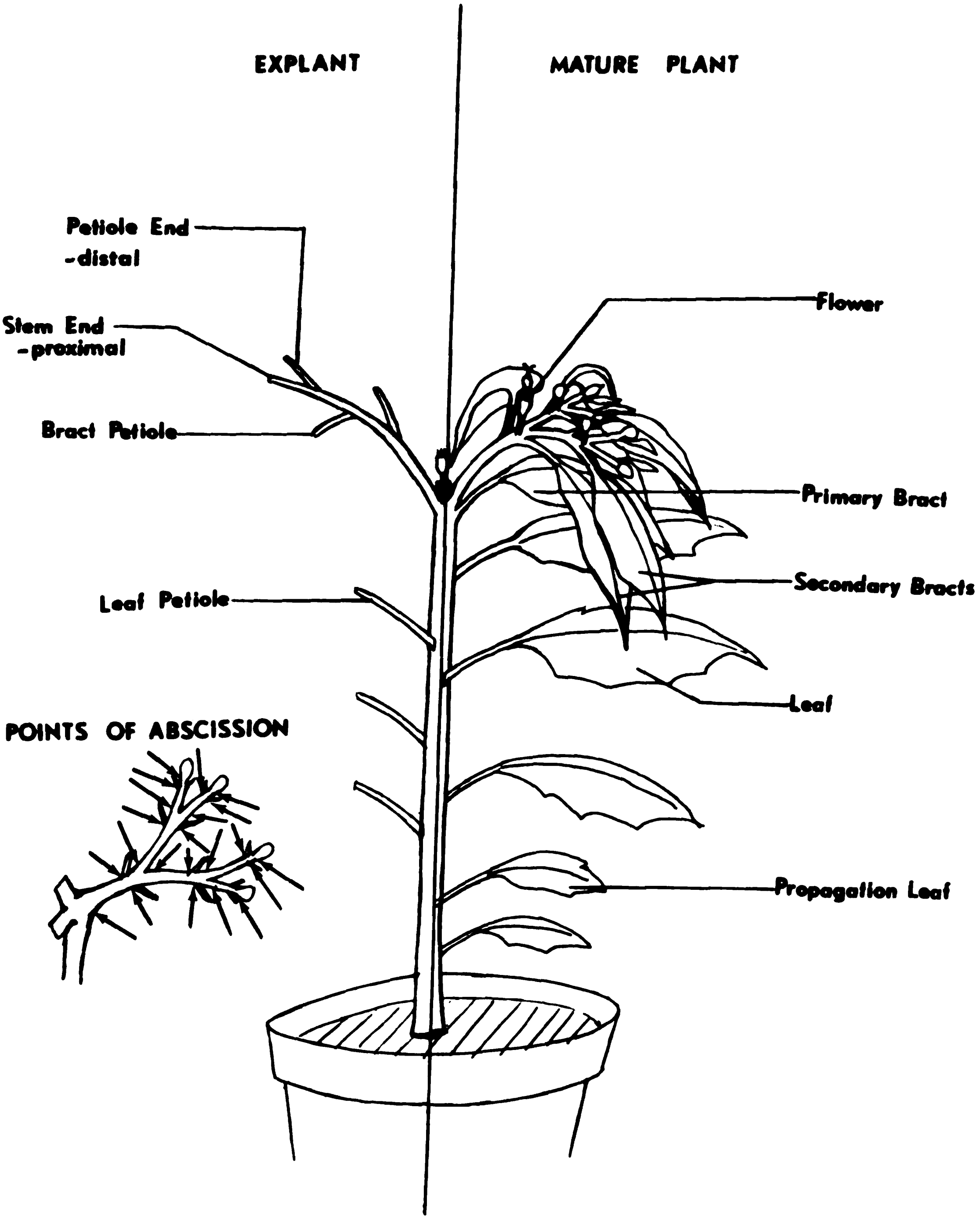
MATERIALS AND METHODS

Plants of the poinsettia cultivars Paul Mikkelsen, White Ecke, New Ecke Pink and Barbara Ecke Supreme were obtained as rooted cuttings in the fall or winter and grown as single stem plants to anthesis in 5 inch clay pots using standard greenhouse practices. At anthesis the plants were given treatments and transferred either to a laboratory simulating a home environment for intact plants, 21° and 50 ft-c of light for 12 hrs, or a dark room maintained at 21° for explants.

A poinsettia explant is illustrated in Figure 1. All cyathia, growing points and primary bracts were removed. The lower 3 to 4 leaves were also removed as they were formed while the plant was under propagation stress and it was found in a preliminary study that they abscised abnormally early. Remaining leaves and bracts were removed to leave only 3 bracts on each of the 3 flowering stems arising from the first floral division and 6 leaves. These were then debladed by cutting directly below the leaf or bract blade.

The growth regulators employed were indoleacetic acid (IAA), gibberellic acid (GA), abscisic acid (ABA),

Figure 1. Sketch of a poinsettia plant showing an intact plant, an explant and the points of abscission in the flowering head.


EXPLANT
MATURE PLANT
Petiole End
-distal
Stem End
-proximal
Bract Petiole
Leaf Petiole
POINTS OF ABSCISSION
Flower
Primary Bract
Secondary Bracts
Leaf
Propagation Leaf

ethylene, chloramphenicol or 2,4-dichlorophenol either alone or in combination. Ethylene treatment was applied by holding plants for 24 hrs in a sealed plastic bucket containing 10 ppm ethylene in air. Other treatments were applied in lanolin paste to the petiole or stem stumps of explants or as an aqueous solution with 100 ppm Tween 20 wetting agent applied as a spray to the drip off point on intact plants.

Abscission was recorded on a daily basis and included lightly tapping each petiole to ensure complete separation. The abscission rate was based on the average number of days to abscission for all bract or leaf petioles on a plant. Petioles which had not abscised by the termination date were given the maximum value. Explants held in the dark were measured for 12 days and intact plants held in the simulated home environment were maintained either for 18 days or until obviously senescent.

None of the studies were replicated with time. Routinely 3 to 5 individual plant replicates were included for each treatment in each study and added confidence was achieved through the use of nontreated and IAA treated controls added to each of the explant studies.

RESULTS

Abscission of untreated and ethylene treated intact and explant poinsettia leaves and bracts. The cultivars studied exhibited a wide range of keeping times when held in a simulated home environment. In general, the rate of bract abscission determined keeping time. Paul Mikkelsen and Ecke White held their bracts the longest while New Ecke Pink was intermediate and Barbara Ecke Supreme lost its bracts the earliest. Leaf abscission appeared to be independent of bract abscission with little difference among cultivars.

Abscission rates of bract petioles from explants differed among cultivars (Table 1). The relative order of abscission among cultivars was the same as for intact plants although the rate was accelerated. The abscission rates of explant leaf petioles did not vary among cultivars. In all cultivars prepared as explants, leaf petioles abscised sooner than bract petioles. Chronologically, bracts are the more juvenile tissue; however, both bracts and leaves were mature when debladed. Moreover, there was no apparent relationship between position on the stem and the time to abscission for either leaves or bracts.

Table 1. The effect of 10 ppm ethylene treatment on days to 50% abscission of debladed or intact leaves and bracts of 4 poinsettia cultivars.

Cultivar		Intact		Debladed	
		Ethylene	Control	Ethylene	Control
White Ecke	bracts	11.6	12.0	6.6	6.5
	leaves	12.0	12.0	4.6	4.8
Paul Mikkelsen	bracts	12.0	12.0	7.3	7.2
	leaves	12.0	12.0	5.4	5.0
New Ecke Pink	bracts	11.6	11.2	5.5	5.6
	leaves	9.8	10.0	4.5	4.5
Barbara Ecke Supreme	bracts	10.8	11.2	5.7	5.8
	leaves	8.6	9.8	4.8	4.9

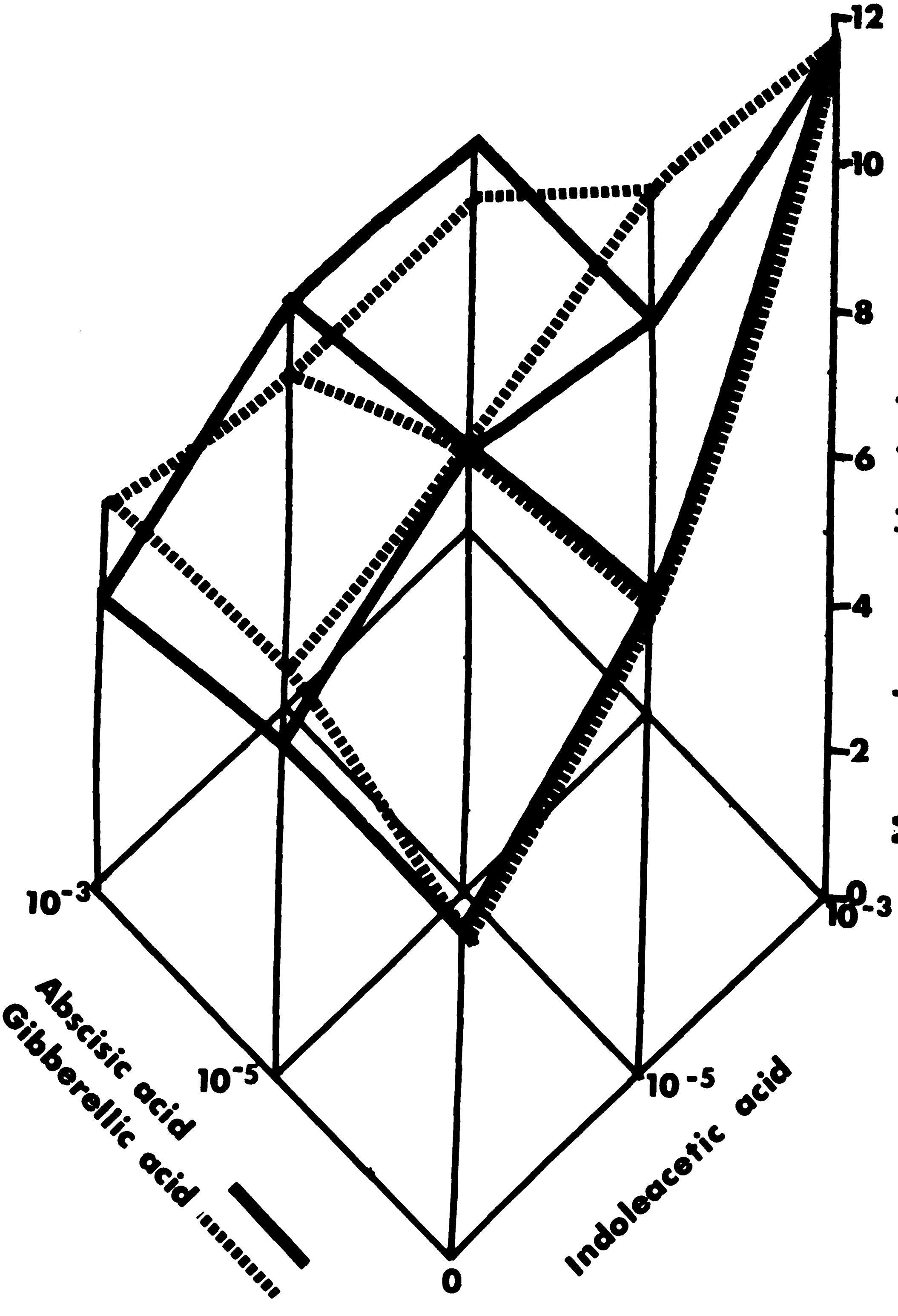
The results of the study on exogenous treatment with ethylene are in Table 1. Ethylene had no effect on abscission of leaves or bracts, whether intact or debladed in any of the cultivars. Over the length of the experiment few leaves or bracts abscised from intact plants, thus possibly masking a belated effect.

A repetition of the experiment using the same design but with aged plants of 2 cultivars, Paul Mikkelsen and Barbara Ecke Supreme, also gave negative results for an ethylene effect although there was considerable abscission among both control and treated intact and debladed plants. Epinasty was observed in many leaves and bracts treated with ethylene. This was more pronounced in explants and occurred least in the aged intact plants.

The effect of indoleacetate acid, abscisic acid and gibberellic acid on bract abscission of explants. The bract petioles of pionsettia explants of the cultivars Paul Mikkelsen and Barbara Ecke Supreme were treated immediately after deblading with IAA applied distally and either ABA or GA proximally. Two concentrations, 10^{-5}M and 10^{-3}M of each chemical were used and all controls, single chemical treatments and interactions between IAA and the other 2 were included.

The results of this study are presented in Figure 2. Although there were significant differences between

Figure 2. The interaction of 2 concentrations of IAA applied distally with 2 concentrations of GA or ABA applied proximally to debladed poinsettia bract petioles on the rate of bract petiole abscission.



Molar concentration
of growth regulator

the cultivars there was no interaction between cultivar and chemical treatment so treatment effects are presented as the average of both cultivars.

Both main order and interaction effects between chemicals may be seen. Indoleacetic acid retarded the rate of abscission, more so at the higher concentration but also at the lower. Neither ABA nor GA at either concentration had any effect when used alone or with the lower concentration of IAA. However when interacted with the higher concentration of IAA, both ABA and GA at either concentration reduced the effectiveness of IAA in delaying abscission to approximately that of the lower concentration of IAA alone. Epinasty was observed in all treatments that included IAA.

<u>The effect of concentration and point of application of indoleacetic acid and gibberellic acid on leaf and bract abscission of explants</u>. Paul Mikkelsen and Barbara Ecke Supreme were treated immediately after deblading with IAA or GA at 10^{-3} or 10^{-5}M applied either to the leaf petiole stump or the bract petiole stump (distal) or to the flowering stem terminal stump (proximal). Lanolin alone was applied to stumps not treated with chemical and to control plants.

The results of this study are presented in Table 2. There was no interaction between cultivars and

Table 2. The effect of growth regulator concentration and point of application on days to 50% abscission of debladed bract and leaf petioles.

Point of Application	Indoleacetic Acid		Gibberellic Acid	
	10^{-3}M	10^{-5}M	10^{-3}M	10^{-5}M
		Bract Abscission		
Control	6.4			
Leaf Petioles	6.8	6.0	6.6	6.3
Bract Petioles	11.6	9.3	5.6	5.8
Stem Terminals	8.8	7.3	6.8	6.4
		Leaf Abscission		
Control	5.1			
Leaf Petioles	9.2	6.7	4.7	4.6
Bract Petioles	4.7	4.6	4.8	5.2
Stem Terminals	5.3	5.0	5.0	4.7

LSD .05% - bracts 1.0
- leaves .7

chemical treatment so the values were averaged across both cultivars. There were significant differences between chemicals, points of application and their interaction. Indoleacetic acid delayed the rate of bract abscission of applied to the bract petiole stump or the stem terminal. Proximal application was effective in delaying abscission only at the higher concentration. Application to the leaf petioles did not affect the rate of bract petiole abscission. Indoleacetic acid delayed leaf petiole abscission only if applied at the higher rate directly to the leaf petiole stump. Gibberellic acid did not significantly affect abscission in any of the treatments studied although there appeared to be a consistent tendency for it to enhance bract abscission when applied distally to the bracts. It did not affect the leaf petiole abscission rate. In no case did IAA accelerate or GA slow the rate of abscission.

The effect of indoleacetic acid, gibberellic acid and abscisic acid on leaf and bract abscission from intact plants. Paul Mikkelsen and Barbara Ecke Supreme plants were prepared as explants except that the remaining 6 leaves and 9 bracts were not debladed. The plants were then sprayed with either IAA, GA or ABA at 10^{-3}M or water and held in a simulated home environment for 18 days.

The first apparent result was pronounced epinasty of IAA treated plants which continued for the duration of the experiment. The effect of these treatments on abscission is summarized in Table 3. Because of the relatively short time span under observation, plants in several treatments had little or no abscission. This may have masked differences due to treatment as, for example, bract abscission of Paul Mikkelsen.

There were however, certain striking differences in abscission rates as a result of treatment. Abscisic acid tended to accelerate the rate of leaf abscission while having little or no effect on the bracts. Indoleacetic acid had the apparent effect of delaying leaf abscission in the cultivar Paul Mikkelsen while accelerating it in Barbara Ecke Supreme. Both IAA and GA tended to delay bract abscission in Barbara Ecke Supreme.

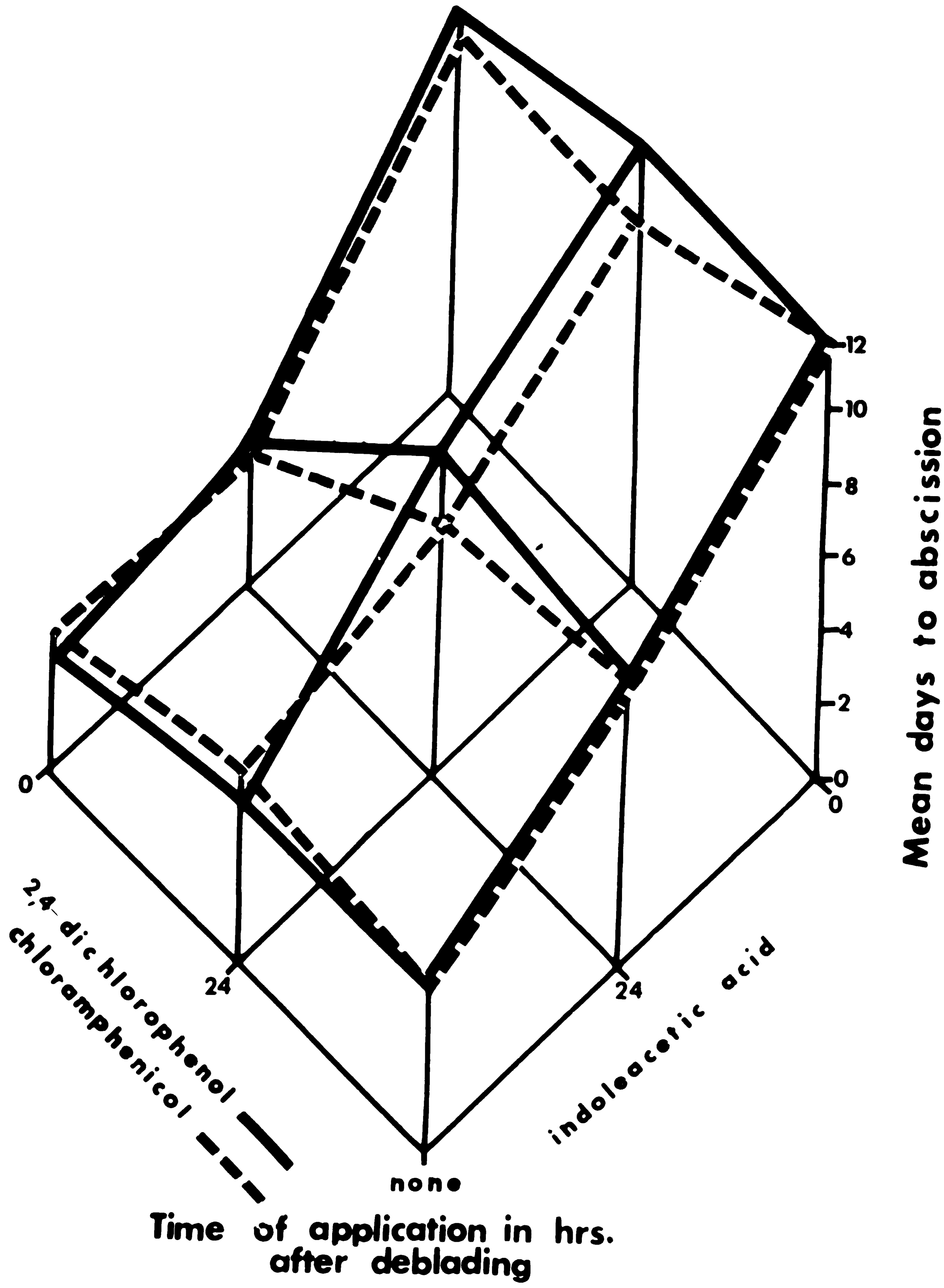
The effect of time of application of indoleacetic acid, chloramphenicol and 2,4-dichlorophenol on bract abscission of explants. Explants of the cultivar White Ecke were treated either immediately after preparation or 24 hrs later with IAA applied distally to the bract petiole stumps and either 2,4-dichlorophenol or chloramphenicol proximally to the stem terminal. All chemical treatments were at 10^{-3}M. Each alone and all possible combinations between IAA and the others were tested.

As shown in Figure 3, IAA delayed abscission irrespective of time of application. However, the later

Table 3. The effect of 10^{-3} M IAA, GA, and ABA on mean number of days to abscission of leaves and bracts of 2 cultivars of poinsettia.

	Control	IAA	GA	ABA
Paul Mikkelsen				
Bracts	18.0	18.0	18.0	17.6
Leaves	15.4	18.0	17.1	10.2
Barbara Ecke Supreme				
Bracts	14.7	18.0	18.0	16.9
Leaves	12.6	7.6	11.8	4.1

Figure 3. The interaction of IAA applied distally immediately after deblading or 24 hours later with 2,4-dichlorophenol or chloramphenicol applied proximally immediately after deblading or 24 hours later to debladed poinsettia bract petioles on the rate of bract petiole abscission.


12
10
8
6
4
2
0
0
0
24
24
none
Mean days to abscission
2,4-dichlorophenol
chloramphenicol
indoleacetic acid
Time of application in hrs. after deblading

application had a lessened effect, approximately equal to a 10^{-5}M IAA treatment applied immediately after deblading. Neither 2,4-dichlorophenol nor chloramphenicol alone had any effect on the abscission rate. Neither did they modify the effect of IAA applied at the time of deblading. However, if 2,4-dichlorophenol or chloramphenicol were applied immediately after deblading the effect of IAA added 24 hours later was nullified. In no case was the rate of abscission accelerated over the control.

DISCUSSION

The 4 cultivars studied exhibited a wide range in keeping time as evidenced by their differential rates of bract abscission. Paul Mikkelsen and Barbara Ecke Supreme which were chosen for more intensive study represent the extremes of commercial cultivars.

The rate of bract petiole abscission from explants differed among cultivars but leaf abscission did not. This would indicate that leaves and bracts are under different control systems or a system that is differentially active in the 2 tissues. If it is one system then it is active in both the blade and the petiole since removing the blade hastened the rate of abscission yet left a residual varietal difference. While both leaves and bracts were fully mature the leaves were older. This relative difference in age in a mature flowering poinsettia may account for a portion of the different responses of leaf and bract petioles after deblading.

The effect of 10^{-3}M IAA applied immediately after deblading in delaying abscission of petioles confirms an accepted fact based on observations of the responses of many plants (11). However, the delay of abscission by proximal application, treatment after 24 hours aging with

10^{-3}M IAA or immediately with 10^{-5}M IAA has not been so universally recognized. Gaur and Leopold (9) showed that dilute concentrations of IAA (10^{-5}M) stimulate abscission while 10^{-3}M IAA that will delay abscission if applied distally to fresh explants may accelerate it if applied proximally (3) or if applied distally to aged explants (16). Later verification (1) has shown that it is primarily a question of auxin transport to the site of abscission as influenced by point of application, petiole length and environmental conditions.

There was no apparent relationship between petiole length or distance from proximal treatment site and petiole response. This agrees with the findings of Lewis and Bakhshi (12) who used seedling orange explants analogous to those in this study. No attempt was made to monitor hormone transport rates in the poinsettia but it may be hypothesized that there is a sufficiently free flow of auxin basipetally to and across the abscission zones. The delay of abscission by proximal application of a high concentration of IAA would also indicate a functional acropetal transport. However, in the study on debladed leaf petioles application of auxin proximally either to the stem terminal or the bract petiole terminals had no effect on the rate of abscission. Either adequate transport does not continue down the stem or there is an active auxin destruction mechanism.

Neither GA nor ABA had any effect when applied alone to poinsettia explants. The effect of GA as an abscission accelerator has been debatable. According to Jacobs (11) the effect is slight and confined to young tissue. In these studies bracts and leaves were fully mature which may explain the lack of response. The ineffectiveness of ABA in promoting abscission is surprising in view of its effect on other plants (8). In part this may have been the result of proximal application only which is less effective than distal treatment (11).

Although neither GA nor ABA was active as an abscission regulator either alone or in combination with 10^{-5}M IAA they both interacted in decreasing the effectiveness of 10^{-3}M IAA to approximately that of 10^{-5}M IAA. Thus it would appear that both GA and ABA are effective in altering the abscission rate of debladed poinsettia bracts but only under conditions favoring maintained growth and maximum longevity.

The results of spray application of IAA, GA, and ABA to intact plants are anomolous with those from the explant studies and with those from other studies. El-Antably, Wareing and Hillman (8) found very little activity when intact plants from a number of species were treated with ABA. Insufficient work has been done with ABA to allow an interpretation of these data although it again illustrates the inherent difference between bracts and leaves. The effect of IAA in stimulating abscission in one instance is difficult to explain in view of a consistent inhibition of abscission on explants. The fact that the same treatment stimulated leaf abscission and delayed bract abscission on the same plants would argue against this being an artifact; however, the study was not repeated to confirm the observation. More likely, variable penetration and transport combined with endogenous growth regulators and uncontrolled variables in the environment resulted in this apparent discrepancy. It does support the observations made by other investigators that under some conditions auxin may stimulate abscission (3).

Protein synthesis inhibitors have been reported to promote abscission (18) but were not effective on poinsettia bracts. Proximal application at a distance from the abscission zone could account for the lack of effect for chloramphenicol alone. However, the negation of the delaying effect of IAA added 24 hours later would indicate that chloramphenicol had some activity. Some possible pathways could be: interference with transport, action or destruction of auxin or the inhibition of protein formation either necessary for auxin activity or normally formed as an integral part of the auxin effect. The lack of effect from simultaneous application may be the result of differential transport times to the abscission zone.

The IAA-oxidase co-factor 2,4-dichlorophenol was similar to chloramphenicol in action. Schwertner and Morgan (17) found that IAA-oxidase co-factors as exemplified by 2,4-dichlorophenol could accelerate abscission of explants. The most feasible explanation to account for the effect of 2,4-dichlorophenol would be that prior application of the enzyme co-factor resulted in increased IAA-oxidase activity at the abscission zone sufficient to decrease the level of auxin from the later treatment to below an effective concentration.

Several current theories regarding abscission control have attempted to explain several diverse observations through a common channel; that of ethylene biosynthesis (1,4). In vegetative poinsettias Gawadi and Avery (10) found that 2.8 ppm ethylene chlorohydrin caused intact poinsettia leaves to abscise as rapidly as deblading. Among studies of plants in flower Phan (14) working on iris and tulip and Nichols (13) on carnations found considerable ethylene evolution and a surge associated with wilting and petal drop.

In view of the many positive results for an ethylene effect the lack of stimulation of poinsettia leaf or bract abscission is surprising. Similar experiments were conducted on 3 separate occasions; twice using plants at anthesis and once with senescing plants and in no case was a positive result found. Bract and leaf petioles on treated explants had an epinastic response typical of ethylene treatment.

The results of this study apparently are in sharp contrast to those of Gawadi and Avery (10). The 2 methodologies differed in several respects and may be responsible for the anomalous results. The earlier work applied ethylene chlorohydrin to actively growing vegetative plants while the present study used flowering plants with mature leaves and bracts and applied gaseous ethylene. It is possible that ethylene chlorohydrin and ethylene do not act the same particularly as they found that ethylene chlorohydrin could overcome the abscission delay resulting from a previous treatment with 1% NAA and also that it induced abscission of younger leaves first. These effects are not typical for ethylene as found in other studies (2,16,15).

One possible reason for the lack of stimulation by ethylene may be that ethylene was always added immediately after deblading and discontinued after 24 hours. Rubinstein and Abeles (15) found that ethylene became effective in stimulating abscission of bean petioles only after explants had aged for 12 hours. This agrees with the 2 - stage process of abscission (16) in which ethylene is effective only during the second stage. It is conceivable that after 24 hours the poinsettia was still in stage 1. The diminished delay of abscission resulting from the application of auxin 24 hours after deblading would support this. The only effect common to poinsettias, both bracts and leaves, and other explants was a delay of abscission with auxin. Belated application of IAA may act in slowing the rate of abscission development already initiated. The ineffectiveness of abscission accelerators when applied alone or with lower rates of IAA could be a result of transport time to the site of activity being sufficiently long that the auxin level has dropped below the threshold value.

One of the aims of this study was to elucidate, by analogy, a possible endogenous abscission control system which could explain the differential rates of abscission found among poinsettia cultivars. Secondary division, prerequisite for separation, has been completed in the mature leaf or bract (10). When an explant is made by removing the leaf blade there is insufficient auxin in the petiole to delay the onset of the abscission process for a significant length of time. In the bract petiole there is sufficient endogenous auxin easily transported to the abscission zone to delay abscission in proportion to the limited amount of auxin. This could account for the increased longevity of bracts over leaves and also the residual varietal difference in bract petiole abscission.

In every explant study each cultivar responded the same relative to its own control. This would argue for one common control system. Therefore, a system with auxin as the central control and differences in the amount of diffusible auxin among cultivars would appear to be the most feasible. Above a certain threshold concentration auxin delays the onset of abscission of a bract. When the effective concentration of diffusible auxin falls below the threshold at any of the well defined abscission zones then abscission results. This could come about through decreased synthesis, impaired transport or increased enzymatic destruction.

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ARTICLE II

REGULATION OF ENDOGENOUS INDOLEACETIC ACID AND KEEPING QUALITY OF POINSETTIA

Regulation of Endogenous Indoleactice Acid and Keeping Quality of Poinsettia

Abstract. A study was made of the changes with aging in the level of endogenous auxin and the activity of IAA-oxidase in the bracts of 2 poinsettia cultivars.

The auxin level decreased with time in both cultivars but faster in the poor keeper. The activity of the IAA-oxidase system and the level of hydrogen peroxide increased with aging. The auxin level could be related to the activity of IAA-oxidase. The IAA-oxidase activity could be related to the appearance of new forms of peroxidase. A hypothesis is developed to explain differences in keeping quality among poinsettia cultivars based on changes in the level of endogenous auxin.

INTRODUCTION

Keeping quality of poinsettias is dependent on the rate of bract abscission and wide differences exist with respect to this factor among cultivars. Indoleacetic acid (IAA) at concentrations above 10^{-5}M applied to the petiole stump has been found to delay abscission of explants (10). This is also true in poinsettias for leaves and bracts. Studies with intact plants have indicated a close relationship between the level of endogenous diffusible IAA and the propensity to abscise (19). A decrease in the level of auxin induces abscission. Several hypotheses have been advanced to explain this auxin effect *in vivo* subscribing to a direct auxin effect (5), auxin interacting with aging and senescence (2), or auxin interacting with ethylene (1). The level of diffusible auxin may be regulated through snythesis, transport, or destruction. Control of abscission in cotton has been related to the activity of the IAA-oxidase system (18).

This study was conducted to determine endogenous auxin relationships in the poinsettia. Experiments were designed to measure changes in the level of growth regulators and especially IAA with normal aging and after experimental treatment. A study of the IAA-oxidase system was undertaken to ascertain its role in the regulation of the level of IAA.

MATERIALS AND METHODS

The poinsettia cultivars Paul Mikkelsen, White Ecke, New Ecke Pink and Barbara Ecke Supreme were used in these studies. Paul Mikkelsen, a good keeping cultivar, and Barbara Ecke Supreme, a poor keeping type, were used in more intensive studies. Cultural methods, experimental methods, and preparation of explants were as described in Article 1.

Both extractable and diffusible growth regulators were assayed by the *Avena* coleoptile straight growth bioassay. Five gram samples of both healthy and senescent freeze dried leaf and bract tissue from Paul Mikkelsen and Barbara Ecke Supreme were extracted 24 hrs at 0° with 2x 75 ml aliquots of methanol and rinsed with a third. The methanol extracts were combined, dried in a flash evaporator and redissolved in 75 ml water. The pH was adjusted to 3.0 and extracted with 5x 50 ml aliquots of peroxide-free ether. The ether extract was taken just to the point of dryness in a flash evaporator and the final solution made to 1 ml with ethanol.

Diffusible growth regulators were determined on either individual entire bracts or flowering stems cut directly above the primary branch point. They were embedded in 1% agar at 21° and 100% RH in the dark and left

to diffuse for 24 hrs. The agar was extracted 3 times with 50 ml aliquots of methanol and subsequent concentration was the same as for the extractable growth regulators.

Duplicate 100 µl samples of each extract were spotted on Whatman No. 1 paper and developed in descending chromatography with isopropanol:ammonium hydroxide: water, 10:1:1 v/v for 20 cm. Each chromatogram was cut into 10 equal sections and eluted in the bioassay tube with 1 ml 0.1 M phosphate-citrate buffer pH 5.0 with 2% sucrose added. The coleoptile straight growth bioassay using <u>Avena sativa</u> cv. Brighton was done according to Nitsch and Nitsch (15). All steps were carried out at 21°.

Peroxidase or IAA-oxidase enzyme studies were carried out using fresh or freeze dried tissue. Freeze dried material was extracted for 2 hrs at 0° with sufficient 0.1 M phosphate-citrate buffer pH 6.0 to give a final protein concentration of approximately 1 mg/ml (usually 25 ml/gm tissue). For analyses using fresh tissue only the petioles were used. They were coarsely chopped then homogenized in a glass homogenizer at 0° with 5 volumes of phosphate-citrate buffer. In either case the extracts were given minimal further treatment as recommended by Steward and Barber (21). This usually involved a 3,000 x g centrifugation for 5 min followed by a second centrifugation at 100,000 x g, for 30 min all at 0°. The supernatent was used directly for enzyme activity or gel electrophoresis studies.

For studies on the kinetics of IAA-oxidase the method of Meudt and Gaines (14) was followed. The best results were found when 0.5 ml of the enzyme extract was incubated for 30 min in the dark at 21° with 1.5 ml of the incubation mixture which contained 0.5 mM IAA, 0.2 mM 2,4-dichlorophenol and 0.1 mM H_2O_2 in 0.01 M phosphate-citrate buffer pH 6.1. The reaction was stopped after 30 min with the addition of 1 ml of 1% p-dimethylamino-cinnamaldehyde in 2N HCl and the color read at 562 mμ after a further 30 min.

The method for vertical disc gel electrophoresis was based on that of Davis (3). Routinely 0.2 ml enzyme extract was run in 7% polyacrylamide gel at pH 8.3 with 2.5 m amps/gel. After running, peroxidase activity was developed by incubating the gels in a mixture of 5 mM guaiacol and 1 mM H_2O_2 in 0.1 M phosphate buffer pH 6.1. Color developed rapidly and the reaction was stopped by flooding with 10% acetic acid.

Perioxide assay was based on the method of MacNevin and Urone (13). Fresh petioles were homogenized in excess cold ethanol. Peroxide was complexed by the addition of 0.5 ml of 12.5% $TiCl_4$ and precipitated with excess NH_4OH. After centrifugation for 5 min at 2,000 x g the supernatent was discarded and the precipitate solubilized in 10 ml of 3.0 N HCl. The yellow color was read at 405 mμ.

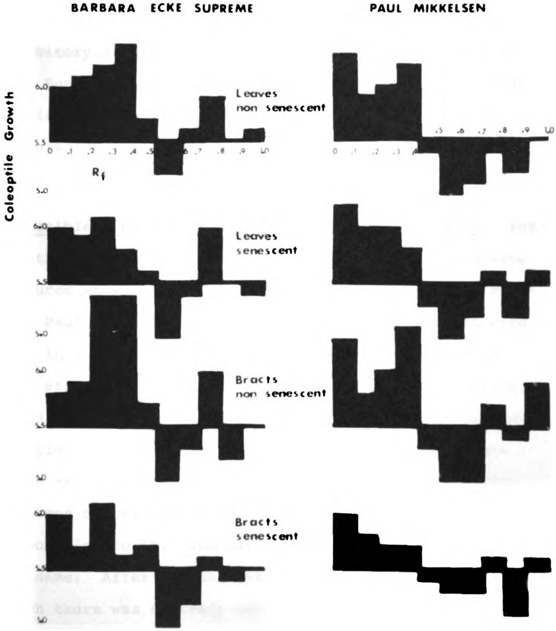
RESULTS

Extractable growth regulators. The results of this study are shown in Figure 1. The extracts contained a complex of substances affecting coleoptile growth. There were differences between cultivars, between leaves and bracts, and between healthy and senescent tissue. Pure IAA spotted on the chromatogram for standardization was found to have an R_F of 0.3 to 0.4 with a tail in the 0.2 to 0.3 region. The equivalent region of the extract chromatograms caused stimulated coleoptile elongation and showed variation among samples. In general there was greater activity in extracts from healthy tissue as compared to senescent tissue. There was also more growth stimulation from extracts of the healthy tissue of Barbara Ecke Supreme than of Paul Mikkelsen. Healthy bracts showed greater activity than corresponding leaves. Differences between cultivars and tissues were not as apparent in the extract from senescing material.

Other zones of promotion also showed differential activity. In the zone R_F 0 to 0.1 the extracts of Paul Mikkelsen showed greater activity while in the zone 0.7 to 0.8 Barbara Ecke Supreme had the greater activity.

Figure 1. Extractable growth regulators from leaves and bracts of 2 cultivars of poinsettia at anthesis and the time of abscission measured as the effect on the growth of *Avena* coleoptile sections.

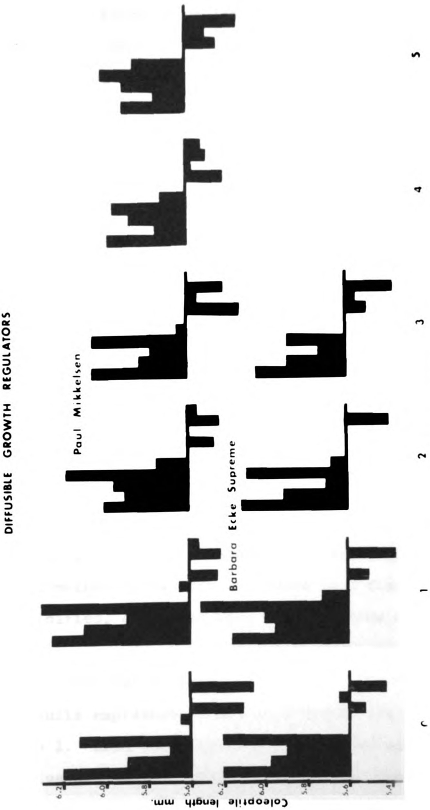
EXTRACTABLE GROWTH REGULATORS



There were zones of active inhibition and these too showed differences among samples. The zone from 0.6 to 0.7 showed less activity in the leaf samples of Barbara Ecke Supreme while the zone 0.8 to 0.9 showed greater inhibitory activity in Paul Mikkelsen. There were more inhibitory substances in Paul Mikkelsen than in Barbara Ecke Supreme. In comparing mature healthy and senescent abscised tissue the complex of stimulatory substances diminished while the inhibitory substances remained relatively static.

Diffusible growth regulators in the course of aging. The growth regulators diffusing from the flowering head were measured at weekly intervals on 2 cultivars of poinsettia, Paul Mikkelsen and Barbara Ecke Supreme, which were held in a simulated home environment. The results of this study are presented in Figure 2. There are definite zones of stimulation and inhibition on the chromatograms. Relative to the study on extractable growth regulators there are fewer, more clearly defined zones of activity. The zone corresponding to IAA, R_F 0.3 to 0.4, stimulated coleoptile growth. The pattern for both cultivars was the same. After 1 week there was a small increase after which there was a steady decline. Extracts from Barbara Ecke Supreme declined in activity more rapidly. A second major zone of stimulation 0 to 0.1 also changed with time.

Figure 2. Diffusible growth regulators from flowering heads of poinsettia sampled at weekly intervals after anthesis measured as the effect on the growth of *Avena* coleoptile sections.


DIFFUSIBLE GROWTH REGULATORS
Paul Mikkelsen
Barbara Ecke Supreme
Coleoptile length mm.
6.2
6.0
5.8
5.6
6.2
6.0
5.8
5.6
5.4
C
1
2
3
4
5
Weeks in home environment

In both cultivars it steadily declined at approximately the same rate. The zones 0.1 to 0.2 and 0.2 to 0.3 could be separate growth regulators or tails of the 2 major peaks. The zones 0.6 to 0.7 and 0.8 to 0.9 were the only ones consistently active as inhibitors of coleoptile growth. No gross difference was apparent between the cultivars nor was there any consistent change with time for the inhibitors.

Evidence to support the hypothesis that stimulation in the zone R_F 0.3 to 0.4 was due to endogenous IAA was obtained by colorimetric measurement of diffusate after reaction with Salper reagent (7). Twenty bracts were sampled at 6 day intervals from plants of Paul Mikkelsen and Barbara Ecke Supreme held in a home environment from the time of anthesis. Half the plants of each cultivar had been given a single spray with 10^{-3} M IAA at anthesis. Bracts were cut at the base of the petiole and diffused into 1% agar for 24 hours. The diffusate was eluted with methanol, reduced in volume in a flash evaporator, acidified, extracted with peroxide-free ether, dried and finally taken up in 1 ml methanol which was used directly in the colorimetric reaction.

The results expressed in IAA equivalents are presented in Table 1. Both the untreated and treated samples showed a relative decline in diffusible IAA with time. The treated samples showed higher levels of IAA throughout but the treatment differential decreased with time in both

Table 1. Diffusible IAA from bracts of 2 poinsettia cultivars at different sampling times after anthesis in μg IAA/10 bracts. Half the plants were sprayed with 1 mM IAA at anthesis.

	Days after Anthesis			
Treatment	0	6	12	18
Paul Mikkelsen				
untreated	6.1	6.7	5.3	4.9
treated		9.2	8.0	5.4
Barbara Ecke Supreme				
untreated	5.9	5.8	4.4	3.0
treated		7.6	6.0	4.5

cultivars. Between cultivars, the IAA level in Barbara Ecke Supreme declined more rapidly.

IAA-oxidase activity. The use of crude enzyme preparations from red or pink bracted cultivars gave a color to the final solution that was impossible to distinguish from a positive test for IAA degradation products. Activity was therefore expressed as the difference in optical density, after incubation, between an active enzyme preparation and a second aliquot of the same enzyme inactivated by holding for 3 min in boiling water. The addition of H_2O_2 to the incubation mixture resulted in what was apparently considerable non-enzymatic destruction of IAA. For studies involving the addition of H_2O_2, activity was based on the difference between an active and an inactive enzyme preparation both with added H_2O_2. Data showing the inactivated enzyme, peroxide, and active enzyme components in IAA destruction are presented in Table 1 of the appendix.

Preliminary experiments using freeze dried bract material of Paul Mikkelsen, White Ecke, New Ecke Pink and Barbara Ecke Supreme indicated different activities of the enzyme among cultivars and at different sampling times (Table 2). There was also a marked differential in response to added peroxide. Both at anthesis and senescence Paul Mikkelsen and White Ecke had a lower level of enzyme

Table 2. IAA-oxidase activity in bracts of 4 poinsettia cultivars sampled at anthesis and senescence with and without H_2O_2 in the incubation solution. Activity measured in ΔOD at 562 mμ after reaction with p-dimethylaminocinnamaldehyde.

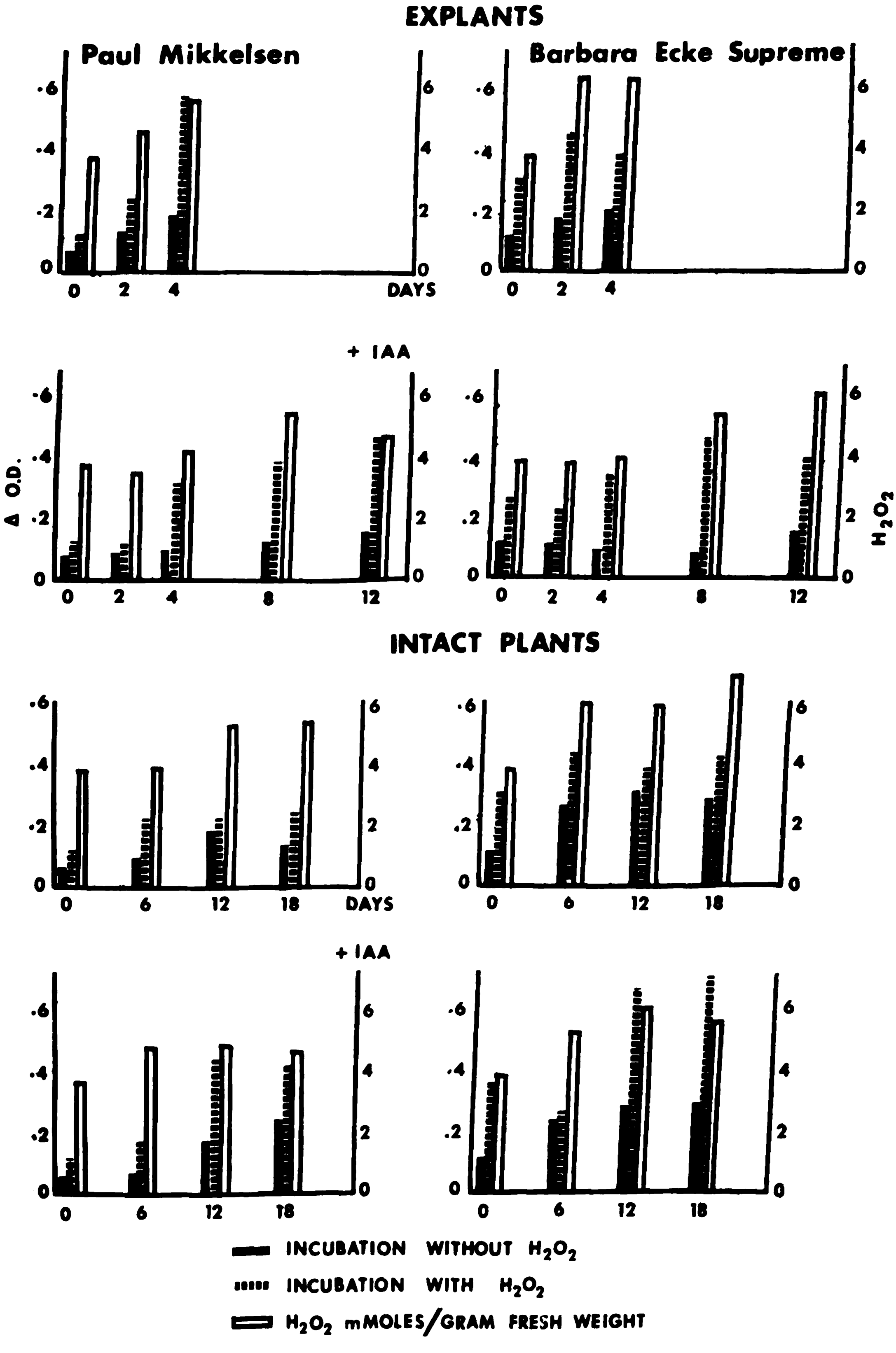
Cultivar	Anthesis		Senescence	
	Control	H_2O_2	Control	H_2O_2
Paul Mikkelsen	0.21	0.38	0.19	0.71
White Ecke	0.18	0.35	0.15	0.74
New Ecke Pink	0.32	0.46	0.39	1.13
Barbara Ecke Supreme	0.28	0.45	0.26	0.94

activity than the other 2 cultivars either with or without added peroxide. New Ecke Pink had the highest level of activity and was the only cultivar to show an increase in IAA-oxidase between anthesis and senescence without the addition of peroxide. The others showed a slight decline in activity. The addition of 0.1 mM H_2O_2 increased the activity of IAA-oxidase and had a much greater activating effect on extracts from senescent tissue.

The time course relationship for the activity of IAA-oxidase and the level of endogenous peroxide in intact and explant poinsettias was studied using freshly harvested bract petioles. The cultivars Paul Mikkelsen and Barbara Ecke Supreme were prepared as explants and held in the dark or as intact plants held in a simulated home environment. Half the explants were treated with 1 mM IAA in lanolin applied to the bract petiole stumps and half the intact plants were given an initial spray of 1 mM IAA. Duplicate measurements of IAA-oxidase activity were made at 6 day intervals with and without 1 mM H_2O_2 in the incubation mix. The results are shown in Figure 3.

The peroxide level showed a general increase with time. Explants and intact plants treated with IAA had a slower rate of increase. Although the 2 cultivars had the same level at anthesis Barbara Ecke Supreme increased

Figure 3. The activity of IAA-oxidase and the level of endogenous hydrogen peroxide in explants and intact plants of 2 cultivars of poinsettia sampled at various times after anthesis. IAA treatment was applied at anthesis.


EXPLANTS
Paul Mikkelsen
Barbara Ecke Supreme
+ IAA
INTACT PLANTS
+ IAA
Δ O.D.
H_2O_2
0
·2
·4
·6
0
2
4
6
DAYS
0
2
4
8
12
0
6
12
18
INCUBATION WITHOUT H_2O_2
INCUBATION WITH H_2O_2
H_2O_2 mMOLES/GRAM FRESH WEIGHT

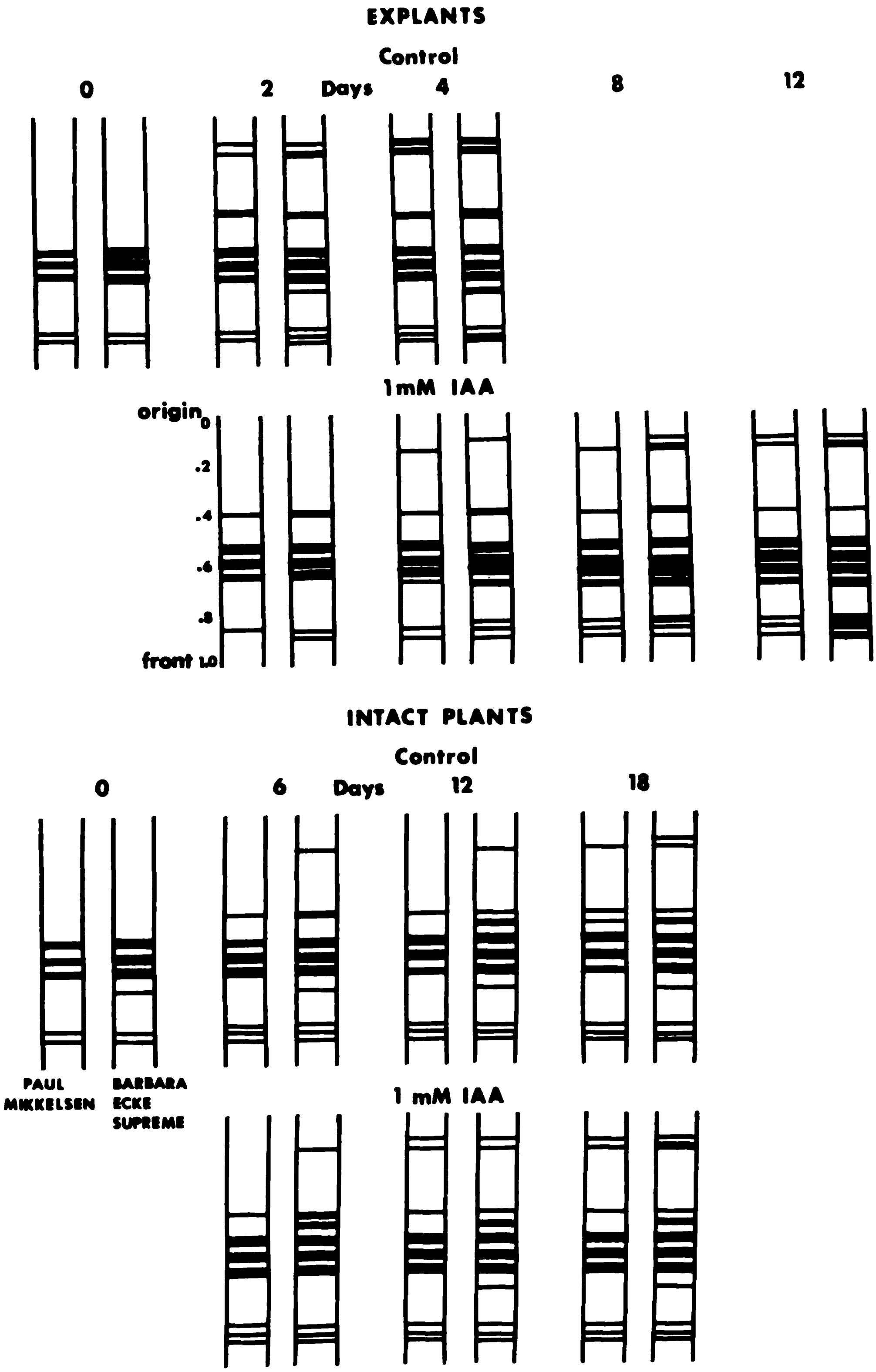
more rapidly and had a higher level at the termination of sampling.

For IAA-oxidase without added peroxide the general trend was for a small increase in activity with increasing age. In IAA treated explants however, there was no apparent trend. The addition of peroxide during incubation resulted in increased activity in all samples and the trend with time was for a general increase disproportionately larger than without peroxide. Initial treatment with IAA delayed the surge of IAA-oxidase activity with peroxide in intact plants.

The poor keeping cultivar, Barbara Ecke Supreme, had a higher initial activity than did Paul Mikkelsen. This was the case both with and without peroxide. Explants of Paul Mikkelsen appeared to lag, equalling the IAA-oxidase activity of Barbara Ecke Supreme but at a later sampling time.

Gel electrophoresis of peroxidase enzyme. Aliquots of the enzyme extract were run on acrylamide disc gels and developed with guaiacol, peroxide and IAA. The addition of 5 mM IAA to the incubation mix did not reveal any new bands nor noticeably alter the relative intensity of staining but did appear to result in sharper, more clearly defined bands. The zymogram of the electrophoretic patterning is shown in Figure 4. The approximate intensity

Figure 4. Zymogram of the forms of peroxidase of 2 cultivars of poinsettia prepared as explants or left intact sampled at various times after anthesis. IAA treatments was applied at anthesis.


EXPLANTS
Control
0
2
Days
4
8
12
1mM IAA
origin 0
.2
.4
.6
.8
front 1.0
INTACT PLANTS
Control
0
6
Days
12
18
PAUL MIKKELSEN
BARBARA ECKE SUPREME
1 mM IAA

of the staining of an isoenzyme band is indicated by the width of the line in the zymogram.

In all cases there were multiple forms of the enzyme and the greatest number visible in one gel was 11. As a general rule the number of isoenzymes and relative intensity of staining increased with age for all samples. Three heavy staining bands at R_F 0.55, 0.60, 0.65 appeared in every instance as did, in general, the light staining bands at R_F 0.85 to 0.90. Other bands, notably at R_F 0.10 to 0.15, 0.40 to 0.45 and 0.70 varied with cultivar, treatment and age. The single band R_F 0.70 was confined to the cultivar Barbara Ecke Supreme except for IAA treated plants where it was most intense and in both cultivars. The 1 or 2 bands at R_F 0.40 to 0.45 appeared after anthesis and developed more intense staining with time in Barbara Ecke Supreme. The second band appeared in intact plants of Barbara Ecke Supreme only. The 2 bands at R_F 0.10 to 0.15 were absent at anthesis but developed into intensively stained bands with time. They were more prominent in explants than in intact plants. Prior treatment with IAA delayed the development of these bands in explants but stimulated their development in intact plants, particularly in the cultivar Paul Mikkelsen.

In overall isoenzyme patterning, explants showed fewer bands but more intensive staining. The cultivar

Barbara Ecke Supreme showed more bands and deeper staining than did Paul Mikkelsen. Treatment with IAA appeared to delay the development of new bands in explants and to stimulate their formation in intact plants.

DISCUSSION

The composite results of this research provide sufficient basis for the development of a hypothesis to explain the control of abscission in poinsettia bracts and hence differences in keeping quality. Auxin above a certain level results in bract retention; when it falls below this level senescence and abscission result. Studies of the enzymatic destruction of IAA by IAA-oxidase indicated a differential enzyme activity between cultivars that could account for the observed differences in auxin level.

Data from studies involving exogenous application of growth regulators support this thesis. The only regulator tested that had any consistent effect was IAA. It could substitute for the bract blade in inhibiting abscission of debladed petioles.

The data from the study on extractable growth regulators showed considerable differences in the IAA zone. Other zones active in the bioassay likely contain complexes of hormones, anti-metabolites, and other compounds capable of affecting coleoptile growth but less likely to exert any considerable influence on abscission *in vivo*.

The study on diffusible growth regulators substantiated the preliminary findings and gave a measure of the relative rate of decline in functional auxin. The colorimetric analysis of diffusible IAA provided further verification by a different technique. Both colorimetric analysis of control samples and zone R_F 0.3 to 0.4 of the chromatograms from the diffusible growth regulator study gave very similar patterns. They showed a maintenance or slight increase in auxin over the first week after anthesis followed by a steady decline. It is in the rate of decline that the major difference between cultivars is apparent. Barbara Ecke Supreme had a more rapid loss of IAA than did Paul Mikkelsen. Jacobs (10) showed a negative correlation between diffusible auxin level and propensity to abscise. This, together with the present evidence would seem to justify assigning control of abscission of poinsettia bracts to endogenous diffusible IAA.

The zones R_F 0 to 0.1 and 0.1 to 0.2 of the diffusible growth regulator study stimulated coleoptile growth. The pattern with time was similar to that of the IAA zone. It is possible that these zones contain a bound form of auxin.

The results indicated a differential capacity between cultivars for enzymatic oxidation of IAA which may account for the differential decline in auxin level. The activity of IAA-oxidase must be considered together with the effect of added hydrogen peroxide and the endogenous concentration of this compound. Although the level of enzyme activity for the 2 cultivars at anthesis was the same without peroxide the addition of peroxide resulted in considerably more activity in extracts from Barbara Ecke Supreme.

The addition of peroxide exaggerated differences in enzyme activity while in itself it was subject to differential increase with time. The increase in peroxide may be an indication of incipient senescence. Peroxide may act in enzymatic destruction as a co-factor for IAA-oxidase, in non-enzymatic destruction of IAA and in the appearance of new forms of peroxidase through substrate derepression. Its effect as an enzyme co-factor is apparent from the results of the present study and previous investigation (9, 17) and its ability to non-enzymatically degrade IAA was observed in enzyme blank samples as well as being documented in other tissues (20, 22). The possibility that peroxide controls the formation of new isoenzymes of IAA-oxidase or peroxidase is demonstrated in a comparison of the changes with time in the level of peroxide, the activity of IAA-oxidase and the forms of peroxidase in any sample. An increase in endogenous peroxide was coupled with the appearance of new enzyme forms and in increased enzyme activity particularly with added H_2O_2 as co-factor.

There does not appear to be as strict a relationship between the behavior of explants and intact plants of 1 cultivar as between the 2 cultivars when both were either intact or prepared as explants. The increased peroxide level in aged intact bract petioles was associated with a smaller response to H_2O_2 in terms of increased activity and fewer new isoenzymes as compared with aging explants. This could be interpreted as a modifying effect of the bract blade.

The difference between explants and intact plants both with and without prior treatment with IAA would obviate a simple hypothesis with one control for peroxidase enzyme. Buildup of free peroxide could induce the formation of new forms of peroxidase and hence increased enzyme activity through substrate derepression or co-factor activation. Ockerse, Siegel and Galston (16) and Lavee and Galston (11) have shown that the addition of IAA to cultured pith tissue can both inhibit and stimulate the formation of new peroxidase bands and that it acts through repression-derepression. They suggest that it may act through ethylene mediation but the results of this study present the alternative that it may be acting through control of the peroxide level. A primary effect of auxin is to delay aging (2) and one effect of the aging process is the uncoupling of oxidative systems and the accumulation of free peroxide. It is suggested that prior treatment of explants with IAA which delayed abscission acted through this scheme.

In intact plants the situation is somewhat altered. The poor keeping cultivar, Barbara Ecke Supreme, was shown to build up higher levels of peroxide and a more active IAA-oxidase system and to show the appearance of new forms of peroxidase sooner when compared to the good keeping cultivar, Paul Mikkelsen. However quantitatively similar increases in peroxide level did not elicit a quantitatively concomitant increase in enzyme activity or new isoenzymes when compared to explants. The difference between explants and intact plants may be explained on the assumption of a second compound diffusing from the bract blade and able to modify the effect of peroxide. This could be through repression or inactivation. There is no real evidence as to the nature of this hypothetical second compound; however, the similarity between aging nontreated intact plants and IAA treated explants would suggest that it could be endogenous IAA. This assumption would give IAA a dual role in inhibiting abscission: delaying the accumulation of free peroxide through maintained growth, and inhibiting the formation of new forms of IAA-oxidase possibly through gene repression.

Prior treatment of intact plants with IAA results in apparently conflicting evidence. In this case there is a smaller increase in peroxide yet an increased enzyme activity and more isoenzymes developed relative to respective control samples. The new peroxidase forms appear electrophoretically identical to those induced by accumulation of peroxide. Thus, it would appear that high levels of IAA and/or peroxide are able, either directly or indirectly, to induce the formation of the same new forms of peroxidase which would then function in decarboxylating the former and reducing the latter.

Endo (4) has shown that IAA-oxidase and peroxidase may be both qualitatively and quantitatively different on a zymogram and that an analysis based solely on peroxidase may be misleading. Attempts to assay for IAA-oxidase activity on a gel by adapting the p-dimethylaminocinnamaldehyde method of Meudt and Gaines (14) did not prove consistent enough for routine experimental analysis but those observations which were possible showed IAA-oxidase activity in the 3 consistently heavy staining bands R_F 0.55 to 0.65 and, in older tissue, also in the bands R_F 0.10 to 0.15. If this does indicate the actual case for IAA-oxidase in poinsettia then it would only serve to fortify the hypothesis since the zymogram bands R_F 0.10 to 0.15 showed a close parallel to changes in the peroxide level and IAA-oxidase activity.

The IAA-oxidase system in poinsettia appears to differ from that in other plants in its requirement for a phenolic co-factor. In oat coleopitles (8), raw extract from peas (6), or cotton (18), and purified extract from horseradish (9) the IAA-oxidase system requires a monophenol such as 2,4-dichlorophenol as co-factor but not hydrogen peroxide. Raw extracts from corn leaves (12) have an inhibitor which stops any reaction from proceeding. After removal of the inhibitor the purified extract requires both a monophenol and H_2O_2 for activity. Raw extract from poinsettia did not respond to added 2,4-dichlorophenol. However if the extract were purified by passing through a column of Sephadex G-25 then 2,4-dichlorophenol was necessary for activity (Table 2 in appendix). The inference that may be drawn from this is that raw extract and the in vivo system contains sufficient phenolic co-factor that it is not limiting. Conceivably this phenol could account for the distinct zone of inhibition R_F 0.8 to 0.9 (Figure 2).

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APPENDIX

GENERAL INTRODUCTION

The poinsettia Euphorbia pulcherrima Willd ex. Klotzsch is an economically important container grown flower crop. Recently it has become the subject of considerable research, both on cultural practices and fundamental plant physiology. A problem of interest from both aspects is that of keeping quality. The recent introduction of several new cultivars with improved keeping quality has revitalized the importance of this plant as a desirable ornamental and directed attention to determining the heritability and physiology of bract and leaf retention.

Keeping quality in the poinsettia is a matter of bract and leaf retention. Color fading, particularly bract greening of white bracted cultivars, may be observed under high light intensity but rarely is the primary cause of discarding a plant. The true inflorescences, cyathia, tend to abscise within a few days to a few weeks under home conditions leaving an unattractive center on the plant. This loss of cyathia is not usually considered critical from the keeping quality standpoint. A third undesirable aspect of aging poinsettia plants is the propensity for the leaf axillary buds to commence growing. These are normally removed from a well tended plant.

As used in this research, keeping quality is defined as the time for sufficient bracts and/or leaves to abscise from a container grown plant held under defined environmental conditions for that plant to be considered undesirable.

Since abscission is the major contributing factor to the keeping time of a poinsettia plant the pattern of leaf and bract drop should be described. Careful examination of a plant reveals two classes of leaves and two Classes of bract. Each has a unique pattern of abscission and the same classes were found in all cultivars studied. Class I are the propagation leaves that were either developed or undergoing rapid expansion while the vegetative cutting was being rooted. They are smaller, thicker and a lighter green in color and abscise soon after the plant is mature.

Class II leaves are those which are developed after the rooted cutting is transplanted to the container. They are typically large, dark green, succulent and comprise the majority of the foliage on a mature plant. These leaves generally remain on the plant longer and abscise at random, irrespective of their position on the stem.

Class I bracts, herein termed primary bracts, are those which subtend the first inflorescences. They occur as a whorl of 3 to 6 bracts below the first floral branch of the stem. In general they are larger, thicker bracts and the last to abscise. Class II or secondary bracts are those borne singly on the flowering branches. These bracts account for most of the appeal of the plant. There is a tendancy for them to abscise in the same order as they were developed, from the center of the plant toward the growing point but this may vary considerably.

Leaf abscission occurs in one manner but two distinct types of bract abscission have been observed. In the normal course, leaf abscission is characterized by a loss of green color from the leaf blade, followed quickly by separation at the base of the petiole (23). Bracts may abscise either singly or in groups. Single abscission is typified by loss of color and flacidity of the bract followed by separation at the base of the petiole. The multiple loss of bracts is as a secondary result of flowering stem abscission. In this case the stem separates at a point immediately distal to any inflorescence or branch point. The bracts do not usually show the senescence syndrome characteristic of the single abscission type. Primary bracts abscise singly while secondary bracts may fall either way. Inflorescences also abscise either singly or attached to an abscising stem.

Keeping quality is under genetic control and is a major aspect of any breeding program. The research which has been done to elucidate the nature of this genetic control has shown that keeping time is inherited quantitatively (53) and that there is independent inheritance for leaves and bracts. Good keeping quality does not show linkage with other known loci.

Cultivars vary considerably in their keeping times. Environment and handling also plays a part but, in general, in a home environment, the largest component is the heritable one. The cultivar "White Ecke," a seedling of unknown parentage, has been the source of good keeping in all the new cultivars. The wild poinsettia does not have good bract holding ability. Breeding for improved keeping quality has been a matter of selection based on empirical response in a simulated home environment test. This form of test is costly, time consuming and inaccurate unless adequate control plants are used. A more complete understanding of the nature and control of the abscission process in poinsettia may lead to a more critical and less time consuming test.

The poinsettia has not been used extensively as a test plant in fundamental research on abscission. There are arguments to support a greater emphasis on this species. It is an economically important crop. It also offers the possibility of conducting simultaneous experiments on two different tissues, leaves and bracts. Clearly defined abscission zones are fully developed in a mature poinsettia, requiring only a trigger to initiate the abscission process. Finally, there are cultivars encompassing the gamut of holding times and within each cultivar genetic consistency is maintained through vegetative propagation.

The chosen approach to the problem was to study selected cultivars with a wide range of keeping times; following their endogenous regulating mechanisms in relation to abscission rates and studying their responses to experimental treatment. The nature of the abscission control mechanism was the ultimate goal of this research.

Metabolic Changes in the Poinsettia in Relation to Aging, Senescence, and Abscission

INTRODUCTION

Abscission is the natural termination of the process of aging and senescence. As used in this study aging is defined as the normal development of a leaf or bract with respect to time and senescence as the irreversible breakdown of normal cellular functions leading to death. Abscission is the separation of the leaf or bract from the stem which, in poinsettia, occurs at the base of the petiole. Abscission is not a passive stage but rather an active one usually requiring secondary cellular division in the abscission zone, dissolution of the middle lamella, separation, and subsequent secondary thickening of the abscission scar. Gawadi and Avery (23) found this to be the case in the poinsettia.

The relationship between aging and abscission would appear to be the gradual development of an increased propensity to abscise with age. Chatterjee and Leopold (15) showed decreased response to abscission inhibition with auxin in older leaves. According to de la Fuente and Leopold (19) the correlation between senescence and abscission may be the result of lowered auxin levels,

lowered auxin responsiveness, depression of distal growth and/or the production of abscission accelerators. Thus not senescence per se, but rather some corollary of senescence is the abscission inducer.

The senescence syndrome is characterized by several physiological changes which have been causally related to abscission. Many of these may be only indirectly related to the final abscission. In general, there is a decrease in dry weight, chlorophyll, respiration rate, protein and RNA, gradually with aging and more rapidly with the advent of senescence (4,49).

Free amino acids accumulate during aging and senescence and when applied exogenously have been found to accelerate abscission. Valdovinos and Muir (56) suggested that abnormal levels of amino acids may act as anti-metabolites in normal protein synthesis. Aging leaves may also accumulate diffusible abscission stimulating substances such as abscisic acid (17,41). Another aspect of senescence is the mobilization of nutrients. Manipulation of localized centers of accumulation may either hasten or retard abscission (42,49). Accumulation proximal to the abscission zone and depletion on the distal side leads to localized cellular senescence and abscission. During the aging process mobilization occurs and, as senescence is approached, the movement is largely inward from the leaf blade (19).

Abscission mediated by exogenous ethylene is relatively independent of aging, senescence and mobilization. Gawadi and Avery (23) showed that young leaves of poinsettia could be induced to abscise without atrophy. Abeles, Holm and Gahagan (4) found that ethylene did not stimulate mobilization while stimulating abscission. Recent studies (11,19) indicate that the surge of ethylene synthesis that accompanies the preparation of explants probably acts in promoting aging which is necessary for the transition from stage I (47) which is insensitive to abscission promoters to stage II which is irreversibly induced to abscise and highly subject to stimulation.

Ethylene is normally present in many vegetative and flowering plants (10,44). It is often present at a level below the response threshold but may increase in response to some stimulus to a concentration able to initiate development. In flowering iris and tulip plants the proponderance of ethylene emanation is associated with the reproductive organs, although the accessory organs also produce a significant amount (44). There is usually a rise in the level of ethylene as cut flowers age. In carnation, a surge of ethylene is associated with the wilting and drop of the petals (39).

Although aging and senescence are considered degradative processes, abscission is a well integrated and active process. Respiration increases in the abscission zone coincident with abscission (34), and abscission is inhibited by respiratory poisons or the absence of oxygen (13). Specific m-RNA and protein synthesis is apparently necessary for abscission (2).

A recent hypothesis by Abeles (1,2) to explain abscission control involved aging and ethylene. As a leaf ages it becomes increasingly sensitive to endogenous ethylene until a threshold is reached, at which time ethylene initiates the synthesis of specific m-RNA and protein required to carry out cell wall breakdown. In his hypothesis aging and senescence are considered indirect initiates of abscission and inhibitors act through maintaining tissue in a juvenile condition. This hypothesis was the target of a series of analyses designed to monitor changes in metabolic rates and substrate levels for leaves and bracts of several cultivars of poinsettia as they underwent aging, senescence and abscission under home conditions.

MATERIALS AND METHODS

The poinsettia cultivars Paul Mikkelsen, White Ecke, New Ecke Pink, Barbara Ecke Supreme and MSU 64-5 used in this study represent a broad range of keeping times in a home environment. Rooted cuttings were transplanted 3 to a 6 inch clay pot and grown to flowering during the winter using recommended procedures, in a greenhouse at 18° NT-20° minimum DT. The plants were arranged in a random block design. When the plants reached anthesis each replicate was split with half the plants remaining in the greenhouse while half were moved to a simulated home environment. The latter was achieved by spacing the pots on benches in a laboratory with a constant temperature of 21° and 50 ft-c fluorescent and natural light.

A daily record was kept of leaf and bract abscission. Observations were made separately for propagation leaves which had expanded on the plant prior to transplanting as preliminary observation had indicated that those leaves which underwent the shock of rooting and transplanting were more prone to abscise.

Measurements were made of the respiration rate, protein and soluble nonprotein nitrogen fractions and carbohydrate reserves at 5 and 10 day intervals for the home and greenhouse environments respectively. Endogenous ethylene was monitored at irregular intervals.

Respiration was measured on leaf discs 10 mm in diameter in a standard Warburg apparatus at 21°. The discs were cut at random from the leaf blade, rinsed in distilled water and weighed. Respiration was measured over a period of 2 hr and expressed as µl O_2/hr/gm dry wt.

For nitrogen and carbohydrate analyses several leaf or bract blades, excepting the midribs, were oven dried, bulked, ground in a 40 mesh Wiley mill and 100 mg sub-samples weighed out for analysis.

Nitrogenous compounds were fractionated into three components; alcohol soluble, alcohol insoluble-base soluble and base insoluble. Samples were extracted twice with 5 ml aliquots of 80% ethanol and centrifuged at 2,000x g. The supernatent solutions were combined to form the alcohol soluble fraction. The precipitate was twice extracted with 5 ml aliquots of 1N NaOH and centrifuged at 2,000x g. The supernatent solutions made up the alcohol insoluble-base soluble fraction. All samples were then taken to dryness in a vacuum oven, digested with 3 ml concentrated H_2SO_4 containing 0.3 mM $CuSO_4$ and the nitrogen was measured in a semi-micro Kjeldhal apparatus. The nitrogen distilled over was captured in 4% boric acid indicator solution and back titrated to the methyl red-methyl blue endpoint with .01N HCl.

Sugars and starch were analysed using the anthrone test (16). Samples were extracted twice with 5 ml of 80% ethanol at 50°. After centrifugation at 2,000x g the supernatent solutions were combined, the alcohol driven off in a vacuum oven and the residue made to volume. The precipitate was gently hydrolyzed twice with 50% perchloric acid and centrifuged at 10,000x g. The supernatent was tested for sugars resulting from the hydrolysis of starch. Two ml test solution was mixed with 10 ml anthrone reagent, heated in a boiling water bath and the green color read at 630 mμ. Colorimeter readings were converted to sugar equivalents by comparison to a sucrose standard curve.

Endogenous ethylene was monitored by 2 methods. One was based on that of Young, Pratt and Biale (57). A measured volume of air, approximately 300 ml/min, was passed across intact potted poinsettia plants or explant material held in a tightly closed container. The efflux, dispersed through scintered glass, was bubbled for various lengths of time through a column of 0.25 M mercuric perchlorate in 2.0 M perchloric acid, maintained at o°. Aliquots of the perchlorate solution, containing ethylene in complex, were pipetted into serum bottles which were then sealed and the ethylene liberated by the addition of an excess of 2.0 N LiCl. Quantitative measurement of ethylene was done on a gas chromatograph. Intact poinsettia plants and leaves or flowering heads with attendant bracts removed from the plant were measured using this method.

The second method involved the extraction and direct measurement of the internal atmosphere of poinsettia leaves or bracts. A known weight of tissue was submerged in water containing Tween 20 wetting agent. The internal atmosphere was withdrawn from the plant material by exhaustive vacuum and collected by water displacement. This was then read directly in the gas chromatograph.

RESULTS

The 5 cultivars showed different patterns for leaf and bract fall. One expression of differential abscission is presented in Table 1. The 50% abscission date was based on all leaves or bracts which fell during the course of the study plus those on the plant the day it was discarded. All the leaves were developed at the time the study was begun but the flowering stems continued to grow at a reduced rate in the home environment and the greenhouse.

The 5 cultivars fell into 3 distinct keeping groups. The good keeping group consisted of the cultivars Paul Mikkelsen and White Ecke while New Ecke Pink was intermediate and Barbara Ecke Supreme and MSU 64-5 were poor keepers. The same groups and order were maintained both in days to 50% abscission and in days to discarding.

In each case a cultivar was discarded for having too few bracts. Leaf drop over the span of keeping time was largely confined to propagation leaves. Non propagation leaves which abscised did so irrespective of their position on the stem and the behavior of adjacent leaves. Most bracts abscised singly by separation at the base of the petiole. Only in the terminal stages when apparent

Table 1. Number of days to 50% bract and leaf abscission and number of days to discarding for 5 poinsettia cultivars in a simulated home environment.

Cultivar	Days to 50% Leaf and Bract Fall		Days to Discarding
	Bracts	Leaves	
Paul Mikkelsen	52	20	60
White Ecke	50	24	60
New Ecke Pink	20	14	33
Barbara Ecke Supreme	14	18	20
MSU 64-5	16	16	20

growth had ceased did stem abscission occur. All cyathia present when the plants were moved into the home environment abscised within 2 weeks. Abscission among the greenhouse plants was confined to cyathia and propagation leaves of all cultivars and a few scattered bracts from Barbara Ecke Supreme and MSU 64-5.

The pattern of leaf and bract fall with respect to time for all 5 cultivars in a home environment is depicted in Figures 1-5. In each cultivar the difference in abscission rate between propagation and normal leaves may be seen. Abscission of the propagation leaves began shortly after the plants were moved to the home environment, reached a peak between 10 and 20 days then declined until nearly all leaves had fallen by 40 days. Normal leaves abscised erratically and slower than propagation leaves. First abscission was also later; after 10 days for Barbara Ecke Supreme and after 20 days for the remaining cultivars. No peak in the time course of leaf abscission was discerned and no difference in rate among the several cultivars was apparent.

Bract abscission varied among the cultivars and it was on this basis that they were classified. Paul Mikkelsen and White Ecke, which were classified as good keepers did not commence bract drop until after 10 days and did not reach peak abscission until 20 to 40 days. Then the rate was less than 0.5 bracts abscissed per plant

Figure 1. Leaf and bract abscission rate in a home environment with respiration rate and nitrogen and carbohydrate pools in a home and greenhouse environment in days after anthesis for the poinsettia cultivar Paul Mikkelsen.

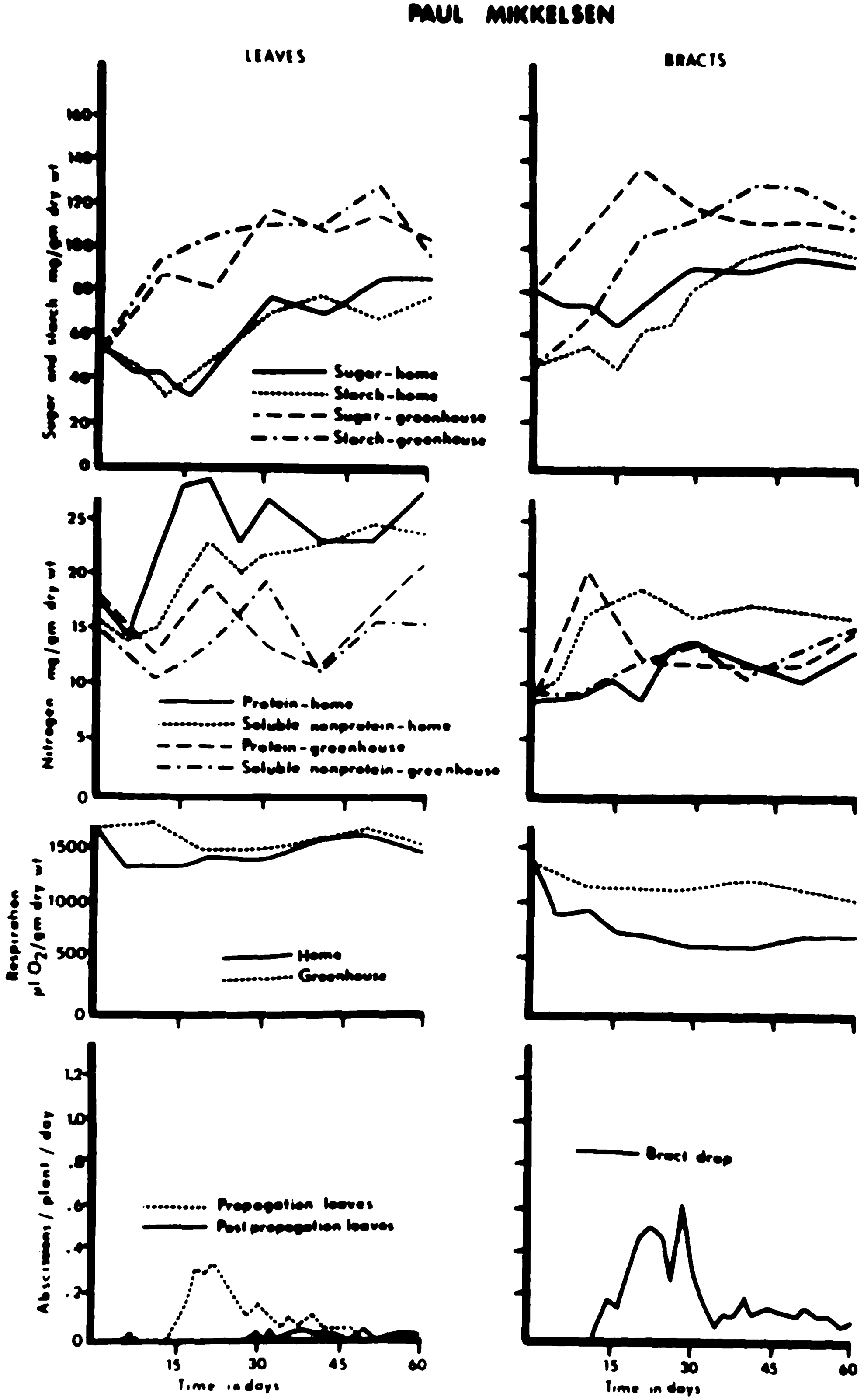

PAUL MIKKELSEN
LEAVES
BRACTS
Sugar and starch mg/gm dry wt
0
20
40
60
80
100
120
140
160
Sugar-home
Starch-home
Sugar-greenhouse
Starch-greenhouse
Nitrogen mg/gm dry wt
0
5
10
15
20
25
Protein-home
Soluble nonprotein-home
Protein-greenhouse
Soluble nonprotein-greenhouse
Respiration µl O2/gm dry wt
0
500
1000
1500
Home
Greenhouse
Abscissions / plant / day
0
.2
.4
.6
.8
1.0
1.2
Propagation leaves
Post propagation leaves
Bract drop
15
30
45
60
Time in days
15
30
45
60
Time in days

Figure 2. Leaf and bract abscission rate in a home environment with respiration rate and nitrogen and carbohydrate pools in a home and greenhouse environment in days after anthesis for the poinsettia cultivar White Ecke.

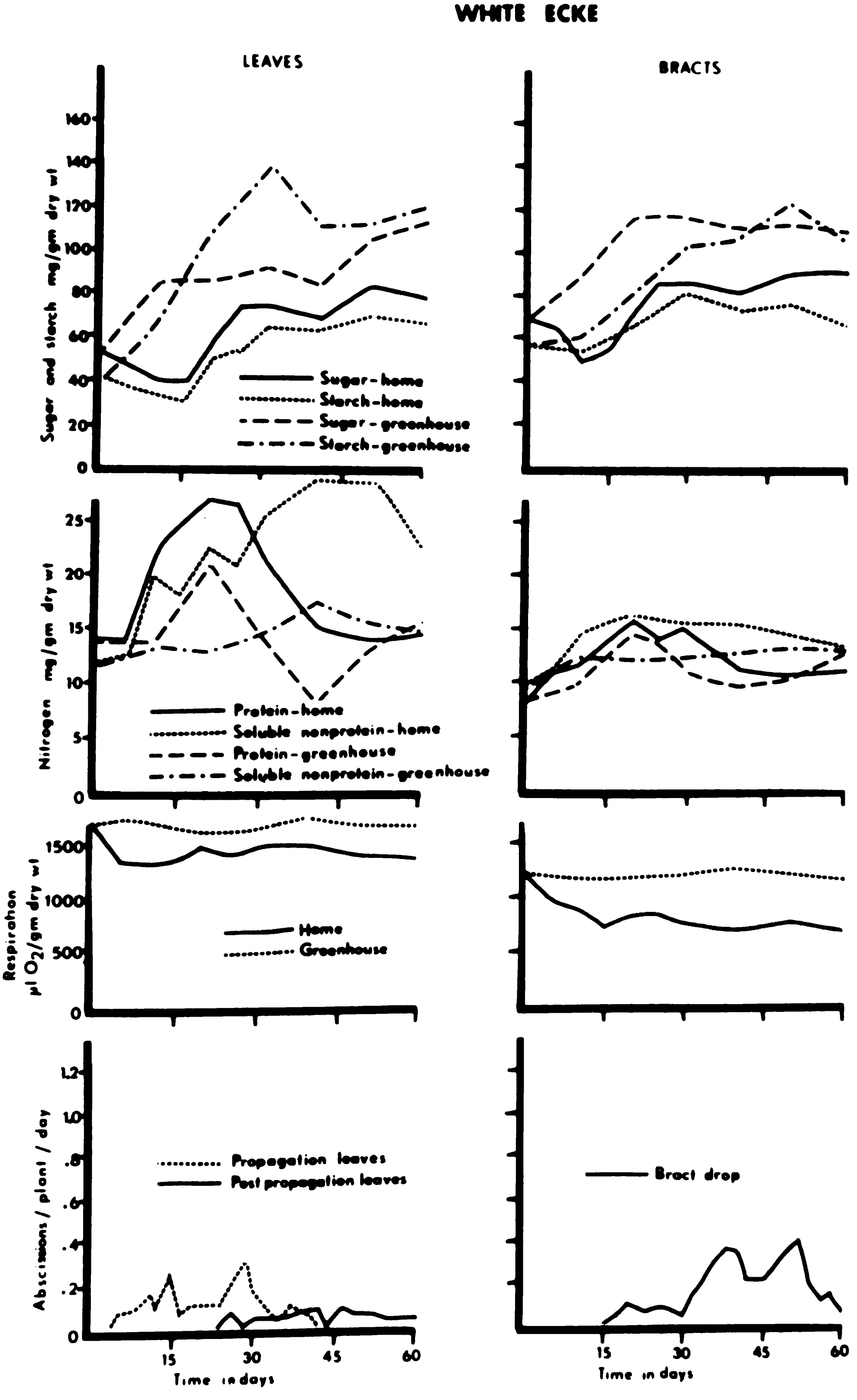
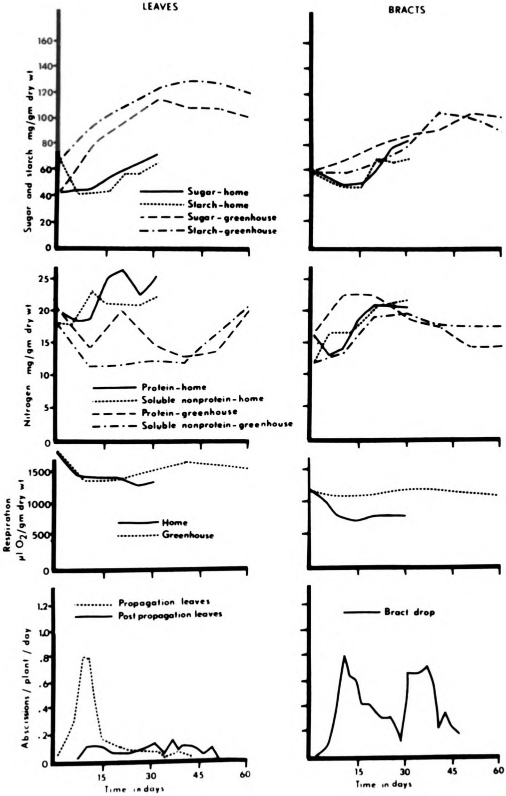

WHITE ECKE
LEAVES
BRACTS
Sugar and starch mg/gm dry wt
160
140
120
100
80
60
40
20
0
Sugar-home
Starch-home
Sugar-greenhouse
Starch-greenhouse
Nitrogen mg/gm dry wt
25
20
15
10
5
0
Protein-home
Soluble nonprotein-home
Protein-greenhouse
Soluble nonprotein-greenhouse
Respiration µl O2/gm dry wt
1500
1000
500
0
Home
Greenhouse
Abscissions / plant / day
1.2
1.0
.8
.6
.4
.2
0
Propagation leaves
Post propagation leaves
Bract drop
15
30
45
60
Time in days

Figure 3. Leaf and bract abscission rate in a home environment with respiration rate and nitrogen and carbohydrate pools in a home and greenhouse environment in days after anthesis for the poinsettia cultivar New Ecke Pink.



NEW ECKE PINK

Figure 4. Leaf and bract abscission rate in a home environment with respiration rate and nitrogen and carbohydrate pools in a home and greenhouse environment in days after anthesis for the poinsettia cultivar Barbara Ecke Supreme.

BARBARA ECKE SUPREME

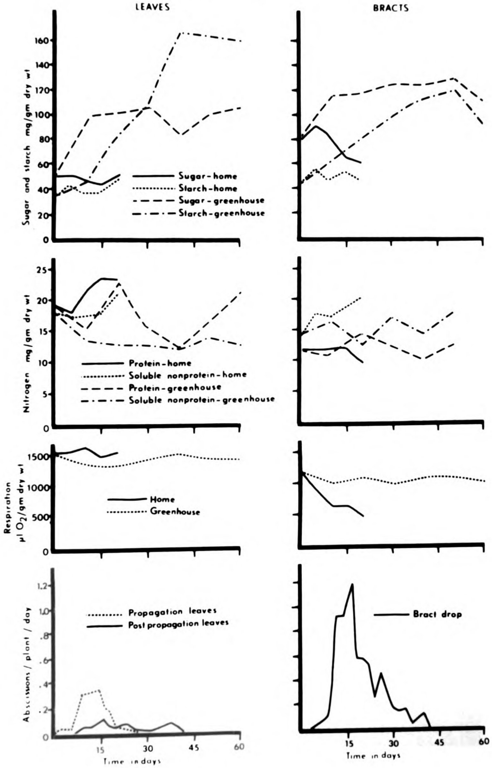
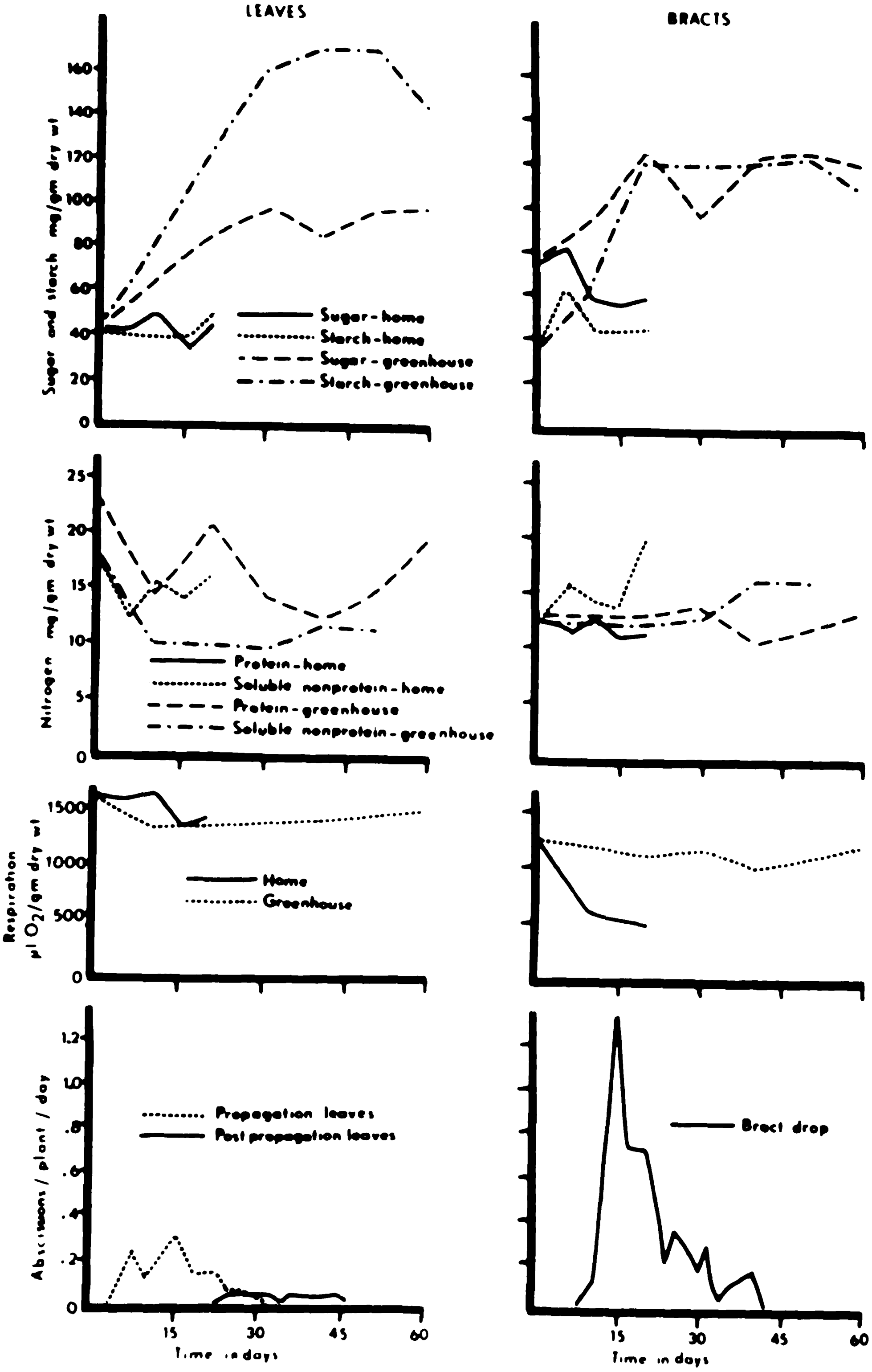


Figure 5. Leaf and bract abscission rate in a home environment with respiration rate and nitrogen and carbohydrate pools in a home and greenhouse environment in days after anthesis for the poinsettia cultivar MSU 64-5.

64-5


LEAVES
BRACTS
Sugar and starch mg/gm dry wt
160
140
120
100
80
60
40
20
0
Sugar-home
Starch-home
Sugar-greenhouse
Starch-greenhouse
Nitrogen mg/gm dry wt
25
20
15
10
5
0
Protein-home
Soluble nonprotein-home
Protein-greenhouse
Soluble nonprotein-greenhouse
Respiration µl O2/gm dry wt
1500
1000
500
0
Home
Greenhouse
Abscissions / plant / day
1.2
1.0
.8
.6
.4
.2
0
Propagation leaves
Post propagation leaves
Bract drop
15
30
45
60
Time in days
15
30
45
60
Time in days

per day. Barbara Ecke Supreme and MSU 64-5 were grouped as poor keeping types. In both, abscission began by 5 days, and reached a peak by 15 days with the rate exceeding 1.0 bracts abscised per plant per day. New Ecke Pink was intermediate in bract abscission. It began bract drop when the poor keepers did but at half the rate which it sustained up to 40 days. This cultivar maintained a reasonable appearance for 30 days even though abscission occurred for 25 of those days. New Ecke Pink characteristically forms numerous small narrow bracts and continues to develop more in a home environment.

The parameters of metabolic activity are graphically presented in Figures 1-5. Each figure is a summation of all the data for 1 cultivar and each graph depicts the changes with time for 1 or 2 related parameters, comparing plants held in the simulated home with those in the greenhouse. For bracts, data are based on samples taken at random from all bracts, irrespective of age or position. Leaf smpling was confined to post propagation leaves.

The data on respiration rate indicated consistent differences between bracts and leaves, between plants in the greenhouse and home environments, and among cultivars. The respiration rate of leaves or bracts on plants held in the greenhouse was steady while in the home environment it changed in rate with time. Each cultivar, irrespective of the environment, had a higher respiration rate in leaves than in bracts. There was no difference among cultivars for leaf or bract respiration when held in the greenhouse but there were cultivar differences in the home environment. Bract respiration was consistently less in the home than in the greenhouse but leaf respiration was not. Paul Mikkelsen and White Ecke had a lower but steady bract and leaf respiration rate. The intermediate cultivar, New Ecke Pink, had a lower bract respiratory pattern but a leaf respiratory level similar to its own greenhouse control. Barbara Ecke Supreme and MSU 64-5 had a bract respiration rate that declined steadily over the keeping period while leaf respiration was steady and above that of the greenhouse.

The nitrogen levels varied with time, environment, tissue and cultivar. Differences were also observed between protein N and soluble non protein N among the previously mentioned variables. Because all measurements were done by the Kjeldahl method, protein values are expressed in terms of protein N rather than total protein. Variability between sampling times was more apparent with nitrogen than any other analyses. Thus, without a fitted line, trends were difficult to establish. No undue variability was found between replicates of any one sample so there were probably inconsistencies in the extraction procedures between sampling times.

Leaf protein N increased with time in the home in 3 cases. The exceptions were White Ecke, where the level declined in the terminal stages and MSU 64-5 where the level remained relatively constant. In the greenhouse, leaf protein N remained relatively constant but fluctuated between individual samplings. The protein N level of the bracts in the home showed differences among the cultivars. In New Ecke Pink there was a pronounced increase following an initial drop. In the 2 poor keepers a constant level was maintained and the good keepers had small increases. In the greenhouse, bract protein N did not fluctuate except for New Ecke Pink which initially increased then declined.

The levels of soluble non-protein N increased with time in both leaves and bracts in the home environment. It was most apparent during the terminal stages in the 2 good keeping cultivars. In the greenhouse the same trend was not apparent. Rather, the levels tended to remain constant to a slight increase in the bracts.

Carbohydrate reserves varied among cultivars, and between environments though little difference was found between leaves and bracts. The sugar and starch levels may be directly compared since they are both expressed as sucrose equivalents. In every case the sugar and starch level in any cultivar-environment responded the same with time regardless of the direction of the trend.

Both starch and sugar accumulated in the leaves and bracts with time in the greenhouse plants. Large differences among cultivars were not apparent except in the greater accumulation of starch in the leaves of the poor keepers, MSU 64-5 and Barbara Ecke Supreme. In the home environment the general pattern for both sugars and starch in both leaves and bracts was an initial decline followed by accumulation. The poor keeping cultivars did not accumulate a carbohydrate reserve but, at the equivalent time, neither had the good keeping types which only had an increase in carbohydrate later. Greenhouse plants accumulated more carbohydrate, both sugar and starch, than the corresponding home samples.

As an adjunct to the study, recently abscised senescent samples of both leaves and bracts of all cultivars were collected from the plants held in the home environment and subjected to the routine analyses. The results comparing the values at anthesis and abscission are in Table 2. Many of the differences observed are typical of senescing tissue although some differences may be found among cultivars. Comparison may also be made between terminal values and trends for aging to determine if aging is a gradual approach to senescence. The respiration rate of senescent tissue was markedly lower than healthy tissue. This reinforced the trend in bracts but indicated an abrupt change in the metabolism of leaves.

Table 2. Metabolic activity and substrate levels of healthy mature (H) and abscised senescent (S) leaves and bracts of 5 poinsettia cultivars. Respiration is measured in µl O_2/gm dry wt/hr and nitrogen and carbohydrate in mg/gm dry wt.

Cultivar	Respiration		Protein N		Soluble N		Sugar		Starch	
	H	S	H	S	H	S	H	S	H	S
Paul Mikkelsen										
bracts	1355	285	8.4	4.4	9.6	4.8	52	87	49	84
leaves	1670	867	17.0	6.7	15.7	10.8	55	66	52	72
White Ecke										
bracts	1225	222	8.4	4.4	10.0	5.4	68	89	58	87
leaves	1766	938	14.1	6.8	12.0	11.2	52	74	40	77
New Ecke Pink										
bracts	1158	340	16.9	4.9	12.2	7.7	59	53	60	98
leaves	1748	790	20.6	5.6	19.2	9.7	42	62	67	102
Barbara Ecke Supreme										
bracts	1175	152	11.9	2.6	14.0	5.1	81	66	43	70
leaves	1526	914	19.4	7.7	18.9	8.4	49	75	35	86
MSU 64-5										
bracts	1232	167	12.2	3.1	11.9	5.3	77	71	37	70
leaves	1605	1010	16.3	6.0	16.8	7.9	42	72	35	92

The level of protein N decreased markedly in each case. This was not typical of the aging trends where protein either increased or maintained an overall constancy. Soluble non-protein N did not respond the same in different tissues or cultivars. In all cases it declined but most in the bracts of the poor keeping cultivars and least in the leaves of the good keepers. The terminal values are again not in proportion and in some cases not in the direction of the trends during aging.

Sugar accumulated in all leaves but only in bracts of the good keepers while it declined in the others. Starch, on the other hand, accumulated in every instance. In all cases where carbohydrates accumulated it was proportionately less than the trends during aging would have indicated.

Correlation regression analysis was performed to establish possible relationships between 1 or more of the indices of metabolic activity and differences in keeping time. Values for each parameter at each sampling date were compared with the rate of bract abscission for that date. Comparisons were made over all cultivars and for each individual cultivar except for Barbara Ecke Supreme and MSU 64-5 which were pooled since they responded similarly and each alone provided an insufficient sample. Correlations were also run between the same parameters and the rate of abscission 5 days later.

Preliminary tests had shown that a debladed bract abscises approximately 5 days later and it was felt that the condition of the bract 5 days prior to abscission would provide a more realistic comparison. Results of this analysis, expressed as coefficients of correlation are summarized in Table 3.

The results indicated a very poor degree of correlation and an inconsistency in the direction of the regression. Correlation over all samples gave a value intermediate to the individual cultivars and in no case was it a better fit than the best individual cultivar. In general the best fit was with respiration where in every case negative regression was found. Starch was also found to be negatively correlated. Sugars were generally positively correlated with the exception of Paul Mikkelsen. Soluble non-protein N appeared to be positively correlated with abscission in the good keepers and negatively correlated in the poor keepers. Protein N was negatively correlated in the poor keeping cultivars and gave the worst fit to abscission among all parameters. Very few of the correlations were significant and those which were did not indicate any particular pattern but were scattered among all parameters and cultivars. Correlation between the parameters and abscission 5 days later did not appear to describe as close a fit as with abscission on the day of sampling.

Table 3. Coefficients of correlation between parameters of metabolism and the rate of bract abscission at the time of sampling and 5 days later.

Cultivar	Respiration	Protein N	Soluble N	Sugar	Starch
Abscission					
Paul Mikkelsen	-.673	-.051	+.770*	-.615	-.021
White Ecke	-.887*	-.086	+.634	+.813*	-.874
New Ecke Pink	-.666	-.204	-.623	+.448	-.491
Barbara Ecke Supreme and MSU 64-5	-.262	+.305	-.507	+.125	-.828
Total	-.677*	-.012	+.165	+.352	-.575*
Abscission 5 Days Later					
Paul Mikkelsen	-.416	-.433	+.509	-.744*	-.674
White Ecke	-.814*	-.392	+.802*	+.849*	-.889
New Ecke Pink	-.644	-.452	+.053	+.311	-.631
Barbara Ecke Supreme and MSU 64-5	-.123	+.514	-.347	+.418	-.596
Total	-.481	-.019	+.182	-.275	-.555*

*Significant at the 5% level.

Exhaustive attempts to monitor the level of endogenous ethylene in the poinsettia at different stages of aging and senescence gave only negative results indicating no detectable endogenous ethylene. Extraction of the internal atmosphere from poinsettia bracts or leaves and control extraction of air from a water sample all indicated a trace of ethylene not greater than 0.01 ppm of the gas sample. Moreover the level of ethylene bore no apparent relationship to the size of sample nor to the time of sampling but was constant and indicative of the ambient level in the external environment.

Long term collection of ethylene from air passed over poinsettia material in a closed system indicated ethylene levels not different from blank controls. Variability was found between replications. Part of this variability could have been due to faulty apparatus allowing gas leaks or variable pressure altering the flow rate. However, the system was not so insensitive as to mask any large difference in ethylene concentration particularly between plant sample and blank. Therefore, the conclusion drawn from both methods is that the poinsettia does not have an endogenous ethylene concentration greater than that of the ambient atmosphere.

DISCUSSION

Changes in metabolism with time could be associated with differences among poinsettia cultivars with respect to keeping quality. There were several differences between good and poor keepers which may bear a relation to their behavior in a home environment. The assumption on which the study was based was that there were differential rates of bract or leaf aging among different cultivars of poinsettia.

The respiration rate as a function of time most nearly describes the theoretical situation when comparisons are made among cultivars and between samples from plants held in a greenhouse and a home environment. The reason for the differences in bract respiration is not completely clear. Conceivably it could be the result of limiting substrate level, limiting enzyme catalysts, or the buildup of rate limiting anti-metabolites. The first possibility may be eliminated on the basis of the data which shows a maintenance in the levels of both sugars and starch. Protein N maintained a steady level in the bracts of all cultivars though quantitative uniformity does not imply strict continuity of the same enzymes and as no qualitative protein studies were undertaken this aspect must remain in doubt. Similarly the accumulation of anti-metabolites was not measured directly except in the case of soluble non-protein N. Valdovinos and Muir (56) showed that accumulation of amino acids and especially those of the D-form could stimulate senescence and abscission through an inhibition of protein synthesis. There was an increase in the soluble non-protein N fraction in the bracts of all cultivars which was more pronounced in the poor keepers. This increase could conceivably indicate an increase in amino acids which, on being mobilized, could induce abscission.

The possible importance of mobilization in bract abscission is apparent in the comparison of healthy and abscised bracts. Considerable mobilization of soluble nitrogenous compounds, both protein and non-protein N, occurred as the bracts senesced. It did not occur during the aging phase when there was accumulation with no apparent movement out of the bract. Mobilization was not apparent for either sugars or starch. Scott and Leopold (49) suggested that the mobilization of nutrients prior to abscission could accelerate senescence and abscission. More recent work by Abeles (2) on ethylene stimulated abscission has shown that mobilization is not necessarily an integral part of abscission. The present study does not elucidate the possible role of mobilization. It was shown to be a factor in senescence in poinsettia bracts but whether it is a result of senescence, corollary with abscission, or whether it plays a role in enhancing the abscission process is speculative.

The absence of measurable ethylene evolution at any sampling time and for any cultivar leaves open the question of the role of ethylene in abscission of intact plants. The methods employed were designed to measure gross ethylene. It is possible that ethylene evolved in or near the abscission zone at the time of senescence would have been masked by the bulk of non-ethylene evolving tissue measured in the same sample. Most of the studies on ethylene mediated abscission have employed explants as their plant material and it has been shown (11) that the preparation of an explant induces a surge of ethylene which could possibly result in a shift in the metabolic pathways to one which is more receptive to additional ethylene.

One problem in approaching a study on aging by the methods employed in this series of experiments is the introduction of a sampling bias which must be considered in any interpretation. Poinsettia plants in the home continue to develop more bracts, albeit at a reduced rate, and senescence and abscission occurs randomly over an extended period of time. The sampling method measured the overall state of the leaves or flowering head. It did not take into account any differences among cultivars with respect to continued growth nor did it account for the portion of the original sample already abscised. This latter aspect would be critical if it could be shown that differences in abscission among cultivars were not the result of differential aging rates but rather the effect of a second factor inducing abscission at different relative degrees of aging. The results showing decreasing respiration rates and faster aging in the bracts of the poor keeping cultivars would argue that abscission is related to a certain degree of aging.

A second aspect of the sampling dilemma is the interaction with nutrients mobilized out of senescing tissue and accumulating in the remaining bracts or leaves. Where mobilization was shown to occur, as for nitrogenous compounds, it could alter the pattern with aging and make comparisons with studies using explants where there is no mobilization into distal tissue more difficult.

ADDITIONAL RELEVANT DATA ON
IAA-OXIDASE ACTIVITY

Table 4. Optical density at 562 mμ after incubation of active and inactivated by boiling extracts of the bracts of 2 poinsettia cultivars with and without the addition of H_2O_2. Incubation was for 0 and 30 min and color development was read 30 min later.

	Paul Mikkelsen		Barbara Ecke Supreme	
	Inactive	Active	Inactive	Active
Time 0				
without H_2O_2	.061	.041	.046	.046
with H_2O_2	.131	.137	.155	.149
Time 30				
without H_2O_2	.066	.208	.066	.229
with H_2O_2	.208	.357	.222	.432

Table 5. Optical density at 562 mμ after incubation for 30 min of active and inactive extracts of the bracts of 2 cultivars of poinsettia before and after passing through a column of Sephadex G-25. Complete incubation mix contained 0.5 mM IAA, 0.2 mM 2,4-dichlorophenol, 0.2 mM $MnCl_2$ in 0.01 M phosphate-citrate buffer pH 6.1 with or without 0.1 mM H_2O_2 added.

	Paul Mikkelsen		Barbara Ecke Supreme	
	Inactive	Active	Inactive	Active
Before Spehadex				
Without H_2O_2				
complete	.041	.208	.066	.237
less Mn	.046	.215	.071	.244
less 2,4-DCP	.032	.187	.046	.276
With H_2O_2				
complete	.168	.357	.174	.432
less Mn	.187	.377	.194	.398
less 2,4-DCP	.161	.387	.155	.432
After Sephadex				
Without H_2O_2				
complete	.000	.071	.000	.092
less Mn	.000	.086	.000	.086
less 2,4-DCP	.000	.000	.000	.000
With H_2O_2				
complete	.081	.155	.092	.174
less Mn	.102	.143	.092	.161
less 2,4-DCP	.076	.071	.081	.086

GENERAL DISCUSSION

Results of the experiments conducted in this study indicated differences between good and poor keeping cultivars of poinsettia which could be related to observed differences in abscission rates. There are many recent historic and current hypotheses dealing with the physiological basis for abscission control and involving the aging process or the action of growth regulators such as auxin or ethylene. It is necessary to examine the data in the light of these hypotheses to establish a comprehensive hypothesis to explain abscission control in poinsettia and cultivar differences with respect to this character.

Gaur and Leopold (22) proposed that the concentration of auxin at the abscission zone determines whether a leaf will remain or abscise. A high concentration of auxin inhibits abscission while a low concentration promotes it. The application of exogenous IAA to poinsettia explants confirmed that a high concentration did inhibit abscission but no stimulation was found at lower concentrations. In the normal aging of intact leaves or bracts it is difficult to say whether the normally reduced auxin level promotes the abscission process or merely allows it to proceed.

Jacobs (32) has proposed that auxin delays abscission indirectly by maintaining growth. Elongation measurements were not made of intact or debladed poinsettia bracts but there was no observable growth in explants except where some treatments induced epinasty and bracts were retained on good keeping cultivars which had long ceased growth in the home environment. Perhaps it is more a matter of growth maintaining a high auxin level *in vivo* than the other way.

Jacobs (30) and Addicott *et al*. (8) proposed similar hypotheses explaining abscission control as the result of auxin-auxin interactions at the zone of abscission. Called respectively the auxin-auxin balance hypothesis and the auxin gradient hypothesis they both theorize that the auxin ratio across the abscission zone is the controlling factor. If the balance favors the distal side or the gradient is from the distall to the proximal then the leaf remains attached. However if the balance or gradient is reversed, irrespective of quantity, then abscission occurs. Attempts to establish reverse gradients or an unfavorable balance by proximal application of IAA to poinsettia explants resulted in a slight inhibition of abscission rather than the theoretically expected promotion.

Rubinstein and Leopold (47) further elaborated on the control of abscission by auxin in proposing the

2-stage theory for auxin action. In explants there is a first stage or induction period where abscission is inhibited by auxin followed by a second stage which is promoted by the same concentration of auxin. Attempts to illustrate the 2-stage auxin action on poinsettia explants were not successful.

Several hypotheses have as their basis for abscission control the interaction between auxin and ethylene. Gawadi and Avery (23) hypothesized that abscission was dependent on the balance of auxin and ethylene. They further suggested that ethylene could be active in hastening leaf aging at low auxin levels. Hall (26) considered that when auxin synthesis is reduced or ethylene evolution increased abscission results. Attempts to reproduce the findings of Gawadi and Avery (23), using basically similar methods though differing in several details, failed to show any promotion of abscission with ethylene. Moreover, ethylene was not found to be evolved from normally aging intact poinsettia plants.

Abeles and his group have continually stressed the importance of ethylene as the primary mechanism of abscission control while other factors may interact with the ethylene response or cause stimulation or inhibition of ethylene evolution. Abeles and Rubinstein (5) found a 2 stage ethylene action similar to that for auxin and also a positive correlation between auxin and ethylene levels. They hypothesized that the auxin effect in abscission is ethylene mediated. Similarly (1) a wide range of abscission stimulators were found to have one common basis: that of stimulation of ethylene evolution. According to Abeles and Holm (3) ethylene has its effect in regulating protein synthesis through the stimulation of specific m-RNA's and r-RNA's. More recently Abeles (4) has expanded this hypothesis to include an interaction between aging and ethylene which could serve as a basis for abscission control in intact plants and explants. The aging-ethylene hypothesis states that the role of ethylene is to accelerate the formation of enzymes responsible for abscission but ethylene cannot act while there is a continued supply of a juvenility factor such as auxin. A decrease in IAA concommitant with aging would result in a shift to the ethylene sensitive stage. Again the problem of interpreting poinsettia bract abscission in terms of Abeles hypotheses has been the lack of response by intact or debladed poinsettia bracts to ethylene.

Burg (11) has suggested a hypothesis dealing with auxin and ethylene effects on abscission in which the primary effect of ethylene in promoting abscission is in limiting the synthesis, transport and/or destruction of auxin. The decrease in effective auxin causes the onset of senescence and abscission. Ethylene may have a second effect in accelerating abscission through a number of possible systems. This hypothesis is compatible with the findings for poinsettia if it is assumed that ethylene is not essential for the observed decrease in auxin. Ethylene could conceivably have different catalytic properties depending on the plant species and tissue involved.

Other hypotheses have based abscission control on other growth regulators in interaction with auxin. Osborne (41) found a diffusible substance, capable of accelerating abscission, to increase in senescing leaves. She hypothesized that the increase in this senescence factor combined with the decrease in auxin in aging leaves is responsible for abscission. No experiments were devised to measure senescence factors directly in poinsettia; however, no diffusible substance inhibitory in the *Avena* coleoptile straight growth test was found to increase markedly in aging bracts.

Carns (12) suggested that gibberellic acid plays a role in abscission. He proposed that auxin, gibberellin and the senescence factor interact in one common mechanism which regulates abscission. The fault with this sort of hypothesis is that it is necessarily vague and much easier to formulate than substantiate. Exogenous gibberellin did not exert any pronounced effect on poinsettia bract abscission.

Addicott et al. (6) described the senescence factor as abscisic acid and linked this hormone to abscission regulation. Abscisic acid did not influence the rate of abscission of intact poinsettia bracts though it did stimulate leaf abscission.

Several hypotheses have based abscission on mobilization phenomena with depletion or accumulation of metabolites or anti-metabolites. Osborne and Moss (42) showed that artificially induced mobilization could affect abscission and hypothesized that alterations in the balance of growth regulators _in vivo_ could induce mobilization resulting in localized senescence. Valdovinos and Muir (56) hypothesized that certain amino acids which may be mobilized at senescence could induce abscission through an anti-metabolite action interfering with normal protein synthesis. Mobilization, particularly of the amino acid fraction, was observed in senescent intact poinsettia bracts. No experiments were undertaken to test the possible implications of this observation but, based on the hypothesis of Valdovinos and Muir (56) and supported by observations by Rubinstein and Leopold (47), this aspect could conceivably play a role in stimulating abscission of poinsettia bracts.

Scott and Leopold (49) showed that stage 1 of bean explant abscission could be associated with the establishment of a nutrient imbalance across the abscission zone and supported the hypothesis that mobilization and subsequent cellular senescence provide a component in abscission. The absence of a demonstrable 2-stage relationship in poinsettia explants may possibly be explained in terms of this hypothesis. One possible assumption is that mobilization is slower in aged, debladed poinsettia bract petioles. However, a more attractive assumption is that the debladed petiole has few mobilizable reserves relative to the vast proximal sink that is the main stem. In this view there is not a depletion-accumulation phenomenon but rather the gradual depletion of nutrients in the petiole which would accelerate the senescence process initiated by a primary fundamental component.

Horton and Osborne (29) have hypothesized that senescence distal to the abscission zone gives rise to diffusion or mobilization products which initiate an increase in cellulase activity in the separation zone. The action of cellulase is to effect cell wall dissolution leading directly to abscission while the action of growth, anti-metabolites or growth regulators such as auxin or ethylene is to hasten or retard senescence. A hypothesis of this type in which growth regulator action is indirect and the only critical aspect is senescence would appear to best fit the poinsettia data. Additional circumstantial evidence comes from the study of Gawadi and Avery (23) who showed that poinsettia leaves undergo secondary division forming a complete separation layer before the leaves have achieved full size and require only dissolution of the middle lamella for abscission.

The foregoing hypotheses have attempted, by and large, to explain the primary cause and mechanism of abscission. This study was designed to test for differences among poinsettia cultivars that could serve as a basis to explain differential abscission rates. There was no elaboration as to what mechanism may be involved in poinsettia bract abscission but the results did not indicate the presence of more than one system. The general lack of an ethylene effect, the absence of any interaction between cultivar and exogenous growth regulator and differences in rate of change of endogenous factors rather than qualitative differences all point to one common mechanism but do not add to the elucidation of that mechanism.

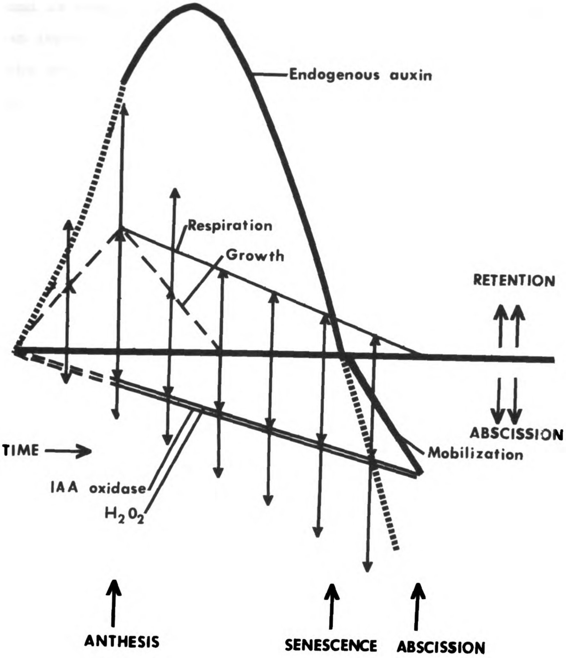
What the results do indicate is that differences in abscission rate among cultivars is the result of the differential appearance with time of the primary cause that initiates abscission and further that the primary cause of abscission in poinsettia is a decrease in diffusible auxin below a certain level at which point senescence is initiated in the abscission zone.

The most characteristic internal changes in the bracts were a decrease in the level of diffusible auxin, a decrease in the rate of respiration, an increase in the capacity of the IAA-oxidase enzyme system, an increase in the IAA-oxidase co-factor, peroxide and mobilization of soluble nitrogenous compounds. From these observations it is possible to formulate the hypothesis that senescence of poinsettia bracts is the result of a decrease in diffusible auxin below a certain critical level and that abscission is triggered by senescence and further hastened by senescence dependent mobilization. Within the framework of this hypothesis alterations in the respiration rate, the activity of IAA-oxidase, or the level of peroxide may all be interpreted as indirectly affecting abscission through a direct effect on the level of endogenous auxin. It is an assumption that the respiration rate is a valid estimate of the rate of auxin synthesis which was not measured directly. The hypothesis does not extend to the mechanism by which senescence and mobilization act in effecting abscission.

A sketch of the ways in which these factors may interact in governing abscission is presented in Figure 6. It illustrates the hypothesis that senescence is based on the level of endogenous auxin which in turn is a combined function of auxin synthesis and destruction. The method chosen to illustrate this may be termed cumulative vector analysis. The sketch is designed to represent the principle rather than any particular case. The 4 independent variables; respiration, growth, IAA-oxidase, and peroxide have each been assigned the same maximum and are assumed to run from zero to unity.

Figure 6. The theoretical interrelationships among factors regulating endogenous auxin, senescence and abscission in poinsettia bracts.

COMPONENTS OF ABSCISSION



Assumed parameters and unmeasured values are presented as dotted lines while observed values are in the solid lines. Although the effect of growth was not measured and is completely speculative, conceivably it could play an important role during the early development stages of the bract. The dependent variable, endogenous IAA, is portrayed as the cumulative mean of the independent variables. The derived shape of the auxin curve fits closely to that observed for diffusible auxin. Finally, the point of senescence initiation is at an arbitrarily selected point on the auxin curve. The sketch has been interpreted as representing the mean of the entire flowering head. It could also be interpreted as representing a single bract.

The hypothesis may be visualized as one of developments among the quantitative relationships rather than qualitative differences or abrupt changes. In this way it is possible to account for observable differences among cultivars with respect to abscission rate. A change in the level of any factor controlling the level of auxin would influence the approach to senescence and a change in more than one factor would have either a cumulative or compensating effect. What was observed were relatively small increments in all factors between good and poor keeping cultivars. However, in every case the increment of the poor keepers was in the direction favoring lower auxin levels thus leading to a magnified cumulative effect and a disproportionate increment in diffusible auxin.

The time scale, which is the measure of keeping quality, is directly dependent on the increment, inflection point and slope of the auxin curve and the differences among cultivars may be a reflection of any or all of these. The data would indicate that the slope of decrease after inflection showed the greatest variation. Thus, the slower rate of decrease in diffusible auxin for the good keeping cultivar Paul Mikkelsen when compared to the poor keeper Barbara Ecke Supreme was the factor primarily responsible for the characteristic of improved bract holding ability.

The hypothesis explaining the physiological basis for abscission control may be related to the inheritance pattern for keeping quality. According to Stewart (53) and observations made by the author keeping quality is inherited quantitatively and is not linked to any other known character in poinsettia. The quantitative inheritance pattern would be expected in view of the role of 3 independent factors in governing the auxin level. The large number of peroxidase isoenzymes alone would predicate quantitative inheritance or at least quantitative modification. Bempong and Sink (9) showed that the poinsettia may contain multiple loci making genetic analysis more complex.

Crosses between cultivars with different keeping times usually give progeny intermediate in nature or somewhat favoring either parent. One notable exception to this is MSU 64-5 a triploid seedling from a cross between the diploid, White Ecke and the tetraploid, Barbara Ecke Supreme. In keeping time and metabolic characteristics it was very similar to Barbara Ecke Supreme, probably as a result of a gene dosage effect.

Stewart (53) has found independent inheritance for keeping quality between leaves and bracts. The present study which showed large differences in bract abscission and internal factors but no difference in leaf abscission or internal relationships among the cultivars studied would support this finding. Clearly then, the hypothesis explaining keeping quality differences must be limited in application to the bracts. Further investigation will be necessary to discover the underlying control of poinsettia leaf abscission.

One of the anticipated benefits of this study was the development of an objective method to assess potential keeping quality in a breeding program. Analysis of the diffusible auxin level at anthesis and again after a predetermined time of holding in a home environment would serve this end.

More elaborate analysis of respiration rate, IAA-oxidase activity and peroxide level would give an even clearer picture of the situation and allow better parental matching for mutual compensation. The disadvantage of this type of assay is that it entails sophisticated equipment and a great deal of time in performing the analysis and the plants must be kept nearly as long as for subjective analysis.

Preliminary investigation of the behavior of debladed bract petioles suggests that this may serve as a method of assaying for keeping quality. Although the differential was shortened, debladed bracts of Paul Mikkelsen took longer to abscise than those of Barbara Ecke Supreme. The main advantages to this assay would be its ease and rapidity in gaining results. A plant to be tested could be taken at anthesis, stripped of all cyathia, growing points and all but 10 bracts and/or leaves. The remaining leaves or bracts would be trimmed at the base of the blade, the plant placed in a dark place, and daily counts made of the petioles abscised. Within a week results should be known, the plants discarded and crosses planned for other specimens of the same material grown at the same time. This must be taken as a qualified recommendation based on scanty evidence. Further study will be necessary to test its universal applicability.

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