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FLOWER FORCING IN BANANA SHRUB (*Michelia skinneriana* Dunn.) AND BOUGAINVILLEA (*Bougainvillea* Wild.)

By

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FLOWER FORCING IN BANANA SHRUB (*Michelia skinneriana*

Dunn.) AND BOUGAINVILLEA (*Bougainvillea* Wild.)

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Flower forcing to meet holiday market can increase the value of potted flowering plants. This study was to investigate the effects of chilling and post-chilling photoperiod treatments on flowering of banana shrub and the effects of water stress, daminozide, chlormequat, and ethephon on flowering of bougainvillea. Experiments were conducted at R.R. Foil Plant Science Research Center and MSU Dorman Greenhouse at Mississippi State University (MSU), Mississippi State, MS. Results from banana shrub experiment indicated that 8-week chilling at 8 ºC was necessary for banana shrub to break bud dormancy and a tank mix of daminozide and chlormequat was able to increase the number of flower buds on banana shrub. Results from bougainvillea experiment indicated that water stress, daminozide, and chlormequat enhanced flowering. In conclusion, this study provided fundamental information to future research on flower forcing of banana shrub and bougainvillea.

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TABLE OF CONTENTS

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LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Banana shrub (*Michelia skinneriana* Dunn.), an evergreen shrub native to Southern China, belongs to the Magnoliaceae and flowers from May to June (Liu et al., 2002). Like other commercial flowering crops, flowering of banana shrub involves a complex series of stages, including flower induction, initiation, and development. Spring flowering plants require vernalization and proper daylength to flower (Sung et al., 2006), and banana shrub may have similar requirements. Plant growth regulators (PGR) are widely used to increase the number of flowers in azalea, a spring flowering plant. However, there are no studies investigating the chilling and photoperiod requirements for banana shrub flowering or the feasibility of applying PGRs to increase the number of flowers in banana shrub.

Bougainvillea (*Bougainvillea spectabilis* Wild.) is a very popular tropical and subtropical evergreen landscape plant with showy, vibrantly colorful petaloid bracts used in most areas with warm climates. As the inflorescence is the most valuable aesthetic part in bougainvillea, flowering is of great importance. Water stress advanced flowering on bougainvillea (Tang et al., 2000). Use of PGRs to promote flowering is easier and more consistent than using water stress, however, the effective application rates are cultivar

dependent. Few studies have investigated the effects of combining water stress and PGRs on flowering of bougainvillea.

There were four hypotheses in this study: 1) chilling, photoperiod are necessary for flower initiation and development in banana shrub; 2) daminozide and chlormequat can increase the number of flowers in banana shrub; 3) gibberellic acid can produce uniform flowering on banana shrub; 4) daminozide, chlormequat, ethephon and water stress promote flower induction of bougainvillea.

The objectives of this study were:

1. To determine the effects of chilling treatment and post-chilling photoperiod on flowering in banana shrub.
2. To determine the effects of daminozide, chlormequat, and GA applications on banana shrub flower bud initiation and development.
3. To investigate the effects of daminozide, chlormequat, ethylene, and water stress on flowering and growth of bougainvillea.

CHAPTER II

LITERATURE REVIEW

Flower forcing is a process to create off-season blooming on plants. It is an important component of the floriculture industry which had its inception in the US in the early 19th century in the vicinity of Philadelphia (Laurie et al., 1969).

Potted plants targeting the holiday market play a substantial role in today's green industry. Under the category of floriculture crop wholesale value, the percentage of wholesale value of potted flowering plants increased from 16.2% to 17.3% since 2006 in "15 states program for operations with $100,000+ sales" (USDA-NASS, 2007, 2008, 2009a). Most of the potted plants are produced for a specific season of the year or for certain holidays (Laurie et al., 1969). Nowadays, the most popular potted plants are African violets (*Saitpaulia ionantha* Wendl.), azaleas (*Rhododendron* L.), chrysanthemums (*Dendranthema* x *grandiflorum* [Ramat.] Kitam), Easter lilies (*Lilium longiflorum* Thunb.), and poinsettias (*Euphorbia pulcherrima* Willd. ex Klotzsch) (USDA-NASS, 2009b). Recent surveys indicated that Christmas, Mother's Day, Valentine's Day, Easter, and Thanksgiving rank as the top five calendar occasions (SAF, 2008b), which cover 13.5% of the American floral purchase occasions. Poinsettias dominate Christmas flower sales and Easter lilies ranked first as the most promoted product for Easter (SAF, 2008a). Based on the percentage of wholesale values and needs

of floral product during holidays, the value of plants may be increased if flowering could be promoted or delayed to meet the holiday markets.

Biological mechanism of flowering

Flowering of most plant species requires the transition to the mature phase from the juvenile phase. Different species have different lengths of the juvenile phase. The length of the juvenile phase is 20-30 days for hybrid tea rose (*Rosa hybrida*), 1 year for grape (*Vitis spp*.), and 5-8 years for citrus (*Citrus spp*.) (Clark, 1983). Flowering can be accelerated if the juvenile phase is shortened. Involvement of endogenous gibberellic acid (GA) can accelerate reproductive formation in conifers. Treatments, such as water stress, root removal, and nitrogen starvation, can build up GAs in plants so that they can substitute involvement of GAs in pines (Taiz and Zeiger, 2006).

After transition to the adult phase, there are two stages in floral evocation: competence and determination (Taiz and Zeiger, 2006). A plant is competent if it is able to flower when given the appropriate internal and external development signals. A bud is determined if it progresses to the next developmental stage even after being removed from its normal context. Plants have different stimuli controlling flower initiation, which could be categorized into three main groups (Meilan, 1997): 1) environmental factors, including photoperiod and temperature; 2) cultural manipulations, such as water stress treatment (Levy and Dean, 1998); 3) plant growth regulator (PGR) treatments, including GA, ethylene, cytokinins, and plant growth retardants. In addition to the factors in these

three main categories, flower initiation might also be affected by plant size or the number of vegetative nodes (Levy and Dean, 1998).

Effects of temperature on flowering

The effects of temperature on flowering include two aspects: effects of low temperature and effects of high temperature. Vernalization is defined as the acquisition or acceleration of the ability of plants to flower by a chilling treatment (Chouard, 1960). Chilling treatment is perceived by stem apices, buds, and leaves (Curtis and Chang, 1930; Wellensiek, 1961). Effective chilling temperatures to induce flowering range from 1ºC to 7 ºC in most plants, but are species dependent. For example, celery (*Apium graveolens* L.) requires a chilling temperature as low as -6 ºC while 18 ºC is low enough for vernalizing the biennial plant stock (*Matthiola incana* L.) (Bernier et al., 1981). Plants can be facultative or obligate in requiring vernalization to induce flowering (Michaels and Amasino, 2000). Biennial and perennial plants generally have obligate vernalization requirements while most annuals are facultative vernalization plants (Bernier et al., 1981). Easter cactus (*Rhipsalidopsis gaertneri* J.), Chinese astilbe (*Astilbe chinensis* Maxim.), and geranium (*Pelargonium hybrid* L'Hér) are vernalization obligate plants (Runkle et al., 1998; Lewis et al., 1998), whereas double meadow-rue (*Thalictrum delavayi* Tourn. 'Hewitt's Double') is a vernalization facultative plant (Huang et al., 1999).

Vernalization suppresses the expression of the floral inhibitor *flowering locus C*, which could inhibit transcription of a positive regulator of flowering: *flowering locus T* (*FT*), at the molecular level in *Arabidopsis thaliana*, thus vernalization encourages the

expresstion of *FT* and results in accelerating plants' competence to flowering (Searle et al., 2006).

Chilling treatment is important in breaking perennial woody plants bud dormancy (Frenguelli et al., 1992). During their annual cycle, most woody plants, both coniferous and dicotyledonous species, have an active growth in spring and summer, and a dormancy phase in autumn and winter, together with the development of dormant buds (Wareing and Philips, 1978). Plants in dormancy as well as the dormant buds need to be exposed to a period of winter chilling to overcome bud dormancy.

If vernalization is involved in flower initiation process, then high temperature is more likely involved in flower opening, which was supported by research on telosma (*Telosma cordata* Merill.), chocolate cosmos [*Cosmos atrosanguineus* (Hook.) Voss, S. obalata,], apricot (*Prunus armeniaca* L.), Chinese wild peach (*Prunus davidiana* L.), and black locust (*Robinia pseudoacacia* L.) (Richard, 1995; Kanellos and Pearson, 2000; Lu et al., 2006). Advanced flowering by higher temperature can be explained by heat-unit concept. Heat units, expressed as growing degree-days (GDD), are frequently used to describe the timing of biological processes including flower opening. The basic equation used is GDD = $[(T_{MAX} + T_{MIN})/2]-T_{BASE}$, where T_{MAX} and T_{MIN} are daily maximum and minimum air temperatures, respectively, and T_{BASE} is the base temperature for plant growth (McMaster and Wilhelm, 1997). Relative events for every developmental stage of the plant only occurs after a specific heat unit requirement is satisfied, so does flowering, thus, the higher the T_{MAX} and T_{BASE}, the shorter the GDD will be, and the earlier the plant will flower.

Effects of photoperiod on flowering

Photoperiod is another important environmental stimulus controlling flowering, which was reported as early as 1920 (Garner and Allard, 1920). Based on plants' photoperiodic responses to flowering, plants are classified as: short day plants (SDP), long-day plants (LDP), and day-neutral plants (DNP). Those that only flower or flower more rapidly when the day length is shorter than a critical duration are SDPs; whereas those that only flower or flower more rapidly, when the day length is longer than a critical duration are LDPs. Those whose flowering is not affected by day length are called DNPs. For example, SDP poinsettia (*Euphorbia pulcherrima* Willd. ex Klotzsch) initiates flower buds when daylength is or less than 12 h. (Joiner and Harrison, 1967). 'Goldsturm' rudbeckia (*Rudbeckia fulgida* L.), flowering only under 14-h photoperiod or with 4-h night interruption, is a LDP (Runkle, 1999). Rockcress (*Arabis sturii* Mottet), flowers under 16-h, 12-h, and 8-h photoperiod, is a DNP (Zhang, 1999). The difference among the three classification is caused by changes in gene expression, led by phytochrome, a cytosolic polypeptide, functioning as a photosensor based on its capacity for reversible interconversion between the red (R)-absorbtion phytochrome (P)r form and the far-red (FR)-absorbing Pfr form upon sequential absorption of R and FR light (Quail et al., 1995).

Effects of water stress on flowering

Of all the resources that plants need to grow and function, water is the most abundant and at the same time the most limiting for agricultural productivity (Taiz and Zeiger, 2006). Water stress (WS), caused by insufficient soil water availability to plants,

plays a substantial role in flowering of some ornamental plants and has an effect on controlling plant growth. The water-plant relation was studied first in arid area (Alvim, 1960). Water stress has been successfully used to promote flowering on tropical and subtropical fruit trees (Southwick and Davenport, 1986; Chaikiattiyos et al., 1994). Modification in plant hormonal balance induced by WS, leading to earlier flower induction, might be the reason for flowering advancement on loquat (*Eriobotrya japonica Lindl.*) (Cuevas et al., 2007). Leaf water potential (LWP), stomatal resistance, transpiration rate, net photosynthesis rate, canopy temperature, canopy minus air temperature, crop water stress index, and visual leaf rolling score have been used to asses water stress and to develop quantitative methods (Toole et al., 1984). Water stress causes reduction in plant growth rate by reducing leaf area controlled by hormonal signals generated in roots (Munns, 2002). From an ecological point of view, flower promoting by WS is an escape strategy adopted by plants, which relies on successful reproduction before the onset of severe stress (Levitt, 1972; Turner, 1988).

Effects of plant growth regulators on flowering

Gibberellic acid (GA) is a PGR produced in the roots and fruits of higher plants. Experimental evidence shows that the application of GAs causes reproductive structures to form in juvenile plants of several conifer families, but they seem to have little effect on floral initiation in woody angiosperms (Taiz and Zeiger, 2006). Gibberellic acid application has also been reported to delay flowering in red and yellow pytaya (*Hylocereus undatus* [Haworth] Britton and Rose; *Selenicereus megalanthus* [K.

Schumann ex Vaupel] Ralf Bauer) (Khainov and Mizrahi, 2006), instead of promoting it. In azalea, GA helps in substituting for chilling and producing uniform flower development (Larson, 1993). Plant growth retardants, most of which act by inhibiting GA biosynthesis, have effects on flowering. For example, chlormequat and daminozide enhanced flowering on bougainvillea (Tang et al., 2007). Paclobutrazol and uniconazole application successfully improved mountain laurel (*Kalmia latifolia* L.) flower bud development (Banko and Stefani, 1995).

Cytokinin treatment to the shoot apex reduced time for the first node to flower on 'San Diego Red' bougainvillea (Tse et al., 1974; Ramina et al., 1979; Sachs et al., 1979). Zeatin (a naturally occurring cytokinin) promoted flowering in apples (*Malus demostica* Borkh.) (Ramirez and Hoad, 1979), and PBA (6-benzylamino-9 -(tetrahydropyran 2-yl)-9H-purine, a synthetic cytokinin) invoked an inflorescence in 4-week-old grape seedlings while flowering normally does not occur until grape plant is 3-5 years of age (Srinivasan and Mullins, 1978). CPPU (N-(2-chloro-4-pyridinyl)-N-phenylurea), a cytokinin-active compound, promoted flowering in red and yellow pitaya (Khaimov and Mizrahi, 2006).

Banana shrub and bougainvillea

Banana shrub (*Michelia skinneriana* Dunn.), an evergreen shrub native to Southern China, belongs to the Magnoliaceae and flowers from May to June (Liu et al., 2002). The flower of banana shrub smells like ripe banana fruits, is cream yellow, and has five petals edged with maroon, multiple yellow stamens and light-green pistils. Like other commercial flowering crops, flowering of banana shrub involves a complex series

of stages, including flower induction, initiation, and development. Spring flowering plants require vernalization and proper daylength to flower (Sung et al., 2006), and banana shrub may have the similar requirements. However, there are no studies investigating the factors that affect flowering in banana shrub.

Bougainvillea (*Bougainvillea spectabilis* Wild.) is a very popular tropical and subtropical evergreen landscape plant with showy, vibrantly colorful petaloid bracts used in most areas with warm climates, including Australia, India, Malaysia, the Mediterranean region, Mexico, South Africa, and many states in the United States (Arizona, California, Florida, Hawaii, and southern Texas). Bougainvillea could be used to decorate fences and arbors with explosions of color in the house corridor, office and play ground. Bougainvillea is also a great vine for large containers to decorate patios and plazas (Sharif Hossain et al., 2007). As the inflorescence is the most valuable aesthetic part in bougainvillea, flowering is of great importance to be studied. Water stress advanced flowering on bougainvillea (Tang et al., 2000). Use of PGRs to promote flowering is easier and more consistent than using water stress. Some studies done on effects of PGRs including chlormequat, ancymidol, dikegulac, and paclobutrazol on growth and flowering of bougainvillea (Kobayashi et al., 2007; Shao et al., 2006; Dierking and Sanderson, 1985; Tang et al., 2006), however, the effective application rates were cultivar dependent. In Shao and Tang's reports, the application rates of plant growth retardants were not clear (Shao et al., 2006; Tang et al., 2006). Besides, few studies investigated the effects of combination of WS and plant growth retardants on flowering of bougainvillea (Kobayashi et al., 2007).

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CHAPTER III

EFFECT OF CHILLING, POST-CHILLING PHOTOPERIOD, AND PLANT GROWTH REGULATORS ON FLOWERING OF BANANA SHRUB (*Michelia skinneriana* Dunn.)

Abstract

Scheduling flowering is a process to create off-season blooming on plants and plants value could be increased if flowering meets holiday markets. The objective of this study was to investigate the effects of chilling and post-chilling photoperiod, tank mix of daminozide and chlormequat and sequent GA application on flowering of banana shrub (*Michelia skinneriana* Dunn.). Experiment 1 was conducted in a polycarbonate-covered quonset greenhouse located at R.R. Foil Plant Science Research Center Mississippi State University (MSU), MS. Chilling treatment was conducted in a cooler at Natchez Trace Greenhouses, Kosciusko, MS, where plants were chilled at 8 °C for 0, 6, 8 or 12 weeks and then moved to the photoperiod regimes of 8, 10, 14 or 16-h daylength on 12 Sept. or 19 Sept. 2008. Results indicate that banana shrub requires at least 8-weeks chilling to break flower bud dormancy. Photoperiod treatments did not affect the number of days to the first flower and the number of flowers/pot. In Experiment 2, plants were treated with a tank mix of daminozide and chlormequat (0/0 ppm, 2,500/1,250 ppm or 5,000/1,500 ppm) and were moved to an 8 °C growth chamber on 3 June 2009 for 4 or 8-week

chilling. Plants treated with 4 week chilling were followed by four foliar applications of 2,500 ppm GA. Results indicate that a hight rate of tank mix of daminozide and chlormequat increased the number of flower buds, however, GA could not substitute for chilling treatment to produce uniform flowering.

Introduction

Banana shrub (*Michelia skinneriana* Dunn.), an evergreen shrub native to Southern China, belongs to the Magnoliaceae and flowers from May to June (Liu et al., 2002). The flower of banana shrub smells like ripe banana fruits, is cream yellow, and has five petals edged with maroon, multiple yellow stamens and light-green pistils. Like many commercial flowering crops, flowering of banana shrub involves a complex series of stages, including flower induction, initiation, and development. Spring flowering plants require vernalization and proper daylength to flower (Sung et al., 2006), and banana shrub may have the similar requirements. However, there are no studies investigating the factors that affect flowering in banana shrub.

Vernalization is defined as the acquisition or acceleration of the ability of plants to flower by a chilling treatment (Chouard, 1960). Typically, when young plants are subject to winter cold temperatures, flowering will be induced. Chilling is perceived by stem apices, buds, and leaves (Curtis and Chang, 1930; Wellensiek, 1961). Effective chilling temperatures to induce flowering vary from 1 to 7ºC in most plants, but are species dependent. For example, celery (*Apium graveolens* L.) requires a chilling temperature as low as -6ºC while 18ºC is low enough for vernalizing the biennial plant

stock (*Matthiola incana* L.) (Bernier et al., 1981). Plants can be facultative or obligate in their requirement for vernalization to induce flowering (Michaels and Amasino, 2000). Biennial and perennial plants generally have obligate vernalization requirements while most annuals are facultative vernalization plants (Bernier et al., 1981). Cactus (*Rhipsalidopsis gaertneri* J.), Chinese astilbe (*Astilbe chinensis* Maxim.), and geranium (*Pelargonium hybrid* L'Hér) are obligate vernalization plants (Runkle et al., 1998; Lewis et al., 1998), whereas double meadow-rue (*Thalictrum delavayi* Tourn. 'Hewitt's Double') is a facultative vernalization plant (Huang et al., 1999).

Chilling also plays a substantial role in breaking woody plants' bud dormancy (Frenguelli et al., 1992). Most woody plants, both coniferous and deciduous dicotyledonous species, show, during their annual cycle, an active growth in spring and summer, and a dormancy phase in autumn and winter, together with the development of resting buds (Wareing and Philips, 1978). They need to be exposed to a period of winter chilling to overcome the dormancy of their buds.

Photoperiod is another important environmental stimulus controlling flowering, which was reported as early as 1920 (Garner and Allard, 1920). Based on plants' photoperiodic responses to flowering, plants were classified as: short-day plants (SDP), long-day plants (LDP), and day-neutral plants (DNP). Those that only flower or flower more rapidly when the day length is shorter than a critical duration are SDPs; whereas those that only flower or flower more rapidly, when the day length is longer than a critical duration are LDPs. Those whose flowering is not affected by day length are called DNPs. For example poinsettia (*Euphorbia pulcherrima* Willd. Ex Klotzsch) initiates

flower buds when daylength is or less than 12 h (Joiner and Harrison, 1967). ‘Goldsturm’ rudbeckia (*Rudbeckia fulgida* L.), flowered only under a 14-h photoperiod or with 4-h night interruption and is a LDP (Runkle, 1999). Rock cress (*Arabis sturii* Mottet), flowering under 16-h, 12-h, or 8-h photoperiod is a DNP (Zhang, 1999).

Gibberellic acid (GA) is a PGR produced in the roots and fruits of higher plants. In azalea (*Rhododendron* L.), GA helped in producing uniform flowering (Larson, 1993). Plant growth retardants, most of which act by inhibiting GA biosynthesis, were reported to promote flowering. For example, chlormequat and daminozide enhanced flowering on bougainvillea (*Bougainvillea spectabilis* Wild) (Tang et al., 2007). Daminozide increased the number of flowers in magnolia (Matysiak, 2002), while paclobutrazol and uniconazole successfully improved mountain laurel (*Kalmia latifolia* L.) flower bud development (Banko and Stefani, 1995).

Understanding the effects of chilling and photoperiod would be a fundamental advance to determine the mechanisms controlling flowering in a subtropical species. In investigating the effects of a tank mix of daminozide and chlormequat, and sequent GA on flowering of banana shrub helps producing marketable potted plants for buyers. This knowledge can be used to produce off-season flowering banana shrub with flowers for special days such as Thanksgiving, Christmas, Valentines’ Day, and Easter. Most research in flower forcing has focused on a limited number of herbaceous species or woody plants that have been cultivated for many decades, such as poinsettia and azalea. However, relatively few studies have considered development of practical methods for controlling flowering in woody angiosperm species (Henriod et al., 2000). The objectives

of this study were to investigate the effects of chilling and photoperiod on flowering of banana shrub, the feasibility of applying tank mix of a daminozide and chlormequat to increase flower numbers, and sequent GA to substitute for chilling.

Materials and Methods

Experiment 1: Effect of chilling and post-chilling photoperiod on flowering of banana shrub

Plant materials

Fifteen cm tall rooted vegetative banana shrub cuttings (2 cuttings per cell) were obtained from a commercial nursery (van der Giessen Nursery, Semmes, AL). Two cuttings were planted into 15-cm azalea pots in Sunshine Mix1 (SunGro Hort., Bellevue, WA) on 20 Mar. 2008. Plants were grown in a polycarbonate-covered quonset greenhouse located at R.R. Foil Plant Science Research Center, Mississippi State University, Mississippi State, MS (33° 27' 1" N / 88° 49' 5" W) under full sun and natural daylength for three months before the treatments. Plants were fertilized at each watering with 200 mg N· L^{-1}20N−4P−6K (Peters® 20−10−20, Scotts Professional, Allentown, PA). Before chilling treatment, plants shoot height ranged from 17-22cm and 19-26 cm after chilling treatment. Plants were well branched with profuse mature leaves.

Chilling treatment

A total of 96 pots were randomly selected for the experiment and divided into Block A and B. Within each block, plants were randomly divided into 4 groups of 12 plants. One group of plants in Block A was randomly selected and moved to a cooler at Natchez Trace Greenhouses (Kosciusko, MS) for chilling treatment on 16 June 2008. Two other groups in Block A were moved to the cooler on 14 July and 28 July, respectively. The fourth group of plants in Block A remained in the MSU greenhouse. Plants in Block B were treated in a similar manner and three groups of plants were moved to Natchez Trace Greenhouses on 23 June, 21 July, and 4 August, respectively. Block A plants were all moved from the cooler to the greenhouse on 12 September to receive photoperiod treatments and Block B on 19 September, so that there was one group of plants within each block receiving 12, 8, 6, and 0 weeks of chilling. Plants without chilling were grown continuously in the greenhouse.

The environmental conditions in the cooler at Natchez Trace Greenhouses was set at 8 ºC (varied from 5.7 ºC to 11.3 ºC), and 60% relative humidity (varied from 51.1% to 84.3%), which were monitored by a Watchdog data logger 450 (Spectrum Technologies Inc., Plainfield, IL), as well as 12 h photoperiod. Two 40-w fluorescent lights were evenly placed 1 m above the 72 chilled plants. Sufficient tap water was provided to each pot once a week.

Post-chilling photoperiod treatment

Plants were moved back to the greenhouse where they were before chilling treatment. Eight and 10-h photoperiod were created by covering plants with black cloth from 17:00 h to 09:00 h and from 17:00 h to 07:00 h, respectively. Fourteen-hour was created by turning on incandescent bulbs from 17:00 h to 21:00 h in addition to natural day light from 07:00 h to 17:00 h while 16-h photoperiod was created by turning on incandescent bulbs from 17:00 h to 23:00 h in additional to natural day light from 07:00 h to 17:00 h.

Data collection

On 13 Sept. 2008, flower buds from each treatment were collected and fixed in formalin before dehydration. Bud samples were treated in a series of the following porocedures: 1) 0.1 M phosphate buffer (pH = 7.2) for 10 min and repeated for six times, 2) 2% OsO_4 - 0.1 M phosphate buffer until buds color changed to black, 3) distilled water for 10 min and repeated 6 times 4) 35% EtOH for 10-15 min twice, 5) 50% EtOH for 10-15 min twice, 6) 95% EtOH 10-15 min twice 7) 100% EtOH for 15 min four times, 8) Dried in a polaron E-300 critical point dryer (Polaron Equipment Ltd, Holywell Industrial, Estate, Watford) until dry. The last step was to examine buds via Scanning Electron Microscopy (JOEL 6500F field emission (FE)-SEM, Oxford Instruments, England) to observe bud development and identify stage of floral development. Data were collected on the number of flowers and days to first flower (DTF) after chilling. The experiment was arbitrarily terminated 80 days after chilling.

Experimental design and analysis

The experiment was conducted as a split-plot design with post-chilling photoperiod (8, 10, 14, and 16 h) as the main plot factor and chilling (0, 6, 8, and 12 weeks) as the sub plot factor with three replications. The entire experiment was repeated over time (Block A and B). Statistical analyses were conducted using SAS System (V9.12) statistical package (SAS Institute Inc., Cary, NC). Tests for significance of chilling and photoperiod effects were executed by ANOVA and means were separated by LSD ($p < 0.05$).

Experiment 2: Effect of tank mix of daminozide and chlormequat and sequent GA on flowering of banana shrub

Plant materials

Eighteen plants were randomly selected from plants treated with 8-week chilling treatment in Experiment 1, pruned to 25 cm on 12 Jan. 2009 and grown under long day (LD) in the same greenhouse as in Experiment 1 until 24 Feb. 2009 for 6 weeks. Long day conditions were created by turning on a 20-W incandescent bulb from 17:00 h to 21:00 h in addition to natural day light from 07:00 h to 17:00 h. Same cultured practices were applied to the plants as in Experiment 1.

Plant growth retardant application

Eighteen plants were randomly and divided into three groups of six plants and moved to natural daylength on 24 Feb. 2009. The plants were treated with 0/0, 2,500/1,250, or 5,000/1,500 ppm daminozide and chlormequat (Dazide™, Fine Americas Inc., Walnut Creek, CA; Cycocel®, OHP, Inc., Mainland, PA). Ten ml treatment solution was foliar sprayed on banana shrub with a trigger sprayer on 6 Mar. and again on 13 Mar. 2009. Plants were moved to 10-h short day (SD) on 31 Mar. until 3 June. The 10-h SD was created by covering plants with black cloth from 17:00 h to 7:00 h.

Chilling treatment

On 3 June 2009, all the plants were transferred into a growth chamber (CMP3244, Conviron, Canada). The environmental conditions in the growth chamber were set at 8 ºC and 80% relative humidity as well as 10-h SD. Lighting was provided by 4 incandescent bulbs and 4 fluorescence lamps. Tap water was applied to plants as necessary. Three out of 6 plants in each PGR treatment were treated with 4-week chilling treatment and moved to MSU Dorman greenhouse where the temperature was set at 28/24 ºC (day/night) on 3 July. The other three plants in each PGR treatment remained in the growth chamber for a total of 8 weeks and then moved to the MSU Dorman greenhouse on 29 July 2009.

GA application

Plants treated with only 4-week chilling treatment were foliar sprayed with 2,500 ppm GA (ProGibb®, Valent U.S.A. Corporation, Walnut Creek, CA) on 8, 15, 22, and 29

July, respectively. Each time, 30 ml GA solution was sprayed on each plant with a CO_2 pressure backpack sprayer (nexAir, Memphis, TN).

Data collection

The total number of flower buds and the number of flowers were recorded. To count the total number of flower buds, all the buds on plants were collected into a zip-lock bag and examined under a dissecting microscope on 25 Sept. 2009. Only buds with visible petals, pistils, and stamens were counted as a flower bud. The number of flowers was counted based on the number of senesced flowers on the plants.

Experiment design and data analysis

It was a split-plot design with chilling-treatment (8-week and 4-week) as the main plot factor and PGR (tank mix of damonizide and chlormequat (2,500/1,250 ppm, 5,000/1,500 ppm or control) as the sub plot factor with three replicates. Statistical analyses were conducted using SAS System (V9.12) statistical packages (SAS Institute Inc., Cary, NC). Tests for significance of PGR and duration of chilling treatment effects were executed by ANOVA and means were separated by LSD ($p = 0.05$).

Results and Discussion

Experiment 1

Scanning electron microscopic (SEM) pictures taken immediately after chilling treatment confirmed flower initiation in each chilling treatment (Figure 3.1 a and b),

which were distinguished from vegetative buds (Figure 3.1 c and d). Bracts, petals, and stigmas were easily identified from peripheral to central area. Percentage of flowering varied when plants were treated with different combination of chilling and photoperiod (Table 3.1). Banana shrub flowers opened under 10, 14, or 16-h photoperiod regimes after 8 or 12- week chilling. Plants without chilling or with 6- week chilling treatment did not flower (Table 3.1). Plants did not flower under 8-h photoperiods regardless of chilling treatment. One half of the plants flowered in treatment of 10-h photoperiod after 8-week chilling. Thirty-three percent of plants flowered in treatments of 14-h photoperiod after 8-week chilling or in the treatment of 16-h photoperiod after 12-week chilling. Seventeen percent of plants flowered in the treatment of 16-h photoperiod after 8-week chilling. There were no significant interaction effect between chilling and photoperiod for days to first flower (DTF) or the average number of flowers/pot (Table 3.2). The data means for each chilling and post-chilling treatment are listed in Table 3.3 and 3.4.

Specific temperature and duration of chilling to initiate flowers or to break flower bud dormancy varies between species (Moncur, 1992; Moncur and Hasan, 1994; Day et al., 1994; Sytsema and Ruesink, 1996). The least chilling duration to initiate flowering in Shasta daisy (*Leucanthemum* x *superbum* D.H. Kent 'Snowcap' D.H. Kent) is 6 weeks, Easter cactus (*Rhipsalidopsis gaetneri* (Regel) Vaupel 'Crimson Giant') 8 weeks, and Chinese astilbe (*Astilbe chinensis* (Maxim.) Franch. and Sav.) 15 weeks (Runkle et al., 1998; Lewis et al., 1998). To break bud dormancy, 50 h for some cultivars of quince (*Cydonia oblonga* Mill.) and almond (*Prunus dulcis* [Mill.] A.Webb) while more than 2,000 h is needed for some grape and apple cultivars when chilling requirement was

measured by the number of hours at low temperature (Westwood, 1978; Faust, 1989). In this study, flower initiation occurred in all plants with or without chilling as evidenced by SEM pictures of flower buds in all treatments. At least 8 weeks chilling was needed for banana shrub to break flower bud dormancy at 8ºC to produce flowers.

Studies on pōhutukawa (*Metrosideros excels* Gaertn.) (Henriod et al., 2000) showed that plants exposed to longer durations of cold temperatures flowered earlier than those exposed to shorter durations of cold temperatures, which was different from banana shrub in this experiment. Longer chilling duration of 12 weeks slightly delayed plant flowering compared to 8 weeks.

When applied at the right time, photoperiod may have an effect on both flower initiation and flower development. Twelve-hour or less photoperiod is necessary for poinsettia flower bud initiation (Joiner and Harrison, 1967). The result that all the post-chilling photoperiod except 8-h produced flowers suggests that post-chilling photoperiod influenced flower development in banana shrub.

Short day initiates flower buds in pōhutukawa (Henriod et al., 2000), azalea (Larson, 1993), and Japanese andromeda (*Pieris japonica* D.Don ex G.Don ‘Debutante’, Sytsema and Ruesink, 1996), which are all spring flowering plants. Banana shrub may have the same photoperiod requirement for flower initiation, thus, the small number of flowers/pot in banana shrub was probably due to SD is more productive of flower bud initation in banana shrub than LD.

Experiment 2

There was interaction between tank mix of daminozide and chlormequat and chilling treatment on the total number of flower buds/pot and the number of flowers/pot (Table 3.5). Within plants treated with 8-week chilling treatment, PGR level significantly affected the total number of flower buds and the number of flowers (Table 3.6). There were more flowers and more flower buds produced on plants treated with daminozide and chlormequat (5,000/1,500 ppm) than flowers on plants treated with daminozide and chlormequat (2,500/1,250 ppm) and control. However, no difference on the number of flowers and the number of flower buds was observed between daminozide/chlormequat (2,500/1,250 ppm) and control. Within plants treated with 4-week chilling treatment, PGR level did not significantly affected the total number of flower buds and the number of flowers, means was listed in Table 3.6.

Flowers on plants receiving post-chilling GA applications after 4-week chilling were morphologically different from the flowers on plants treated with only 8-week chilling treatment (Figure 3.2a). Former ones either open with linear petals (Figure 3.2b) or did not fully open before withering away (Figure 3.2c).

Plant growth retardants are applied to enhance flowering on many woody plants. For example, dikegulac increased the number of inflorescences on ‘Barbara Karst’ and ‘Rainbow Gold’ bougainvillea (Norcini et al., 1992). Daminozide successfully increased the number of flowers on magnolia and azalea (Matysiak, 2002; Larson, 1993). In this experiment, high rate of tank mix of daminozide/chlormequat (5,000/1,500 ppm) increased the total number of flower buds/pot and the number of flowers/pot in plants

chilled for 8 weeks. A tank mix of daminozide/chlormequat (5,000/1,500 ppm) has great potential to increase the number of flowers on banana shrub although the mechanism needs investigation.

Eight-week chilling at 8 ºC broke bud dormancy in banana shrub in Experiment1, which was confirmed in Experiment 2 by the number of flowers/pot. Although 4-week chilling followed by 4 applications of GA at 2,500 ppm was able to break flower bud dormancy to some degree, which was supported by the flowers observed on corresponding plants, it could not substitute for 8-week chilling to produce mature flower buds. Gibberellic acid was commercially applied to produce uniform flowering on azaleas (Larson, 1993) but it did not show a similar effect on banana shrub in this experiment. A low percentage of flowering and irregular flowers were observed on plants treated with GA. These two negative aspects of flowering make it undesirable to apply post-chilling GA on plants if the chilling requirements were not adequate for banana shrub.

The time of full bloom depends on two factors: the chilling requirements and growing degree hours celsius (GDH ºC) required after endo-dormancy for reaching full bloom (Raseira, 1986; Aslamarz et al., 2009). In this experiment, the fact that the total number of flower buds/pot was much more than the number of flowers/pot suggests a non full-bloom on banana shrub. As indicated in Raseira and Aslamarz's paper, more GDH ºC can be obtained by increasing air temperature can be applied to produce uniform bloom. Non-uniform developmental stage of flower buds might be another reason for

non-uniformity of bloom. It was not clear that either GDH ºC or developmental stages or both aspects contribute to this non-uniformity of bloom in this experiment.

In conclusion, flowering in banana shrub requires a minimum of eight weeks of chilling to break flower bud dormancy in order to produce flowers. A tank mix of daminozide and chlormequat (5,000/1,500 ppm) could be potentially applied on banana shrub to increase the number of flower buds. Post-chilling GA spray is not desirable to substitute part of chilling requirements to create uniform blooming on banana shrub. Banana shrub has the potential to be considered as a potted flowering plant due to its fragrance, attractive flowers, and evergreen growth habit for holiday market.

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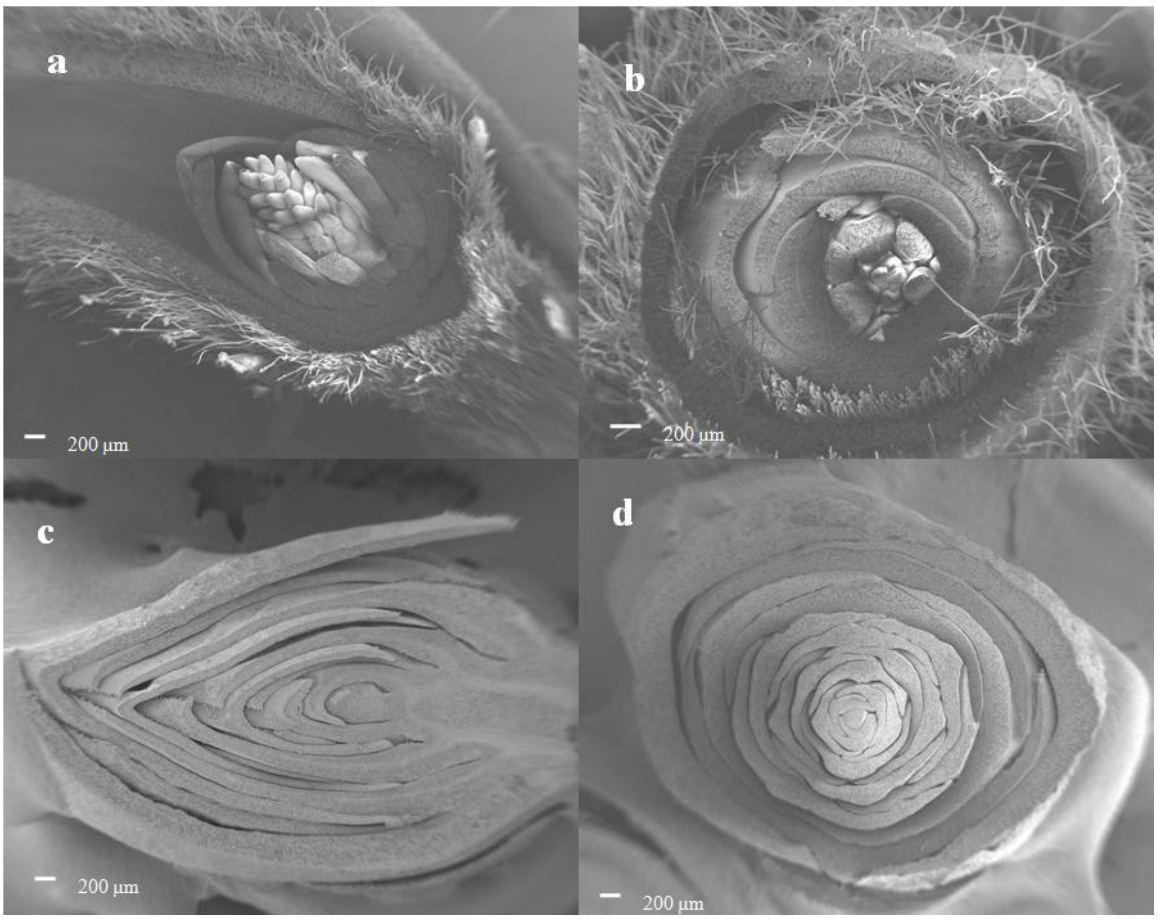

Figure 3.1. Scanning electron micrographs of banana shrub (*Michelia skinneriana*).flower buds (a-longitudinal section; b-transverse section) and vegetative buds (c-longitudinal section; d-transverse section) showing the differences between vegetative and reproductive stages of bud development.

Figure 3.2. Photographs showing (a) Normal flower of banana shrub (*Michelia skineriana*); (b) and (c) abnormal flowers from plants treated with post-chilling GA foliar application at 2,500 ppm. Photographs of a and b were taken on 30 Aug. 2009 and, c was taken on 7 Aug.2009.

Table 3.1. The percentage of banana shrub (*Michelia skinneriana*) flowering in each of the sixteen combinations of chilling treatments at 8 ºC and post-chilling photoperiod treatments.

	Percentage of flowering plants			
Photperiod	Chilling treatment			
	0-week	6-week	8-week	12-week
8 h	0	0	0	0
10 h	0	0	50%	0
14 h	0	0	33%	0
16 h	0	0	17%	33%

Table 3.2. Analysis of variance of effect of chilling and post-chilling photoperiod treatments on banana shrub (*Michelia skinneriana*) flowering with regard to days to the first flower (DTF[z]) and the number of flowers.

Treatment	*P* value	
	DTF	The number of flowers
Chilling	0.16	0.23
Photoperiod	0.15	0.75
Chilling*photoperiod	NS[y]	0.08

[z] Days between the date of transferring to the greenhouse after chilling treatment and the days to first flower.
[y] Missing data caused interaction to lose.

Table 3.3. Effect of chilling treatment on days to first flower (DTF[z]) and the number of flowers/plant after 96 pots of banana shrub (*Michelia skinneriana*) were chilled at 8 ºC for different durations.

Chilling duration (week)	DTF	The number of flowers
0	-	0
6	-	0
8	36.7 NS[y]	0.6 NS
12	46.5 NS	0.2 NS

[z] Days between the date of transferring to the greenhouse after chilling treatment and the date to the first flower.
[y] NS= no significant difference

Table 3.4. Effect of post-chilling photoperiod treatment on days to the first flower (DTF[z]) and the number of flowers on banana shrub (*Michelia skinneriana*).

Photoperiod (h)	DTF	The number of flowers
8	-	0
10	43.0 NS[y]	0.5 NS
14	46.5 NS	0.3 NS
16	46.3 NS	0.4 NS

[z] Days between the date of transferring to the greenhouse after chilling treatment and the date to the first flower.
[y] NS = no significant difference.

Table 3.5. Analysis of variance of the effect of chilling duration and daminozide/chlormequat (PGR) treatments on the total number of flower buds/pot and the number of flowers/pot of banana shrub (*Michelia skinneriana*).

Treatment	*P* value	
	The total number of flower buds/pot	The number of flowers/pot
PGR	0.04	0.004
Chilling	0.01	0.01
Chilling*PGR	0.02	0.005

Table 3.6. Effect of pre-chilling tank mix of daminozide/chlormequat on the number of flowers/pot and the total number of flower buds/pot on banana shrub (*Michelia skinneriana*) in 2008. Data were collected on the total of 18 plants on 25 Sept. 2009.

Chilling duration	PGR treatment (ppm)	The number of Flowers /pot	The total number of flower buds/pot
	Control	0 b	10.8 b
8-week	Daminozide/chlormequat (2,500/1,250)	0.3 b	1.7 b
	Daminozide/chlormequat (5,000/1,500)	4.7 a[y]	37 a
4-week[z]	Control	1.7 NS[x]	4.0 NS
	Daminozide/chlormequat (2,500/1,250)	0 NS	3.7 NS
	Daminozide/chlormequat (5,000/1,500)	0 NS	1.0 NS

[z] Four-week chilling treatment was followed by 4 times of 2,500 ppm GA spray on 8 July, 15 July, 22 July, and 29 July 2009, respectively.
[y] Means followed by the same letter within each column were not significantly different (LSD, $p<0.05$, n=3).
[x] NS means no significant difference.

CHAPTER IV

EFFECTS OF WATER STRESS AND PLANT GROWTH RETARDANTS ON FLOWERING AND GROWTH OF BOUGAINVILLEA (*Bougainvillea* Wild.)

Abstract

Bougainvillea (*Bougainvillea spectabilis* Wild.) is a very popular evergreen landscape plant in tropical and subtropical areas but its vigorous growth habit requires a lot of pruning when being used as a potted plant. Water stress and some plant growth retardants (PGR) have been reported to be effective in enhancing flowering and controlling bougainvillea, but there were little research conducted on the effective rates of daminozide, chlormequat, ethephon and a combination of water stress and PGRs on flowering and growth. The objective of this study was to investigate the effect of daminozide, chlormequat, ethephon, water stress either alone or in the combination of water stress and PGRs on flowering and growth in 'Raspberry Ice' bougainvillea as a potted plant. Two experiments were conducted at Mississippi State University Greenhouse in summer 2008 and 2009. In Experiment 1, water stress, three rates of PGRs (daminozide, chlormequat, ethephone) and water stress combined with each PGR and three rates of tank mix of daminozide and chlormequat were applied. All the treatments except 600 ppm chlormequat and tank mix of daminozide/chlormequat (2,500/2,500 ppm) had a greater percentage of flowering pots compared to control. In Experiment 2, 3 levels

of water stress, daminozide, chlormequat, and tank mix of daminozide/chlormequat were applied. Results indicated that none of the treatments affected days to first flower and all the treatments except 600 ppm chlormequat and tank mix of daminozide and chlormequat (2,500/2,500 ppm) had a greater percentage of flowering pots compared to control.

Introduction

Bougainvillea (*Bougainvillea spectabilis* Wild.) is a very popular tropical and subtropical evergreen landscape plant with showy, vibrantly colorful petaloid bracts and used in many areas with warm climates, including Australia, India, Malaysia, the Mediterranean region, Mexico, South Africa, and Arizona, California, Florida, Hawaii, and southern Texas in the United States. Bougainvillea can be used to decorate fences and arbors with explosions of color in corridor, office and play ground. Bougainvillea is also a great vine for large containers to decorate patios and plazas (Sharif Hossain, 2007). Flowers are the most important aesthetic part for bougainvillea. As a SDP (Chen et al., 1979), bougainvillea blooms in fall on apical panicles formed on current year wood.

Water stress (WS), caused by insufficient soil moisture availability to plants, was reported to play a substantial role in plant flowering on some ornamental plants and have an effect on controlling plant growth. The water-plant relation was studied first in arid areas (Alvim, 1960). Water stress has been successfully used to promote flowering in tropical and subtropical fruit trees (Southwick and Davenport, 1986; Chaikiattiyos et al., 1994). Modification in plant hormonal balance by WS, leading to earlier flower induction, might be the reason for flowering advancement on loquat (*Eriobotrya japonica Lindl.*)

(Cuevas et al., 2007). Leaf water potential (LWP), stomatal resistance, transpiration rate, net photosynthesis rate, canopy temperature, canopy minus air temperature, crop water stress index, and visual leaf rolling score have been used to asses water stress and to develop quantitative methods (Toole et al., 1984). Water stress reduced plant growth rate by reducing photosynthetic leaf areas controlled by hormonal signals generated in roots (Munns, 2002). From an ecological point of view, flower promoting by WS is an escape strategy adopted by plant, which relies on successful reproduction before the onset of severe stress (Levitt, 1972; Turner, 1988).

Myriad processes including flowering and plant growth, which are important to horticultural crops, are regulated by plant growth regulators (PGRs) (Malladi and Burns, 2007). Use of PGRs to promote flowering is easier and more consistent than using water stress. Chlormequat delayed flowering when plants were treated under SD (Tang et al., 2006). Dikegulac at 400 to 1,600 ppm could enhanced flowering on ‘Barbara Karst’ and ‘Rainbow Gold’ bougainvillea in both late spring to early summer and late summer to mid-fall despite the reduced bracteole size in the late spring to early summer when temperature is high (Norcini et al., 1993).

Because of the vigorous growth habit, bougainvillea needs a lot of labor-intensive pruning when used as a potted plant. Plant growth regulators could be used as alternatives to frequent pruning on bougainvillea to reduce labor cost. Daminozide, paclobutrazol, chlormequat and dikegulac have been reported to effectively control plant height in bougainvillea although the effectiveness was cultivar dependent (Kobayashi et al., 2007). Most growth retardants act by inhibiting gibberellin (GA) biosynthesis. Four different

types of inhibitors are known: (a) Onium compounds, such as chlormequat, blocks the cyclases copalyl-diphosphate synthase and ent-kaurene synthase involved in the early steps of GA metabolism; (b) Structural mimics of 2-oxoglutaric acid, like daminozide, inhibits GA by blocking particularly 3ß-hydroxylation, thereby inhibiting the formation of highly active GAs from inactive precursors; (c) Compounds with an N-containing heterocycle, e.g. paclobutrazol and uniconazole-P. These retardants block cytochrome P450-dependent monooxygenases, thereby inhibiting oxidation of ent-kaurene into ent-kaurenoic acid; (d) 16, 17-Dihydro-GA5 and related structures act most likely by mimicking the GA precursor substrate of the same dioxygenases (Rademacher, 2000).

Effective rates on flowering enhancement and growth control are influenced by cultivar, PGR type, and environmental conditions such as temperature and humidity (Kobayashi et al., 2007). Water stress was successfully applied to induce flowering and control growth of bougainvillea, however, there was no research conducted on examining the effects of combination of WS and plant growth retardants. It is important to fill the niche to select proper plant growth retardants for greenhouse potted plant production on bougainvillea and to investigate the effects of the combination of WS and plant growth retardants on flowering and growth of bougainvillea. The objective of the study was to examine the effects of daminozide, chlormequat, ethephon, WS either alone or in combination on flowering and growth of ‘Raspberry Ice’ bougainvillea.

Materials and methods

Experiment 1: Effects of daminozide, chlormequat, ethephon, and water stress on flowering and growth of bougainvillea

Plant materials

One hundred and eighty four 10-cm tall rooted 'Raspberry Ice' bougainvillea cuttings were obtained from Vista Farms S.E. (Vista Farms S.E, Juana Diaz, Puerto Rico). One cutting was potted into 15-cm azalea pot with Sunshine Mix1 (SunGro Hort., Bellevue, WA) on 16 Jan. 2008 and grown in Mississippi State University Greenhouse. Plants were fertilized at each watering at 200 mg $N \cdot L^{-1}$ 20N−4P−6 K (Peters® 20−10−20, Scotts Professional, Allentown, PA). All plants were pruned to approximately 15 cm tall on 9 May 2008.

Treatments application

The experiment was started on 13 May and terminated on 8 Aug. 2008. There were 23 treatments including control, WS, chlormequat (Cycocel®, OHP, Inc., Mainland, PA) at 600, 1,200, and 1,800 ppm, daminozide (Dazide™, Fine Americas Inc., Walnut Creek, CA) at 1,250, 2,500, and 3,750 ppm, ethephon (Florel®, Southern Agricultural Insecticides, Inc., Boone, NC) at 500, 1,000, and 1,500 ppm, combinations of WS and each PGR treatment, and combination with WS and tank mix of daminozide/chlormequat (800/1,000, 1,250/1,250, and 2,500/2,500 ppm). Water stress was induced in the plants by withholding water from 13 May to 24 May until mild wilt occurred. Supplementary

watering started on 24 May by applying 50 ml water per pot every other day from 24 May to 6 June. For treatments of combination of PGR and WS, PGRs were applied on 6 June and again on 20 June. Plants treated with chlormequat and ethephon alone were foliar sprayed with 12.5 ml PGR solution with surfactant (Spreader sticker, Voluntary Purchasing Group, Inc., Bonham, TX) using a trigger sprayer. Daminozide was applied by drenching 100 ml PGR solution per pot. Substrates in pot were kept relatively dry before drenching to minimize leaching from the pot. Treatments with PGR alone were applied on 13 May and 26 May. The average temperature in the greenhouse was 35 °C/25 °C (day/night).

Data collection

The number of inflorescences/plant (bracteole >2 cm), were taken weekly, and plant height was measured every other week. Also, the number of structural branches/plant (length>2.5 cm long) were counted before and 4 weeks after the experiment started.

Experimental design and data analysis

It was a randomized complete design with 23 treatments including control, WS, 3 levels of chlormequat (600, 1,200, or 1,800 ppm), 3 levels of daminozide (1,250, 2,500, or 3,750 ppm), 3 levels of ethephon (500, 1,000, or 1,500 ppm) and combination of WS and each PGR treatment, and 3 levels of combination of WS and tank mix of daminozide/chlormequat (800/1,000, 1,250/1,250, or 2,500/2,500 ppm). Each treatment

had five replicates. Statistical analyses were conducted using SAS System (V9.12) statistical packages (SAS Institute Inc., Cary, NC). Tests for significance of PGR and WS treatment effects were executed by ANOVA and data means were separated by LSD (p=0.05).

Experiment 2: Effect of three levels of daminozide, chlormequat, and water stress on flowering of bougainvillea

Plant materials

One hundred and ninety five 12-cm tall 'Raspberry Ice' bougainvillea cuttings were obtained from the same commercial nursery as in Experiment 1 on 1 April 2009 and three cutting were potted per 20-cm azalea pots by Sunshine Mix1 on the same day. Cultural practices were similar to Experiment 1. All the plants were moved to Mississippi State University Dorman greenhouse on 3 June and pruned to 15 cm in height on 5 June.

Treatments application

There were thirteen treatments including three levels of WS (2-week, 3-week, and 4-week), three levels of daminozide (1,250, 2,500, or 3,750 ppm), three levels of chlormequat (Citadel®, Fine Americas Inc., Walnut Creek, CA; 300, 600, or 900 ppm) and tank mix of daminozide/chlormequat (800/1,000, 1,250/1,250, or 2,500/1,500 ppm) in this experiment. Water was withheld from WS plants from 8 June to 17 June until mild wilting occurred. One hundred and fifty ml tap water was provided to each WS plants

from 18 June. Plants treated with two-week, three-week, and four-week WS were well watered starting from 2 July, 8 July, and 15 July, respectively. Saucers were placed under pots treated with WS to ensure the 150 ml water was fully absorbed by plants. Plant growth retardants treatments combinations were foliar sprayed with a trigger sprayer at 20 ml PGR solution/pot on 8 June. Control plants were not subject to PGR or WS treatment. The experiment was terminated on 20 Aug. 2009.

Data collection

Days to the first flower and the number of flowers/pot were recorded when bloomed. The number of branches with flowers/pot and the total number of branches were counted on 1 July 2009. Shoot heights were measured on 15 July.

Soil moisture of plants under three levels of WS and control were monitored by placing one watermark soil moisture sensor (Spectrum Technologies Inc., Plainfield, IL) in one pot of each treatment. A sensor was placed in the center of each selected pot from each treatment where the sensor was inserted 7 cm in depth from the soil surface. Readings were taken every half an hour by Watchdog data logger 450 (Spectrum Technologies Inc., Plainfield, IL) and exported with Spec 7 Pro software (Spectrum Technologies Inc., Plainfield, IL). Midday leaf water potential (LWP) was defined as the LWP between 12:30 h to 13:30 h in this experiment and was measured weekly on three matured, fully expanded leaves from each pot treated with WS and control with a portable plant water status console (Soilmoisture Equipment Corp., Santa Barbara, CA) according to Turner (1988). Midday canopy temperature was defined as the canopy

temperature between 12:30 h to 13:30 h and was measured on three leaves from each pot treated 3-week, 4-week WS and control with an infrared temperature gun (Omega Engineering Inc., Stamford, CT) on 8 July and 15 July 2009 before plants were well-watered.

Experimental design and data analysis

It was a randomized complete design with 13 treatments including control, three levels of WS (2-week, 3-week, or 4-week), three levels of chlormequat (300, 600, or 900 ppm), three levels of daminozide (1,250, 2,500, or 3,750 ppm), and three levels of combination of WS and tank mix of daminozide/chlormequat (800/1,000, 1,250/1,250, or 2,500/1,500 ppm). Each treatment had five replicates. Statistical analyses were conducted using SAS System (V9.12) statistical packages (SAS Institute Inc., Cary, NC). Tests for significance of PGR and WS treatment effects were executed by ANOVA and data means were separated by LSD ($p = 0.05$).

Results

The midday average LWPs on control plants were greater than LWP on WS plants before well-watering. Leaf water potential on control plants ranged from -0.78 to -1.2 MPa and LWP on WS plants before well-watering ranged from -1.38 to -1.9 MPa (Figure 4.1). Leaf water potential for each WS treatment was approximately 0.6 MPa less than the control (Figure 4.1). On 8 July, one week after plants treated with 2-week WS were well watered, LWP increased from -1.8 to -0.7 MPa, which was not different from

control. Leaf water potential on plants treated with 3-week or 4-week WS were still around -1.7 MPa. On 10 July, 2 days after well-watering 3-week WS plants, LWP was -1.19 MPa and was not different from control plants. On 17 July, two days after well-watering 4-week WS plants, LWP was -0.9 MPa and was not different from control plants. Average difference between canopy and air temperature (DCT) and average LWP are two parameters to indicate WS levels. There is a negative correlation between DCT and LWP (Figure 4.2b). In Figure 4.2a, DCT on plants treated with 3-week and 4-week WS was 15. 8 ºC and 11 ºC, both of which were significantly larger than from the DCT on control plants 5.2 ºC.

All the treatments except 600 ppm chlormequat and tank mix of daminozide/chlormequat (2,500/2,500 ppm) had greater percentage of flowering pots compared to the control (Figure 4.3). Water stress had 40-60% flowering pots, daminozide had 40-80% flowering pots, and 1,250 ppm daminozide and tank mix of daminozide and chlormequat (800/1,000 ppm) had 80% flowering pots.

There was no difference among PGR treatments (Table 4.1 and 4.2), therefore the 3 levels of each PGR treatment were combined and analyzed as one treatment (Table 4.3 and 4.4), to investigate the effect of PGR or WS instead of PGR rates or WS durations. Treatments did not affect DTF or percentage of branches with flowers (Table 4.2 and 4.4).

Of all the treatments applied in Experiment 1 and 2, only WS, combination of WS/chlormequat, WS/tank mix of daminozide and chlormequat in Experiment 1 and daminozide in Experiment 2 produced more flowers/pot than the control (Table 4.3 and 4.4). Water stress in Experiment 1 produced 7 more flowers/pot than the control.

Daminozide applied as substrate drench did not have any effect on flowering in Experiment 1 (Table 4.3), however, daminozide applied as foliar spray in Experiment 2 produced more flowers/pot than all the other treatments and produced 5 more flowers/pot than the control (Table 4.4). Application of tank mix of daminozide/chlormequat after WS increased the number of flowers/pot in Experiment 1 resulting in 4.1 flowers/pot while control plants only produced 0.4 flowers/pot (Table 4.3). Tank mix of daminozide/chlormequat alone showed little difference from control in Experiment 2 (Table 4.4). The number of flowers was 1.1 flower/pot on plants treated with tank mix of daminozide/chlormequat, and control plants had 0.6 flower/pot.

Only ethephon, WS/ethephon, and WS/tank mix of daminozide and chlormequat inhibited branching on 'Raspberry Ice' bougainvillea (Table 4.3). They had 1.5, 0.3 or 1.3 branches increased, respectively, which were less than the 3.6 branches increased on control plants. Height on plants treated with ethephon and WS/ethephon were shorter than the control plants.

Discussion

Gibberellic acid inhibited flower development on bougainvillea (Steffen et al., 1988), by causing a diversion of essential photosynthetic assimilates away from the shoot apex where the bloom formed (King et al., 2000). Since PGRs act by inhibiting GA, the application of PGR reduces GA synthesis (Rademacher, 2000), which may promote flowering in bougainvillea. Therefore, the facts that the PGRs promoted the number of flowers/pot and percentage of flowering pots could be explained by more photosynthetic

assimilates used in reproductive growth of the shoot apex in plants treated with PGRs than the control plants.

Previous studies indicated that dikegulac reduced the height of 'Raspberry Ice' and 'San Diego Red' bougainvillea under short days without enhancing flowering (Dierking and Sanderson, 1985). Besides, benzyladenine at 50-100 ppm induced lateral branching as well as retarded growth. Chlormequat, ancymidol, and paclobutrazol were reported to be effective to slow bougainvillea growth (Kobayashi et al., 2007; Shao et al., 2006; Tang et al., 2006). However, PGRs in the current experiment did not show a strong effect in controlling growth of 'Raspberry Ice' bougainvillea, which may be due to the timing of PGR application.

Daminozide had a different effect on flowering in Exp 1 and 2, which may have resulted from the PGR application methodology. Daminozide applied by soil drench in Experiment 1 resulted in minimal effect on flowering and growth compared to control while foliar application of daminozide on bougainvillea in Experiment 2 showed significant difference from control on the number of flowers. Although daminozide is rapidly absorbed through the leaves, roots, and stems of plants (Oregon State University et al., 1996), its target tissue is species specific. In bougainvillea, daminozide was more effective when it was foliar applied.

Water stress has been successfully used to promote flowering on bougainvillea and loquat (Tang et al., 2006; Cuevas et al., 2007), which was consistent with results from this study. Promoting flowering by WS probably was due to ABA function as previous research found that ABA synthesis would be increased when plants are under

WS (Taiz and Zerger, 2006). Absicisic acid is a plant hormone to control leaf stomata closure. When ABA increases, leaf stomata will close, resulting in an increased canopy temperature and acceleration heat accumulation expressed in growing degree-day (GDD). Growing degree day will be shortened when plants are under WS than plants are adequately watered.

Results in this study found that DCT was significantly different between WS plants and well-watered plants. The greater the DCT is or the lower the LWP, the more severe the WS. Since LWP was correlated with DCT, DCT may be used to evaluate WS severity instead of LWP, which is destructive and time-consuming.

On loquat, the greater the water shortage, the earlier the trees bloomed (Cuevas et al., 2007). However, 'Raspberry Ice' bougainvillea did not show similar results in this experiment. It could be the level of WS, instead of the duration of WS, that affects flower initiation in bougainvillea. Flower initiation may be involved in hormone modification by WS while flower opening is more related with GDD.

In conclusion, PGR treatments daminozide, chlormequat, and tank mix of daminozide/chlormequat promoted flowering on bougainvillea when they were foliar sprayed. However, they did not affect plant height significantly when applied in summer. Additional PGR application may be needed to control plant growth and to promote more uniform flowering. When used for controlling growth in bougainvillea, ethephon should not be applied at high concentration (>1,000ppm) due to the possibility of reduced aesthetic value from defoliation. Water stress can be applied to enhance flowering on

bougainvillea and further studies are needed find the proper level of WS that is effective in promoting bougainvillea flowering.

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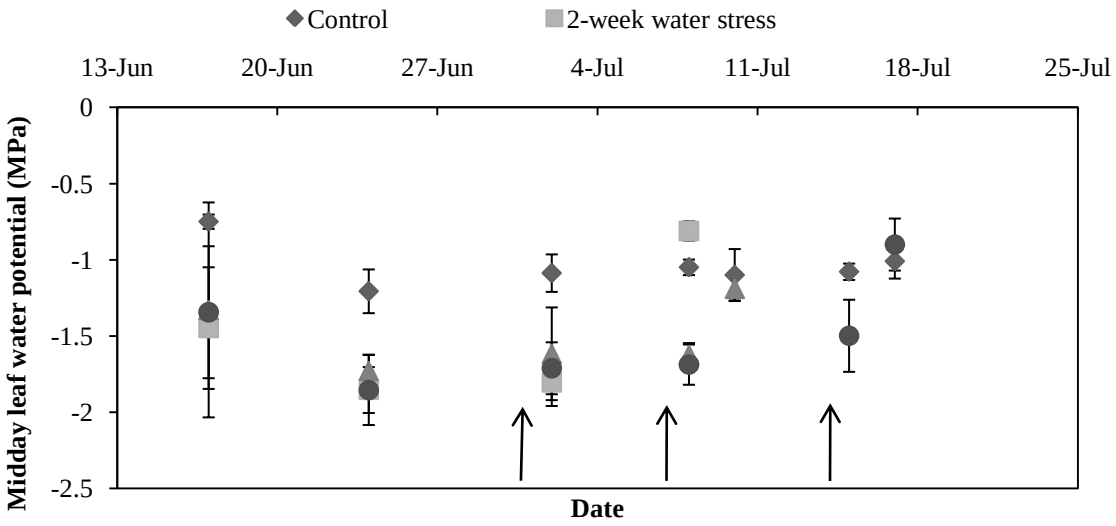

Figure 4.1. Midday average leaf water pontential (LWP, MPa) of 'Raspberry Ice' bougainvillea for each water stress (WS) treatment and control in 2009. Leaf water potential was measured at a weekly-interval on newly mature and fully-expanded leaves. Each treatment had 5 replicates with 3 subsamples. Arrows indicate that plants in 2-week WS, 3-week WS and 4-week WS were well watered starting from 2 July, 8 July, and 15 July, respectively.

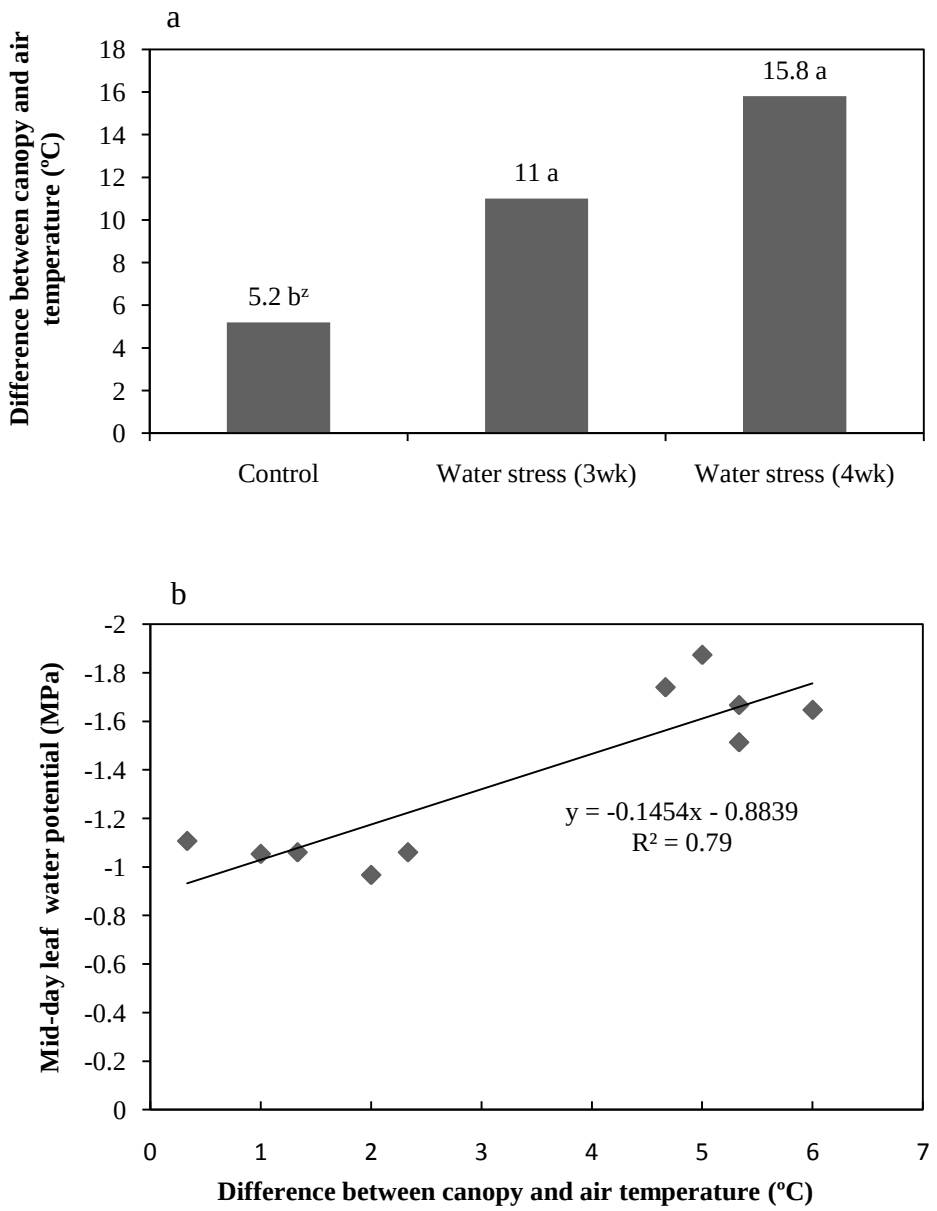

Figure 4.2. (a) Average temperature difference between canopy and air temperature on 8 July 2009 for control, and 'Raspberry Ice' bougainvillea treated with 3-week, and 4-week WS. Means indicated by the same letter are not significantly different (LSD, $p<0.05$, n=5) (b) The correlation between mean difference between canopy and air temperature and leaf water potential for control and 4-week water stressed plants on 8 July 2009.

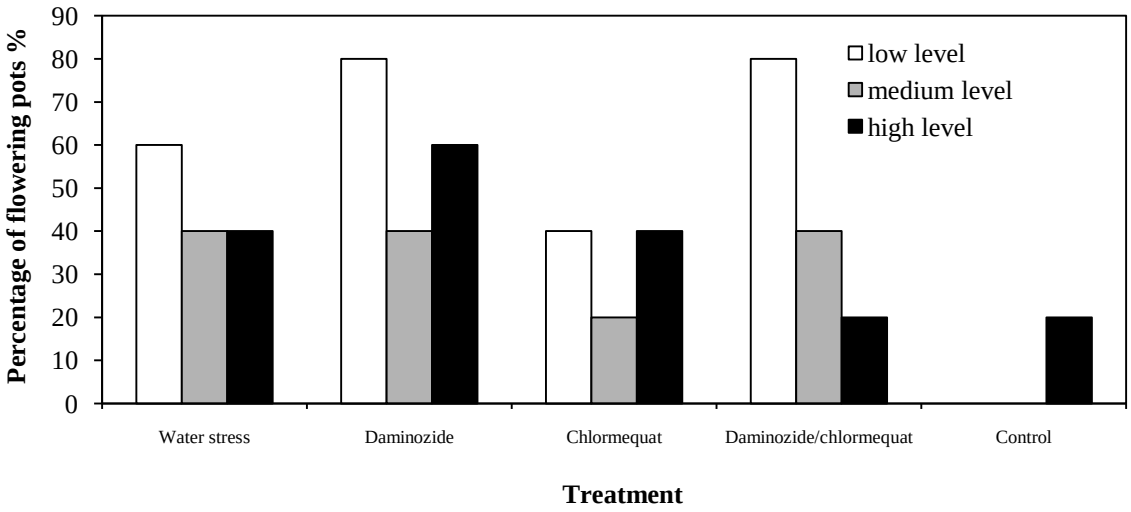

Figure 4.3. Percentage of flowering pots of 'Raspberry Ice' bougainvillea treated with WS, daminozide, chlormequat, tank mix of daminozide/chlormequat in 2009. Low, medium, and high levels of WS treatment were 2-week, 3-week, 4-week, for daminozide were 1,250 ppm, 2,500 ppm, and 3,750 ppm, for chlormequat were 300 ppm, 600 ppm, and 900 ppm, and for daminozide/chlormequat were 800/1,000 ppm, 1,250/1,250 ppm, and 2,500/2,500 ppm.

Table 4.1. Effects of water stress (WS) and plant growth regulators including chlormequat, daminozide, and ethephon on growth and flowering of 'Raspberry Ice' bougainvillea in 2008.

Treatment (ppm)	Shoot height[z] (cm)	Number of flowers /plant[y]	Increased number of branches [x]/plant
Control	5.8ab[w]	0.4d	3.6a
WS	3.6abcde	5.6a	2.1abc
Chlormequat[v] (600)	6.5a	4.9abc	2.5abc
Chlormequat (1,200)	4.6abc	1.1cd	3.3ab
Chlormequat (1,800)	2.6bcde	1.8bcd	1.9abc
WS + chlormequat (600)	2.9bcde	4.1abcd	2.0abc
WS + chlormequat (1,200)	4.9abc	3.0abcd	2.4abc
WS + chlormequat (1,800)	3.6abcde	3.4abcd	2.5abc
Daminozide[u] (1,250)	5.0abc	0.1d	3.4a
Daminozide (2,500)	4.9abc	2.0bcd	3.0ab
Daminozide (3,750)	3.6abcde	0.7cd	3.0ab
WS + daminozide (1,250)	3.4abcde	1.8bcd	2.9ab
WS + daminozide (2,500)	5.6ab	3.5abcd	2.4abc
WS +daminozide (3,750)	4.8abc	0.5cd	2.9ab
WS +Daminozide/chlormequat (800/1,000)	3.5abcde	3.3abcd	1.1bcd
WS +Daminozide/chlormequat (1,250/1,250)	4.8abc	3.5abcd	1.1bcd
WS +Daminozide/ chlormequat (2,500/2,500)	4.0abcd	5.6ab	1.8abc
Ethephon (500)	3.4abcde	0.0d	2.1abc
Ethephon (1,000)	0.8de	0.0d	1.9abc
Ethephon (1,500)	0.4e	1.0cd	0.7cd
WS + ethephon (500)	2.9bcde	0.9cd	2.3abc
WS + ethephon (1,000)	1.6cde	0.0d	0.0d
WS + ethephon (1,500)	2.8bcde	0.0d	0.0d

[z] Shoot height was measured on 8 Aug. 2008.

[y] Number of flowers (bracteole>2 cm) was counted on 17 July 2008

[x] Increased number of branches (length>2cm) was counted on 11 June 2008.

[w] Means followed by the same letters within each column are not significantly different (LSD, p=0.05, n=8).

Table 4.2. Effects of water stress (WS) and plant growth regulators on flowering of 'Raspberry Ice' bougainvillea in 2009. There were 13 treatments with 5 replicates.

Treatment (ppm)	DTF[z] (day)	The number of flowers /plant[y]	Percentage of flowering branchesr[x]/plant
Control	20 NS [w]	0.6 NS	1.6 NS
Two-week WS	25 NS	2.0 NS	4.4 NS
Three-week WS	35 NS	2.6 NS	7.6 NS
Four-week WS	61 NS	2.8 NS	7.1 NS
Daminozide (1,250)	33 NS	2.4 NS	3.8 NS
Daminozide (2,500)	19 NS	5.4 NS	3.3 NS
Daminozide (3,750)	16 NS	9.2NS	11.4 NS
Chlormequat (300)	47 NS	0.4 NS	2.3 NS
Chlormequat (600)	16 NS	0.4 NS	0.8 NS
Chlormequat (900)	45 NS	0.8 NS	1.7 NS
Daminozide/chlormequat (800/1,000)	18 NS	2.2 NS	4.3 NS
Daminozide/chlormequat (1,250/1,250)	23 NS	0.2 NS	0.9 NS
Daminozide/chlormequat (2,500/2,500)	18 NS	0.8 NS	1.9 NS

[z] Days to first flower from end of treatment.
[y] The number of flowers (bracteole>2 cm) was recorded throughout the experiment.
[x] Number of branches (length > 5 cm) was counted on 1 July 2009.
[w] Means followed by NS within each column were not significant difference (LSD, p=0.05, n=5).

Table 4.3. Effects of water stress (WS) and plant growth regulators (PGR) including chlormequat, daminozide, and ethephon on growth and flowering of 'Raspberry Ice' bougainvillea transplanted in 2008. PGR rates[z] were combined for analysis.

Treatment (ppm)	Shoot height[y] (cm)	Number of flowers /plant[x]	Increase number of branches [w]/plant
Control	18.4 a[v]	0.4 d	3.6 a
WS	17.6 ab	7.4 a	2.1 ab
Chlormequat	17.7 ab	2.6 bcd	2.5 ab
WS + chlormequat	16.7 abc	3.5 bc	2.3 ab
Daminozide	17.3 abc	1.0 cd	3.1 a
WS + daminozide	17.7 ab	1.9 bcd	2.7 ab
WS +daminozide+chlormequat	17.5 ab	4.1 b	1.3 bc
Ethephon	15.4 c	0.5 cd	1.5 bc
WS + ethephon	15.8 bc	0.3 d	0.3 c

[z] Chlormequat (600, 1,200, or 1,800 ppm); Daminozide (1,250, 2,500, or 3,750 ppm); Ethphon (500, 1,000, or1,500 ppm).
[y] Shoot height was measured on 8 Aug. 2008.
[x] Number of flowers (bracteole>2 cm) was recorded on 17 July 2008.
[w] Increased number of branches (length>2cm) was counted on 11 June 2008.
[v] Means followed by the same letters within each column are not significantly different (LSD, p=0.05, n=8 for control and WS and n=24 for other treatments, respectively).

Table 4.4. Effects of Water stress (WS) and plant growth regulators on flowering of 'Raspberry Ice' bougainvillea in 2009. PGR rates[z] were combined for analysis.

Treatment (ppm)	DTF[y] (day)	The number of flowers /plant[x]	Percentage of flowering branches[w]/plant
Control	20 NS[v]	0.6 b[u]	1.6 NS
WS	41.13 NS	2.5 ab	6.4 NS
Daminozide	24.44 NS	5.7 a	6.2 NS
Chlormequat	40 NS	0.5 b	2.3 NS
Daminozide/chlormequat	19.43 NS	1.1 b	1.6 NS

[z] WS (2-week, 3-week, or 4-week); Daminozide (1,250, 2,500, or 3,750 ppm); Chlormequat (300, 600, or 900 ppm); Daminozide/chlormequat (800/1,000, 1,250/1,250, or 2,500/2,500 ppm).

[y] Days to first flower.

[x] The number of flowers (bracteole>2 cm) was recorded throughout the experiment.

[w] Number of branches (length > 5 cm) was counted on 1 July 2009.

[v] Means followed by NS within each column were not significant different (LSD, p=0.05, n=15 and n=5 for control, respectively).

[u] Means followed by same letter within each column are not significantly different (LSD, p<0.05, n=5 for control and n=15 for the other treatments, respectively).

CHAPTER V

CONCLUSION

In banana shrub, 8 weeks of chilling at 8 ºC is the least duration required for banana shrub to break flower bud dormancy after flower bud initiation. Ten or greater post-chilling photoperiods were required to produce flowers. Pre-chilling SD are more productive in banana shrub flower initiation than LD. A foliar spray of a tank mix of daminozide and chlormequat (5,000/1,500 ppm) before chilling increased the number of flower buds/pot. This is recommended to use in commercial production to increase the aesthetic value of banana shrub. However, post-chilling applications of 2,500 ppm GA did not substitute for part of the chilling treatment or produce uniform flowering and even caused irregular flowers. Timing and dose of application of GA need further investigation to produce uniform flowering in banana shrub.

In ‘Raspberry Ice’ bougainvillea, water stress enhanced flowering. Chlormequat enhanced flowering in 2008 while showed little effect on promoting flowering in 2009. The reason for the inconsistency is unknown. Daminozide is more effective when foliar sprayed than soil drenched on promoting flowering. Ethephon caused severe defoliation instead of promoting flowering and is not recommended for inducing flowering in ‘Raspberry Ice’ bougainvillea.