

EFFECTS OF CYTOKININ AND ANCYMIDOL ON
POINSETTIA PLANT DEVELOPMENT,
COMPOSITION AND PERFORMANCE

By

ROBERT EARL MOON

Bachelor of Science
Oklahoma State University
Stillwater, Oklahoma
1969

Master of Science
Oklahoma State University
Stillwater, Oklahoma
1971

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
DOCTOR OF PHILOSOPHY
July, 1974

Thesis
1974D
M818e
cop. 2

MAR 13 1975

EFFECTS OF CYTOKININ AND ANCYMIDOL ON
POINSETTIA PLANT DEVELOPMENT,
COMPOSITION AND PERFORMANCE

Thesis Approved:

Leroy J. Crox

Thesis Adviser

R N Payne

Earl E. Whitcomb

W R Kays

Robert Terry

N N Durbin

Dean of the Graduate College

902150

PREFACE

The influence of chemical growth regulators has been a significant factor in improving plant quality commercially for several years. As more growth regulating chemicals are developed, more research is needed to determine their possible commercial value. This study was concerned with two of these chemicals and their effect on poinsettia production.

I would like to express my sincere appreciation to the many people who helped make the completion of this study possible. I have truly enjoyed all my personal contacts and will remember the kindness and friendship shown me.

I am greatly indebted to Dr. R. N. Payne and Dr. L. I. Croy for their valuable guidance, counseling, persistent patience and understanding, and constant encouragement throughout my graduate program. Valuable suggestions and comments from the other members of my committee, Professor W. R. Kays, Dr. C. E. Whitcomb, and Dr. H. R. Terry are also appreciated.

Special appreciation is extended to Mr. H. W. Macklin, Greenhouse Foreman, for his assistance throughout this research project. Appreciation is extended to the entire Agronomy and Horticulture staffs who contributed time and energy to assist me.

My greatest love and appreciation goes to my wife, Suzanne, daughter, Christee, and family for their patience, confidence and encouragement.

I will always thank God for the opportunities I have had here at Oklahoma State University to work with these individuals, make friends, and to expand my knowledge.

TABLE OF CONTENTS

Chapter	Page
I. INTRODUCTION	1
II. REVIEW OF LITERATURE	3
Cytokinins	3
Ancymidol	6
Foliar Analysis	8
Stock Plant Cutting Production	9
Soluble Carbohydrates - Total Sugars	13
III. MATERIALS AND METHODS	14
Stock Plants	14
Foliar Analysis	16
Soluble Carbohydrates	17
Rooting	17
Christmas Crop	17
IV. RESULTS	19
Stock Plants	19
Symptoms	19
Stem Diameter of Cuttings	19
Cuttings Harvested	21
Macronutrients	27
Anthrone Detectable Sugars	27
Root Weight	32
Rooting	32
Stem Diameter	32
Days to Root	32
Christmas Crop	37
V. DISCUSSION	43
Stock Plants	43
Symptoms	43
Stem Diameter of Cuttings	44
Cuttings Harvested	45
Macronutrients	46
Anthrone Detectable Sugars	46
Rooting	47
Stem Diameter and Days to Root	47

Chapter	Page
Christmas Crop.	47
SELECTED BIBLIOGRAPHY	49
APPENDIX - EXPERIMENTAL PROCEDURE ANTHRONE METHOD	54

LIST OF TABLES

Table	Page
I. Mineral Analysis Interpretation Key for Poinsettias	10
II. Theoretical Stock Plant Production.	11
III. Stock Plant Cutting Production, 'Eckespoint C-1'.	12
IV. Influence of Cytokinin and Ancymidol on Cutting Stem Diameter Over a Five Week Harvest Period.	22
V. Effect of Cytokinin for all Levels of Ancymidol on Cutting Stem Diameter Over a Five Week Harvest Period.	23
VI. Effect of Ancymidol for all Levels of Cytokinin on Cutting Stem Diameter Over a Five Week Harvest Period.	24
VII. Number of Cuttings Harvested Over a Five Week Period as Influenced by Cytokinin and Ancymidol.	25
VIII. Effect of Cytokinin Over all Levels of Ancymidol on Number of Cuttings Produced Over a Five Week Period.	26
IX. Influence of Ancymidol for all Levels of Cytokinin on Number of Cuttings Produced Over a Five Week Period.	28
X. Effect of Cytokinin and Ancymidol on Macronutrients and Anthrone Detectable Sugars.	29
XI. Macronutrients and Anthrone Detectable Sugars as Influenced by Cytokinin Over all Levels of Ancymidol	30
XII. Influence of Ancymidol for all Levels of Cytokinin on Macronutrients and Anthrone Detectable Sugars	31
XIII. Effect of Cytokinin on Root Dry Weight of Stock Plants.	33
XIV. Influence of Cytokinin and Ancymidol Treatments on Stem Diameter of August 24 Cuttings and Rooting Quality	34

Table	Page
XV. Stem Diameter of August 24 Cuttings and Rooting Quality as Influenced by Cytokinin Over all Levels of Ancymidol	35
XVI. Effect of Ancymidol for all Levels of Cytokinin on Stem Diameter of August 24 Cuttings and Rooting Quality	36
XVII. Influence of Cytokinin and Ancymidol Treatments on the Development of the Christmas Crop	38
XVIII. Development of the Christmas Crop as Influenced by Ancymidol Over all Levels of Cytokinin.	39
XIX. Effect of Cytokinin for all Levels of Ancymidol on the Development of the Christmas Crop	40

LIST OF FIGURES

Figure	Page
1. Effect of Cytokinin (SD8339) on Growth of Randomly Selected Stock Plants. Upper - Four Days After the Last Application. Lower - 18 Days After the Last Application. Left to Right: 0-None; 1X-100 ppm 1 Time; 2X-100 ppm 2 Times; and 3X-100 ppm 3 Times.	20
2. Comparative Growth of Randomly Selected Plants From the Christmas Crop. Upper - 4 Levels of Cytokinin No Ancymidol. Lower - 4 Levels of Ancymidol, No Cytokinin	41
3. Residual Effects of Cytokinin (SD8339) on the Christmas Crop. Upper - Chemical Pinching Effect. Middle - Blindness Effect. Lower - Temporary Short Internode Elongation Effect	42

CHAPTER I

INTRODUCTION

Chemical growth regulators are used by plant growers to hasten root formation, inhibit growth, stimulate growth, control abscission, hasten flowering and fruiting, and delay senescence [44]. Commercial poinsettia (Euphorbia pulcherrima Willd.) production has been improved by the use of growth regulators; in propagation [6], and height control [22]. The poinsettia is an important florist crop propagated by cuttings from stock plants. Commercial greenhouse operators usually obtain new stock plants yearly from specialists. Since this represents an annual expenditure, it is important that the operators obtain the maximum number of high quality cuttings from each stock plant. Therefore, if the number of quality cuttings could be increased by using chemical growth regulators, the operator's profits could be increased.

The purpose of this study was to investigate the effects of a synthetic cytokinin and a chemical growth inhibitor on poinsettia stock plants.

Specific objectives were to determine the effects of a synthetic cytokinin (SD8339)¹ and ancymidol (A-Rest)² on development of

¹SD8339 (PBA)-6-benzylamino-9-(tetrahydropyran-2-yl)-9H-purine supplied by Shell Development Company, Modesto, California, as an experimental compound.

²Ancymidol- α -cyclopropyl- α -(4 methoxyphenyl)-5-pyrimidinemethanol supplied by Elanco Products Company, Indianapolis, Indiana.

'Eckespoint C-1' stock plants relative to (a) number of usable propagation branches developing from pinches; (b) stem diameter of cuttings; (c) soluble carbohydrate and macronutrient (percent of dry weight, N, P, K, Ca, Mg, and $\text{NO}_3\text{-N}$ ppm) contents of cutting foliage; (d) rooting quality of cuttings without use of a rooting hormone; (e) residual effects of treatments if any on stock plants (number and stem diameter of all cuttings produced for five weeks); and (f) performance and residual effects in the rooted cuttings used for producing plants for Christmas sales.

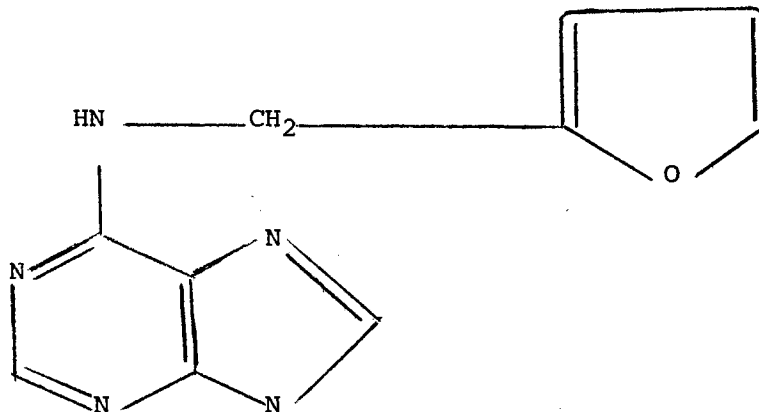
CHAPTER II

REVIEW OF LITERATURE

Cytokinins

Research by J. Van Overbeek in 1941 suggested the presence of kinetin [54]. Cytokinins were first discovered by Professor Folke Skoog as a direct outcome of tissue culture studies [47]. The substance was first isolated in December of 1955 by Carlos Miller [31].

The structure of kinetin is [29, 30]:



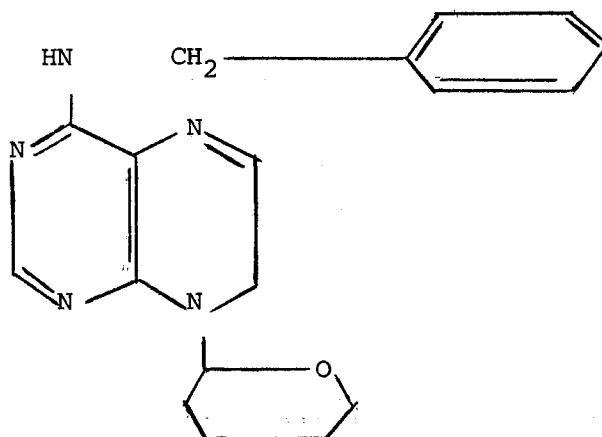
Kinetin - 6-furfurylamino-purine

The class of products were first named kinetin. However, this proved to be an unsatisfactory name because it conflicted with another class already named by the animal physiologists. Skoog in 1965 proposed the name be changed to cytokinins and this has met the widest acceptance [48].

Cytokinins are thought to affect plant growth by increasing mitosis and cell division [31]; stimulating cell enlargement [42]; initiating morphogenesis [19]; breaking dormancy [32, 37]; delaying senescence [39]; retarding apical dominance [10, 40]; and promoting fasciated growth [53].

Interaction between cytokinins, auxins, and gibberellins regulate cell division, cell enlargement, and cell differentiation. Application of cytokinins will break apical dominance in some plants. Apical dominance is thought to depend on an antagonism between the inhibiting influence of auxin and the promoting influence of the cytokinins. When the growing apex of a shoot is intact the auxin inhibits lateral shoots because there is more auxin present than cytokinin. However, when synthetic cytokinins are applied or the apex removed, the cytokinins become greater than the auxins and lateral branching occurs [41]. Therefore, synthetic cytokinins could be beneficial to plant growers by increasing the number of cuttings per stock plant and the number of flowers on flowering plants.

Previous research conducted on synthetic cytokinins indicates growth was modified for several floricultural plants. The structure for synthetic cytokinin is [46]:



SD8339 or PBA - 6-benzylamino-9(tetrahydropyran-2-yl)-9H-purine (PBA)

Synthetic cytokinins have increased branching of poinsettias, roses, chrysanthemums, petunias, azaleas, carnations and geraniums. It has significantly increased the number of lateral branches produced after pinching [20]. Foliar applications have been more effective than other methods of application [20]. Some plants are moderately tolerant of synthetic cytokinin, showing no phytotoxicity to applications of 2000 ppm while other plants are sensitive to applications of 50 ppm. Results show [20] that several applications of SD8339 at low concentration are more effective than single treatments at a higher concentration. Synthetic cytokinin stimulates early flowering and causes shorter plants [20].

Carpenter's results on chrysanthemums [9] showed plants treated with synthetic cytokinin increased branching, reduced stem length, delayed flowering and increased cutting production without affecting rooting. The 200 ppm spray was more effective than the 100 ppm spray. More branches were developed from the application made at time of pinching than two weeks before or two weeks after the pinch.

Research conducted on geraniums indicated synthetic cytokinin used at 75 ppm will promote lateral branching and have no significant effect on flowering. Plants treated two weeks before pinching had the largest number of branches from 2.5-7.5 cm., while plants treated on the date of pinching induced more branches greater than 7.5 cm. in length [8].

Williams' [55] data showed synthetic cytokinin applied to new leaf buds caused actively growing 'Golden Delicious' and 'Red Delicious' apple shoots to develop into spurs and lateral branches ten to fourteen days after treatments.

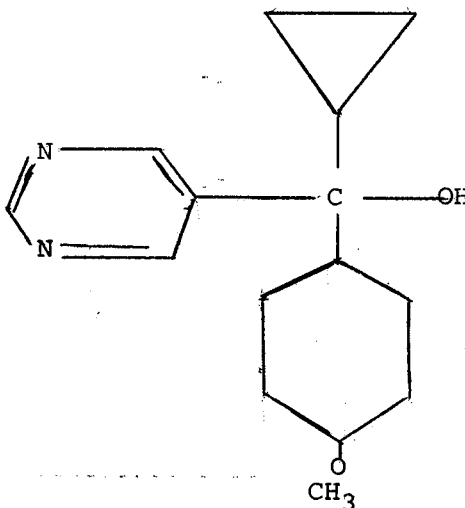
'Eckespoint C-1' poinsettias were treated with synthetic cytokinin (6-benzylamino purine) at 500 and 1000 ppm three, two or one week before soft pinching, at pinching, and one or two weeks after pinching. The 6-benzylamino purine improved branching at all treatment times over plants not treated, but the best branching resulted when sprays were applied prior to pinching [7].

Fox and Weis [17] reported that kinetin is slowly translocated in plants and as much as 40 percent of 6-benzylamino purine was metabolized before it was translocated.

Milbocker [28] found that poinsettias respond to low concentrations of kinetin when applied directly to the buds. Foliar application of kinetin had to be of higher concentrations than direct bud application because the cytokinin must be transported to the buds.

Ancymidol

Ancymidol (A-Rest) is a plant growth inhibitor which has been shown to reduce internode elongation. The structure of ancymidol is as follows [16]:



Ancymidol - α -cyclopropyl- α -(4 methoxyphenyl)
-5-pyrimidinemethanol

Ancymidol is effective on a large number of commercial greenhouse plants. This is the main advantage ancymidol has over other growth retardants. When applied at effective dosages (usually low) the chemical has controlled the height of many tropical and bedding plants [18]. It has also been used effectively on chrysanthemums, Easter lilies, and poinsettias.

Larson [22, 24] presented data on the effectiveness of ancymidol on chrysanthemums and Easter lilies. Height was effectively controlled with both foliar sprays and soil drenches. Chrysanthemums showed no adverse effects relative to flower or leaf number, but flowering was delayed when high (250 and 500 ppm) concentrations were used as a foliar spray two weeks after the pinch. Easter lilies with 125 ppm foliar spray and the 0.25 and 0.50 mg. drench had excellent height control with no adverse effects. The chemical was applied February 11 for those potted December 31. Higher concentrations caused injury to the lilies.

Satisfactory height control has been achieved on flowering poinsettias with ancymidol. Soil drench applications work more efficiently than foliar sprays. Foliar sprays tend to cause leaf injury on poinsettias [12, 3, 36]. Data indicate that a single drench application in the range of 0.25-1.0 mg. per 6-inch pot was necessary [36]. Cathey [11] reported the diameter of poinsettia bracts was reduced, but branching was increased more on plants treated with ancymidol than on untreated plants.

Very little has been published concerning the effect of ancymidol on poinsettia stock plants, the residual effect on cuttings, or temperature effects on stock plants and cuttings. However, there has been

research conducted in these areas with other growth retardants [6, 33, 34, 38, 44]. Larson [23] found that cycocel controlled height on stock plants and had some residual effect on cuttings. However, the best plants were produced by a second treatment of cycocel after cuttings were established. Love [26] found little difference in plant height between different night temperature regimes.

The interaction effects of temperature relative to effectiveness of cytokinin and ancymidol are not clear. Rates of application that sufficiently control flowering poinsettias might have less effect on stock plants grown in higher temperatures. Other questions such as ancymidol's effectiveness as a residual growth retardant, subsequent rooting of cuttings, and influence on plant carbohydrate and nutrient levels have not been documented.

Foliar Analysis

Foliar analysis is a fairly precise method of estimating the plant's nutrient status. The plant is a good indicator of all the factors which influence plant growth. If the factors influencing plant growth are negative or positive the tissue nutrient content should show this in the analysis [13, 35]. Smith [49] showed that foliar analysis is based on the uptake and distribution of nutrients and the interaction between absorbed nutrients and growth.

The mineral nutrient content varies with different plant parts. Foliage, petioles, stems, flowers and fruits all contain different amounts of an element. Therefore, when comparing values, it is necessary to compare similar plant parts. In order to utilize the foliar analysis technique effectively, considerable care must be taken in

this area. Carlson and Sink [5] have proposed procedures for sampling poinsettias. They suggest using the most recently mature, fully-expanded leaves.

Table I expresses suggested mineral element levels for poinsettias [15]. There are several standard tables available. This one was selected because the range expressed encompasses most of the other standard tables.

Very little has been published concerning the effect of ancymidol and cytokinin upon nutrient content of the plant. However, there has been research conducted on the effect of cycocel on nitrogen, phosphorus, and potassium levels of poinsettia tissue. Kiplinger [21] and Brown [4] showed that cycocel treated plants had a higher leaf content of nitrogen, phosphorus, and potassium than did untreated plants.

Stock Plant Cutting Production

The number of cuttings produced by a stock plant varies considerably depending upon cultivar, potting date, pot size, number of pinches, and plant nutrition. Tables II and III were reprinted from The Poinsettia Manual [15] to show the theoretical and actual stock plant cutting production as influenced by potting date, pot size, and number of pinches. The cutting production was greater than theoretical cutting production because of a higher number of breaks occurring after the first pinch (Tables II and III). The number of cuttings produced can also be influenced by nutrition. Shanks [43] showed that nitrogen, phosphorus and potassium significantly increased the number of terminal cuttings produced.

TABLE I
MINERAL ANALYSIS INTERPRETATION KEY FOR POINSETTIAS^a

Element	Critical Level	Normal Range	Toxic Level
		<u>Percentage</u>	
Nitrogen	3.0	4.0-6.0	>7.3
Phosphorus	0.2	0.3-0.7	>0.7
Potassium	1.0	1.5-3.5	4.0
Calcium	0.5	0.7-2.0	
Magnesium	0.2	0.4-1.0	
Sulfur as Sulfate	b	b	b
Sulfur as Total	b	b	b
Sodium		0.0-0.4	0.5
Chloride		0.0-1.5	3.0
		<u>PPM</u>	
Copper	1	2-10	
Zinc	15	25-60	
Manganese	30	45-300	650
Iron	50	100-300	
Boron	20	30-300	700
Molybdenum	0.5	1-5	

^aYoungest mature leaves including petiole. Approximately 20 leaves required per sample.

^bNo critical values available.

Source: The Poinsettia Manual [15].

TABLE II
THEORETICAL STOCK PLANT PRODUCTION

	<u>Date of Pinch</u>			
Plant Liners	March 15	April 15	May 15	June 15
1st Pinch (at 2 weeks) 3 Breaks	March 30	April 29	May 29	June 29
2nd Pinch (at 6 weeks) 6 Breaks	April 29	May 27	June 26	July 27
3rd Pinch (at 10 weeks) 12 Breaks	May 25	June 24		
4th Pinch (at 14 weeks) 24 Breaks	June 22			
	<u>Number of Cuttings</u>			
Harvest 1st Cuttings, August 13	24	12	6	3
Harvest 2nd Cuttings, September 17	48	24	12	6
Total Cuttings/Stock Plant	72	36	18	9

Source: The Poinsettia Manual [15].

TABLE III
STOCK PLANT CUTTING PRODUCTION, 'ECKESPOINT C-1'^a

Pinching Dates	Planting Date			
	March 15	April 15	May 15	June 15
1	March 30	April 30	May 30	June 30
2	April 30	May 30	July 6	
3	May 30	July 6		
4	July 6			

Cutting Harvest	Per Plant	Acc. ^b	Per Plant	Acc. ^b	Per Plant	Acc. ^b	Per Plant	Acc. ^b
1 Aug. 7	15.0		9.4		10.7		6.0	
2 Aug. 14	19.1	34.1	20.8	30.2	3.3	14.0	0.0	6.0
3 Aug. 21	16.7	50.8	5.0	35.2	5.8	19.8	0.8	6.8
4 Aug. 28	1.7	52.5	1.7	36.9	10.0	29.8	0.0	6.8
5 Sept. 5	21.7	74.2	15.0	51.9	20.9	50.7	15.9	22.7
6 Sept. 11	26.7	100.9	23.3	75.2	6.7	57.4	3.3	26.0
7 Sept. 18	25.9	126.8	13.3	88.5	10.0	67.4	2.5	28.5

^a2¼-inch liners planted into 12-inch plastic pots, 1/pot, 6 plants/planting date.

^bAccumulative yield.

Source: The Poinsettia Manual [15].

Soluble Carbohydrates - Total Sugars

Soluble carbohydrate levels are influenced by light intensity [2], temperature [52], nitrogen [1], seasonal variation [14], various chemicals [45], and sampling locations [56]. There are several methods of determining soluble carbohydrates. The anthrone assay procedure was used in this study [27, 57]. For the estimation of soluble sugars in plant extracts the method yields results comparable with those obtained with the copper reagent, but includes the sugars of stable glycosides which may constitute a large proportion of the soluble carbohydrates in some plant tissue [57].

CHAPTER III

MATERIALS AND METHODS

Stock Plants

The experimental design was a randomized complete block with a 4 x 4 factorial set of treatment combinations and 10 blocks [50, 51]. There were four levels of cytokinin applied as a foliar spray¹ (45 ml. per plant) seven days prior to the second pinch, on the day of the second pinch, and seven days following the second pinch. Also there were four levels of ancymidol applied as a soil drench (100 ml. per pot) seven days prior to August 24 propagation. Each block had 16 experimental units which represent one experimental unit per treatment. The four different levels of cytokinin were none, 100 ppm one time (1X), 100 ppm two times (2X), and 100 ppm three times (3X) and levels of ancymidol were none, 0.125 mg./pot, 0.250 mg./pot and 0.500 mg./pot.

The 16 treatments were:

1 - 1, 1	none	Cytokinin	none	Ancymidol
2 - 1, 2	none	"	0.125	"
3 - 1, 3	none	"	0.250	"
4 - 1, 4	none	"	0.500	"
5 - 2, 1	1X	"	none	"
6 - 2, 2	1X	"	0.125	"
7 - 2, 3	1X	"	0.250	"
8 - 2, 4	1X	"	0.500	"

¹Tween 20 was used as a surfactant at the rate of 1.5 ml./500 ml.

9 - 3, 1	2X	Cytokinin	none	Ancymidol
10 - 3, 2	2X	"	0.125	"
11 - 3, 3	2X	"	0.250	"
12 - 3, 4	2X	"	0.500	"
13 - 4, 1	3X	"	none	"
14 - 4, 2	3X	"	0.125	"
15 - 4, 3	3X	"	0.250	"
16 - 4, 4	3X	"	0.500	"

The 'Eckespoint C-1 Red' rooted cuttings were potted in 6-inch clay pots using a growing medium of 1 soil, 1 sphagnum peat moss, 1 perlite, and seven pounds dolomite/cu. yd. by volume, on June 4, 1973. The plants were spaced 41 x 41 cm. in a glass greenhouse.

A uniformity trial was conducted in the glass greenhouse before the study was initiated. The trial showed there was less variation in the center of the house than on the outer edges. Therefore, the experimental units for this research were located in the center of the glass greenhouse. Wire platforms were used for the benches. These platforms were used because uniform air movement could be maintained around the plants. Each plant was on an individual platform.

Customary stock plant cultural procedures were used [15, 25] with plants receiving 12 oz. of soluble 200 ppm N, 88 ppm P, and 166 ppm K at every watering. Ferrous sulfate was applied at the rate of 1 oz./2 gal. if the pH was 7.0 or above. Supplementary N was added with NH_4NO_3 as soil tests indicated the need [43]. The temperature was maintained approximately 18° C nights and 25° C days. Two weeks after potting (June 18), the plants were soft-pinned. The plants receiving one, two, and three applications of cytokinin (100 ppm) were treated July 9. The plants were pinched the second time July 16 and plants receiving two and three applications of cytokinin (100 ppm) were treated. Plants receiving three applications of cytokinin (100 ppm)

were treated July 23 (seven days after the pinch). This concluded all applications of cytokinin.

The different rates of ancymidol were applied August 17, seven days prior to taking of cuttings. Every potential cutting was harvested for a five week period, August 24 - September 21 at seven day intervals. Before a shoot could be classified as a potential cutting, it had to measure 9 cm. from the growing tip to the base and still have two basal leaves on a shoot for future breaks, except for the last cutting date (anything over $7\frac{1}{2}$ cm.).

The number of cuttings and the cutting diameter² were recorded for each experimental unit. Cuttings taken August 24 were used for foliar analysis, carbohydrate analysis, and rooting study. Cuttings were taken from approximately the same location on the stock plant for each test. Cuttings taken August 31, September 7, 14, and 21 were discarded after number and stem diameter were recorded.

Foliar Analysis

Three cuttings from each experimental unit (each stock plant) were taken for the foliar analysis. The recently matured leaves, with petioles, were removed from the three cuttings, dried and put together for a composite sample of each experimental unit. Macronutrients were analyzed by the following methods: $\text{NO}_3\text{-N}$ - specific ion electrode; percent N - Micro-Kjeldahl; percent P - wet digestion and colorimetric procedure; percent K - wet digestion and flame photometry; and Ca and Mg - wet digestion and atomic absorption spectrophotometry.

²Stem diameter measurements were taken with a caliper at the base of the cutting without compressing the stem.

Soluble Carbohydrates

Two cuttings from each stock plant were frozen to prevent any change in the enzymes for the quantitative analysis of soluble carbohydrates total sugars anthrone assay (see Appendix). Two cuttings were used to form a composite sample for each experimental unit. Soluble sugars were extracted with hot 80% ethanol and recorded.

Rooting

Four cuttings from each stock plant were randomly selected for the rooting study. The cuttings were rooted in 2 $\frac{1}{4}$ -inch plastic pots using a growing medium of sphagnum peat moss-horticultural perlite mix by volume. The cuttings were spaced 12.7 x 12.7 cm. in a fiberglass greenhouse with a 21° C minimum night temperature. Each cutting's identification was maintained by labeling it and placing it in the proper block location under the mist. Customary propagation cultural procedures were used [15, 25]. The cuttings were kept in their original stock plant block, and randomized in that block. The experimental design was a 4 x 4 factorial with 4 sub-samples per treatment arranged in 10 blocks.

Rooting evaluation was recorded in days from time cuttings were taken until first roots were visible on the outside of the soil ball.

Christmas Crop

Three of the four rooted cuttings were randomly selected for the Christmas crop. The experimental design was a randomized complete block arranged in a 4 x 4 factorial with 3 sub-samples arranged in 10

blocks. The treatments were kept in their original stock plant blocks. The rooted cuttings were potted in 5½-inch clay pots using 1 soil, 1 sphagnum peat moss, and 1 perlite, on October 10, 1973.

The plants were spaced 25 x 25 cm. in a fiberglass greenhouse on redwood benches. Plants received 12 oz. of soluble 500 ppm N, 220 ppm P, and 415 ppm K weekly. The night temperature was maintained as close as possible to 18° C. Data were taken when half the cyathia showed pollen. Days to maturity, height (cm.) from the medium surface to the top of the plant, bract diameter (cm.), and stem diameter (cm.) half way up the plant were recorded on each treatment.

CHAPTER IV

RESULTS

Stock Plants

Symptoms

Immediately following the application of cytokinin, chlorosis, stunting and distortion were observed on the leaves and stems. The distortion appeared as manifestations of fasciated growth, development of split or compound leaves, two prominent glands at the base of some leaves, and crowded crowns. Plants receiving higher rates of cytokinin had more drastic symptoms than did plants receiving lower rates as shown in the upper photograph of Figure 1. The lower photograph of Figure 1 shows plants starting to recover from the chlorosis about 18 days after the last application of cytokinin. The stunted growth and distortion remained on the stock plants and on the cuttings taken for the Christmas crop for the entire study.

Stem Diameter of Cuttings

Average stem diameter of cuttings in all treatments harvested August 24 and September 21 were larger than those harvested August 31, September 7, and September 14. There was a significant increase in stem diameter as the level of cytokinin was increased. The one, two and three application rates of cytokinin significantly increased stem

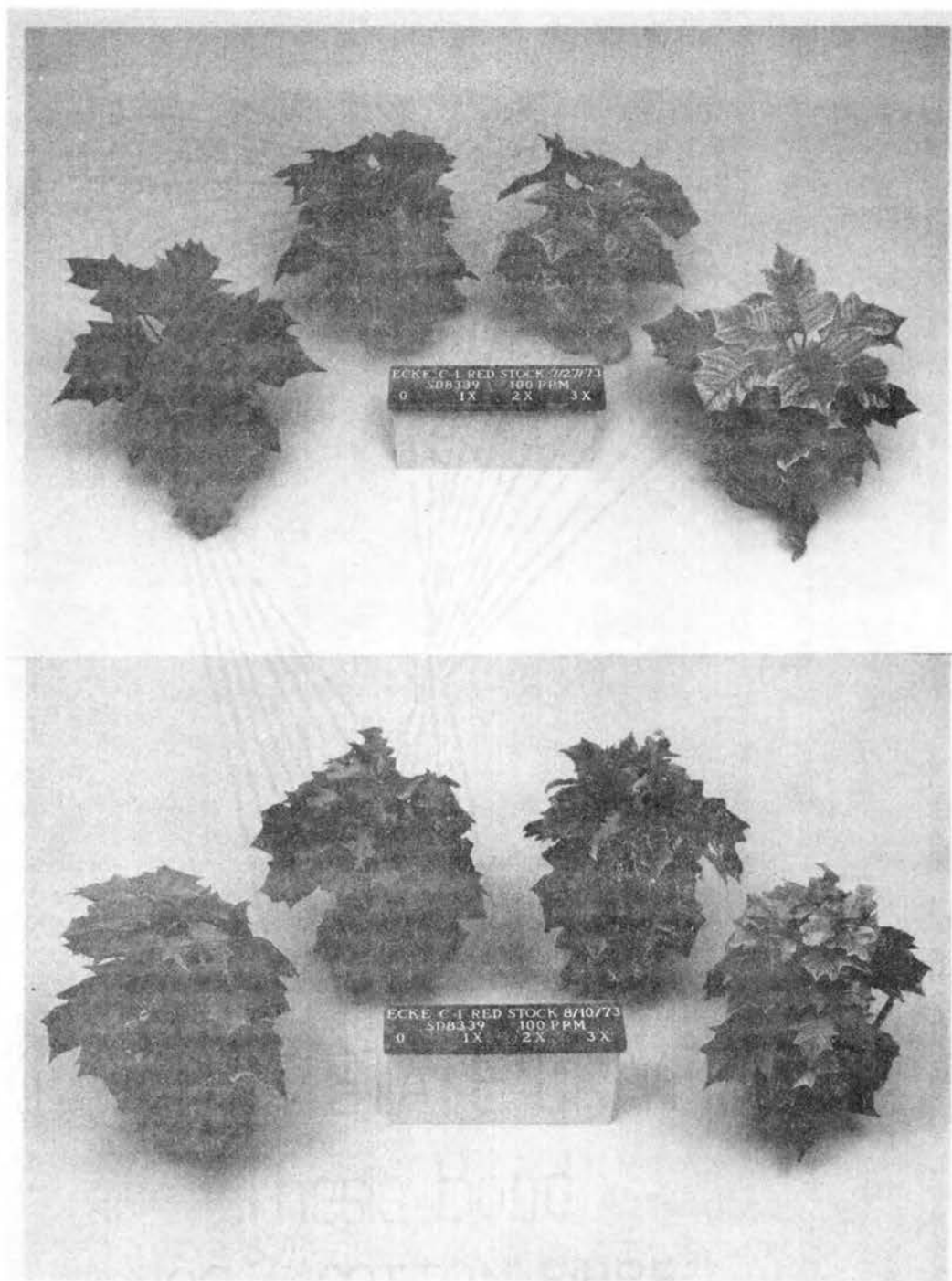


Figure 1. Effect of cytokinin (SD8339) on growth of randomly selected stock plants. Upper - four days after the last application. Lower - 18 days after the last application. Left to right: 0-none; 1X-100 ppm 1 time; 2X-100 ppm 2 times; and 3X-100 ppm 3 times.

diameter over the control August 24, August 31 and combined harvest dates (Table IV). The ancymidol influenced treatments August 24 and September 21. August 24 and September 21, the 0.500 rate of ancymidol increased stem diameter over the control in several instances.

Cytokinin treatments resulted in significantly larger stem diameter than the untreated plants with differences more pronounced at the higher cytokinin levels August 24, September 21 and combined harvest dates (Table V). The ancymidol showed significant differences August 31 and September 21 harvest dates. On those dates, cuttings in the 0.500 ancymidol treatment had significantly greater stem diameter than the control plants. There was a gradual increase in stem diameter as ancymidol rate increased for the September 21 propagation (Table VI).

Cuttings Harvested

The greatest cutting production occurred August 24 and September 21. There were significant differences among the 16 treatments but no trends were established (Table VII).

Over all levels of ancymidol, plants treated with cytokinin produced significantly more cuttings for the August 24 harvest date than the untreated plants (Table VIII). However, when considering the total number of cuttings produced during the five week harvesting period, plants treated with cytokinin produced no more cuttings than untreated plants.

The ancymidol drenches caused a gradual reduction in number of cuttings produced on August 24 and September 21. The number of cuttings for the ancymidol increased on the other three dates. Differences usually were not significant; however, ancymidol treatments resulted in a

TABLE IV

INFLUENCE OF CYTOKININ AND ANCYMIDOL ON CUTTING STEM DIAMETER
OVER A FIVE WEEK HARVEST PERIOD^a

Treatment		Stem Diameter of Cuttings Harvested (cm.)					Average Stem Diameter (cm.)
Cytokinin (100 ppm)	Ancymidol (mg./pot)	Aug. 24	Aug. 31	Sept. 7	Sept. 14	Sept. 21	
None	None	0.69bcd ^b	0.29abc ^b	0.33a ^b	0.26a ^b	0.56ab ^b	0.63a ^b
None	0.125	0.67ab	0.31abc	0.37a	0.40a	0.60abc	0.64ab
None	0.250	0.66a	0.30abc	0.39a	0.20a	0.67def	0.65abc
None	0.500	0.68ab	0.37bc	0.29a	0.47a	0.66cdef	0.67c
1X	None	0.77fg	0.19ab	0.38a	0.36a	0.55a	0.68c
1X	0.125	0.77fg	0.13a	0.30a	0.45a	0.61abcd	0.71ef
1X	0.250	0.71cde	0.42c	0.53a	0.33a	0.63bcde	0.67bcd
1X	0.500	0.73de	0.43c	0.50a	0.42a	0.65cdef	0.68d
2X	None	0.83hi	0.29abc	0.39a	0.21a	0.57ab	0.75g
2X	0.125	0.77fg	0.38bc	0.38a	0.51a	0.63bcde	0.73fg
2X	0.250	0.74ef	0.38bc	0.50a	0.39a	0.63bcde	0.70de
2X	0.500	0.76fg	0.32abc	0.44a	0.48a	0.71f	0.73fg
3X	None	0.84i	0.30abc	0.38a	0.30a	0.61abcd	0.76g
3X	0.125	0.80gh	0.43c	0.41a	0.38a	0.67cdef	0.75gh
3X	0.250	0.81hi	0.37bc	0.40a	0.29a	0.69ef	0.77h
3X	0.500	0.81hi	0.51c	0.46a	0.36a	0.70ef	0.76g
LSD (.05)		0.03	0.23	0.29	0.33	0.07	0.03

^aThe data represent the mean of 10 plants. There was no interaction between cytokinin and ancymidol.

^bMeans within a column followed by the same letters do not differ significantly at the .05 level.

TABLE V
 EFFECT OF CYTOKININ FOR ALL LEVELS OF ANCYMIDOL ON
 CUTTING STEM DIAMETER OVER A FIVE
 WEEK HARVEST PERIOD^a

Treatment Cytokinin (100 ppm)	Stem Diameter of Cuttings Harvested (cm.)					Average Stem Diameter (cm.)
	Aug. 24	Aug. 31	Sept. 7	Sept. 14	Sept. 21	
None	0.67a ^b	0.32a ^b	0.34a ^b	0.33a ^b	0.63ab ^b	0.64a ^b
1X	0.74b	0.29a	0.43a	0.39a	0.61a	0.68b
2X	0.77c	0.34a	0.43a	0.41a	0.64ab	0.72c
3X	0.81d	0.40a	0.41a	0.33a	0.66ab	0.76d
LSD (.05)	0.02	0.12	0.14	0.16	0.03	0.02

^aThe data represent the mean of 40 plants. There was no interaction between cytokinin and ancymidol.

^bMeans within a column followed by the same letters do not differ significantly at the .05 level.

TABLE VI
 EFFECT OF ANCYMIDOL FOR ALL LEVELS OF
 CYTOKININ ON CUTTING STEM DIAMETER
 OVER A FIVE WEEK HARVEST PERIOD^a

Treatment Ancymidol (mg./pot)	Stem Diameter of Cuttings Harvested (cm.)					Average Stem Diameter (cm.)
	Aug. 24	Aug. 31	Sept. 7	Sept. 14	Sept. 21	
None	0.78c ^b	0.27a ^b	0.37a ^b	0.28a ^b	0.57a ^b	0.70a ^b
0.125	0.75ab	0.31ab	0.36a	0.44a	0.62b	0.70a
0.250	0.73a	0.37ab	0.45a	0.30a	0.65bc	0.69a
0.500	0.75ab	0.41ab	0.42a	0.43a	0.68c	0.71a
LSD (.05)	0.02	0.12	0.14	0.16	0.03	0.02

^aThe data represent the mean of 40 plants. There was no interaction between cytokinin and ancymidol.

^bMeans within a column followed by the same letters do not differ significantly at the .05 level.

TABLE VII

NUMBER OF CUTTINGS HARVESTED OVER A FIVE WEEK PERIOD AS
INFLUENCED BY CYTOKININ AND ANCYMIDOL^a

Treatment		Cuttings Harvested					Average Number of Cuttings
Cytokinin (100 ppm)	Ancymidol (mg./pot)	Aug. 24	Aug. 31	Sept. 7	Sept. 14	Sept. 21	
None	None	13.1abcd ^b	1.1ab ^b	1.0ab ^b	0.7a ^b	10.0i ^b	25.9e ^b
None	0.125	12.8abc	1.6b	0.9ab	1.3a	8.3ghi	25.0cde
None	0.250	12.8ab	1.2ab	1.0ab	0.4a	6.5def	21.1a
None	0.500	11.6a	1.3ab	0.9ab	1.2a	6.2bcde	21.2a
1X	None	13.7bcd	0.3a	1.1ab	0.7a	9.1hi	24.9cde
1X	0.125	13.8bcd	0.2a	0.5a	0.9a	7.3efg	22.7abcde
1X	0.250	14.2cd	2.1b	1.1ab	0.6a	7.9fgh	25.9e
1X	0.500	13.1abcd	1.3ab	1.4ab	1.1a	7.3efg	24.2abcde
2X	None	15.0d	1.0ab	0.9ab	0.4a	6.3cdef	23.8abcde
2X	0.125	14.3cd	1.2ab	0.8ab	1.2a	5.2abcd	22.7abcde
2X	0.250	14.2cd	1.7b	1.7b	0.9a	5.6abcd	24.1abcde
2X	0.500	14.8cd	1.2ab	1.2ab	1.3a	4.9abcd	23.1abcde
3X	None	14.9d	1.3ab	0.8ab	0.5a	6.3cdef	23.8abcde
3X	0.125	14.1cd	1.5b	1.1ab	0.9a	4.8abc	22.4abcde
3X	0.250	14.4cd	1.3ab	0.9ab	0.9a	4.4a	21.9abc
3X	0.500	13.0abcd	1.7b	1.6b	0.6a	4.6ab	21.5a
LSD (.05)		2.1	1.1	0.9	0.9	1.6	3.2

^aThe data represent the mean of 10 plants. There was no interaction between cytokinin and ancymidol.

^bMeans within a column followed by the same letters do not differ significantly at the .05 level.

TABLE VIII
EFFECT OF CYTOKININ OVER ALL LEVELS OF ANCYMIDOL
ON NUMBER OF CUTTINGS PRODUCED
OVER A FIVE WEEK PERIOD^a

Treatment Cytokinin (100 ppm)	Cuttings Harvested					Average Number of Cuttings
	Aug. 24	Aug. 31	Sept. 7	Sept. 14	Sept. 21	
None	12.4a ^b	1.3a ^b	0.9a ^b	0.9a ^b	7.7b ^b	23.3ab ^b
1X	13.7b	1.0a	1.0a	0.8a	7.9b	24.4b
2X	14.6b	1.3a	1.1a	0.9a	5.5a	23.4ab
3X	14.1b	1.4a	1.1a	0.7a	5.0a	22.4a
LSD (.05)	1.0	0.6	0.5	0.5	0.8	1.6

^aThe data represent the mean of 40 plants. There was no interaction between cytokinin and ancymidol.

^bMeans within a column followed by the same letters do not differ significantly at the .05 level.

significant decrease in cuttings harvested September 21, and the 0.500 rate caused fewer total cuttings to be produced over the five week period (Table IX).

Macronutrients

Macronutrient levels as influenced by the cytokinin-ancymidol treatments were measured in leaves of cuttings taken from stock plants August 24. Leaf concentrations of percent N, P, K, Ca, and Mg for the 16 treatments were significantly different in some areas; however, no trends were established. The $\text{NO}_3\text{-N}$ leaf content increased as the cytokinin levels were increased. There was a significant increase in $\text{NO}_3\text{-N}$ and in K in plants treated three times with cytokinin (Table X).

The highest (3X) rate of cytokinin over all levels of ancymidol was significantly greater than the control in the percent $\text{NO}_3\text{-N}$, K, Ca, and Mg (Table XI).

The ancymidol over all levels of cytokinin showed no significant trends for the macronutrients (Table XII).

Anthrone Detectable Sugars

The anthrone detectable sugars for the 16 treatments showed significant differences but no definite trends were established (Table X). The cytokinin treatments over all levels of cytokinin did not significantly affect percent sugar (Table XI). However, when the plants treated with ancymidol were examined over all levels of cytokinin, the percent sugar decreased as the ancymidol levels were increased (Table XII).

TABLE IX
 INFLUENCE OF ANCYMIDOL FOR ALL LEVELS OF CYTOKININ
 ON NUMBER OF CUTTINGS PRODUCED
 OVER A FIVE WEEK PERIOD^a

Treatment Ancymidol (mg./pot)	Cuttings Harvested					Average Number of Cuttings
	Aug. 24	Aug. 31	Sept. 7	Sept. 14	Sept. 21	
None	14.2a ^b	0.9a ^b	0.9a ^b	0.6a ^b	7.9b ^b	24.6b ^b
0.125	13.7a	1.1ab	0.8a	1.1a	6.4a	23.2ab
0.250	13.7a	1.6b	1.2a	0.7a	6.1a	23.2ab
0.500	13.2a	1.4ab	1.3a	1.0a	5.7a	22.5a
LSD (.05)	1.0	0.6	0.5	0.5	0.8	1.6

^aThe data represent the mean of 40 plants. There was no interaction between cytokinin and ancymidol.

^bMeans within a column followed by the same letters do not differ significantly at the .05 level.

TABLE X

EFFECT OF CYTOKININ AND ANCYMIDOL ON MACRONUTRIENTS AND ANTHRONE DETECTABLE SUGARS^a

Treatment		Percent N	PPM NO ₃ -N	Percent P	Percent K	Percent Ca	Percent Mg	Percent Sugar
Cytokinin (100 ppm)	Ancymidol (mg./pot)							
None	None	4.0bcdef ^b	575.0a ^b	0.606abcd ^b	3.113a ^b	0.219a ^b	0.341ab ^b	1.90ab ^b
None	0.125	3.8abc	650.0a	0.636cd	3.132a	0.199a	0.338a	1.98bc
None	0.250	4.1cdef	612.5a	0.631cd	3.145a	0.239ab	0.360abcd	1.8ab
None	0.500	4.2ef	722.5a	0.609abcd	3.127a	0.241ab	0.367abcd	1.82ab
1X	None	4.1def	547.5a	0.655d	3.202ab	0.294b	0.371abcde	2.12c
1X	0.125	4.0bcdef	510.0a	0.651d	3.115a	0.235ab	0.383cde	1.99bc
1X	0.250	3.9abcde	459.8a	0.566ab	3.070a	0.228a	0.353abc	1.92abc
1X	0.500	4.0bcdef	477.5a	0.628cd	3.062a	0.248ab	0.367abcd	1.73a
2X	None	4.0bcdef	802.5ab	0.596abcd	3.140a	0.257ab	0.380cde	1.99bc
2X	0.125	3.8abc	630.0a	0.627abcd	3.237ab	0.250ab	0.379bcde	1.87ab
2X	0.250	4.3f	802.5ab	0.579abc	3.170ab	0.295b	0.405e	1.79ab
2X	0.500	4.1cdef	788.7ab	0.586abc	3.157ab	0.258ab	0.377bcde	1.90ab
3X	None	3.6a	1294.4c	0.564a	3.387bc	0.244ab	0.363abcd	1.93abc
3X	0.125	3.8abc	1089.8bc	0.625bcd	3.475c	0.240ab	0.361abcd	1.87ab
3X	0.250	3.7ab	1206.4c	0.634cd	3.517c	0.298b	0.395de	1.82ab
3X	0.500	3.9abcd	1417.5c	0.617abcd	3.522c	0.248ab	0.366abcd	1.82ab
LSD (.05)		0.3	359.8	0.060	0.238	0.064	0.038	0.21

^aThe data represent the mean of 10 plants. There was no interaction between cytokinin and ancymidol.

^bMeans within a column followed by the same letters do not differ significantly at the .05 level.

TABLE XI
 MACRONUTRIENTS AND ANTHRONE DETECTABLE SUGARS AS INFLUENCED
 BY CYTOKININ OVER ALL LEVELS OF ANCYMIDOL^a

Treatment Cytokinin (100 ppm)	Percent N	PPM NO ₃ -N	Percent P	Percent K	Percent Ca	Percent Mg	Percent Sugar
None	3.99 ^b	640ab ^b	0.620a ^b	3.129a ^b	0.225a ^b	0.351a ^b	1.8a ^b
1X	4.04b	499a	0.625a	3.112a	0.251ab	0.368ab	1.9a
2X	4.06b	756b	0.597a	3.176a	0.265b	0.385b	1.8a
3X	3.77a	1252c	0.610a	3.476b	0.257b	0.371b	1.8a
LSD (.05)	0.15	180	0.030	0.118	0.032	0.019	0.1

^aThe data represent the mean of 40 plants. There was no interaction between cytokinin and ancymidol.

^bMeans within a column followed by the same letters do not differ significantly at the .05 level.

TABLE XII
 INFLUENCE OF ANCYMIDOL FOR ALL LEVELS OF CYTOKININ ON
 MACRONUTRIENTS AND ANTHRONE DETECTABLE SUGARS^a

Treatment Ancymidol (mg./pot)	Percent N	PPM NO ₃ -N	Percent P	Percent K	Percent Ca	Percent Mg	Percent Sugar
None	3.96a ^b	805a ^b	0.605ab ^b	3.211a ^b	0.253ab ^b	0.364a ^b	2.0b ^b
0.125	3.87a	729a	0.635b	3.240a	0.231a	0.365a	1.9ab
0.250	4.01a	770a	0.602a	3.226a	0.265b	0.378a	1.8a
0.500	4.02a	852a	0.610ab	3.217a	0.249ab	0.369a	1.8a
LSD (.05)	0.15	180	0.030	0.119	0.032	0.019	0.1

^aThe data represent the mean of 40 plants. There was no interaction between cytokinin and ancymidol.

^bMeans within a column followed by the same letters do not differ significantly at the .05 level.

Root Weight

Periodic observations indicated that cytokinin treated plants had a smaller root system than untreated plants. After the five week harvesting period had been completed, dry weight root readings were taken on plants treated with the different levels of cytokinin (no ancymidol). The root weight decreased dramatically as the cytokinin levels increased (Table XIII).

Rooting

Stem Diameter

Stem diameter readings for this section of the study were taken August 24. Four cuttings were harvested from the upper third of each plant to be used for the rooting study. These average stem diameter measurements were greater than for the other cuttings taken from the stock plants because of the location and quantity of cuttings harvested. Over-all measurements representing the stock plants were lower because they were an average of all cuttings harvested for the five week period. Stem diameter gradually increased over the 16 treatments as the cytokinin levels were increased (Table XIV).

Main effects of cytokinin showed an increase in stem diameter (Table XV). The ancymidol treated plants over all levels of cytokinin were significantly smaller in stem diameter than the untreated plants (Table XVI).

Days to Root

There were significant differences among the 16 treatments but the

TABLE XIII
EFFECT OF CYTOKININ ON ROOT DRY
WEIGHT OF STOCK PLANTS^a

Treatment Cytokinin (100 ppm)	Mean Root Dry Weight (grams)
None	37.12c ^b
1X	29.87b
2X	28.70b
3X	17.50a

^aThe data represent the mean of 10 plants.

^bMeans within a column followed by the same letters do not differ significantly at the .05 level.

TABLE XIV
 INFLUENCE OF CYTOKININ AND ANCYMIDOL TREATMENTS
 ON STEM DIAMETER OF AUGUST 24 CUTTINGS
 AND ROOTING QUALITY^a

Treatment		Cutting Stem Diameter (cm.)	Days to Root
Cytokinin (100 ppm)	Ancymidol (mg./pot)		
None	None	0.79abcd ^b	28.3ab ^b
None	0.125	0.75ab	29.0ab
None	0.250	0.74a	28.0a
None	0.500	0.77abc	28.3a
1X	None	0.86efg	29.0ab
1X	0.125	0.88fg	29.6ab
1X	0.250	0.80bcd	29.6ab
1X	0.500	0.82cde	29.7ab
2X	None	0.96i	31.3b
2X	0.125	0.89gh	28.9ab
2X	0.250	0.83de	30.4b
2X	0.500	0.83def	29.0ab
3X	None	0.93hi	29.4ab
3X	0.125	0.89gh	29.9ab
3X	0.250	0.88fgh	29.2ab
3X	0.500	0.88fgh	29.4ab
LSD (.05)		0.05	2.1

^aThe data represent the mean of 40 plants. There was no interaction between cytokinin and ancymidol.

^bMeans within a column followed by the same letters do not differ significantly at the .05 level.

TABLE XV
STEM DIAMETER OF AUGUST 24 CUTTINGS AND ROOTING
QUALITY AS INFLUENCED BY CYTOKININ
OVER ALL LEVELS OF ANCYMIDOL^a

Treatment Cytokinin (100 ppm)	Cutting Stem Diameter (cm.)	Days to Root
None	0.77a ^b	28.5a ^b
1X	0.84b	29.5ab
2X	0.87c	29.9b
3X	0.89c	29.5ab
LSD (.05)	0.02	1.0

^aThe data represent the mean of 160 plants.
There was no interaction between cytokinin and ancy-
midol.

^bMeans within a column followed by the same let-
ters do not differ significantly at the .05 level.

TABLE XVI

EFFECT OF ANCYMIDOL FOR ALL LEVELS OF CYTOKININ
ON STEM DIAMETER OF AUGUST 24 CUTTINGS
AND ROOTING QUALITY^a

Treatment Ancymidol (mg./pot)	Cutting Stem Diameter (cm.)	Days to Root
None	0.88a ^b	29.4a ^b
0.125	0.85b	29.4a
0.250	0.81c	29.1a
0.500	0.82c	29.1a
LSD (.05)	0.02	1.0

^aThe data represent the mean of 160 plants.
There was no interaction between cytokinin and ancymidol.

^bMeans within a column followed by the same letters do not differ significantly at the .05 level.

treated plants did not differ significantly from the control treatment when considering days to root. The maximum number of days to root was 31 and the minimum was 28 (Table XIV).

The cytokinin treatments examined over all levels of ancymidol showed some significant differences but the differences were so small they would not be important for commercial purposes (Table XV). Ancymidol treatments over all levels of cytokinin did not significantly affect days to root (Table XVI).

Christmas Crop

Days to bloom, plant height, bract diameter, and stem diameter were not significantly different for any of the treatments (Tables XVII, XVIII, XIX). Figure 2 shows randomly selected plants treated with cytokinin without ancymidol and ancymidol without cytokinin. There were very few differences among the treatments except for chemical damage.

The distortion mentioned on the stock plants was still noticeable on the plants that had been treated with cytokinin. Chlorosis and fasciated growth were gone but the short internodes, glands, and split leaves were still present at the base of the plants. New symptoms had developed on some of the cytokinin treated plants. The chemical caused a few plants to branch and a few others to go blind. These symptoms are shown in Figure 3.

TABLE XVII
 INFLUENCE OF CYTOKININ AND ANCYMIDOL TREATMENTS ON
 THE DEVELOPMENT OF THE CHRISTMAS CROP^a

Treatment		Days to Bloom	Plant Height (cm.)	Bract Diameter (cm.)	Stem Diameter (cm.)
Cytokinin (100 ppm)	Ancymidol (mg./pot)				
None	None	70.9a ^b	43.9a ^b	36.7a ^b	.92a ^b
None	0.125	70.4a	43.7a	36.6a	.90a
None	0.250	70.7a	42.1a	36.8a	.90a
None	0.500	70.4a	43.5a	36.2a	.90a
1X	None	72.1a	42.2a	37.1a	.90a
1X	0.125	70.3a	43.5a	37.7a	.93a
1X	0.250	71.0a	41.9a	35.2a	.90a
1X	0.500	70.4a	42.6a	36.5a	.92a
2X	None	71.7a	44.2a	35.7a	.93a
2X	0.125	71.4a	41.2a	35.4a	.90a
2X	0.250	72.0a	41.8a	36.8a	.92a
2X	0.500	70.7a	41.3a	36.7a	.91a
3X	None	72.2a	41.3a	37.7a	.90a
3X	0.125	70.6a	42.8a	36.8a	.90a
3X	0.250	71.2a	42.6a	37.5a	.92a
3X	0.500	71.7a	41.4a	36.0a	.90a
LSD (.05)		1.9	2.9	2.5	.03

^aThe data represent the mean of 30 plants. There was no interaction between cytokinin and ancymidol.

^bMeans within a column followed by the same letters do not differ significantly at the .05 level.

TABLE XVIII

DEVELOPMENT OF THE CHRISTMAS CROP AS INFLUENCED
BY ANCYMIDOL OVER ALL LEVELS OF CYTOKININ^a

Treatment Ancymidol (mg./pot)	Days to Bloom	Plant Height (cm.)	Bract Diameter (cm.)	Stem Diameter (cm.)
None	71.7a ^b	43.0a ^b	36.8a ^b	.91a ^b
0.125	70.8a	42.7a	36.6a	.91a
0.250	71.2a	42.1a	36.6a	.91a
0.500	70.8a	42.0a	36.4a	.90a
LSD (.05)	0.9	1.4	1.2	.02

^aThe data represent the mean of 120 plants. There was no interaction between cytokinin and ancymidol.

^bMeans within a column followed by the same letters do not differ significantly at the .05 level.

TABLE XIX
 EFFECT OF CYTOKININ FOR ALL LEVELS OF ANCYMIDOL
 ON THE DEVELOPMENT OF THE CHRISTMAS CROP^a

Treatment Cytokinin (100 ppm)	Days to Bloom	Plant Height (cm.)	Bract Diameter (cm.)	Stem Diameter (cm.)
None	70.6a ^b	43.3a ^b	36.5a ^b	.91a ^b
1X	70.9a	42.5a	36.6a	.92a
2X	71.4a	42.2a	36.1a	.92a
3X	71.4a	41.7a	37.0a	.90a
LSD (.05)	0.9	1.4	1.2	.02

^aThe data represent the mean of 120 plants. There was no interaction between cytokinin and ancymidol.

^bMeans within a column followed by the same letters do not differ significantly at the .05 level.

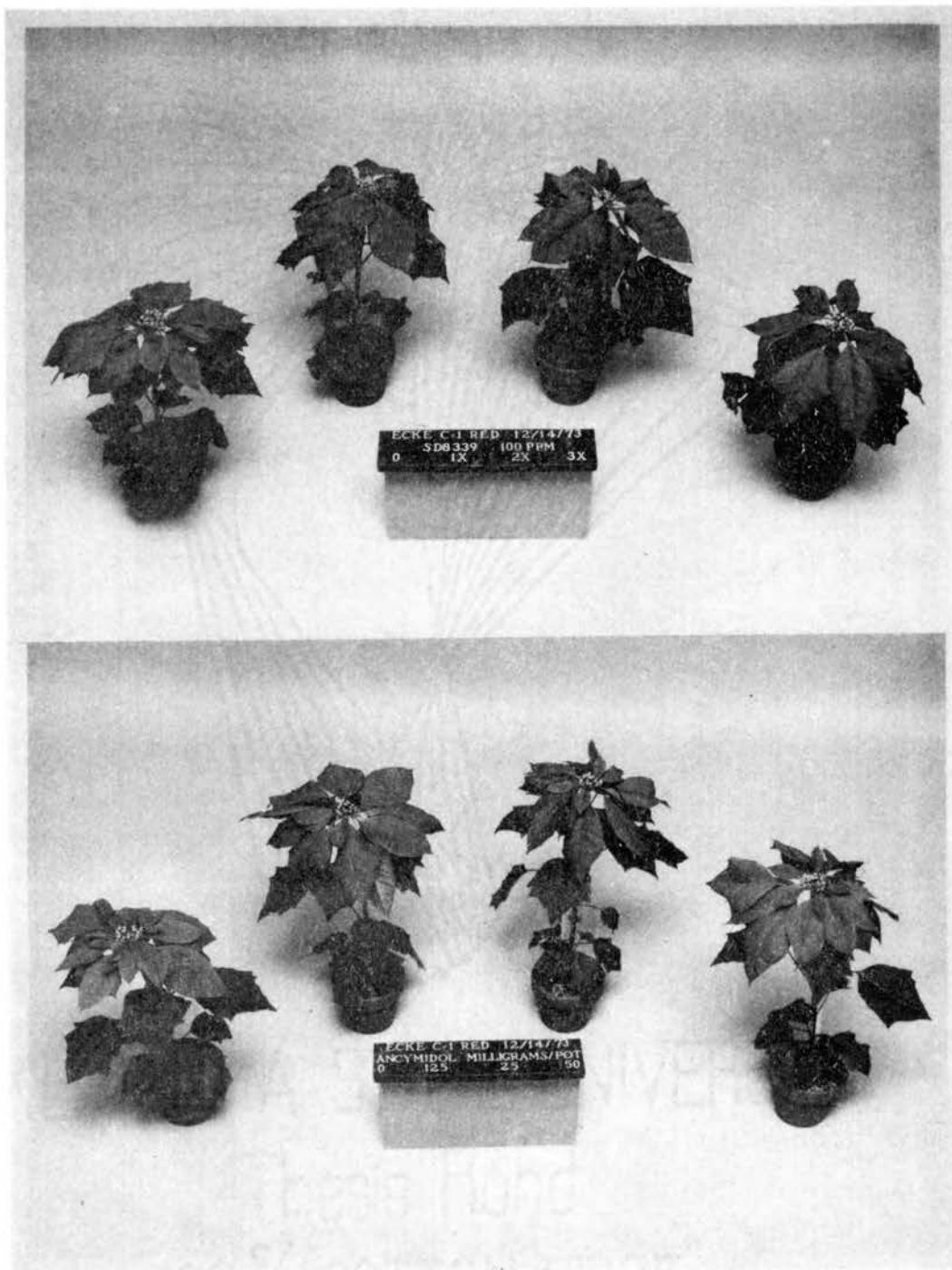


Figure 2. Comparative growth of randomly selected plants from the Christmas crop. Upper - 4 levels of cytokinin, no ancymidol. Lower - 4 levels of ancymidol, no cytokinin.

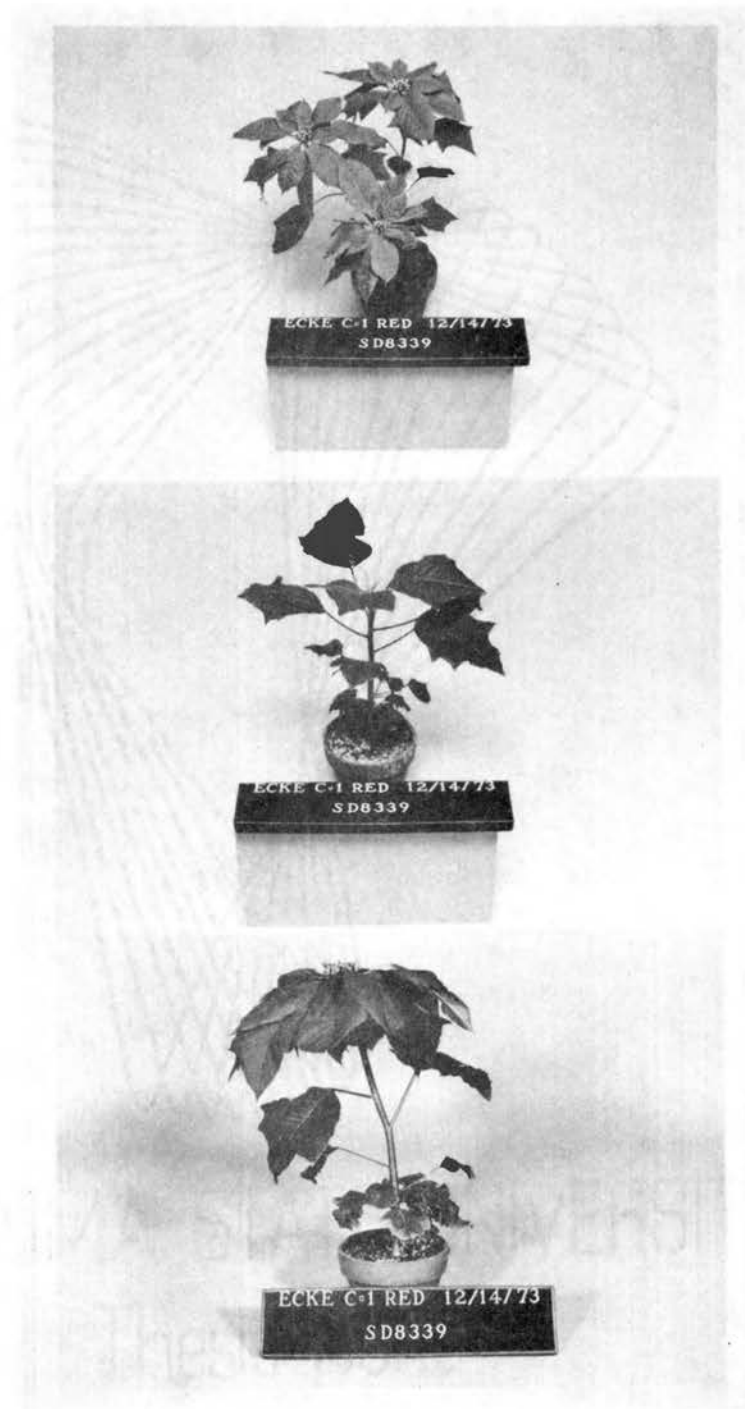


Figure 3. Residual effects of cytokinin (SD8339) on the Christmas crop. Upper - chemical pinching effect. Middle - blindness effect. Lower - temporary short internode elongation effect.

CHAPTER V

DISCUSSION

Stock Plants

Symptoms

Plants treated with cytokinin showed varying levels of chlorosis, stunting and distortion. The chlorosis could be attributed to foliar damage caused by foliar application of the cytokinin or by reduced nutrient uptake. Surfactants could also cause chlorosis at concentrations used in this study. Cytokinin treatments decreased root dry weight as application rates were increased (Table XIII). It was initially thought that the decreased root volume caused a decreased nutrient uptake; therefore, chlorosis resulted. Cytokinin did influence the root system on the stock plants (Table XIII); but the macronutrient studies indicated that nutrient uptake was not reduced by high application rates of cytokinin. However, this could be due to the concentration effect explained in the macronutrient section of this chapter. Previous research indicates that cytokinin decreased root formation because of the auxin imbalance [41]. The more cytokinin applied to the plant the greater the auxin imbalance. Plants started to recover from the chlorosis 18 days after the last application of cytokinin. The plant growth regulators or the surfactant damage could have become more normal or stable after the 18 days.

Stock plants treated with cytokinin also were shorter than untreated plants. Previous work by Jackson [20] and Carpenter [9] indicates that cytokinins reduce plant height.

The distortions included fasciated growth, formation of compound leaves, prominent glands and crowded crowns. These distortions were probably caused by a plant growth regulator imbalance created by the treatment applications. Cytokinins have been reported to stimulate fasciated growth [53].

Stem Diameter of Cuttings

Cytokinin treatments resulted in significantly larger stem diameter cuttings than untreated plants especially for the August 24 harvest date (Tables IV and V). The cytokinin forced the lateral branches to come out faster, therefore, the cuttings harvested August 24 obtained a larger caliper. However, after this initial fast break the controls started to catch up with the treated plants.

The ancymidol at the rate of 0.500 mg. showed significantly greater differences August 24 and September 21 from the control in stem diameter of cuttings (Tables IV and VI). Stem diameters on the other harvesting dates were smaller because cuttings harvested were on the lower, weaker branches. There could be some question whether the ancymidol had sufficient time to influence the August 24 harvest date but the results for the September 21 harvest date could be attributed to the ancymidol. Other studies have shown that growth retardant substances increase stem diameter.

Cuttings Harvested

There were more cuttings harvested over all stock plant treatments August 24 and September 21 than August 31, September 7 and September 14 (Tables VII, VIII, IX). Due to the date of pinching, more cuttings were harvested on those dates because a large number of cuttings were ready to harvest August 24 and by five weeks later (September 21) another large number of harvestable cuttings were available.

Total cutting production was not increased by cytokinin (Table VIII). There was a slight increase August 24, indicating that if plants were sprayed (SD8339) near every major pinch or propagation date, more total cutting production might be achieved. Also, high temperature at the time SD8339 was applied may have affected absorption of cytokinin (rapid evaporation). Fewer cuttings were produced on the September 21 harvest date because the influence of the cytokinin had started to wear off. The cytokinin caused an initial flush after treatment but after the August 24 harvest the controls started to catch up with the treated plants. The ancymidol drenches caused a slight decrease in the number of cuttings produced September 21 and the 0.500 mg. rate of ancymidol caused significantly fewer cuttings to be produced over all levels of cytokinin (Table IX). Stem elongation was slower on the ancymidol treated plants, therefore, fewer cuttings were harvested because they were too short. It was observed that these plants had many short unharvestable branches. The total number of cuttings produced by all treatments compared favorably with the June planting date explained by Ecke (Table III).

Macronutrients

Leaf nutrient concentrations obtained in this experiment were compared to those reported by Ecke [15] (Table I). The N, P, K and Mg were in or very close to the normal ranges. However, the Ca levels were low in all treatments. Cultivar differences, sampling procedures, stage of development, and testing procedures could account for the low Ca readings.

Nutrient concentrations of percent N, P, K, Ca and Mg for the 16 treatments were significantly different, however, only one treatment established a definite trend (Table X). The $\text{NO}_3\text{-N}$ leaf content increased as the cytokinin levels were increased. There was a significant increase in $\text{NO}_3\text{-N}$, K, Ca and Mg for plants treated three times with cytokinin (Table XI). The increase of $\text{NO}_3\text{-N}$, K, Ca and Mg for plants treated three times was probably caused by the reduced leaf size. For this reason, it would take more leaves to make up the weight sample and higher macronutrients could result because of the different concentration. The ancymidol over all levels of cytokinin showed no significant trends on the macronutrients (Table XII). It is probable that the seven days that elapsed from time of ancymidol application to cutting harvest was not an adequate period of time for manifestation of more definite effects of the growth retardant.

Anthrone Detectable Sugars

Cytokinin and ancymidol had little effect on the sugar content of the recently harvested August 24 cuttings. There were significant differences between treatments; however, no trends were established

(Table X). Table XI shows that cytokinin over all levels of ancymidol caused no significant differences in sugar levels. Sugar content of plants in the ancymidol treatments decreased as the ancymidol levels increased (Table XII). This decrease in sugars due to ancymidol treatment may have been caused by decreased photosynthesis. There was less photosynthesis surface on the ancymidol treated plants. These differences were very small and did not influence the performance of the plant in any other area.

Rooting

Stem Diameter and Days to Root

The stem diameter had no significant commercial application because the rooting range average was 28 days minimum to 31 days maximum and performance of all plants was similar for the Christmas crop (Table XIV). The cytokinin did not adversely affect the rooting of the cuttings (Table XV), and the ancymidol had no effect on the rooting (Table XVI). It is possible that the ancymidol had insufficient absorption and translocation time for the physiological effects to show up because cuttings were harvested too soon after application. Foliar application or a longer waiting period after applying ancymidol would probably have a different effect on the plants.

Christmas Crop

There were no differences among treatments in any of the data recorded (Tables XVII, XVIII, XIX and Figure 2). The only differences among treatments were the chemical damages (Figure 3). The ancymidol had no residual effect on the plant height.

These chemicals, as used in this study, would not be recommended for commercial purposes, however, the information could be valuable in further research with these chemicals. Rates and times of applications could be varied to try to improve the effect of these chemicals on poinsettias. If the cytokinin was applied near each pinch and after each harvest date and the same trend held for that established on the August 24 harvest date, it is possible that cutting production could be increased. Better results might have been obtained if the chemical had been applied at night (cooler temperature) for better absorption. Past research indicates translocation is directly affected by the method of application and rate [17]. Ancymidol rates, method, and time of application could be varied for better results.

SELECTED BIBLIOGRAPHY

- [1] Adegbola, A. and C. M. McKell. "Effect of Nitrogen Fertilization on the Carbohydrate Content of Coastal Bermudagrass." Agron. J., 58:60-64, 1966.
- [2] Alberda, T. "The Effect of Cutting, Light Intensity and Night Temperature on Growth and Soluble Carbohydrate Content of Lolium perenne L." Plant and Soil, 8:199-229, 1957.
- [3] Besemer, S. T. "Comparison of Quel and Cycocel for Reducing Height of Two Poinsettia Cultivars -- Progress Report." Florists' Review, 150(3892):37-38, 77, 1972.
- [4] Brown, H. C. "The Effect of Cycocel ([2-chlorethyl] trimethylammonium chloride) Applied to the Soil on the Inhibition of Growth and Mineral Element Content of the Foliage of Potted Poinsettias (Euphorbia pulcherrima Willd.)." Diss. Abst., 24:4335, Ohio State University, Columbus, Ohio, 1964.
- [5] Carlson, W. H. and K. C. Sink. "The Effect of Soil Mixes on Poinsettia Variety Paul Mikkelsen." Quart. Bull., Mich. Agri. Exp. Sta., 50:20-24, 1967.
- [6] Carlson, W. H. and W. J. Carpenter. "Drenches of Plant Growth Regulators Improve Poinsettia Rooting." Florists' Review, 149(3863):25, 64-65, 1971.
- [7] Carpenter, W. J. and W. H. Carlson. "Improved Branching of Poinsettia Planting Sprayed with Cytokinins PBA and N⁶BA." HortScience, 6(3):289, 1971.
- [8] Carpenter, W. J. and W. H. Carlson. "Improving Geranium Branching with Growth Regulator Sprays." Mich. State Coop. Ext. Flower Notes, 4(3):4-6, 1972.
- [9] Carpenter, W. J. and W. H. Carlson. "The Effect of Growth Regulators on Chrysanthemums." Mich. Florist, 508:19-21, 1973.
- [10] Carpenter, W. J., R. C. Rodriguez, and W. H. Carlson. "Growth Regulator Induced Branching of Non-Pinched Poinsettias." HortScience, 6(5):457-458, 1971.
- [11] Cathey, H. M. and Howard E. Heggestad. "Effects of Growth Retardants and Fumigations with Ozone and Sulfur Dioxide on Growth and Flowering of Euphorbia pulcherrima Willd." J. Amer. Soc. Hort. Sci., 98:3-7, 1973.

- [12] Coorts, G. C. and D. E. Schrader. "Effectiveness of Quel on Mikkelsen Poinsettias." Florists' Review, 149(3853):30-31, 81-82, 1971.
- [13] Criley, R. A. and W. H. Carlson. "Tissue Analysis Standards for Various Floricultural Crops." Florists' Review, 146(3771): 19-20, 70-74, 1970.
- [14] Dowler, W. M. and F. D. King. "Seasonal Changes in Starch and Soluble Sugar Content of Dormant Peach Tissue." Proc. Amer. Soc. Hort. Sci., 89:80-84, 1966.
- [15] Ecke, Paul, Jr. and O. A. Matkin (Ed.). The Poinsettia Manual. Paul Ecke Poinsettias, Encinitas, California, 1971.
- [16] Eli Lily and Company. Personal Correspondence with the Eli Lily Company, June, 1973.
- [17] Fox, J. E. and J. S. Weis. "Transport of the Kinin N⁶ Benzyl-adenine: non Polar or Polar?" Nature, 206:678, 1965.
- [18] Furuta, T., W. C. Jones, W. Humphrey, and J. Breece. "Ancymidol Retards Growth of Many Plants." Florists' Review, 150(3879): 23, 45, 1972.
- [19] Harvis, G. P. and E. M. Hart. "Regeneration From Leaf Squares of Peperomia sandersii A, DC: A Relationship Between Rooting and Budding." Ann. Bot., 28:509-526, 1964.
- [20] Jackson, E. K. and J. C. Lingle. "Use of Cytokinin SD8339 (PBA) for Branching Floricultural Crops." HortScience, 6(2):88, 1971.
- [21] Kiplinger, D. C. "1962 Tests With Cycocel on Poinsettias." Ohio Flor. Assoc. Bull., 407:2-6, 1963.
- [22] Larson, R. A. "Chemical Growth Regulators and Their Effects on Poinsettia Height Control." N. Car. Agr. Exp. Sta. Tech. Bul., 180:1-27, 1967.
- [23] Larson, R. A. and M. L. McIntyre. "Residual Effect of Cycocel on Poinsettia Height Control." Proc. Amer. Soc. Hort. Sci., 93:667-672, 1968.
- [24] Larson, R. A. and R. K. Kimmins. "Results With a New Growth Regulator." Florists' Review, 148(3841):22-23, 54, 1971.
- [25] Laurie, A., D. C. Kiplinger, and K. S. Nelson. Commercial Flower Forcing. 7th Edition, New York: McGraw Hill, 1968.

- [26] Love, J. W., R. A. Larson, and B. G. Hillard. "The Effects of Growth Retardants and Temperature on the Growth and Flowering of Poinsettia cvs. 'Annette Hegg' and 'Eckespoint C-1'." HortScience, 5:482-483, 1970.
- [27] McCready, R. M., J. Guggolz, V. Silveria, and H. S. Owens. "Determination of Starch and Amylose in Vegetables." Anal. Chem., 22:1156-1158, 1950.
- [28] Milbocker, D. C. "Axillary Shoot Stimulation in Poinsettia With Kinetin." HortScience, 7(5):483-484, 1972.
- [29] Miller, C. O., F. Skoog, F. S. Okumura, M. H. Von Saltza, and F. M. Strong. "Structure and Synthesis of Kinetin." J. Am. Chem. Soc., 77:2662, 1955.
- [30] Miller, C. O., F. Skoog, F. S. Okumura, M. H. Von Saltza, and F. M. Strong. "Isolation, Structure and Synthesis of Kinetin, a Substance Promoting Cell Division." J. Am. Chem. Soc., 78:1375-1380, 1956.
- [31] Miller, C. O., F. Skoog, M. H. Von Saltza, and F. M. Strong. "Kinetin, a Cell Division Factor From Deoxyribonucleic Acid." J. Am. Chem. Soc., 77:1329, 1955.
- [32] Miller, C. O. "The Relationship of the Kinetin and Red Light Promotions of Lettuce Seed Germination." Plant Physiol., 33:115-117, 1958.
- [33] Mitlehner, A. W. "Retardants on Poinsettias." Florists' Review, 136(3530):21-22, 67, 1965.
- [34] Nell, T. A. and K. C. Sanderson. "Effects of Several Growth Regulators on the Rooting of Three Azalea Cultivars." Florists' Review, 150(3891):21-22, 54, 1972.
- [35] Ohlrogge, A. J. "Soil and Plant Tissue Test." Purdue Univ. Agri. Exp. Sta. Bull. 635, 1962.
- [36] Payne, R. N. "Effects of Quel Treatments on 'C-1' Poinsettias." Okla. Greenhouse Growers Newsletter, No. 18:8-26, 1972.
- [37] Poll, L. "The Effect of Cytokinin N⁶-Benzyladenine on Bud Break of Fruit Trees." Horticultura, 22:1-16, 1968.
- [38] Read, P. E. and V. C. Hoysler. "Stimulation and Retardation of Adventitious Root Formation by Application of B-Nine and Cycocel." J. Amer. Soc. Hort. Sci., 94 314-316, 1969.
- [39] Richmond, A. and A. Lang. "Effect of Kinetin on Protein Content and Survival of Detached Xanthium Leaves." Sci., 125:650-651, 1957.

- [40] Sachs, T. and K. V. Thimann. "Release of Lateral Buds From Apical Dominance." Nature, 201:939-940, 1964.
- [41] Sachs, T. and K. V. Thimann. "The Role of Auxins and Cytokinins in the Release of Buds from Dominance." Amer. J. Bot., 54: 136-144, 1967.
- [42] Scott, R. A. and J. L. Liverman. "Promotion of Leaf Expansion by Kinetin and Benzylaminopurine." Plant Physiol., 31:321-322, 1956.
- [43] Shanks, J. B. and Conrad B. Links. "Poinsettia Stock Plant Nutrition in Relation to Production, Rooting, and Growth of Cuttings." Proc. Amer. Soc. Hort. Sci., 69:513-522, 1952.
- [44] Shanks, J. B. "Chemical Growth Regulators for Floricultural Crops." Maryland Florist, 164:1-8, 1970.
- [45] Singh, B., O. P. Vadhura, M. T. Wue, and D. K. Salunkhe. "Effects of Foliar Application of S-Triazines on Protein, Amino Acids, Carbohydrates, and Mineral Composition of Pea and Sweet Corn Seed, Bush Bean Pods, and Spinach Leaves." J. Agri. Food Chem., 20:1256-1259, 1972.
- [46] Skelsey, J. J. Personal Correspondence with Mr. J. J. Skelsey, Field Representative for the Shell Development Company, June, 1973.
- [47] Skoog, F. and C. Tsui. "Chemical Control of Growth and Bud Formation in Tobacco Stem Segments and Callus Cultured in Vitro." Am. J. Bot., 35:782-787, 1948.
- [48] Skoog, F., F. M. Strong, and C. O. Miller. "Cytokinins." Sci., 148:532, 1965.
- [49] Smith, P. F. "Mineral Analysis of Plant Tissue." Ann. Rev. Plant Physiol., 13:81-108, 1962.
- [50] Snedecor, G. and W. G. Cochran. Statistical Methods. 6th Edition, Ames, Iowa: The Iowa State Univ. Press, 1971.
- [51] Steel, G. D. and J. H. Torrie. Principles and Procedures of Statistics. New York: McGraw-Hill, 1960.
- [52] Sullivan, J. T. and V. G. Sprague. "The Effect of Temperature on the Growth and Composition of the Stubble and Roots of Perennial Ryegrass." Plant Physiol., 24:706-719, 1949.
- [53] Thimann, K. V. and T. Sachs. "The Role of Cytokinins in the 'Fasciation' Disease Caused by Corynebacterium fascians." Am. J. Bot., 53:731-739, 1966.

- [54] Van Overbeek, J., M. E. Conklin, and A. F. Blakeslee. "Factors in Coconut Milk Essential for Growth and Development of Datura embryos." Sci., 94:350, 1941.
- [55] Williams, M. W. and E. A. Stahly. "Effect of Cytokinins on Apple Shoot Development from Axillary Buds." HortScience, 3(2): 68-69, 1968.
- [56] Worley, R. E. "Pecan Nutlet Set and Carbohydrate Level of Various Tissue in the Spring as Affected by Fungicidal Sprays." J. Amer. Soc. Hort. Sci., 98:68-71, 1973.
- [57] Yemm, E. W. and A. J. Willis. "The Estimation of Carbohydrates in Plant Extracts by Anthrone." Biochem. J., 57:508-514, 1954.

APPENDIX

EXPERIMENTAL PROCEDURE ANTHRONE METHOD

Sample Preparation and Extraction

Samples of freshly harvested plant material were sealed in plastic bags and immediately frozen. The samples were stored at -10°C until analyzed. Extraction of the soluble sugars from the plant tissue was initiated by weighing 1 gram of the frozen leaf sample and placing it into 10 ml. of 80% ethanol. The samples were then put into an oven (82°C) for 12 hours. The extract was removed and another 10 ml. of 80% ethanol was added to the sample. The sample was then placed into a boiling water bath for 25 minutes. This extract was removed and added to the first extract. The second extraction process was repeated. A marble chip was added to the total extract and the extract was placed into the boiling water bath again until approximately 4 ml. of the extract was left. The concentrated extract was centrifuged at 10,000 rpm for 20 minutes to remove the chlorophyll. It was then filtered, exudate was washed gently twice with distilled water and then filtered into a 50 ml. volumetric flask. The extract was brought to 50 ml. volume with distilled water. One ml. of this was removed and 4 ml. of distilled water was added. One ml. of the diluted extract was added to 5 ml. of anthrone reagent and thoroughly mixed. The sample was then placed in a boiling water bath for 15 minutes and immediately cooled for 20 minutes. The readings were taken on a Bausch and Lomb

spectrophotometer absorbance 620 μ . The readings were compared to standard glucose solutions.

To prepare 500 ml. of anthrone reagent add 140 ml. water and 360 ml. concentrated sulfuric acid. Allow boiling to subside, then add, while swirling, 0.250 grams anthrone and 5 grams of thiourea. Store in refrigerator.

VITA

Robert Earl Moon

Candidate for the Degree of

Doctor of Philosophy

Thesis: EFFECTS OF CYTOKININ AND ANCYMIDOL ON POINSETTIA PLANT
DEVELOPMENT, COMPOSITION AND PERFORMANCE

Major Field: Crop Science

Biographical:

Personal Data: Born in Walters, Oklahoma, June 21, 1947, the son
of Mr. and Mrs. C. A. Moon.

Education: Graduated from Walters High School in 1965; attended
Cameron College from 1965-1967; received Bachelor of Science
degree from Oklahoma State University in 1969 with a major
in Agricultural Education; received Master of Science in
Horticulture in 1971; completed requirements for the Doctor
of Philosophy degree at Oklahoma State University, July,
1974.

Professional Experience: Graduate Teaching Assistant and Graduate
Research Assistant, Department of Horticulture, Oklahoma
State University, 1969-1974.