COMMERCIAL PRODUCTION OF *CHRISTIA SUBCORDATA* MOENCH BY ESTABLISHING CULTURAL PRACTICES AND BY APPLYING PLANT GROWTH REGULATORS

by

PAULA A. WHITING

(Under the Direction of Svoboda V. Pennisi)

ABSTRACT

Cultural guidelines for greenhouse production of *Christia subcordata* were established using two irradiance levels (low and high) and five (100, 200, 300, and 400 mg/L) nutrient levels. No significant difference found in height and number of branches between plants grown under low irradiance and those plants grown under high irradiance. Biweekly fertilization at a rate of 100 to 200 mg/L nitrogen between 400-600 μmol $m^{-2}s^{-1}$ produced optimal plant growth and healthy, marketable plants. Plant growth regulator (PGR) studies were conducted using four commercial chemicals: benzyladenine, chlormequat chloride, dikegulac-sodium, and ethephon. Ethephon was most effective for controlling growth and producing marketable plants. Ethephon-treated plants exhibited anatomical differences in leaf and stem tissue and cell diameter when compared with untreated plants.

INDEX WORDS: Benzyladenine, chlormequat chloride, dikegulac-sodium, ethephon, foliage plants, *Christia subcordata*, irradiance, shade, fertilizer rate, plant growth regulator, lateral branching, compact

COMMERCIAL PRODUCTION OF *CHRISTIA SUBCORDATA* MOENCH BY ESTABLISHING CULTURAL PRACTICES AND BY APPLYING PLANT GROWTH REGULATORS

by

PAULA A. WHITING

B.S.C.E., Florida A & M University, 1995

B.S.A., University of Georgia, 2006

A Thesis Submitted to the Graduate Faculty of the University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2007

COMMERCIAL PRODUCTION OF *CHRISTIA SUBCORDATA* MOENCH BY ESTABLISHING CULTURAL PRACTICES AND BY APPLYING PLANT GROWTH REGULATORS

by

PAULA A. WHITING

Major Professor: Svoboda V. Pennisi

Committee: David A. Knauft
Paul A. Thomas
Zheng-Hua Ye

Electronic Version Approved:

Maureen Grasso
Dean of Graduate School
The University of Georgia
December 2007

DEDICATION

To the glory of God, from whom all blessings flow.

ACKNOWLEDGEMENTS

Above all, I thank my Father God and Lord Jesus, for protection and provision in all my needs. Let this accomplishment be to His Glory.

Unlimited and overflowing thanks to my advisor Dr. Bodie Pennisi for her guidance, patience, understanding, weekend phones and home-cooked meals.

Thank you to my committee of Dr. David Knauft, Dr. Paul Thomas and Dr. Zheng-Hua Ye for being available to me. I appreciated knowing I could knock on your doors at anytime.

Thank you to Pamela Lewis, Georgia Smith and their greenhouse crew. I greatly appreciated your technical assistance, laughter and baked goods over the last two years.

Thank you to Dr. John Shields, Center for Ultrastructure Research for unlimited patience and use of the microscopy lab.

Thank you to my godmother, Gladys Weston, my mother, Dianne Whiting and my brother and sister-in-law for the words of encouragement, faith and love.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

Bedding plants are the number one wholesale and retail floriculture crop in the United States, followed by potted plants and foliage plants, respectively. According to the USDA *Floriculture Crops 2006 Summary* (USDA NASS, 2006), demand for these three commodities has increased. There is a constant need to introduce rare and fascinating material into the market. *Christia* has an uniquely colored butterfly-shaped foliage that appeals to the customers of ornamental potted plants.

The genus *Christia* was first documented as the genus *Hedysarum* by João de Loureiro in *Flora Cochinchinensis* (de Loureiro, 1790; Merrill, 1935). The same year, Natalis Joseph Necker established the genus of *Lourea* for certain species of the genus *Hedysarum* (Babu and Thothathri, 1971). In 1802, Conrad Moench discovered a species identified from *Hedysarum vespertilionis* and applied the genus *Christia*, which became an accepted name replacing *Lourea* (Ali, 1965; McVaugh, 1968; Rudd, 1970; Babu and Thothathri, 1971).

Christia is an ornamental legume in the Fabaceae family consisting of 13 species in tropical Asia and five species found in China. Its distribution in tropical and subtropical Asia, includes Ryukyu Island, Taiwan, Indochina, Malaysia, Indonesia, Vietnam, Laos, Cambodia, Thailand, India, China and northern Australia. The genus is also naturalized in Fiji and on some Caribbean islands including St. Vincent, Martinique, Jamaica and St. Kitts (Locke and Heald, 1994). *Christia* spp. typically inhabit dry, grassy areas, sandy soils, and roadsides. The genus

Christia, commonly called the island pea (USDA, 2006), is a non-climbing perennial herb used as an ornamental in cultivated gardens in southeast Asia because of its uniquely shaped leaves, drought tolerance, and nitrogen-fixing ability (van Meeuwen et al., 1961; Ohashi, 1977).

Market Value to Industry. Although the genus has been documented since 1802, the species *Christia subcordata* is rarely marketed, and does not have documented cultural guidelines for industry use in the United States. The better-known species of this genus *C. vespertilionis* (van Meeuwen et al., 1961), is propagated by seed or cuttings in a peat-free mix under full sun, high humidity, moist media and a minimum temperature of 21ºC (Barham, 1996). *Christia vespertilionis* grows to a height of 1 m. It has slender stems with trifoliate leaves. Juvenile leaves have a purple tint and mature dark green with pale green stripes along prominent veins.

Christia obcordata, formerly known as *Ploca humilis, Hedysarum obcordatum, Desmodium obcordatum*, or *Lourea obcordata* (Bentham and Mueller, 1864; Baker, 1879; Bailey, 1900; Backer and van den Brink, 1963; Huang, 1993; Locke and Heald, 1994; Baker, 2007), also has slender stems and trifoliate leaves. Leaf background is light green in juvenility and becomes bright green with dark burgundy stripes at maturity. The leaves are wide reniform or kidney-shaped and flutter like butterfly wings in a breeze, hence the common name, Butterfly Plant. *Christia subcordata* has the slender stems and trifoliate leaves typical of the genus. Juvenile leaves are green and mature to burgundy with darker burgundy stripes. Growth habit is similar to *C. verpertilionis*.

Christia constricta, C. obcordata and *C. vespertilionis* are prized for their medicinal qualities in Indochina and Japan. *Christia obcordata* is used to treat urinary blockage, and acute and chronic nephritis. *Christia vespertilionis* is used mixed with water to treat tuberculosis,

bronchitis and inflamed tonsils, colds, muscle weakness and poor blood circulation (Nippon, 2006). *Christia obcordata* was also evaluated as a treatment for malaria but has been found ineffective (Nguyen-Pouplin et al, 2007).

Members of the genus *Christia* have a creeping, non-vining habit which makes it difficult to control in the landscape while in production. Growth control is required to achieve more marketable plants with upright and compact habit. Natural height controls include selective pruning, moisture stress and decreased fertilization. Early induction of the reproductive state may also decrease height (Allbritton et al., 2002). However, the use of natural height control is time- and labor-intensive and result in increased production costs. Therefore chemical growth regulators may be a better alternative for commercial production.

Limited information is available on applying chemical growth regulators (PGRs) to *Christia* species. Two commonly used growth regulators, paclobutrazol and ethephon, were not effective in reducing height or increasing number of branches (K. Steincamp, Agristarts, Inc., 2006, personal communication). However, other growth regulators may offer better results including benzyladenine, a synthetic cytokinin that induces branching, and two chemical pinchers, chlormequat chloride and dikegulac sodium that induce lateral branching. Because plants treated with branching agents are typically more compact than untreated plants, these agents may be used to control growth habit.

LITERATURE REVIEW

Botany of the Genus *Christia*

The sub-family Papilionoideae of the Fabaceae, known as Leguminosae, has a high level of variety and specialized functions (Ohashi et al., 1981; Lavin, 2006). The Desmodieae tribe of this subfamily consists of trifoliate herbs or shrubs with a distinctive loment fruit (Bailey et al, 1997). The genus *Christia*, formerly known as *Lourea*, is one of two genera that have stipels present and loments with two or more segments folded and enclosed in the calyx (Keng et al, 1993). The *Christia* species are erect or diffuse herbs or sub-shrubs that have both trifoliate compound leaves and simple leaves on the same plant. All leaves are arranged alternately with small stipule-like structure at the base (Fig. 1.2, 1.3, and 1.4). The panicles or racemes are occasionally axillary. They have small, white flowers (Fig. 1.1, 1.3). Bell-shaped membranous calyx, which enlarge with age, have five ovate-lanceolate lobes, which are approximately as long as the corolla and slightly broader. The corolla has a standard width with the base tapering gradually to a narrow width. The floral tube has a large, central obtuse petal with a distinct crease and two adjacent, wing-like petals with blunt or rounded apices (Moench, 1794; Moench, 1802; Bentham and Mueller, 1864; Baker, 1879; Forbes and Hemsley, 1886; Hitchcock, 1893; Bailey, 1900; Backer and van den Brink, 1963; Ohashi, 1977; Ohashi et al, 1981; Huang, 1993; Ohashi and Huang, 1993; Keng et al, 1993; Davidson and Davidson, 1993; Chapman and Wang, 2002; Klitgaard and Bruneau, 2003; Lewis et al, 2005; Puhua and Ohashi, 2006; Baker, 2007).

Irradiance and Nutrient Effects on Morphology and Anatomy

Before large-scale commercial production of *C. subcordata* can be implemented guidelines need to be established for optimal plant growth. Areas that lend themselves to further investigation include (1) the optimal growth environment (irradiance and nutrition); (2) physiological responses (response to various chemicals at different rates) and (3) anatomical features affecting (physiological responses shoot cuticle and trichomes that may block the absorption of PGRs).

Climate for cultivation. The native Southeast Asia habitats of *Christia* have a constant tropical climate with an average temperature of 26-27°C and 80% humidity. This region has two distinct seasons. The first season, ranging from June to September is the dry monsoonal season and the second, ranging from December to March, is the rainy season. Southeastern Asia and northern Australia where *Christia* is native, has an average temperature range of 5 to 31°C with the minimum and maximum temperatures of 28 and 33°C. Except in a few mountainous areas, snow is rare.

The USDA Hardiness Zones for Southeastern Asia and Australia range from 8 to 11. These zones have low temperatures extending from -12.2°C to above 4.5°C. *Christia* is naturalized and distributed in high temperature and high humid. Therefore, the Southeast United States [which has a similar hardiness zones to Southeast Asia ranging from 7b to 11, -17.7 to 4.5°C (minimum average temperature ranges)] is optimally suited for *Christia* production. Similar to Southeast Asia, the Southeast United States has high levels of humidity, heat and moisture.

Irradiance. In native settings, *Christia* is found in open grasslands, dry sandy soils, thickets, sparse forests and on beaches and roadsides (Puhua and Ohashi, 2006). Therefore, the

irradiance level for *Christia* would depend on growth location. Comparatively, tropical rain forest plants grow under lower light intensities versus plants in open areas (Manaker, 1997). Wong and Wilson (1980) noted that the photosynthetic capacity of tropical legumes becomes light saturated at 50% full sun but it is adapted to photosynthesize better in shade. In tropical climates, lighting conditions for low growing erect or diffuse herbs typically range from 14 μmol $m^{-2}s^{-1}$ in a forest understory to 720 μmol $m^{-2}s^{-1}$ in open savannas (Joiner, 1981). In commercial production, deeply shaded plants receive 36 to 72 μmol $m^{-2}s^{-1}$ on average.

Tropical plants such as *Christia* undergo morphological and physiological changes based on the ambient light level. Plants grown in shade have wider, thinner, darker leaves and thicker stems due to decreased photosynthetic rate and carbohydrate production, which reduces growth (Wong and Wilson, 1980; Joiner, 1981; Nelson, 1998; Baruch et al, 2000) than plants exposed to ambient light. Increased leaf area and decreased specific leaf mass under low light is a reaction to increased carbon capture and a means to regain internal carbon balances (Baruch et al., 2000). Taiz and Zeiger (1991) referencing Ehleringer et al (1976) stated that pubescence, salt glands, cuticular waxes and other leaf area characteristics influence the reflection of light. Below the epidermis are the palisade parenchyma cells, spongy mesophyll cells, the xylem and the phloem (Taiz and Zeiger, 1991; Mauseth, 2003). The palisade cells, the uppermost photosynthetic layer of cells, are arranged in parallel columns that maybe up to 3 layers thick. When excess light is absorbed through the epidermis to the palisade cells the chlorophyll rearranges into clusters in the chloroplasts to limit the amount of light absorbed. This also allows the light entrance into intercellular spaces of the leaf. The spongy mesophyll absorbs and scatters the light allowed in by the palisade to other mesophyll cells (Taiz and Zeiger, 1991; Baruch et al, 2000; Mauseth, 2003). Maximum light absorption for photosynthesis occurs when the chloroplasts within the

leaf surface are perpendicular to the light sources and minimum absorption when the chloroplasts are parallel to the light sources.

Plant growth is largely determined by the amount of light available to the canopy. Plant species with colored leaves often require higher light intensities than plants with green foliage (Manaker, 1997). Plants grown in shaded conditions develop mechanisms to capture the maximum amount of light possible throughout the course of the day (Baruch et al, 2000; Taiz and Zeiger, 1991). The mechanisms developed by shade grown plants allow enough light absorption so that photosynthesis can sustain plant growth. Shaded leaves are located in areas with higher concentrations of far-red light, which gives the plants elongated internodes, higher content of chlorophyll (Taiz and Zeiger, 1991) longer, broader, and thinner leaves with a low specific leaf mass and increased leaf area (Wong and Wilson, 1980). Low light availability increases the amount of biomass allocated to leaves to compensate for reduced carbon fixation, increases plant height, increases specific leaf mass, decreases growth rate in leaf numbers, decreases leaf production costs and absorption rate on leaf area and has higher adaptation capacity to limited light in four invasive Melastomataceae, two shade-intolerant herbs (*Arthrostema ciliatum* and *Tibouchina herbacea*) and two shade-tolerant woody species (*Clidemia hirta* and *Miconia calvescens*) (Baruch et al., 2000). Conversely, in their study, Baruch et al (2000) found that shade and water stress reduced biomass, specific leaf mass and root-shoot ratio in all species used, especially in the herbaceous plants. Light wavelength and intensity influences the effects of hormones such as cytokinins, auxins and ethylene. This can be seen in a study conducted where low light intensity decreased the cytokinin content in *Xanthium* leaves (Evans, 1984).

Nutrition. Nitrogen, phosphorus and potassium are required for normal physiological processes. Proteins, nucleic acids, chlorophyll and other cell components require nitrogen to function. Phosphorus is a constituent of cell components and is central to the ATP molecules that provide energy for cell processes. Potassium is used as an enzyme activator in the cells (Manaker, 1997). Fertilization rate and frequency are related to the rate of plant growth and dry matter accumulation (Kang and van Iersel, 2004; Kessler, 2007). High nutrient concentrations reduced plant size, lowered plant fresh and dry weight, and reduced leaf number and area. Ehret et al. (2004) found that nutrient concentrations in pineapple sage significantly affected plant fresh mass, dry mass, leaf area, chlorophyll content and shelf life. Research has also shown that total leaf area increased with increasing fertilizer concentrations in *Tradescantia* (White, 2003). The fertility requirements of various plants vary seasonally, so that changes to the nutrient programs should be made on a seasonal basis (Kessler, 2007).

Wong and Wilson (1980) and Wilson (1996) found that nitrogen levels and dry matter production in *Mawoptilium atropurpureum* cv. Siratro, green panic (*Panicum maximum var. trichoglume*), buffel (*Cenchrus ciliaris*), Rhodes (*Chloris gayana*), and speargrass (*Heteropogon contortus*) increased as irradiance decreased. White (2003) noted in Spiderwort (*Tradescantia virginiana* L.) that as nitrogen rates increased plant height, plant width, shoot dry weight and media electrical conductivity increased. The author also found that pH decreased as nitrogen rates increased. Also, as excess nitrogen was applied, foliage chroma and hue decreased. Foliage became darker, and plant variegation became greener. Plant size, total leaf area and foliage color are affected by nutrient availability (Havlin et al 2005). Wen (1990) found that as nitrogen applications increased, yield days to flower and days to pod maturity increased in soybean. Increasing nitrogen applications on soybeans was also associated with increased plant

height, dry matter, number of branches, pods number per plant and seeds per pod. Ronzhina (2002) related that an increase in nutrients to leaves is associated with a change in dry weight. As the rate of nitrogen fertilization increased in *Vicia faba* L. total biomass, total plant leaf area, and total nitrogen accumulation also increased (Jia and Gray, 2004).

Plant Growth Regulators

Under these categories fall many of the commonly used PGRS such as cytokinins, ethylene inducers such as ethephon, and growth retardants such as chlormequat chloride and dikegulac-sodium (Figure 1.2). Many PGRs are plant hormones. They can be either naturally occurring or synthetic chemicals. Often they act as retardants, slowing cell division and elongation especially in the apical meristem (Joiner, 1981; Taiz and Zeiger, 1991). Commercially produced ornamental plants, which have not been treated with PGRs often exhibit elongated growth that makes them susceptible to breakage during shipping and reduces available packing space (Latimer et al., 2001). Untreated plants often require pruning to be marketable, which increases labor costs. By using PGRs, the grower can produce a healthier plant with a more marketable size and shape, darker leaf color and a longer shelf life (Schnelle et al, 1996).

Benzyladenine. Benzyladenine (BA) is a synthetic cytokinin with a chemical composition of N^6-benzyladenine. BA can be used to increase the size of fruit. It also induces lateral bud break and promotes lateral shoot elongation, resulting in increased flower number and decreases time it takes between flowering (Kochankov et al., 1989; USEPA, 1994; Arteca, 1996, Kays and Paull, 2004). BA also a) controls leaf formation, and delays leaf yellowing and senescence (Tayama et al., 1992; Stern, 2003; Kays and Paull, 2004), b) promotes cell division, cell differentiation and organogenesis in plant tissue cultures (Laloue et al., 1978; Stern, 2003;

Kays and Paull, 2004), c) opens and closes stomates, translocates nutrients and organic substances to cytokinin-concentrated tissues (Arteca, 1996), d) delays protein and chlorophyll degradation in detached leaves and prolongs the storage life in green vegetables (Halvey et al., 1966; Stern, 2003), e) stimulates seed germination, f) stimulates formation of seedless fruit, g) breaks seed dormancy, and h) alters fruit growth. BA binds ribosomal RNA and ribosomal proteins in the cytoplasm for leaf senescence (Laloue et al., 1978). BA is normally applied weekly to plants in plug trays, liners or pots. It may be applied as either a drench or a spray. A surfactant may be added to the spray mix for some crops (Tayama et al., 1992).

Chlormequat chloride. [(2-chloroethyl) trimethylammonium chloride or (2-Chloroethyl-N,N,N-trimethyl-ammonium chloride] is a gibberellin biosynthesis inhibitor which reduces stem length by inhibiting internode elongation (Tanaka and Tolbert, 1966; Aphalo et al,1997; Nelson, 1998; Bora and Sarma, 2006).

Dikegulac-sodium. [2,3:4,6-bis-O-(1-methylethylidine)-alpha-L-xylo-2-hexulofuranosonic acid sodium salt] is gibberellin synthesis inhibitor and a systemic PGR that works by inhibiting plant hormones, interrupting apical dominance and inhibiting shoot elongation (Thomas et al, 1992; Schnelle et al, 1996; Fain et al, 2001a; Fain et al, 2001b; Puglisi, 2002; Pozo et al, 2004;). In commercial production dikegulac-sodium is used to chemically prune, (Thomas et al, 1992; Clark et al, 2004), and to increase lateral branching (Thomas et al, 1992; Fain et al, 2001a; Fain et al, 2001b; Puglisi, 2002; Pozo et al, 2004) inducing axillary bud growth (Fain et al, 2001a; Fain et al, 2001b; Puglisi, 2002) and to promote mature fruit abscission (Pozo et al, 2004). However, high concentrations (>2000 mg-L^{-1}) of dikegulac-sodium causes abscission of flowers, young leaves and fruit, especially when applied to susceptible species during early season growth (Pozo et al, 2004).

Ethephon. (2-chloroethylphosphonic acid), is an ethylene-releasing compound that: a) promotes the production of short, thick stems; promotes seed germination (Kepczynski, 1989; Arteca 1996), b) breaks dormancy, induces and/or inhibits flowering, increases branching and controls height, and causes defoliation prior to dormancy (Kepczynski, 1989; Tayama et al., 1992; Arteca, 1996; Nelson, 1998), and c) enhances coloring in fruit and speeds ripening (Nelson, 1998). In its liquid form, ethephon action is triggered by elevated pH, which causes release of ethylene gas, especially when plants are under stress (Tayama et al., 1992; Arteca, 1996). When sprayed on foliage, ethephon catalyzes chemical reactions inside plant tissues leading to ethylene formation (Arteca, 1996). Rounkova (1989) showed that ethephon inhibits growth for a 6-week interval after a foliar spray application. After this 6-week interval, regular growth resumes so that treated and untreated plants ultimately attain the same height.

PGR Effects on Plant Morphology and Anatomy

When cytokinins are not present, apical dominance increases inhibiting lateral buds (Stern, 2003; Sinha, 2004). However, if the terminal bud is removed the lateral buds will grow within hours. If the terminal bud is left intact and cytokinin is applied lateral buds will develop. Studying auxin/cytokinin ratios, Macháčková (1992) found that cytokinin stimulates protein production, which is required for cell division. Therefore, high cytokinin levels promote the initiation and growth of apical buds. Evans (1984) found that cytokinins stimulate cell expansion in dicotyledons, especially young bean plants. Cytokinins also slow the senescence and degradation of chlorophyll, nucleic acids and proteins (Macháčková, 1992; Sinha, 2004); stimulate germination by breaking dormancy in seeds (Macháčková, 1992; Sinha, 2004); regulate chloroplast development (Macháčková, 1992; Sinha, 2004); regulate water movement

by inducing transpiration and opening the stomata, regulate fruit setting and ripening (Macháčková, 1992); increase cotyledon expansion via cell enlargement; promote pigment production; determine specific organ formation; tuber formation (Sinha, 2004) and inhibit stem and root cell elongation (Evans, 1984; Sinha, 2004).

Foliar application of cytokinins causes the translocation of nutrients and amino acids to the treated areas. Thus, the application of cytokinins creates strong nutrient sink (Weaver and Johnson, 1985; Macháčková, 1992; Ronzhina, 2002; Kays and Paull, 2004). Weaver and Johnson (1985) and Ronzhina (2002) found that nutrients accumulated in leaves and to the plant tissues sprayed with cytokinins. Fully expanded grape leaves generally export nutrients, but when treated with BA, the leaves darkened and/or the base of the shoots develop rings where ^{14}C-photosynthate is imported (Weaver and Johnson, 1985). Spray application of BA to young grape cuttings caused the nutrients to move into the upper portion of the cuttings (Weaver and Johnson, 1985). Cytokinins are immobile in the shoot after application. However, their presence directs and stimulates the movement of nutrients from the leaves resulting in an increase in shoot dry weight (Ronzhina, 2002). Evans (1984) found that cytokinin application caused expansion through new cell formation in soybean hypocotyls. As a result, cytokinin application increased the fresh weight of soybean hypocotyls. Evans (1984) described BA induced cell elongation and growth under the influence of ethylene production, and when this process is interrupted cell elongation is inhibited.

Unlike cytokinins, chlormequat chloride does not produce a sink that pulls the nutrients into treated shoots. Instead chlormequat causes plant organs that are usually sinks to become sources, giving up their nutrients. Furthermore, the application of chlormequat chloride on young plants and fully expanded leaves of Thompson seedless grapes resulted in a downward

movement of nutrients making it act as source (Weaver and Johnson, 1985). Chlormequat chloride thickens stems and inhibits the formation of vegetative shoots during flower bud development. Since chlorophyll is denser in compacted cells, chlormequat chloride application also deepens leaf color (Nelson, 1998). Sinha (2004) observes the following effects after an application of chlormequat chloride: delayed senescence, suppressed vegetative growth, increased flower growth in short day plants only, reduced and/or induced fruit set depending upon species, and increased plant tolerance to drought, frost and excess watering.

Dikegulac-sodium inhibits gibberellin biosynthesis (Ebrahim, 2004), temporarily halts shoot elongation, promotes lateral branching, and prevents flowering and fruiting in certain species (Nelson, 1998). Concentrations of dikegulac sodium applied to *Zantedeschia aethiopica* cv. Spreng increases shoot and roots number, shoot and root length, shoot fresh weight and rhizome formation. Treatment with dikegulac sodium causes inhibition of apical dominance, which induces axillary branching, allowing for the growth of the lateral shoots. However, concentrations above 1.69 μM decrease growth. Only low dosages promote growth (Ebrahim, 2004). Excess applications retard growth, inhibit the production of new leaves, distort the young shoots by inhibiting expansion, and cause temporary chlorosis of young tissues (Arzee, 1977).

Ethylene inhibits cell division and stems elongation (Evans, 1984; Macháčková, 1992). It induces stem thickening, and accelerates lateral growth (Sinha, 2004; Macháčková, 1992). Although ethylene does not affect the total cell volume, it causes reorientation of the cellulose microfibrils and microtubules (Macháčková, 1992). Macháčková (1992) found that ethylene caused a loss of geotropism. Ethylene also plays a central role in epinasty, fruit ripening, leaf abscission (Taiz and Zeiger, 1991; Macháčková, 1992; Sinha, 2004), and seedling growth (Taiz and Zeiger, 1991). In addition ethylene inhibits hook opening in the dark (Evans, 1984;

Macháčková, 1992), breaks seed and bud dormancy (Taiz and Zeiger, 1991; Sinha, 2004), promotes stem elongation in some monocots (Taiz and Zeiger, 1991), induces root formation (Taiz and Zeiger, 1991; Sinha, 2004), inhibits root elongation (Evans, 1984), induces and inhibits flowering depending upon species, causes flower and leaf senescence (Taiz and Zeiger, 1991; Sinha, 2004) and reduces of male flower formation (Sinha, 2004).

LITERATURE CITED

Ali, S. I. 1965. Proposal for the conservation of the generic name Lourea Desv. Regnum vegetabile 40: 25-26.

Allbritton, G., J.G. Norcini, and J.H. Aldrich. 2002. Natural height control of container grown *Eupatorium fistulosum.* J. Env. Hort. 20 (4): 232-235.

Aphalo, P.J., R. Rikala and R.A. Sanchez. 1997. Effect of CCC on the morphology and growth potential of containerised silver birch seedlings. New Forests, 14:167–177.

Arteca, R.N. 1996. Plant growth substances: principles and applications. Chapman & Hall, NY.

Arzee, T., H. Langenauer, and J. Gressel. 1977. Effects of Dikegulac, a New Growth Regulator, on Apical Growth and Development of Three Compositae. Botanical Gazette, 138(1): 18-28.

Babu, C. R. and K. Thothathri. (323) Proposal to Conserve the Generic Name Sub 3899 Maughania ("Moghania") J. St.-Hil. (Jan. 1813) [FABACEAE] against Flemingia Roxb. ex W. Ait. (1812) and Lourea ("Luorea") Neck. ex J. St.-Hil. (Dec. 1812) [FABACEAE]. Taxon, Vol. 20, No. 4. (Aug., 1971), pp. 645-647.

Backer, C.A. and R.C.B. van den Brink, Jr. 1963. Flora of Java, Vol.1. N.V.P. Noordhoff. Pp 602-611.

Bailey, F.M. 1900. The Queensland Flora, Part 2. HJ Diddams and Co., Brisbane. Pp 410-417.

Bailey, C.D., J.J. Doyle, T. Kajita, T. Nemoto and H. Ohashi. 1997. The Chloroplast rpl2 Intron and ORF184 as Phylogenetic Markers in the Legume Tribe Desmodieae Systematic Botany, 22(1): 133-138.

Baker, J. G. Leguminosae. 07 July 2007. <http://www.botanicus.org/primeocr/botanicus1/b11816351/21753000004415/21753000004415_0158.txt>

Baker, J.G. 1879. 45. Lourea, Neck. In: J.D. Hooker (ed.), Flora of British India, Vol. 2. L. Reeve and Co, Ltd, Ashford. Pp 154-156.

Barham, J.M. 1996. Christia vespertilionis var. verspertilionis Leguminose. Curtis's Bot. Mag. 13 (1): 19-21.

Baruch, Z., R.R. Pattison, and G. Goldstein. 2000. Responses to Light and Water Availability of Four Invasive Melastomataceae in the Hawaiian Islands. Int. J. Plant Sci. 161(1):107–118.

Bentham, G. and F. Mueller. 1864. Flora Australiensis: A description of the plants of the Australian Territory, Vol. 2. Lovell Reeve and Co, London. Pp 229-238.

Chapman, G.P. and Y.Z. Wang. 2002. The plant life of China: Diversity and distribution. Springer-Verlang, Berlin.

Davidson, B.R. and H.F. Davidson. 1993. Legumes: The Australian experience, the botany, ecology and agriculture of indigenous and immigrant legumes. Taunton: Research Studies Press LTD.

Ebrahim, M.K.H. 2004. Comparison, determination and optimizing the conditions required for rhizome and shoot formation, and flowering of in vitro cultured calla explants. Scientia Horticulturae, 101: 305–313.

Ehret, D.L., F. Vanel, P. Bowen, T. Helmer; B. Frey, and K. Usher. 2004. Demonstration of nutrient concentration effects on pineapple sage with the aid of automated crop monitoring. J. hortic. sci. biotechnol, 79(4):619-626.

Ehleringer, J., O. Bjorkman, and H.A. Mooney. 1976. Leaf pubescence: Effect on absorptance and photosynthesis in desert shrub. Science 192: 376-377.

Evans, M.L. 1984. Functions of Hormones at the Cellular Level of Organization, p. 23-79. In: T.K. Scott (ed.). Hormonal Regulation of Development II: The Functions of Hormones from the Level of the Cell to the Whole Plant. Springer-Verlag, Berlin.

Fain, G.B., C.H. Gilliam, and G.J. Keever. 2001a. Response of Lagerstroemia x 'Tuscarora' to Pistill and Atrimmec. J. Environ. Hort. 19(3):145–149.

Fain, G.B., C.H. Gilliam, and G.J Keever. 2001b. Effects of Pistill and Atrimmec on Production of Lagerstroemia. Combined Proceedings-International Plant Propagators Society, 51: 553-557.

Forbes, F.B. and W.B. Hemsley. 1886. An enumeration of all the plants known from China Proper, Formosa, Hainan, Corea, the Luchu Archipelago, and the Island of Hong Kong, together with their distribution and synonymy. London: The Journal of the Linnean Society, Volume XXIII, pp 178.

Halevy, A.H., D.R. Dilley, and S.H. Wittwer. 1966. Senescence inhibition and respiration induced by growth retardants and ^{6}N-Benzyladenine. Plant Physiol. 41(7): 1085–1089.

Havlin, J.L., J.D. Beaton, S.L. Tisdale and W.L. Nelson. 2005. Soil Fertility and Fertilizers An Introduction to Nutrient Management, 7th Edition. Pearson-Prentice Hall, New Jersey.

Hitchcock, A. S. 1893. List of Plants Collected in the Bahamas, Jamaica and Grand Cayman. Missouri Botanical Garden Annual Report, 1893: 46-180.

Huang, T-C. 1993. Flora of Taiwan, 2nd Edition, Vol. 3. Editorial Committee of the Flora of Taiwan 2nd Edition, Taipei, Taiwan. Pp 211-214.

Jia, Y. and V.M. Gray. 2004. Interrelationships between nitrogen supply and photosynthetic parameters in Vicia faba L. Photosynthetica 41(4): 605-610.

Joiner, J.N., ed. 1981. Foliage Plant Production. Prentice-Hall, Englewood Cliffs, NJ.

Jones, D.G. and D.R. Davies. 1983. Temperate Legumes: Physiology, Genetics and Nodulation. Boston: Pitman Advanced Publishing Program.

Kang, J-G. and M.W. van Iersel. 2004. Nutrient Solution Concentration Affects Shoot: Rot Ratio, Leaf Area Ratio, and Growth of Subirrigated Salvia (*Salvia splendens*). HortScience 39(1):49-54.

Kays, S.J. and R.E. Paull. 2004. Postharvest Biology. Exon Press, Athens, GA.

Keng, H., D.Y. Hong and C.J. Chen. 1993. Orders and Families of Seed Plants of China. World Scientific Publishing, Singapore.

Kepczynski, J. 1989. Effects of ethephon and acc on germination of Amaranthus caudatus l. seeds preincubated in 2.5-norbornadiene. Acta Hort. 251:41-46.

Kessler, J.R., Jr. 2007. Greenhouse Light Quality and Intensity. Landscape Horticulture, Auburn University. 12 July 2007. <http://www.ag.auburn.edu/hort/landscape/lightintensityquality.html>

Klitgaard, B. and A. Bruneau. 2003. Advances in Legume Systematics: Part 10- Higher Level Systematics. The Royal Botanic Gardens, Kew.

Kochankov, V.G., E.L. Milyaeva, E.A. Zhyvukhina, and M.K.H. Chailakhyan. 1989. Effect of 6-benzylaminopurine on stem formation and flower bud initiation in rudbeckia bicolor plants of different ages under non-inductive conditions. Acta Hort. 251:25-34.

Laloue, M., M.K.C. Menon, and D. Klambt. 1978. Functions of cytokinins [and discussion]. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences. The Biochemical Functions of Terpenoids in Plants; 284(1002): 449-457.

Latimer, J.G., H.L. Scoggins, and T.J. Banko. Using Plant Growth Regulators on Containerized Herbaceous Perennials. Publication Number 430-103, December 2001.

Lavin, M. Fabaceae. 6 August 2006. BookRags. http://www.bookrags.com/research/fabaceae-plsc-02/

Lewis, G., B. Schrire, B. Mackinder, and M. Lock (eds.). 2005. Legumes of the World. Royal Botanic Gardens, Kew.

Lock, J.M. and J. Heald. 1994. Legumes of Indo-China – A Checklist. Royal Botanic Gardens, Kew. Pp 66.

de Loureiro, J. 1790. Flora Cochinchinensis: sistens plantas in regno Cochinchina nascentes. Quibus accedunt aliae observatae in Sinensi imperio, Africa orientali, Indiaeque locis variis. Omnes dispositae secundum systema sexuale Linnaeanum. i-xx, 1-744 errata 11.

Macháčková, I. 1992. Growth and growth regulators, p. 215-271. In: J. Šebánek (ed.). Plant Physiology, Developments in Crop Science 21. Elsevier, Amsterdam.

Manaker, G.H. Interior Plantscapes Installation, Maintenance, and Management, 3rd Edition. Prentice Hall, New Jersey.

Mauseth, J.D. 2003. Botany, An Introduction to Plant Biology, 3rd Edition. Jones and Bartlett Publishers, Sudbury.

McVaugh R. 1968. Report of the Committee for Spermatophyta, Conservation of Generic Names, X. Taxon, 17(4):462-466.

Merrill, E.D. 1935. A Commentary on Loureiro's "Flora Cochinchinensis". Transactions of the American Philosophical Society, New Ser., 24, (2):1-445.

Moench, C. 1794. 'Methodus Plantas horti botanici et agri Marburgensis'. P.39-40.

Moench, C. 1802. Supplementum ad Methodum Plantas a Staminum situ Describendi. Marburgi Cattorum.

Necker, Natalis Joseph. 1790. Elementa botanica : genera genuina, species naturales omnium vegetabilium.

Nelson, P.V. 1998. Greenhouse Operation and Management, Fifth Edition. Prentice-Hall, Upper Saddle River, NJ.

Nguyen-Pouplin, J., H. Tran, H. Tran, T.A. Phan, C. Dolecek, J. Farrar, T.H. Tran, P. Caron, B. Bodo and P. Grellier. 2007. Antimalarial and cytotoxic activities of ethnopharmacologically selected medicinal plants from South Vietnam. Journal of Ethnopharmacology 109(3): 417-427.

Nippon Shinyaku Co., LTD. 2006. Japanese Greenhouse New Medicine Corporations Herbs - 41. The leaf is similar to the airplane, "[hikoukisu]", Christia vespertilionis (L.f.) Bakh.f. (Legumes). 04 October 2006. <www.nippon-shinyaku.co.jp/herb/syokubutsu/005/index.html>.

Niumsakul, S. 2001. Chemical constituents of leaves of Christia vespertilionis Bakh. f. Bangkok : Ramkhamhaeng University, 109 p. (T E18535)

Ohashi, H. 1977. Leguminosae. Flora of Taiwan, Volume Three, Angiospermae. Epoch Publishing, Taiwan.

Ohashi, H. and T.C. Huang. 1993. Leguminosae. Flora of Taiwan, Second Edition, Volume Three, Angiosperms-Dicotyledons. Lungwei Printing Company, Taiwan.

Ohashi, H., R.M. Polhill and B. Raven 1981. Desmodieae, p. 292-300. In: Polhill, R.M. and P.H. Raven (eds.). Advances in Legume Systematics, Part 1. Royal Botanic Gardens, Kew.

Pozo, L., A. Redondo, U. Hartmond, W.J. Kender and J.K. Burns. 2004. Dikegulac Promotes Abscission in Citrus. HortScience 39(7): 1655-1658.

Puglisi, S.E. 2002. Use of Plant Growth Regulators to Enhance Branching of Clematis spp. Virginia Polytechnic Institute and State University, Blacksburg, MS Thesis.

Puhua H. and H. Ohashi. 2006. Fabaceae [Draft], Trib. Desmodieae (Bentham) Hutchinson. Flora of China Editorial Committee, eds. In Preparation. Flora of China. Vol. 10 (Fabaceae). Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis.

Ronzhina, E.S. 2002. Sink Activity of Cytokinins and Plant Productivity, p.43-44. In: R. Dris and C. Barry-Ryan (eds.). Plant Physiology Characteristics, Breeding and Genetics. Science Publishers, Inc., Enfield, NH.

Rounkova, L.V. 1989. Effect of ethylene producing substances and cultar on some ornamental plants. Acta Hort. (ISHS) 251:281-288.

Rudd, V.E. 1970. (303) Proposal to Conserve the Generic Name 3899 Flemingia Roxburgh ex W. T. Aiton (1812) (Leguminosae) vs. 7914 Flemingia Roxburgh ex Rottler (1803)(Acanthaceae) and Lourea ('Luorea') Necker ex Jaume St.-Hilaire (Dec. 1812)(Leguminosae). Taxon, 19(2):294-297.

Schnelle, M.A., B.D. McCraw, and J.M. Dole. 1996. Height Control of Flowering Crops and Vegetable Transplants. Oklahoma Coop. Ext. Ser., Div. of Agr. Sci. Nat. Res. Bul. F-6714.

Sinha, R.K. 2004. Modern Plant Physiology. Alpha Science International Ltd, Pangbourne. Pp 461-512.

Steinkamp, K. 2006. Personal Communication.

Stern, K.R. 2003. Introductory Plant Biology, Edition Nine. McGraw-Hill, New York.

Taiz, L. and E. Zeiger. 1991. Plant Physiology. The Benjamin/Cumming Publishing Company, Inc. Redwood City, CA.

Tanaka, K. and N.E. Tolbert. 1966. Effect of Cycocel Derivatives and Gibberellin on Choline Kinase and Choline Metabolism'. Plant Physiol. 41: 313-318.

Tayama, H.K., R.A. Larson, P.A. Hammer and T.J. Roll. 1992. Tips on the Use of Chemical Growth Regulators on Floriculture Crops. Ohio Florists' Association, Columbus, OH.

Thomas, P.A., J.G. Latimer, D. Zhang, and S. Hartzler. 1992. Effects of Plant Growth Regulators on Lateral Branching and Shoot Growth of Hypericum. SNA Research Conference - Vol. 37-1992.

United States Environmental Protection Agency (USEPA). 1994. EPA R.E.D. Facts: N6-Benzyladenine.

USDA Natural Resources Conservation Service. 2006. Classification for Kingdom Plantae down to genus *Christia* Moench. 19 August 2006. <http://plants.usda.gov/java/ClassificationServlet?source=display&classid=CHRIS2>

USDA National Agricultural Statistics Service. 2006. Floriculture Crops 2005 Summary, Sp Cr 6-1 (06).

van Meeuwen, M.S., C.G.G.J. van Steenis, and J. Stemmerik. 1961. Prelimnary revisions of some genera of Malaysian Papilionaceae II. Reinwardtia 6: 85-108.

Weaver, R.J. and J.O. Johnson. 1985. Relation of Hormones to Nutrient Mobilization and the Internal Environment of the Plant: The Supply of Mineral Nutrients and Photosynthate, p.

3-36. In: R.P. Pharis and D.M. Reid (eds.). Hormonal Regulation of Development III: Role of Environmental Factors. Springer-Verlag, Berlin.

Wen, Tu Xue. 1990. Effect of nitrogen fertilizer level on soybean yield. AVRDC-ARC, China.

White, S.A. 2003. Nutrition and Plant Growth Regulator Rates for High Quality Growth of Containerized Spiderwort (*Tradescantia virginiana* L.). Thesis, Virginia Polytechnic Institute and State University.

Wilson, J.R. 1996. Shade-stimulated growth and nitrogen uptake by pasture grasses in a subtropical environment. Aust. J. Agric. Res., 47:1075-93.

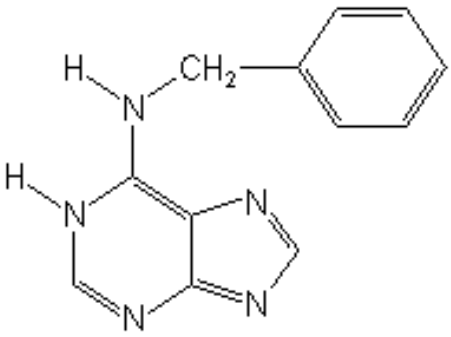
Wong, C.C. and J.R. Wilson. 1980. Effects of Shading on the Growth and Nitrogen Content of Green Panic and Siratro in Pure and Mixed Swards Defoliated at two Frequencies. Aust. J. Agric. Res., 31: 269-85.

FIGURE 1.1: *Christia subcordata* Moench raceme with flowers and seed pods.

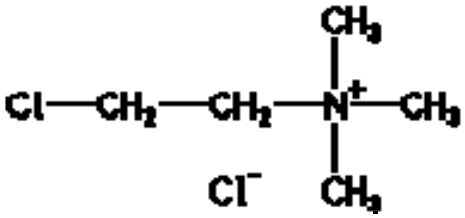
FIGURE 1.2: *Christia subcordata* Moench foliage.

FIGURE 1.3: Elongated growth of *Christia subcordata* Moench stems.

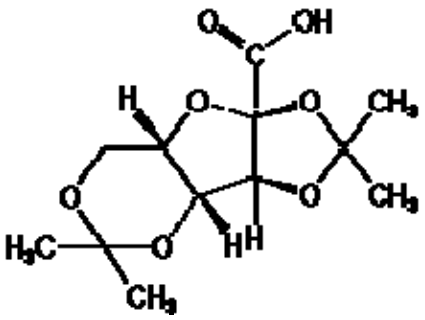
FIGURE 1.4: New leaves and terminal raceme of *Christia subcordata* Monech.



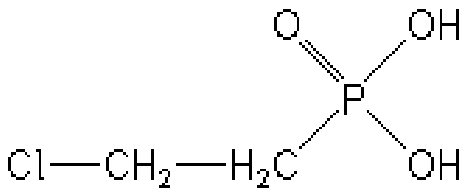
BENZYLADENINE



CHLORMEQUAT CHLORIDE



DIKEGULAC-SODIUM



ETHEPHON

FIGURE 1.5: Chemical structures of select Plant Growth Regulators

CHAPTER 2

MORPHOLOGICAL AND ANATOMICAL CHANGES IN *CHRISTIA SUBCORDATA* MOENCH FOLLOWING APPLICATION OF BENZYLADENINE, CHLORMEQUAT CHLORIDE, DIKEGULAC-SODIUM OR ETHEPHON[1]

[1] Whiting, P.A., S.V. Pennisi and P.A. Thomas. To be submitted to *HortScience*.

Subject Category: Growth Regulators

Morphological and Anatomical Changes in *Christia subcordata* Moench Following Application of Benzyladenine, Chlormequat chloride, Dikegulac-sodium or Ethephon

Additional Index Words: *Christia*, Plant Growth Regulator, Benzyladenine, Chlormequat Chloride, Dikegulac-sodium, Ethephon, Lateral branching, Compaction

ABSTRACT

Christia subcordata was grown under various nutritional regimes and PGRs to determine the most effective PGRs and rates for producing optimal growth while producing a compact, marketable plant. Ethephon was consistently more effective in reducing height, shortening internodes, and inducing lateral branches in *C. subcordata* when compared to dikegulac-sodium. The plants treated with ethephon had the lowest leaf area and shoot dry weight, but the highest leaf area ratio. Ethephon-treated plants were more compact than plants treated with dikegulac-sodium. Plants treated with 500 mg/L chlormequat chloride showed a significant reduction in plant height, internode length leaf area and leaf area ratio, when compared with plants receiving 750, 1000, or 1250 mg/L chlormequat chloride. Benzyladenine (BA) had no statistically significant effect on growth parameters. Fertilizer rate (100, 175, 250 mg/L) had no significant effect on lateral branching and leaf area ratio. The 100 mg/L treatment had the highest growth parameters. From the four chemicals tested, ethephon was found to be most effective in controlling growth and producing marketable *Christia subcordata.*

Chemical Names:

N^6-benzyladenine (or *N*-benzyl-1*H*-purin-6-amine)

(2-chloroethyl) trimethylammonium chloride

2,3:4,6-bis-O-(1-methylethylidine)-alpha-L-xylo-2-hexulofuranosonic acid sodium salt

2-chloroethylphosphonic acid

INTRODUCTION

The floriculture industry uses plant growth regulators (PGRs) to control plant height, stimulate lateral branching, and improve marketability. PGRs have been documented to affect plant morphology by creating a compact plant with shorter internodes, darker green leaves, increased or decreased leaf area, accelerated or delayed flowering, dry matter accumulation and partitioning, increased drought tolerance, and slowing cell division and elongation (Joiner, 1981). Plant response to PGRs is not consistent; while some species and cultivars are responsive to low doses of PGRs, others are not.

PGRs fall in six general classes: i) auxins promote shoot elongation, thin tree fruit, and increase rooting and flower formation; ii) gibberellins stimulate cell division and elongation; increase stalk length, and increase flower and fruit size; iii) cytokinins stimulate cell division, bud initiation and root growth and prolonging storage life of flowers and vegetables; iv) ethylene generators ripen and induce uniform ripening in fruit and vegetables; v) growth inhibitors stop growth and promote flower production by shortening internodes; and vi) growth retardants slow growth (Fishel, 2006).

One of the classes of PGRs is cytokinins. Cytokinins stimulate cell division and differentiation, cell enlargement, floral bud dormancy, vegetative bud break, seed germination, fruit set and delaying of senescence (Joiner, 1981; Taiz and Zeiger, 1991). They can be naturally occurring or synthetically produced like N^6-Benzyladenine, which promotes lateral bud and leaf growth (Cline et al, 2006).

Chlormequat chloride reduces internode elongation (Tanaka and Tolbert, 1966; Bora and Sarma, 2006), and sometimes the plant size altogether (Bora and Sarma, 2006).

Dikegulac-sodium is a gibberellin biosynthesis inhibitor and systemic PGR that works by blocking plant hormones, interrupting apical dominance and temporarily stopping shoot elongation (Thomas et al, 1992; Schnelle et al, 1996; Fain et al, 2001a; Fain et al, 2001b; Puglisi, 2002; Pozo et al, 2004). Dikegulac-sodium mechanically prunes (Thomas et al, 1992; Clark et al, 2004), increases lateral branching (Thomas et al, 1992; Fain et al, 2001a; Fain et al, 2001b; Puglisi, 2002; Pozo et al, 2004) induces axillary bud growth (Fain et al, 2001a; Fain et al, 2001b; Puglisi, 2002) and to promote mature fruit abscission (Pozo et al, 2004). However, high concentrations (>2000 mg-L^{-1}) of dikegulac-sodium may cause abscission of flowers, young leaves and fruit, especially when applied to susceptible species of *Citrus sinensis* L. Osbeck during early season growth (Pozo et al, 2004).

Another commonly used PGR is ethylene, commercially available as ethephon. Ethephon is sprayed as an aqueous solution that upon absorption releases ethylene which stimulates lateral branching and reduces stem elongation (Schnelle et al, 1996; Bailey and Whipker, 1998; Puglisi, 2002). Effects of ethylene are not consistent across species. Fain et al. (2001a) citing Kiyomoto (1997) found that ethephon stimulated shoot elongation in *Kalmia latifolia*. Conversely, Puglisi (2002) found that in Clematis ethephon suppressed lateral branching and stem elongation over time with an application rate of 500 mg/L.

Little is known about *C. subcordata* culture. Optimal nutrition regimes have not been established. Although its foliage has considerably strong ornamental potential, *Christia* exhibits strong apical dominance, leading to its sprawling growth habit. The four PGRs chosen are known for their ability to inhibit internode elongation and induce lateral branching. This research was undertaken to investigate the response of *C. subcordata* to various PGRs and nutritional regimes. The objectives were threefold, 1) to determine the most effective PGRs and rates for

controlling growth of *Christia subcordata*, and 2) to determine optimal nutritional regime, and 3) to investigate the anatomical mechanism via which plant morphology may be affected.

MATERIALS AND METHODS

Production Environment. The experiment was conducted spring and summer of 2007. Plants were grown in a double-polyethylene Quonset-style greenhouse. The greenhouse temperatures were set at 21/18°C day/night (Wadsworth Systems; Arvada, CO). A layer of 60% shade cloth was placed over each of the greenhouse benches on which the plants were grown. Previous investigations indicated that better plant performance of *C. subcordata* was obtained under irradiance level higher than 180 $\mu mol \cdot m^{-2}s^{-1}$ (Appendix A). Therefore, in these studies, the production irradiance level was approximately 600 $\mu mol \cdot m^{-2}s^{-1}$ under the shade cloth and measured with a handheld meter at 12:00 pm.

Plant Material. Tissue culture liners of *Christa subcordata* (Agri-Starts Inc., Apopka, FL) were transplanted into 10-cm pots using a soilless peat-based medium (Fafard Medium Weight #3-B, Fafard, Anderson, SC) and placed on ebb-and-flow benches (1.2 x 2.4 m^2, Midwest GroMaster, St. Charles, IL).

Treatments. Nutrition rates. Plants were sub-irrigated with 150, 175 or 250 mg/L N [Peters Excel Cal-Mag 15-5-15 (15N-2.2P-12.6K); Scotts, Maryville, OH]. Fertilizer solutions were stored in plastic barrels (210 L) and pumped into the watertight trays of the ebb-and-flow system using submersible pumps (NoKorode#2; Little Giant, Oklahoma City, OK). The bottoms of the pots were immersed in the fertilizer solution for about 13 minutes (5 minutes for pumping and 8 minutes for draining). Fertigation was administered once per week for the first two weeks, followed by three times per week for the reminder of the experiment. pH and electrical

conductivity of the substrate in each treatment were monitored weekly using the pour-through analysis (VTEM, Yeager et al., 1997).

Plant Growth Regulators. Four PGRs were utilized: 1) Benzyladenine (N^6-benzyladenine, Cole-Parmer Instrument Company, Vernon Hills, IL), 2) Atrimmec (dikegulac-sodium, PBI/Gordon, Kansas City, MO), 3) Cycocel (chlormequat chloride, OHP, Inc., Mainland, PA), and 4) Florel (ethephon, Lawn and Garden Products, Inc, Fresno, CA). There were two experimental setups, herein referred to as Study I and Study II. Study I, using ethephon and dikegulac-sodium, ran for 5 weeks in May 2007 while Study II, using benzyladenine and chlormequat chloride, ran for 5 weeks in June 2007. Dikegulac and ethephon were applied at three rates, while benzyladenine and chlormequat chloride were applied at four rates. Preliminary tests conducted in Winter 2006 indicated that ethephon reduced stem elongation and promoted lateral branching in *C. subcordata* (personal communication). Conversely, the effect of benzyladenine or chlormequat on *C. subcordata* have not been studied, so that a wider range of rates was evaluated. PGRs were applied as a spray to the foliage. Prior to PGR treatment, plants were trimmed to a uniform height of 7 to 8 cm. In Study I, 150 ml-volume sprays of dikegulac-sodium [Atrimmec 2, 3, 4 mg/L of active ingredient (a.i.)] or ethephon [Florel 250, 500, 750 mg/L of active ingredient (a.i.)] were applied uniformly over 1 m^2. In Study II, 207 ml-volume sprays of benzyladenine [50, 100, 150, 200 mg/L of active ingredient (a.i.)] or chlormequat [Cycocel 500, 750, 1000, 1250 mg/L of active ingredient (a.i.)] were applied uniformly over 1 m^2. In both studies, control plants received a spray of de-ionized water.

Measurements. At the each end of experiment (3 weeks after PGR treatment) the following morphological data was taken: plant height, plant width (two width measurement perpendicular to one other), length of the 4th internode counting from the apex, number of

branches, leaf area and shoot dry weight. Plant leaf area was taken with a leaf area meter (Model 3100 Leaf Area Meter; LI-COR, Lincoln, NE), and leaf area ratio [(LAR), leaf area divided by total plant dry mass] was calculated. The leaf area was measured as an average of three recently matured leaves. The shoots were placed separately in paper bags and dried in a forced-air oven maintained at 80ºC for a week. Representative dried tissue samples were sent to Micro Macro Labs (Athens, GA) for macro- and micronutrient analysis.

Experimental Design. There were three ebb-and-flow tables for each of the three nutrition rates for a total of 9 tables. Study I using dikegulac-sodium and ethephon had three sub-repetitions randomized within each table (35 plants/table). A sub-repetition consisted of 35 plants: five plants from each the three rates of dikegulac-sodium, five plants from each of three rates of ethephon and five control plants. The total plant number for Study I was 315 plants. Study II using benzyladenine and chlormequat had three sub-repetitions randomized within each table (45 plants/table). A sub-repetition consisted of 45 plants: five plants from each the four rates of benzyladenine, five plants from each of four rates of chlormequat and five control plants. The total plant number for Study II was 405 plants. The experimental design was a completely randomized split plot with 9 whole plots (tables) with the variables of fertilizer rate, PGR type and rate.

Data was analyzed in SAS (version 9.1, SAS Institute, Cary, NC) testing for main effects as well as two-way and three-way interactions and significant correlations ($P< 0.05$ were considered statistically significant). Means separation analysis [Fisher's protected least significant difference (LSD)] was used to further analyze the data. Significance of the main effects (fertilizer rate, PGR type and rate) and their interaction were determined using analysis of variance.

Anatomy. Tissue samples from the widest diameter stem were taken from the first mature internode below the apex. Samples of midrib and lamina tissue from the most recently matured leaf were taken from the widest part of the lamina, between the mid-rib and the leaf margin. Tissue samples of leaves and stems, were fixed in Histochoice (Amresco, Solon, OH), an aldehyde-based fixative. The tissue samples were dehydrated in an ascending series of alcohol, using standard histology protocols and embedded in paraffin. Tissue samples were sectioned with an ultramicrotome to 40 μm thickness (Reichert-Jung Ultracut E, C. Reichert Optische Werke AG, Wien, Austria). Transverse sections of leaf and median longitudinal sections of stem tissue were stained with 0.5% Toluidine Blue, mounted on poly-lysine coated slides and examined with a Leica DMLB light microscope (Leica Microsystems, Inc., Wetzlar, Germany). Photomicrographs were taken with a Spot Insight Color Mode 3.2.0 digital camera (Diagnostic Instrument, Inc., Sterling Heights, MI). Cell length and width measurements were averaged from 100 cells per tissue sample. Data was analyzed using pairwise T-test ($P< 0.05$ were considered statistically significant).

RESULTS and DISCUSSION

STUDY I: Plant Growth Regulators. Dikegulac-sodium and ethephon. Ethephon was more effective than dikegulac-sodium in reducing height, and shortening internodes in *Christia subcordata* (Figure 2.1a, c). However, the difference in lateral branching between ethephon and dikegulac-sodium was not significantly different from each other or the control plants. The plants treated with ethephon had the lowest leaf areas and shoot dry weights, but the highest leaf area ratio (LAR) (Table 2.1). The most consistent rate in reducing height, internode length,

shoot dry weight, and leaf area was ethephon at 500 mg/L treatment followed closely by ethephon at 750 mg/L. The 500 mg/L also had the highest LAR.

Interestingly, the 750 mg/L ethephon treatment yielded the highest number of branches, while the 500 mg/L ethephon treatment yielded the lowest. Clematis exhibited increased branching when treated with 500 mg/L ethephon, compared to 1000 mg/L (Puglisi 2002). The author also noted that a concentration of 750 mg/L produced no axillary shoot breaks and suppressed the main leader (Puglisi 2002). In this study, *C. subcordata* was more responsive in branching to 750 mg/L than 500 mg/L. Krug et al. (2006) found ethephon to be most effective in controlling height in *Narcissus pseudonarcissus* not during production but during post-harvest when it enhanced the consumer perception of plant quality.

Plants treated with dikegulac-sodium at a rate of 2 mg/L were tallest, with longest internodes and highest dry weight and leaf areas. They also had the second highest LAR at the concentration of 3 mg/L (Table 2.1). The ethephon treatment, the dikegulac-sodium treatment and the control were each significantly different in LAR. Ethephon treatments produced visibly more compact plants, while the dikegulac-sodium treatments had little effect and exceeded growth beyond the control plants (Figure 2.1). The 2 mg/L dikegulac-sodium treatment was the least effective in reducing stem elongation and increasing lateral branching (Figure 2.1a, b). Clark et al. (2004) noted that as dikegulac-sodium application rates increased from 1500–4500 PPM stem elongation linearly decreased. High dikegulac-sodium concentrations damaged the plants causing severe stunting and chlorosis so that plants were not marketable for several months following treatment. Puglisi (2002) found that dikegulac-sodium at a rate of 800 mg/L produced the highest number of branches in Clematis and plant height and dry weight decreased

linearly as concentrations increased. As no phytotoxicity was observed in *Christia* treated with dikegulac-sodium in this study, further research should explore higher application rates.

There was a distinct difference in plant width between dikegulac-sodium and ethephon. The width of plants treated with dikegulac-sodium was not significantly different than the control (0 mg/L) plants. Examining each dikegulac-sodium treatment individually revealed significant differences between the concentrations, with the 2 mg/L plants yielding the widest plants followed by the 4 mg/L. The 3 mg/L plants were the most compact. The width of ethephon-treated plants, however, was significantly different from that of both the dikegulac-sodium and the control plants. The plants treated with ethephon were the most compact, with 500 and 750 mg/L treatments producing the smallest width (Table 2.1).

Fain et al (2001a) studying the effects of ethephon (Pistill) and dikegulac-sodium on *Lagerstroemia x* 'Tuscarora' found that ethephon-treated plants had a higher number of new lateral branches than dikegulac-sodium treated and control plants. However, number of branches was not significantly different between ethephon-treated plants and manually pruned plants. No specific rate of ethephon yielded more new shoots. These results were consistent with the results of Study I, since ethephon treated plants yielded the most new branches. However, Thomas et al. (1992) demonstrated that dikegulac-sodium linearly increased lateral branching in *Hypericum calycinum* as PGR concentration increased, with the concentration of 3200 PPM yielding the most lateral branches. Bruner et al. (2002) also found with increased dikegulac-sodium concentrations, stem elongation in *Lonicera* × *heckrottii* was reduced and lateral branching increased, especially if the plants were hand pruned prior to application.

Nutrition. A fertilization rate of 100 mg/l nitrogen produced significantly greater plant height, width, internodes, leaf area, and shoot dry weight (Table 2.1). White (2003) determined

that rates of 100 and 200 mg/L were acceptable for quality growth and increases beyond these rates in nutrient application caused stunting in the growth of *Tradescantia*. The 175 and 250 mg/L N treatments were not significantly different in growth parameters, and growth had reached a plateau at 250 mg/L N. The lateral branching and LAR were not significantly different between the fertilization rates.

STUDY II: Plant Growth Regulators. Benzyladenine and Chlormequat chloride. Chlormequat chloride caused some reduction in plant height, internode length, leaf area and LAR (Table 2.2). However, only plants treated with the lowest rate were significantly shorter that plants from other treatments, including the control. These plants also had lowest internode length, plant width, leaf area, and shoot dry weight. The second highest rate for chlormequat chloride (1000 mg/L) produced the highest LAR. Benzyladenine (BA) was found to have no statistically significant effect on any of the growth parameters (Table 2.2). Gianfagna and Merritt (1998) reported similar results for BA in their study on *Aquilegia*. Leopold and Kawase (1964) found that the application of BA to the primary leaflet in a trifoliate leaf of *Phaseolus vulgaris* L. suppressed the growth of the remaining leaflets.

Bora and Sarma (2006) found that chlormequat chloride linearly reduced shoot growth of *Pisum sativum* L.; the higher the concentration, the greater the growth reduction, with 1000 µg/mL (equivalent to 1000 mg/L) being the highest concentration used. Chlormequat chloride had no effect on number of branches. Bora and Sarma (2006) concluded regardless of the chlormequat chloride concentration applied, the number of branches increased for the two cultivars of *Pisum sativum*. However, at higher concentrations phytotoxicity was observed. Similarly, Tanaka and Tolbert (1966) found that chlormequat chloride reduced stem elongation up to 10% in squash.

Schon and Blevins (1990) conducted a study on soil and foliar applications of boron to soybeans in the field. In their literature review, they referenced Pilbeam and Kirkby (1983) and Wagner and Michael (1971) and found that auxins and cytokinin levels are influenced by application of boron. Schon and Blevins (1990) studying soil and foliar applications of boron field-grown soybeans discovered that boron deficiency reduces the activity of the auxins and cytokinins that regulate apical dominance. Low boron-PGR interaction results in the release of apical dominance, which in turn induces branching (Schon and Blevins, 1990).

The foliar nutrient levels of *Christia subcordata* shown in Table 2.3 for Study I and Study II show that boron levels 31.29 mg/L compared to another container-grown perennial legume *Lathyrus latifolius* the boron levels of *C. subcordata* are within recommended range (Mills and Jones, 1996). Boron levels were not sufficient in *C. subcordata* for BA to trigger release of apical dominancy inducing lateral branching.

Shoot dry weight and plant width under the chlormequat chloride treatments did not vary in effect from the control and benzyladenine. BA and chlormequat chloride did not appear to increase lateral branching, reduce stem elongation or increase LAR. Chlormequat chloride at 500 mg/L was the most consistent PGR rate in reducing plant height, internode length, leaf area, plant width and shoot dry weight. The 100 mg/L treatment of BA yielded the highest LAR of the study, followed by the 1000 mg/L of chlormequat chloride. However, variations were not significant enough to be visible when compared with the other PGR treatments (not shown).

Nutrition. There was no significant difference between the growth parameters of the 100 and 175 mg/L N fertilizer rates. The nutrient effects on the plants in these treatments were inconsistent with the 100 mg/L N having the highest internode length, leaf area and dry weight, while the 175 mg/L N had the highest height, number of branches and LAR. The fertilizer rate

of 250 mg/L nitrogen was consistently the lowest producer for each parameter (Table 2.2). The 250 mg/L rate generated the lowest lateral number of branches, although test showed the reduction was not statistically significant. The LAR was not significantly different between the treatments affected by the various nutrition treatments.

Macro- and micronutrient levels in the *Christia* tissue were found to be within appropriate ranges, based on general recommendations for perennial legumes (Mills and Jones, 1996) (Tables 2.3a-b). The foliar nutrient levels differed between the Study I and Study II control plants. It is plausible that plants in Study I, conducted during spring, had different nutrient needs than plants in Study II, conducted during summer. Elevated air temperatures during Study II possibly caused general increase in plant size (Tables 2.1-2). However, no concomitant increase in dry mass occurred, leading to the conclusion that the increase was due to higher water content in the tissues of plants grown during the summer. During the spring (Study I) the leaf content of nitrogen, magnesium and sulfur was higher while conversely in the summer (Study II) the leaf content of potassium and calcium was slightly higher.

In the irradiance and nutrition study (Appendix A), fertilizer application was intermittent (manual watering) while these studies had continuous liquid feed (CLF). The difference in growth is considerable with the plants under CLF being the twice the height of the intermittent plants. An additional consideration was time of year for production with the spring and summer months producing taller plants with more leaf area. The lower fertilization rates applied under continuous liquid feed resulted in enhanced growth.

Anatomical Response of C. subcordata to PGRs. It was assumed that at least one of the treatment combinations would be effective in controlling growth of *Christia subcordata*. In order to glean the mechanism via which *C. subcordata* responded to the chemical application,

anatomical analysis of stem and leaf tissues was performed. Samples were taken from the fourth node from the apex (tissue sectioned was equidistant from the nodes), and the leaf subtending the fourth node. Tissue samples were taken from untreated and ethephon-treated (Florel at 750 mg/L) plants grown under a fertilizer regime of 250 mg/L. This PGR treatment was selected because it had proven effective in controlling plant growth. Tissue was examined via light microscopy.

Stem. Figures 2.2-2.3 show *C. subcordata* tissue from untreated and ethephon-treated plants (Florel at 750 mg/L a.i.). A median longitudinal section of an internodal segment revealed the stem tissues in the following: uniseriate epidermis, multilayered cortex, vascular tissue present as a continuous ring, and central pith (Figure 2.2). Observations were focused on leaf and stem cells of *Christia subcordata*, since they were most likely to exhibit altered anatomy, i.e., parenchymatous tissue of the cortex and pith in the stem and palisade and spongy mesophyll in the leaf.

Cells of the untreated stem had the following average dimensions (width x length): cortical parenchyma 12 µm by 23 µm, and pith parenchyma 45 µm by 70 µm (Table 2.4, Figures 2.2. and 2.3). Cells of the ethephon-treated stems had the following average dimensions (width x length): cortical parenchyma 8 µm by 11 µm, and pith parenchyma 35 µm by 52 µm. Morphologically, there was a difference in the length of 4th internode (Table 2.1) between treated and untreated stems, with the former being 23% shorter than the latter. The probable cause of this reduction is the effect of ethephon on the parenchyma tissue of the stem cortex and pith. Similar reductions occurred in the treated stem cells where the cortical parenchyma was reduced by 34% and the pith parenchyma was reduced by 25%. Ethephon appears to have inhibited cell elongation in the stem cortical and pith parenchyma.

Leaf. Transverse sections of *Christia subcordata* leaves revealed the following tissues: uniseriate adaxial epidermis, palisade, and spongy mesophyll layers, and uniseriate abaxial epidermis. Xylem and phloem tissue was found in vascular bundles (Figure 2.4). The untreated leaf appeared to have increased intercellular spaces in the spongy mesophyll tissue as well as more loosely arrayed palisade cells (Figure 2.5). The leaf thickness of an untreated leaf was 142 □m, with a palisade layer of 58 □m and a spongy mesophyll layer of 52 □m. Leaf thickness of ethephon-treated leaf was 119 □m with palisade layer of 38 □m and spongy mesophyll layer of 52 □m (Table 2.4). Palisade cells of untreated leaves displayed typical morphology, being elongated and easily defined within the leaf tissue, while palisade cells of ethephon-treated leaves exhibited were shorter and less well-defined within the leaf tissue (Figure 2.5). The anatomy correlated with morphology in that the treated plants had reduced leaf area. Steinkamp et al. (1991) citing Johnson et al. (1982) found that in *Ficus benjamina* ethephon-treated plant tissue had reduced intercellular spaces in the palisade and spongy mesophyll cells, especially near leaf margins. Bosabalidis and Exarchou (1995) reported similar anatomical results when applying GA3 to the leaves of *Origanum x intercedens* Rech.

The anatomical analysis of *C. subcordata* supports the morphological data and reports in literature regarding plant response to ethephon treatments, namely reduced cell length in internodal stem parenchymatous tissue (cortex and pith), and reduced intercellular spaces and leaf thickness, and reduced palisade and spongy mesophyll cell lengths.

CONCLUSION

Plant growth regulators may be applied to control plant height, stimulate lateral branching, and improve marketability of *Christia subcordata.* Of the four PGRs tested, ethephon

was found to be most effective in controlling growth of *C. subcordata.* Plants treated with ethephon were shorter and had enhanced branching. In addition to reduced height and width, *C. subcordata* treated with ethephon exhibited other features typical of PGR-treated plants, including reduced leaf area and dry weight. Anatomical analysis of the leaf and stem tissue supported the morphological effects of the ethephon applications. Ethephon-treated plant had reduced cell length in internodal stem parenchyma tissue (cortex and pith), and reduced intercellular spaces and leaf thickness, and reduced palisade and spongy mesophyll cell length. With respect to nutrition, optimal pant growth of *C. subcordata* can be obtained with a rate of 100 to 175 mg/L nitrogen. Further studies are needed to elucidate if higher application rates of ethephon, and/or other plant growth regulators may result in enhanced growth control of *Christia subcordata.*

LITERATURE CITED

Adhikari, K.N., N.W. Galwey and M. Dracup. 2001. The genetic control of highly restricted branching in narrow-leafed lupin (Lupinus angustifolius L.). Euphytica 117:261–274.

Bailey, D.A. and B.E. Whipker. 1998a. Height Control of Commercial Greenhouse Flowers. NC Cooperative Ext. Ser. Hort. Info. Leaflet 528.

Bailey, D.A. and B.E. Whipker. 1998b. Best Management Practices for Plant Growth Regulators Used in Floriculture. NC Cooperative Ext. Ser. Hort. Info. Leaflet 529.Bosabalidis, A. M. and F. Exarchou. 1995. Effect of NAA and GA3 on Leaves and Glandular Trichomes of Origanum x Intercedens Rech.: Morphological and Anatomical Features. Int. J. Plant Sciences, 156 (4): 488-495.

Bora, R.K. and C.N. Sarma. 2006. Effect of Gibberellic Acid and Cycocel on Growth, Yield and Protein Content of Pea. Asian J. of Plant Sci. 5(2): 324-330.

Bruner, L.L., G.J. Keever, J.R. Kessler, C.H. Gilliam, 2002. Atrimmec Suppresses Shoot Length and Promotes Branching of Lonicera x heckrottii `Goldflame' (Goldflame Honeysuckle). J. Env. Horticulture, 20(2): 73-76.

Clark, J.B., G.J. Keever, and T.A. Morrison. 2004. Growth Regulation of Vinca minor. SNA Research Conference - Vol. 49 – Growth Regulators Section.

Cline, M.G., M. Thangavelu and K. Dong-Il. 2006. A possible role cytokinin in mediating long-distance nitrogen signaling in the promotion of sylleptic branching in hybrid poplar. J. Plant Physiology, 163:684-688.

Fain, G.B., C.H. Gilliam, and G.J. Keever. 2001a. Response of Lagerstroemia x ‘Tuscarora’ to Pistill and Atrimmec. J. Environ. Hort. 19(3): 145–149.

Fain, G.B., C.H. Gilliam, and G.J Keever. 2001b. Effects of Pistill and Atrimmec on Production of Lagerstroemia. Combined Proceedings-International Plant Propagators Society, 51: 553-557.

Fishel, F.M. 2006. Plant Growth Regulators. Document PI-139, Pesticide Information Office, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida.

Gianfagna, T.J. and R.H. Merritt. 1998. $GA_{4/7}$ promotes stem growth and flowering in a genetic line of Aquilegia x hybrida Sims. Plant Growth Regulation 24:1–5.

Havlin, J.L., J.D. Beaton, S.L. Tisdale and W.L. Nelson. 2005. Soil Fertility and Fertilizers An Introduction to Nutrient Management, 7th Edition. Pearson-Prentice Hall, New Jersey.

Johnson, C.R., D.B. McConnell and J.N. Joiner. 1982. Influence of ethephon and light intensity on growth and acclimatization of Ficus benjamina. HortScience 17(4):614-615.

Joiner, J.N., ed. 1981. Foliage Plant Production. Prentice-Hall, Englewood Cliffs, NJ.

Kiyomoto, R.K. 1997. Use of ethephon treatments to reduce seed set and stimulate shoot production in Kalmia latifolia. Proc. Intern. Plant. Prop. Soc. 47:592–595.

Krug, B.A., B.E. Whipker, I. McCall and J.M. Dole. 2006. Narcissus Response to Plant Growth Regulators. HortTechnology, 16(1): 129-132.

Leopold, A.C. and M. Kawase. 1964. Benzyladenine Effects on Bean Leaf Growth and Senescence. Am. J. of Botany, 51(3):294-298.

Pilbeam, D.J., and E.A. Kirkby. 1983. The physiological role of boron in plants. J Plant Nutr 6: 563-582.

Pozo, L., A. Redondo, U. Hartmond, W.J. Kender and J.K. Burns. 2004. Dikegulac Promotes Abscission in Citrus. HortScience 39(7): 1655-1658.

Puglisi, S.E. 2002. Use of Plant Growth Regulators to Enhance Branching of Clematis spp. Virginia Polytechnic Institute and State University, Blacksburg, MS Thesis.

Schnelle, M.A., B.D. McCraw, and J.M. Dole. 1996. Height Control of Flowering Crops and Vegetable Transplants. Oklahoma Coop. Ext. Ser., Div. of Agr. Sci. Nat. Res. Bul. F-6714.

Schon, M.K. and D.G. Blevins. 1990. Foliar Boron Applications Increase the Final Number of Branches and Pods on Branches of Field-Grown Soybeans. Plant Physiol. 92:602-607.

Steinkamp, K., C.A. Conover, and R.T. Poole. 1991. Acclimatization of *Ficus benjamina*: A Review. University of Florida Central Florida Research and Education Center – Apopka CFREC-A Research Report RH-91-5.

Taiz, L. and E. Zeiger. 1991. Plant Physiology. The Benjamin/Cumming Publishing Company, Inc. Redwood City, CA.

Tanaka, K. and N.E. Tolbert. 1966. Effect of Cycocel Derivatives and Gibberellin on Choline Kinase and Choline Metabolism'. Plant Physiol. 41: 313-318.

Thomas, P.A., J.G. Latimer, D. Zhang, and S. Hartzler. 1992. Effects of Plant Growth Regulators on Lateral Branching and Shoot Growth of Hypericum. SNA Research Conference - Vol. 37-1992.

Wagner, V.H. and G. Michael. 1971. The influence of varied nitrogen supply on the production of cytokinins in the roots of sunflower plants. Biochem Physiol Pflanzen 162: 147-158.

TABLE 2.1: Growth parameters of *Christia subcordata* as affected by fertilizer rate, plant growth regulator type and dosage following five weeks in production. No statistically significant interactions were found between PGR type and rate for any growth parameters. No statistically significant interactions were found between fertilizer rate, PGR type and rate for leaf area.

PGR TYPE	HEIGHT (cm)	WIDTH (cm)	LENGTH OF 4th INTERNODE (cm)	NO. BRANCHES	LEAF AREA (cm^2)	LAR	DRY WEIGHT (g)
CONTROL	[y]41.716a	28.639a	2.7289a	2.4444a	36.39b	27.6c	1.494a
DIKEGULAC-SODIUM	41.941a	28.404a	2.7815a	2.4148a	39.474a	33.9b	1.45926a
ETHEPHON	27.312b	15.133b	2.097b	2.4519a	34.979b	39.9a	1.03252b

PGR RATE (mg/L)	HEIGHT (cm)	WIDTH (cm)	LENGTH OF 4th INTERNODE (cm)	NO. BRANCHES	LEAF AREA (cm^2)	LAR	DRY WEIGHT (g)
CONTROL 0	41.716b	28.75b	2.74ab	2.4222ab	36.603b	27.6c	1.502a
DIKEGULAC-SODIUM 2	46.991a	32.422a	3.0556a	2.4ab	42.807a	28.0c	1.6424a
DIKEGULAC-SODIUM 3	36.209c	24.067c	2.4644b	2.3778ab	34.711bc	42.1ab	1.1891b
DIKEGULAC-SODIUM 4	42.62b	28.756b	2.8489a	2.5111ab	40.673a	31.4c	1.55a
ETHEPHON 250	35.394c	17.678d	2.7356ab	2.4ab	40.557a	35.4bc	1.2551b
ETHEPHON 500	22.918d	13.494e	1.8044c	2.2444b	32.017c	44.2a	0.8478c
ETHEPHON 750	23.14d	14.083e	1.7156c	2.6889a	32.38c	40.5ab	0.9829c

FERTILIZER RATE (mg/L)	HEIGHT (cm)	WIDTH (cm)	LENGTH OF 4th INTERNODE (cm)	NO. BRANCHES	LEAF AREA (cm^2)	LAR	DRY WEIGHT (g)
100 mg/L	38.245a	25.2476a	2.7381a	2.3714a	40.564a	35.8a	1.3829a
175 mg/L	33.46b	21.319b	2.3943b	2.4952a	34.44b	34.8a	1.214b
250 mg/L	35.213b	21.6833b	2.3095b	2.4381a	36.317b	36.1a	1.2461b

[y]Any two values within a column not followed by the same letter are significantly different, at $P < 0.05$, using Fisher's protected LSD.

TABLE 2.2: Growth parameters of *Christia subcordata* as affected by fertilizer rate, plant growth regulator type and dosage following five weeks in production. No significant interactions between fertilizer rate, PGR type and rate for height, internode length or leaf area.

PGR TYPE	HEIGHT (cm)	LENGTH OF 4th INTERNODE (cm)	NO. BRANCHES	LEAF AREA (cm2)	LAR	DRY WEIGHT (g)
CONTROL	[y]50.484a	9.1889a	4.4511ab	2.7556a	48.897a	44.5a
BENZYLADENINE	50.059a	9.2264a	4.5222a	2.8722a	47.556a	45.2a
CHLORMEQUAT CHLORIDE	47.2b	9.4597a	4.25b	2.9a	47.378a	42.6a

PGR RATE (mg/L)	HEIGHT (cm)	LENGTH OF 4th INTERNODE (cm)	NO. BRANCHES	LEAF AREA (cm2)	LAR	DRY WEIGHT (g)
CONTROL	50.484ab	9.1889bc	4.45111ab	2.7556a	48.897a	44.5abc
BENZYLADENINE 50	50.751a	8.7667c	4.5756a	2.9556a	48.442ab	43.2abc
BENZYLADENINE 100	49.253ab	9.6556ab	4.4abc	2.8444a	46.89ab	51.1a
BENZYLADENINE 150	50.531ab	9.3056bc	4.5111ab	2.8a	46.646ab	42.7abc
BENZYLADENINE 200	49.7ab	9.1778bc	4.6022a	2.8889a	48.244ab	43.9abc
CHLORMEQUAT CHLORIDE 500	43.527c	8.7667c	4.0978c	2.8444a	44.392b	46.7abc
CHLORMEQUAT CHLORIDE 750	50.498ab	9.55abc	4.4467ab	2.7111a	49.37a	35.8c
CHLORMEQUAT CHLORIDE 1000	47.2b	9.35abc	4.2489bc	3.0667a	48.306ab	49.0ab
CHLORMEQUAT CHLORIDE 1250	47.576ab	10.1722a	4.2067bc	2.9778a	47.444ab	39.1bc

FERTILIZER RATE (mg/L)	HEIGHT (cm)	LENGTH OF 4th INTERNODE (cm)	NO. BRANCHES	LEAF AREA (cm2)	LAR	DRY WEIGHT (g)
100 mg/L	49.229a	9.4852a	4.46444a	2.9556a	49.338a	45.2a
175 mg/L	50.981a	9.3667a	4.57407a	2.8222a	49.781a	43.8a
250 mg/L	46.297b	9.1259a	4.14148b	2.8370a	43.668b	43.0a

[y]Any two values within a column not followed by the same letter are significantly different, at $P < 0.05$, using Fisher's protected LSD.

TABLE 2.3a: Nutritional analysis of *Christia subcordata* untreated plants at the three fertilization rates in Study I: Dikegulac-sodium and Ethephon.

MACRO NUTRIENTS, %	**100 mg/L**	**175** mg/L	**250** mg/L
Nitrogen (N)	4.08	3.82	4.20
Phosphorus (P)	0.29	0.26	0.27
Potassium (K)	2.22	2.07	2.06
Calcium (Ca)	0.87	0.96	0.86
Magnesium (Mg)	0.37	0.46	0.35
Sulfur (S)	0.25	0.22	0.53
MICRO NUTRIENTS, mg/L			
Iron (Fe)	65.25	63.78	70.30
Manganese (Mn)	58.61	55.81	58.71
Boron (B)	27.49	26.19	29.06
Copper (Cu)	5.67	4.20	4.46
Zinc (Zn)	46.79	41.49	50.21
Molybdenum (Mo)	4.75	1.14	0.78

TABLE 2.3b: Nutritional analysis of *Christia subcordata* untreated plants at the three fertilization rates in Study II: Benzyladenine and Chlormequat Chloride.

MACRO NUTRIENTS, %	**100 mg/L**	**175** mg/L	**250** mg/L
Nitrogen (N)	3.85	4.16	4.55
Phosphorus (P)	0.29	0.28	0.33
Potassium (K)	2.34	2.30	2.51
Calcium (Ca)	1.05	1.03	0.99
Magnesium (Mg)	0.28	0.29	0.28
Sulfur (S)	0.22	0.23	0.26
MICRO NUTRIENTS, mg/L			
Iron (Fe)	57.52	63.86	67.16
Manganese (Mn)	22.93	34.08	28.25
Boron (B)	33.98	28.67	29.58
Copper (Cu)	6.21	4.35	3.75
Zinc (Zn)	62.77	52.13	57.39
Molybdenum (Mo)	6.49	3.18	3.17

TABLE 2.4: Anatomical measurements of *Christia subcordata* untreated and treated with ethephon (Florel at 750 mg/L). Leaf measurements were taken 500 μm from the midrib. Pith parenchyma from the central area of stem tissue and cortical parenchyma of the third sub-epidermal layer were included in the measurements.

Parameter	**Untreated (μm)**	**Ethephon-treated (μm)**
Stem – Pith Parenchyma Cell (Width x Length)	45 x 70a*	35 x 52b
Stem – Cortical Parenchyma Cell (Width x Length)	12 x 23a	8 x 11b
Leaf Thickness	142a	119b
Leaf – Palisade Layer Thickness	58a	38b
Leaf – Spongy Mesophyll Thickness	52a	52a

* Values are averages of measurements from 100 cells per leaf or stem, respectively. Three replicate samples were used in the analysis. Any two values within a column not followed by the same letter are significantly different, at $P < 0.05$, using pairwise t-test.

0 mg/L 250 mg/L 500 mg/L 750 mg/L 2 mg/L 3 mg/L 4 mg/L
Florel Atrimmec

FIGURE 2.1a: Comparison of ethephon (Florel) (250, 500 and 750 mg/L, respectively) with dikegulac-sodium (Atrimmec) (2, 3 and 4 mg/L, respectively) and the control (no PGR added) on *Christia subcordata*. Florel-treated plants were significantly reduced in height, internode length and had increased lateral branching. Atrimmec-treated plants were not affected and shown elongated growth as compared to the control plant. Plants shown were grown with 175 mg/L N.

0 mg/L **2 mg/L** **3 mg/L** **4 mg/L**

FIGURE 2.1b: Application of three rates of dikegulac-sodium (Atrimmec) (2, 3 & 4 mg/L, respectively) to *Christia subcordata* grown at fertilizer rate of 175 mg/L. Atrimmec had no effect on plant height, internode length or branching.

0 mg/L **250 mg/L** **500 mg/L** **750 mg/L**

FIGURE 2.1c: Application of three rates of ethephon (Florel) (250, 500 & 750 mg/L, respectively) to *Christia subcordata* grown at fertilizer rate of 175 mg/L. Visible reduction of height and internodes is evident as well as increased lateral branching compared to untreated plant.

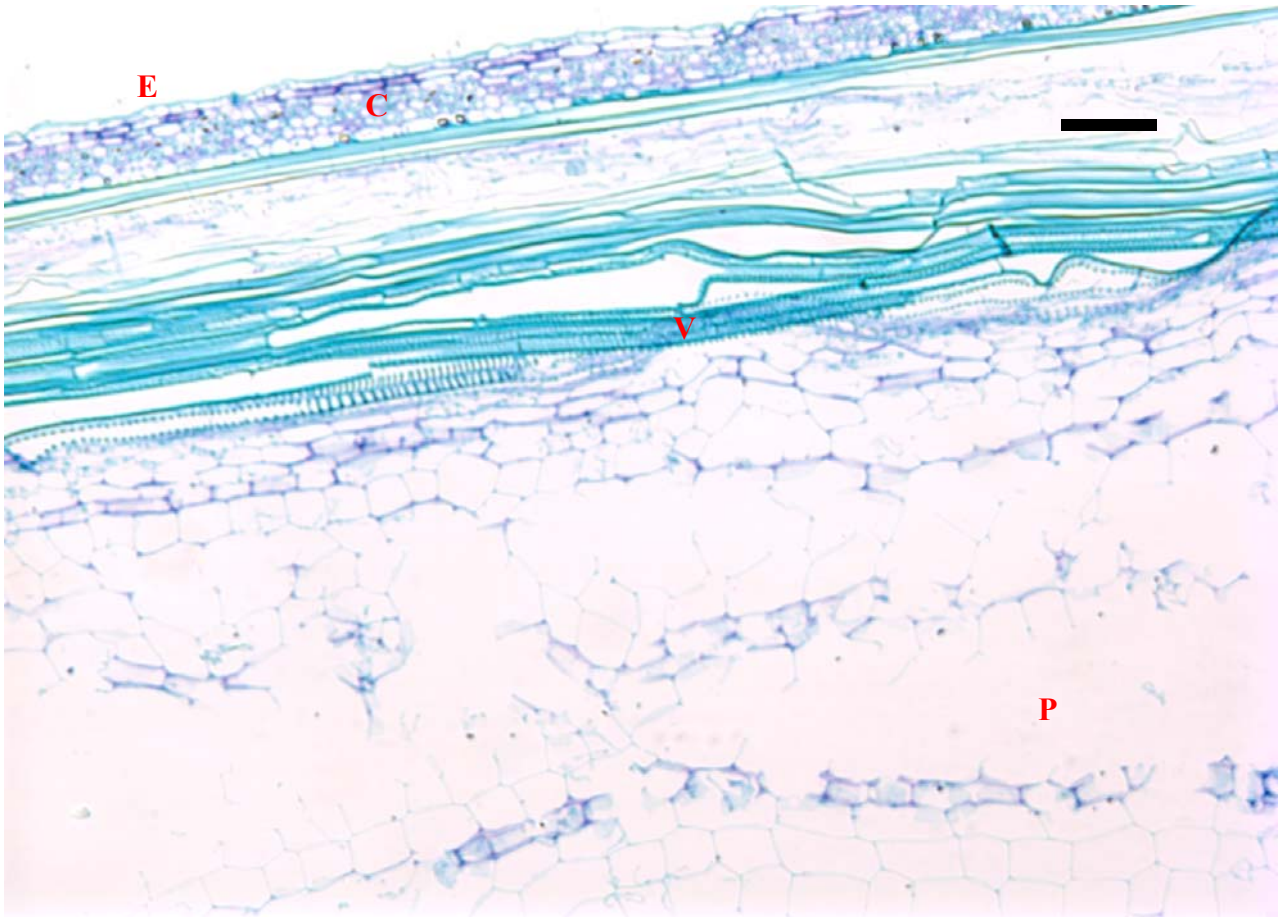


FIGURE 2.2: Median longitudinal section of *Christia subcordata* stem displays the epidermis (E), cortical parenchyma (C), the vascular bundles containing xylem and phloem (V) and the pith parenchyma (P). Samples were taken from the fourth node from the apex (tissue sectioned was equidistant from the nodes). Scale bar = 100µm.

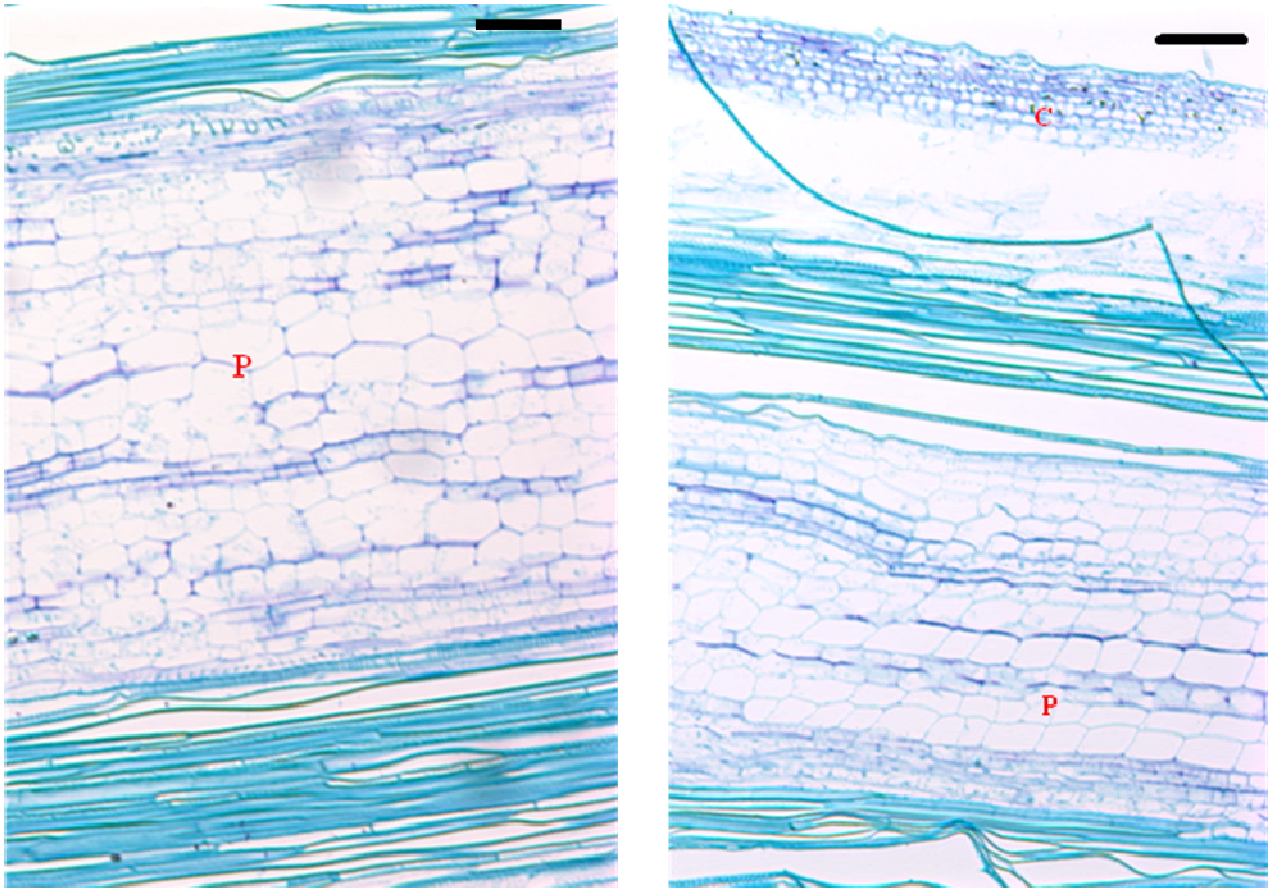


FIGURE 2.3: Median longitudinal section of *C. subcordata* untreated (left) stem and the ethephon-treated (right, Florel, at 750 mg/L). Plants were grown with 250 mg/L nitrogen nutrition rate. Note reduced cell lengths of cortical and pith parenchyma tissue of ethephon-treated plants. Tissues were taken from the fourth node from the apex (tissue sectioned was equidistant from the nodes). Scale bar = 100 µm.

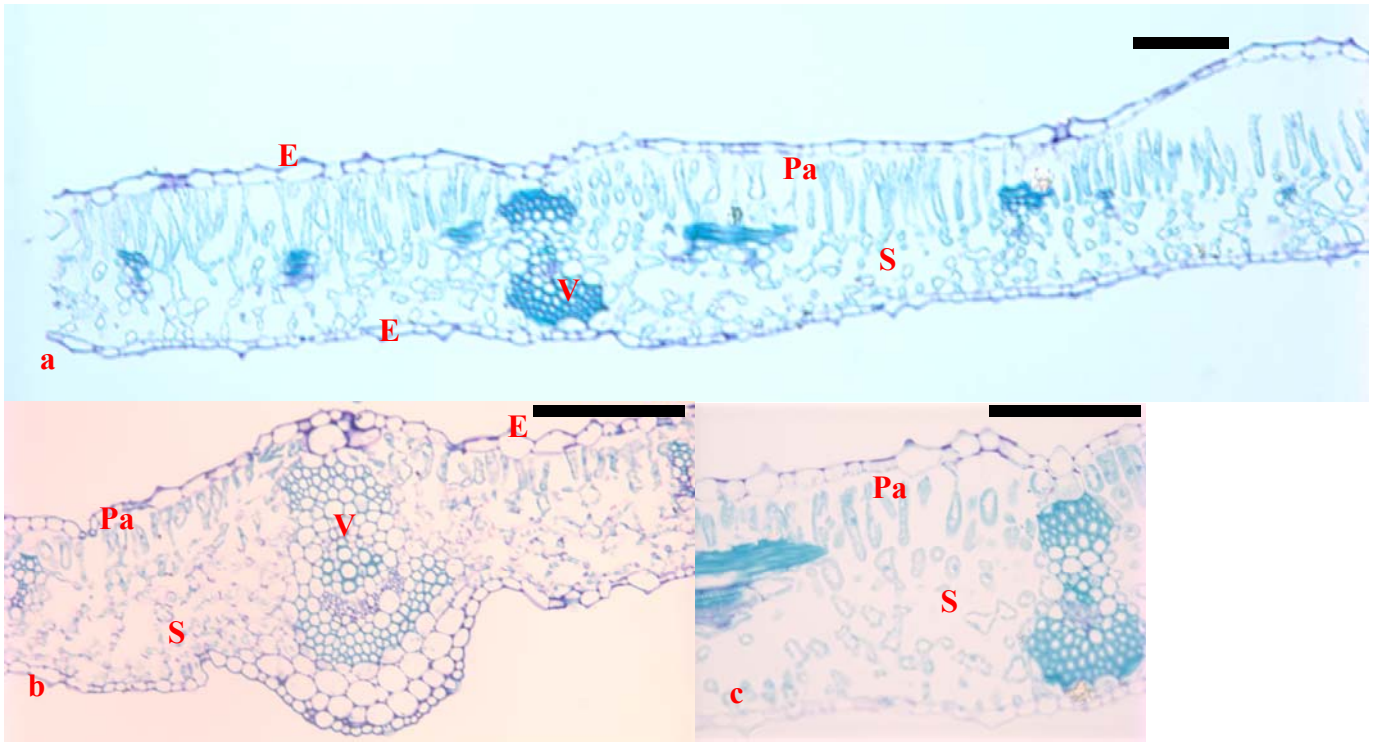


FIGURE 2.4: Transverse section of *C. subcordata* leaf tissue section displays a) the upper and lower epidermis (E), palisade layer (Pa), spongy mesophyll cells (S) and the vascular bundles containing xylem and phloem (V); b) the mid-rib vascular bundle; and c) inset at 400X of the palisade and spongy mesophyll. Samples were taken from the leaf subtending the fourth node. Scale bar = 100 µm.

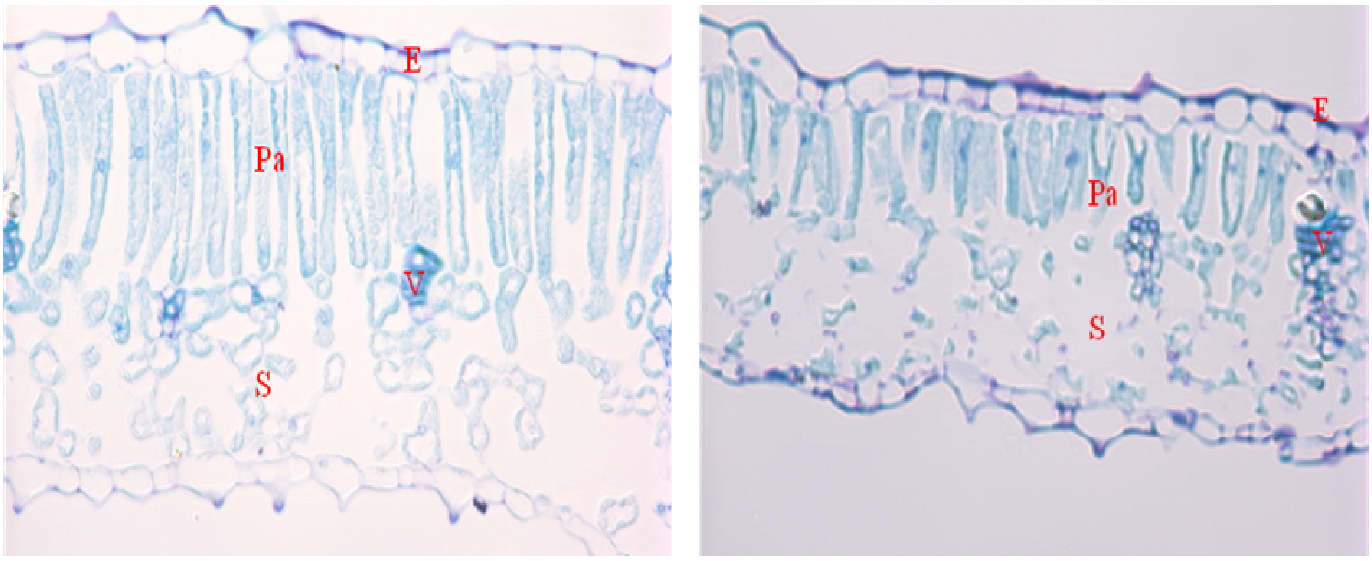


FIGURE 2.5: Transverse section of *C. subcordata* leaf of untreated (left) and treated (right, Florel at 750 mg/L). Note that the ethephon-treated leaf exhibits reduced leaf thickness, intercellular spaces, and palisade cell length. Samples were taken from the leaf subtending the fourth node. Scale bar = 100 µm.

CHAPTER 3

CONCLUSIONS

In the floriculture industry the need to introduce rare and fascinating material into the market is constant. For the foliage plant sector, *Christia subcordata* adds a unique shape and texture suitable for enticing the consumers of ornamental potted plants. However, limited cultural guidelines for the production of new plant material can make the process costly, time consuming and potentially unproductive.

Climate, irradiance and nutritional needs are key factors in developing a healthy and marketable plant. Deviations in these factors can lead to costly trials with no return profit. Knowing the general native locale and growth habit provides a starting place for developing cultural guidelines for commercial production. In adapting to its location of growth, the plant undergoes morphological and physiological changes based on the ambient irradiance level. The growth of a plant is determined by the amount of light available to the canopy. In greenhouse production, irradiance and nutrition levels determine the amount of growth plants achieve. Plants grown under higher irradiance levels generally need more nutrients for optimal growth. Plants generally respond to higher nutrition rates with increased growth. However, too high rates may be inhibiting to plant growth, and unnecessary costly for the producer.

Commercially produced ornamental plants, which have not been treated with PGRs often exhibit elongated growth that makes them susceptible to breakage during shipping as well as reduces available packing space. These plants have reduced marketability until pruned, and then the cost of labor for having to trim the plant has increased its production cost, not guaranteeing

the retailer a fair return. By using PGRs to control stem growth the grower presents to the retailer a product that is healthier with a more marketable size and shape.

Based on the presented studies, it can be concluded that irradiance and nutrition levels are key to establishing the right environment for production of *C. subcordata*. *Christia subcordata* exhibited optimal growth under irradiance levels of 600 μmol·m-2s-1 and nutrient regime of 100 to 175 mg/L nitrogen. Plant size was achieved in the shortest amount of time when plants were grown under elevated air temperatures naturally found in spring and summer.

The effects of four PGRs were tested. Only ethephon visually and effectively resulted plant size and enhanced branching. The other three PGRs, benzyladenine, chlormequat chloride and dikegulac-sodium were ineffective, potentially due to application rates, timing of application, application technique and/or physiological stage of plant development. Future studies are needed to determine if other PGR types and rates on *C. subcordata* can further improve the marketability of this plant.

APPENDIX A

EFFECT OF VARYING NUTRIENT AND IRRADIANCE ON *CHRISTIA SUBCORDATA* MOENCH

METHODS AND MATERIALS

Plant Material. The experiment ran in November 2006. Tissue culture liners of *Christa subcordata* obtained from Agri-Starts Inc. (Apopka, FL) were transplanted to 10-cm pots, planted using a soilless peat-based medium with 50% peat, 30% pine bark, 15% perlite and 10% vermiculite (Fafard Middleweight Mix #3-B, Fafard, Anderson, SC). Plants were initially fertilized with 20-20-20 [(20N-8.7P-16.7K_2O) Jack's Professional water-soluble fertilizer, JR Peters, Allentown, PA)] at a concentration rate of 200 mg/L. The plants were grown in a glass greenhouse with a 21/18°C day/night temperature. Relative humidity, temperature and irradiance data were collected continuously using quantum sensors (QSO-SUN; Apogee Instruments Inc., Logan, UT) connected to dataloggers (HOBO data logger**,** H08-004-02; Onset computer Corporation, Pocasset, MA). The data was then downloaded into a spreadsheet to calculate daily averages.

Irradiance Treatment. There were two irradiance levels, high and low. The low irradiance level was created with a single layer of 63% black shade cloth placed over half of the 14 m^2 of bench space. The remaining half of the bench space received ambient light and was designated as the high irradiance treatment. However there was a single layer of shade clothe over the glass greenhouse as well. The irradiance levels were recorded as instantaneous and continuous measurements using quantum sensors set at pot level on the bench and apart from the plant canopy.

Nutrition Treatment. There were five fertilizer levels based on the nitrogen concentration, 0, 100, 200, 300, and 400 mg/L [(20N-8.7P-16.7K); (Jack's Professional 20-20-20J.R. Peters, Allentown, PA)]. A week prior to treatment each plant received only water to leach out any fertilizer present. During the early stages of growth, plants were fertilized once a week, and then

twice per week as plant size increased. Each fertilizer concentration above 0 mg/L was stored as solution in a labeled 3.78-L container and applied in a 1:100 ratio via a Dosatron Model DI-16 fertilizer injector (Dosatron, Clearwater, FL). Fertility levels were monitored biweekly on a random sample of 24 plants using the pour-through method (Yeager et al., 1997). Electrical conductivity (EC) and pH were measured one hour after irrigation/fertilization using 100 mL distilled water poured into each pot and collecting the leachate. pH and EC were analyzed with a Hanna HI9813 GroChek Meter (Hannah Instruments, Woonsocket, RI) to establish appropriate EC levels (1.3-1.6 dS/m) and pH levels (5.5-6.5) for the growth medium.

Experimental Design. The design was a split plot with replication nested in the main plot treatments with two irradiance levels, low or high, and nutrition with five levels randomized within the irradiance level. There were 20 replicates per treatment combination.

Measurements. At the end of experiment (5 months after initiation) the following morphological data was taken: plant height, number of branches, leaf area, shoot dry weight and root dry weight. Visual observations on plant appearance also were made.

Leaf area was taken with a leaf area meter (Model 3100 Leaf Area Meter; LI-COR, Lincoln, NE), and leaf area ratio [(LAR), leaf area divided by total plant dry mass] was calculated. The shoots and roots were placed in paper bags separately and dried in a forced-air oven maintained at 80ºC for a week. Dry weights of the separated shoots and roots were recorded and later combined for analysis where necessary. Dried tissue samples were sent to Micro Macro Labs (Athens, GA) for analysis after the dry weight values were assessed. Macro- and micronutrient levels in the plant tissue were found to be within appropriate ranges, based on general recommendations for perennial legumes (Mills and Jones, 1996).

Data was analyzed in SAS (version 9.1, SAS Institute, Cary, NC) testing for main effects as well as two-way and three-way interactions and significant correlations ($P< 0.05$ were considered statistically significant). Significance of the main effects and their interaction were determined using analysis of variance. Means separation analysis [Fisher's protected least significant difference (LSD)] was used to further analyze the data.

RESULTS

IRRADIANCE. Christia subcordata plants grown under low irradiance treatment (65 $\mu mol \cdot m^{-2}s^{-1}$, 21.9°C, and 39% humidity) and high irradiance treatment (187 $\mu mol \cdot m^{-2}s^{-1}$, 22.9°C, and 36% humidity) had no significant difference in height and number of branches (Table A-1).

Total dry weight (TDW) was found to be significantly different (Table A-1). The plants responded to varied irradiance level in a typical fashion, i.e. under high irradiance, plants accumulated more biomass [higher shoot dry weight (SDW), total dry weight (TDW), root dry weight (RDW), and root to shoot (R:S) ratio], and less leaf area, which means a lower leaf area ratio (LAR). Conversely, under lower irradiance, plants had low SDW, TDW, RDW, and R:S ratios, and larger but thinner leaves, which resulted in higher leaf area and higher LAR. Because of the increased leaf area there was more available surface to receive light, and higher photosynthetic rate which increased the biomass. Since pH and EC (Table A-2) did not show difference among treatments, irradiance was one of the prevalent factors causing all these changes. During the months of November 2006 to March 2007 light levels outside the greenhouse changed from long days and full sun to shorter days and cloudy skies. Height in the *C. subcordata* was not significantly different between the high and low irradiance plants but

comparable growth was evident in both groups, even though the low irradiance treatment had the higher leaf area.

NUTRITION. The nutrition treatment levels also affected the plants. The data in Table A-1 shows the 100 mg/L producing the tallest plants and the 400 mg/L the shortest, so that the increasing nutrient rate reduced the overall plant height. In addition, there were no significant differences in height and number of branches between the 100, 200, and 300 mg/L nutrient concentrations. Multiple nutrient treatment levels on *Christia subcordata* had no effect on the number of stems per plant.

Plants grown under 400 mg/L have the lowest leaf area. There were no significant differences between the 100, 200 and 300 mg/L treatments in amount of leaf area. However, the control plants (no nutrient added) has significantly reduced leaf area compared to the nutrition-added treatments, with exception of the highest nutrition level. Shoot dry weight yields the highest under the 300 mg/L treatment but still not significantly different enough to differentiate it from the other nutrient treatments. Plants grown with no added nutrients accumulate approximately four times less shoot dry weight compared to the nutrient added treatments (Table A-1).

Application rates of 100, 200 and 300 mg/L nutrients increased height while the 400 mg/L treatment consistently reduces plant growth. Height, number of branches, leaf area, and shoot dry weight were lowest under the control (0 mg/L) than the other treatments. Height and number of branches was higher at the lower nutrient level and decreased as the nutrient concentration increased. In contrast, shoot dry weight increased as nutrient level increased, until it reached 400 mg/L, which caused a reduction in SDW. In *C. subcordata,* growth increased at nitrogen concentrations of 300 mg/L but beyond this concentration growth declined. The growth

outcome of plant height, number of branches, and leaf area decreasing as the nutrient levels increased can be potentially explained by the build up of salinity from the increased fertilization as seen in the increased EC and decreased pH (Table A-2) possibly causing nitrogen phytotoxicity. The increased nitrogen allowed for initial shoot growth but as the plants became saturated, the nitrogen restricted further leaf growth, instead adding more floral stalks. Also the excess nitrogen caused added elongated stem growth and darker green leaf stripes. Tissue analysis in Table A-2 shows the macro- and micro-nutrient levels for the five fertilizer rates in high light level was lower compared to another perennial shade legume, *Baptisia australis* that was container grown in a greenhouse during the summer per recommended standards (Miller and Jones, 1996).

Leaf Area Ratio. The leaf area ratio (LAR) of the 100, 200 and 300 mg/L nutrient treatments were not significantly different from each other, but were higher than the control and the 400 mg/L treatments. Treatments 100, 200 and 300 mg/L N had less overall loss in leaf area per treatment than in the 400 mg/L plants. However, the 400 mg/L treatment plants developed more roots and stems than leaf area, doing the very opposite expected under environmental stress of nitrogen phytotoxicity. The 100, 200 and 300 mg/L nutrient treatment plants had higher leaf area ratios than the 400 mg/L and control treatments resulting in increased plant weight and leaf area allowing for the increase in photosynthesis. The difference between the 100, 200 and 300 mg/L nutrient levels showed little significance in leaf area and overall growth.

The increase in nutrient rate did not always increase the leaf area, hence the LARs were unique per treatment and responded to difference in shoot dry weight. The 100, 200, 300 and 400 mg/L treatments were not significantly different in LAR, but again the control and the 400 mg/L treatments had significantly lower LAR than the other treatments. Since there was not a

significant difference between the two light intensities for the plants, it became the question of the amount of leaf area available to intercept the light and photosynthesize for further plant growth. The plants in both light levels had the root to shoot ratio (Table A-1) decrease as the nutrient treatments increased. The high total dry matter production in *C. subcordata* was a result of increased nutrient treatment, which led to over fertilization and an increase in LAR. The 400 mg/L treatment plants reduced significantly potentially to phototoxicity at a higher nutrient level.

CONCLUSION

For optimal growth, *Christia subcordata* cultured during the winter and early spring months is recommended at a production irradiance level of 180 μmol $m^{-2}s^{-1}$ or higher with a fertilization rate of 100 to 200 mg/L nitrogen administered biweekly would produce optimal plant growth and produce a marketable plant.

Table A-1: Separation of means based on irradiance (upper) and nutrition rate (lower) treatment for growth parameters in *Christia subcordata* Moench. An interaction did not exist between irradiance and nutrition level for plant height and number of branches. SDW (Shoot Dry Weight), Root Dry Weight (RDW), TDW (Total Dry Weight), R:S (Root Shoot Ratio), LAR (Leaf Area Ratio), EC (Electrical Conductivity)

AVERAGE DAY PPF (µmol m^{-2} s^{-1})	**HEIGHT (cm)**	**NO. BRANCHES**	**LEAF AREA (cm^2)**	**SDW (g)**	**RDW (g)**	**TDW (g)**	**R:S**	**LAR**
SHADE – 65	29.7a[y]	3.8a	228.2a	0.99b	0.7b	1.7b	0.9b	136.3a
AMBIENT – 187	27.8a	4.7a	185.8b	1.3a	1.5a	2.8a	1.75a	67.1b
NUTRIENT CONCENTRATION (based on N, mg/L)	**HEIGHT (cm)**	**NO. BRANCHES**	**LEAF AREA (cm^2)**	**SDW (g)**	**RDW (g)**	**TDW (g)**	**R:S**	**LAR**
0	22.9b	1.96b	60.1c	0.37b	1.0a	1.4b	2.7a	71.1b
100	34.2a	5.23a	279.2a	1.5a	1.3a	2.8a	0.9b	109.6a
200	29.9a	5.4a	268.3a	1.5a	1.2a	2.7a	0.9b	109.0a
300	30.4a	5.2a	264.1ab	1.5a	1.3a	2.9a	0.8b	119.9a
400	21.7b	4.75a	192.2ab	1.36a	1.0a	2.4ab	0.7b	92.4ab

[y]Any two values within a column not followed by the same letter are significantly different, at $P < 0.05$, using pairwise t-tests.

Table A-2: EC and pH on irradiance (upper) and nutrition rate (lower) treatment in *Christia subcordata* Moench.

AVERAGE DAY PPF (µmol m^{-2} s^{-1})	**EC**	**pH**
SHADE – 65	0.83074	5.326
AMBIENT – 187	0.90156	5.382
NUTRIENT CONCENTRATION (based on N, mg/L)	**EC**	**pH**
0	0.2341	6.64
100	0.68875	5.895
200	0.8309	5.055
300	1.2147	4.84
400	1.3623	4.34

Table A-3: Tissue analysis of *Christia subcordata* Moench plants grown in a greenhouse and supplied with Jack's Professional 20-20-20 at the five fertilization rates.

MACRO NUTRIENTS, %	**0 mg/L**	**100 mg/L**	**200 mg/L**	**300 mg/L**	**400 mg/L**
Nitrogen (N)	1.63	1.41	1.82	2.17	1.89
Phosphorus (P)	0.29	0.24	0.28	0.48	0.55
Potassium (K)	1.33	1.15	1.11	1.84	1.49
Calcium (Ca)	1.93	1.24	1.33	1.14	0.98
Magnesium (Mg)	0.72	0.45	0.44	0.52	0.48
Sulfur (S)	0.50	0.15	0.20	0.22	0.23
MICRO NUTRIENTS, mg/L					
Iron (Fe)	194.39	76.97	72.37	78.28	55.47
Manganese (Mn)	44.03	59.25	106.65	121.76	92.09
Boron (B)	31.29	23.24	22.19	19.49	17.50
Copper (Cu)	31.14	23.14	15.39	12.63	7.42
Zinc (Zn)	52.04	35.81	33.55	24.40	20.38
Molybdenum (Mo)	12.23	0.55	0.01	0.01	0.01