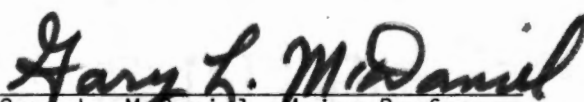
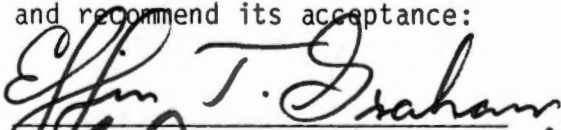
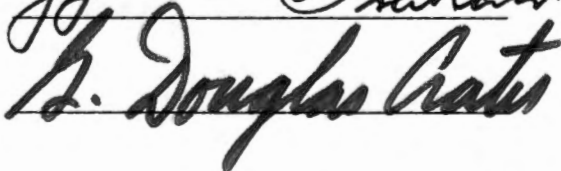


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THE ROLE OF NITROGEN AND CALCIUM ON STEM STRENGTH OF
POINSETTIA (EUPHORBIA PULCHERRIMA, WILLD.)

A Thesis

Presented for the

Master of Science

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ABSTRACT

The role of nitrogen and calcium fertilization in stem strength of potted poinsettia was assessed. 'Annette Hegg Dark Red', 'Gutbier V-14 (Glory)', and 'Eckespoint C-1', were provided with the following fertilizer formulations: 1) 15-16-17 Peter's Peat-lite Special, 2) 15-0-15 Peter's Dark Weather Feed, 3) 15-16-17 plus foliar sprays of calcium chloride, and 4) 15-0-15 plus calcium sprays. The 15-16-17 fertilizer formulation is currently used in commercial poinsettia production. It is a higher ammoniacal nitrogen formulation (21% $\text{NH}_4\text{-N}$, 26% urea, 53% $\text{NO}_3\text{-N}$) than 15-0-15 (13% urea, 87% $\text{NO}_3\text{-N}$, primarily as CaNO_3). Calcium chloride foliar sprays provided additional calcium.

Preliminary results of the first study, in which the plants were fertilized with 20-20-20 Peter's General Purpose Fertilizer (87% $\text{NH}_4\text{-N}$) initially and the above treatments were begun following the pinch date, showed no differences in branch retention due to the nitrogen source or additional calcium. Subsequent studies began nutritional regimes after potting the rooted cuttings. Generally, branch retention is enhanced by use of calcium nitrate as the nitrogen source. Additional calcium as foliar sprays did not significantly increase branch retention. However, further investigations of calcium chloride spray applications at higher rates, more frequent application dates and for a longer duration may be warranted.

Histological investigation of anatomical structure was also conducted. The poinsettia tissue was sampled at maturity when the stems were very woody and processing by the conventional paraffin technique was impractical. A new method of tissue sectioning was developed. Infiltration with polyethylene glycol (PEG) 400 and sectioning with a disposable rotary microtome blade attached to a standard sliding microtome blade provided a satisfactory method of working with this difficult tissue. While sections obtained in this manner were quite thick, (40 micrometers), there was very good differentiation of the vascular, cortical and epidermal tissue systems, nuclei, and starch when the sections were stained with toluidine blue or Gill's hematoxylin counterstained with safranin. Further investigations refining this technique to produce thinner sections and involving histochemical tests and polarized light microscopy are warranted.

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CHAPTER I

INTRODUCTION

The poinsettia, Euphorbia pulcherrima, Willd., is a photoperiod requiring plant having showy red, white, pink, or variegated bracts. It blooms naturally under the longnight conditions of autumn with peak color November through January. It is a Christmas crop of commercial significance for the floral industry.

There are two basic types of poinsettia cultivars: the self-branching types and the standard type. The 'Annette Hegg' and 'Gutbier' cultivars are the most widely grown self-branching types. The 'Eckespoint' cultivars are best known of the standard types. The self-branching plants form lateral shoots from the nodes at an early age. If the apical meristem is removed these plants form more lateral branches than do standards. The self-branching cultivars generally form a more compact plant with a larger number of bracts and having more consumer acceptability (20).

In addition to producing a more compact plant, the self-branching, or multi-flowering, plants have a shorter cropping time of 8-9 weeks versus 10-12 weeks for standard cultivars, allowing the self-branching types to be flowered earlier for a longer sales season. The shorter forcing period also means a savings to growers in terms of time the crop occupies valuable bench space.

These plants can be grown at lower night temperatures, which represents a savings in fuel costs. Other advantages include: less space required to produce the same number of flowers compared to single-stem culture, more flowers per plant, and less growing space required following final pinching (20).

A major production problem with the self-branching types, especially the 'Hegg' cultivars, is these plants have thin stems and weak lateral branches (20, 27). These branches often split from the main stem resulting in a misshapen plant with much less marketability (2, 9, 27). Although the problem of weak lateral branches has been noted for both the 'Hegg' and 'Gutbier' cultivars no previous research report on the specific problem has been found by this author. In fact, no research findings on the strength of lateral branches on any herbaceous plant has been found. Some work has been reported on the effects of chemical growth regulators on stem strength of poinsettia (8, 14). That research does not address the problem of the lateral branches shearing from the main stem.

Tissue culture experiments by developmental morphologists have demonstrated plant phenotype may be altered by the nutritional environment (4, 35, 37, 38). Many environmental factors also come into play in determining plant morphology. Light intensity, temperature, photoperiod, soil type, water quality, media and water pH and management practices all influence plant growth and development. This research examined the effects of nutrition on poinsettia lateral branch strength.

A series of experiments has been conducted to study the problem of lateral branch strength of the self-branching cultivars, the 'Heggs' and 'Gutbiers'. These experiments involved cultural treatments which were followed by histological examination.

The objectives of this research were to:

- 1.) Test the effects of the standard, commercially used fertilizer, 15-16-17 Peter's Peat-lite Special (26% $\text{NH}_4\text{-N}$, 21% urea, 53% potassium nitrate) against the calcium nitrate formulation, 15-0-15 Peter's Dark Weather Feed (87% nitrate-N, primarily as calcium nitrate, 13% urea) on the ultimate branch strength of poinsettia.
- 2.) Determine if branch strength can be enhanced by supplemental calcium as calcium chloride foliar sprays.
- 3.) Conduct a histological investigation of the anatomical structure of the representative poinsettia cultivars.

CHAPTER II

LITERATURE REVIEW

A. POINSETTIA CULTIVARS

In poinsettia cultivar trials three distinctly different growth habits have been observed among those studied. These differences can be grouped according to cultivar classes of 1) 'Annette Hegg', (2) 'Eckespoint', and (3) 'Gutbier' (40).

The 'Annette Hegg' cultivars are of the self-branching or multi-flowering type. These plants produce comparatively slender stems which tend to cascade, making them especially suitable for hanging basket plantings (20). The 'Heggs' are noted for exceptional leaf and bract retention and the ability to produce six to eight branches on a pinched stem. However, the bracts of some cultivars may droop at maturity and cyathia drop early (27).

In addition, the 'Hegg' group has been noted to have weak lateral branches which split from the main stem (2, 9). Some growers avoid the 'Heggs' while others tie the weak branches to stakes for support (2, 9). The 'Annette Hegg' cultivars can, and perhaps should, be grown at cool temperatures of 16-18 C (60-65 F) nights until bracts are developed, then may be grown as cool as 14 C (58 F) night temperatures. If grown warmer the bracts will be large but the plant will not withstand handling and shipping (2).

The 'Annette Hegg' was among the first multi-flowering poinsettia introduced into the United States (31). It was found in a commercial crop in a greenhouse in Norway (25). The exact parentage is unknown (10). The Norwegian firm of Thormod Hegg and Sonn introduced it at the Sarpsborg Exhibition in 1967 as 'Hegg '67' but later renamed it 'Annette Hegg' (17). Paul Ecke introduced it into the United States in 1968 (10, 17). In 1969, 'Annette Hegg' sported to 'A. Hegg White', 'A. Hegg Marble', and 'A. Hegg Dark Red' (10).

The most widely grown poinsettias of the second group are the 'Eckespoint C-1' cultivars. The 'C-1' plants are characterized by strong, stiff stems. These plants produce large excellent quality bracts. In fact, they are considered to be the standard for quality among florists' poinsettias (31). The 'Eckespoint C-1' plants require a longer growing period of 10 to 12 weeks and higher temperatures to finish the crop than either the 'Hegg' or 'Gutbier' cultivars. The 'C-1's' will produce an average of only three branches when pinched, compared to six to eight or more per plant when the multi-flowering types are pinched (14, 17). The 'C-1' cultivars reportedly have a high light requirement, making them more popular among growers in Florida and the Southeast than in the cloudy weather areas of the North where the 'Heggs' are the backbone of the crop (2).

The 'Eckespoint' cultivars were the first to exhibit the characteristics of good leaf and bract retention. These plants

resulted from sports or mutations of seedling selections from the old 'Oak Leaf' cultivars (33).

The third group, the 'Gutbier' cultivars, is the result of seedling selections with self-branching qualities. These plants were introduced into the United States from West Germany by Paul Ecke in 1977 (27). The most widely grown 'Gutbier' cultivars are 'V-10 (Amy)' and 'V-14 (Glory)'.

The 'Gutbier' cultivars tend to be shorter plants than the previously described cultivar classes. Both cultivars produce a profusion of secondary breaks, whether pinched or unpinched, which may account for the reduced height of the plant (40). Stem development in 'Glory' is noted to be similar to the 'Eckespoint' (40). The 'V-10 (Amy)' has been reported to be of exceptional symmetry but some of the side branches shear from the primary stem when the plant is moved (40). The 'V-14's' have a high light requirement (2). Growers in the Southeastern states and Florida prefer the 'Gutbier' cultivars to the 'Heggs', stating the 'Gutbiers' are sturdier plants (1).

B. NITROGEN

Nitrogen is related to carbohydrate utilization in the plant. When the nitrogen supply is insufficient, carbohydrates will be deposited in vegetative cells causing the cell walls to thicken. When the nitrogen supply is adequate the carbohydrates will combine with the nitrogen, forming amino acids, and ultimately

synthesizing proteins. At high nitrogen levels an increase in protoplasm and a decrease in deposited carbohydrates occurs, resulting in a more succulent plant. In cotton it has been demonstrated that excessive nitrogen leads to weaker fiber (36).

The source of nitrogen is also important in respect to plant growth and development. Several researchers have studied the effects of ammonium and nitrate forms of nitrogen on the growth of the poinsettia. Boodley (1971) found ammonium nitrogen sources least satisfactory for poinsettia. He stated the ammonium ions reduced the ability of the plant to take up water. This reduced water uptake becomes more severe in cases of water stress, such as in high light and high temperature situations and is less severe in shortday, cloudy conditions.

Ecke (1976) reported the poinsettia reacts unfavorably to excess ammonium or urea. He further stated no more than half the nitrogen should be in the form of ammonium and urea should be omitted if possible. This stance is supported by research. In a study on the effects of $\text{NH}_4\text{-N}:\text{NO}_3\text{-N}$ ratio it was also recommended no ammonium be used on poinsettias (7). This research found ammonium injury occurs as a result of the interaction of the concentration of the ammonium ions, the concentration of urea, and the rate of nitrification. Gaffney (1982) also found nitrate nitrogen to be the superior nitrogen form. He stated over half the nitrogen should be in the nitrate form when using fertilizers containing NO_3^- and NH_4^+ . Plants provided only ammonium nitrogen in this study produced stunted root systems and brittle

stems. It has been observed that plants provided ammonium nitrogen are also taller than those given calcium nitrate (10).

Calcium nitrate is recommended as the best nitrogen source for poinsettias (3, 10). Calcium nitrate is 15.5% nitrogen and it supplies all of this nitrogen in the nitrate form (36). It does not supply a reserve or slow release supply of nitrogen but there is no chance for ammonium toxicity (36). Research has shown plants supplied with 266 mg/l calcium nitrate on a weekly basis in a peat-lite medium were superior plants (3). These plants were the most uniform in size and had the best developed, darkest colored bracts; the best developed root system; and did not drop a single leaf. Later work recommended using a constant liquid feed fertilization program at the rate of 6-12 meq/l nitrate nitrogen when growing poinsettias in a sand medium (11).

C. CALCIUM

Clarkson and Hanson (1980) discuss the role of calcium in plant physiology. The review states calcium has a well recognized but unidentified structural role in maintaining membrane integrity. It serves physiologically to ward off membrane damage and leakiness to delay senescence and abscission.

Calcium also strengthens cell wall structure, a result which is particularly noticeable in soft fruits. Calcium is a major cation integral to the protein-pectin "cement" of the middle lamella. It tends to coordinate strongly with a variety of ligands to produce a complex cross-linking which can tie diverse

macromolecules together. The bonds formed then give stability to a variety of cytoplasmic structures and enzymes. As such, calcium has apoplastic importance in cell membranes, walls and adhesions.

In research on the role of calcium in vegetables it was found calcium deficiency is related to disintegration of vascular tissue in celery heart rot (29). This research reiterated the role of calcium as a cell wall component and noted excessive growth of leafy vegetables, especially if promoted by high nitrogen, requires more calcium than can be absorbed and moved to rapidly growing tissue. It was reported calcium chloride sprays can alleviate this problem.

Anatomical studies of calcium deficient poinsettia leaves found the mesophyll cells were very compact with very few intercellular spaces (34). The cells surrounding the vascular bundles had hypertrophied causing obliteration of some phloem cells. Considerable crushing of phloem cells and disorganization of surrounding tissue also occurred. Xylem cells were thin walled and completely disorganized.

D. NITROGEN NUTRITION AND CALCIUM UPTAKE

A strong association exists between nitrogen fertilization and calcium deficiency disorders. Vegetative growth stimulated by nitrogen may induce a stress in calcium sensitive tissues. This has been demonstrated in research on calcium disorders of vegetable heart tissues (29) and apples (6, 21, 32). The increase in growth resulting from nitrogen can bring about a calcium

requirement which cannot be met by the rate of calcium uptake by the roots (19). This situation can be further aggravated by the simultaneous effect of nitrogen depressing root growth and so impairing calcium uptake.

Calcium uptake by plant roots is also dependent on nitrogen form. Calcium deficiency disorders occur more frequently with ammoniacal nitrogen than with nitrate nitrogen fertilization. Depressed calcium levels have been associated with the lower cation composition of NH_4 -fed plants by a number of authors on a number of plant species (18, 22, 23, 24, 39). A competition exists between NH_4^+ and Ca^{++} ions for uptake. The NH_4^+ cation is taken up very rapidly, thereby competing very strongly for uptake with Ca^{++} ions (19).

A useful tool in examining the effects of nitrogen nutrition on calcium translocation involves collection of xylem exudates from decapitated plants (19). Results of these experiments show ammonium nitrogen nutrition depresses both Ca^{++} concentration and the volume of sap exuded. In contrast, nitrate nitrogen has the general effect of stimulating cation uptake (23, 24).

Another important point in relation to nitrogen form is water stress. Water stress can restrict calcium uptake and distribution (19). Ammonium nitrogen has a depressing effect on water uptake (3, 36) and may therefore also impair calcium nutrition.

CHAPTER III

MATERIALS AND METHODS

A. GREENHOUSE

A preliminary experiment was conducted in Spring 1984. Rooted cuttings of the poinsettia cultivars 'Annette Hegg Dark Red', 'Gutbier V-14 (Glory)' and 'Eckespoint C-1' were potted in 15 cm pots in a peat-lite medium containing equal parts by volume of peat moss, perlite, vermiculite, and pine bark. These plants were fertilized with 20-20-20 Peter's General Purpose Fertilizer (20% N, 8.6% P, 16.6% K), at the rate of 66.5 mg/l N until pinch date. Fertilizer treatments at the rate of 133 mg/l N using: (1) 15-16-17 Peter's Peat-lite Special (15% N, 6.9% P; 14.1% K); (2) 15-0-15 Peter's Dark Weather Feed (15% N, 0% P, 12.4% K); (3) 15-16-17 plus 66.5 mg/l calcium as foliar sprays of calcium chloride; or (4) 15-0-15 plus calcium chloride sprays; began at subsequent irrigations.

Temperatures were higher than recommended since the study took place under warmer spring conditions. The plants did not receive photoperiod control. As a result, these plants did not initiate flowers.

The second study took place in the Fall of 1984. The conditions of this study are those commonly encountered in

commercial poinsettia production since this is the time of year poinsettias are normally grown.

Rooted cuttings of 'Annette Hegg Dark Red', 'Gutbier V-14 (Glory)' and 'Eckespoint C-1' were potted September 13, 1984. The plants were grown as a single stem, with one plant to a 15 cm pot. Plants were watered in with 66.5 mg/l N Peter's General Purpose Fertilizer 20-20-20. The primary plant nutrient sources of the fertilizer are urea, ammonium phosphate and potassium nitrate. Nitrogen is supplied as 26% potassium nitrate, 20% ammoniacal nitrogen, and 52% urea.

The potting medium was a peat-lite mix containing equal parts by volume of peat moss, perlite, vermiculite, pine bark. No other soil ammendments were added to this mix. Initial and final pH readings of the medium were taken to determine the effects of the fertilizer programs on pH.

Based on results of the Spring 1984 study, fertilizer treatments were begun earlier in this experiment. Ten days following potting the plants were irrigated with clear water to leach pots. Fertilization began with the next irrigation and continued on a constant feed basis at the rate of 133 mg/l N.

The fertilizers used in this study were: (1) 15-16-17 Peter's Peat-lite Special; (2) 15-0-15 Peter' Dark Weather Feed. The 15-16-17 Peter's Peat-lite Special has nitrate of soda, urea, ammonium phosphate and potassium nitrate as the primary plant nutrient sources. Total nitrogen is 15%. The nitrogen is in the

following forms: 53% nitrate nitrogen as potassium nitrate; 21% ammoniacal nitrogen; 26% urea.

The primary plant nutrient sources of 15-0-15 Peter's Dark Weather Feed are calcium nitrate, urea and potassium nitrate. The nitrogen is 87% nitrate nitrogen, primarily as calcium nitrate, and 13% urea. There is no ammoniacal nitrogen present.

Superphosphate (0-20-0) was added at the rate of 2.7 kg/m^3 to the plants in the 15-0-15 treatment groups. The guaranteed analysis of superphosphate states 20% phosphoric acid is available for plant nutrition. This supplies 8.6% phosphorous. Calcium sulfate comprises 50% of the superphosphate and supplies phosphorous, sulfur and calcium to the plants.

The plants were provided with additional calcium by spraying the foliage of the entire plant with calcium chloride using a hand pump sprayer. Beginning on October 1, 1984, three days following the September 28 pinch date, foliar sprays of calcium chloride were applied weekly. These spray treatments continued for six weeks until bract color was noted. Required spray volume increased as the plants grew. Initial applications of 25 ml calcium chloride per plant increased to 100 ml per plant at the final application. The rate of 66.5 mg/l calcium remained constant throughout the study.

Growing temperatures followed the recommended guidelines with seasonal and climatic variations. The plants were grown at 16°C (60°F) night temperatures prior to flower initiation. However,

there were several (4-6) nights with very warm temperatures, as high as 26 C (78 F), which is typical for autumn months in Tennessee. The average night temperature was $16\text{ C}\pm 1$ ($60\text{ F}\pm 2$). During bract formation and development, night temperatures were increased to $17\text{ C}\pm 1$ ($62\text{ F}\pm 2$). The finishing temperature was 16 C (60 F).

The experiment consisted of four treatments:

- 1) 15-16-17 Peter's Peat-lite Special
(53% $\text{NO}_3\text{-N}$, 47% $\text{NH}_4\text{-N}$ and urea)
- 2) 15-0-15 Peter's Dark Weather Feed
(87% $\text{NO}_3\text{-N}$, primarily as CaNO_3 ; 13% urea)
- 3) 15-16-17 plus calcium chloride sprays
- 4) 15-0-15 plus calcium chloride sprays

These fertility treatments were applied to three poinsettia cultivars: 'Annette Hegg Dark Red'; 'Gutbier V-14 (Glory)'; 'Eckespoint C-1'. These cultivars were chosen as representative of the three poinsettia types previously described. The first two are self-branching types and the last is a standard type. The experiment was a randomized complete block design with eight replications. Analysis of variance was conducted and mean separation was determined using Duncan's new multiple range test.

B. HISTOLOGY

Tissue Preparation

Tissue samples (Fig. 1) were collected from mature plants in which the epidermis was thick and cells were lignified (Fig.2) making sample pieces very tough. Tissues were fixed in 50% FAA (v/v) (Fig. 3) at least 48 hours.

Paraffin histology did not seem feasible because the specimens were naturally very hard. Total dehydration with alcohol and paraffin infiltration would increase the hardness of the tissue even further. However, paraffin sections were tried and proved unacceptable, as predicted. The celloidin method (26), was rejected as too expensive, although technically applicable. An alternative method of infiltrating tissue with polyethylene glycol (PEG) 400 (Fig. 3) was developed for this investigation (13).

Sectioning

A hybrid knife was made by gluing a 75 x 14mm disposable blade for a rotary microtome onto a standard 250mm sliding microtome knife. This allowed sectioning of several tissue specimens without the problem of sharpening the large sliding microtome knife. The specimen was placed in molten PEG 1540 (Fig. 3) on a steel pedestal in a boat which had been made using aluminum tape. When cooled, the solidified PEG 1540 held tissue in place and tape was removed. Specimen on pedestal was mounted on a sliding microtome and sectioned longitudinally at a thickness

Figure 1. Example of tissue specimen for histological sectioning. Note thick epidermis (E) and vascular tissue (V).

Figure 2. Example of tissue specimen with underlying tissue exposed, showing the thickened epidermis (E) and the vascular tissue (V).



Figure 1.



Figure 2.

1. Fixation, 50% FAA (16).

Formula: 225 ml absolute ethanol.
225 ml distilled water.
25 ml glacial acetic acid.
25 ml 37% w/v formalin.

2. Stain, Toluidine Blue in Benzoate Buffer (30).

0.5% toluidine blue in 0.02 M sodium benzoate buffer, pH 4.4.

Formula for buffer: Benzoic acid 0.25 gm
Sodium benzoate 0.29 gm
Water 200 ml
Dissolve and filter.

Dissolve 1.0 gm Toluidine Blue O in 200 ml buffer.

Filter.

3. Stain, Gill's Hematoxylin counterstained with Safranin.

Gill's Hematoxylin (12).

Safranin (16).

Formula for safranin: safranin O 0.1 gm
sodium acetate 0.1 gm
37% v/v formalin 0.5 ml
methyl cellosolve 25 ml
absolute alcohol 25 ml

Bring up to 100ml volume with distilled water.

4. Polyethylene glycol 400 (PEG 400).

5. Polyethylene glycol 1540 (PEG 1540).

Figure 3. Histological reagents and materials.

6. Ethanol Solutions.

(i) To rinse FAA:

50% aqueous ethanol: 300 ml absolute ethanol + 200 ml water

(ii) To dehydrate stained sections:

% alcohol	95% ETOH	TBA	Distilled water
50	250 ml	50 ml	200 ml
70	250 ml	100 ml	150 ml
85	250 ml	175 ml	75 ml
95	250 ml	250 ml	-----
100	125 ml*	375 ml	-----
*Absolute ethanol			
9:1	450 ml TBA	50 ml 2-propanol.	

(iii) To dehydrate sections stained with Gill's hematoxylin counterstained with safranin.

% alcohol	95% ETOH	Distilled water
25	25 ml	70 ml
30	30 ml	65 ml
35	35 ml	60 ml
40	40 ml	55 ml
70	70 ml	25 ml
85	85 ml	10 ml
95	95 ml	-----

7. Clearing solvent: Histoclear, proprietary reagent, International Diagnostics.

8. Coverglass resin: Lipshaw's Mounting Medium,
Lipshaw Manufacturing, Inc.

9. Coverglasses: 18mm, 1 thickness.

Figure 3 (Cont.).

of 40 micrometers. Sections were placed in jars of distilled water to extract the PEG 400 and 1540.

Processing sections

Sections were processed in individual capped jars to maintain serial order. The water was aspirated from the jars and replaced with 10ml of toluidine blue stain (Fig. 3). Sections were immersed in the stain 15 minutes. Following staining the sections were passed through the same TBA dehydration series used in the paraffin method: 50%; 70%; 85%; 95%; 100% and 9:1 TBA:2-propanol (v:v) (Fig. 3). Dehydrated sections were brought through three 15 minute changes of histoclear to remove all residual alcohol. A teflon spatula was found to be essential to successfully transfer sections from jars to resin pools on slides. Each section was covered with an individual 18mm coverglass to alleviate problems with trapped air.

An alternate stain sequence was used on some tissue which involved staining with Gill's hematoxylin counterstained with safranin (Fig. 3). In this method, sections were removed from the knife blade and placed in 25% ETOH to remove residual PEG 400 and 1540. After 5 minutes the alcohol was aspirated from the jars and sections were flooded with 10 ml Gill's hematoxylin (Fig. 3) for 2 minutes. The hematoxylin was withdrawn and sections were passed through a graded series of ethyl alcohols as used in the paraffin method: 30%; 35%; 40% ETOH (Fig. 3). Sections were counterstained in safranin for 30 minutes. Stained sections were dehydrated by

passage through the following graded alcohol series: 70%; 85%; 95%; absolute alcohol (Fig. 3). Dehydrated sections were brought through two changes of 1:1 v/v absolute ethanol:histoclear solution followed by two 15 minute changes of histoclear to remove residual alcohol. Sections were removed to slides as described for toluidine blue stain method above.

Retting

Retting is an old method used to examine gross morphological structure of vascular tissue (28). Large sections of mature poinsettia trunks, including branch stubs, were placed in beakers in tap water and exposed to a natural population of decay organisms. After 10 to 14 days the epidermis was washed away under running tap water since this tissue is more susceptible to decay than the underlying vascular tissue. The vascular tissue was thus exposed for examination.

CHAPTER IV

RESULTS

A. GREENHOUSE NUTRITION EXPERIMENTS

Preliminary results from the spring 1984 study showed no differences in branch retention caused by any of the fertility regimes on any of the cultivars studied. In this study the fertilizations did not begin until after the plants had been pinched. Up until that time the plants were fertilized with 20-8.8-15.7 NPK (20-20-20), which is a high ammoniacal nitrogen formulation fertilizer. From these results it was determined the fertility regimes should begin at potting for plants in subsequent studies. The results of this spring study were also influenced by seasonal variation due to warmer temperatures and lengthening days.

The Fall 1984 study began fertility treatments soon after potting the rooted cuttings. The parameters measured in this study were bract size, plant height, total number of branches produced, and the number of branches which had sheared from the main stem. Since this experiment took place in the autumn when poinsettias are typically produced, these results are considered to be the most representative and valid.

Annette Hegg Dark Red

The plants receiving calcium nitrate fertilizer (15-0-15), containing 15% N, 0% P, 11.8% K, had smaller bracts than those

receiving the higher ammoniacal nitrogen (15-16-17) formulation, which contains 15% N, 7% P, 14.1% K, (Table 1). The addition of calcium as calcium chloride sprays did not limit bract size. Plants receiving 15-0-15 plus calcium chloride sprays had smaller bracts than those provided with 15-16-17. This was true whether the ammoniacal nitrogen was provided alone or in combination with calcium chloride sprays. There were no differences in bract size between plants receiving 15-16-17 only and those fertilized with 15-16-17 plus calcium sprays.

The 'Annette Hegg Dark Red' plants were shorter when provided with additional calcium (Table 1). Plants fertilized with higher ammoniacal nitrogen only were taller than plants in the other treatment combinations (Fig. 4).

While there were no differences in the total number of branches produced due to any fertility regime, there was a difference in the number of broken branches (Table 1). The plants provided calcium nitrate had fewer broken branches than those receiving the higher ammoniacal nitrogen formulation (Fig. 5). The addition of calcium as calcium chloride sprays did not affect the number of broken branches. However, visual inspection showed fewer branches were broken (Figs. 6, 7) which indicates that calcium supplements decrease the number of broken branches when used with either of the nitrogen sources, and especially when used with the higher ammoniacal nitrogen fertilizer formulations (Fig. 7). In addition, when the data were analyzed at the 1% level, there was a highly significant difference in the number of

TABLE 1. Effects of Nitrogen and Calcium Fertilization on Mean Bract Size, Height, and Number of Broken Branches of 'Annette Hegg Dark Red' Poinsettias.

Treatment	Bract Size (cm)	Height (cm)	Broken Branches
Ammoniacal-N (15-16-17)	45.6 a ^z	71.7 a	3.2 a* ^y
NH ₄ -N + CaCl ₂	46.3 a	64.3 b	2.2 a
Calcium Nitrate (15-0-15)	42.1 ab	61.1 b	0.9 b
CaNO ₃ + CaCl ₂	39.1 b	63.0 b	0.3 b*

^zDuncan's new multiple range test, 5% level.

^y*Denotes significance at the 1% level, Duncan's new multiple range test.

- Figure 4. Photograph of 'Annette Hegg Dark Red' plants showing all fertilization treatments.
- Figure 5. Comparison of plants fertilized with Peter's Dark Weather Feed (15-0-15) with plants receiving Peter's Peat-lite Special (15-16-17).
- Figure 6. Comparison of plants fertilized with calcium nitrate (15-0-15) with and without calcium chloride sprays.
- Figure 7. Comparison of plants fertilized with the higher ammoniacal nitrogen formulation (15-16-17) with and without calcium chloride spray applications.



Figure 4.



Figure 5.



Figure 6.



Figure 7.

broken branches between plants receiving 15-16-17 and those supplied 15-0-15 plus calcium chloride sprays. The plants receiving higher amounts of calcium had fewer broken branches.

Gutbier V-14 (Glory)

There were fewer differences observed among treatments in the 'Gutbier V-14' cultivar (Fig. 8). There were no differences in bract diameter or number of broken branches. However, the 'V-14' plants lost very few branches and plants receiving calcium spray supplements lost fewer branches than plants not supplied with the spray treatments (Table 2). The nitrogen source alone did not affect the number of broken branches.

Plant height was affected by the different fertility regimes (Table 3). The plants fertilized with higher ammoniacal nitrogen were taller than those receiving additional calcium as calcium nitrate and/or calcium chloride sprays (Fig. 8).

There was a difference in the number of branches produced by the plants (Table 2). The plants given additional calcium had fewer branches than plants fertilized with 15-16-17 only.

Eckespoint C-1

In the 'Eckespoint C-1' plants no difference in the total number of branches produced was found among treatments. Differences in bract size, plant height and number of broken branches are shown in Table 3.



Figure 8. Photograph of 'Gutbier V-14' plants showing the effects of the fertilization treatments. Plants supplied 15-16-17 were taller than those in the other fertility treatments.

TABLE 2. Effects of Nitrogen and Calcium Fertilization on Height and Total Branches of 'Gutbier V-14' Poinsettias.

Treatment	Height (cm)	Total Branches
Ammoniacal-N ($\text{NH}_4\text{-N}$) (15-16-17)	56.4 a ^z	5.5 a
$\text{NH}_4\text{-N} + \text{CaCl}_2$	52.4 b	6.2 b
Calcium nitrate (CaNO_3) (15-0-15)	52.6 b	6.2 b
$\text{CaNO}_3 + \text{CaCl}_2$	50.0 b	7.0 b

^zDuncan's new multiple range test, 5% level.

TABLE 3. Effects of Nitrogen and Calcium Fertilization on Mean Bract Size, Height, and Number of Broken Branches of 'Eckespoint C-1' Pointsettias.

Treatment	Bract Size (cm)	Height (cm)	Broken Branches
Ammoniacal-N (15-16-17)	42.1 a ^z	60.3 a	1.0 a
NH ₄ -N + CaCl ₂	38.9 ab	56.9 ab	0.4 b
Calcium Nitrate (15-0-15)	38.9 ab	57.1 ab	0.1 b
CaNO ₃ + CaCl ₂	34.9 b	52.7 b	0.1 b

^zDuncan's multiple range test, 5% level.

Plants fertilized with higher ammoniacal nitrogen had larger bracts than those receiving calcium nitrate supplemented with calcium chloride sprays (Table 3). There were no differences in bract sizes among plants in the higher calcium treatments.

Plants supplied 15-16-17 fertilizer alone were taller than those given the highest calcium levels, provided by calcium nitrate fertilization plus calcium chloride sprays (Table 3). There were no differences in height among plants in the higher ammonium, ammonium plus calcium chloride and calcium nitrate only treatments (Fig. 9).

The 'Eckespoint C-1' cultivars receiving additional calcium in the form of calcium nitrate or calcium chloride sprays had fewer broken branches than plants supplied the higher ammoniacal nitrogen alone (Table 3). There were no differences found among these higher calcium treatments. However, visual observation indicated addition of calcium in the form of calcium chloride sprays to the higher ammoniacal nitrogen formulation of fertilizer enhanced branch retention. A similar observation was not evident between plants receiving calcium nitrate and those given calcium nitrate plus calcium sprays.

Effects on pH

The initial pH was 6.0 for all treatments. Final pH was 6.5 for ammoniacal-N fertilized plants, 6.3 for $\text{NH}_4\text{-N}$ plus calcium sprays, 6.8 for calcium nitrate fed plants, and 6.5 for plants receiving $\text{CaNO}_3\text{-N}$ plus calcium sprays.



Figure 9. Photograph comparing the effects of the fertilization treatments on 'Eckespoint C-1' poinsettias. Height was reduced in plants receiving 15-0-15 + calcium chloride and branch retention was increased in those fertilized with additional calcium.

Cutting production

During the course of this study plants were propagated by stem tip cuttings to maintain a supply of plant material for destructive histological sampling. It was noted that cuttings obtained from stock plants fertilized with calcium nitrate rooted much faster and produced thicker root systems under mist than did propagules from stock plants fertilized with the higher ammoniacal nitrogen formulation (Fig. 10).

B. HISTOLOGY

Sections

The sections obtained were 40 μ m thick as is typical of sliding microtomy. Such sections are disadvantageous for high power brightfield microscopy but can be viewed and photographed with excellent resolution up to 120x magnification on a stereoscope. Clear views of stained tissue systems were available in sections 40 micrometers thick.

Stains

Both stain techniques provided good discrimination of the epidermal, cortical and vascular tissue systems (Fig. 11, 12). Toluidine blue O provided good metachromatic staining. The starch granules and middle lamella were stained a purplish red; lignified cell walls stained blue-green; cytoplasm, and nuclei stained violet; nucleoli stained purple; and cellulose cell walls were variable from purplish red to violet. Sections stained with Gill's hematoxylin counterstained with safranin showed purplish

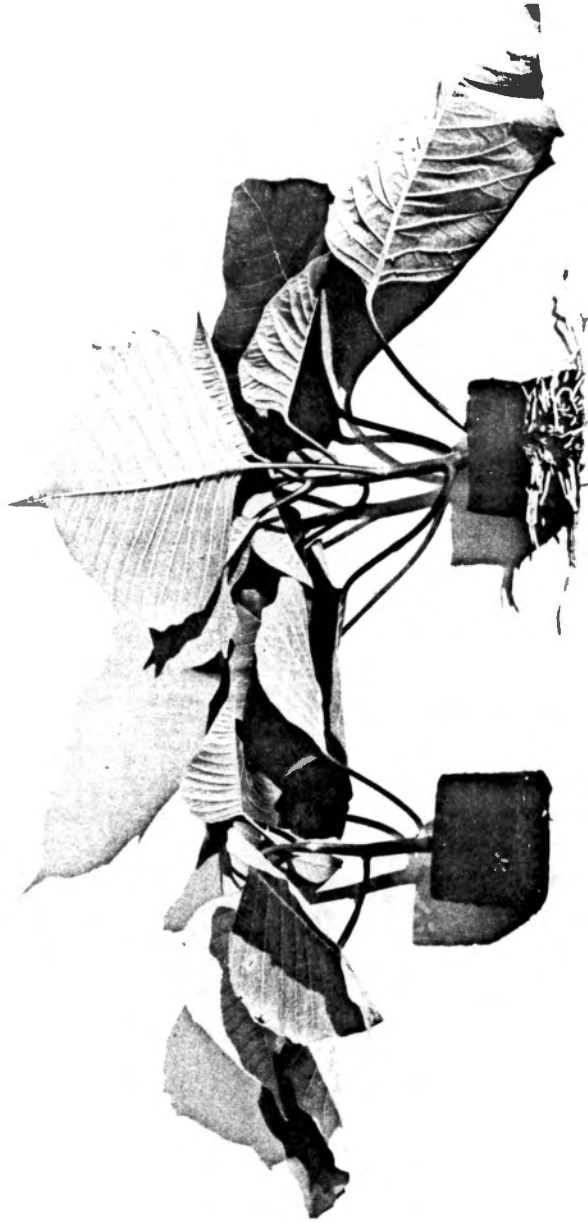


Figure 10. The effect of calcium nitrate (right) versus ammoniacal nitrogen (left) on the production of rooted cuttings under mist. Cuttings from stock plants fertilized with calcium nitrate (15-0-15) produced thicker root systems than cuttings from stock fertilized with higher ammoniacal nitrogen (15-16-17).

Figure 11. Photomicrograph of poinsettia near median longitudinal section showing tissue stained with Toluidine Blue O. (7.5 X Magnification).

Figure 12. Photomicrograph showing the results of the hematoxylin counterstained with safranin stain technique on a near median longitudinal section of poinsettia tissue. (7.5 X Magnification).



Figure 11.

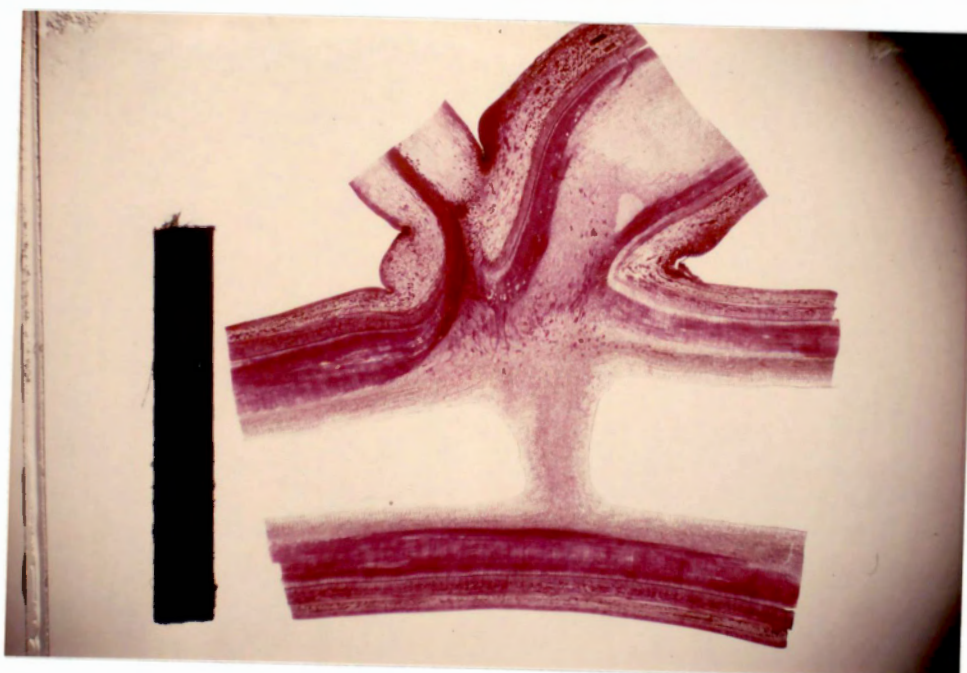


Figure 12.

nuclei and starch; deep red cells scattered throughout the tissue; dark red lignified cell walls; and lighter red to pinkish red nonlignified cell walls. Stain results were equally clear in both the 'Annette Hegg' and 'C-1' tissue samples.

A mass of cells was noted in the branch axis of some of the specimens (Fig. 13). These cells appear to be undifferentiated parenchyma. Nuclei could not be discerned. Cells of this type were observed more frequently in 'Annette Hegg' specimens but were also observed in some 'C-1' sections. These cells reacted differently to both stain methods than did those in the rest of the tissue. In toluidine blue these cells stained greenish aqua while in Gill's hematoxylin-safranin stain they turned blood red.

Anatomical structure

Comparisons of the anatomical structure of the two cultivars did not discern any gross differences in the vascular tissue systems. The 'C-1' tissue sections were larger than the 'Annette Hegg' but tissue distribution and patterns of lignification were similar. There were no discernable differences in the connection of the lateral branches to the main stem when viewed in median longitudinal sections.

Retting experiments did show differences in the branch attachments between the two cultivars. The connection in 'Annette Hegg' is constricted at the point of attachment (Fig. 14). In the 'C-1' the connection between the lateral branch and main stem is much more substantial and there is no constriction (Fig. 14).

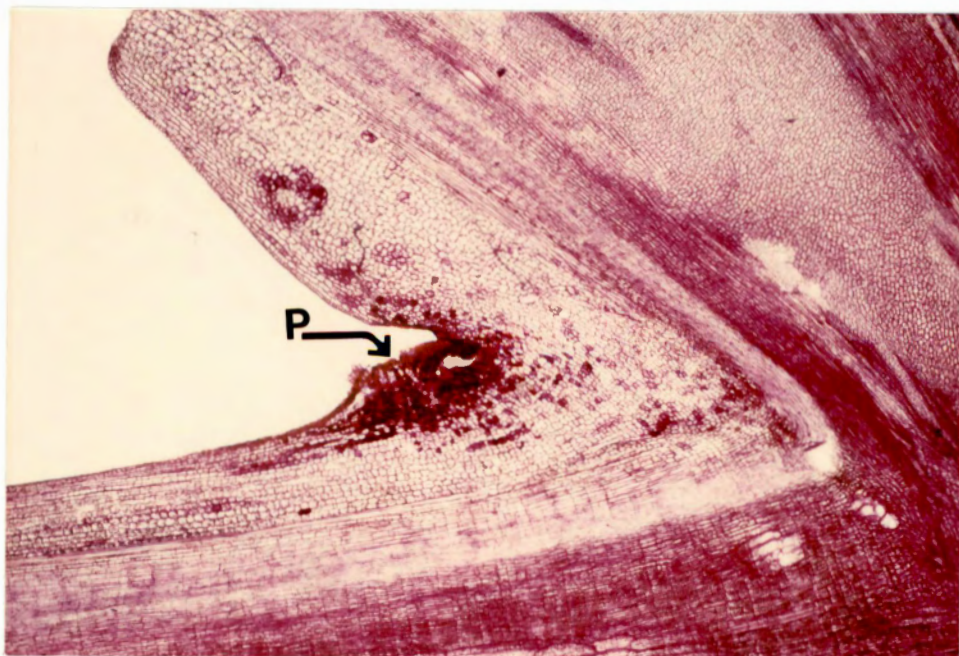


Figure 13. Photomicrograph of undifferentiated parenchyma cells (P) that were noted in the branch axis of some plants. (37.5 X Mag.).

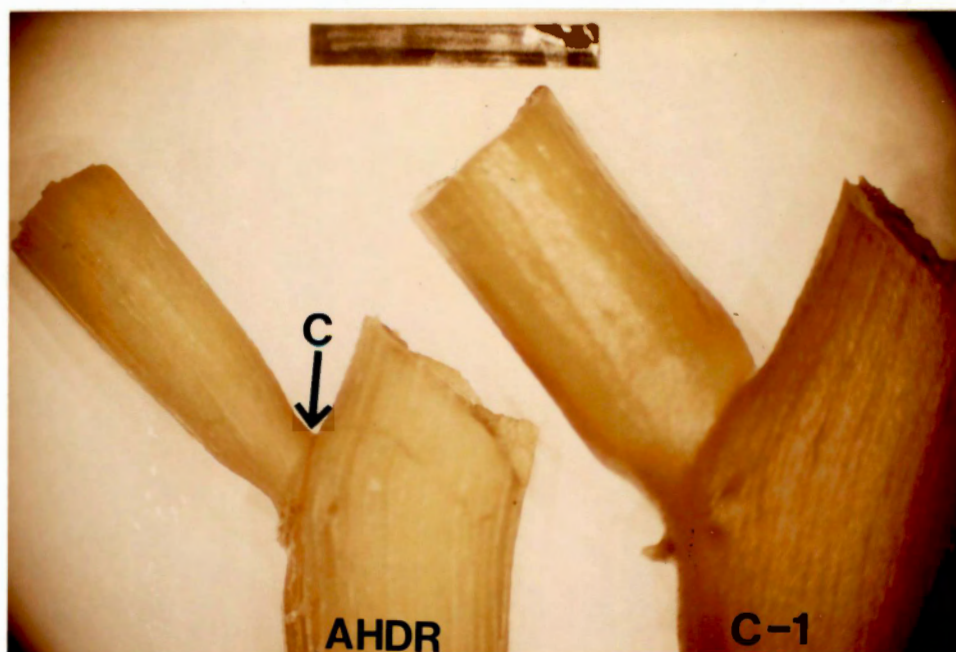


Figure 14. Photomicrograph of retted tissue of 'Annette Hegg Dark Red' (AHDR) and 'Eckespoint C-1' (C-1) showing the constriction (C) of the lateral branch at the junction with the main stem in AHDR but not in the C-1 cultivar. (1.5 X Magnification).

CHAPTER V

DISCUSSION

A. GREENHOUSE

The primary objective of this study was to determine the effects of nitrogen and calcium fertilization on branch retention of standard and self-branching poinsettia cultivars. The cultivars tested were: 'Eckespoint C-1' (standard); 'Annette Hegg Dark Red' (self-branching); and 'Gutbier V-14 (Glory)' (self-branching).

The results showed calcium nitrate fertilization enhanced branch retention in 'Eckespoint C-1' and 'Annette Hegg Dark Red' poinsettias. Bract size of 'Annette Hegg Dark Red' and height of both cultivars were reduced. Bract size of 'Eckespoint C-1' was reduced when plants received calcium nitrate fertilization plus calcium chloride sprays. However, the reduced bract size was more than off-set by the increased symmetry of the plant. Since most to all of the branches are retained on these plants the overall result is a higher quality poinsettia despite reduced bract size.

Branch retention was not enhanced by calcium nitrate fertilization in the 'Gutbier V-14 (Glory)'. However, these plants lost very few branches compared to the other two cultivars. Height was reduced in calcium nitrate fertilized plants but bract size was not.

These results are in agreement with those of previous researchers. Calcium nitrate fertilization has been shown to

produce plants with reduced height (3, 10); larger, more productive root systems (3, 11); and more vigorous growth (3, 7, 11).

Enhanced branch retention may be related to more efficient calcium uptake in calcium nitrate fertilized plants. Calcium has been shown to have an integral structural role in maintaining membrane integrity (29). Depressed calcium levels have been associated with high levels of nitrogen fertilization (29, 36) and the lower cation composition of NH_4 -fed plants (18, 19, 24). Calcium cannot be taken up as rapidly as required when plants are grown under high nitrogen levels (29) and a competition exists between NH_4^+ and Ca^{++} ions for uptake (19, 24). In addition, high nitrogen levels inhibit root growth which in turn impairs calcium uptake (19, 36). Also, ammoniacal nitrogen can induce water stress in poinsettias, a condition under which calcium uptake becomes inefficient (6, 11, 19).

Supplying nitrogen as calcium nitrate may therefore alleviate these problems in the following ways:

- 1) There is a constantly available supply of calcium in the soil in the form of calcium nitrate.
- 2) The higher level of nitrate ions improve efficiency of Ca^{++} uptake.
- 3) Lower levels of NH_4^+ ions are present to compete with the Ca^{++} ions for uptake.
- 4) Water stress is not associated with nitrate-N fertilizer as is ammoniacal-N.

5) Root growth is enhanced in calcium nitrate fertilized poinsettias.

Calcium is rapidly immobilized in the plant and cannot be taken up rapidly enough by the roots to meet plant requirements under high nitrogen conditions (19, 29). Foliar spray applications have been demonstrated to provide increased calcium in a number of species (19, 21, 29, 32, 39, 41). It was therefore decided supplying additional calcium in this manner may improve branch retention in plants receiving the higher ammoniacal nitrogen formulation fertilizer, 15-16-17, and may further enhance branch retention in calcium nitrate fertilized (15-0-15) plants.

Results showed the addition of calcium as foliar sprays of calcium chloride at the rate of 66.5 mg/l on a weekly basis did not enhance branch retention when used with calcium nitrate liquid fertilization for any of the cultivars tested. Branch retention was increased when spray applications were made on 'Eckespoint C-1' fertilized with 15-16-17 (21% urea, 26% ammoniacal-N, 53% nitrate-N).

However, in this study the spray applications began following pinching and were terminated when bract color was noted to prevent possible damage to the developing bracts. Recent research on marginal bract necrosis on 'Gutbier V-14 (Glory)' reported successful use of calcium chloride sprays at higher concentrations beginning when bract color was noted and continuing until anthesis (41). These results suggest further research on the use of

calcium chloride sprays at higher rates and continuing for a longer duration is in order. More frequent application dates could also prove more effective in improving branch retention.

An additional benefit of calcium nitrate fertilization was noted in production of rooted cuttings. When placed under mist, cuttings taken from stock plants receiving calcium nitrate produced a thicker, more vigorous root system faster than did those from plants supplied with the higher ammoniacal nitrogen formulation. Apparently increased calcium levels in the stock plants enhances the ability of propagules to produce roots.

The effect of the different fertilizers on pH did not prove to be significant. While the pH of the media in the calcium nitrate fertilized plants was higher (6.8) than that of the other treatments, it was still in the acceptable range. The rise in pH in both types of fertilizers may be attributed to the pH of the irrigation water. The water was found to have an average pH greater than 7.

B. HISTOLOGY

Polyethylene glycol infiltration provided an acceptable method of sectioning the tough tissue by keeping the tissue partially hydrated. Total dehydration was avoided and the fixed tissue remained naturally pliable. This made possible the production of large sections of whole trunk and branch crotches of mature poinsettia plants. The water soluble property of PEG

makes extraction by immersing the sections in water or 25% aqueous ethanol a simple task.

The development of the hybrid microtome blade enhanced efficiency of sectioning with the sliding microtome. The blades remained sharp enough to section several tough specimens (4-6) and changing the dull blade was much simpler than sharpening the sliding microtome knife.

The clear metachromasy of the toluidine blue stain provided a simple, relatively quick method of differentiating the tissue systems. The results obtained were equal to those obtained with the longer, more painstaking Gill's hematoxylin-safranin stain method. However, a variety of other stain techniques may also be appropriate and should be investigated.

While no anatomical differences between the cultivars were observed in the median longitudinal sections obtained, more studies on this aspect are needed. The undifferentiated cells in the stem axis observed in some of the specimens should be more fully investigated. Refinement of the sectioning technique to obtain thinner sections and the use of histochemical tests to determine: the proportion of vascular tissue types and cell distribution; cell wall thickness; quantitative characteristics of cambial activity; and proportions of lignified and nonlignified fiber and support cells are warranted.

The retting technique is a very old method used in plant anatomy. Sharman (1941) retted mature crowns of maize to

demonstrate vascular architecture. In this study retting proved to be a simple, effective way of revealing structural differences in the branch attachment between the poinsettia cultivars tested. The results indicate the vascular connection between the branch and main stem is more substantial in the 'C-1' cultivar. This connection in the 'Annette Hegg' is constricted and appears much weaker.

Retting experiments in future studies should be aimed at examining any differences in this connection that may be caused by the various fertilization treatments. The effects of calcium nitrate fertilization and supplemental calcium chloride sprays on this attachment should be determined.

CHAPTER VI

SUMMARY AND CONCLUSIONS

A. GREENHOUSE

The main purpose of this research was to determine the effects of nitrogen and calcium fertilization on stem strength (shearing of lateral branches from the main stem) of three representative poinsettia cultivars: 'Eckespoint C-1' (standard type); 'Annette Hegg Dark Red' (self-branching type); and 'Gutbier V-14 (Glory)' (self-branching type). These poinsettias were provided one of the following fertilizer regimes:

- 1) Peter's Peat-lite Special 15-16-17 (21% $\text{NH}_4\text{-N}$, 26% urea, 53% $\text{NO}_3\text{-N}$).
- 2) Peter's Dark Weather Feed 15-0-15 (87% $\text{NO}_3\text{-N}$, 13% urea).
- 3) 15-16-17 plus foliar sprays of calcium chloride.
- 4) 15-0-15 plus calcium chloride sprays.

Liquid fertilization was supplied as constant feed at the rate of 133 mg/l nitrogen. Best results were obtained when this fertilization began upon potting the rooted cuttings. Calcium chloride sprays at the rate of 66.5 mg/l calcium began following pinch date and continued until bract color was noted.

Calcium nitrate fertilization resulted in plants with enhanced stem strength. The height of all cultivars was reduced as was the bract size of 'Annette Hegg Dark Red' plants. However, the smaller bracts were more than off-set by the improved

symmetry and overall quality of the plants due to the increased number of branches. The 'Gutbier V-14 (Glory)' plants did not exhibit enhanced branch retention but these plants lost very few branches in any of the treatments.

Supplemental calcium as calcium chloride foliar sprays enhanced branch retention when used on 'Eckespoint C-1' plants fertilized with 15-16-17, the higher ammoniacal nitrogen formulation. It did not improve stem strength of 'C-1' when used with calcium nitrate fertilization or of 'Annette Hegg Dark Red' or 'Gutbier V-14 (Glory)' when used with either nitrogen fertilizer. However, recent research results (41) indicate the merits of further studies using more frequent applications of calcium chloride sprays at higher rates for a longer duration.

Further research is also warranted on the role of nitrogen and calcium fertilization on cutting production. Stem cuttings obtained from stock plants fertilized with calcium nitrate rooted faster and more vigorously under mist than did those from the higher ammoniacal nitrogen fed plants.

B. HISTOLOGY

No anatomical differences were noted between the standard poinsettia type, 'Eckespoint C-1', and the self-branching type poinsettia, 'Annette Hegg Dark Red', when median longitudinal sections of the specimens were examined. Anatomical differences could be noted when the thick epidermis was removed and the underlying vascular tissue was exposed by the retting technique.

Further anatomical and histological studies to quantify and determine the exact nature of these differences are in order. Some areas of investigation would be to determine: the proportional areas occupied by vascular and nonvascular supporting tissues; proportions of lignified and cellulose support fibers; and the frequencies of different cell types in the different tissue systems. The significance of the mass of undifferentiated parenchyma cells in the axis of some of the sections should be ascertained. Use of different stain and histochemical techniques and refinement of the sectioning method to produce thinner sections are warranted.

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