2009

Induction of acquired stress tolerance for improving landscape survivability of Petunia x hybrida

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INDUCTION OF ACQUIRED STRESS TOLERANCE FOR IMPROVING LANDSCAPE SURVIVABILITY OF *PETUNIA X HYBRIDA*

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of
requirements for the degree of
Master of Science

in

The School of Plant, Environmental, and Soil Sciences

by
Jennifer Mader
B.S., Louisiana State University and Agricultural and Mechanical College, 2007
August 2009

ACKNOWLEDGEMENTS

I am very grateful for many people who have supported me and my research over the past few years. I would like to thank my advisor, Dr. Jeff S. Kuehny for his guidance, patience, and valuable advice as a mentor and friend. I would also like to thank my committee members, Dr. James Board and Dr. Jeff Beasley, for their continued support, comments and important direction of my research. I would also like to acknowledge Dr. Kevin McCarter who has been extremely helpful with my statistical analysis. I would also like to thank Dr. Charles Johnson and Bob Mirabello for their guidance and encouragement during both my undergraduate and graduate studies. I would like to thank Victoria Hurst for always helping me with the littlest tasks and being a constant ear and companion over the past six years. I greatly appreciate my lab members See Teng Soo and especially Miao Liu who was always willing to help and give advice in every area of my research.

I would like to dedicate this thesis to my parents, Beverley and Doug Mader, who have supported and encouraged me my whole life and never let me quit, even when I didn’t think I could make it. To my brothers, Stephen and Jonathan, and my grandfather, Harry Shiach, who have helped me become the person I am today. I would also like to thank my best friends Rita Bridges, Katie Garcia and Jamie Hilderbrand for their unconditional support, advice, patience, encouragement, sense of humor and irreplaceable friendship that will last all of our lives.

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ABSTRACT

Heat stress is one of the greatest challenges affecting growth and development of bedding plants during greenhouse production and in the landscape. Inducing an acquired stress tolerance during production may greatly improve postproduction marketability and survival in the landscape when exposed to heat stress. Few researches have investigated the morphological effects of bedding plants during heat shock or enduring heat stress preconditioning in the greenhouse and subsequent landscape performance. The present objectives were to quantify morphological and physiological responses to heat stress and use this information to develop a greenhouse protocol for inducing acquired thermotolerance for improved landscape survivability using *Petunia x hybrida*.

Preliminary studies revealed petunia Dreams ‘Midnight’ grown at 35/25 or 40/30ºC caused desirable traits such as compact growth for improved landscape performance but also decreased flowering during production resulting in poor marketable quality. Heat shock at 35 or 40ºC for 2 h every 7 d did not significantly effect petunia growth and development.

Determination of optimum heat shock temperature and duration for development of acquired thermotolerance revealed that heat shock every 3 d or enduring heat stress was most effective at 45ºC. However, the critical duration or frequency of exposure necessary for promotion of a heat tolerant marketable plant at 45ºC was not fully elucidated within the treatments investigated. Chlorophyll fluorescence (maximum quantum efficiency of PSII - Fv/Fm) was measured in young and mature leaves to investigate stress response and photosynthetic performance of petunia pre and post acquired thermotolerance test. Fv/Fm ratios indicated the heat shock or enduring heat stress treatments did not cause permanent damage to photosynthetic apparatus.

Nineteen previously evaluated petunia cultivars from three plant classes were heat shocked at 45ºC for 4 h every 3 d during greenhouse production followed by landscape evaluation. Greenhouse and field results indicated the heat shock treatment did not significantly promote heat tolerance compared to control.

The critical temperatures used in this study were effective for promoting heat tolerance in petunia, but specific durations or frequency of exposure at 45ºC should be further investigated in order to define an effective acquired thermotolerance protocol to improve landscape survivability.

CHAPTER 1. INTRODUCTION

Floriculture in the United States is a billion dollar industry that is comprised of a variety of flowering and foliage plants. Within the industry, bedding plants represent herbaceous annual or perennial flowering plants typically used for color in maintained landscapes. According to the USDA 2007 Floriculture Crops Summary, the wholesale value in sales of bedding plants was $1.26 billion and accounted for the largest percent of wholesale sales of all floriculture crops (U.S. Department of Agriculture, 2008). Bedding plant production is greatest in early spring and late summer to early fall depending on species and climate. Because of their potential for year round production and generally short production time, bedding plants are widely grown for their high economic yield and efficiency. With high demand from consumers in retail nurseries and landscape contractors, bedding plants remain the primary crop for growers within the industry. This high demand can also be reflected in the numerous breeding programs focused on development of new and 'improved' cultivars.

Bedding plants are typically propagated by seed or vegetative cutting and produced as plugs for greenhouse production (Styer and Koranski, 1997). Plugs are grown in trays consisting of numerous cells ranging in soil volume from 21.74 to 3.5 cm^3, with the most common sizes being 288 and 512 cells per tray (Hamrick, 1989). Bedding plant plugs are commonly transplanted into larger cell packs or containers during production which will then be sold to the consumer.

Achieving optimum plant quality during greenhouse production and in the landscape is a shared goal for plant breeders, growers and landscape contractors. However, as with any crop, efficient growth and stability of bedding plants during production and postproduction can be problematic due to unpredictable and sometimes uncontrollable growing environments. The growth and development of a plant includes highly sensitive morphological and physiological processes within the plant (Pollock et al., 1993). There are many abiotic factors that may limit optimum growth and development of a plant, some of which include nutrient availability, light quality and quantity, drought stress and heat stress (Alscher and Cumming, 1990; Hall, 2001). Advances in technology and proper management practices have helped to alleviate many of these stresses during production. However, due to increasing air temperatures and energy costs, heat stress in particular continues to be one of the greatest challenges during greenhouse production of bedding plants and also during postproduction in the landscape. Prolonged exposure to heat stress can cause permanent damage to the growth and development of the plant (Hall, 2001). Symptoms of heat stress are described as extreme wilt, yellowing or senescence of leaves, leaf curl, stunted growth and underdeveloped flowers or fruit (Dole and Wilkins, 2004; Kuroyanagi and Paulsen, 1988; Larcher, 1995; Lohar and Peat, 1998). Research by Natarajan (2005) found that exposure of *Salvia splendens* F. Sellow ex Roem & Schult.'Sizzler Red' to short duration temperature stress of 35°C for 3h every 3d resulted in marginal leaf burning and necrosis of the leaves while exposure to 40°C caused reduction in plant height, shoot and root dry weight. With such constant demand for quality plants, addressing the issue of heat stress has been a priority for research within the industry. Currently, much of the research concerning high temperature stress focuses on many horticultural and agronomic crops but not bedding plants (Adedipe et al., 1971; Knight and Ackerly, 2003; Liu and Huang, 2002; Valladares and Pearcy, 1997; Wright *et al*., 2001). Research conducted on bedding plants pertains more to breeding programs using inefficient field trials and selections based primarily on aesthetics. While strong aesthetic quality of bedding plants is an important goal, there are many physiological and morphological traits equally important to consider when breeding a 'heat tolerant' plant. Because of this indiscretion, many of the bedding plants marketed for such heat tolerance are not truly 'tolerant' and inevitably become a disappointment in postproduction (Natarajan, 2005).

A plant's ability to acclimate and perform necessary metabolic processes during heat stress is important for assigning heat tolerance and is crucial for survivability in stressful environments (Hale and Orcutt, 1997; Lichtenthaler, 1996). A plant's natural tolerance to heat has much to do with its native climate and optimal growing temperature. In nature, the geographical distribution of plants is strongly linked to their adaptability to a particular climate (Mahan et al., 1997). However, mass production of non-native plants occurs in areas where the growing environment is considered to be undesirable. This is especially true in the southern United States where many cool-seasoned plants are being produced under much higher temperatures and oftentimes at the expense of plant quality or landscape longevity. For example, petunia is a very popular bedding plant and has an optimum growing temperature of approximately 24°C (Ball Horticultural Company, West Chicago, IL). Production is ideal for temperate climates; however, petunia is also commonly produced in subtropical regions for late summer and early fall planting, but can be very challenging due to temperatures that can easily approach 40°C in the greenhouse and in the landscape. In addition, most growing recommendations do not include guidelines for production under heat stress conditions (Hancheck and Cameron, 1995). Under these circumstances, promoting acquired thermotolerance during production would be beneficial to the plant as well as the grower and landscaper.

Acquired thermotolerance may be obtained through temperature preconditioning using supraoptimal temperatures for specific durations (Natarajan, 2005). Growers would be able to use high temperatures to their advantage during production and induce thermotolerance in plants resulting in a marketable plant in the greenhouse and improved landscape survivability. Using an acquired thermotolerance protocol in the greenhouse would also promote more sustainable production practices and help alleviate energy costs used to cool the greenhouse. There is little research on the morphological and physiological effects of acquired thermotolerance on bedding plants during production and their resulting landscape performance. Therefore, the objectives of this research were to quantify morphological and physiological responses to heat stress and use this information to develop a greenhouse protocol for inducing acquired thermotolerance for improved landscape survivability. This was achieved through the following studies using *Petunia x hybrida*: 1) determine the effect of enduring high temperature stress and short duration heat shock (preconditioning) on growth and development*;* 2) determine optimum heat shock temperature and duration for development of acquired thermotolerance and to test thermotolerance through subsequent heat stress; 3) use determined heat shock temperature and duration for induction of acquired thermotolerance of several petunia plant classes and evaluate subsequent landscape performance.

1.1 LITERATURE CITED

Adedipe, N.O., L.A. Hunt, and R.A. Fletcher. 1971. Effect of benzyl adenine on photosynthesis, growth and senescence of the bean plant. Physiol. Plant. 25:151-153.

Alscher, R.G. and J.R. Cumming. 1990. Stress Responses in Plants: Adaptation and Acclimation Mechanisms. Wiley-Liss, Inc., New York.

Ball®. 2009. GrowerFacts *Petunia x hybrida*. Ball Horticultural Company, West Chicago, IL

Dole, J.M. and H. F. Wilkins. 2004. Petunia, pp. 744-748 in *Floriculture : principles and species*, 2^{nd} ed. D. Yarnell, K. Yehle, L. Dalberg, M. Carnis, editors. Pearson Education, Inc., Upper Saddle River, New Jersey.

Hale, M.G. and D.M. Orcutt. 1997. The physiology of plants under stress. Wiley New York.

Hall, A.E. 2001. Crop Response to Environment. CRC Press LLC, Boca Raton, FL.

Hamrick, D. 1989. The three cent bedding plant plug. Grower Talks 53(8):44-46.

Hancheck, A.M. and A.C. Cameron. 1995. Harvest date and storage quality of herbaceous perennials. HortSci. 30: 573-576.

Knight, C.A. and D.D. Ackerly. 2003 Evolution and plasticity of photosynthetic thermal tolerance, specific leaf area and leaf size: congeneric species from desert and coastal environments. New Phytologist 160:337–347.

Kuroyanagi, T., and G.M. Paulsen. 1988. Mediation of high-temperature injury by roots and shoots during reproductive growth of wheat. Plant Cell Environ. 11: 517–523.

Larcher, W. 1995. Stress due to extreme temperatures. In: Physiological Plant Ecology, 3rd ed. pp. 340-354.

Lichtenthaler, H.K., 1996. Vegetation stress: an introduction to the stress concept in plants. J Plant Physiol. 148, 4–14.

Liu X, B. Huang, and G. Banowetz. 2002. Cytokinin effects on creping bentgrass responses to heat stress: I. Shoot and root growth. Crop Sci. 42:457-465.

Lohar, D.P. and W.E. Peat. 1998. Floral characteristics of heat-tolerant and heat-sensitive tomato (*Lycopersicon esculentum* Mill.) cultivars at high temperature. Scientia Horticulturae 73:53-60.

Mahan, J.R., B.L. Mc Michael, and D.F. Wanjura. 1997. Reduction of high temperature stress in plants, p. 137-150.In: A.S. Basra and R.K. Basra (eds). Mechanism of environmental stress resistance in plants. Harwood Academic Publishers, The Netherlands.

Natarajan S. 2005. High temperatures stress responses of *Salvia splendens* and *Viola x wittrockiana.* Ph.D Diss.

Pollock, C.J., C.F. Eagles, C.J. Howarth, P.H.D. Schunmann, and J.L. Stoddart. 1993. Temperature Stress, pp. 109-127 in Plant Adaptation to Environmental Stress. L. Fowden, T. Mansfield, and J. Stoddart, editors. Chapman & Hall, London.

Styer, R., and D. Koranski. 1997. Plug and Transplant Production. Ball Publishing, Batavia, IL. 400 p.

United States Department of Agriculture. 2008. Floriculture crops 2007 summary. Sp Cr 6-1 (08) pp. 9-29.

Valladares, F. and R.W. Pearcy. 1997. Interaction between water stress, sun-shade acclimation, heat tolerance and photoinhibition in the sclerophyll Heteromeles arbutifolia. Plant, Cell and Environ. 20:25-36.

Wright, I.J., P.B. Reich, and M. Westoby. 2001. Strategy shifts in leaf physiology, structure and nutrient content between species of high and low rainfall and high and low nutrient habitats. Funct. Ecol.15:423–434.

CHAPTER 2. LITERATURE REVIEW

The effect of temperature on plants can be divided into three categories: air, leaf, and soil temperature. These temperatures are not always consistent with each other and may differ under certain environmental conditions (i.e. humidity, light intensity, and air circulation). Air temperature is more readily monitored therefore growing recommendations usually refer to day and night air temperatures (Dole and Wilkins, 2004). Temperature can affect a plant in many ways and growers have been manipulating temperatures for many years in order to achieve certain characteristics. In order to regulate height in some plant species, growers sometimes use a concept known as DIF (Myster and Moe, 1995). This term refers to the difference between day and night temperatures (day – night = DIF), where higher day temperatures (+DIF) promote stem elongation and higher night temperatures reduce stem elongation (Erwin et al., 1989; Warner and Erwin, 2001).

Temperature may not always be easily manipulated or controlled, especially during the summer months or in hotter climates. When plants are exposed to higher temperatures than their optimum range, there may be damaging effects to plant function resulting in reduced growth, development and yield (Gusta et al., 1997; Harding et al., 1989). The term heat stress is defined as when exposure of plants to high temperatures for a specific length of time will cause *irreversible* damage to plant metabolism and overall development (Hall, 2001). Metabolic processes tend to be highly sensitive to temperature. Fluctuations in temperature may affect not only the type of protein being synthesized, but also the amount being produced or cause inactivation and denaturation of enzymes and proteins (Pollock et al., 1993; Nakamoto and Hiyama, 1999). A temperature that is considered to cause heat stress in a plant is related to a species optimum temperature and may also differ depending on the stage of growth or development at which the plant is exposed (Burke, 1990).

2.1 EFFECTS OF HEAT STRESS ON PLANT REPRODUCTIVE TISSUE

Heat stress can be a major challenge for production of fruits, vegetables and floriculture crops. Reproductive stages of a plant can be highly sensitive and can affect the development of reproductive organs in many different ways. Flower response to heat stress is dependent on the species and even some cultivars within a species may differ in flower characteristics when exposed to high temperatures. Past research has indicated that the response of flower number in tomato (*Lycopersicon esculentum* Mill.) to heat stress differs greatly among cultivars, even those labeled as 'heat tolerant' (Abdul-Baki, 1991; Charles and Harris, 1972). However, some characteristics like association of total flower bud and flower production may be a more acceptable standard when selecting for heat tolerance (Lohar and Peat, 1998). According to a study by Bjorkman and Pearson (1998), broccoli (*Brassica oleracea* var. *italica* L.) was exposed to heat stress at different developmental stages to study the effect on broccoli inflorescence. The exposure to heat stress resulted in variation of flower bud size. The 'Galaxy' cultivar of broccoli at different developmental stages were exposed to 35/22°C day/night for one week in a growth chamber. Results indicated a stronger response to heat stress during reproductive stage. The authors concluded that the uneven size in flower buds was due to a delay in bud development rather than a direct inhibition of bud initiation.

There have been many studies that have investigated the effect of heat stress on tomato (*Lycopersicon esculentum* Mill.) which is in the same family as *Petunia x hybrida* hort. Vilm.-Andr., Solanaceae. Lohar and Peat, (1998) studied floral characteristics of known heat-sensitive and heat-tolerant cultivars of tomato (*Lycopersicon esculentum* Mill.) when exposed to 15/10, 22/17, 28/23, and 35/30°C day/night air temperatures. Results showed that the heat tolerant cultivar, when exposed to 35/30°C, displayed earlier flowering. The heat tolerant cultivar also produced more flower buds than the heat sensitive cultivar when exposed to the three warmer temperature treatments. Anthesis at 35/30°C in the heat-sensitive cultivar was greatly reduced but may be due to elevated night temperature (30°C) rather than the higher day temperature (35°C). This is consistent with other reports where heat stress is applied at night as well as during the day as seen in pea (*Pisum sativum* L.) (Guilioni et al., 1997). Research by Lohar and Peat (1998) also found that morphology of the flower was also greatly affected by heat stress in both cultivars. Heat sensitive cultivar at 35/30°C had only a calyx and no petals while flowers of the heat tolerant cultivar were not aborted but displayed “underdeveloped inner whorls restricted within the calyx” (Lohar and Peat, 1998). Stigma exertion is also a characteristic of heat stressed flowers but does not greatly affect pollination and should not always be seriously considered when selecting for heat tolerance of a cultivar (El Ahmadi and Stevens, 1979). Other reports indicate that the sequence of flowering or the presence of older reproductive structures on a plant can affect the development of future flowers or fruit when exposed to heat stress (Aloni et al., 1991; Guilioni et al., 1997; Sato et al., 2001).

As mentioned earlier, some research has indicated that elevated night temperatures or mean day/night temperature can have a greater affect on flowering than high day temperature alone. However, the flowering response may differ depending on species, photoperiod requirements, duration and time of heat stress exposure. Some reports using cowpea (*Vigna unguiculata* (L.) Walp.) showed that moderate heat stress of at least 20°C at night can be more damaging to reproductive development than when the plants were exposed to 40°C day temperature (Warrag and Hall, 1984a, b). A study by Cockshull and Kofranek (1993) indicated that flower bud initiation in chrysanthemum (*Chrysanthemum x grandiflorum* L.) was delayed when exposed to elevated night temperature (32°C) for a short period of time at the beginning of (SD) while bud formation was delayed when exposed for longer durations. The authors also noted that high night temperatures (32°C) caused leaves to become chlorotic and delay in stem elongation. Willits and Bailey (2000), studied exposure of high night (20 to 26°C) and mean diurnal temperatures (22 to 27°C) over a 4 yr period to heat-sensitive and heat-tolerant cultivars of chrysanthemum (*Chrysanthemum x grandiflorum* L.) and found that heat-sensitive cultivars showed a greater difference in the inflorescence diameter compared to more heat-tolerant cultivars.

2.2 VEGETATIVE AND OTHER PHYSIOLOGICAL RESPONSES

Plant exposure to heat stress may result in foliar chlorosis, necrosis and underdeveloped growth of a plant and flowers (Dole and Wilkins, 2004; Hall, 2001; Larcher, 1995). However, these characteristics are often an indirect result due to increased transpiration and water stress associated with high temperatures (Burke, 1990). When exposed to high temperatures, the symptoms expressed in shoot and leaf tissue may be directly associated with damage to the root system, photosynthetic apparatus, and inhibition of water and nutrient uptake (Ashraf and Hafeez, 2004; Berry and Bjorkman, 1980; Aldous and Kaufmann, 1979; Gur and Shulman, 1979). A study by Huang and Xu (2000) investigated the effects of high air and soil temperatures on creeping bentgrass (*Agrostis palustris* Huds.). The study revealed that a combination of high soil and air temperatures (35/35°C) caused greater damage to root growth, photosynthetic activity and turf quality than either stressful temperature alone. The study also showed that higher soil temperatures (20/35°C) compared to higher air temperatures (35/20°C) caused a significant decrease in not only root growth but photosynthetic activity and shoot growth as well. The effect of heat stress temperatures of 23, 29, or 32ºC exposed to perennial grass, *Lolium perenne* (Trin.) Tzvel., was investigated by measuring photosynthetic gas exchange, the maximal efficiency of PSII photochemistry determined by leaf chlorophyll fluorescence measurements, nitrogen level, and lipid peroxidation. Results indicated that high temperature decreased plant biomass, inhibited nutrient uptake, and had detrimental effect on plant qualities (Xu and Zhou 2006).

Other studies have indicated that distribution of photoassimilates can be inhibited at high temperatures (Ewing, 1981; Jiao and Grodzinski, 1996). Also, membrane fluidity is considered to be a factor in a plant's ability to sense changes in temperature (Sung et al., 2003). Membrane stability at high temperatures is crucial for normal plant function and survivability (Raison et al., 1980). There have been numerous studies on the effects of heat stress and membrane stability where results indicate a strong connection between tolerance to high temperatures with increased membrane stability as seen with kentucky bluegrass (*Poa pratensis* L.) (Marcum, 1998); wheat (*Triticum aestivum* L.) (Ibrahim and Quick, 2001); soybeans (*Glycine max* (L.) Merr.) (Martineau, 1979); and tomato (*Lycopersicon esculentum* Mill.) (Chen et al., 1982).

Photosynthesis is a very heat-sensitive process that can have reversible effects on rate when exposed to temperatures ranging from 10°C to 35°C, but may cause permanent damage to the photosynthetic apparatus at temperatures below or above this range (Berry and Bjorkman, 1980; Burke, 1990). Heat stress causes changes in membrane structures and interferes with protein-lipid relations within the chloroplast (Gounaris et al., 1983). Results from Smillie et al. (1978) indicated that chloroplast biogenesis was inhibited when barley (*Hordeum* L.) plants were grown at temperatures greater than 32°C. Photosynthetic yield and efficiency is dependent on thermostable interactions between light harvesting pigments and photosystem reaction centers (Armond et al., 1978). Photosystem II is considered the most heat-sensitive mechanism of photosynthesis (Berry and Bjorkman, 1980; Havaux, 1993). Stidham et al., (1982) used wheatgrass (*Triticum aestivum* L.) to show that the effects of heat stress on light reactions were significantly correlated to the duration of pre-exposure to temperatures greater than 35°C. Burke (1990) studied acclimation of the photosynthetic electron transport chain at PSII in wheat (*Triticum aestivum* L.) by monitoring changes in chlorophyll fluorescence. Results showed a reduction in *Fv/Fo* ratio of seedlings grown at control (22°C) after subsequent heat stress treatment at 42°C, while the ratio remained steady in wheat seedlings with previous heat shock treatment of 40°C before exposure to 42°C heat stress, indicating an acquired thermotolerance of PSII. When using chlorophyll fluorescence to study the effects of environmental stresses on the photochemical efficiency of PSII or underlying damage to the photosynthetic apparatus, the most common measurement used in research is the maximum quantum efficiency of PSII (Fv/Fm) determined by the ratio of variable fluorescence (Fv) to maximal fluorescence (Fm) in dark-adapted leaves (Maxwell and Johnson, 2000).

2.3 ACQUIRED THERMOTOLERANCE

Plants have the capacity to cope with environmental stresses using various morphological and physiological mechanisms (Hall, 2001). These mechanisms have been the interest of many researches and plant breeders over the past few decades in an effort to promote higher performing crops in variable environments (Maestri et al., 2002; Queitsch et al., 2000; Ismail and Hall, 1999). A plant's ability to acquire tolerance to a particular stress depends on the genotype, level of susceptibility and optimum growing environment (Jones and Jones, 1989; Ketring, 1984). For tolerance of high temperature stress, this process can be performed through long term pre-exposure, commonly known as acclimation or 'heat-hardening', or through acute short duration exposure known as heat shock. The latter process is more definitive of an acquired thermotolerance, where pre-exposure to supraoptimal temperatures for a relatively short period of time triggers internal mechanisms that enable a plant to survive during lethal temperatures (Sung et al., 2003).

A study by Wu and Wallner (1984), focused on using heat shock (38°C for 20 min) and elevated growing temperature (30°C) on suspension-cultured pear (*Pyrus communis* (L.) cv Bartlett) cells to induce heat tolerance. Following pretreatment, treated cells were exposed to 43°C for 20 min heat stress and returned to 22°C control temperature for 10 d before taking %

viability tests. Regrowth (culture growth 10 d after heat stress), electrolyte leakage, and TTC (triphenyltetrazolium chloride) reduction measurements were taken to determine % viability and subsequent heat tolerance. The results indicated that heat shock at 38°C for 20 min had the highest % heat tolerance when pear cells were exposed to 43°C heat stress after heat shock treatment and regrown at control (22°C) for 10 d. In addition cells grown in control temperature showed less than 15% viability when exposed to same heat stress of 43°C for 20 min. Over the ten day period, it was also noted that the heat tolerance declined after about 3 d which seemed to be consistent with studies that show a decrease in heat shock protein synthesis upon return to a more optimal temperature (Key et al., 1981). This study also looked at inducing heat tolerance by growing in a supraoptimal temperature of 30°C, a temperature considered stressful but is less than the temperature used for heat shock. These results showed that heat tolerance was induced after 3 d and declined after 6 d. The treated cells also had a higher % viability (60-65%) when compared to the control but were significantly less when compared to % viability of heat shocked cells (90-95%). However, the decline in heat tolerance did not occur the same way in both treatments; those acclimated at 30°C lost tolerance in the first two days while those heat shocked retained it for the initial 2 d. Also, this study compared cells grown at 30°C to cells grown at 30°C with an additional heat shock before exposure to heat stress. These results indicate that after 24 h, cells grown at only 30°C had a significantly less survival % than those with the additional heat shock. It was also noted that viability tests for heat shocked cells at 38°C for 20 min and those hardened at 30°C were significantly different when compared to control cells. While the hardened cells %viability increased in all three tests (TTC reduction, electrolyte leakage, and regrowth potential), cells that were heat shocked also increased in % viability but had lower TTC and EC than hardened cells. However, heat shocked cell regrowth potential was significantly higher than the cells grown at 30°C. Thus, both treatments provide some type of heat tolerance but seem to induce tolerance differently from each other. It may be that heat shock and heat injury are dependent on a temperature and duration interaction (Levitt, 1980). Research by Ortiz and Cardemil (2001) studied two leguminous plants with known acquired thermotolerance. These plants were evaluated at the seedling and mature plant stages. Seeds from *Prosopis chilensis* (Mol.) Stuntz and *Glycine max* (L.) Merr. were germinated at 25 or 35°C and were subjected to heat shock treatments in a growth chamber at 25, 30, 35, 40, 45, 50°C or 35, 40, 45, 50°C respectively, for 2 h. The response to relative growth of embryo axis' length between species was evaluated and results indicated that *P. chilensis* (Mol.) Stuntz germinated at 25°C increased relative growth at 30, 35, and 40°C but decreased at 45°C when compared to control. Seedling growth temperature was found to be lethal at 50°C. *Glycine max* (L.) Merr. seeds germinated at 25°C (control) increased relative growth at 30 and 35°C but decreased at 40 and 45°C and 50°C was lethal. However, when seedling were germinated at 35°C, both species were able to survive at 50°C and *P. chilensis* (Mol.) Stuntz increased relative growth rate (RGR) at 35, 40 and 45°C compared to the control while RGR of *Glycine max* (L.) Merr. decreased at all higher temperatures compared to control at 25°C. Natarajan and Kuehny (2008) exposed heat sensitive and tolerant cultivars of *Salvia splendens* F. Sellow ex Roem & Schult. to heat shock treatments of 35°C every three days until flowering. Significant differences were observed between heat sensitive and heat tolerant cultivars for leaf size and thickness as well as gas exchange and transpiration.

One physiological mechanism strongly linked to plant thermotolerance is the induction of specific proteins known as heat-shock proteins (HSPs) (Vierling, 1991). These special proteins are initiated rapidly during short duration pre-exposure to supraoptimal temperatures when normal protein synthesis is inhibited. HSPs have been associated with stabilizing proteins, chaperone function, protein folding and transport, as well as keeping steady membrane state

(Balogi, 2003; Hassane et al., 2002; Ellis, 1987). The temperature that provokes synthesis of HSPs is species dependent but is generally induced at temperatures that are 10°C higher than the optimal temperature of that species (Pollock et al., 1993; Parsel and Lindquist, 1993). Acquired thermotolerance associated with HSPs exists only when plants are re-exposed to optimal temperatures after heat shock exposure. During this time, the heat-shock initiated HSPs genes are fully synthesized resulting in thermotolerance (Howarth, 1991). It should also be noted that the rate, time and amount of HSPs synthesis is species dependent and not necessarily a permanent response that can be prolonged through longer heat shock treatments (Pollock et al., 1993; Kimpel et al., 1990; Necchi et al., 1987). Natarajan (2005) investigated acquired thermotolerance and synthesis of heat shock proteins for two cultivars of *Salvia splendens* F. Sellow ex Roem & Schult., heat tolerant ‘Vista’ and heat sensitive ‘Sizzler’. Both cultibars were either grown at 25/18°C (control) or preconditioned at 35°C for 3 h every 3 d before exposure to 30/23°C or 35/28°C. Results indicated that synthesis of heat shock proteins increased in heat tolerant ‘Vista’ in both control and preconditioned plants resulting in better plant performance. However, sHSP in ‘Sizzler’ control plants were not affected and were increased in preconditioned plants but the increased synthesis of sHSP showed no direct relation to plant performance.

2.4 CHLOROPHYLL FLUORESCENCE

Heat tolerance has also been strongly associated with increased thermotolerance of the photosynthetic apparatus and PSII (Berry and Bjorkman, 1980). A very effective and non-invasive approach to estimating photochemical efficiency and investigating stress to the photosynthetic apparatus is by measuring chlorophyll fluorescence. When leaf chlorophyll molecules absorb light, the excited electron may return to the ground state using one of three mechanisms. Energy may relax and be given off as heat, be reemitted as light (fluorescence), or most importantly used for photochemical reactions to drive photosynthesis (Buchanan et al., 2000). These mechanisms for relaxation compete with one another so that an increase in efficiency of one mechanism can be observed as decreases in yields for the other mechanisms (Maxwell and Johnson, 2000). When PSII absorbs light, a series of electron transfers occur through photochemical reactions within the reaction center. A reaction center is referred to as 'closed' when an electron has already been accepted and has yet to be transferred to another carrier. The reaction center is unable to accept another electron until this transfer occurs resulting in decreased photochemical efficiency and an increase in chlorophyll fluorescence (Maxwell and Johnson, 2000; Rohacek and Bartak, 1999). Therefore, a sustained reduction of photochemical efficiency as reflected by changes in chlorophyll fluorescence yields can be inferred as injury or stress to the photosynthetic apparatus (Bilger et al., 1995).

Although there has been vast research on chlorophyll fluorescence for over 50 years, the applied science, instrumentation, methodology and standardized nomenclature were lacking until recently (van Kooten and Snel, 1990; Weis and Berry, 1987). This progress has led to a greater availability and efficiency of measuring photosynthesis in the lab and under field conditions resulting in a very effective means for investigating the effects of environmental stress on photosynthetic activity (Havaux and Tardy, 1999; Elhani et al., 2000). Chlorophyll fluorescence is sometimes considered to be a more appropriate measurement of photosynthesis under heat stress compared to CO_2 exchange; which can be altered by stomata closure provoked by many environmental conditions and not just heat (Larcher, 1994). When studying stress tolerance or damage to photosynthetic apparatus, measurements should be taken in dark-adapted and light-adapted states to estimate the extent or lack of damage (Maxwell and Johnson, 2000). The data collected from fluorescence measurements can be relative and inferred differently depending on the particular mechanism being investigated. These data are currently standardized fluorescence terminology used to identify specific chlorophyll fluorescence measurements or combination of measurements (van Kooten and Snel, 1990). The term *Fs* is the steady state of fluorescence in the light. The term *Fo* represents the minimal fluorescence and is measured in the dark when the photosynthetic membrane is in a non-energized state and all PSII reaction centers are open. The term *Fm* represents maximum fluorescence yield which measures the fluorescence intensity when all PSII reaction centers are closed in the dark adapted state and non-photochemical quenching is low. The term *Fv* is the difference between the non-energized or open state of PSII (*Fo*) and the highly energized or closed state (*Fm*). The maximum quantum efficiency of PSII is expressed as the ratio *Fv/Fm*, or (*Fm – Fo*)/*Fm*, and is taken when the plant is in the dark-adapted state. Efficiency of heat dissipation is measured as changes in non-photochemical quenching (*NPQ*). The term *NPQ* or (*Fm – F'm*)/*F'm*, refers to non-photochemical quenching in the light where *F'm* represents the light adapted fluorescence maximum and is relative to the dark-adapted value of *Fm.* To investigate heat stress, Gamon and Pearcy (1989) studied dark-adapted *Fv/Fm* and increased *Fo* to measure injury. Other chlorophyll fluorescence measurements like *NPQ*, quantum efficiency, and electron-transport rates are also useful estimates of photoinhibitory damage to PSII (Bilger et al., 1995). Ortiz and Cardemil (2001), who studied the acquired thermotolerance of *P. chilensis* (Mol.) Stuntz and *Glycine max* (L.) Merr. also evaluated chlorophyll fluorescence between these species. When measuring the maximum photochemical efficiency (*Fv/Fm*) of Photosystem II, leaves from both species were dark adapted for 30 min and then measured fluorescence followed by heat shock for 2 h at 35°C and then dark adapted at 25°C and measured fluorescence after 1, 2, 3 or 5 h. Results indicated a decrease in *Fv/Fm* for both species between 40 and 45°C but was reversed after dark adaption at 25°C for 3 h. Law and Crafts-Bradner (1999) studied inhibition and acclimation of photosynthesis to heat stress associated with activation state of Rubisco in intact cotton (*Gossypium hirsutum* L.) and wheat (*Triticum aestivum* L.). The study investigated non-photochemical chlorophyll fluorescence quenching (qN) and maximum quantum efficiency of PSII (*Fv/Fm*) when cotton and wheat plants were rapidly heat stressed at a rate of 1°C min^{-1} to 45°C or gradually heat stressed by increasing temperature in 2.5°C increments (at rate of 1°C min^{-1}) and remained for 1 h at each temperature. Results showed increased qN at leaf temperatures of 30°C for wheat and 35°C for cotton for both rapid and gradual stress treatments resulting in inhibition of CO_2-exchange rate, but qN levels were less in plants gradually stressed. For maximum quantum efficiency, results indicated steady *Fv/Fm* ratio until 40°C in cotton and wheat that were gradually heat stressed, while decreases in *Fv/Fm* occurred around 35°C for plants rapidly heat stressed, indicating an acquired thermotolerance of PSII can be achieved through gradual acclimation. Similar reports of acquired thermotolerance of PSII have been found by measuring chlorophyll fluorescence in potato leaves (*Solanum tuberosum* L.) (Havaux, 1993).

2.5 *PETUNIA X HYBRIDA*

Petunia x hybrida hort. Vilm.-Andr. is a hybrid cross between *P. axillaris* (Lam.) and *P. integrifolia* (Hook.). Native to Argentina and Brazil, petunia is also a member of the Solanaceae family and includes over 35 species within its genus (Dole and Wilkins, 2004). Petunias are considered an annual bedding plant but may perennialize in warmer climates (Armitage, 1985; Bailey and Bailey, 1976). *Petunia x hybrida* is grouped by several classifications which include: grandiflora, multiflora, floribunda, milliflora and spreading (Dole and Wilkins, 2004; Kelly et al., 2007). Propagation of hybrid petunia is typically by seed (8,600 to 10,000 seeds/g) with an optimum germination and growing temperature of 20 to 26°C (Dole and Wilkins, 2004; Holcomb and Mastalerz, 1985). However, there are other hybrid cultivars like ‘Supertunia’, which produce little seed and are vegetatively propagated by cuttings (Weidner, 1994). Petunia vegetative and reproductive stages are greatly affected by photoperiod and temperature (Adams et al., 1998;

Piringer and Cathey, 1960). Petunias will flower under different photoperiods; longer photoperiods (critical night length of 10 to 13 h), higher light intensities and warmer temperatures will cause flowering to occur earlier and more rapidly (Karlsson, 1996; Piringer and Cathey, 1960; Wilkins and Pemberton, 1981). Also, research has shown that petunias under LD will have more upright growth while SD results in more compact growth with more branching (Adams et al., 1998; Merritt and Kohl, 1982; Piringer and Cathey, 1960). Similar results of petunia growth habit were found with warmer and cooler temperatures (10 to 30°C), where plant height increased and the number of lateral shoots decreased as day temperature increased (Kaczperski et al., 1991).

Petunia x hybrida is considered to be an acceptable model system for comparative research, mainly due to its diversity and genetic transferability among different species (Gerats and Vandenbussche, 2005). Being a member of the Solanaceae family, it is closely related to other genome mapped species including tomato (*Lycopersicon esculentum* Mill.), potato (*Solanum tuberosum* L.), tobacco and nicotiana (*Nicotiana spp*. L.) (Dole and Wilkins, 2004). Information regarding growth and development of these species may also be helpful for petunia research. Petunia is one of the most popular bedding plants today and also proves to have staying power in the market since it is thought to be one of the original cultivated bedding plants (Gerats and Vandenbussche, 2005). Along with petunia marketability, petunia is a beneficial model for bedding plant research due to its leaf tissue quality for biochemical analysis, efficient tissue culture, and macromolecule purification (Ausubel et al., 1980). Over the last 50 years, research using petunia as a model system has resulted in useful information regarding flavonoid synthesis, genetic behavior and other molecular interactions, and more recently floral development (Gerats and Vandenbussche, 2005). This information has benefitted plant breeding research leading to great advancements in the bedding plant industry (Kelly et al., 2007; Craig, 2003). The genetics of petunia are also being studied by University of Florida (Clevenger *et al*., 2004) and Ohio State University (Jones *et al*., 2005) particularly with petunia sensitivity or insensitivity to ethylene.

Petunia cultivars are very diverse considering the multiple classes, forms and colors available. Plant breeding programs use field trials for evaluating new cultivars, but results may be ambiguous considering there has been no class standard cultivar used as comparison and also due to climate specific field trials (Kelly et al., 2007). Recently, the University of Florida evaluated 125 petunia cultivars in multiple field trials to establish petunia class standards that have been lacking in the industry. Cultivars were grouped by plant class, plant height, and flower color and pattern and were then evaluated and compared within these groups (Kelly et al., 2007). The authors chose a cultivar as a class standard using results of overall landscape performance ratings ($\geq$5.5 using scale 1 – 7), based on combination of foliage, flower, insect and disease symptom ratings. (Foliage ratings: 7=all plants in a plot had full uniform foliage, plants were free of arthropod and disease symptoms and abnormalities or weaknesses such as lodging; 4=average foliage density, minimal lodging, or some insect damage but foliage was still acceptable; 1=foliage sparse, stem lodging, or unacceptable pest damage making plants undesirable. Flower ratings: 7=flowers numerous, uniformly distributed overall plants, flowers were free from arthropod and disease symptoms; 4=average floral display, may have some pest damage but not severe enough to cause flowers to be unacceptable; 1=unacceptable flower number or display, or pest damage severe resulting in unattractive flowers) (Kelly et al., 2007). Results from this research will be helpful for future studies when choosing cultivars that are relevant and beneficial to the bedding plant industry.

2.6 LITERATURE CITED

Abdul-Baki, A.A., 1991. Tolerance of tomato cultivars and selected germplasm to heat stress. J. Am. Soc. Horticultural Sci. 116(6), 1113–1116.

Adams, S.R., P. Hadley, and S. Pearson. 1998. The effects of temperature, photoperiod, and photosynthetic photo flux on the time to flowering of petunia ‘Express Blush Pink.’ *Journal of the American Society for Horticultural Science* 123: 577-580.

Aldous, D.E. and J.E. Kaufmann. 1979. Role of root temperature in shoot growth of two Kentucky bluegrass cultivars. Agron J. 71: 545–547.

Aloni B., Pashkar T. and Karni L. 1991. Partitioning of [^{14}C] sucrose and acid invertase activity in reproductive organs of pepper plants in relation to their abscission under heat stress. Ann. Bot. 67, 371-377.

Armitage, A.M. 1985. Petunia, pp. 41-46 in *Handbook of Flowering*, vol. IV, A.H. Halevy, editor. CRC Press, Boca Raton, Florida.

Armond, P.A., U. Schreiber, O. Bjorkman. 1978. Photosynthetic acclimation to temperature in the desert shrub, *Larrea divaricata*: II. Light-harvesting efficiency and electron transport. Plant Physiol 61:411-415.

Ashraf, M. and M. Hafeez. 2004. Thermotolerance of pearl millet and maize at early growth stages: growth and nutrient relations. *Biologia Plantarum* 48 (1):81-86.

Ausubel, F.M., K. Bahnsen, and M. Hanson. 1980. Cell and tissue culture of haploid and diploid Petunia Mitchell. *Plant Mol. Biol. Newsletter* 1, 26-31.

Bailey, L.H. and E.Z. Bailey. 1976. Petunia, pp. 850-851 in *Hortus Third: A Concise Dictionary of Plants Cultivated in the United States and Canada*. Macmillan, New York.

Balogi, Z. 2003. Small heat shock proteins can stabilize heat stressed membranes. Acta Biol Szegediensis. 47: 53.

Berry, J and O. Bjorkman.1980. Photosynthetic response and adaptation to temperature in higher plants. Ann Rev Plant Physiol. 31: 491-543.

Bilger, W., U. Schreiber, M. Bock. 1995. Determination of the quantum efficiency of photosystem II and of non-photochemical quenching of chlorophyll fluorescence in the field. *Oecologia* 102:425-432.

Bjorkman, T. and K. Pearson. 1998. High temperature arrest of inflorescence development in broccoli (Brassica oleracea var. italica L.). J. of Exp. Botany, Vol. 49 (318):101–106.

Burke, John J. 1990. High Temperature Stress and Adaptations in Crops, pp.295-307 in *Stress Responses in Plants: Adaptation and Acclimation Mechanisms*. R.G. Alscher and J.R. Cumming, editors. Wiley-Liss, Inc. New York.

Charles, W.B. and R.E. Harris. 1972. Tomato fruit-set at high and low temperatures. Can. J. Plant Sci. 52,497–506.

Chen, H. H., Z.Y. Shen and P.H. Li. 1982. Adaptability of crop plants to high temperature stress. Crop Sci. 22: 719–725.

Clevenger, D.J., J.E. Barrett, H.J. Klee, and D.G. Clark. 2004. Factors affecting seed production in transgenic ethylene-insensitive petunias. J. Amer. Soc. Hort. Sci. 129:401-406.

Cockshull, K.E. and A.M. Kofranek. 1994. High night temperatures delay flowering, produce abnormal flowers and retard stem growth of cut-flower chrysanthemums. Scientia Horticulturae 56:217-234.

Craig, R. 2003. Creating a more beautiful world: A century of progress in the breeding of floral and nursery plants. *Hort-Technology* 30:928-936.

Dole, J.M. and H. F. Wilkins. 2004. Petunia, pp. 744-748 in *Floriculture : principles and species*, 2nd ed. D. Yarnell, K. Yehle, L. Dalberg, M. Carnis, editors. Pearson Education, Inc., Upper Saddle River, New Jersey.

El Ahmadi, A.B., Stevens, M.A., 1979. Reproductive responses of heat tolerant tomatoes to high temperatures. J. Am. Soc. Horticultural Sci. 104-5: 686–691.

Elhani, S., Y. Rharrabti, L.F. Roca and L.F. García del Moral. 2000. Evolution of chlorophyll fluorescence parameters in durum wheat as affected by air temperature. CIHEAM-IAMZ, p.275-277: Seminar on durum wheat improvement in the Mediterranean region: New Challenges, 04:12-14, Zaragoza, Spain.

Ellis, J 1987. Proteins as molecular chaperones. Nature. 328: 378-79.

Erwin, J., R. Heins, R. Berghage, and W. Carlson. 1989. How can temperatures be used to control plant stem elongation? Minnesota State Florists Bulletin 38(3):1-5.

Ewing, E.E. 1981. Heat stress and the tuberization stimulus. Am Potato J. 58: 31-49.

Gamon, J.A. and R.W. Pearcy. 1989. Leaf movement, stress avoidance and photosynthesis in *Vitis californica*. *Oecologia* 79:475-481.

Gerats, T. and M. Vandenbussche. 2005. A model system for comparative research: Petunia. *Trends in Plant Sci*. 10:251-256.

Guilioni, L., J. Wery and F. Tardieu. 1997. Heat stress-induced abortion of buds and flowers in pea: Is sensitivity linked to organ age or to relations between reproductive organs? Ann Bot. 80: 159-168.

Gounaris, K., W.P. Williams, P.J. Quinn. 1983. Heat stress induces non-bilayer lipid structures in chloroplast membranes. Biochem Soc Trans 11:388-389.

Gur, A.J. and Y. Shulman. 1979. The influence of root temperature on apple trees. IV. The effect on the mineral nutrition of the tree. J Hortic Sci. 54: 313–321.

Gusta, L.V., B.J. O'Connor and R.S. Bhatty. 1997. Flax (*Linum usitatissimum* L.) responses to chilling and heat stress on flowering and seed yield. Can. J. Plant Sci. 77:97-99.

Hall, A.E. 2001. Crop Response to Environment. CRC Press LLC, Boca Raton, FL.

Harding, S.A., J.A. Guikema, and G.M. Paulsen. 1990. Photosynthetic decline from high temperature stress during maturation of wheat. Plant Physiol. 92:654-658.

Hassane, S. M, E.K. Dodson and H.A. Koteiche 2002. Mechanism of Chaperone Function in Small Heat Shock Proteins J Biol Chem. 277: 40557-40566.

Havaux, M. 1993. Characterization of thermal damage to the photosynthetic electron transport system in potato leaves. Plant Sci. 94: 19–33.

Havaux, M. and F. Tardy. 1999. Loss of chlorophyll with limited reduction of photosynthesis as an adaptive response of Syrian barley landraces to high-light and heat stress. Aust. J. Plant Physiol. 26:569-578.

Holcomb, E.J. and J.W. Mastalerz. 1985. Seedling and seedling production, pp. 87-125 in *Bedding Plants III*, J.W. Mastalerz and E.J. Holcomb, editors. Pennsylvania Flower Growers, University Park, Pennsylvania.

Howarth, C.J. 1991. Molecular responses of plants to an increased incidence of heat-shock. *Plant Cell Environ.* 14: 831-841.

Huang, B., and Q.Xu. 2000. Root growth and nutrient element status of creeping bentgrass cultivars differing in heat tolerance as influenced by supraoptimal shoot and root temperatures. J Plant Nutr. 23: 979-990.

Ibrahim A M H and J S. Quick. 2001. Heritability of heat tolerance in winter and spring wheat. Crop Sci. 41:1401-1405.

Ismail, A.M., A. E. Hall and T.J. Close. 1999. Allelic variation of a dehydrin gene cosegregates with chilling tolerance during seedling emergence. Proc Natl Acad Sci (USA). 96: 13566-13570.

Jiao, J and B. Grodzinski 1996. The effect of leaf temperature and photorespiratory conditions on export of sugars during steady state photosynthesis in *Salvia splendens*. Plant Physiol. 111: 169-178.

Jones, H.G. and M.B. Jones. 1989. Introduction: some terminology and common mechanisms, pp. 1-10 in *Plants Under Stress*. Society for experimental biology, seminar series:39. H.G. Jones, T.J. Flowers and M.B. Jones, editors. Cambridge University Press, Cambridge.

Jones M.L., G.S. Chaffin, J.R. Eason, and D.G. Clark. 2005. Ethylene-sensitivity regulates proteolytic activity and cysteine protease gene expression in petunia corollas. J. Exp. Bot. 56:2733-2744.

Kaczperski, M.P., W.H. Carlson, and M.G. Karlsson. 1991. Growth and development of *Petunia x hybrid*a as a function of temperature and irradiance. *Journal of the American Society for Horticultural Science* 116:232-237.

Karlsson, M. 1996. Control of flowering in petunia by photoperiod and irradiance. *HortScience* 31:681. (Abstract)

Kelly, R.O., Z.N. Deng, and B.K. Harbaugh. 2007. Evaluation of 125 petunia cultivars as bedding plants and establishment of class standards. HortTechnology 17: 386-396.

Ketring, D.L. 1984. Temperature effects on vegetative and reproductive development on peanut. Crop Sci. 24:877-882.

Key J.L., C.Y. Lin, Y.M. Chen. 1981. Heat shock proteins of higher plants. *Proc Natl Acad Sci* USA 78: 3526-3530.

Kimpel, J.A., R.T. Nagao, V. Goekjian and J.L. Key. 1990. Regulation of the heat-shock response in soybean seedlings. *Plant Physiol*. 94: 988-995.

Larcher, W. 1994. Photosynthesis as a tool for indicating temperature stress events, pp.261-277. In, Ecophysiology of photosynthesis. Ecological studies 100. E.D. Schulze and M.M. Caldwell, editors. Springer-Verlag, Berlin.

Larcher, W. 1995. Stress due to extreme temperatures. In: Physiological Plant Ecology, 3rd ed. pp. 340-354.

Law, R.D. and S.J. Crafts-Brandner. 1999. Inhibition and acclimation of photosynthesis to heat stress is closely correlated with activation of ribulose-1,5-bisphosphate carboxylase/oxygenase. Plant Physiology (120):173–181.

Levitt, J. 1980. Responses of plants to environmental stresses. Volume I Chilling, Freezing and High Temperature Stress. Academic Press, New York

Lohar, D.P. and W.E. Peat. 1998. Floral characteristics of heat-tolerant and heat-sensitive cultivars of tomato (*Lycopersicon esculentum* Mill.) cultivars at high temperature. Scientia Horticulturae 73:53-60.

Maestri E., N. Klueva, C. Perrotta, M. Gulli, H.T. Nguyen and N. Marmiroli. 2002. Molecular genetics of heat tolerance and heat shock proteins in cereals. Plant Molecular Biology 48: 667-681.

Malkin, R. and K. Niyogi. 2000. Photosynthesis, pp.568-627 in *Biochemistry and Molecular Biology of Plants*. B. Buchanan, W. Gruissem, R.L. Jones, editors. American Society of Plant Physiologists, Maryland.

Marcum Kenneth B. 1998. Cell membrane thermostability and whole-plant heat tolerance of Kentucky bluegrass. Crop Sci. 38:1214–1218.

Martineau, J. R., J.E. Specht, J. H. Williams, and C.Y. Sullivan. 1979. Temperature tolerance in soybeans. I. Evaluation of a technique for assessing cellular membrane thermostability. Crop Sci. 19: 75–78.

Maxwell, K. and G.N. Johnson. 2000. Chlorophyll fluorescence – a practical guide. Journal of Exp Botany, vol.51 345:659-668.

Merritt, R.H., and J.C. Kohl, Jr. 1982. Effect of root temperature and photoperiod on growth and crop productivity efficiency of petunia. *Journal of the American Society for Horticultural Science* 107: 997-1000.

Myster, J., and R. Moe. 1995. Effect of diurnal temperature alterations on plant morphology in some greenhouse crops – a mini review. Scientia Horticulturae 62:205-215.
Nakamoto, H. and T. Hiyama. 1999. Heat-shock proteins and temperature stress, pp.399-416 in *Handbook of Plant and Crop Stress*. M. Pessarakli, editor. Marcel Dekker, New York.

Natarajan S. and J.S.Kuehny. 2008. Morphological, physiological, and anatomical characteristics associated with heat preconditioning and heat tolerance in *Salvia Splendens.* J. Amer. Soc. Hort. Sci. 133:527-534.

Necchi, A., N.E. Pogna and S. Mapelli. 1987. Early and late heat-shock proteins in wheats and other cereal species. *Plant Physiol*. 84: 1378-1390.

Ortiz, C. and L. Cardemil 2001. Heat-shock responses in two leguminous plants: a comparative study. J. Exp. Bot. 52:1711-1719.

Parsel, D. A and S. Lindquist. 1993. The function of heat shock proteins in stress tolerance: degradation and reactivation of damaged proteins. Ann Rev Genet. 27: 437-496.

Piringer, A.A., and H.M. Cathey. 1960. Effect of photoperiod, kind of supplemental light and temperature on the growth and flowering of petunia plants. *Proceedings of the American Society for Horticultural Science* 76: 649-660.

Pollock, C.J., C.F. Eagles, C.J. Howarth, P.H.D. Schunmann, and J.L. Stoddart. 1993. Temperature Stress, pp. 109-127 in Plant Adaptation to Environmental Stress. L. Fowden, T. Mansfield, and J. Stoddart, editors. Chapman & Hall, London.

Queitsch, C., S.W. Hong, E. Vierling, and S. Lindquist. 2000. Heat Shock Protein 101 plays a crucial role in thermotolerance in arabidopsis. Plant Cell. 12: 479–492.

Raison, J.K., J.A. Berry, P.A. Armond, and C.S. Pike. 1980. Membrane properties in relation to the adaptation of plants to temperature stress. p. 261–273. *In* N.C. Turner and P. J. Kramer (ed.) Adaptation of plants to water and high temperature stress. John Wiley & Sons, New York.

Rohacek, K. and M. Bartak. 1999. Technique of the modulated chlorophyll fluorescence: basic concepts, useful parameters and some applications. Photosynthetica 37 (3):339-363.

Sato, S., M. Peet and R. Gardner. 2001. Formation of parthenocarpic fruit, undeveloped lowers and aborted flowers in tomato under moderately elevated temperatures. Scientia Horticulturae 90:243-254.

Smillie, R.M., C. Critchley, J.M. Bain, R. Nott. 1978. Effect of growth temperature on chloroplast structure and activity in barley. Plant Physiol 62:191-196.

Stidham, M.A., E.G. Uribe, G.J. Williams. 1982. Temperature dependence of photosynthesis in *Agropyron smithii* Rydb: II. Contribution from electron transport and photophosphorylation. Plant Physiol 69:929-934.

Sung, D., F. Kaplan, K. Lee and C. L. Guy. 2003. Acquired tolerance to temperature extremes. *TRENDS* in Plant Sci. vol.8 (4):179-187.

van Kooten O. and Jan F.H. Snel. 1990. The use of chlorophyll fluorescence nomenclature in plant stress physiology. *Photosynthesis Research* 25:147-150.

Vierling, E. 1991 The roles of heat-shock proteins in plants. Annu Rev Plant Physiol Plant Mol Biol. 42: 579-620.

Warner, R.M., and J.E. Erwin. 2001. Temperature, pp. 10-17 in *Tips on Regulating Growth of Floriculture Crops*, M.L. Gaston, L.A. Kunkle, P.S. Konjoian, and M.F. Wilt, editors. Ohio Florists' Association Services, Columbus, Ohio.

Warrag, M.O.A. and A.E. Hall. 1984a. Reproductive responses of cowpea [*Vigna unguiculata* (L.) Walp.] to heat stress. I. Responses to soil and day air temperatures. *Field Crops Res*. 8: 3-16.

Warrag, M.O.A. and A.E. Hall. 1984b. Reproductive responses of cowpea [*Vigna unguiculata* (L.) Walp.] to heat stress. II. Responses to night air temperatures. *Field Crops Res*. 8: 17-33.

Weidner, E. 1994. Supertunias™ - More than just a petunia. *Ohio Florists' Association Bulletin* 777: 4-5.

Weis E. and J.A. Berry. 1987. Quantum efficiency of photosystem II in relation to 'energy' dependent quenching of chlorophyll fluorescence. Biochim Biophys Acta 894: 198-208.

Wilkins, H.F., and H.R. Pemberton. 1981. Interaction of growth regulators and light. *Proceedings 14th International Bedding Plant Conference*, Seattle, Washington, 14:182-188.

Willits, D.H. and D.A. Bailey. 2000. The effect of night temperature on chrysanthemum flowering: heat-tolerant versus heat-sensitive cultivars. Scientia Horticulturae 83:325-330.

Wu, M. and S. Wallner. 1984. Heat stress responses in cultured plant cells: heat tolerance induced by heat shock *versus* elevated growing temperature. Plant Physiol. 75:778-780.

Xu, Z.Z. and G.S. Zhou, 2006. Combined effects of water stress and high temperature on photosynthesis, nitrogen metabolism and lipid peroxidation of a perennial grass *Leymus chinensis*. Planta 224:1080-1090.

CHAPTER 3. EFFECT OF HIGH TEMPERATURE STRESS AND HEAT HARDENING (PRECONDITIONING) ON GROWTH AND DEVELOPMENT OF PETUNIA

3.1 INTRODUCTION

Bedding plants continue to have great economic value in the Floriculture industry. The ability to have multi-seasonal production throughout the year is a profitable investment for growers and retailers. However, production times and availability differs greatly among bedding plant species and is greatly influenced by the location and climate of growers (Mahan et al., 1997). Species with unique growing recommendations and temperature requirements may be problematic for some producers. High temperature stress is one of the greatest challenges negatively affecting production of bedding plants (Natarajan, 2005). Plant growth, development and yield can be greatly reduced due to exposure from heat stress (Gusta et al., 1997; Harding et al., 1990). Heat stress has been a hindrance to many producers where proper growing recommendations are sometimes unachievable. This is especially true during the late summer months and for nursery producers and greenhouse growers in the southern United States (Wehner and Watschke, 1987). Markets in these regions have evolved to produce bedding plants best suited to their environment or at least limiting production to cooler months of the year. However, spring and early fall continue to be the seasons with the highest demand for plant material and therefore, the highest profit potential. Heat stress is a primary factor during production in most all regions during late summer but growers must still produce plants for the landscape that have cooler optimum growing temperatures for fall planting. However, production quality of these plants and ultimate landscape performance is usually decreased in these circumstances. Effects of heat stress and plant tolerance is vital for survivability in stressful environments (Lichtenthaler, 1996). There are few research based recommendations on beneficial production practices where environmental stresses are an issue (Hancheck and Cameron, 1995). Acquired thermotolerance for bedding plants would be most useful in regions where high temperatures negatively affect greenhouse production and landscape performance. From a consumer standpoint, acquired thermotolerance would not only improve landscape performance but also plant survivability during prolonged heat stress. Production practices that induce acquired thermotolerance would be beneficial by decreasing cooling costs and allow growers to take advantage of the ‘stressful’ growing environment. Using resources that are naturally available would also promote a more sustainable approach in greenhouse production.

There has been little research in quantifying physiological and morphological responses to heat stress on bedding plants. Research by Natarajan (2005) found whole plant responses in *Salvia splendens* F. Sellow ex Roem & Schult. of adaptable morphological and physiological characteristics associated with heat tolerance. However, this information has not been investigated for other bedding plants in a greenhouse or landscape setting. Evaluating responses of bedding plants to prolonged or short duration heat stress during greenhouse production is crucial before attempting a protocol for inducing acquired thermotolerance. Understanding and quantifying these responses will determine which morphological traits are most sensitive and if they are beneficial or deleterious to marketability and survivability of a plant. *Petunia x hybrida* ‘Midnight’ from the Dreams series was chosen as the model bedding plant for this research. Petunia is a popular bedding plant that has had more genome mapping than any other bedding plant and information may be essential to geneticist and easily transferred between related important horticultural species (Gerats and Vandenbussche, 2005). The objective of these experiments was to determine the effect of enduring high temperature stress and short duration heat shock (preconditioning) on growth and development of *Petunia x hybrida*.

3.2 MATERIALS AND METHODS

3.2.1 Plant Material

Petunia x hybrida was chosen as a model for the following experiments. Plugs (288 cell tray) (4.9 cm^3) of the petunia variety Dreams™ ‘Midnight’ (Ball Plug Network, Ball Horticultural Company, West Chicago, IL) were obtained for the use of this study. Petunia Dreams™ ‘Midnight’ (Ball Horticultural Company, West Chicago, IL) is a grandiflora class petunia with large dark purple flowers that bloom spring to fall and has a mounding growth habit. The average mature height and width is 25-38 cm and 30-45 cm respectively. This variety of petunia has an optimum growing temperature range of 22-26°C (d) and full sun requirement (PanAmerican Seed Company, West Chicago, IL). Upon arrival, plug trays were immediately removed from packaging and maintained in a greenhouse for 5 d at 26°C day/night before transplanting.

3.2.2 Greenhouse Establishment Prior to Treatments

Greenhouses used for this study were located at Campus Greenhouses 440-7 and 440-8 at Louisiana State University, 30° N 91° W Baton Rouge, Louisiana. A total of three polycarbonate covered greenhouses with 40% shade cloth (1000 µmol m^{-2} s^{-1}) were used for each temperature treatment. Each greenhouse has respective automated heating and cooling systems using Wadsworth STEP© Control 50A (Wadsworth Control Systems Incorporated, Arvada, CO) with day/night 12 h temperature settings. Prior to transplanting, the day/night temperatures of control greenhouse was recorded for temperature consistency for one week using HOBO® Pro Series™ data logger (Onset Computer Corporation, Bourne, MA) (Figure 3.1) and also daily visual recordings of minimum and maximum thermometer temperatures. On 15 August 2007, petunia Dreams ‘Midnight’ plugs were transplanted into (650 cm^3) plastic pots using a middleweight media Fafard™4M Mix (Conrad Fafard, Incorporated, Agawam, MA) media. One plant was transplanted into each pot and was placed on greenhouse benches. Broad spectrum fungicide was applied as a drench to each pot after one week transplant at a rate of 19.5 ml/L (Banrot® 8G, a.i. 3% Etridiazole, a.i. 5% Thiophanate-methyl, Scotts-Sierra, Marysville, OH). Petunias were grown at 30/20°C and fertigated using Hozon™ Brass Siphon Mixer (1:16) (Phytotronics, Incorporated, Earth City, MO) with 200 ppm N 15N-2.2P-12.4K (15-5-15 Cal Mg, Scotts-Sierra, Marysville, OH) daily for two weeks to allow for root growth and proper establishment within the pot.

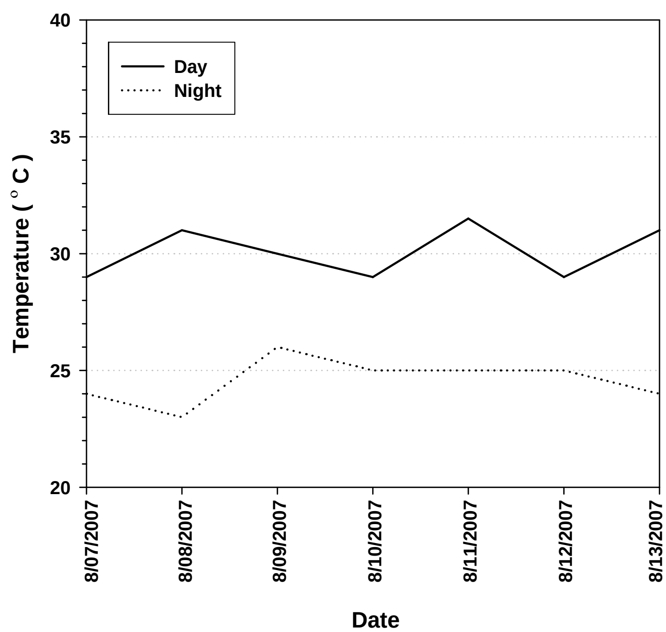

Figure 3.1 Average day/night temperatures of control greenhouse (30/25°C) for one week prior to transplanting petunia.

3.2.3 Enduring High Temperature Stress

Enduring heat stress treatments were applied to petunia after two weeks growth at 30/25°C in the greenhouse. Petunia optimum growing temperature is 26°C; however, due to difficulty to maintain consistent cool temperatures in greenhouse with seasonal Louisiana heat, the average day/night control temperature was 30/25°C. This temperature was the closest consistent temperature to optimum growing temperature and was an ideal representative growing temperature for greenhouse growers in the southern United States at this time of year. Heat stress challenging temperatures were chosen in 5°C increments increasing from the control temperature, 30/25°C. Heat stress treatment includes control temperature 30/25°C day/night or enduring heat stress at 35/25 or 40/30°C day/night for 5 weeks. A total of 252 petunias were exposed to each heat stress treatment where 36 of these plants were transplanted after 5 weeks exposure into the field for observation. The other set of 212 plants were divided into weekly harvests to determine growth and development for a total 5 week exposure period in the greenhouse. Temperature treatments were applied in respective greenhouses and recorded using HOBO® Pro Series™ data logger (Onset Computer Corporation, Bourne, MA) (Figure 3.2 A, B & C). Weather records, including temperature (°C) and natural irradiance (μmol m^{-2} s^{-1} PPFD), were also taken daily for the duration of the experiments (Louisiana Agriclimatic Information Systems, LSUAgCenter, BAE) (Figure 3.3 A & B).

3.2.4 Short Duration Heat Shock (Preconditioning)

Petunias were grown for 2 weeks after transplant at 30/25°C prior to exposure to heat shock treatments. Petunias are kept in 25 cm x 50 cm plastic trays with 4 pots per tray for treatment efficiency. Plants in trays were manually moved between greenhouse sections that were set to a constant specific heat shock treatment temperature. Heat shock treatment includes petunia grown at 30/25°C where plants in trays were removed and exposed to 35 or 40°C for 2 h every 7 d for 5 weeks. After 2 h heat shock duration, plants were returned to 30/25°C and remained until following weekly exposure. A total of 252 petunias were exposed to each heat stress treatment where 36 of these plants were transplanted after 5 weeks exposure into the field for observation. The other set of 212 plants were divided into weekly harvests to determine growth and development for a total 5 week exposure period in the greenhouse. During the continuous heat stress treatments and prior to heat shock exposure, plants were irrigated to minimize water stress during treatment. Plants were destructively harvested weekly 3 d after heat shock for data collection.

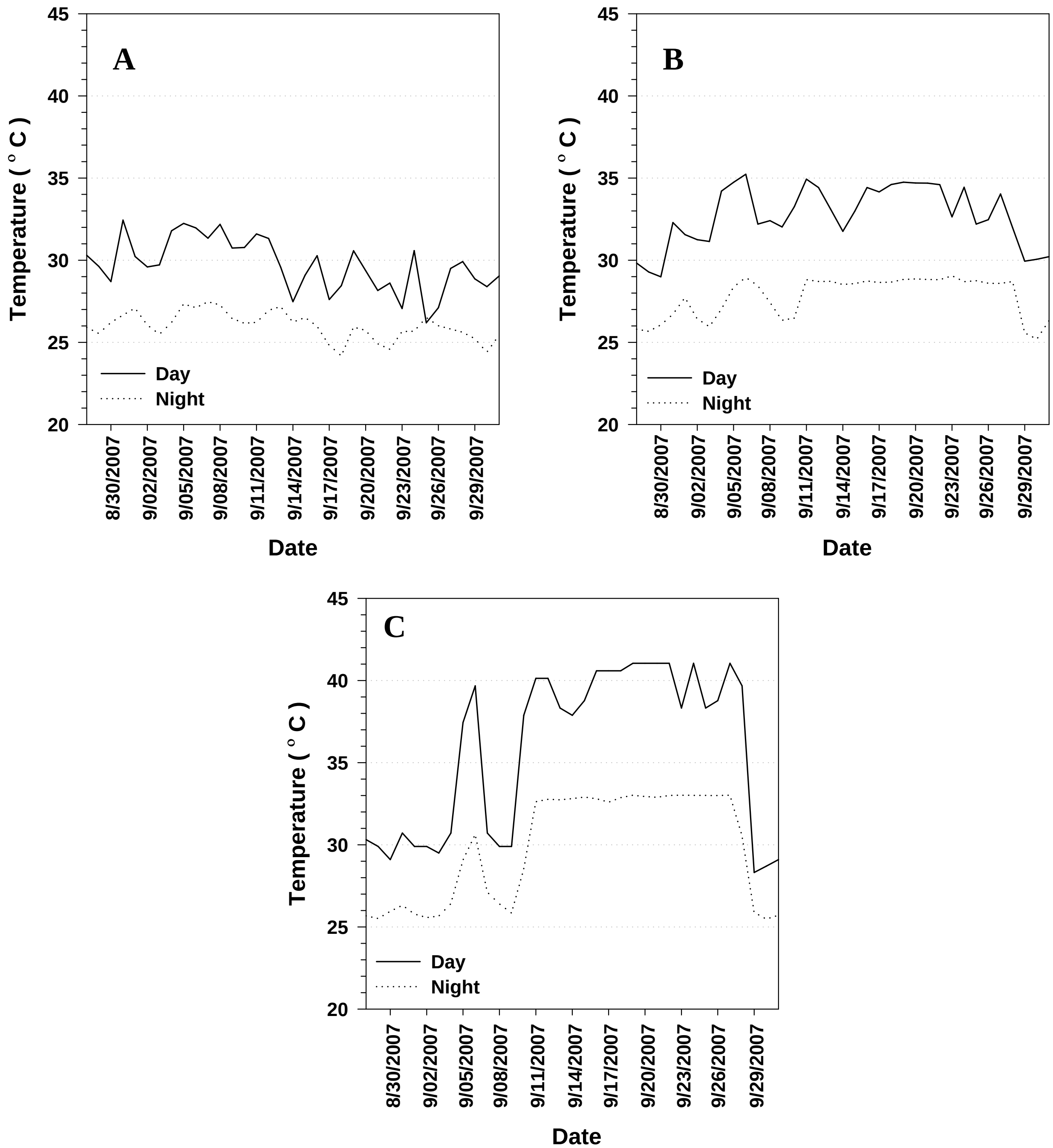

Figure 3.2 Average day/night temperatures (°C) recorded in greenhouses for each temperature treatment over 5 week course of experiment. A) Control 30/25°C B) 35/25°C C) 40/30°C

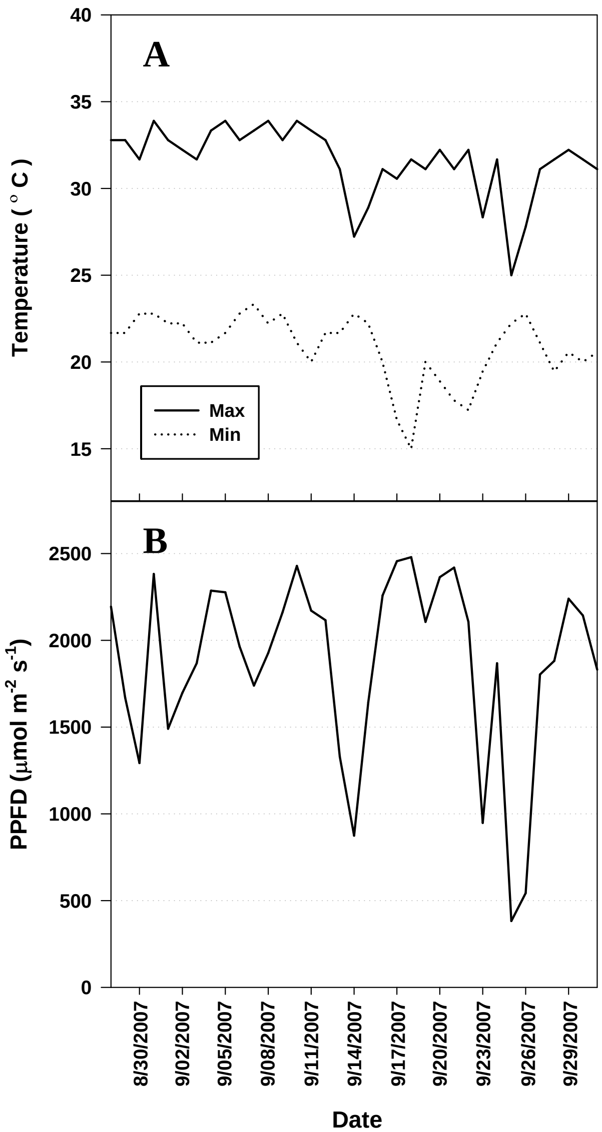

Figure 3.3 Average daily weather reports for Baton Rouge, Louisiana during enduring heat stress and heat shock experiments. A) minimum and maximum daily temperatures (°C) and B) natural irradiance (μ mol m^{-2} s^{-1} PPFD).

3.2.5 Measurement of Growth and Development in the Greenhouse

For both studies described above a set of plants from each study were destructively harvested every 7 d for 5 weeks. Petunia growth and development was quantified by measuring number of flowers per plant, average flower size (cm) per plant and shoot dry weight (g). Fully expanded flowers were visually counted and recorded weekly. Flower size was determined by using a handheld metric ruler and measuring the diameter (cm) of one flower visually estimated to be average for that respective plant. After flower measurements were taken, petunia shoots were cut at the soil line in the pot and placed in labeled paper bag. Labeled samples were oven dried at 80°C for 24 h before obtaining dry weights.

3.2.6 Field Study Establishment

Postproduction was evaluated by transplanting heat stressed or heat shocked plants in field trial landscape beds. A broad spectrum fungicide (Banrot® 8G, a.i. 3% Etridiazole, a.i. 5% Thiophanate-methyl, Scotts-Sierra, Marysville, OH) was applied in the greenhouse 3 d prior to field transplant. Petunias were transplanted immediately after greenhouse treatments on 1 October 2007. The field trial was located at LSU AgCenter Burden Center, 30° N 91° W Baton Rouge, Louisiana. The landscape trial was implemented in raised beds (1.5 m wide by 43 m long) which consisted of an Olivier silt loam soil amended with composted pine bark. Petunia were planted using 30 x 30 cm spacing. Irrigation was used around the edges of the raised bed where spray nozzles were spaced 1 m apart. Plants were irrigated as needed. Weather records were taken daily from the Burden Center weather station for the duration of the field trial (Louisiana Agriclimatic Information Systems, LSUAgCenter, BAE) (Figure 3.4 A & B). Petunias were allowed to establish one week before data measurements were taken.

3.2.7 Field Data Collection

Growth and development and landscape performance of treated petunias were evaluated over a 3 week period where measurements were taken weekly. Data collected included number of flowers per plant, average flower size (cm), cross-sectional diameter of whole plant, and quality ratings. Quality rating scale ranged from 1 to 5, where 1=dead and 5= optimum performance. Quality is based on a combination of plant flowering, leaf color and compactness

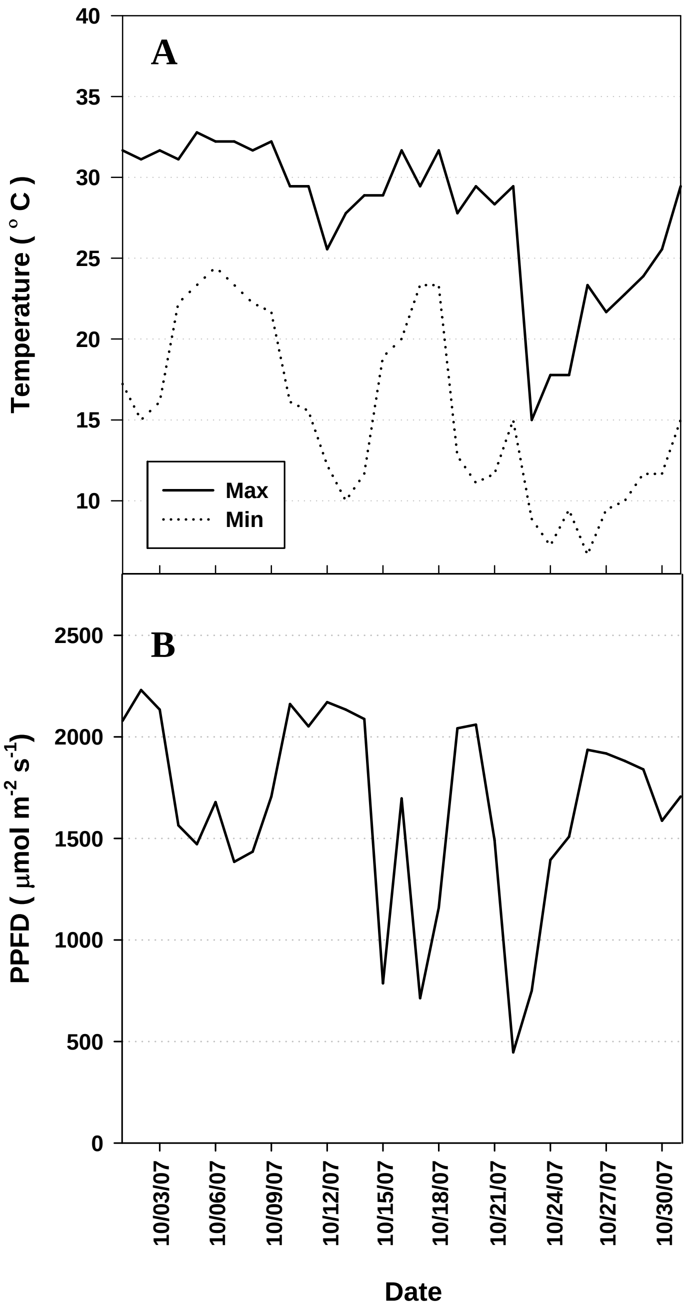

Figure 3.4 Average daily weather reports for Burden Center, Baton Rouge, Louisiana during field evaluation of heat stressed and heat shocked *Petunia x hybrida* Dreams 'Midnight'. A) minimum and maximum daily temperatures (°C) and B) natural irradiance (µ mol m^{-2} s^{-1} PPFD).

of plant. Quality scores; 5 to 4.5 = excellent plants with healthy green leaves, compact uniform growth and good inflorescence, 4.5 to 3.5 = green healthy foliage with moderate flowers, 3.5 to 2.5 = plants with chlorotic leaves and poor inflorescence, 2.5 to 1.5 = plants with necrotic or dried leaves with terminal bud damage and poor flower set, <1.5 = dead. Due to problems with the trial beds for those plants treated with heat shock, field data was collected on only the heat stressed plants.

3.2.8 Experimental Design and Statistical Analysis

Petunia plants grown for greenhouse experiments were arranged in a complete randomized block design on benches. Four blocks were used each consisting of six replicate plants. Plants exposed to heat shock treatment were arranged randomly within trays that were also completely randomized on the benches. Statistical analysis was performed using SAS ProcMixed Procedure (Statistical Analysis Software, version 9.1, Cary, NC).

3.3 RESULTS

3.3.1 Effect of Enduring High Temperature Stress on Growth and Development of Petunia during Greenhouse Production

- **Flowers per Plant**

Petunia Dreams 'Midnight' grown at 30/25, 35/25 or 40/30°C showed significant differences in flower count over a 5 week period (Fig 3.5). Flower count increased at all temperatures after the first week. However, petunias grown at 30/25°C had significantly more flowers per plant for all weeks. After the first week, there was no difference in flower count in petunias grown at 35/25 and 40/30°C. After two weeks of heat stress exposure, differences in flower count appeared at the higher temperatures. Flower count of petunias grown at 35/25°C increased at weeks 3 and 4 while those grown at 40/30°C decreased. Flower count at control temperature (30/25°C) remained significantly higher than 35/25 and 40/30°C during this time. After week 4, there was a significant decrease in flower counts for petunias grown at 30/25 and 35/25°C while flower count at 40/30°C remained unchanged. At 5 weeks, continuous exposure to 30/25°C had significantly higher flower counts than the higher temperatures; however counts decreased and resembled flower numbers recorded at week 3. At 5 weeks, flower counts of

35/25°C grown petunias significantly decreased and showed no difference between flowers counts of petunias grown at 40/30°C.

- **Average Flower Size**

Temperature had a significant effect on the average flower size of petunia Dreams 'Midnight'. After 5 weeks exposure to enduring temperatures of 30/25, 35/25 or 40/30°C, petunias grown at 30/25°C had a significantly larger flower size compared to petunias grown at higher temperatures (Fig. 3.6). Similar to flower count results, flower size was not significantly different between petunia grown at 35/25 and 40/30°C. Petunia Dreams 'Midnight' also displayed underdeveloped, misshaped and longer corolla tubes in some flowers grown at 40/30°C with some flowers showing bleaching of dark purple petal pigmentation (Fig. 3.7 A and B).

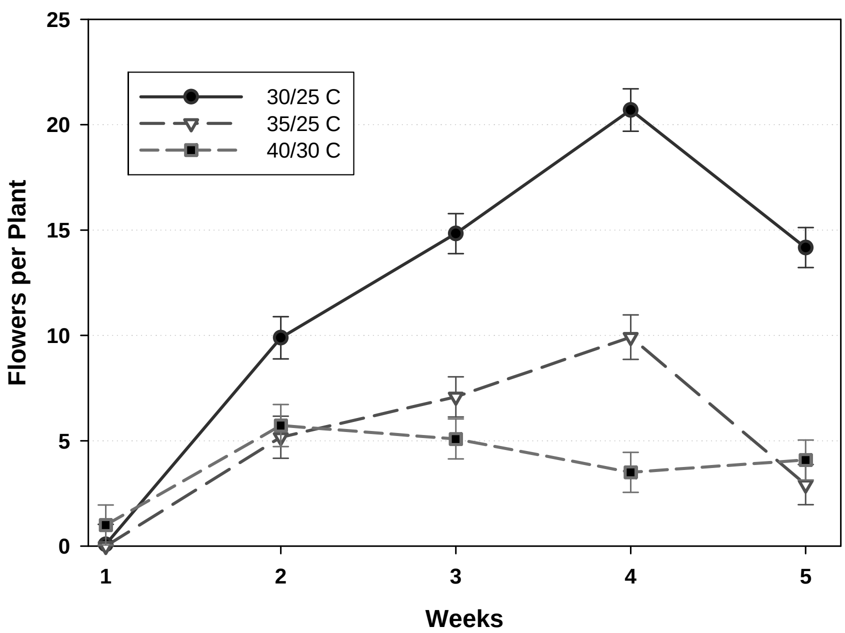

Figure 3.5. The effect of enduring high temperature stress on flower production during greenhouse production of *Petunia x hybrida* Dreams 'Midnight'. Petunias were exposed to 30/25, 35/25 or 40/30°C day/night for a 5 week duration. Error bars represent mean of six measurements ± standard error.

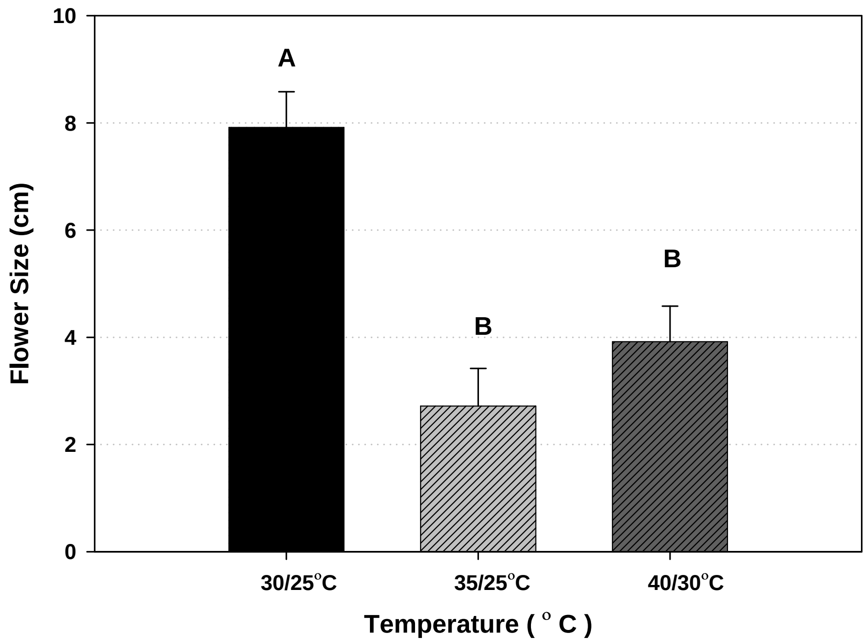

Figure 3.6. Average flower size (cm) of *Petunia x hybrida* Dreams 'Midnight' when exposed to 30/25, 35/25 or 40/30°C day/night for 5 weeks in the greenhouse. Means with different letters are significantly different at $P<0.05$ (Tukey's Test).

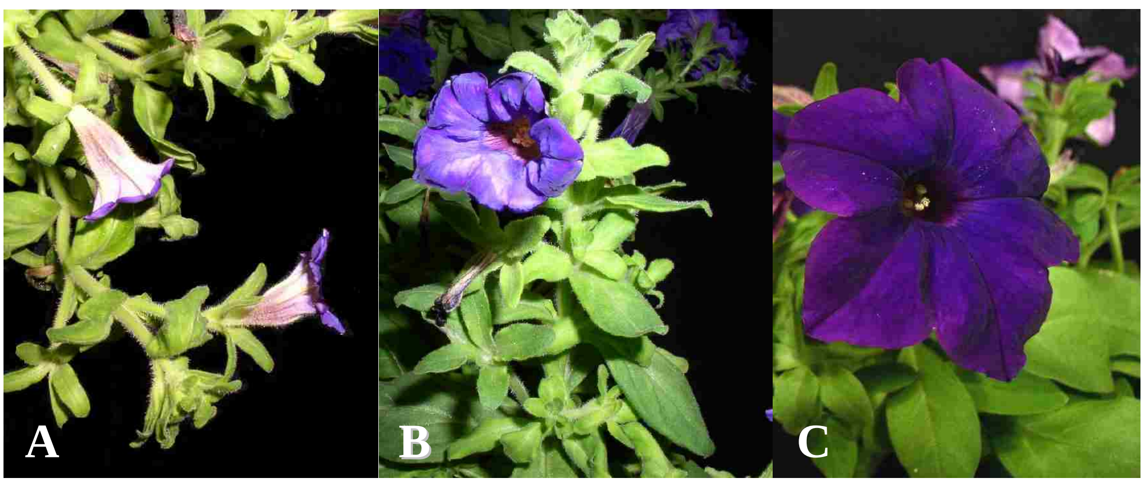

Figure 3.7. Effect of heat stress on flower development in *Petunia x hybrida* Dreams 'Midnight' after 5 weeks of exposure. A) Elongated corolla tube with smaller petal width observed in petunia grown at 40/30°C, B) Asymmetric and non-fully expanded petals accompanied by bleaching and striations of petal pigmentation in petunia grown at 40/30°C, C) Flower of petunia grown at 30/25°C.

- **Shoot Dry Weight**

Petunia Dreams ‘Midnight’ showed an increase in shoot dry weight (g) over the 5 week duration (Fig.3.8). Petunias grown at 30/25 and 35/25°C displayed similar trends but showed no significant difference in shoot dry weight. Petunias grown at 40/30°C also shared a similar trend to 30/25 and 35/25°C during the first 4 weeks, but significantly decreased shoot dry weight at the end of 5 weeks. Although shoot dry weight did not show a significant difference until the last week, petunias grown at 35/25 and 40/30°C appeared much smaller in biomass with shortened internodes and smaller leaves (personal observation).

3.3.2 Short Duration Heat Shock (Preconditioning)

Results from petunias grown at 30/25°C and heat shocked at 35 or 40°C for 2 h every 7 d showed few differences among growth parameters measured (Table 3.1). All flower counts increased over the first 4 weeks and decreased at week 5 (Table 3.2). However, petunias heat shocked at 35 or 40°C showed no difference in number of flowers per plant when compared to control temperature. Similar results were found for average flower size of heat shocked plants. Results indicated no significant difference between flower size of plants grown at 30/25°C and plants heat shocked at 35 or 40°C. Shoot dry weight increased over the 5 week period for all plants, but no significant difference was observed between the three temperature treatments (Table 3.2).

Table 3.1. The effect of heat shock treatment on *Petunia x hybrida* Dreams ‘Midnight’ flower count, flower size and shoot dry weight as indicated by significance of fixed effects and interactions.

Effect	Temperature	Week	Temperature*Week
Flower Count	0.2667 NS	0.0135 *	0.1364 NS
Flower Size	0.5619 NS	NS	NS
Shoot Dry Weight	0.3556 NS	<0.0001*	0.8698 NS

Values significant (*) or not significant (NS) at the 5% level by the lsmean procedure in SAS with Tukey’s correction.

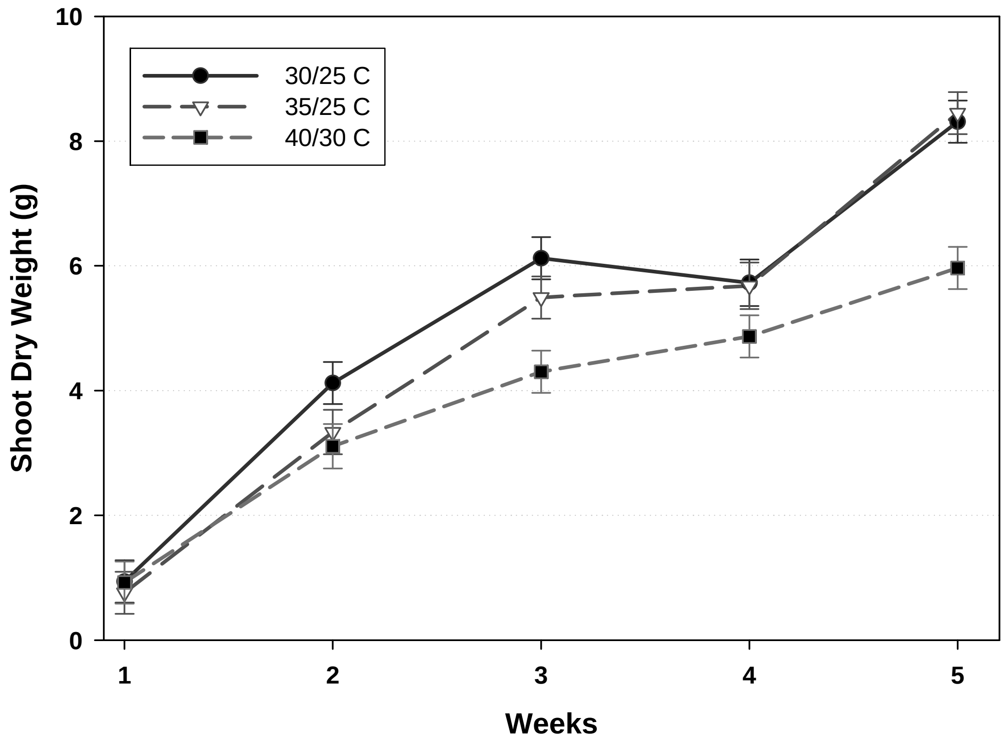

Figure 3.8. Effect of enduring high temperature on shoot dry weight (g) of *Petunia x hybrida* Dreams 'Midnight' when exposed to 30/25, 35/25 or 40/30°C day/night for 5 weeks in the greenhouse. Error bars represent mean of six measurements ± standard error.

Table 3.2. The effect of heat shock treatment on *Petunia x hybrida* Dreams 'Midnight' flower count and shoot dry weight (g) at each week of production in the greenhouse.

Week	Flowers per Plant	Shoot Dry Weight (g)
1	[Y]0.99 d[X]	0.89 d
2	10.10 bc	2.56 c
3	11.40 b	3.95 c
4	16.00 a	5.16 b
5	14.13 b	8.03 a

(Heat shock treatment included growth at 30/25°C (control) and heat shock at 35 or 40°C for 2 h every 7 d for 5 weeks).

[X] Means within columns followed by the same letters are not significantly different at 5% by lsmean procedure in SAS with Tukey's correction.

[Y] Values in the table are averages (n=18).

3.3.3 Field Study

- **Flowers per Plant**

Petunias Dreams ‘Midnight’ exposed to enduring high temperatures in the greenhouse showed differences in the number of flowers per plant after 3 weeks observation in the landscape. Flowers counts of petunias grown at 40/30°C in the greenhouse significantly decreased in the field (Fig. 3.9). At the end of the field trial, petunias grown at 30/25 and 35/25°C had significantly greater flower counts compared to petunias grown at 40/30°C. However, no difference in the number of flowers per plant was indicated between plants grown at 30/25 and 35/25°C.

No field measurements were taken on petunias exposed to short duration heat shock due to poor field establishment.

- **Average Flower Size (cm)**

Petunias exposed to heat stress during greenhouse production displayed an increase in average flower size during the first two weeks in the field followed by a decrease in size at the third week for all temperature treatments. After 3 weeks observation in the field, overall average flower size was not significant between petunias grown at enduring 30/25, 35/25 or 40/30°C in the greenhouse (Table 3.3).

Table 3.3. The effect of enduring heat stress during greenhouse production on average flower size after 3 weeks in the landscape.

Week	**Temperature (°C)**		
	30/25°C	35/25°C	40/30°C
1	[Y]7.00 a[X]	6.66 abc	6.91 ab
2	6.55 abc	6.66 abc	7.00 a
3	6.33 abc	5.66 bc	5.41 c

[X]Mean values followed by different letters indicate significant difference at $P<0.05$ using Tukey’s Test.
[Y]Values in table are averages (n=6).

- **Cross-Sectional Diameter of Whole Plant**

Cross-sectional diameter of petunias increased during the 3 weeks in the field for all temperature treatments. Petunia cross-sectional diameter at week 1 was significantly less than petunias after 3 weeks for all temperature treatments (Table 3.4). Cross-sectional diameter was not significant between temperatures within each week measured. However, petunias grown at 30/25°C appeared to show a more rapid increase in size after week 1 then slowed after week 2, while petunias grown at 35/25°C slowed after week 1 then increased more rapidly after week 2 (Table 3.5). Petunias grown at 40/30°C in the greenhouse did not vary as greatly in cross-sectional diameter in the landscape as the other temperatures.

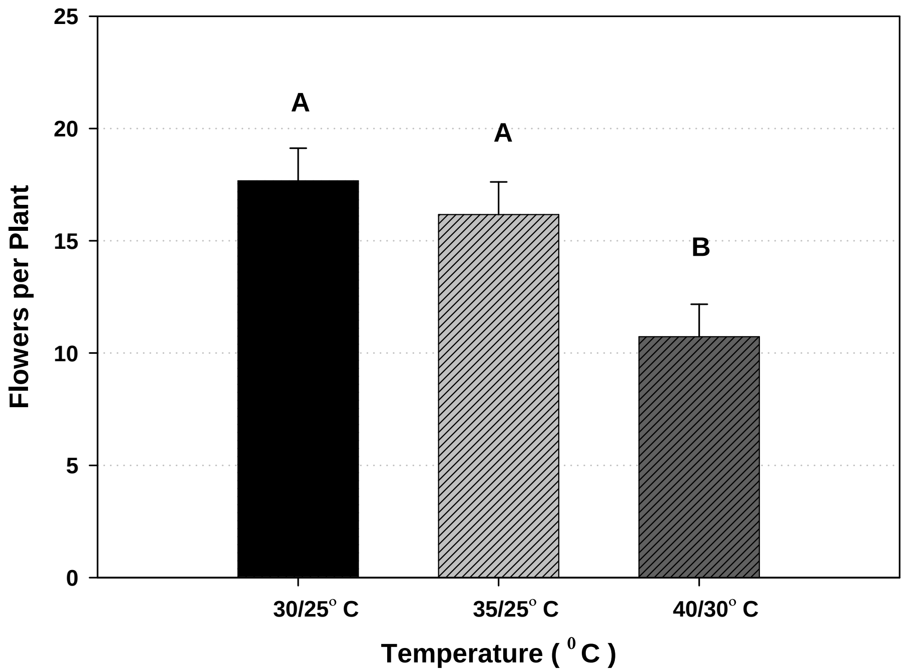

Figure 3.9. The effect of enduring high temperatures (30/25, 35/25 or 40/30°C) during greenhouse production on flower count of field transplanted *Petunia x hybrida* Dreams 'Midnight'. Flower counts from field petunias exposed to respective greenhouse temperatures were taken over a 3 week period. Means with different letters are significantly different at P<0.05 (Tukey's Test).

Table 3.4. Cross-sectional diameter (cm) for all temperature treatments of *Petunia x hybrida* Dreams 'Midnight' after 3 weeks in the landscape.

Week	Cross-sectional diameter
1	[Y]19.55 b[X]
2	21.69 ab
3	22.52 a

[X]Mean values followed by different letters within columns are significantly different at P<0.05 using Tukey's Test.
[Y]Values in table are averages (n=18).

Table 3.5. The effect of enduring heat stress during greenhouse production on *Petunia x hybrida* Dreams 'Midnight' cross-sectional diameter over 3 weeks in the landscape.

Week	Temperature (°C)		
	30/25°C	35/25°C	40/30°C
1	[Y]19.00 b[X]	20.50 ab	19.16 b
2	23.25 ab	21.08 ab	20.75 ab
3	23.33 ab	24.58 a	19.66 ab

[X]Mean values followed by different letters are significantly different at P<0.05 using Tukey's Test.
[Y]Values in table are averages (n=6).

- **Field Quality Ratings**

Petunias exposed to enduring high temperatures in the greenhouse showed differences in quality after transplant in the field. Petunias grown at 30/25 and 35/25°C exhibited significantly higher quality ratings in the field than plants grown at constant 40/30°C (Fig. 3.10). Quality ratings were also significant from week to week between temperatures, with week 1 showing the greatest effect of enduring heat stress (Table 3.6). Petunia grown at 30/25°C had significantly increased quality at week 1, decreased at week 2 and was not significant at the third week. Petunia grown at 35/25°C showed no difference in quality at each week. Similarly, plant quality of petunia grown at 40/30°C was not affected between weeks but displayed significantly decreased quality at week one compared to petunia grown at control (30/25°C).

Table 3.6. The effect of enduring heat stress during greenhouse production on plant quality at each week in the landscape.

Week	Temperature (°C)		
	30/25°C	35/25°C	40/30°C
1	[Y]3.75 a[X]	3.16 ab	2.33 b
2	2.50 b	3.25 ab	2.50 b
3	3.25 ab	2.83 ab	2.83 ab

[X]Mean values followed by different letters within columns are significantly different at P<0.05 using Tukey's Test.
[Y]Values in table are averages (n=6).

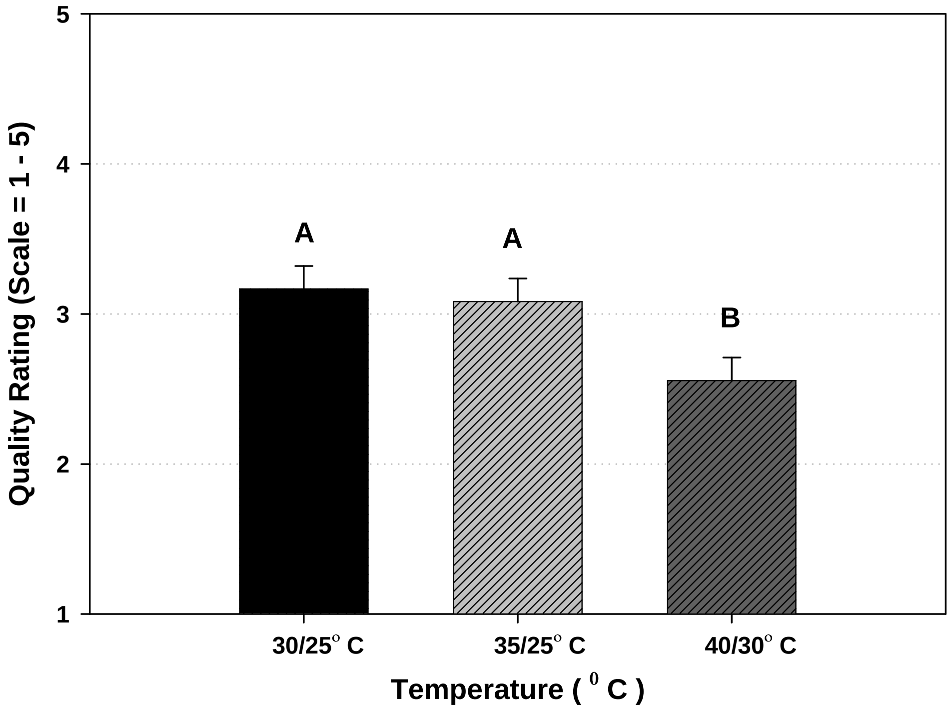

Figure 3.10. Effect of greenhouse temperature on plant quality after 3 weeks in the field. Quality ratings 1 – 5 where 1=dead and 5=optimum field performance. Means with different letters are significantly different at P<0.05 (Tukey's Test).

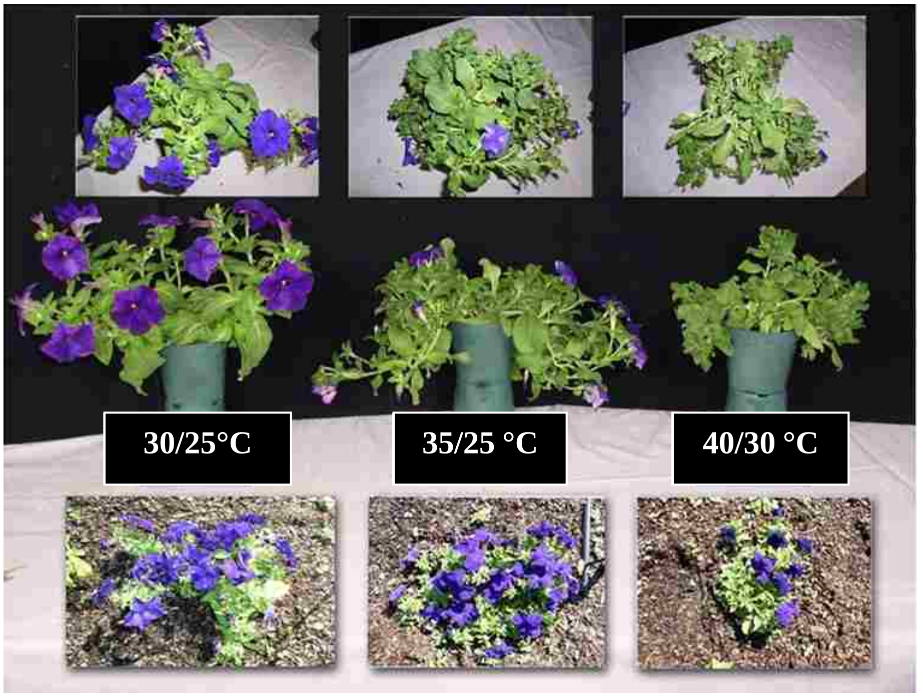

Figure 3.11. Pictorial representation of the effect of heat stress on petunia after 5 weeks treatment in the greenhouse and after 3 weeks transplanted in the landscape. Top and middle side views of petunia grown at 30/25, 35/25 and 40/30°C respectively, for 5 weeks in the greenhouse. Bottom pictures represent greenhouse grown (30/25, 35/25 and 40/30°C) petunias after 3 weeks transplant in the landscape.

- **Greenhouse Production vs. Landscape Performance**

Temperature treatments of 30/25, 35/25, or 40/30°C had a significant effect on growth and development of petunia Dreams 'Midnight' over 5 weeks in the greenhouse but had a different effect in the landscape (Fig. 3.11). Petunias grown at 30/25°C performed best in the greenhouse with significantly more flowers per plant and larger average flower size. In the landscape, petunia grown at 30/25 and 35/25°C showed no significant difference in flower count or quality while there was no significant difference in average flower size for all temperature treatments

3.4 DISCUSSION

Enduring temperature stress during greenhouse production had significant morphological effects on petunia Dreams 'Midnight'. Results indicated that petunia growing at 30/25°C had the greatest number of flowers per plant while flower counts of petunias at the 40/30°C were severely reduced. These results are consistent with Guilioni et al. (1997) who found an increase in bud and flower abortion in peas (*Pisum sativum* L.) when exposed to heat stress (moderate stress: 31°C for 6 h during day and 13°C at night for 4 d or severe stress: 33/30°C day/night for 2 d followed by 4 d of moderate stress treatment). Interestingly, the study by Guilioni et al. (1997) found that severe stress treatment with 30°C night temperatures caused rapid flower abortion while moderate stress treatment did not directly cause flowers to abort, but rather accelerated the natural termination of the flowering process in plants. Petunias grown at 40/30°C day/night had consistently fewer flowers over the 5 weeks which may indicate a more direct interruption of flowering due to heat stress (Figure 3.5). However, petunias grown at 35/25°C and 30/25°C did increase in flower count but decreased abruptly after the fourth week. This may be due to an increased speed in flowering termination associated with heat stress. Flower abortion before anthesis has also been described as being caused by competition for assimilates between previous initiated inflorescence and vegetative apex (Bertin, 1995).

Underdeveloped or smaller flowers observed in petunias grown at 35/25 and 40/30°C in the greenhouse may also be due to water deficiency within flower buds. Tsukaguchi e al. (2003) studied water status and high temperature (32/26°C d/n) in flower buds of heat-sensitive and tolerant snap beans (*Phaseolus vulgaris* L.) under non-drought conditions and concluded that the heat-tolerant cultivar displayed a higher water conductance and less water stress in flower buds under heat stress, resulting in less damage to pollen compared to heat-sensitive cultivar.

Some flowers of petunia Dreams ‘Midnight’ grown at 40/30°C also displayed bleaching or striations in petal pigmentation (Fig. 3.7, B). Petunia Dreams ‘Midnight’ is a dark purple flower whose color is influenced by anthocyanin content within the petal tissue. Anthocyanin synthesis is strongly affected by temperature where higher temperatures greatly reduce anthocyanin concentration (Zhong and Yoshida, 1993). Dela et al. (2003) showed that exposure of roses (*Rosa x hybrida* Schleich.) to 39°C for 3 d resulted in decreased anthocyanin levels in flowers. The study also revealed that the reduction in anthocyanin concentration was directly related to heat stress of the flower buds and not a response from whole plant stress where flower buds were initially removed. Dela et al. (2003) also indicated that when roses were removed from heat stress, anthocyanin synthesis recovered and eventually increased in concentration, which would explain the recovery of petunia Dreams ‘Midnight’ pigmentation after transplant in the field.

A review of flower development in petunia by van der Krol and Chua (1993) discusses specific floral homeotic and nonhomeotic genes and their function in petunia floral organ identity. According to the authors, environmental and physiological factors can affect gene function and alter organ differentiation resulting in variations of floral development. Although, the present study does not investigate gene expression or function, some deformed or severely reduced flower size observed under heat stress (Fig. 3.7, A) may have been due to alterations in the activity of enzymes at high temperatures that influence the expression of the floral homeotic genes as described in the review by van der Krol and Chua (1993).

Shoot dry weight of petunia Dreams ‘Midnight’ was significantly reduced in plants grown at 40/30°C for 5 weeks (Fig. 3.8). Ashraf and Hafeez (2004) found similar results when studying growth and nutrient relations related to thermotolerance of maize (*Zea mays* L.) and pearl millet (*Pennisetum glaucum* (L.) R. Br.). Shoot dry weight was found to be reduced in maize grown at 38°C, but was unaffected in pearl millet. Furthermore, the authors also indicate reduced net-assimilation rate in maize compared to an increase in relative growth and net-assimilation rates in pearl millet when grown at 38°C. The authors conclude that the reduced biomass and net-assimilation rates found in the maize studied is associated with a decreased thermotolerance compared to the pearl millet. Research by Natarajan (2005), where two cultivars of *Salvia splendens* F. Sellow ex Roem & Schult. were exposed to heat shock every 3 days for 3 hours at 30, 35, 40 and 45°C, also resulted in decreased root and shoot dry weight as temperature increased. Reduced biomass of petunia Dreams 'Midnight' may be due to increased transpiration and respiration rates and decreased net photosynthesis at higher temperatures as was found in redbud (*Cercis canadensis* L.) (Griffin et al., 2004), spinach (*Spinacia oleracea* L.) (Brooks and Farquhar, 1985), and *Salvia splendens* F. Sellow ex Roem & Schult. (Jiao and Grodzinski, 1996).

The results from the present heat shock experiments showed no significant difference between any of the temperature treatments for all growth measurements. This may be due to the duration and frequency chosen for heat shock rather than the temperatures used. Morphological effects of whole plant petunia Dreams 'Midnight' may be limited to longer and/or more frequent durations at these temperatures rather than the 2 h duration once per week heat shock used in this experiment. Lin et al. (1984) studied acquired thermotolrance in soybean (*Glycine max* L. Merr.) seedlings and found differences in seedling length of control (28°C) and heat shocked plants at 40°C for 2h before subsequent heat stress of 45°C for 2 h. The authors also found that thermotolerance could be achieved in seedlings heat shocked for only 15 min at 40°C followed by recovery period of 2 to 4 h at 28°C before heat stress exposure to 45°C. Similar temperature and duration heat shock treatment were used in the present study; however, the species and age of plant at time of heat shock are different and sensitivity of these plants may vary accordingly. Natarajan (2005), showed differences in growth of *Salvia splendens* F. Sellow ex Roem & Schult. another popular bedding plant, when heat shocked every 3 d for 3 h at 30, 35, 40 and 45°C indicating an increase in duration time and frequency for the present study may result in more profound differences in petunia growth.

After heat stress treatments in the greenhouse, petunia Dreams ‘Midnight’ showed dramatic morphological differences between temperatures (Figure 3.11). Petunias grown at 30/25°C displayed more characteristics of a desirable plant including higher flower count, flower size and larger biomass compared to plants grown at 35/25 and 40/30°C which showed significant reductions in these measurements. However, once transplanted into the field, petunias grown at 35/25°C displayed similar growth and flowering habits as petunias grown at 30/25°C. Average flower size was unaffected in the landscape between all temperatures while the flower size was severely reduced at 35/25 and 40/30°C in the greenhouse. These results indicate that reproductive tissue may be acutely affected by heat stress but do not have permanent effects on future floral development once removed from sustained heat stress.

Although plants grown at 35/25°C are less marketable after greenhouse production, they are capable of recovering in overall quality in the landscape. This is also demonstrated by the more compact and uniform growth and darker leaves in the landscape of petunias grown at this temperature (personal observation) (Fig.3.11). These characteristics are similar to other reports describing morphology of heat tolerant species such as shorter or compact growth, thicker stems, and smaller, darker, and thicker leaves (Beadle, 1981; Natarajan, 2005). The compact growth of petunia Dreams ‘Midnight’ grown at 35/25 and 40/30°C is considered to be a desirable trait for heat tolerance as was discovered in cowpea (*Vigna unguiculata* (L.) Walp**.**), where plants with longer internodes were found to be more heat-susceptible (Ismail et al., 2000).

After 3 weeks in the landscape, petunias grown at 30/25 and 35/25°C showed no difference in quality ratings (Fig. 3.10). Petunia grown at 30/25°C did decrease in quality at 2 weeks in the landscape while plants grown at 35/25 and 40/30°C slightly increased, though the difference was not significant. Differences between the temperature treatments may be more pronounced in the landscape over a longer period of time than 3 weeks.

In conclusion, petunia Dreams 'Midnight' were significantly affected by enduring heat stress of 35/25 and 40/30°C in the greenhouse but appeared to adapt to these excessive temperatures. However, heat shock treatments of 35 and 40°C for 2 h once per week proved insignificant for inducing similar morphological effects in petunia during greenhouse production. Although, enduring stress of 35/25 and 40/30°C caused desirable traits such as compact vegetative growth for improved landscape performance, these temperatures also caused detrimental effects to petunia flowering habit during greenhouse production. Poor flowering or damaged flower tissue is not ideal for marketing bedding plants. Therefore, 35/25 and 40/30°C would not be a recommended growing regime for petunia Dreams 'Midnight' intended for market. The morphological characteristics displayed under enduring heat stress experiments should be considered if less extreme flowering and growth effects could be achieved. Further heat shock treatments including a higher range in temperature, durations and frequency should be investigated for similar but less extreme morphological characteristics that would produce a marketable plant and induce thermotolerance for improved landscape survivability.

3.5 LITERATURE CITED

Ashraf, M. and M. Hafeez. 2004. Thermotolerance of pearl millet and maize at early growth stages: growth and nutrient relations. *Biologia Plantarum* 48 (1):81-86.

Beadle, N.C.W. 1981. The vegetation of Australia. Cambridge: Cambridge University Press.

Bertin, N. 1995. Competition for assimilates and fruit position affect fruit set in indeterminate greenhouse tomato. Ann. Bot. 75:55-65.

Brooks, A and G.D. Farquhar. 1985. Effect of temperature on the CO2/O2 specificity of ribulose-1,5-bisphosphate carboxylase/oxygenase and the rate of respiration in the light. Planta. 165: 397-406.

Dela, G., E. Or, R. Ovadia, A. Nissim-Levi, D. Weiss and M. Oren-Shamir. 2003. Changes in anthocyanin concentration and composition in 'Jaguar' rose flowers due to transient high temperature conditions. Plant Science 164(3): 333- 340.

Gerats, T. and M. Vandenbussche. 2005. A model system for comparative research: Petunia. *Trends in Plant Sci*. 10:251-256.

Griffin, J.J, T.G.Ranney, and D.M. Pharr. 2004. Heat and drought influence photosynthesis, water relation and soluble carbohydrates of two ecotype of Redbud (*Cercis canadensis*). J Amer Soc Hort Sci. 129: 497-502.

Guilioni, L., J. Wery and F. Tardieu. 1997. Heat stress-induced abortion of buds and flowers in pea: Is sensitivity linked to organ age or to relations between reproductive organs? Ann Bot. 80: 159-168.

Gusta, L.V., B.J. O'Connor and R.S. Bhatty. 1997. Flax (*Linum usitatissimum* L.) responses to chilling and heat stress on flowering and seed yield. Can. J. Plant Sci. 77:97-99.

Hancheck, A.M. and A.C. Cameron. 1995. Harvest date and storage quality of herbaceous perennials. HortSci. 30: 573-576.

Harding, S.A., J.A. Guikema, and G.M. Paulsen. 1990. Photosynthetic decline from high temperature stress during maturation of wheat. Plant Physiol. 92:654-658.

Ismail, A. M, A. E. Hall and J.D. Ehlers. 2000. Delayed-leaf-senescence and heattolerance traits mainly are independently expressed in cowpea. Crop Sci. 40: 1049-1055.

Jiao, J and B. Grodzinski. 1996. The effect of leaf temperature and photorespiratory conditions on export of sugars during steady-state photosynthesis in *Salvia spiendens*. Plant Physiol. 111: 169-1 78.

Lichtenthaler, H.K., 1996. Vegetation stress: an introduction to the stress concept in plants. J Plant Physiol. 148, 4–14.

Lin, C., J. K. Roberts and J.L. Key. 1984. Acquisition of thermotolerance in soybean seedlings: synthesis and accumulation of heat shock proteins and their cellular localization. Plant Physiol. 74:152-160.

Mahan, J.R., B.L. Mc Michael, and D.F. Wanjura. 1997. Reduction of high temperature stress in plants, p. 137-150.In: A.S. Basra and R.K. Basra (eds). Mechanism of environmental stress resistance in plants. Harwood Academic Publishers, The Netherlands.

Natarajan S. 2005. High temperatures stress responses of *Salvia splendens* and *Viola x wittrockiana.* Ph.D Diss.

Tsukaguchi, T., Y. Kawamitsu, H. Takeda, K. Suzuki and Y. Egawa. 2003. Water status of flower buds and leaves as affected by high temperature in heat-tolerant and heat-sensitive cultivars of snap bean (*Phaseolus vulgaris* L.). Plant Prod. Sci. 6(I):24-27.

van der Krol, A.R. and N. Chua. 1993. Flower Development in Petunia. The Plant Cell, vol. 5:1195-1203.

Wehner, D.J., and Watschke T.L. 1987 Heat tolerance of Kentucky bluegrasses, perennial ryegrass, and annual bluegrass. Agron J: 73: 79-84.

Zhong, J. and T. Yoshida. 1993. Effects of temperature on cell growth and anthocyanin production in suspension cultures of *Perilla frutescens* (J. Ferment). Bioeng. 76:530-531.

CHAPTER 4. DETERMINING OPTIMUM CHALLENGING TEMPERATURES AND DURATIONS FOR INDUCING ACQUIRED THERMOTOLERANCE IN *PETUNIA X HYBRIDA*

4.1 INTRODUCTION

Heat stress is a major challenge in bedding plant production, primarily during the late spring and summer months and for growers in the southern United States (Koh, 2002; Mahan et al., 1997). Plant growth and development is greatly affected by high temperature stress and may cause irreversible damage depending on the plant species, temperature and duration of exposure (Hall, 2001; Burke, 1990). High temperature stress is reported to be one of the greatest causes for bedding plant loss during postproduction (Armitage, 1989), where plants can face inconsistent environments and stressful conditions.

A plant's ability to acclimate and maintain physiological functions under stressful conditions is crucial to plant survival when exposed to extreme high temperature (Hale and Orcutt, 1997; Lichtenthaler, 1996). Plant acquired thermotolerance may be achieved through heat shock or short exposure to supraoptimal temperatures (Sung et al., 2003). Plant adaptive responses to heat stress associated with acquired thermotolerance can vary by species; however, research has indicated that development of smaller and thicker leaves, thicker stems, and shortened internodes (Beadle, 1981; Natarajan, 2005; Ismail et al., 2000), as well as increased membrane thermostability (Yeh and Hsu, 2004), and increased synthesis of heat shock proteins (Park et al., 1996; Vierling, 1991) are characteristics that improve heat tolerance.

Photosynthesis is a heat-sensitive process that can result in negative whole plant responses depending on the extent of damage to the photosynthetic apparatus (Berry and Bjorkman, 1980; Larcher, 1994). PSII is considered to be one of the most heat-labile mechanisms of photosynthesis (Berry and Bjorkman, 1980; Heckathorn et al., 1998; Weis and Berry, 1987). Measuring chlorophyll fluorescence in the light and dark is an effective and nondestructive means to determine quantum efficiencies of PSII, which can be inferred as tolerance or damage to the photosynthetic apparatus (Bilger et al., 1995; Maxwell and Johnson, 2000). Havaux (1993) used chlorophyll fluorescence measurements on leaf discs taken from mature potato leaves (*Solanum tuberosum* L.) to study photosynthetic adaptation to heat stress when grown at 25°C (control) or pre-heated at 35°C. The study determined that potato pre-exposed to 35°C for 2 h resulted in increased tolerance of PSII when the temperature was increased at a rate of 1°C min^{-1} to 40°C for 40 min compared to potato grown at control temperature (25°C).

Standard greenhouse production protocol may provide for alleviating heat stress if closely monitored. However, inducing acquired thermotolerance of bedding plants during greenhouse production may greatly improve heat tolerance postproduction. There has been little research on critical temperatures and durations for inducing acquired thermotolerance during greenhouse production of bedding plants and achieving adaptive characteristics while still maintaining marketability. Further investigation of whole plant adaptive characteristics related to photochemical efficiency could be an effective tool for determining the ability of bedding plants to develop acquired thermotolerance in the greenhouse. Therefore, the objective of this study was to determine optimum heat shock temperature and duration for development of acquired stress tolerance of *Petunia x hybrida* Dreams 'Midnight' and to test for thermotolerance through subsequent heat stress and measurement of fluorescence.

4.2 MATERIALS AND METHODS

4.2.1 Plant Material

Petunia x hybrida Hort. Ex Vilm. variety Dreams™ 'Midnight' (Ball Horticultural Company, West Chicago, IL) was chosen as a model for the following experiments. Petunia seeds were planted 25 March 2008 into 288 (4.9 cm^3) plug tray using lightweight media Fafard™2M Mix (Conrad Fafard, Incorporated, Agawam, MA) and germinated in a growth chamber (EGC, Chagrin Falls, OH) maintained at 80% RH with a 12-h photoperiod of 3-4 umoles^{-1}m^{-2}s^{-1} from very high output fluorescent, light bulbs (OERAM Sylvania, Danvers, MA). Seedlings were grown in the growth chamber for six weeks before transplant into the greenhouse.

4.2.2 Greenhouse Establishment Prior to Treatments

The greenhouses were located at the LSU AgCenter Campus Greenhouses numbers 440-7 and 440-8 at Louisiana State University, 30° N 91° W Baton Rouge, Louisiana. A total of three polycarbonate covered greenhouses with 40% shade cloth (1000 µmol m^{-2} s^{-1}) were used to complete each temperature treatment. Each greenhouse had respective automated heating and cooling systems using Wadsworth STEP© Control 50A (Wadsworth Control Systems Incorporated, Arvada, CO) with day/night 12-h temperature settings. On 5 May 2008, petunia Dreams 'Midnight' plugs were transplanted into plastic pots (650 cm^3) using a middleweight media Fafard™4M Mix (Conrad Fafard, Incorporated, Agawam, MA). One plant was transplanted into each pot and was placed in trays on greenhouse benches. Broad spectrum fungicide was applied as a drench to each pot after one week transplant at a rate of 19.5 ml/L (Banrot® 8G, a.i. 3% Etridiazole, a.i. 5% Thiophanate-methyl, Scotts-Sierra, Marysville, OH). Petunias were grown at 30/25°C day/night and fertigated using a Hozon™ Brass Siphon Mixer (1:16) (Phytotronics, Incorporated, Earth City, MO) with 200 ppm N 15N-2.2P-12.4K (15-5-15 Cal Mg, Scotts-Sierra, Marysville, OH) daily for two weeks before beginning heat shock treatments to allow for root growth and proper establishment within the pot.

4.2.3 Heat Shock/Stress Treatments

Heat shock/stress treatments were applied to petunia after two weeks growth at 30/25°C in the greenhouse. Challenging temperatures were chosen in 5°C increments increasing from the control temperature, 30°C. Heat shock treatments included short duration heat shock at 35, 40 or

45°C for 2, 4 or 6 h every 3 d for 4 weeks. Enduring heat stress treatments included 24-h duration continuous exposure to 35/25, 40/30 or 45/35°C day/night for 4 weeks (Table 4.1).

Table 4.1 Heat shock/stress treatments of given temperature and duration.

Temperature (°C)	Duration (hours)
30/25°C	24 h
35/30°C	2, 4, 6, 24 h
40/35°C	2, 4, 6, 24 h
45/40°C	2, 4, 6, 24 h

For heat shock treatments, petunias were grown at 30/25°C day/night and kept in 25 cm x 50 cm plastic trays with 4 pots per tray for treatment efficiency. Plants in trays were manually moved every 3 d between greenhouse sections that were set to a constant specific heat shock treatment temperature (35, 40 or 45°C) which remained for the respective duration time (2, 4 or 6 h) and returned to 30/25°C until the following heat shock exposure (staring at 1000 HR and removed at 1200, 1400 and 1600 HR, respectively). For heat stress treatments, petunia were exposed for 24 h duration and remained in respective temperature treatments for the 4 week period. Prior to heat shock exposure and during heat stress, plants were irrigated to maximum water holding capacity to minimize water stress during treatment. Temperature treatments were applied in respective greenhouses and recorded using HOBO® Pro Series™ data logger (Onset Computer Corporation, Bourne, MA)(Figure 4.1. A, B, C, D). Weather records for Baton Rouge, Louisiana, including temperature (°C) and irradiance (μ mol m^{-2} s^{-1} PPFD), were recorded daily for the duration of the experiments (Louisiana Agriclimatic Information Systems, LSUAgCenter, BAE) (Figure 4.3. A & B). A total of 360 petunias were exposed to heat shock/stress treatments where 282 plants were divided into weekly destructive harvests to determine growth and development over a 3 week period and 78 plants were kept for subsequent acquired thermotolerance test.

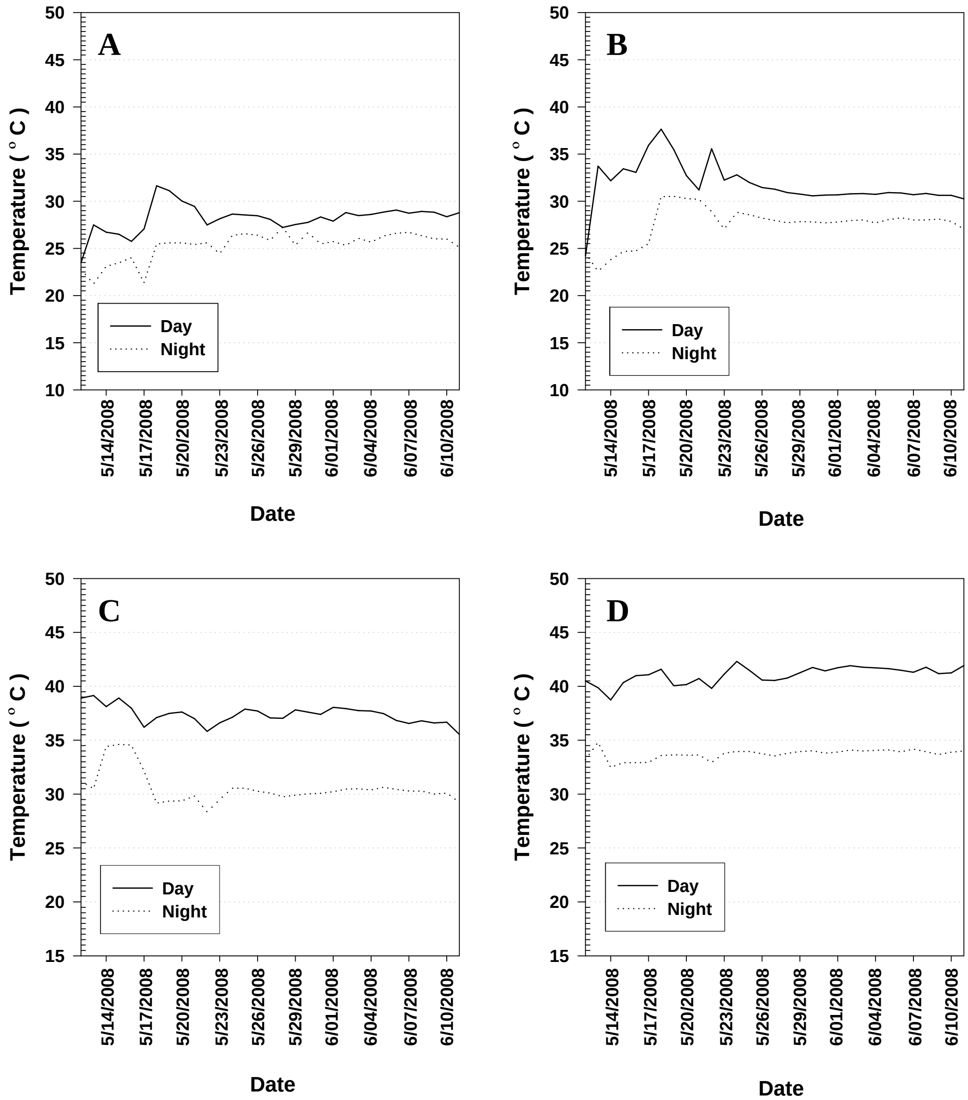

Figure 4.1 Average day/night temperatures (°C) A) Control 30/25°C B) 35/25°C C) 40/30°C D) 45/35°C recorded in greenhouses for each temperature treatment over a 4 week course of experiment.

4.2.4 Acquired Thermotolerance Test

Following a 4 week exposure to heat shock/stress treatments, all treated *Petunia x hybrida* Dreams 'Midnight' were exposed to subsequent heat stress of 45/35°C day/night for one week in the greenhouse. Temperature in the greenhouse was recorded using HOBO® Pro Series™ data logger (Onset Computer Corporation, Bourne, MA) (Figure 4.2). Chlorophyll fluorescence measurements were recorded after 4 weeks of heat shock/stress treatments, 3 and 7 d after the acquired thermotolerance test. Quality ratings (1 to 5) were also taken on treated petunias before and after acquired thermotolerance test followed by destructive harvest for data collection. Overall marketable quality of plants was assessed based on 1 to 5 scale with 5 being the best and 1 being the worst. Marketable quality scores; 5 to 4.5 = excellent plants with healthy green leaves, compact uniform growth and good inflorescence, 4.5 to 3.5 = green healthy foliage with moderate flowers, 3.5 to 2.5 = plants with chlorotic leaves and poor inflorescence, 2.5 to 1.5 = plants with necrotic or dried leaves with terminal bud damage and poor flower set, <1.5S = dead.

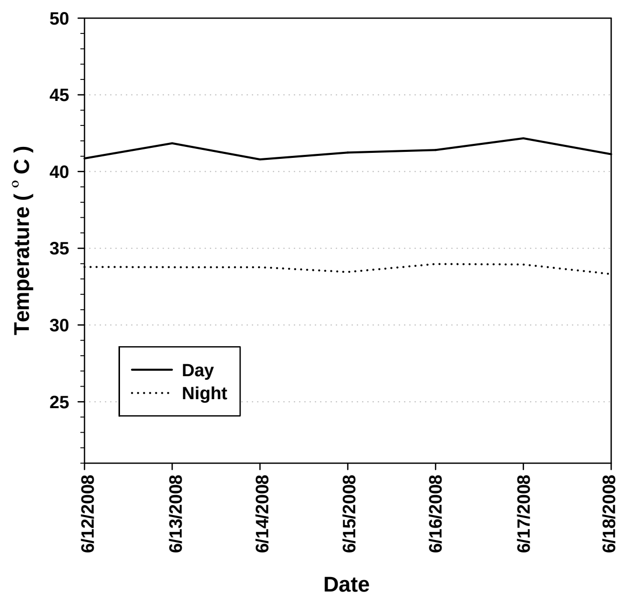

Figure 4.2 Average day/night temperatures (45/35°C) in greenhouse during one week acquired thermotolerance test.

4.2.5 Measurement of Growth and Development in the Greenhouse

Treated plants were destructively harvested for data collection every 7 d for 3 weeks followed by a fourth harvest after acquired thermotolerance test. *Petunia x hybrida* growth and development was quantified by measuring number of flowers per plant, average flower size (cm) per plant, chlorophyll concentration, average internode length (cm), total leaf area per plant

(mm^2), and shoot dry weight (g). Fully expanded flowers were visually counted and recorded weekly. Flower size was determined by using a handheld metric ruler and measuring the diameter (cm) of one flower visually estimated to be average for that respective plant. Average chlorophyll content was determined by taking two measurements per plant using Minolta SPAD-502 chlorophyll meter (Spectrum Technologies, Inc., Plainfield, IL). Average internode length was determined by measuring lengths of first 3 internodes from newest true leaves on one branch per plant. Total leaf area per plant was measured using LI-3100C leaf area meter (LICOR Biosciences, Lincoln, NE). Shoot dry weights (g) were obtained after oven drying at 80°C for 24 h.

4.2.6 Chlorophyll Fluorescence Measurements

Chlorophyll fluorescence was measured using the FMS2 field-fluorescence monitoring system (Hansatech Instruments Ltd., England) on dark adapted leaves after four weeks of heat shock/stress and 3 and 7 d after plants were subjected to acquired thermotolerance test (45/35°C for one week). The FMS2 uses a modulating beam (<0.05 µmol m^{-2} s^{-1}) and short duration light pulses (1.6 sec) to determine leaf fluorescence characteristics. Maximum quantum efficiency of PSII was calculated using the following formula: (Fm - Fo/Fm) or (Fv/Fm) where Fm = maximum fluorescence yield, Fo = fluorescence yield after dark-adaptation when all reaction centers are “opened”, and Fv calculated as the difference between Fo and Fm known as variable fluorescence. Dark adapted light measurements were taken on fully expanded young and mature leaves for each plant after a 12 h dark period. The intensity and duration of the saturating light pulse used to determine Fm was based on preliminary experiments (data not shown).

4.2.7 Experimental Design and Statistical Analysis

Heat shock treatments were arranged as a strip-split plot design in the greenhouse. Six replicate plants were used for each temperature/duration treatment for each of the 4 harvests.

Plants exposed to heat shock treatment were arranged in trays according to temperature and duration with 4 harvests per tray. Statistical analysis was performed using SAS ProcMixed Procedure (Statistical Analysis Software, version 9.1, Cary, NC).

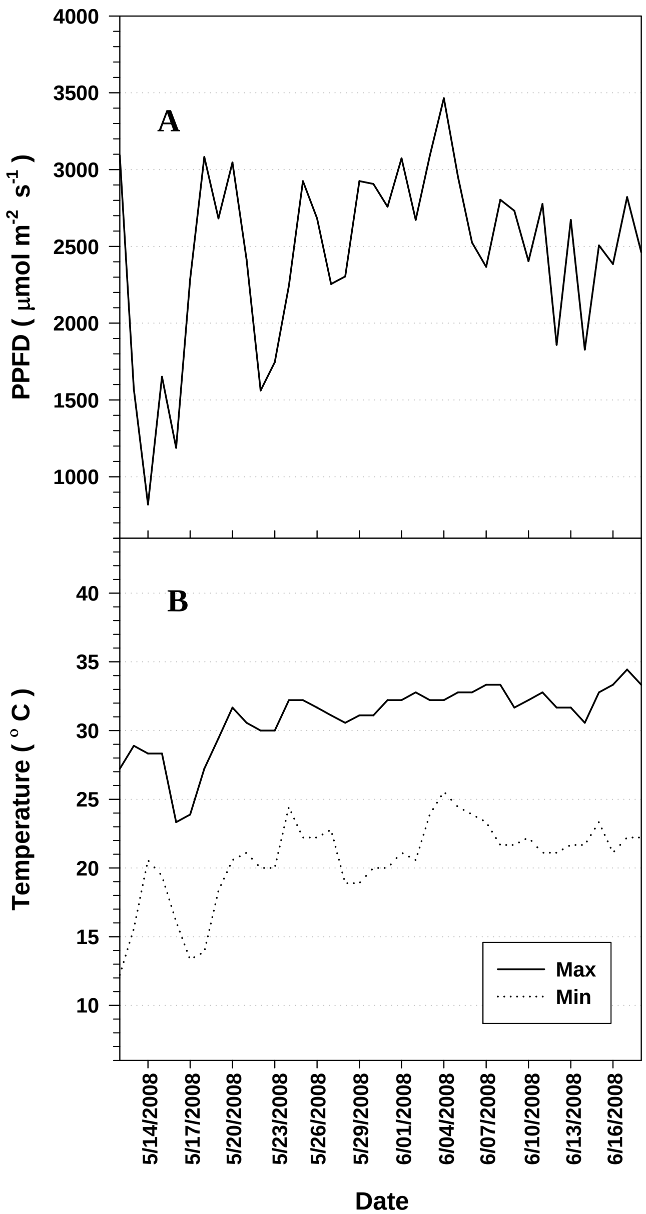

Figure 4.3 Average daily weather reports A) natural irradiance (µ mol m^{-2} s^{-1} PPFD), B) minimum and maximum daily temperature (°C) for Baton Rouge, Louisiana during heat shock/stress treatments and acquired thermotolerance test.

4.3 RESULTS

4.3.1 Effect of Heat Shock and Enduring Heat Stress

- **Flowers per Plant**

Temperature, duration and week had a significant effect on the number of flowers per plant in *Petunia x hybrida* Dreams 'Midnight'. Petunia heat shocked at 35°C for 6 h had significantly increased flower count compared to petunia exposed to enduring heat stress (24h) at 40/30 and 45/35°C (Table 4.2). Petunia flower count increased for all temperatures and durations over the 3 week treatment period (Table 4.3). Heat shock/stress treatments did not significantly effect flower counts at week 1 (Table 4.4) while petunia heat shocked at 35°C for 6 h had higher flower counts than all other treatments except petunia heat shocked at 40°C for 4 or 6 h (Table 4.5). At week 3, prior to acquired thermotolerance test, petunia exposed to enduring heat stress at 45/35°C (24 h) had fewer flowers than all other treatments (Table 4.6). Petunia grown at 30/25°C or exposed to heat treatment at 35 or 40°C had a greater number of flowers compared to petunia exposed to 45°C after 3 weeks (Fig. 4.4 A). Also, petunia exposed to enduring heat stress (24 h duration) had significantly less flowers per plant compared to heat shock for 2, 4 or 6 h, regardless of temperature (Fig. 4.5 A).

- **Average Flower Size**

Heat shock had the greatest effect on the average flower size of petunia after the first week (Table 4.3). At week 1, flower size was significantly smaller in petunia exposed to enduring heat stress at 35/25°C (24 h) compared to control or heat shock at 35°C for 2, 4 or 6 h or 40°C for 4 or 6 h or 45°C for 6 h (Table 4.4). At week 2 petunia exposed to heat shock were significantly larger than petunia exposed to enduring heat stress (Table 4.5) while after 3 weeks of treatment only petunia grown at 45/35°C had significantly smaller flower size (Table 4.6). Average flower size in petunia grown at 30/25°C was significantly larger than petunia exposed to

40 or 45°C (Fig. 4.4 B). The effect of duration was similar to flower count results where petunia exposed to heat stress for 24 h had significantly decreased flower size (Fig.4.5 B).

Table 4.2. The effect of combined heat shock every 3 d and enduring heat stress temperature and duration on growth and development of *Petunia x hybrida* Dreams 'Midnight' after three weeks in the greenhouse.

Temperature (°C)	Duration (hours)	Effect				
		Flowers per Plant	Flower Size (cm)	Shoot Dry Weight (g)	Leaf Area (mm^2)	Internode Length
30/25°C	24 h	[Y]9.94 abc[X]	7.01 ab	3.80 ab	628.88 a	1.33 a
35°C	2 h	10.83 ab	6.60 ab	3.92 ab	648.97 a	1.24 ab
	4 h	9.27 abc	6.77 ab	3.56 ab	581.62 ab	1.33 a
	6 h	12.94 a	6.76 ab	4.31 a	711.86 a	1.19 ab
35/25°C	24 h	8.83 bc	4.46 cd	3.81 ab	726.56 a	1.23 ab
40°C	2 h	9.33 abc	5.23 abcd	3.78 ab	610.17 a	1.22 ab
	4 h	11.11 ab	6.81 ab	3.85 ab	628.02 a	1.11 abc
	6 h	11.00 ab	7.20 a	4.23 a	714.68 a	1.17 ab
40/30°C	24 h	6.00 cd	5.06 bcd	3.02 bc	494.37 ab	1.07 abc
45°C	2 h	9.66 abc	6.16 abc	3.43 ab	570.33 ab	1.15 ab
	4 h	8.48 abc	5.79 abcd	3.31 abc	554.26 ab	1.28 a
	6 h	10.68 ab	6.99 ab	3.57 ab	555.16 ab	0.98 bc
45/35°C	24 h	3.44 d	3.67 d	2.35 c	375.05 b	0.84 c
Temp. x Duration		*	*	*	*	*
Temp. x Dur. x Week		*	*	NS	NS	*
Temperature		*	*	*	*	*
Duration		*	*	*	NS	*
Week		*	*	*	*	*

Values significant (*) or not significant (NS) at the 5% level by the lsmean procedure in SAS with Tukey's correction.

[X] Means within columns followed by the same letters are not significantly different at 5% by lsmean procedure in SAS with Tukey's correction.

[Y] Values in the table are averages (n=18).

- **Shoot Dry Weight**

Heat shock temperature and duration had a significant effect on shoot dry weight of petunia Dreams 'Midnight'. Shoot dry weight increased for both heat shock and enduring heat stress treatments over the 3 week period (Table 4.3). However, at week 1 there was no significant difference in petunia shoot dry weights (Table 4.4) while petunia heat shocked at

35°C for 6 h had greater shoot dry weight compared to petunia grown at 40/30 or 45/35°C (24 h) or heat shocked at 45°C for 4 or 6 h at week 2 (Table 4.5). At week 3, petunia exposed to enduring heat stress at 45/35°C (24 h) weighed significantly less than petunia grown at 35/25°C (24 h) or heat shocked at 35°C for 2 h or 40°C for 4 or 6 h (Table 4.6). Shoot dry weight was significantly decreased in petunia exposed to 45°C compared to petunia exposed to 30 or 35°C (Fig. 4.4 C). Exposure of 24 h heat stress reduced shoot dry matter accumulation compared to heat shock for 2, 4 or 6 h over all temperatures (Fig. 4.5 C).

Table 4.3. The effect of combined heat shock and enduring heat stress treatments on growth and development of *Petunia x hybrida* Dreams 'Midnight' at each week.

	Effect				
Week	Flowers per Plant	Flower Size (cm)	Shoot Dry Weight (g)	Leaf Area (mm^2)	Internode Length (cm)
1	[Y]2.05 c[X]	4.99 b	1.46 c	292.25 c	0 c
2	9.10 b	6.67 a	3.32 b	608.00 b	1.94 a
3	17.22 a	7.01 a	6.16 a	915.99 a	1.65 b

(Heat treatments included 30/25°C (control) and heat shock at 35, 40 or 45°C for 2, 4 or 6 h every 3 d or enduring heat stress at 35/25, 40/30 or 45/35°C.)

[X] Means within columns followed by the same letters are not significantly different at 5% by lsmean procedure in SAS with Tukey's correction.

[Y] Values in the table are averages (n=78).

- **Total Leaf Area**

Total leaf area of petunia Dreams 'Midnight' increased for all temperatures and durations over the 3 week period (Table 4.3). At week 1 there were no differences in petunia leaf areas (Table 4.4) but was significantly greater in petunia heat shocked at 35°C for 6 h compared to petunia heat shocked at 45°C for 4 h or enduring heat stress at 45/35°C at week 2 (Table 4.5). Week 3 results showed that petunia exposed to enduring heat stress at 35/25°C (24 h) had significantly increased leaf area compared to enduring heat stress at 45/35°C (Table 4.6).

Table 4.4. The effect of heat shock/stress temperatures and durations on growth and development of *Petunia x hybrida* Dreams 'Midnight' after one week of treatment in the greenhouse.

Temperature (°C)	Duration (hours)	Week 1				
		Flowers Per Plant	Flower Size (cm)	Shoot Dry Weight (g)	Leaf Area (mm^2)	Internode Length (cm)
30/25°C	24 h	[Y]2.50 a[X]	6.51 a	1.54 a	314.23 a	N/A
35°C	2 h	2.00 a	5.21 a	1.57 a	305.20 a	N/A
	4 h	1.66 a	5.81 a	1.21 a	281.39 a	N/A
	6 h	2.00 a	5.66 a	1.67 a	315.56 a	N/A
35/25°C	24 h	0.83 a	0.00 b	1.31 a	273.55 a	N/A
40°C	2 h	2.50 a	2.25 ab	1.82 a	322.01 a	N/A
	4 h	2.50 a	6.25 a	1.40 a	294.13 a	N/A
	6 h	2.50 a	6.81 a	1.89 a	382.01 a	N/A
40/30°C	24 h	1.16 a	3.38 ab	1.19 a	239.93 a	N/A
45°C	2 h	2.00 a	4.48 ab	1.16 a	228.34 a	N/A
	4 h	1.90 a	3.07 ab	1.28 a	256.40 a	N/A
	6 h	2.52 a	6.87 a	1.57 a	282.43 a	N/A
45/35°C	24 h	1.33 a	4.06 ab	1.23 a	235.44 a	N/A

[X] Means within columns followed by the same letters are not significantly different at 5% by lsmean procedure in SAS with Tukey's correction.

[Y] Values in the table are averages (n=6).

Treatment temperature had a significant effect on petunia leaf area where petunia exposed to 45°C greatly reduced total leaf area compared to cooler temperatures (30, 35 or 40°C) (Fig.4.4 D). Leaf area was also significantly greater in petunia heat shocked for 6 h compared to petunia kept in enduring heat stress for 24 h duration (Fig. 4.5 D).

- **Average Internode Length**

Temperature, duration and week had a significant effect on internode length of petunia Dreams 'Midnight' (Table 4.2). Internode length increased after week 1, and then decreased after week 3 for all heat shock and enduring heat stress treatments (Table 4.3). There was no measurable differences internode length at week 1 (Table 4.4). However, at week 2, petunia exposed to enduring heat stress at 40/30 or 45/35°C (24 h) had significantly shorter internodes compared to petunia heat shocked at 40°C for 4 h (Table 4.5). At week 3, petunia heat shocked at 45°C for 4 h had significantly increased internode length compared to petunia exposed to

Table 4.5. The effect of heat shock/stress temperatures and durations on growth and development of *Petunia x hybrida* Dreams 'Midnight' after two weeks of treatment in the greenhouse.

Temperature (°C)	Duration (hours)	Week 2				
		Flowers Per Plant	Flower Size (cm)	Shoot Dry Weight (g)	Leaf Area (mm^2)	Internode Length (cm)
30/25°C	24 h	[Y]8.16 bcd[X]	6.91 a	3.37 abcd	586.24 abc	2.17 ab
35°C	2 h	9.66 bcd	7.20 a	3.46 bcd	658.13 abc	1.85 abc
	4 h	8.16 bcd	7.25 a	3.07 bcd	558.38 abc	2.10 ab
	6 h	16.66 a	7.21 a	4.84 a	915.31 a	2.01 abc
35/25°C	24 h	8.00 bcd	6.20 ab	3.34 bcd	643.29 abc	1.71 abc
40°C	2 h	8.50 bcd	5.98 ab	3.46 abcd	605.48 abc	1.83 abc
	4 h	11.66 abc	6.93 a	3.42 abcd	631.85 abc	2.16 a
	6 h	13.50 ab	7.26 a	4.16 ab	795.49 ab	1.82 abc
40/30°C	24 h	6.50 cd	5.70 ab	2.45 cd	501.00 abc	1.66 bc
45°C	2 h	9.16 bcd	7.00 a	3.42 abc	687.26 abc	2.05 ab
	4 h	7.16 cd	6.73 a	2.90 bcd	497.75 bc	1.96 abc
	6 h	9.66 bc	7.05 a	3.07 bcd	517.62 abc	1.66 abc
45/35°C	24 h	4.33 d	4.53 b	2.08 d	371.47 c	1.51 c

[X] Means within columns followed by the same letters are not significantly different at 5% by lsmean procedure in SAS with Tukey's correction.

[Y] Values in the table are averages (n=6).

Table 4.6. The effect of heat shock/stress temperatures and durations on growth and development of *Petunia x hybrida* Dreams 'Midnight' Pre-ATT (after 3 weeks of heat shock and enduring heat stress treatments and before exposure to acquired thermotolerance test).

Temperature (°C)	Duration (hours)	Week 3 (Pre-ATT)				
		Flowers Per Plant	Flower Size (cm)	Shoot Dry Weight (g)	Leaf Area (mm^2)	Internode Length (cm)
30/25°C	24 h	[Y]19.16 ab[X]	7.60 a	6.51 ab	986.19 ab	1.81 abc
35°C	2 h	20.83 a	7.38 a	6.73 a	983.59 ab	1.86 ab
	4 h	18.00 ab	7.26 a	6.41 ab	905.10 ab	1.89 ab
	6 h	20.16 ab	7.41 a	6.44 ab	904.70 ab	1.57 abcd
35/25°C	24 h	17.66 ab	7.18 a	6.75 a	1262.84 a	1.99 a
40°C	2 h	17.00 ab	7.46 a	6.07 ab	903.03 ab	1.85 ab
	4 h	19.16 a	7.25 a	6.75 a	958.09 ab	1.18 cd
	6 h	17.00 ab	7.51 a	6.66 a	966.55 ab	1.70 abc
40/30°C	24 h	10.33 bc	6.11 a	5.42 ab	742.19 ab	1.55 abcd
45°C	2 h	17.83 ab	7.00 a	5.71 ab	795.38 ab	1.42 abcd
	4 h	16.33 ab	7.58 a	5.72 ab	907.91 ab	1.88 a
	6 h	20.00 ab	7.08 a	6.12 ab	863.42 ab	1.28 bcd
45/35°C	24 h	4.66 c	2.41 b	3.73 b	518.25 b	1.02 d

[X] Means within columns followed by the same letters are not significantly different at 5% by lsmean procedure in SAS with Tukey's correction.

[Y] Values in the table are averages (n=6).

enduring heat stress at 35/25 or 45/35°C or heat shocked at 40 or 45°C for 4 or 6 h, respectively (Table 4.6). After 3 weeks of heat shock and heat stress, average internode length was significantly reduced in petunia exposed to 45°C compared to petunia exposed to 30 or 35°C (Fig. 4.4 E). Internode length was also significantly reduced in petunia exposed to 24 h duration compared to 2 or 4 h durations (Fig. 4.5 E).

4.3.2 Effect of Heat Shock

Further investigation of the effect of heat shock temperature and duration alone on petunia Dreams 'Midnight' every 3 d for 3 weeks proved insignificant for most growth measurements (Table 4.7). However, heat shock treatments did have a significant effect on

Table 4.7. The effect of heat shock temperature and duration on growth and development of *Petunia x hybrida* Dreams 'Midnight' over 3 weeks.

Temperature (°C)	Duration (hours)	Effect				
		Flowers Per Plant	Flower Size (cm)	Shoot Dry Weight (g)	Leaf Area (mm^2)	Internode Length (cm)
30/25°C (Control)		[Y]9.94 ab[X]	7.45 a	3.80 a	628.88 a	1.99 a
35°C	2 h	10.83 ab	6.95 a	3.92 a	648.97 a	1.86 ab
	4 h	9.27 ab	7.16 a	3.56 a	581.62 a	2.00 a
	6 h	12.94 a	7.13 a	4.31 a	711.86 a	1.79 ab
40°C	2 h	9.33 ab	7.09 a	3.78 a	610.17 a	1.84 ab
	4 h	11.11 ab	7.22 a	3.85 a	628.02 a	1.67 ab
	6 h	11.00 ab	7.20 a	4.23 a	714.68 a	1.76 ab
45°C	2 h	9.66 ab	6.91 a	3.43 a	570.33 a	1.73 ab
	4 h	8.34 b	7.23 a	3.31 a	554.53 a	1.92 a
	6 h	10.70 ab	6.99 a	3.58 a	554.76 a	1.47 b
Temp. x Duration		NS	NS	NS	NS	*
Temp. x Dur. x Week		NS	NS	NS	NS	*
Temp. x Week		NS	*	NS	NS	NS
Duration x Week		NS	NS	NS	NS	NS
Temperature		NS	NS	NS	NS	*
Duration		NS	NS	NS	NS	NS
Week		*	*	*	*	*

Values significant (*) or not significant (NS) at the 5% level by the lsmean procedure in SAS with Tukey's correction.

[X] Means within columns followed by the same letters are not significantly different at 5% by lsmean procedure in SAS with Tukey's correction.

[Y] Values in the table are averages (n=18).

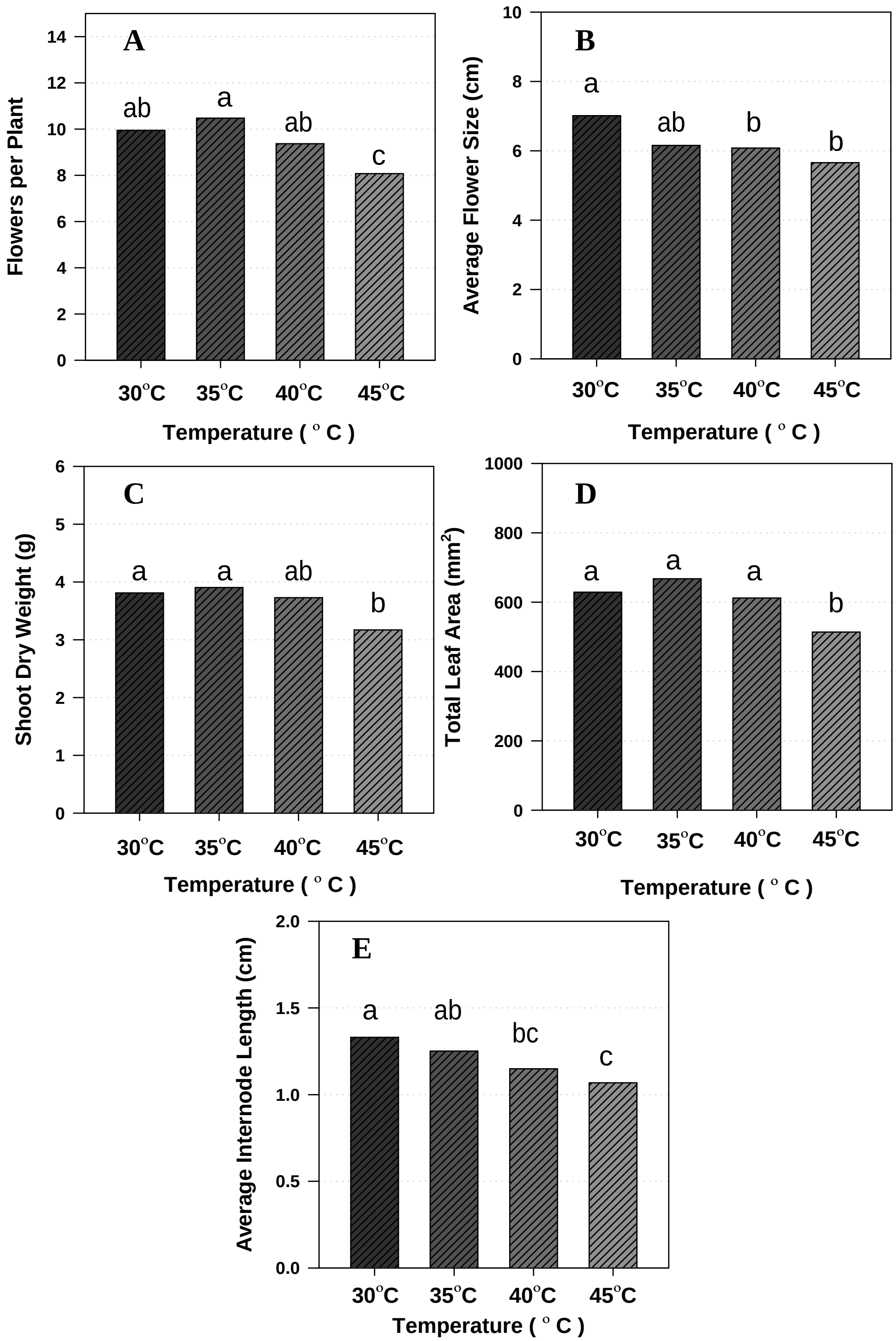

Figure 4.4. The effect of heat shock and enduring heat stress temperatures (°C) on *Petunia x hybrida* Dreams 'Midnight' A) flowers per plant B) average flower size (cm) C) shoot dry weight (g) D) total leaf area (mm^2) and E) average internode length (cm) after 3 weeks grown at 30°C or heat shock every 3 d at 35, 40 or 45°C for all durations (2, 4, 6 or 24h). Means within each effect with different letters are significantly different at $P<0.05$ (Tukey's Test).

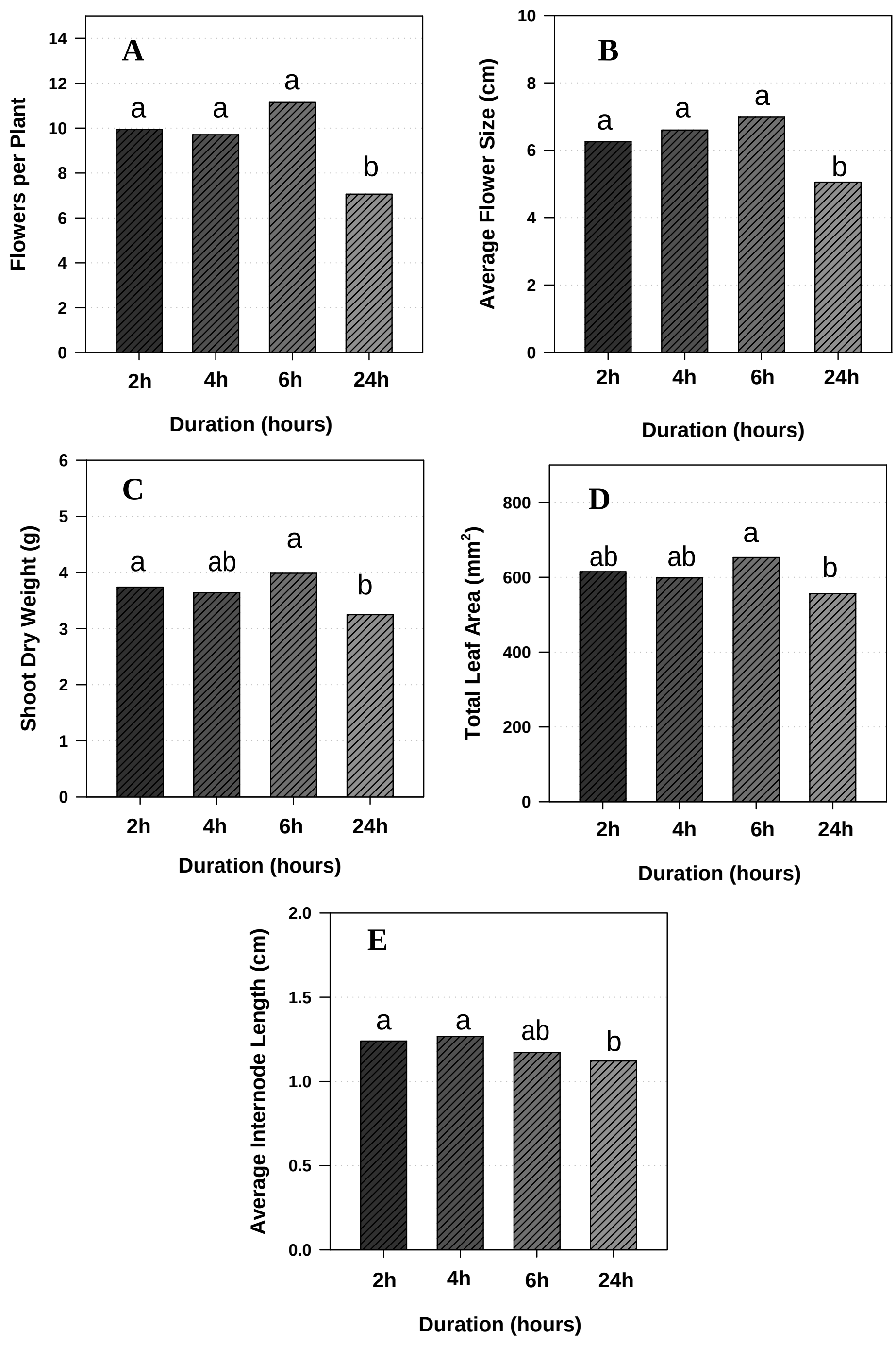

Figure 4.5. The effect of duration (hours) (for heat shock and enduring heat stress treatments) on *Petunia x hybrida* Dreams 'Midnight' A) flowers per plant B) average flower size (cm) C) shoot dry weight (g) D) total leaf area (mm^2) and E) average internode length (cm) after 3 weeks grown at 30°C or heat shock every 3 d for all temperatures (35, 40 or 45°C). Means within each effect with different letters are significantly different at P<0.05 (Tukey's Test).

petunia average internode length. Petunia exposed to heat shock temperature of 45°C over 3 weeks showed a significant decrease in internode length compared to petunia grown at control temperature (30/25°C), regardless of duration (Fig.4.6). Average internode length also decreased significantly after the second week for all heat shock treatments (Table 4.8). Petunia exposed to 40°C for 4 h or 45°C for 2 h having the greatest decrease in internode length by 46 or 31%, respectively (Fig.4.7). Although overall heat shock temperature was insignificant for petunia flower size, there was a significant difference between temperatures for each week. Average flower size of petunia grown at 30/25°C (control) decreased significantly (40%) after week 1, while flower size was not significantly different for heat shock temperatures (35, 40, or 45°C) for all weeks (Fig 4.8).

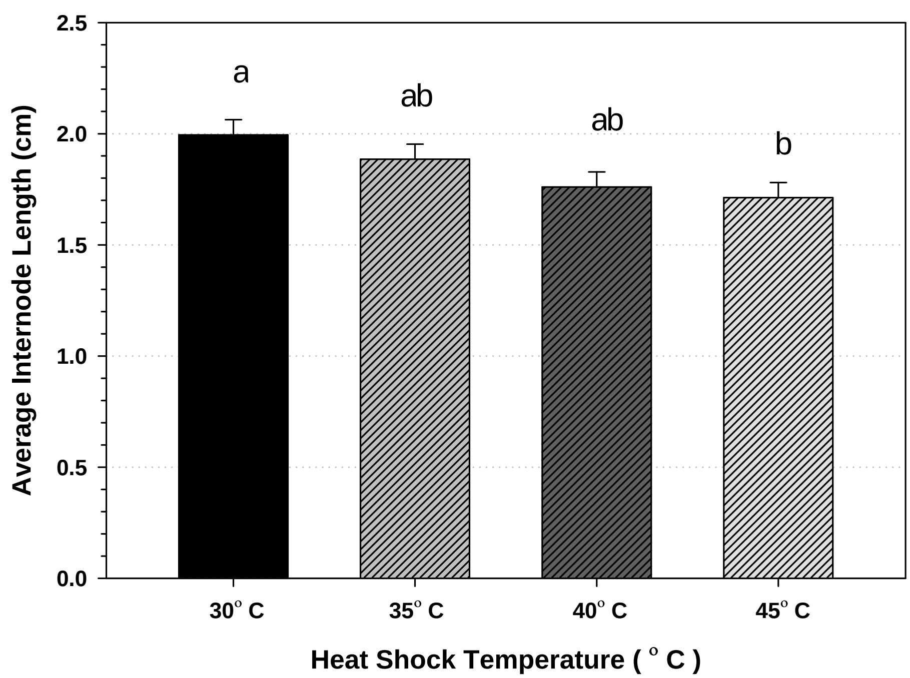

Figure 4.6. The effect of heat shock temperature on average internode length of *Petunia x hybrida* Dreams 'Midnight' over 3 weeks of treatment. Error bars represent means of fifty-four observations ±SE.

Table 4.8. The effect of combined heat shock treatments on growth and development of *Petunia x hybrida* Dreams 'Midnight' for each week.

Week	**Effect**				
	Flowers per Plant	Flower Size (cm)	Shoot Dry Weight (g)	Leaf Area (mm^2)	Internode Length (cm)
1	[Y]2.25 c[X]	7.13 ab	1.51 c	301.04 c	0 c
2	9.85 b	7.04 b	3.49 b	635.50 b	2.00 a
3	18.65 a	7.39 a	6.34 a	928.86 a	1.67 b

(Heat shock treatments included 30/25°C (control) and heat shock at 35, 40 or 45°C for 2, 4 or 6 h every 3 d).

[X] Means within columns followed by the same letters are not significantly different at 5% by lsmean procedure in SAS with Tukey's correction.

[Y] Values in the table are averages (n=60).

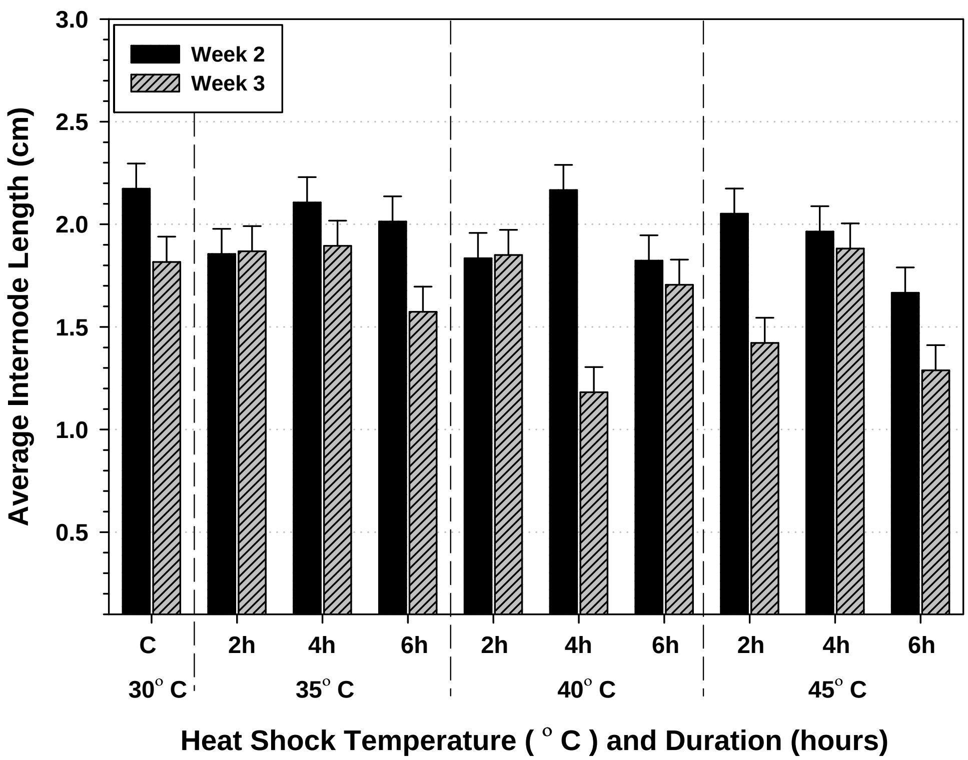

Figure 4.7. The effect of heat shock temperature and duration on average internode length of *Petunia x hybrida* Dreams 'Midnight' from week 2 to week 3. Error bars represent means of six observations ±SE.

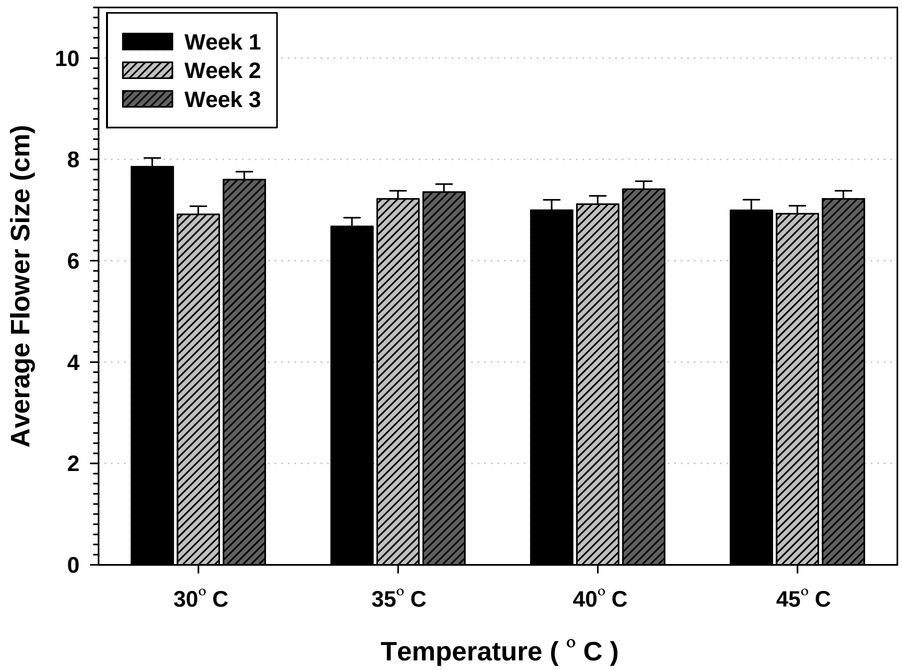

Figure 4.8. The effect of heat shock temperature on the average flower size of *Petunia x hybrida* Dreams 'Midnight' at each week. Error bars represent means of eighteen observations ±SE.

4.3.3 Effect of Enduring Heat Stress

Growth and development of petunia Dreams 'Midnight' was significantly affected by exposure to enduring heat stress for 3 weeks. Petunia grown at 35/25°C had the greatest effect on flower count after the first week while flower count of petunia grown at 40/30°C and 45/35°C were significantly reduced after 3 weeks of exposure (Table 4.9). These results are similar for average flower size of petunia exposed to enduring heat stress. Petunia grown at 35/25°C also had the greatest effect on flower size after week 1 and exposure to 45/35°C after week 2 (Table 4.9). Shoot dry weight of petunia increased for all heat stress temperatures over the 3 weeks but was significantly reduced for petunia grown at 45/35°C after week 2 compared to cooler temperatures (30/25, 35/25, or 40/30°C) (Table 4.9). Enduring heat stress did not have a significant effect on leaf area of petunia Dreams 'Midnight' after 2 weeks exposure. However, after 3 weeks exposure, growth at 40/30 or 45/35°C significantly decreased total leaf area in

Table 4.9. The effect of enduring heat stress on growth and development of *Petunia x hybrida* Dreams 'Midnight' for each week of exposure in the greenhouse.

Temperature (°C)	Week	Effect				
		Flowers Per Plant	Flower Size (cm)	Shoot Dry Weight (g)	Leaf Area (mm^2)	Internode Length (cm)
30/25°C	1	[Y]2.50 cd[X]	6.51 abc	1.54 d	314.23 d	0 c
	2	8.16 bc	6.91 ab	3.37 bc	586.2 bcd	2.17 a
	3	19.16 a	7.60 a	6.51 a	986.19 ab	1.81 ab
35/25°C	1	0 e	0 e	1.31 d	273.55 d	0 c
	2	8.00 bc	6.20 abc	3.34 bc	643.3 bcd	1.71 ab
	3	17.66 a	7.18 ab	6.75 a	1262.84 a	1.99 ab
40/30°C	1	1.16 d	3.38 cd	1.19 d	239.93 d	0 c
	2	6.50 bcd	5.70 abcd	2.45 bcd	501.00 cd	1.66 ab
	3	10.33 b	6.11 abc	5.42 a	742.19 bc	1.55 bc
45/35°C	1	1.33 d	4.06 bcd	1.23 d	235.44 d	0 c
	2	4.33 bcd	4.53 abcd	2.08 cd	371.47 cd	1.51 bc
	3	4.66 bcd	2.41 de	3.73 b	518.25 cd	1.02 c
Temperature		*	*	*	*	*
Week		*	*	*	*	NS
Temperature x Week		*	*	*	*	*

Values significant (*) or not significant (NS) at the 5% level by the lsmean procedure in SAS with Tukey's correction.

[X] Means within columns followed by the same letters are not significantly different at 5% by lsmean procedure in SAS with Tukey's correction.

[Y] Values in the table are averages (n=18).

Table 4.10. The effect of combined enduring heat stress treatments on growth and development of *Petunia x hybrida* Dreams 'Midnight' after 1, 2 or 3 weeks of exposure.

Week	**Effect**				
	Flowers per Plant	Flower Size (cm)	Shoot Dry Weight (g)	Leaf Area (mm^2)	Internode Length (cm)
1	[Y]1.45 c[X]	3.49 b	1.31 c	265.79 c	0 b
2	6.75 b	5.83 a	2.81 b	525.50 b	1.76 a
3	12.95 a	5.82 a	5.60 a	877.37 a	1.59 a

(Heat stress treatments included 30/25°C (control), 35/25, 40/30 or 45/35°C.)

[X] Means within columns followed by the same letters are not significantly different at 5% by lsmean procedure in SAS with Tukey's correction.

[Y] Values in the table are averages (n=24).

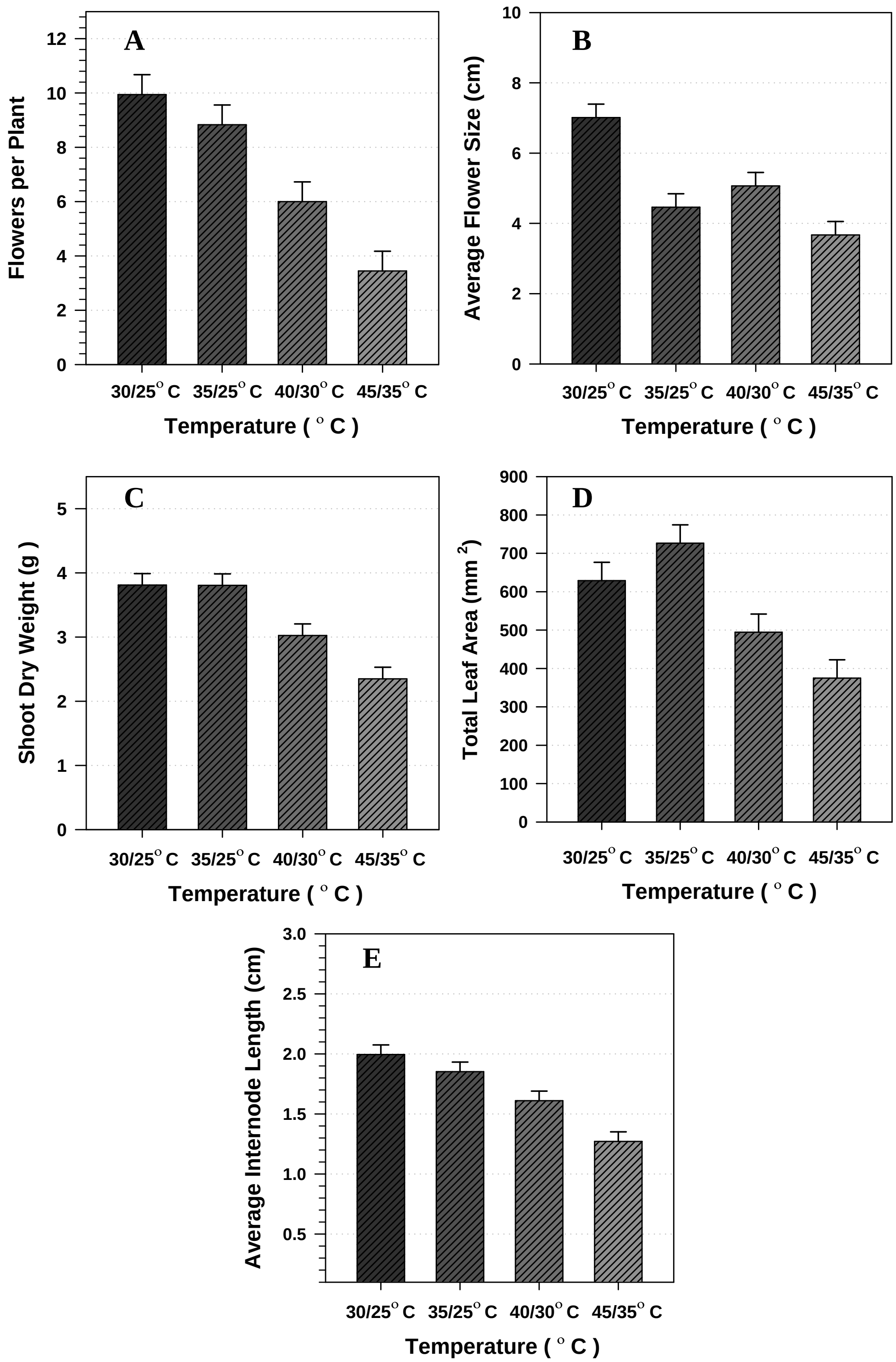

Figure 4.9. The effect of enduring heat stress on *Petunia x hybrida* Dreams 'Midnight' A) flowers per plant B) average flower size (cm) C) shoot dry weight (g) D) total leaf area (mm^2) and E) average internode length (cm) after 3 weeks grown at 30/25°C (control) or 35/25, 40/30 or 45/35°C. Error bars represent means of eighteen observations ±SE.

petunia (Table 4.9). Average internode length was significantly reduced in petunia grown at 45/35°C compared to control (30/25°C) at week 2 and 3, 35/25°C at week 3 and 40/30°C at week 2 (Table 4.9).

The effect of enduring heat stress temperature overall after 3 weeks exposure was also significant in petunia Dreams ‘Midnight’. Petunia grown at 40/30 or 45/35°C significantly reduced the number of flowers per plant by 40 and 66%, respectively, compared to control (30/25°C) (Fig.4.9 A). Average flower size was significantly larger for petunia grown at control temperature by an average of 62% compared to all other heat stress temperatures (Fig.4.9 B). Shoot dry weight was significantly reduced in petunia grown at 40/30 or 45/35°C by an average of 30% compared to 35/25°C or control (30/25°C) (Fig.4.9 C). Total leaf area was also significantly reduced in petunia grown at 45/35°C by an average of 45% compared to petunia grown at 35/25°C or control (30/25°C) (Fig.4.9 D). Petunia grown at 45/35°C had the greatest effect on average internode length by reducing lengths by 36% compared to control and 21% compared to petunia grown at 40/30°C. However, petunia grown at 40/30°C was also significantly reduced by 19% compared to control temperature (Fig.4.9 E). Overall growth and development of petunia for all temperatures did increase in flower count, flower size, shoot dry weight, and leaf area each week except average internode length which was not affected after 2 or 3 weeks of exposure (Table 4.10).

4.3.4 Acquired Thermotolerance Test (ATT)

After 3 weeks of exposure to heat shock or heat stress treatments, petunia Dreams ‘Midnight’ were subsequently exposed to 45/35°C for one week to test for acquired thermotolerance. Both heat shock and heat stress treatments had a significant effect on the growth and development of petunia after exposure to the ATT (Table 4.11). For combined treatments, petunia flower count, average flower size and average internode length significantly decreased after exposure to ATT, while shoot dry weight increased and total leaf area was not significantly affected (Table 4.12).

- **Flowers per Plant**

Petunia Dreams 'Midnight' grown at control temperature (30/25°C) or exposed to 35/25 or 40/30°C for 2, 4, 6, or 24 h durations did not have as significant effect on the number of flowers per plant. However, petunia exposed to heat stress of 45/35°C (24h duration) did show a

Table 4.11. The effect of heat stress/shock temperatures and durations on growth and development of *Petunia x hybrida* Dreams 'Midnight' after acquired thermotolerance test (ATT, exposure to 45/35°C for one week following heat treatments).

Temperature (°C)	Duration (hours)	Effect				
		Flowers Per Plant	Flower Size (cm)	Shoot Dry Weight (g)	Leaf Area (mm^2)	Internode Length (cm)
30/25°C	24 h	[Y]14.91 ab[X]	6.35 a	7.84 a	980.68 a	1.21 ab
35°C	2 h	15.00 ab	5.96 a	8.03 a	991.04 a	1.35 ab
	4 h	14.83 ab	6.10 a	7.36 a	900.92 ab	1.42 ab
	6 h	17.41 ab	6.35 a	8.27 a	953.37 ab	1.34 ab
35/25°C	24 h	15.83 ab	6.18 a	8.79 a	1159.00 a	1.48 ab
40°C	2 h	14.25 ab	6.18 a	7.63 a	986.32 a	1.41 ab
	4 h	15.25 ab	5.56 a	6.70 ab	848.84 ab	1.00 b
	6 h	15.08 ab	6.23 a	8.38 a	1035.50 a	1.21 ab
40/30°C	24 h	11.16 ab	5.68 a	7.18 ab	899.55 ab	1.48 ab
45°C	2 h	17.58 a	5.95 a	7.22 ab	834.13 ab	1.37 ab
	4 h	17.08 a	6.03 a	7.37 a	926.50 ab	1.56 a
	6 h	17.16 a	5.91 a	8.16 a	978.48 a	1.25 ab
45/35°C	24 h	9.50 b	3.69 b	5.02 b	628.04 b	1.06 b
Temp. x Duration		*	*	*	*	*
Temp. x Dur. x ATT		NS	*	NS	NS	*
Temperature x ATT		*	*	NS	NS	*
Duration x ATT		*	*	NS	NS	NS
Temperature		NS	*	*	*	NS
Duration		*	*	*	NS	NS
ATT		*	*	*	NS	*

Values significant (*) or not significant (NS) at the 5% level by the lsmean procedure in SAS with Tukey's correction.

[X] Means within columns followed by the same letters are not significantly different at 5% by lsmean procedure in SAS with Tukey's correction.

[Y] Values in the table are averages (n=12).

significant decrease in flower count compared to petunia heat shocked at this temperature (Table 4.11). Post-ATT, there was no significant difference in petunia flower counts for any heat shock/stress temperature and duration (Table 4.13). However, treatment temperature had a significant effect on petunia flower count post-ATT. There was no significant difference between Pre-ATT flower counts for all temperature treatments. Flower counts of petunia pre-exposed to 40 or 45°C were not affected by the ATT, while flower count of petunia pre-exposed to 30 or 35°C decreased significantly by 46 or 36%, respectively (Table 4.14). Treatment duration also had a significant effect on petunia flower count. Flower count of petunia Dreams 'Midnight' exposed for enduring heat stress (24 h duration), regardless of temperature, did not significantly decrease after exposure to the ATT (Table 4.15). However, petunia exposed for 2, 4, or 6 h decreased flower counts by 35, 29, and 31%, respectively.

- **Average Flower Size**

Petunia Dreams 'Midnight' grown at control temperature (30/25°C) or exposed to 35/25 or 40/30°C for 2, 4, 6, or 24 h durations did not have as significant effect on the average flower size (cm) per plant (Table 4.11). However, petunia exposed to heat stress of 45/35°C (24 h duration) did show a significant decrease in average flower size compared to petunia exposed to all other temperatures and durations. These results are similar for the effect of combined heat shock /stress treatment on average flower size pre and post-ATT (Fig. 4.10 A). While petunia exposed to heat stress of 45/35°C (24 h) was the only temperature and duration to have significantly reduced flower size pre-ATT (Table 4.6), there was no significant difference in flower size for any temperature or duration post-ATT (Table 4.13). However, average flower size did significantly decrease post-ATT for all temperatures and durations by an average of 30%, except petunia pre-exposed to 40/30°C which was unaffected or 45/35°C for 24 h which significantly increased by 104% (Fig. 4.10 A).

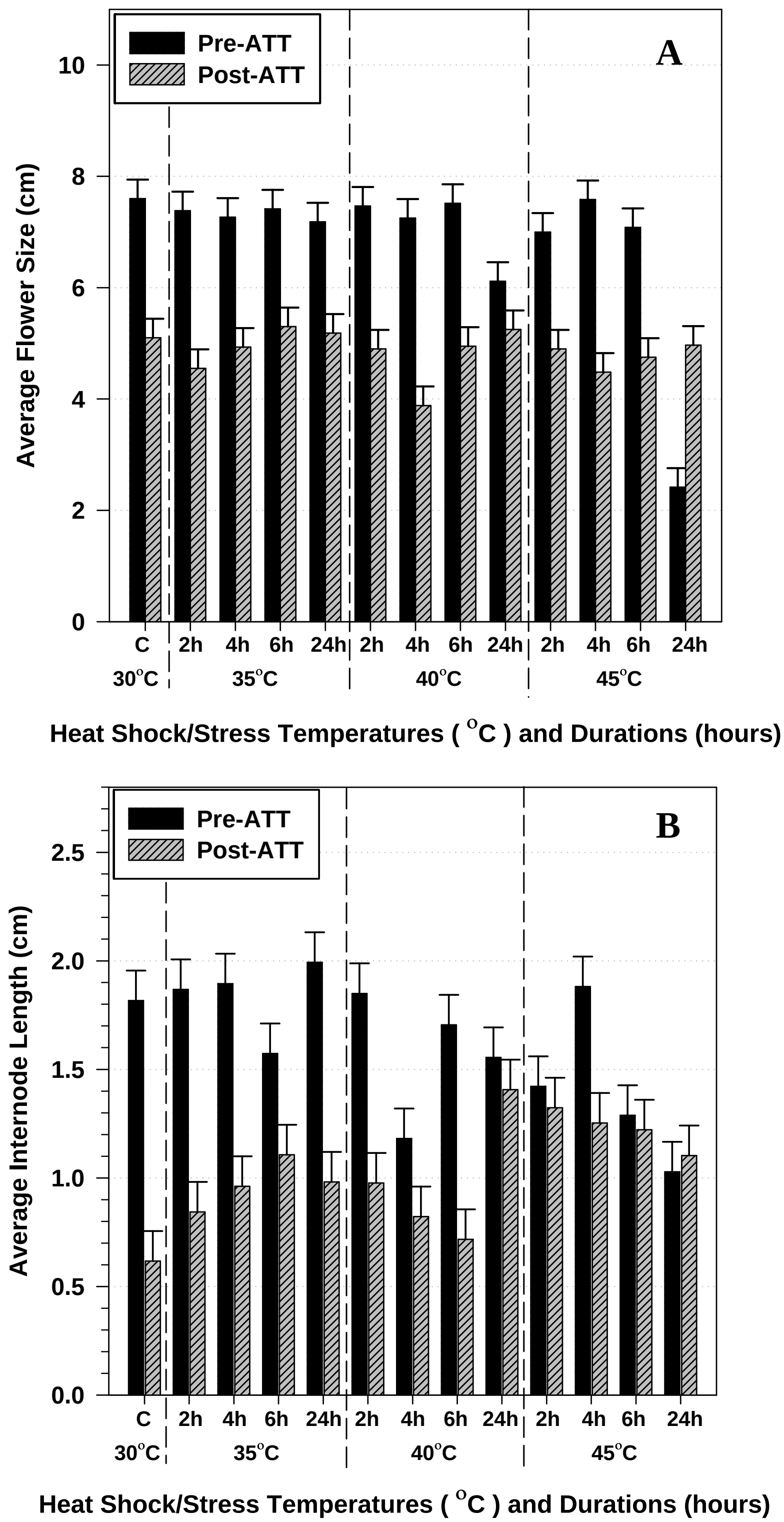

Figure 4.10. The combined effect of heat shock and heat stress treatments on *Petunia x hybrida* Dreams ‘Midnight’ A) average flower size (cm) and B) average internode length, before one week exposure to 45/35°C, (Pre-ATT) and after exposure (Post-ATT). Error bars represent means of six observations ±SE.

Table 4.12. The effect of combined heat shock and heat stress treatments on growth and development of *Petunia x hybrida* Dreams ‘Midnight’ after 3 weeks treatment in the greenhouse (Pre-ATT) and post acquired thermotolerance test (ATT, after one week exposure to 45/35°C).

Test	**Effect**				
	Flowers per Plant	Flower Size (cm)	Shoot Dry Weight (g)	Leaf Area (mm^2)	Internode Length (cm)
Pre-ATT	[Y]17.22 a[X]	7.01 a	6.16 b	915.99 a	1.65 a
Post-ATT	12.75 b	4.90 b	9.03 a	967.06 a	0.94 b

(Heat treatments included growth at 30/25°C (control) and heat shock at 35, 40 or 45°C for 2, 4 or 6 h every 3d or heat stress of 35/25, 40/30 or 45/35°C).

[X] Means within columns followed by the same letters are not significantly different at 5% by lsmean procedure in SAS with Tukey’s correction.

[Y] Values in the table are averages (n=78).

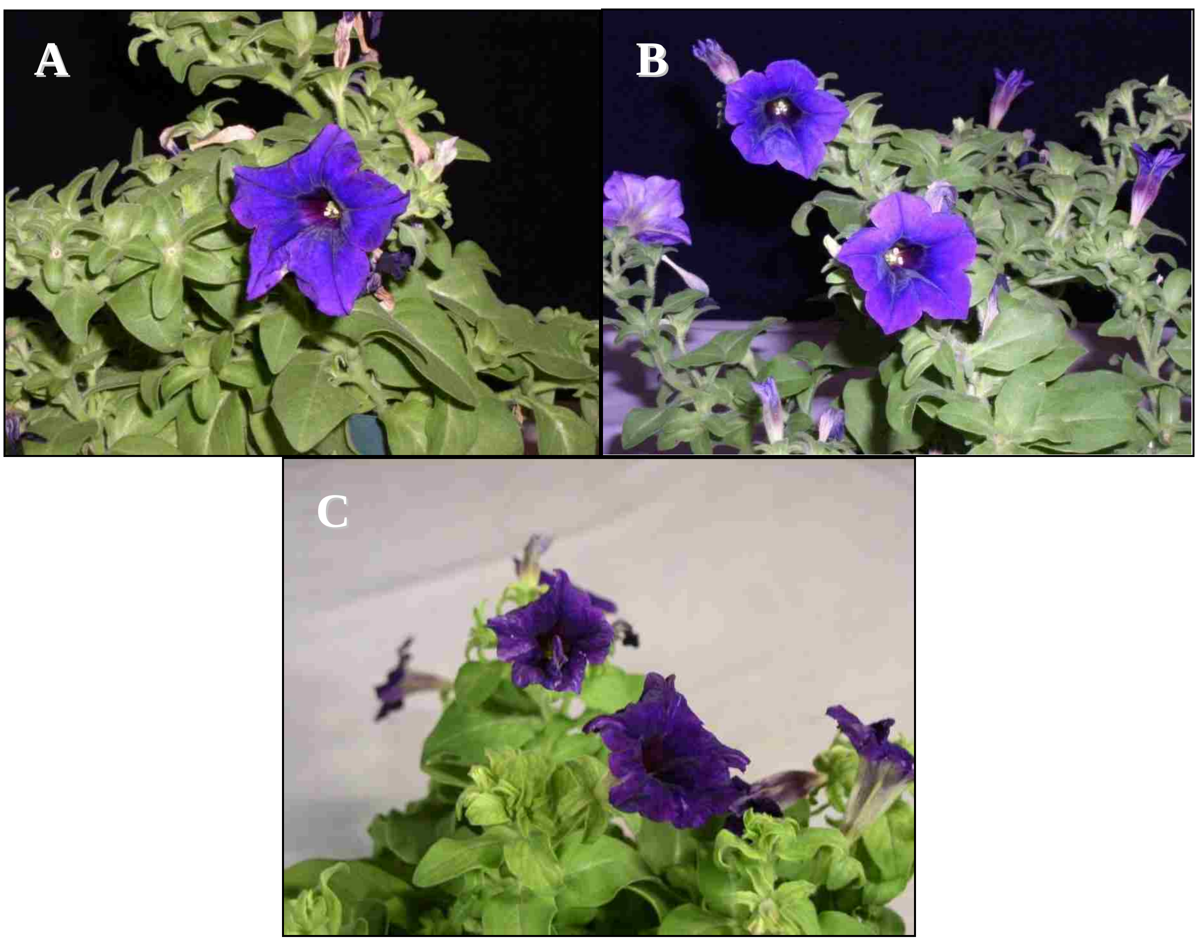

Figure 4.11. Pictorial representation of flower structures of *Petunia x hybrida* Dreams ‘Midnight’after acquired thermotolerance test (45°C for one week) for A) petunia grown at 30/25°C for 4 weeks, B) petunia heat shocked at 45°C for 4h every 3d for 4 weeks, and C) petunia grown at 45/40°C for 4 weeks.

Treatment temperature, regardless of duration, also had a significant effect on petunia flower size pre-ATT, where petunia exposed to highest temperature, 45°C, had significantly reduced flower size. However, while flower size did decrease from pre-test to post-test, there was no difference among post-ATT for all temperature treatments (Table 4.14). These results are also similar to the effect of treatment duration on petunia flower size pre-ATT, where petunia exposed 24 h had significantly reduced flower size. Post-ATT, average flower size was unaffected at the 24 h duration while petunia pre-exposed to 2, 4 or 6 h decreased flower size by 34, 38 or 38%, respectively (Table 4.15). A pictorial representation of the effects of heat shock/stress treatments on petunia grown at 30/25, 45/35°C or heat shocked at 45°C for 4 h post-ATT are depicted in Figure 4.11, A, B & C.

- **Shoot Dry Weight**

Compared to flower count and average flower size, heat shock/stress temperatures and duration had similar effects on shoot dry weight (g) of petunia Dreams 'Midnight' both pre and postATT. Averages from pre and postATT showed shoot dry weight significantly decreased at 45°C for 24 h compared to the control and 35°C for 2, 4, 6 or 24 h, 40°C for 2 or 6 h, or 45°C for 4 or 6 h (Table 4.11). Post-ATT shoot dry weight was less for petunia grown at enduring heat stress of 45/35°C (24 h) compared to enduring heat stress at 35/25°C (24 h) or heat shock at 35, 40 or 45°C for 6 h (Table 4.13). For both pre and postATT, shoot dry weight was significantly reduced in petunia exposed to 45°C compared to 30 or 35°C, and also significantly decreased for petunia exposed for 24 h compared to 6 h duration (Table 4.16).

- **Total Leaf Area**

Leaf area of petunia was significantly reduced when exposed to 45°C for 24 h compared to petunia grown at control (30/25°C) or exposed to 35°C for 2 or 24 h, 40°C for 2 or 6 h, or 45°C for 6 h for pre and post-ATT (Table 4.11). However, total leaf areas of petunia Dreams

Table 4.13. The effect of heat shock/stress temperatures and durations on growth and development of *Petunia x hybrida* Dreams 'Midnight' Post-ATT (preconditioned petunia after exposure to ATT, 45/35°C day/night for one week).

Temperature (°C)	Duration (hours)	**Post-ATT**				
		Flowers Per Plant	Flower Size (cm)	Shoot Dry Weight (g)	Leaf Area (mm^2)	Internode Length (cm)
30/25°C	24 h	[Y]10.66 a[X]	5.10 a	9.18 abc	975.17 a	0.61 b
35°C	2 h	9.16 a	4.55 a	9.33 abc	998.50 a	0.84 ab
	4 h	11.66 a	4.93 a	8.31 abc	896.73 a	0.96 ab
	6 h	14.66 a	5.30 a	10.09 ab	1002.04 a	1.10 ab
35/25°C	24 h	14.00 a	5.18 a	10.82 a	1055.14 a	0.98 ab
40°C	2 h	11.50 a	4.90 a	9.19 abc	1069.61 a	0.97 ab
	4 h	11.33 a	3.88 a	6.65 bc	739.59 a	0.82 ab
	6 h	13.16 a	4.95 a	10.10 a	1104.44 a	0.71 b
40/30°C	24 h	12.00 a	5.25 a	8.93 abc	1056.91 a	1.40 a
45°C	2 h	17.33 a	4.90 a	8.74 abc	872.88 a	1.32 ab
	4 h	17.83 a	4.48 a	9.02 abc	945.08 a	1.25 ab
	6 h	14.33 a	4.75 a	10.21 ab	1093.53 a	1.22 ab
45/35°C	24 h	14.33 a	4.96 a	6.32 c	737.82 a	1.10 ab

[X] Means within columns followed by the same letters are not significantly different at 5% by lsmean procedure in SAS with Tukey's correction.

[Y] Values in the table are averages (n=6).

Table 4.14. The effect of combined heat shock/stress temperature on number of flowers per plant, average flower size, and average internode length of *Petunia x hybrida* Dreams 'Midnight' after 3 weeks exposure to heat preconditioning (Pre-ATT) and after one week exposure to 45/35°C (Post-ATT).

Temperature (°C)	**ATT**	**Effect**		
		Flowers per Plant	Flower Size (cm)	Internode Length (cm)
30°C (Control)	Pre-ATT	[Y]19.16 a[X]	7.60 a	1.81 a
	Post-ATT	10.66 b	5.10 c	0.61 e
35°C	Pre-ATT	19.16 a	7.31 a	1.83 a
	Post-ATT	12.37 b	4.99 c	0.97 d
40°C	Pre-ATT	15.87 ab	7.08 a	1.57 ab
	Post-ATT	12.00 b	4.74 c	0.98 d
45°C	Pre-ATT	14.70 ab	6.02 b	1.40 bc
	Post-ATT	15.95 ab	4.77 c	1.22 cd

[X] Means within columns followed by the same letters are not significantly different at 5% by lsmean procedure in SAS with Tukey's correction.

[Y] Values in the table are averages (n=24).

‘Midnight’ were not significantly different post-ATT (Table 4.13) or between pre- and post-ATT for treatment temperatures, durations, or combination of temperatures and durations, respectively. However, petunia exposed to specific treatment temperature for both pre and post-ATT measurements did have a significant effect on total leaf area. Petunia exposed to 45°C showed significantly reduced leaf area compared to petunia exposed to 35°C (Fig. 4.12).

Table 4.15. The effect of combined heat shock/stress durations on number of flowers per plant and average flower size of *Petunia x hybrida* Dreams ‘Midnight’ after 3 weeks exposure to heat preconditioning (Pre-ATT) and after one week exposure to 45/35°C (Post-ATT).

Duration (hours)	**ATT**	**Effect**	
		Flowers per Plant	Flower Size (cm)
2h	Pre-ATT	[Y]18.70 a[X]	7.36 a
	Post-ATT	12.16 b	4.86 c
4h	Pre-ATT	18.16 a	7.42 a
	Post-ATT	12.87 b	4.6 c
6h	Pre-ATT	19.08 a	7.40 a
	Post-ATT	13.20 b	5.02 c
24h	Pre-ATT	12.95 b	5.82 b
	Post-ATT	12.75 b	5.12 bc

[X] Means within columns followed by the same letters are not significantly different at 5% by lsmean procedure in SAS with Tukey’s correction.

[Y] Values in the table are averages (n=24).

Table 4.16. The effect of combined duration or temperature on *Petunia x hybrida* Dreams ‘Midnight’ shoot dry weight (g) after acquired thermotolerance test (exposure to 45/35°C for one week following heat treatments).

Effect	**Temperature(°C)**				**Duration (hours)**			
	30°C	35°C	40°C	45°C	2 h	4 h	6 h	24 h
Shoot Dry Weight	[Y]7.84 a[X]	8.11 a	7.47 ab	6.94 b	7.68 ab	7.32 ab	8.16 a	7.21 b

[X] Means within rows, for each effect, followed by the same letters are not significantly different at 5% by lsmean procedure in SAS with Tukey’s correction.

[Y] Values in the table are averages (n=48).

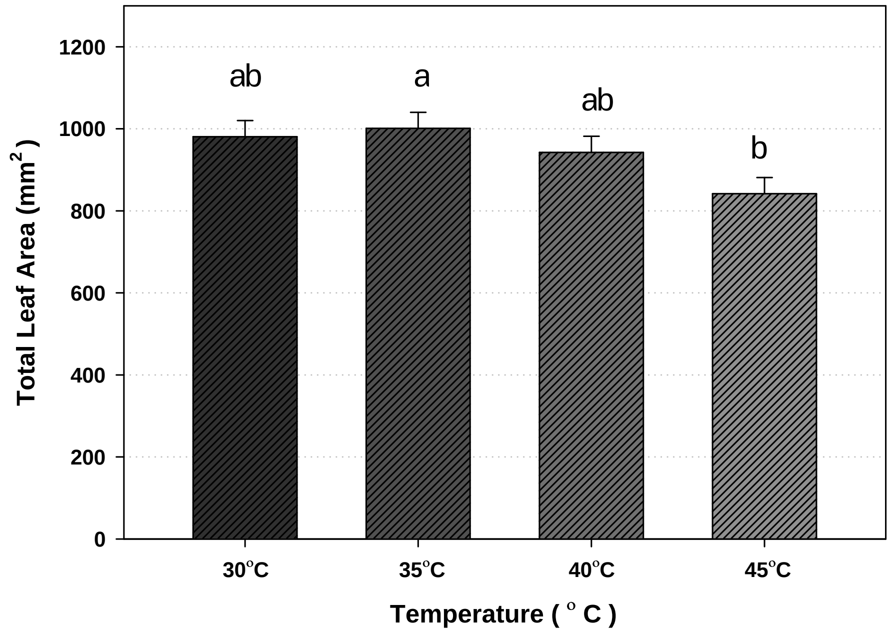

Figure 4.12. The effect of combined heat shock/stress temperature on total leaf area (mm^2) of *Petunia x hybrida* Dreams 'Midnight' for both pre and post-acquired thermotolerance test. Error bars represent means of forty-eight observations ±SE.

- **Average Internode Length**

The average internode length (cm) of petunia Dreams 'Midnight' was significantly reduced when exposed to 40°C for 4 h and 45°C for 24 h compared to petunia exposed to 45°C for 4 h for pre and post-ATT (Table 4.11). Post-ATT, petunia grown at control (30/25°C) or heat shocked at 40°C for 6 h had significantly reduced internode length compared to enduring heat stress at 40/30°C (24 h) (Table 4.13). Average internode length was significantly reduced post-ATT compared to internode length before the test for all heat treatments (Table 4.14). Petunia grown at 30/25°C (control), 35/25°C or heat shocked at 40°C for 2 or 6 h had the greatest effect on internode length by (-66, -50, -47 and -58%, respectively after ATT (Fig. 4.10 B).

- **Relative Chlorophyll Content**

Heat shock/enduring heat stress treatments had a significant effect on the relative chlorophyll content of petunia Dreams 'Midnight' pre and post-ATT. Petunia grown at control temperature 30/25°C or heat shocked at 35°C for 4 h or 45°C for 6 h had significantly lower chlorophyll content than petunia exposed to enduring heat stress at 35/25, 40/30 or 45/35°C which had significantly higher relative chlorophyll contents (Fig. 4.13).

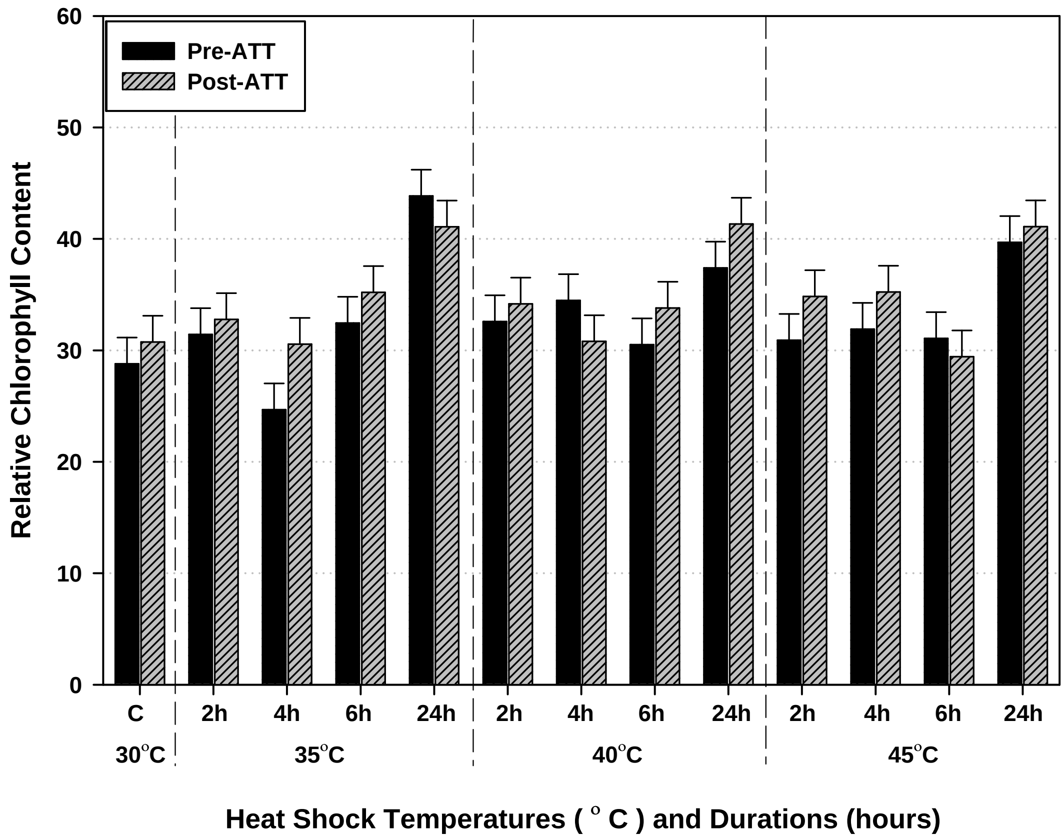

Figure 4.13. The effect of heat shock treatment (35, 40 or 45°C for 2, 4 or 6 h every 3 d) and heat stress (35/25, 40/30 or 45/35°C, 24 h duration) on relative chlorophyll content of *Petunia x hybrida* Dreams 'Midnight' prior to (pre-ATT) and after (post-ATT) one week exposure to 45°C (ATT - acquired thermotolerance test). Error bars indicate means of six observations ± SE.

- **Marketable Quality Pre and Post-Acquired Thermotolerance Test**

Heat shock and heat stress treatments had a significant effect on the marketable quality of petunia Dreams 'Midnight' pre- and post-ATT. Prior to the ATT, petunia grown at 35/25°C (24 h) had significantly higher quality ratings than petunia grown at 40/30 or 45/35°C (24 h) or heat shocked at 35°C for 2 or 4 h, 40°C for 4 h, or 45°C for 2 or 6 h (Fig. 4.14). Post-ATT there was no significant difference in petunia quality among any of the heat treatments (heat shock or heat stress).

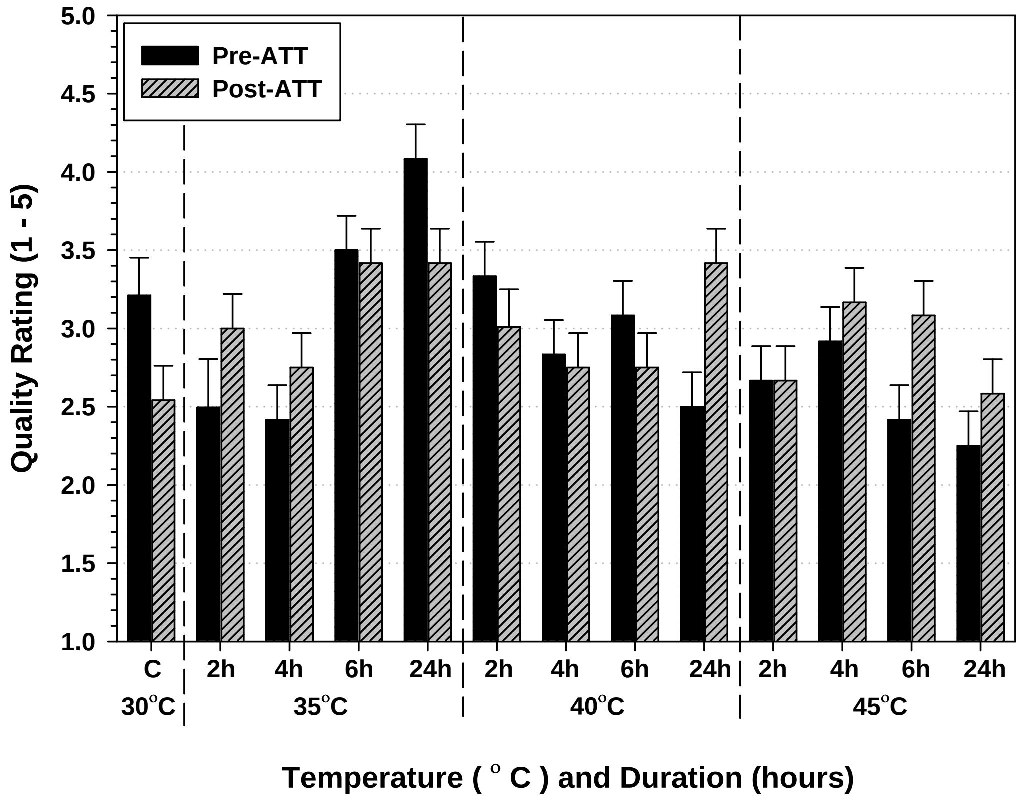

Figure 4.14. The effect of heat shock (35, 40 or 45°C for 2, 4 or 6 h every 3d) or heat stress (35/25, 40/30 or 45/35°C) on plant quality of *Petunia x hybrida* Dreams 'Midnight' after one week exposure to 45°C (acquired thermotolerance test - ATT). Error bars indicate means of six observations ± SE.

4.3.5 Heat Shock Treatment and Acquired Thermotolerance Test

Heat shock temperatures and durations imposed on petunia Dreams 'Midnight' did not have a significant effect on most of the growth measurements taken for pre and post- ATT (45/35°C for one week) (Table 4.17). For heat shock treatment, petunia flower count, average flower size and average internode length significantly decreased after exposure to ATT (-30, -33 and - 44%, respectively), while shoot dry weight increased 45 % and total leaf area was not significantly different (Table 4.18).

Heat shock temperatures did have a significant effect on thermotolerance of petunia flower count. Flower count of petunia grown at control temperature (30/25°C) was significantly decreased by 45% after ATT, while flower count of petunia exposed to 35°C decreased by 40%. Flower counts of petunia exposed to 40 or 45°C were not significantly affected by subsequent exposure to ATT (Fig. 4.15). Heat shock temperature and duration also had a significant effect on thermotolerance of petunia Dreams 'Midnight' average internode length (cm). Average internode length of petunia exposed to 45°C was least affected by the ATT where internode

Table 4.17. The effect of heat shock temperature and duration on growth and development of *Petunia x hybrida* Dreams 'Midnight' after ATT (ATT-acquired thermotolerance test - exposure to 45/35°C for one week following heat shock treatments every 3 d for 4 weeks).

Temperature (°C)	Duration (hours)	Effect				
		Flowers Per Plant	Flower Size (cm)	Shoot Dry Weight (g)	Leaf Area (mm^2)	Internode Length (cm)
30/25°C (Control)		[Y]14.91 a[X]	6.35 a	7.84 a	980.68 a	1.21 a
35°C	2 h	15.00 a	5.96 a	8.03 a	991.04 a	1.35 a
	4 h	14.83 a	6.10 a	7.36 a	900.92 a	1.42 a
	6 h	17.41 a	6.35 a	8.27 a	953.37 a	1.34 a
40°C	2 h	14.25 a	6.18 a	7.63 a	986.32 a	1.41 a
	4 h	16.43 a	5.94 a	7.37 a	924.77 a	1.08 a
	6 h	15.08 a	6.23 a	8.38 a	1035.49 a	1.21 a
45°C	2 h	17.58 a	5.95 a	7.22 a	834.13 a	1.37 a
	4 h	17.08 a	6.03 a	7.37 a	926.50 a	1.56 a
	6 h	17.16 a	5.91 a	8.16 a	978.48 a	1.25 a
Temp. x Duration		NS	NS	NS	NS	NS
Temp. x Dur. x ATT		NS	NS	NS	NS	*
Temp. x ATT		*	NS	NS	NS	*
Duration x ATT		NS	NS	NS	NS	NS
Temperature		NS	NS	NS	NS	NS
Duration		NS	NS	NS	NS	NS
[Z]ATT		*	*	*	NS	*

Values significant (*) or not significant (NS) at the 5% level by the lsmean procedure in SAS with Tukey's correction.

[X] Means within columns followed by the same letters are not significantly different at 5% by lsmean procedure in SAS with Tukey's correction.

[Y] Values in the table are averages (n=12).

[Z] Represents growth measurements taken for pre- (acquired thermotolerance test – ATT) or post-ATT.

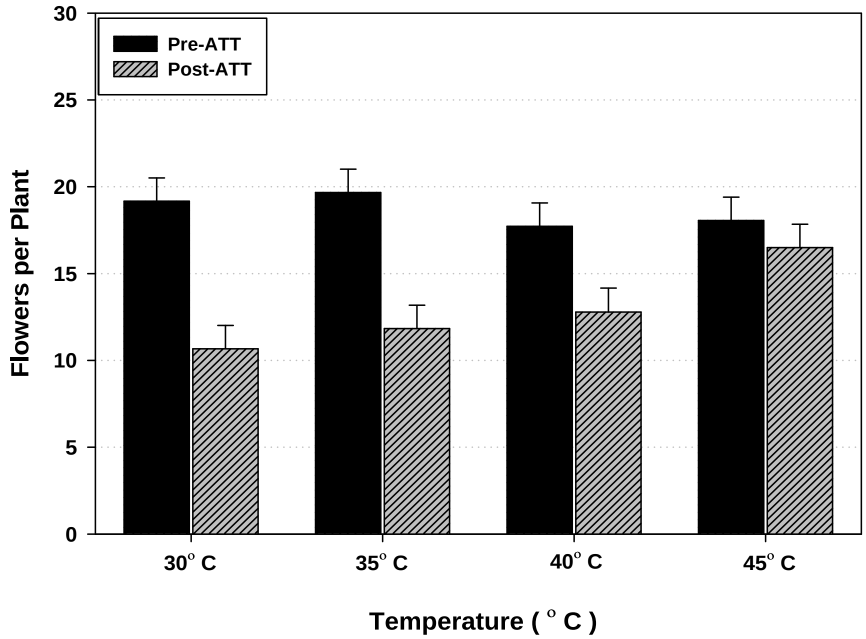

Figure 4.15. The effect of heat shock temperature for all durations (35, 40 or 45°C for 2, 4 or 6 h every 3d) on flower count of *Petunia x hybrida* Dreams 'Midnight' after 3 weeks of heat shock treatment (pre-ATT) and post-ATT (following exposure to 45/35°C for one week). Error bars represent means of eighteen observations ±SE.

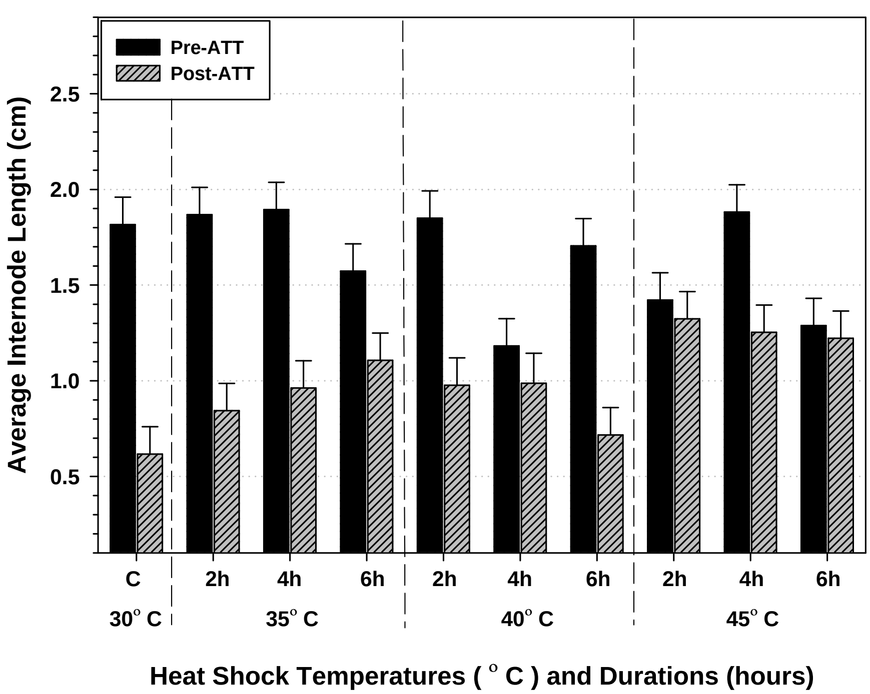

Figure 4.16. The effect of heat shock temperature and duration (35, 40 or 45°C for 2, 4 or 6 h every 3d) on average internode length (cm) of *Petunia x hybrida* Dreams 'Midnight' after 3 weeks of heat shock treatment (pre-ATT) and following exposure to 45/35°C for one week (post-ATT). Error bars represent means of six observations ±SE.

length was not significant by ATT at 2 and 6 h. Petunia internode length was severely affected by exposure to subsequent ATT for petunia grown at control temperature (30/25°C) (- 66%) or heat shocked at 35 or 40°C for 2 (- 54%) or 4 h (- 49%), or 2 (- 47%) or 6 h (- 58%), respectively (Fig.4.16). The reduction of internode growth observed in petunia grown at 30/25°C after one week heat stress at 45°C is depicted in Figure 4.17 A & B.

Table 4.18. The effect of combined heat shock treatments (every 3d for 3 weeks) on growth and development of *Petunia x hybrida* Dreams 'Midnight' (pre-ATT) and following exposure to 45/35°C for one week (post-ATT).

ATT	**Effect**				
	Flowers per Plant	Flower Size (cm)	Shoot Dry Weight (g)	Leaf Area (mm^2)	Internode Length (cm)
Pre-ATT	[Y]18.65 a[X]	7.39 a	6.34 b	928.86 a	1.67 a
Post-ATT	12.94 b	4.89 b	9.21 a	983.31 a	0.93 b

(Heat shock treatments included 30/25°C (control) and heat shock at 35, 40 or 45°C for 2, 4 or 6 h every 3d).

[X] Means within columns followed by the same letters are not significantly different at 5% by lsmean procedure in SAS with Tukey's correction.

[Y] Values in the table are averages (n=60).

Figure 4.17. A pictorial representation of decreased internode length observed in *Petunia x hybrida* Dreams 'Midinght' grown at 30°C after one week exposure to 45°C (acquired thermotolerance test). A) Top view of petunia grown at 30°C after one week exposure to 45°C and B) Close-up view of decreased internode length.

4.3.6 Enduring Heat Stress Acquired Thermotolerance

Enduring heat stress treatments had a significant effect on acquired thermotolerance of petunia Dreams 'Midnight' (Table 4.19). Flower count of petunia was significantly reduced by 55% at control temperature (30/25°C) and significantly increased by 207% at 45/35°C (Fig.4.18 A). Before ATT, average flower size was significantly reduced by an average of 65% when grown at 45/35°C. However, there was no significant difference between flower sizes for all treatment temperatures after ATT. Results show that ATT significantly reduced average flower size by 33% of petunia grown at 30/25°C while average flower size increased over 200% in petunia grown at 45/35°C (Fig.4.18 B). Average internode length of petunia was significantly reduced after ATT grown at 30/25 and 35/25°C by 67% and 51%, respectively. However,

Table 4.19. The effect of enduring heat stress on growth and development of *Petunia x hybrida* Dreams 'Midnight' after acquired thermotolerance test (ATT - exposure to 45/35°C for one week) following heat stress treatments for 4 weeks).

Temperature (°C)	ATT	Effect				
		Flowers per Plant	Flower Size (cm)	Shoot Dry Weight (g)	Leaf Area (mm^2)	Internode Length (cm)
30/25°C	Pre-ATT	[Y]19.16 a[X]	7.60 a	6.51 c	986.19 abc	1.81 ab
	Post-ATT	8.66 bc	5.10 bc	9.18 a	975.17 abc	0.61 e
35/25°C	Pre-ATT	17.66 ab	7.18 ab	6.75 bc	1262.84 a	1.99 a
	Post-ATT	14.00 abc	5.18 bc	10.82 a	1055.14 ab	0.98 de
40/30°C	Pre-ATT	10.33 abc	6.11 abc	5.42 cd	742.19 abc	1.55 abc
	Post-ATT	12.00 abc	5.25 bc	8.93 ab	1056.91 ab	1.40 bcd
45/35°C	Pre-ATT	4.66 c	2.41 d	3.73 d	518.25 c	1.02 cde
	Post-ATT	14.33 ab	4.96 c	6.32 c	737.82 bc	1.10 cde
Temperature		*	*	*	*	*
[Z]ATT		NS	NS	*	NS	*
Temperature x ATT		*	*	NS	NS	*

Values significant (*) or not significant (NS) at the 5% level by the lsmean procedure in SAS with Tukey's correction.

[X] Means within columns followed by the same letters are not significantly different at 5% by lsmean procedure in SAS with Tukey's correction.

[Y] Values in the table are averages (n=6).

[Z] Represents growth measurements taken for pre- (acquired thermotolerance test – ATT) or post-ATT.

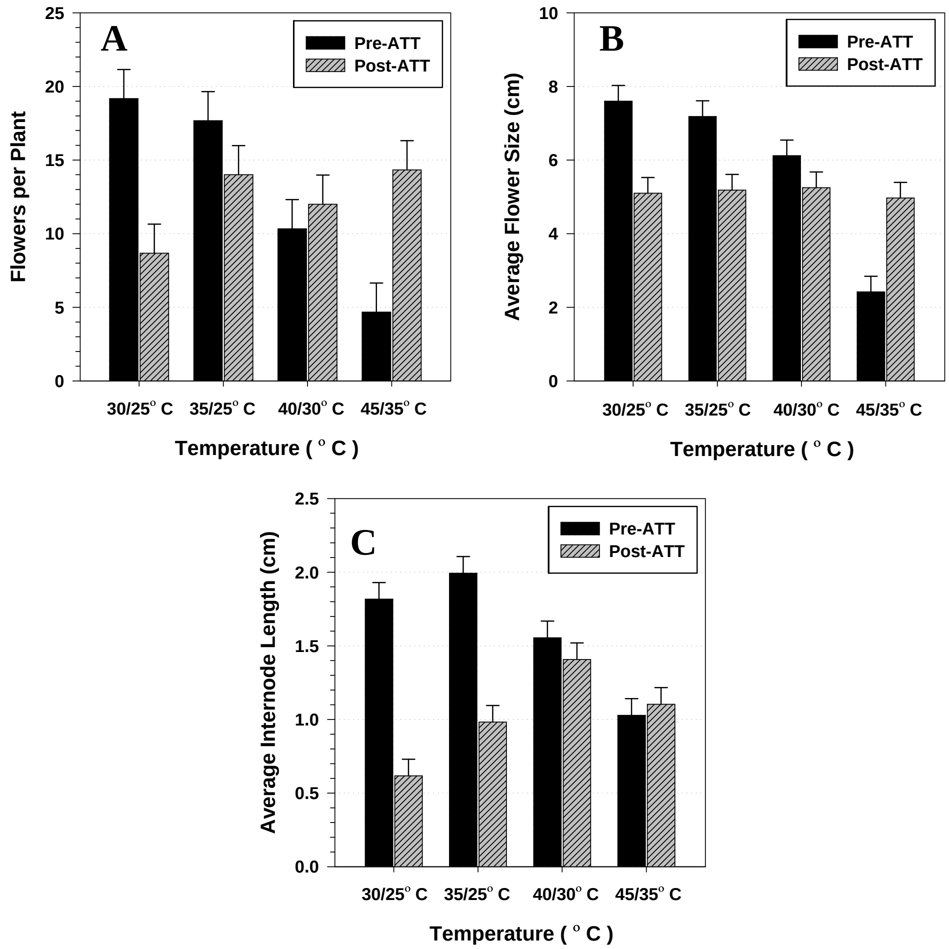

Figure 4.18. The effect of enduring heat stress on *Petunia x hybrida* Dreams 'Midnight' A) flower count, B) average flower size (cm) and C) average internode length (cm) after enduring heat stress for 3 weeks (Pre-ATT) and following exposure to 45/35°C for one week (Post-ATT - acquired thermotolerance test). Error bars represent means of six observations ±SE.

Table 4.20. The effect of enduring heat stress temperatures on overall growth and development of *Petunia x hybrida* Dreams 'Midnight' before (Pre-ATT) and Post-ATT (ATT - acquired thermotolerance test at 45/35°C for one week).

Heat Stress Temperature (°C)	**Effect**				
	Flowers per Plant	Flower Size (cm)	Shoot Dry Weight (g)	Leaf Area (mm^2)	Internode Length (cm)
30/25°C	[Y]13.91 ab[X]	6.35 a	7.84 ab	980.68 a	1.21 ab
35/25°C	15.83 a	6.18 a	8.79 a	1159.00 a	1.48 a
40/30°C	11.16 ab	5.68 a	7.18 b	899.55 ab	1.48 a
45/35°C	9.50 b	3.69 b	5.02 c	628.04 b	1.06 b

(Heat stress treatments included 30/25°C (control), 35/25, 40/30 or 45/35°C.)

[X] Means within columns followed by the same letters are not significantly different at 5% by lsmean procedure in SAS with Tukey's correction.

[Y] Values in the table are averages (n=12).

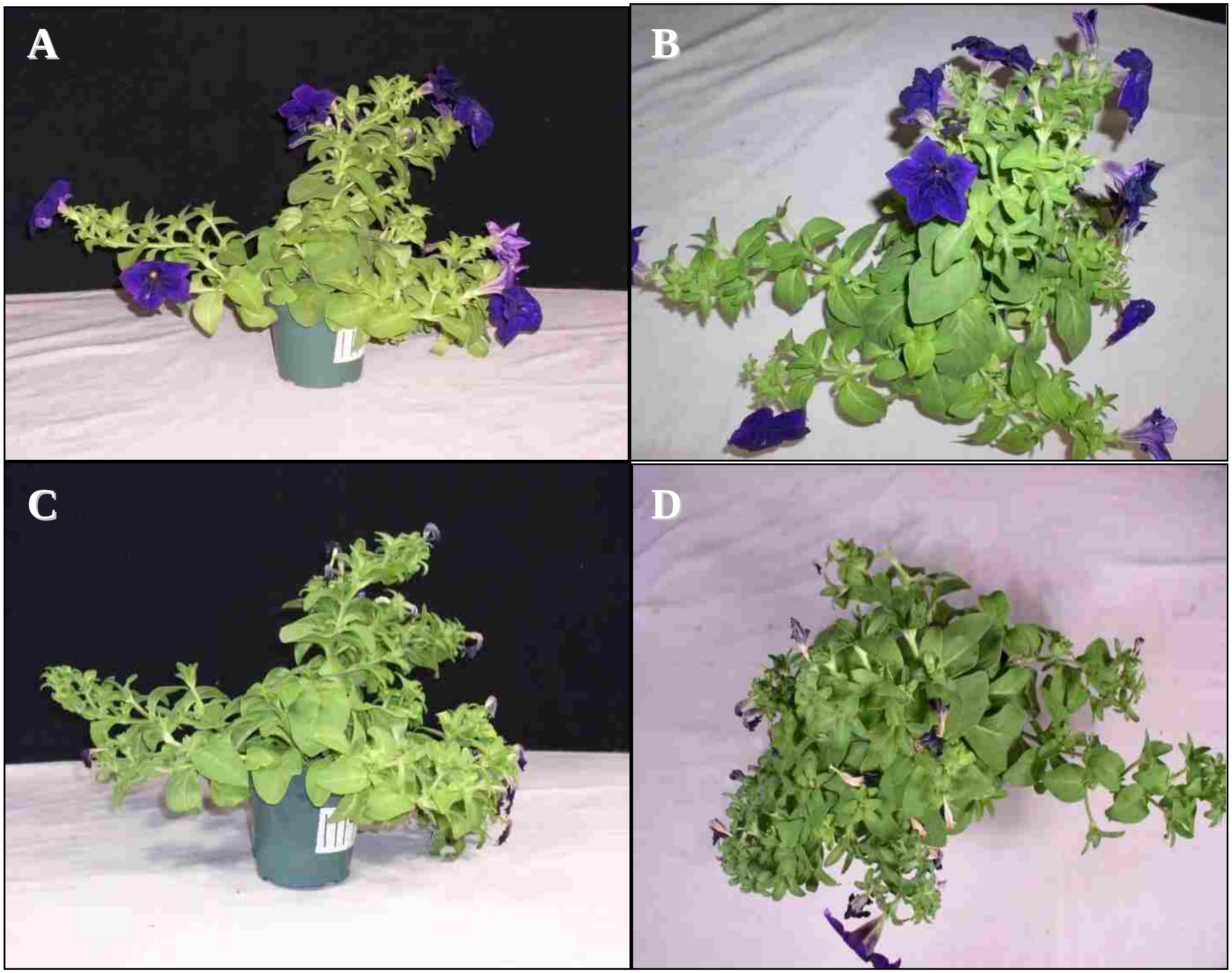

Figure 4.19. Pictorial representation of the effect of one week heat stress at 45°C (acquired thermotolerance test) on non-heat shocked *Petunia x hybrida* Dreams 'Midnight' grown at 30°C. A) Side view of petunia grown at 30°C before exposure to 45°C, B) Top view of petunia grown at 30°C before exposure to 45°C, C) Side view of petunia grown at 30°C after one week exposure to 45°C and D) Top view of petunia grown at 30°C after one week exposure to 45°C.

exposure to ATT did not affect average internode length of petunia grown at 40/30 or 45/35°C (Fig.4.18 C).

Overall heat stress temperatures for both pre and post-ATT measurements had a significant effect on all growth measurements. Results show that petunia grown at 45/35°C had significantly reduced flower count compared to 35/25°C, while average flower size (cm), shoot dry weight and total leaf area (mm^2) were reduced at 45/35°C compared to control temperature (30/25°C) (Table 4.20). Average internode length significantly decreased in petunia grown at 45/35°C compared to 35/25 and 40/30°C. The effect of subsequent acquired ATT on petunia grown at control temperature (30/25°C) is represented in Figure 4.19 A, B, C, & D.

4.3.7 Chlorophyll Fluorescence

- **Heat shock**

Heat shock treatment did not have a significant effect on the maximum quantum efficiency of PSII (Fv/Fm) for either young or mature leaves of petunia Dreams 'Midnight' (Table 4.21). However, heat shock temperature did have a significant effect on Fv/Fm of young leaves, where petunia grown at control temperature (30/25°C) had significantly decreased Fv/Fm of 0.857 compared to petunia heat shocked at higher temperatures (35, 40 or 45°C) (Table 4.22). However, there was no difference between heat shock treatments and chlorophyll fluorescence measurements in young leaves taken before ATT, and 3 or 7 d after ATT. The maximum quantum efficiency of PSII in mature leaves was unaffected by specific heat shock temperatures and durations, but was significantly decreased pre-ATT compared to 3 or 7 d post-ATT (Table 4.21).

- **Enduring Heat Stress**

Enduring heat stress had a significant effect on the maximum quantum efficiency of young and mature leaves of petunia Dreams 'Midnight'. For young leaves, Fv/Fm measurements were not significant between readings but were significantly lower in petunia grown at 30/25°C (Table 4.23). For mature leaves, Fv/Fm was significantly lower for petunia grown at 30/25 and 35/25°C, and was significantly lower in petunia before exposure to ATT (Table 4.23).

Table 4.21. The effect of heat shock temperature and duration on the maximum quantum efficiency of PSII (Fv/Fm) using chlorophyll fluorescence measurements of dark-adapted young and mature leaves of *Petunia x hybrida* Dreams 'Midnight' before acquired thermotolerance test (Pre-ATT), and 3 or 7 d after acquired thermotolerance test (exposure to 45/35°C for one week).

Temperature (°C)	Duration (hours)	**Maximum Quantum Efficiency of PSII (Fv/Fm)**					
		Young Leaf			Mature Leaf		
		Pre-ATT	3 d	7 d	Pre-ATT	3 d	7 d
30/25°C (Control)		0.85 bc[X]	0.85 bc[Y]	0.86 abc	0.83 bcd	0.86 abc	0.86 ab
35°C	2 h	0.86 abc	0.86 abc	0.86 abc	0.81 de	0.85 abc	0.86 ab
	4 h	0.87 abc	0.86 abc	0.86 abc	0.84 bc	0.86 abc	0.87 a
	6 h	0.86 abc	0.86 abc	0.86 abc	0.83 bcd	0.85 abc	0.86 ab
40°C	2 h	0.86 abc	0.86 abc	0.86 abc	0.83 cd	0.85 abc	0.87 a
	4 h	0.87 ab	0.86 abc	0.88 a	0.84 bc	0.85 abc	0.86 ab
	6 h	0.87 abc	0.86 abc	0.87 abc	0.82 cd	0.86 abc	0.86 ab
45°C	2 h	0.87 abc	0.86 abc	0.86 abc	0.82 cd	0.85 abc	0.86 ab
	4 h	0.86 abc	0.86 abc	0.86 abc	0.83 bc	0.85 abc	0.86 ab
	6 h	0.87 abc	0.86 abc	0.86 abc	0.83 bcd	0.86 abc	0.87 a
Temp. x Duration			NS			NS	
Temp. x Dur. x ATT			NS			NS	
Temp. x ATT			NS			NS	
Duration x ATT			NS			NS	
Temperature			*			NS	
Duration			NS			NS	
[Z]ATT			NS			*	

For young or mature leaves, values significant (*) or not significant (NS) at the 5% level by the lsmean procedure in SAS with Tukey's correction.

[X] Means within columns and rows for young leaves or mature leaves followed by the same letters are not significantly different at 5% by lsmean procedure in SAS with Tukey's correction.

[Y] Values in the table are averages (n=6).

[Z] Represents Fv/Fm measurements taken for pre- (acquired thermotolerance test – ATT) or 3 or 7 d after ATT.

Table 4.22. The effect of heat shock treatment temperatures on the maximum quantum efficiency of PSII (Fv/Fm) using chlorophyll fluorescence measurements of dark-adapted young leaves of *Petunia x hybrida* Dreams 'Midnight'.

Heat Shock Temperature (°C)	Maximum Quantum Efficiency of PSII (Fv/Fm) in Young Leaf
30/25°C (Control)	[Y]0.857 b[X]
35/25°C	0.864 a
40/30°C	0.870 a
45/35°C	0.867 a

(Heat shock treatments included 30/25°C (control) and heat shock at 35, 40 or 45°C for 2, 4 or 6 h every 3 d).

[X] Means within columns followed by the same letters are not significantly different at 5% by lsmean procedure in SAS with Tukey's correction.

[Y] Values in the table are averages (n=60).

Table 4.23. The effect of enduring heat stress temperatures on the maximum quantum efficiency of PSII using chlorophyll fluorescence measurements of dark-adapted young and mature leaves of *Petunia x hybrida* Dreams 'Midnight' before acquired thermotolerance test (Pre-ATT), and 3 and 7 d after acquired thermotolerance test (exposure to 45/35°C for one week).

Heat Stress Temperature (°C)	Maximum Quantum Efficiency of PSII (Fv/Fm)					
	Young Leaf			Mature Leaf		
	Pre-ATT	3d	7d	Pre-ATT	3d	7d
30/25°C	[Y]0.855 c[X]	0.855 c	0.861 bc	0.836 b	0.860 ab	0.865 ab
35/25°C	0.878 ab	0.875 abc	0.865 abc	0.806 c	0.848 ab	0.868 a
40/30°C	0.880 ab	0.875 abc	0.868 abc	0.866 a	0.875 a	0.875 a
45/35°C	0.876 ab	0.883 a	0.873 abc	0.870 a	0.875 a	0.873 a
Temperature		*			*	
[Z]ATT		NS			*	
Temp. x ATT		NS			*	

For young or mature Leaves, values significant (*) or not significant (NS) at the 5% level by the lsmean procedure in SAS with Tukey's correction.

[X] Means within columns and rows for young leaves or mature leaves followed by the same letters are not significantly different at 5% by lsmean procedure in SAS with Tukey's correction.

[Y] Values in the table are averages (n=6).

[Z] Represents Fv/Fm measurements taken for pre- (acquired thermotolerance test – ATT) or 3 or 7 d after ATT

4.4 DISCUSSION

Heat preconditioning of petunia through short duration heat shock or enduring heat stress revealed differences in promoting acquired thermotolerance in marketable plants during greenhouse production. Acquired thermotolerance occurs when plants are pre-exposed to varying levels of nonlethal stress and subsequently develop the ability to survive extreme temperatures (Senthil et al., 2003). Certain adaptable characteristics have been associated with acquired thermotolerance in plants, such as smaller and thicker leaves, shortened internodes and increased stability of PSII (Beadle, 1981; Natarajan, 2005; Berry and Bjorkman, 1980). The heat shock and enduring stress treatments used in the present study provide some insight of the temperatures and durations critical to the induction of acquired thermotolerance in petunia, while still producing a marketable plant.

Results indicated that heat shock and enduring heat stress temperature or duration, respectively, had a significant effect on the acquired thermotolerance of all the morphological traits studied. The average of 3 weeks exposure to heat shock or enduring heat stress revealed that the 45°C treatment temperature, regardless of duration, reduced petunia flower count (Fig. 4.4. A). However, prior to the acquired thermotolerance test (ATT), there were no significant differences between petunia flower counts of any heat shock or enduring heat stress temperature, indicating that petunias are still able to maintain flower production after 3 weeks of exposure to temperatures as high as 45°C (Table 4.11). After exposure to ATT, flower count was reduced in petunia grown at control (30/25°C) or exposed to 35°C but was unaffected in petunia preconditioned at 40 or 45°C, revealing an increased heat tolerance when pre-exposed to these temperatures (Table 4.11). Similar results of known heat tolerant and heat sensitive cultivars of tomato (*Lycopersicon esculentum* Mill.) were reported when the heat tolerant cultivar produced a greater number of flower buds and displayed earlier flowering when exposed to enduring

35/30°C compared to heat sensitive cultivar (Lohar and Peat, 1998). The present study only uses one cultivar, Dreams ‘Midnight’, with no predetermined heat tolerance. However, Dreams ‘Midnight’ clearly exhibits the ability to acquire thermotolerance to much higher temperatures similar to the known heat tolerant tomato cultivar used in the study by Lohar and Peat (1998). Enduring heat stress (24 h duration) compared to heat shock at 2, 4 or 6 h, regardless of temperature, significantly decreased the number of flowers per plant after three weeks of treatment and the flower count was unaffected by ATT (Table 4.12). However, flower count of petunia heat shocked for 2, 4, or 6 h decreased significantly post-ATT. These results indicated that petunia flower set is sensitive to continuous high temperatures of greater than 40°C but that exposure to this temperature for shorter durations of heat shock used in this study were not adequate for promoting acquired heat tolerance. Although petunia Dreams ‘Midnight’ proved to be tolerant of 45°C, the reduced flower counts caused by enduring heat stress pre-ATT would not be an ideal growing regime to produce a quality plant. Similar results were observed for petunia flower size, where the average flower size pre-ATT was significantly reduced at 45°C or for 24 h durations at any temperature (Tables 4.11 & 4.12). Post-ATT flower sizes decreased significantly from pre-ATT sizes for all heat shock temperatures but were not significantly different from each other. Petunia pre-exposed to 45°C did not decrease in flower size as much as petunias pre-exposed to lower temperatures indicating a greater tolerance when previously heat shocked or grown at 45°C.

Results revealed that shoot dry weight and total leaf area of petunia exposed to 45°C was significantly less than petunia exposed to 30 or 35°C which may be directly related to inhibition of water and nutrient uptake due to decreased root growth at higher temperatures (Graves et al., 1991). Similarly, shoot dry weight and leaf area decreased in wheat (*Triticum aestivum* L.) grown at 25/35 or 35/35°C shoot/root temperatures compared to regimes with cooler root temperatures (25/25 or 35/25°C) (Kuroyanagi and Paulsen, 1988). Rivero et al. (2003) found similar results in tomato (*Lycopersicon esculentum* Mill.) grown at 10/10, 25/25 (optimal) or 35/35°C day/night temperatures for 30 d, where tomato grown at 35/35°C had significantly reduced shoot biomass compared to plants grown at the optimal 25/25°C. However, shoot dry weight and leaf area increased for all treatments during the 3 weeks of heat shock or enduring heat stress treatments, and continued to increase in shoot dry weight following the acquired thermotolerance test. This may have indicated that metabolism of petunia as related to growth was functional at day temperatures as high as 45°C or night temperatures as high as 35°C even after 4 weeks at this temperature. Compared to the present study, the critical temperature of 35°C found for tomato in the study by Rivero et al. (2003) did not result in significantly reduced shoot dry weight in petunia compared to the optimal temperature (30/25°C), suggesting that petunia Dreams 'Midnight' may be more stress tolerant than other species within the Solanaceae family.

Heat shock and enduring heat stress treatments of 45°C also had the greatest effect on promoting heat tolerance related to petunia internode length pre-ATT and post-ATT (Table 4.11). Pre-exposure to 45°C, regardless of duration, resulted in petunia developing shortened internodes which has been described as an adaptable morphological characteristic associated with improved heat tolerance seen in salvia (*Salvia splendens* F. Sellow ex Roem & Schult.) (Natarajan, 2005) and cowpea (*Vigna unguiculata* (L.) Walp.) (Ismail et al., 2000). The decreased internode length observed over time also resulted in a more compact plant overall, which makes for a more marketable and efficient plant in the landscape. These results were similar to research done on selections and evaluations of lisianthus (*Eustoma grandiflorum* Salisb.) cultivars based on resistance to heat-induced rosetting during development (Harbaugh and Scott, 1999). The authors concluded that the semi-dwarf cultivars 'Florida Pink' and 'Florida Light Blue' were considered more appropriate landscape plants due to their compact growth and diverse flower color (Harbaugh and Scott, 1999). Post-ATT, petunia that had previously developed shortened internodes at higher temperatures and longer durations were unaffected by the subsequent heat stress while control petunia (30/25°C) displayed severely reduced internode growth (- 66%) (Fig. 4.10. B) and resulted in very tight almost whorled appearance of the terminal portions of petunia branches (Fig. 4.17. B).

The maximal quantum efficiency of PSII was investigated using chlorophyll fluorescence yields of leaves pre-ATT, and 3 and 7 d post-ATT that had been dark adapted for 12 h at room temperature. The averaged decreased Fv/Fm ratio observed in young leaves of control (30/25°C) petunias compared to heat shocked leaves at 35, 40 or 45°C (Table 4.21) may be related to the larger leaf area of control plants. Research by Knight and Ackerly (2003) found correlations between reduced specific leaf area and increased thermal tolerance of PSII where species (*Atriplex hymenelytra*, *Encelia farinosa*, *Eriogonum latifolia* and *Salvia mohavensis*) with smaller specific leaf areas could withstand higher temperatures (39 to 46°C) indicated by stability of Fv/Fm at a critical temperature before 50% reduction in the variable to maximal fluorescence ratio. The increased Fv/Fm observed in younger leaves may be associated with smaller leaf areas seen in petunia exposed to higher temperatures (Fig. 4.12). Increased Fv/Fm of mature leaves exposed 40/30 or 45/35°C pre-ATT may be the result of a protective mechanism in heat stressed plants to help dissipate excess excitation energy. Tang et al. (2007) found that spinach (*Spinacia oleracea* L.) leaves subjected to heat stress (25 to 50°C for 30 min) caused an aggregation of the light-harvesting complex of PSII (LHCII) and increased thermal energy dissipation in plants heat stressed above 35°C. The study also associated the LHCII aggregates with reduced susceptibility in heat stressed plants to solubilization of chlorophyll protein complex at high temperatures. The decreased susceptibility of solubilizaton is associated with conformational changes in the chloroplast that occur at temperatures lower than photosynthesis inhibiting temperatures. This was also seen in temperature induced changes in bean (*Phaseolus vulgaris* L.) chloroplasts where the ratio of the quantum efficiencies of PSII and O_2 evolution remained constant when plants were exposed to 20 to 35°C indicating that changes in chloroplast happened before photoinhibition at high temperatures (Pastenes and Horton, 1996). Although the present study does not investigate these protein analyses, increased relative chlorophyll content was found in petunia grown at enduring heat stress 35/25, 40/30 or 45/35°C compared to control and may be related to the LHCII protective mechanism mentioned by Tang et al. (2007). It is also important to note that all Fv/Fm ratios for any leaf are within what is considered to be a healthy range ($\geq$ 0.83) (Bjorkman and Demmig, 1987) and this did not change in petunia previously exposed to any temperature which indicates that photosynthetic decline in petunia does not in occur up to at least 45°C. However, acute decreases in Fv/Fm may have been observed if fluorescence yields were measured during heat stress rather than after recovery at room temperature for 12 h. However, the results indicate that heat shock and enduring heat stress treatments do not cause permanent stress to PSII or irreversible damage to the photosynthetic apparatus.

Investigation of various temperatures and durations to induce acquired heat tolerance revealed that higher temperatures of 45°C or longer durations of 24 h promoted the greatest tolerance. However, the marketable quality of plants grown at 40/30 or 45/35°C was severely reduced and would therefore not be recommended as preconditioning treatment for greenhouse production of petunia. At the same time, the control temperature (30/25°C) which is considered optimal for petunia did not have reduced quality pre-ATT, but had the least heat tolerance when exposed to ATT and should not be considered as an ideal growing temperature when promoting heat tolerance. Figure 4.20 represents petunia pre-exposed to 45°C for 2, 4, 6 or 24 h (A, B, C & D, respectively) and shows the unacceptable quality of petunia at 24 h duration. However, petunia heat shocked at 45°C for 4 h appeared to have the best quality out of the durations used at this temperature due to its compact and uniform growth and acceptable flowering (Fig. 4.20. B), though this was not significantly reflected in the data collected pre or post-ATT. Further investigation using different durations and/or increased frequency of application (i.e. every day rather than every 3 d) at 45°C may produce a greater effect of acquired thermotolerance using a specific temperature and duration combination.

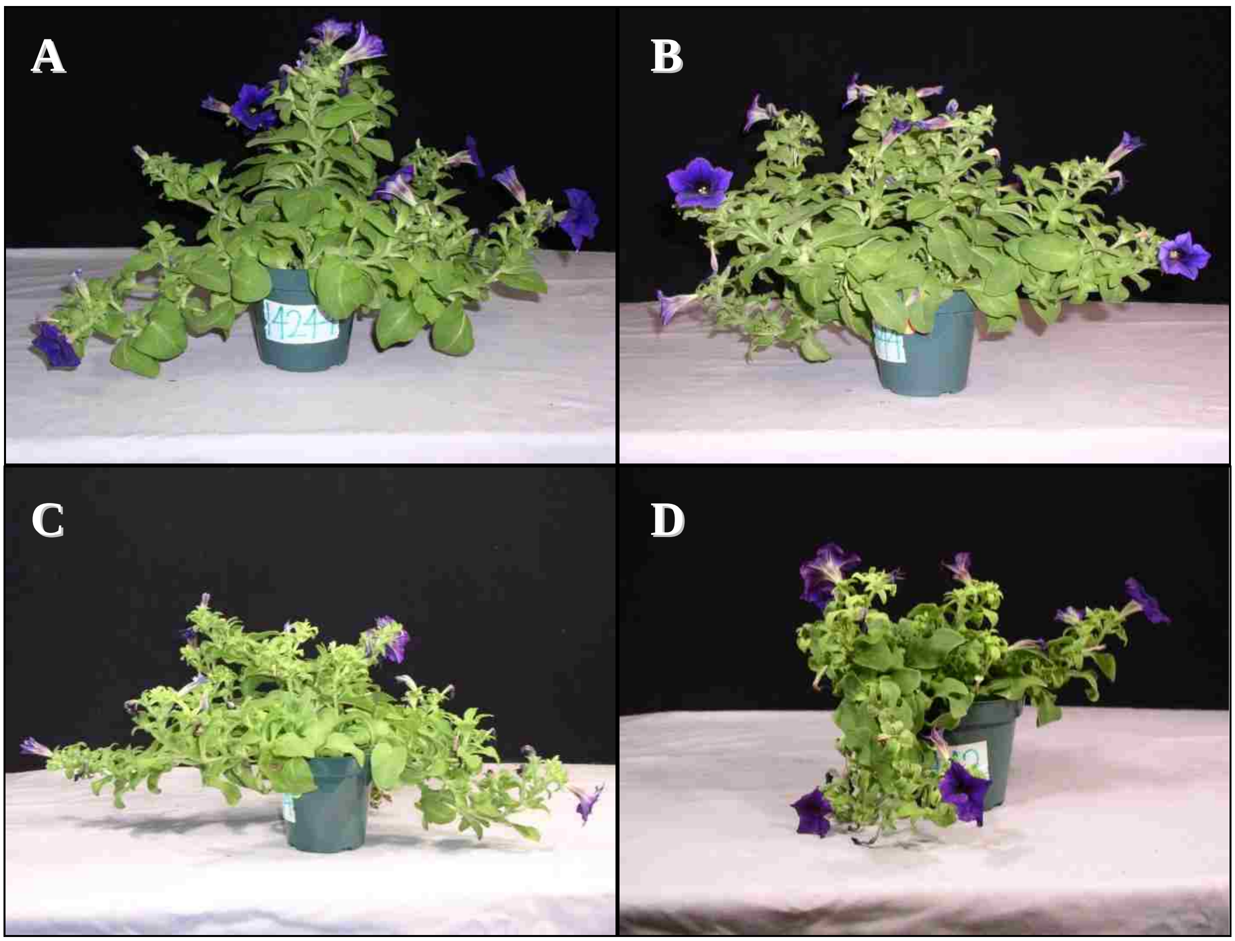

Figure 4.20. Pictorial representation of the effect heat shock at 45°C every 3 d on *Petunia x hybrida* Dreams 'Midnight' for durations of A) 2 h, B) 4 h, C) 6 h or D) 24 h.

4.5 LITERATURE CITED

Armitage, A.M. 1985. Petunia, pp. 41-46 in *Handbook of Flowering*, vol. IV, A.H. Halevy, editor. CRC Press, Boca Raton, Florida.

Beadle, N.C.W. 1981. The vegetation of Australia. Cambridge: Cambridge University Press.

Berry, J and O. Bjorkman.1980. Photosynthetic response and adaptation to temperature in higher plants. Ann Rev Plant Physiol. 31: 491-543.

Bilger, W., U. Schreiber, M. Bock. 1995. Determination of the quantum efficiency of photosystem II and of non-photochemical quenching of chlorophyll fluorescence in the field. *Oecologia* 102:425-432.

Bjorkman, O. and B. Demmig. 1987. Photon yield of O2 evolution and chlorophyll fluorescence at 77k among vascular plants of diverse origins. Planta 170: 489-504.

Burke, John J. 1990. High Temperature Stress and Adaptations in Crops, pp.295-307 in *Stress Responses in Plants: Adaptation and Acclimation Mechanisms*. R.G. Alscher and J.R. Cumming, editors. Wiley-Liss, Inc. New York.

Genty, B., J-M Briantais and N.R. Baker. 1989. The relationship between quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochimica at Biophysica Acta*. 990:87-92.

Graves, W.R., R.J. Joly, and M.N. Dana. 1991. Water use and growth of honey locust and tree-of-heaven at high root-zone temperature. HortSci. 26: 1309–1312.

Hale, M.G. and D.M. Orcutt. 1997. The physiology of plants under stress. Wiley New York.

Hall, A.E. 2001. Crop Response to Environment. CRC Press LLC, Boca Raton, FL.

Harbaugh, B and J.W. Scott. 1999. ‘Florida Pink’ and ‘Florida Light Blue’ – Semi-dwarf Heat-tolerant Cultivars of Lisianthus. HortScience 34(2):364-365.

Havaux, M. 1993. Characterization of thermal damage to the photosynthetic electron transport system in potato leaves. Plant Sci. 94: 19–33.

Ismail, A. M, A. E. Hall and J.D. Ehlers. 2000. Delayed-leaf-senescence and heat tolerance traits mainly are independently expressed in cowpea. Crop Sci. 40: 1049-1055.

Knight, C. and D. Ackerly. 2003. Evolution and plasticity of photosynthetic thermal tolerance, specific leaf area and leaf size: congeneric species from desert and coastal environments. New Phytologist 160:337-347.

Koh Iba. 2002. Acclimative response to temperature stress in higher plants: Approaches of Gene Engineering for Temperature Tolerance. Annu Rev Plant Biol. 53: 225-245.

Kuroyanagi, T., and G.M. Paulsen. 1988. Mediation of high-temperature injury by roots and shoots during reproductive growth of wheat. Plant Cell Environ. 11: 517–523.

Larcher, W. 1994. Photosynthesis as a tool for indicating temperature stress events, pp.261-277. In, Ecophysiology of photosynthesis. Ecological studies 100. E.D. Schulze and M.M. Caldwell, editors. Springer-Verlag, Berlin.

Lichtenthaler, H.K., 1996. Vegetation stress: an introduction to the stress concept in plants. J Plant Physiol. 148, 4–14.

Lohar, D.P. and W.E. Peat. 1998. Floral characteristics of heat-tolerant and heat-sensitive cultivars of tomato (*Lycopersicon esculentum* Mill.) cultivars at high temperature. Scientia Horticulturae 73:53-60.

Mahan, J.R., B.L. Mc Michael, and D.F. Wanjura. 1997. Reduction of high temperature stress in plants, p. 137-150.In: A.S. Basra and R.K. Basra (eds). Mechanism of environmental stress resistance in plants. Harwood Academic Publishers, The Netherlands.

Maxwell, K. and G.N. Johnson. 2000. Chlorophyll fluorescence – a practical guide. Journal of Exp Botany, vol.51 345:659-668.

Natarajan S. 2005. High temperatures stress responses of *Salvia splendens* and *Viola x wittrockiana.* Ph.D Diss.

Park, S.Y., R.Shivaji, J.V.Krans and D.S.Luthe.1996. Heat-Shock response in heat tolerant and nontolerant variants of Agrostis palustris Huds. Plant Physiol. 111: 515-524.

Pastenes, C. and P. Horton. 1996. Effect of High Temperature on Photosynthesis in Beans I. Oxygen Evolution and Chlorophyll Fluorescence. Plant Physiol. 112: 1245-1251.

Rivero, R., E. Sánchez, J. M. Ruiz and L. Romero. 2003. Influence of temperature on biomass, iron metabolism and some related bioindicators in tomato and watermelon plants. J. Plant Physiol. 160: 1065–1071.

Senthil, K.M, V. Srikanthbabu, B. Mohan Raju, N. Ganeshkumar, Shivaprakash and M. Udayakumar. 2003. Screening of inbred lines to develop a thermotolerant sunflower hybrid using the temperature inductionresponse (TIR) technique: a novel approach by exploitingresidual variability J Exper Bot. 54: 2569-2578.

Sung, D., F. Kaplan, K. Lee and C. L. Guy. 2003. Acquired tolerance to temperature extremes. *TRENDS* in Plant Sci. vol.8 (4):179-187.

Tang, Y., X. Wen, Q. Lu, Z. Yang, Z. Cheng and C. Lu. 2007. Heat stress induces an aggregation of the light-harvesting complex of Photosystem II in spinach plants. Plant Physiology 143:629-638.

Vierling, E. 1991 The roles of heat-shock proteins in plants. Annu Rev Plant Physiol Plant Mol Biol. 42: 579-620.

Weis E. and J.A. Berry. 1987. Quantum efficiency of photosystem II in relation to ‘energy’-dependent quenching of chlorophyll fluorescence. Biochim Biophys Acta 894: 198-208.

Yeh, D.M and P.Y. Hsu. 2004. Heat tolerance in English ivy as measured by an electrolyte leakage technique. J Hortic Sci Biotech. 79: 298-302.

CHAPTER 5. INDUCTION OF ACQUIRED THERMOTOLERANCE IN DIFFERENT CULTIVARS OF *PETUNIA X HYBRIDA* AND EVALUATION OF SUBSEQUENT LANDSCAPE PERFORMANCE

5.1 INTRODUCTION

Bedding plants account for some of the largest percent of sales (wholesale) within the floriculture industry and were valued at $1.26 billion in wholesale value in 2007 (U.S. Department of Agriculture, 2008). Bedding plants have great potential profit with the greatest demand coming from the public in retail nurseries followed by an increasing demand by landscape contractors (Copes, 2000; Fossler, 1993). Providing quality plants that perform well in the greenhouse and that also perform well in the landscape is an important goal for plant breeders, growers, and landscape contractors. However, achieving optimum bedding plant quality is highly dependent upon the growing environment, which can have detrimental effects during undesirable conditions (Armitage, 1989).

Heat stress is a major factor affecting bedding plant production, where prolonged exposure can result in irreversible damage to plant function (Hall, 2001) and ultimately decreased growth, development and yield (Gusta *et al.*, 1997; Harding *et al.*, 1990). Heat stress can have strong morphological effects on plants including decreased shoot and root growth, leaf chlorosis and necrosis, abnormal flower development, and increased flower abortion (El Ahmadi and Stevens, 1979; Guilioni et al., 1997; Pollock et al., 1993) as well as physiological effects such as decreased cell membrane stability, stomatal conductance, net photosynthesis, and transpiration (Medina and Cardemil, 1993; Ortiz and Cardemil, 2001; Natarajan and Kuehny, 2008).

Effects of heat stress and plant tolerance is vital for survivability in stressful environments (Lichtenthaler, 1996). Some plants have displayed adaptive responses to heat stress by developing smaller and thicker leaves, shortened internodes (Beadle, 1981; Natarajan, 2005; Ismail et al., 2000), increased membrane stability (Yeh and Hsu, 2004), and induction of heat shock proteins (Park et al., 1996; Vierling, 1991). This acquired thermotolerance in some plant species can be achieved by preconditioning by using supraoptimal temperatures for a specific duration of time, also known as heat shock (Natarajan, 2005; Sung et al., 2003). Effect of heat shock and subsequent heat tolerance varies by species and often between cultivars. There has been little research on inducing acquired thermotolerance during greenhouse production of bedding plants followed by quantitative evaluation of heat tolerance postproduction in the landscape. Therefore, the objective of the present study was to induce acquired thermotolerance in several classes of *Petunia x hybrida* during greenhouse production and to determine heat tolerance by evaluation of landscape performance.

5.2 MATERIALS AND METHODS

5.2.1 Plant Material

Nineteen cultivars of *Petunia x hybrida* Hort. Ex Vilm. (Table 5.1) were used in the present study. *Petunia x hybrida* Dreams 'Midnight' (Ball Horticultural Company, West Chicago, IL) used in previous research (Chapter 3 and 4) was used in this experiment as the standard for comparison. Sixteen additional cultivars were selected based on their overall performance rating as evaluated by Kelly *et al*. (2007). Petunia cultivars were grouped into the following plant classes: floribunda, grandiflora, and spreading. These classes and cultivars can be further separated into eight different series of petunia: Madness, Dreams, Storm, Ultra, Easy Wave, Ramblin, Avalanche, and Wave (Ball Horticultural Company, West Chicago, IL). Within each series, two cultivars were chosen and labeled as either the best or worst rated for overall performance as evaluated by Kelly *et al*. (2007). Two additional cultivars, 'Mitchell Diploid' and '44568' were provided by the University of Florida. The '44568' is transgenic ethylene-insensitive petunia and 'Mitchell Diploid' is its wild type. The cultivars used in this study are labeled by a specific number as indicated in Table 5.1, so that cultivars may be easily referenced in tables and figures. Petunia seeds were planted 5 August 2008 into 288 (4.9 cm^3) plug tray using lightweight media Fafard™2M Mix (Conrad Fafard, Incorporated, Agawam, MA) and germinated at 26°C day/night under intermittent mist (every 2 h for six seconds) in a polycarbonate greenhouse for five weeks.

Table 5.1 The class, number, series and cultivar, and overall performance of selected *Petunia x hybrida* as evaluated by Kelly *et al*. 2007.

Class	No.	Selected Cultivars	Overall Performance
Floribunda	1	'Madness Waterfall Mix'	Best
	2	'Madness Lavender Glow'	Worst
Grandiflora	3	'Dreams Burgundy Picotee'	Best
	4	'Dreams Wild Rose Mix'	Worst
	19	'Dreams Midnight'	Unknown
Grandiflora	5	'Storm Violet'	Best
	6	'Storm Red'	Worst
Grandiflora	7	'Ultra Salmon'	Best
	8	'Ultra Red'	Worst
Grandiflora	17	'Mitchell Diploid'*	Unknown
	18	'44568'*	Unknown
Spreading	9	'Easy Wave Shell Pink'	Best
	10	'Easy Wave Red'	Worst
Spreading	11	'Ramblin Lavender'	Best
	12	'Ramblin White'	Worst
Spreading	13	'Avalanche Lilac'	Best
	14	'Avalanche White'	Worst
Spreading	15	'Wave Pink'	Best
	16	'Wave Blue'	Worst

*'Mitchell Diploid' and '44568' were provided by Dr. David G. Clark from University of Florida (Gainesville, FL). Other seed was contributed by Ball Horticultural Company (West Chicago, IL).

5.2.2 Greenhouse Establishment Prior to Treatments

Greenhouses used in the present study are located at Campus Greenhouses 440-7 and 440-8 at Louisiana State University, 30° N 91° W Baton Rouge, Louisiana. The present study was replicated simultaneously using two greenhouses for each experiment. A total of four polycarbonate covered greenhouses with 40% shade cloth (1000 µmol m^{-2} s^{-1}) were used to complete treatments. Each greenhouse has respective automated heating and cooling systems using Wadsworth STEP© Control 50A (Wadsworth Control Systems Incorporated, Arvada, CO) with day/night 12 h temperature settings. On 10 September 2008, petunia plugs were transplanted into (650 cm^3) plastic pots using a middleweight media Fafard™4M Mix (Conrad Fafard, Incorporated, Agawam, MA) media. One plant was transplanted into each pot and was placed on greenhouse benches. Broad spectrum fungicide was applied as a drench to each pot after transplant at a rate of 19.5 ml/L (Banrot® 8G, a.i. 3% Etridiazole, a.i. 5% Thiophanate-methyl, Scotts-Sierra, Marysville, OH). Petunias were grown at 30/20°C and fertigated using Hozon™ Brass Siphon Mixer (1:16) (Phytotronics, Incorporated, Earth City, MO) with 200 ppm N 15N-2.2P-12.4K (15-5-15 Cal Mg, Scotts-Sierra, Marysville, OH) daily for one week before starting heat shock treatment.

5.2.3 Heat Shock Treatment

Heat shock treatment was applied to all petunia cultivars after two weeks growth at 30/25°C in the greenhouse. Heat shock treatment was chosen based on the optimum temperature and duration determined in Chapter 4. Treatments included continuous exposure to control temperature (30/25°C day/night) or heat shock at 45°C for 4 h every 3 d of plants grown at 30/25°C day/night. Heat shock temperature was established in respective greenhouses by increasing temperature setting on the automated control system (Wadsworth STEP© Control 50A, Wadsworth Control Systems Incorporated, Arvada, CO) from 30 to 45°C at 1000 HR and decreased back to 30°C at 1400 HR every 3 d for 3 weeks. Prior to heat shock exposure, plants were irrigated to maximum water holding capacity to minimize water stress during treatment. Each heat shock experiment used two greenhouses for a total of two control greenhouses and two greenhouses where heat shock was applied. Temperatures in respective greenhouses were recorded using HOBO® Pro Series™ data logger (Onset Computer Corporation, Bourne, MA)

(Figure 5.1 A, B, C & D). Weather records for Baton Rouge, Louisiana, including temperature (°C) and irradiance (μ mol m^{-2} s^{-1} PPFD), were also taken daily for the duration of the experiments (Louisiana Agriclimatic Information Systems, LSUAgCenter, BAE) (Figure 5.2. A & B).

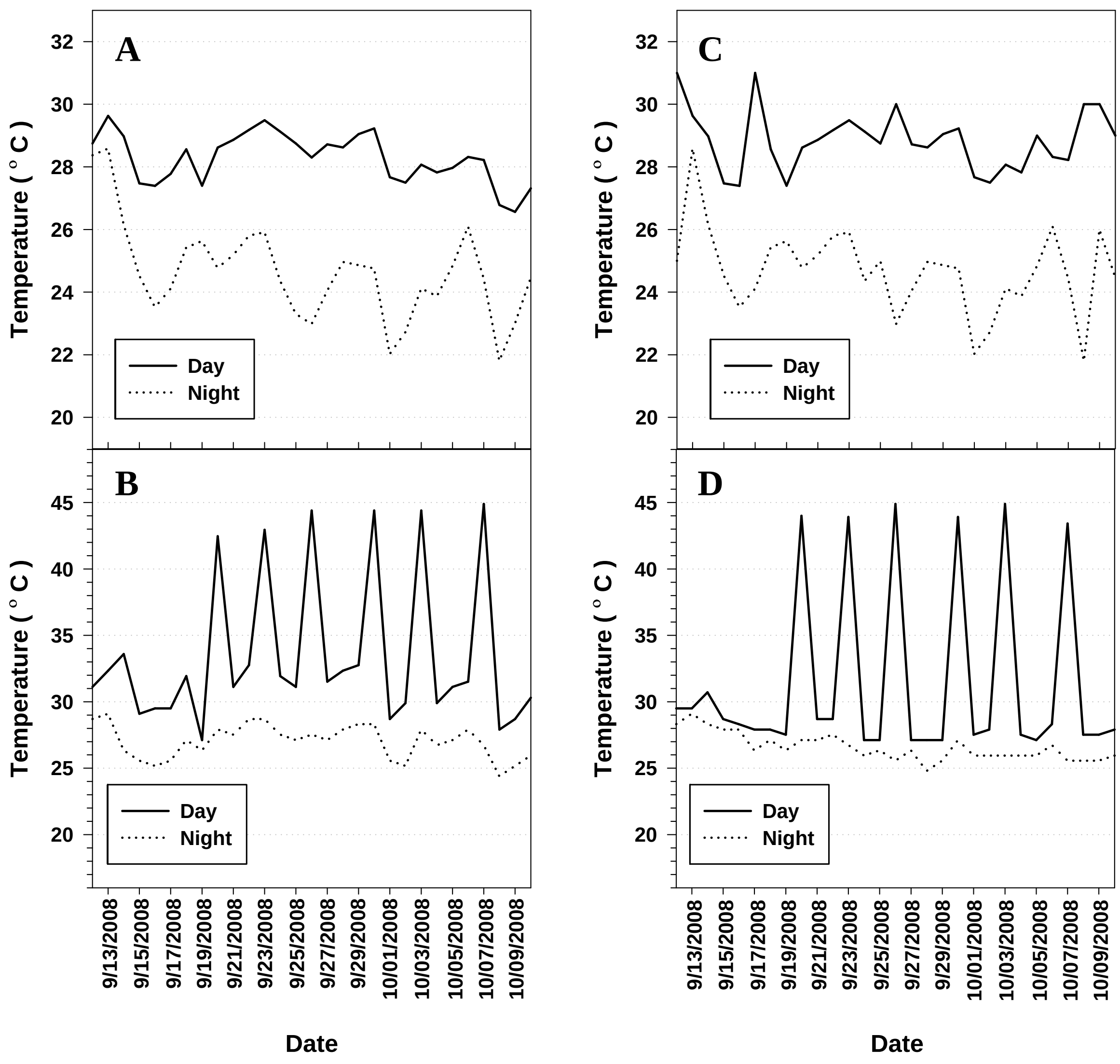

Figure 5.1. Average day/night temperatures (°C) A) Control 30/25°C, B) 30/25°C and heat shock at 45°C for 4h every 3d, C) Control 30/25°C, D) 30/25°C and heat shock at 45°C for 4 h every 3 d recorded in greenhouses for each temperature treatment over a 4 week course of experiment.

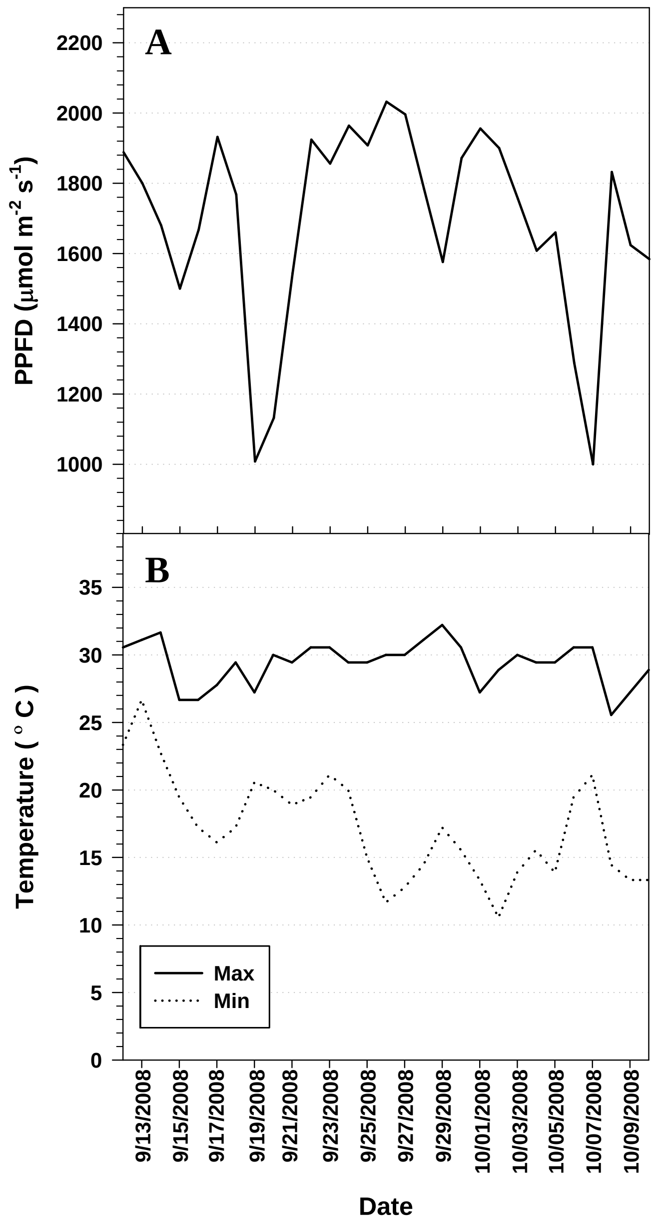

Figure 5.2. Average daily weather reports A) natural irradiance (µ mol m^{-2} s^{-1} PPFD), B) minimum and maximum daily temperature (°C) for greenhouse experiment located at Louisiana State University, Baton Rouge, Louisiana.

5.2.4 Measurement of Growth and Development in the Greenhouse

Petunia cultivars were destructively harvested for data collection every 7 d for 4 weeks with first harvest taken after one week growth at 30/25°C for baseline comparative and the following 3 harvests every 7 d after heat shock treatment began. Petunia growth and development was quantified by measuring number of flowers per plant, average flower size (cm) per plant, relative increase in growth of shoot dry weight (g) or average internode length (cm) expressed in terms of a rate of increase in weight or length per unit of weight or length, relative growth rate (RGR), providing for a more equitable comparison of dry weight where:

$$RGR = (\log_e W_2 - \log_e W_1) / (t_2 - t_1)$$

or internode growth analysis where:

$$RGR = (\log_e L_2 - \log_e L_1) / (t_2 - t_1)$$

That is the natural log of the mean weight (W) or length (L) over the interval of weeks (t) measured (Hunt, 1990). The total leaf area of a plant (mm^2) (L_a) per plant shoot dry weight (W) was also recorded as the leaf area ratio (LAR) and was determined using the following formula (Hunt, 1990):

$$LAR = ([L_{a1}/W_1]) + [L_{a2}/W_2])/2$$

Fully expanded flowers were visually counted and recorded weekly. Flower size was determined by using a handheld metric ruler and measuring the diameter (cm) of one flower visually estimated to be average for that respective plant. Average internode length was determined by measuring lengths of first 3 internodes from newest true leaves on one branch per plant. Total leaf area per plant was measured using LI-3100C leaf area meter (LICOR Biosciences, Lincoln, NE). Shoot dry weights (g) were obtained after oven drying at 80°C for 24 h.

5.2.5 Field Study Establishment

Postproduction was evaluated by transplanting heat shocked and control plants in field trial landscape beds. The transgenic cultivars '44568' and 'Mitchell Diploid' were not evaluated in the field. A broad spectrum fungicide (Banrot® 8G, a.i. 3% Etridiazole, a.i. 5% Thiophanate-methyl, Scotts-Sierra, Marysville, OH) was applied in the greenhouse 3 d prior to field transplant.

Petunias were transplanted on 13 October 2008 into trial beds 3 d after the last heat shock treatment was applied in the greenhouse. The field trial was located at LSU AgCenter Burden Research Station, 30° N 91° W Baton Rouge, Louisiana. The landscape trial was conducted in raised beds (1.5 m wide by 50 m long) and consisted of an Olivier silt loam soil amended with composted pine bark. Petunia were planted using 30 x 30 cm spacing and were irrigated as needed by drip tape placed in between two plants that continued for the entire length of the row. Weather records were taken daily from the Burden Center weather station for the duration of the field trial (Louisiana Agriclimatic Information Systems, LSUAgCenter, BAE) (Figure 5.3 A & B). Petunias were allowed to establish one week before data measurements were taken.

5.2.6 Field Data Collection

Growth and development and landscape performance of treated petunias were evaluated every two weeks after transplant. Data collected included number of flowers per plant, average flower size (cm), and quality ratings. Quality rating scale ranged from 1 to 5, where 1=dead and 5= optimum performance. Quality is based on a combination of plant flowering, leaf color and compactness of plant. Quality scores; 4.5 to 5= excellent plants with healthy green leaves, compact uniform growth and good inflorescence, 3.5 to 4.5= green healthy foliage with moderate flowers, 2.5 and 3.5= plants with chlorotic leaves and poor inflorescence, 1.5 to 2.5= plants with necrotic or dried leaves with terminal bud damage and poor flower set, <1.5 = dead. After six weeks in the field, petunias were destructively harvested and data collected was: number of flowers per plant, average flower size (cm) per plant, average internode length (cm), total leaf area per plant (mm^2), and shoot dry weight (g).

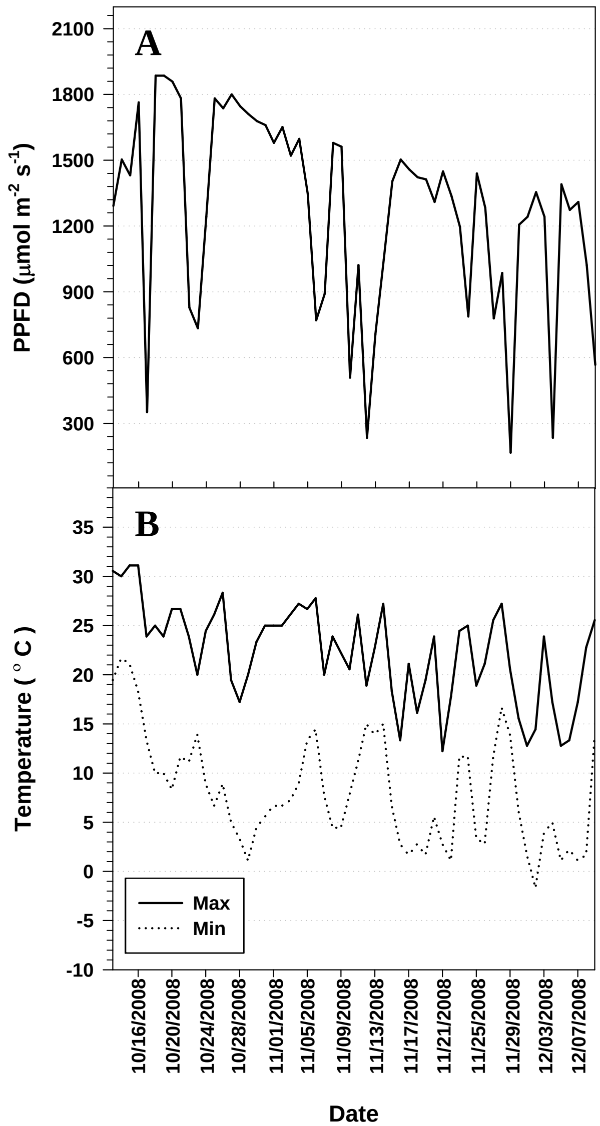

Figure 5.3. Average daily weather reports A) natural irradiance (μ mol m^{-2} s^{-1} PPFD), B) minimum and maximum daily temperature (°C) for field study located at Burden Center, Baton Rouge, Louisiana.

5.2.7 Experimental Design and Statistical Analysis

Heat shock treatments were arranged as randomized complete block design in the greenhouse. The greenhouse experiment was replicated once using three plants (sample units) per treatment for each cultivar and for each of the 4 harvests in the greenhouse and for transplant in the field. Statistical analysis was performed using SAS ProcMixed Procedure (Statistical Analysis Software, version 9.1, Cary, NC).

5.3 RESULTS

5.3.1 Effect of Heat Shock on Growth and Development of Different Petunia Cultivars

Growth and development of several *Petunia x hybrida* plant classes (Floribunda, Grandiflora or Spreading) was not significantly affected by heat shock at 45°C for 4 h every 3 d compared to petunia grown at control temperature (30/25°C) (Fig. 5.4 A, B, C, D & E). However, there were significant differences in some growth measurements between plant types. After 3 weeks of heat shock in the greenhouse, floribunda had a significantly greater flower count compared to spreading when grown at 30/25°C. However, there was no difference in the number of flowers per plant for petunia types that were exposed to heat shock treatment (Fig. 5.4 A). Floribunda and grandiflora plant types had significantly larger flower size than petunia spreading types in both control and heat shocked plants (Fig. 5.4 B). Relative growth rate (based on shoot dry weight) was not significantly different (Fig. 5.4 C) or leaf area ratio (Fig. 5.4 D) in heat shocked or control plants. Grandiflora petunias grown at 30/25°C had a significantly higher relative rate of internode growth compared to spreading exposed to heat shock at 45°C for 4 h every 3 d (Fig. 5.4 E).

When investigating the effect of heat shock during greenhouse production on growth and development of all nineteen *Petunia x hybrida* cultivars, there was no significance between treatments for respective cultivars for flower count, average flower size or relative growth rate (Table 5.2). However, heat shock at 45°C for 4 h every 3 d had a significant effect on the relative rate of internode growth and leaf area ratio for the petunia cultivars ‘Ultra Red’ (8) and ‘44568’(18), respectively, which had reduced growth compared to growth at 30/25°C.

The effect of heat shock was also significant at certain times during treatment in the greenhouse. Heat shock (45°C for 4h every 3 d) did not have a significant effect on flower count after 3 weeks of exposure compared to petunia cultivars grown at 30/25°C day/night. However,

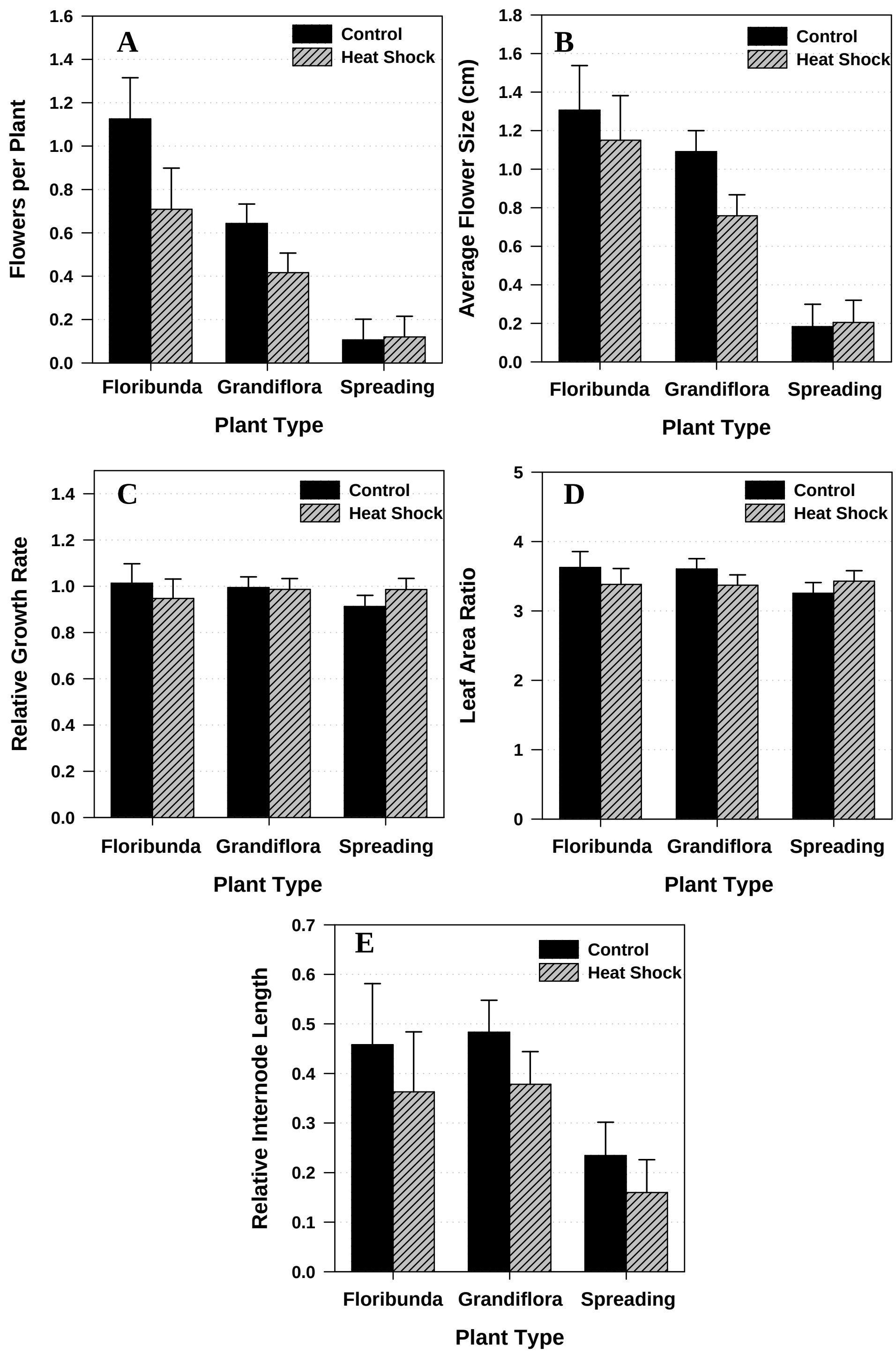

Figure 5.4. The effect of heat shock (45°C for 4 h every 3 d) (Control = 30/25°C day/night) on the A) number of flowers per plant, B) average flower size (cm), C) relative growth rate, D) leaf area ratio and E) relative internode length of nineteen cultivars of *Petunia x hybrida* after 4 weeks in the greenhouse. Error bars represent means of observations ±SE (Floribunda n=48) (Grandiflora n=216) (Spreading n=192).

Table 5.2. Growth and development of nineteen *Petunia x hybrida* cultivars grown at control (30/25°C day/night) or heat shock (45°C for 4 h every 3 d).

Cultivar	Treatment	Flowers per Plant	Flower Size (cm)	RGR (g g^{-1} $week^{-1}$)	LAR (mm^2 mg^{-1})	Internode Length (cm.cm^{-1} $week^{-1}$)
1	Control	[Y]1.83 ab[X]	1.56 abcde	0.93 a	3.44 b	0.47 a
	Heat Shock	1.16 abcd	1.74 abcde	0.91 a	3.44 b	0.39 a
2	Control	0.41 de	1.05 abcde	1.09 a	3.80 ab	0.43 a
	Heat Shock	0.25 de	0.55 cde	0.98 a	3.31 b	0.32 a
3	Control	0.25 de	0.79 cde	0.97 a	3.96 ab	0.47 a
	Heat Shock	0.041 de	0.22 de	1.01 a	3.72 ab	0.48 a
4	Control	1.16 abcd	1.98 abc	0.89 a	3.13 b	0.28 a
	Heat Shock	1.04 abcde	1.52 abcde	0.74 a	3.37 b	0.38 a
5	Control	2.16 a	2.38 a	1.01 a	3.59 b	0.51 a
	Heat Shock	1.58 abc	1.81 abc	1.01 a	3.28 b	0.46 a
6	Control	0 e	0 e	1.12 a	3.61 b	0.62 a
	Heat Shock	0 e	0 e	0.88 a	3.21 b	0.30 a
7	Control	0.95 bcde	2.37 ab	1.01 a	3.27 b	0.80 a
	Heat Shock	0.41 de	1.80 abcd	1.10 a	3.50 b	0.35 a
8	Control	0.41 de	1.05 abcde	1.05 a	3.17 b	0.78 a
	Heat Shock	0.21 de	0.61 cde	0.98 a	3.52 b	0 b
9	Control	0.37 de	0.80 bcde	0.92 a	3.54 b	0.33 a
	Heat Shock	0.75 bcde	1.22 abcde	1.06 a	3.55 b	0.30 a
10	Control	0.05 de	0.20 de	0.86 a	3.16 b	0.21 a
	Heat Shock	0.13 de	0.18 de	0.93 a	3.47 b	0.01 ab
11	Control	0.25 de	0.22 de	0.90 a	3.26 b	0.02 ab
	Heat Shock	0 e	0 e	0.92 a	3.18 b	0.02 ab
12	Control	0 e	0 e	0.90 a	2.94 b	0.38 a
	Heat Shock	0 e	0 e	0.89 a	3.10 b	0.19 a
13	Control	0.16 de	0.22 de	0.87 a	3.19 b	0.25 a
	Heat Shock	0.08 de	0.21 de	1.04 a	3.44 b	0.17 a
14	Control	0 e	0 e	0.91 a	2.94 b	0.09 ab
	Heat Shock	0 e	0 e	0.97 a	2.84 b	0.01 ab
15	Control	0 e	0 e	0.84 a	3.66 b	0.15 a
	Heat Shock	0 e	0 e	0.91 a	4.13 ab	0.03 ab
16	Control	0 e	0 e	1.07 a	3.30 b	0 b
	Heat Shock	0 e	0 e	1.12 a	3.66 b	0 b
17	Control	0 e	0 e	0.97 a	3.36 b	0.13 a
	Heat Shock	0 e	0 e	1.08 a	3.25 b	0.18 a
18	Control	0 e	0 e	1.02 a	5.50 a	0.26 a
	Heat Shock	0 e	0 e	1.04 a	3.44 b	0.31 a
19	Control	0.83 bcde	1.22 abcde	0.87 a	2.82 b	0.57 a
	Heat Shock	0.45 cde	0.83 abcde	1.02 a	2.96 b	0.47 a

[X] Means within columns followed by the same letters are not significantly different at 5% by lsmean procedure in SAS with Tukey’s correction.

[Y] Values in the table are averages (n=24) there was significantly higher flower count in both treatments after 4 weeks of exposure but control petunias (30/25°C) had greater number of flowers compared to petunia exposed to heat shock (Table 5.3). These results are similar to the effect on average flower size and relative growth rate, where heat shocked petunia had significantly decreased flower size and shoot dry weight compared to control after 3 weeks of heat shock exposure (Table 5.3). The leaf area ratio was not affected by heat shock after 4 weeks exposure in the greenhouse, while the internode RGR increased over the 4 weeks but was not significantly different between treatments (Table 5.3).

Table 5.3. The effect of heat shock (45°C for 4 h every 3 d) or control 30/25°C day/night) on growth and development of all cultivars of *Petunia x hybrida* for each week during greenhouse production.

Week	Treatment	Flowers per Plant	Flower Size (cm)	RGR (g g^{-1} $week^{-1}$)	LAR (mm^2 mg^{-1})	Internode Length (cm.cm^{-1} $week^{-1}$)
1	Control	[Y]0 c[X]	0 d	N/A	N/A	N/A
	Heat Shock	0 c	0 d	N/A	N/A	N/A
2	Control	0 c	0 d	1.15 a	3.33 a	0 c
	Heat Shock	0 c	0 d	1.29 a	3.25 a	0 c
3	Control	0.16 c	0.72 cd	1.11 a	3.56 a	0.25 b
	Heat Shock	0.31 c	0.95 c	1.24 a	3.32 a	0.29 b
4	Control	2.33 a	2.71 a	0.64 b	3.58 a	0.95 a
	Heat Shock	1.34 b	1.86 b	0.37 c	3.60 a	0.75 a

(N/A represents no measureable change).

[X] Means within columns followed by the same letters are not significantly different at 5% by lsmean procedure in SAS with Tukey's correction.

[Y] Values in the table are averages (n=144).

5.3.2 Field Study

Heat shock treatment of 45°C for 4 h every 3 d during greenhouse production did not have a significant effect on the number of flowers per plant within petunia cultivars after 6 weeks transplant in the field. However, there was a significant difference between several cultivars for respective treatments. For petunia cultivars grown at 30/25°C, 'Madness Waterfall Mix'(1),

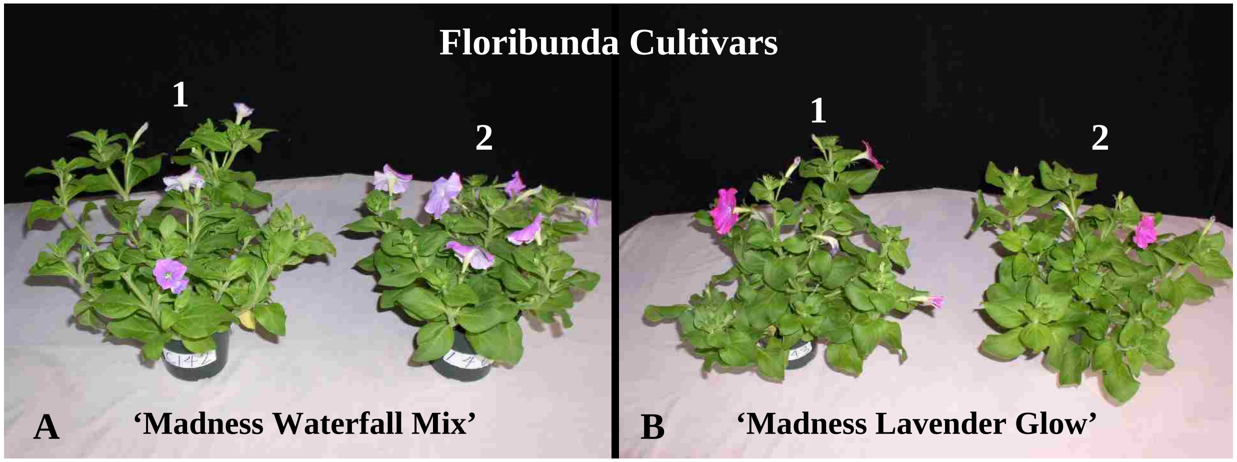

Figure 5.5. A pictorial representation of Floribunda cultivars of *Petunia x hybrida* after 3 weeks growth at 1) control (30/25°C) or 2) exposure to heat shock at 45°C for 4 h every 3 d or for A) 'Madness Waterfall Mix' and B) 'Madness Lavender Glow'.

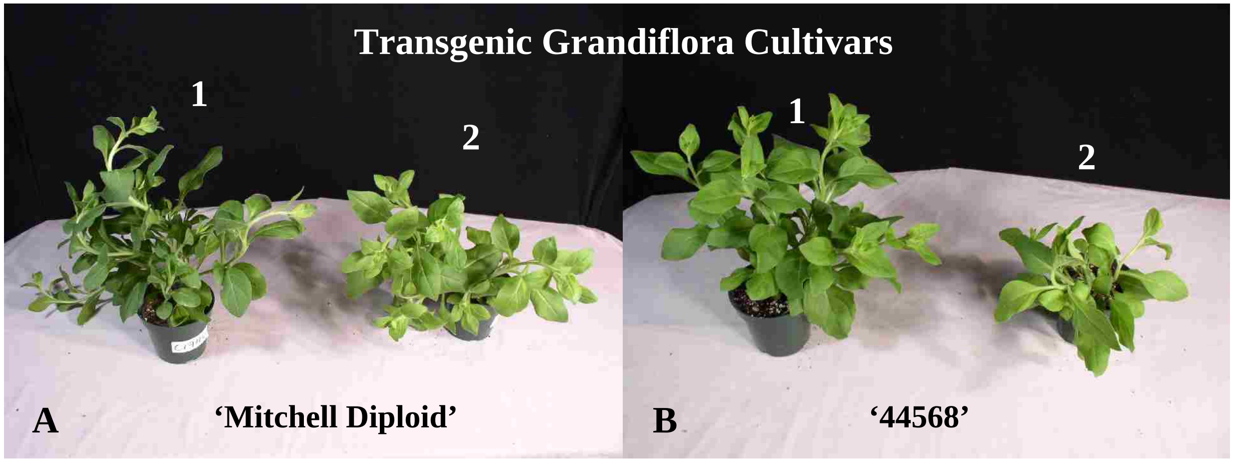

Figure 5.6 A pictorial representation of transgenic grandiflora cultivars of *Petunia x hybrida* after 3 weeks growth at 1) control (30/25°C) or 2) exposure to heat shock at 45°C for 4 h every 3 d or for A) 'Mitchell Diploid and B) '44568'.

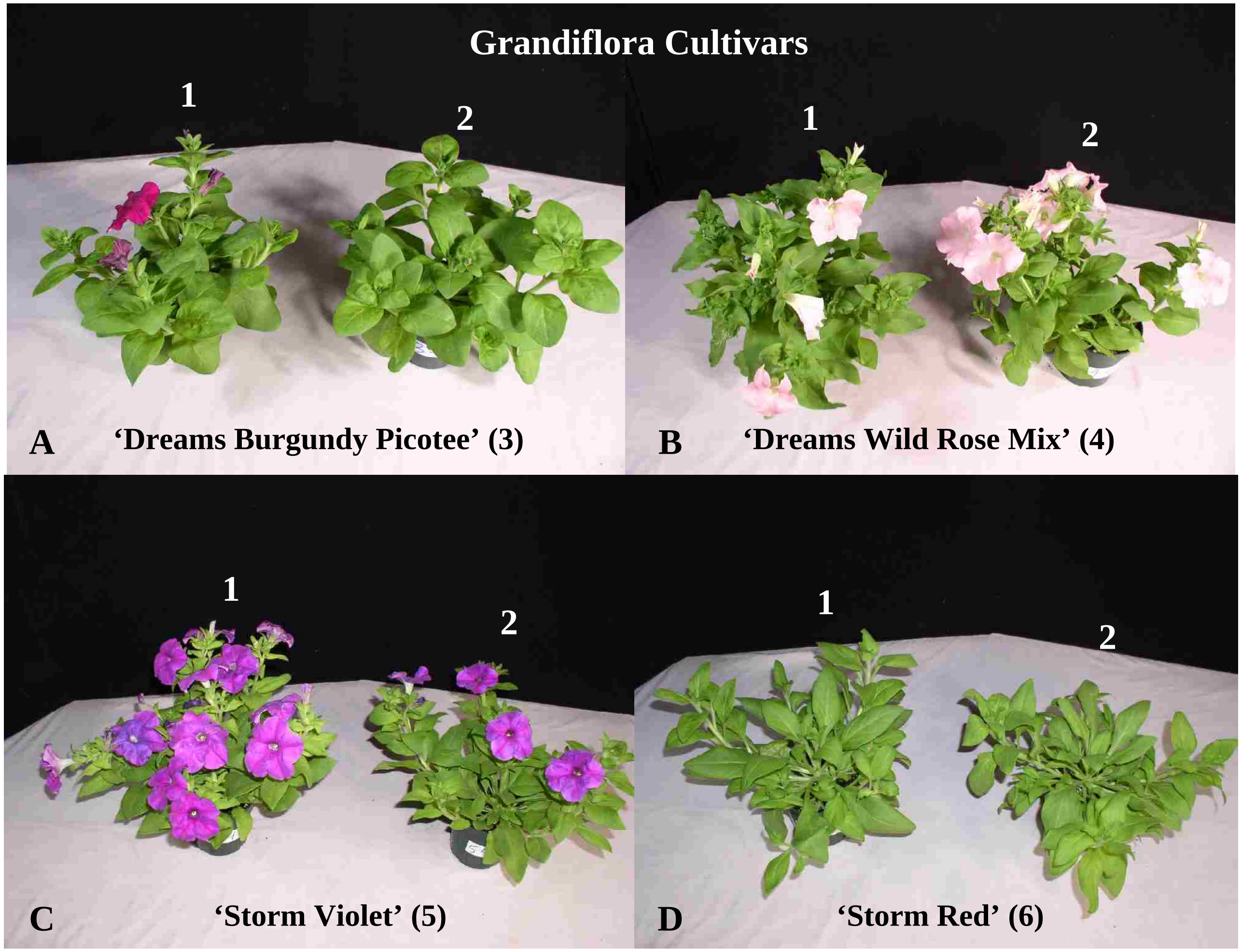

Figure 5.7 A pictorial representation of Grandiflora cultivars of *Petunia x hybrida* after 3 weeks growth at 1) control (30/25°C) or 2) exposure to heat shock at 45°C for 4 h every 3 d or for A) 'Dreams Burgundy Picotee', B) 'Dreams Wild Rose Mix', C) 'Storm Violet' and D) 'Storm Red'.

Figure 5.8 A pictorial representation of Grandiflora cultivars of *Petunia x hybrida* after 3 weeks growth at 1) control (30/25°C) or 2) exposure to heat shock at 45°C for 4 h every 3 d or for A) 'Ultra Salmon, B) 'Ultra Red' and C) 'Dreams Midnight'.

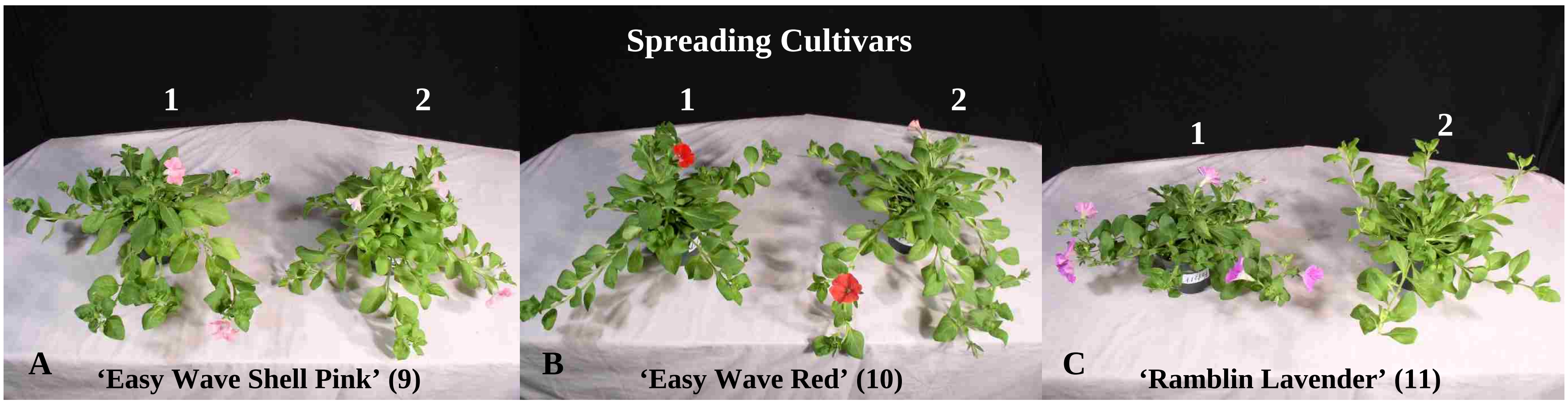

Figure 5.9. A pictorial representation of Spreading cultivars of *Petunia x hybrida* after 3 weeks growth at 1) control (30/25°C) or 2) exposure to heat shock at 45°C for 4 h every 3 d or for A) 'Easy Wave Shell Pink', B) 'Easy Wave Red and C) 'Ramblin Lavender'.

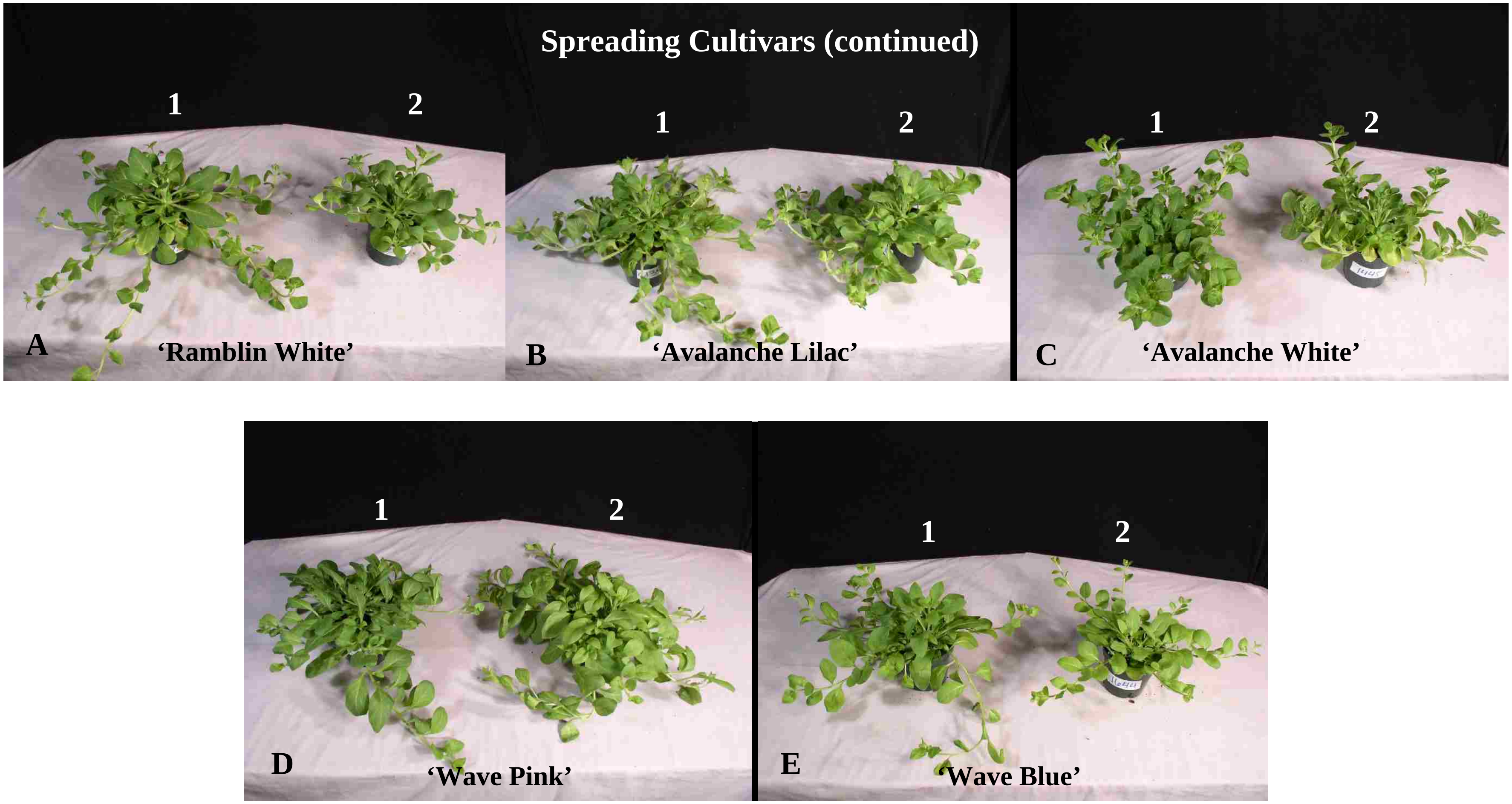

Figure 5.10. A pictorial representation of Spreading cultivars of *Petunia x hybrida* after 3 weeks growth at 1) control (30/25°C) or 2) exposure to heat shock at 45°C for 4 h every 3 d or for A) 'Ramblin White', B) 'Avalanche Lilac', C) 'Avalanche White', D) 'Wave Pink' and E) 'Wave Blue'.

‘Avalanche Lilac’(13) and ‘Dreams Midnight’(19) had significantly greater flower counts in the field compared to cultivar ‘Wave Pink’(15) (Fig. 5.11). For cultivars exposed to heat shock at 45°C for 4 h every 3 d, ‘Wave Pink’(15) had significantly decreased flower counts in the field compared to ‘Madness Waterfall Mix’(1), ‘Storm Violet’(5), ‘Storm Red’(6), ‘Easy Wave Shell Pink’(9), ‘Ramblin Lavender’(11), ‘Avalanche Lilac’(13) and ‘Avalanche White’(14). Petunia cultivar ‘Madness Waterfall Mix’ also had significantly more flowers per plant compared to ‘Ultra Red’(8) and ‘Easy Wave Red’(10) (Fig. 5.11).

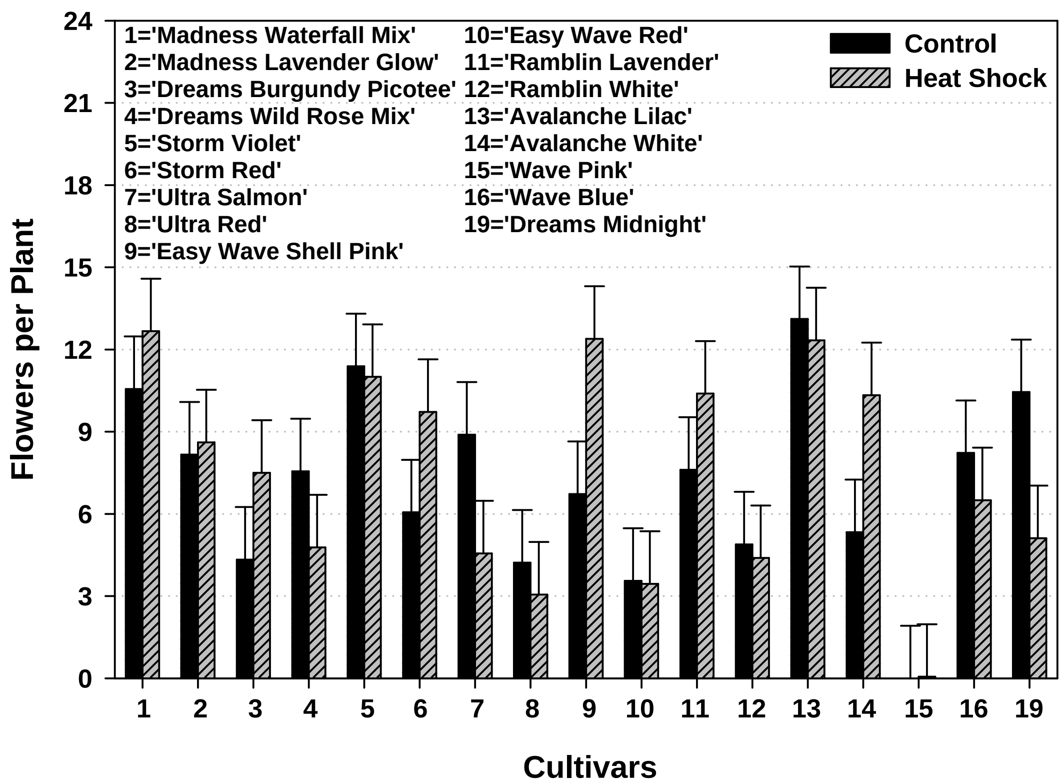

Figure 5.11. The effect of heat shock (45°C for 4h every 3 d) (Control = 30/25°C day/night) applied in the greenhouse on the number of flowers per plant of seventeen cultivars of *Petunia x hybrida* after 6 weeks in the field. Error bars represent means of eighteen observations ±SE.

Results for the effects of heat shock on average flower size in transplanted petunias were very similar to flower count results. There was no significant difference in the average flower size of heat shocked plant compared to plant grown at 30/25°C within a specific cultivar. However, cultivar had a significant effect on flower size in the landscape within each greenhouse treatment. For cultivars grown at 30/25°C day/night, 'Wave Pink'(15) had a significantly smaller flower size compared to 'Madness Waterfall Mix'(1), 'Madness Lavender Glow'(2), 'Dreams Wild Rose Mix'(4), 'Storm Violet'(5), 'Storm Red'(6), 'Ultra Salmon'(7), 'Ultra Red'(8), 'Easy Wave Red'(10), 'Ramblin Lavender'(11), 'Avalanche Lilac'(13) and 'Dreams Midnight'(19) (Fig. 5.12). For cultivars exposed to heat shock, 'Wave Pink'(15) had a significantly smaller flower size compared to 'Madness Waterfall Mix'(1), 'Madness Lavender Glow'(2), 'Dreams Wild Rose Mix'(4), 'Storm Violet'(5), 'Storm Red'(6), Ultra Red'(8), 'Easy Wave Shell Pink'(9), 'Ramblin Lavender'(11), 'Avalanche Lilac'(13) and 'Avalanche White'(14) (Fig. 5.12).

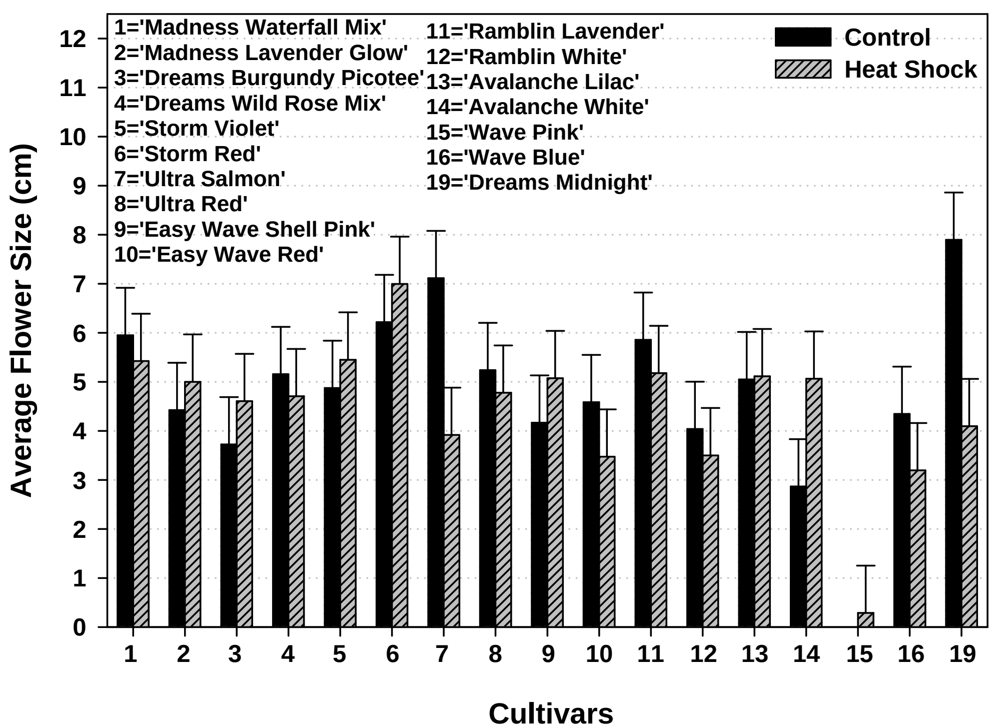

Figure 5.12. The effect of heat shock (45°C for 4 h every 3 d) (Control = 30/25°C day/night applied in the greenhouse) on average flower size (cm) of seventeen cultivars of *Petunia x hybrida* after 6 weeks in the field. Error bars represent means of eighteen observations ±SE.

Heat shock treatment of 45°C for 4 h every 3 d during greenhouse production compared to control (30/25°C) did not have a significant effect on shoot dry weight within petunia cultivars after 6 weeks transplant in the field. However, there was a significant difference between several cultivars for respective treatments. Petunia cultivars ‘Madness Lavender Glow’ (2) and ‘Avalanche Lilac’ (13) exposed to heat shock at 45°C for 4 h every 3 d or control (30/25°C) during greenhouse production had significantly increased shoot dry weights after 6 weeks transplant in the field compared to heat shocked ‘Wave Blue’ (16) (Fig. 5.13).

Results also indicated that for petunias grown at control temperature (30/25°C) in the greenhouse, total leaf area was significantly larger in ‘Avalanche Lilac’ (13) compared to control or heat shocked ‘Avalanche White’ (14) (Fig. 5.14).

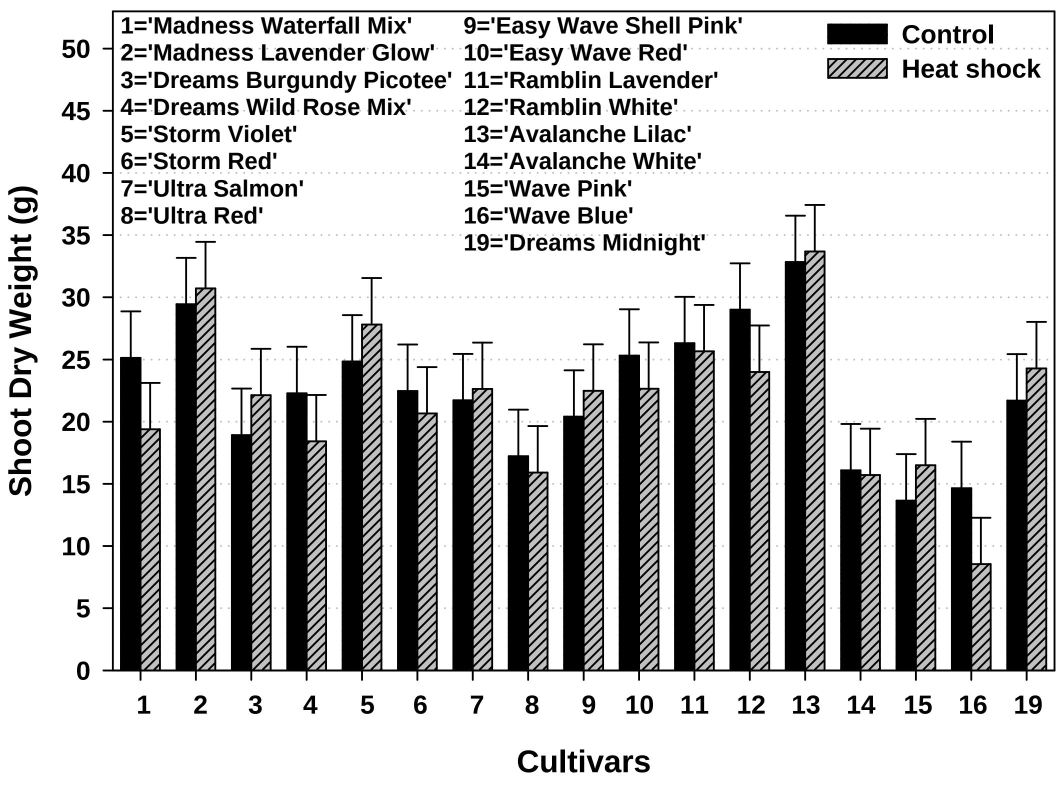

Figure 5.13. The effect of heat shock (45°C for 4 h every 3 d) and Control (30/25°C day/night) applied in the greenhouse on shoot dry weight (g) of seventeen cultivars of *Petunia x hybrida* after 6 weeks in the field. Error bars represent means of eighteen observations ±SE.

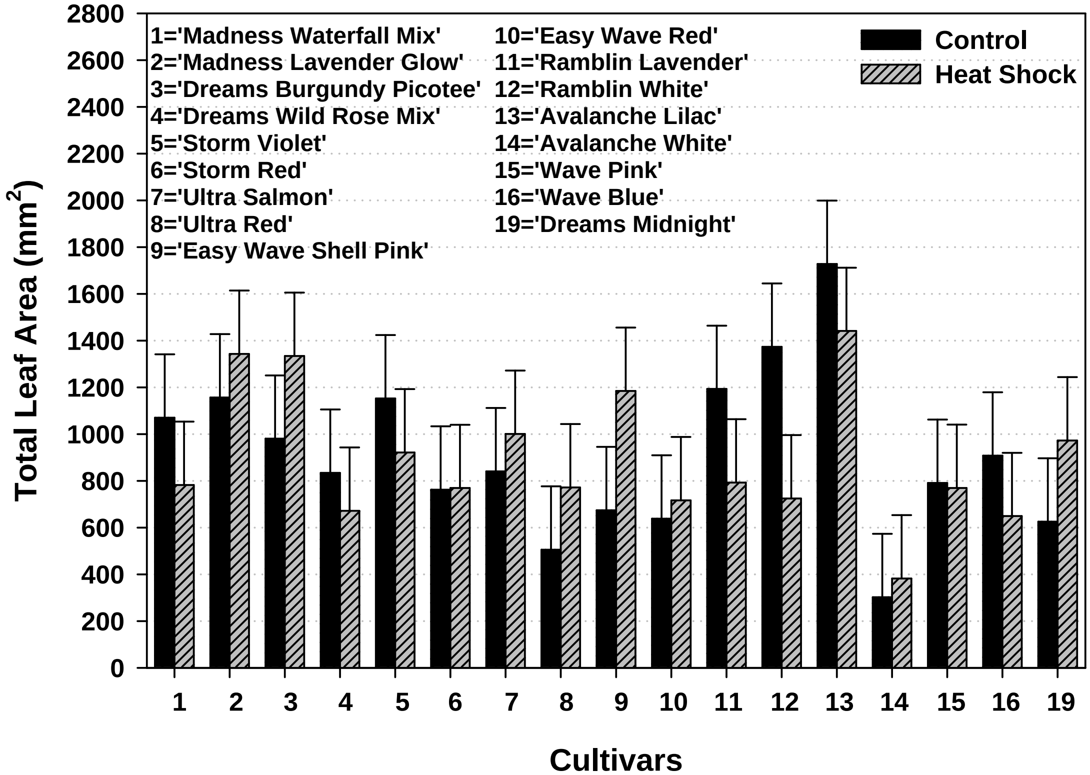

Figure 5.14. The effect of heat shock (45°C for 4 h every 3 d) and Control (30/25°C day/night) applied in the greenhouse on total leaf area (mm^2) of seventeen cultivars of *Petunia x hybrida* after 6 weeks in the field. Error bars represent means of eighteen observations ±SE.

Heat shock at 45°C for 4 h every 3 d during greenhouse production did not have a significant effect on the average internode length (cm) for each cultivar after 6 weeks in the landscape. However, there were differences in internode length between several cultivars for respective treatments. For petunias grown at control (30/25°C), the cultivar ‘Madness Lavender Glow’ (2) had significantly increased internode length compared to ‘Easy Wave Shell Pink’ (9), ‘Ramblin Lavender’ (11), ‘Ramblin White’ (12), ‘Avalanche White’ (14), ‘Wave Pink’ (15) and ‘Wave Blue’ (16) (Fig. 5.15). ‘Madness Lavender Glow’ (2) was unaffected by heat shock but had significantly longer internode lengths compared to heat shocked cultivars ‘Easy Wave Red’

(10), ‘Ramblin Lavender’ (11), ‘Avalanche White’ (14), ‘Wave Pink’ (15) or ‘Wave Blue’ (16) (Fig. 5.15).

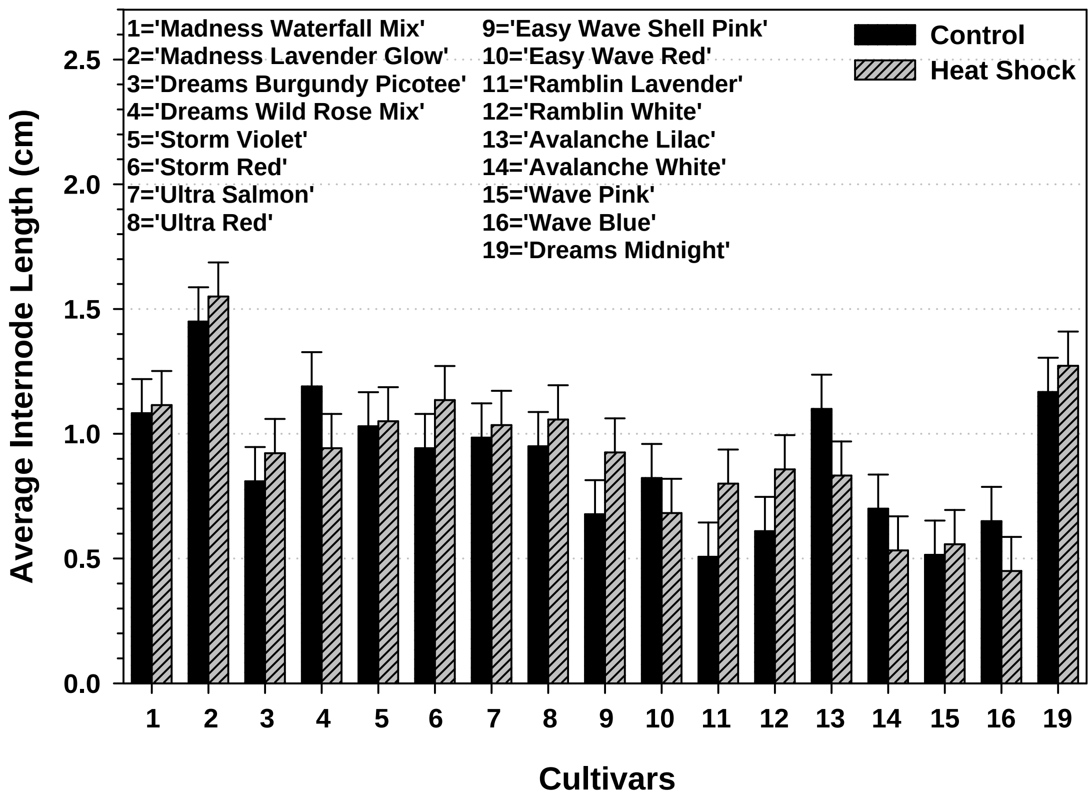

Figure 5.15. The effect of heat shock (45°C for 4 h every 3 d) and Control (30/25°C day/night) applied in the greenhouse on average internode length (cm) of seventeen cultivars of *Petunia x hybrida* after 6 weeks in the field. Error bars represent means of eighteen observations ±SE.

There were no significant differences in plant quality for control and heat shock treated plants after greenhouse production for all petunia cultivars. Petunia cultivars grown at 30/25°C, showed no significant difference in plant quality after 6 weeks in the landscape. However, cultivar had a significant effect on landscape quality of petunias exposed to heat shock (45°C for 4h every 3 d). For heat shocked cultivars, ‘Storm Red’ (6) had significantly higher quality rating than ‘Easy Wave Red’ (10), ‘Ramblin White’ (12) or ‘Wave Pink’ (15) (Fig. 5.15).

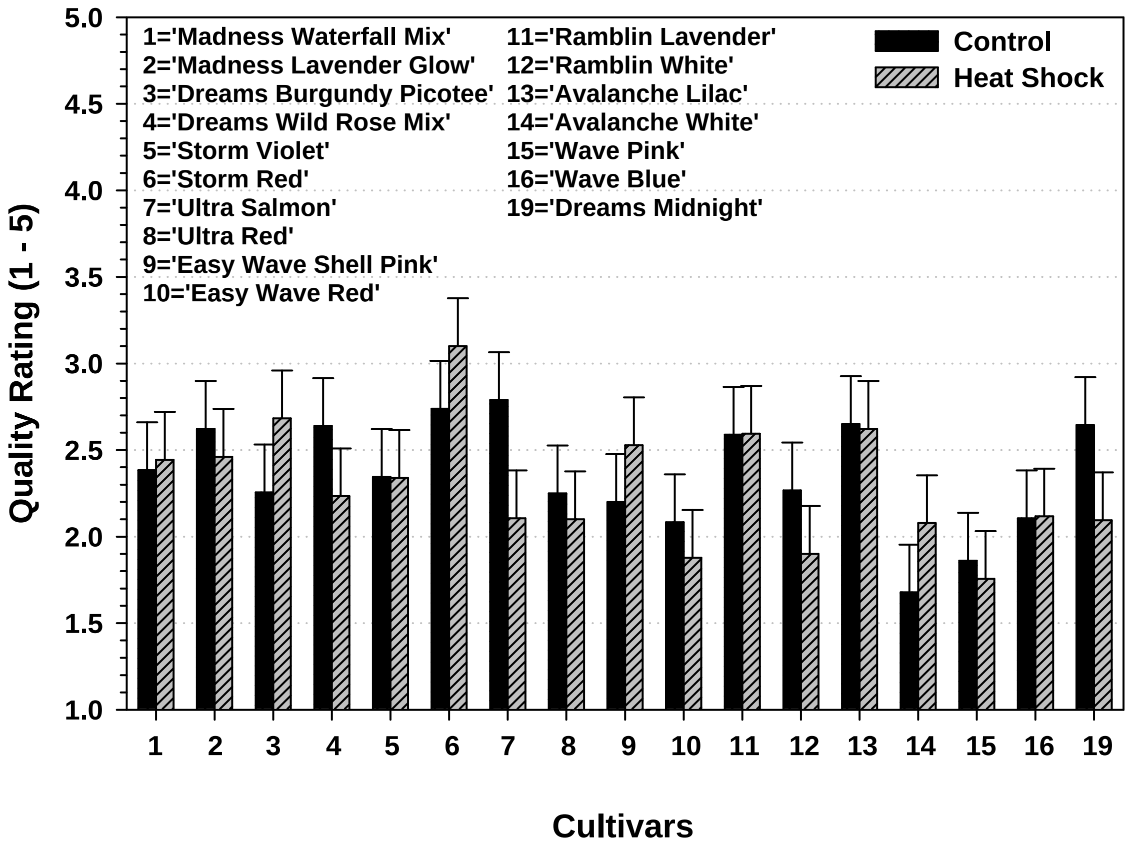

Figure 5.16. The effect of heat shock (45°C for 4 h every 3 d) and Control (30/25°C day/night) applied in the greenhouse on landscape plant quality of seventeen cultivars of *Petunia x hybrida* after 6 weeks in the field. Error bars represent means of eighteen observations ±SE.

5.4 DISCUSSION

Heat shock treatment of 45°C for 4 h every 3 d during 3 weeks greenhouse production was not significant when investigating responses of three *Petunia x hybrida* plant classes (floribunda, grandiflora and spreading). Results indicated that floribunda cultivars displayed a higher flower count than spreading cultivars when grown at control temperature (30/25°C). However, there was no significant difference between plant classes exposed to heat shock, suggesting that there may be significant effects of heat shock for specific cultivars within a plant class. After further investigation of heat shock response of nineteen *Petunia x hybrida* cultivars from these petunia classes, heat shock resulted in reduced relative internode length in 'Ultra Red'

(8) cultivar and reduced leaf area ratio in '44568' (18) compared to respective cultivars grown at control (30/25°C) (Table 5.2). The decreased relative internode length of heat shocked 'Ultra Red' (8) indicated that this cultivar may have a higher capability of withstanding heat stress by developing shorter internodes. Decreased internode length was observed in more heat tolerant cultivars of cow pea (*Vigna unguiculata* (L.) Walp.) (Ismail et al., 2000) and is also an adaptive response that can be developed through heat shock (Natarajan, 2005). The '44568' cultivar is a transgenic ethylene-insensitive petunia that has been used in previous studies where ethylene insensitivity resulted in decreased adventitious root formation (Clark et al., 1999). Decreased root growth as a result of heat stress can strongly affect plant shoot growth and nutrient uptake as seen in wheat (*Triticum aestivum* L.) (Kuroyanagi and Paulsen, 1988) and creeping bentgrass **(***Agrostis palustris* Huds. cv. Penncross) (Huang and Xu, 2000). A decrease in root growth caused by ethylene insensitivity may have further enhanced the effect heat shock at and could be responsible for the reduced LAR observed in '44568'. The number of flowers per plant, average flower size and relative growth rates were not affected by heat shock when averaged after 3 weeks of exposure which indicated that heat shock at this temperature, duration and frequency is not severe enough to cause an immediate response that is sustained over the entire treatment period. Rather, heat shock at 45°C for 4 h every 3 d had an accumulated response, where decreased flower count, flower size and relative growth weights only occurred after being heat shocked for at least 3 weeks (Table 5.3) indicating that these traits are not directly sensitive to the higher temperature but rather the amount or frequency of heat shock exposures. Natarajan (2005) found that *Salvia splendens* F. Sellow ex Roem & Schult. exposed to heat shock every 3 days for 3 hours at 30, 35, 40 and 45°C, resulted in decreased root and shoot dry weight as temperature increased.

Heat shock at 45°C for 4 h every 3 d during 3 weeks greenhouse production did not promote heat tolerance in the landscape. Petunia cultivars grown at control temperature (30/25°C) did not perform any different than the respective heat shocked cultivar for flower count, flower size, shoot dry weight, leaf area, internode length or quality in the landscape. However, results indicated differences between cultivars, irrespective of treatments. After investigating growth responses in the field, petunias in the 'Wave' series, 'Wave Pink' (15) and 'Wave Blue' (16), appeared to have limited overall growth or performance in the field. The flower count, size and quality of 'Wave Pink" was the most significantly affected. These results contradict petunia evaluations by Kelly et al. (2007) who considered 'Wave Pink' to have the best overall landscape performance within spreading class. For some measurements, 'Madness Lavender Glow'(1) and 'Avalanche Lilac' (13) had increased growth effects compared to several other cultivars which does agree with previous landscape evaluations (Kelly et al., 2007; Liu, 2009). Cultivar 'Ultra Red' (8) was not significantly affected in the landscape even though it has been previously evaluated as having poor landscape performance. Decreased internode length observed in 'Ultra Red' (8) during greenhouse production has been considered an adaptive characteristic for heat tolerance and may have had an effect on the subsequent landscape performance.

This study is the first to report on the effects of heat shock during bedding plant production to induce acquired thermotolerance and subsequent growth and development in the landscape. Although heat shock at 45°C for 4 h every 3 d had little effect on plant growth and development in the greenhouse and did not significantly promote heat tolerance in the landscape, an increase in frequency and/or duration of heat shock application may have a more significant effect. Based on the results of this study, further research investigating heat shock frequency and duration should be conducted to develop an effective heat shock protocol in the greenhouse to induce acquired thermotolerance and improve petunia landscape survivability.

5.5 LITERATURE CITED

Armitage, A.M. 1985. Petunia, pp. 41-46 in *Handbook of Flowering*, vol. IV, A.H. Halevy, editor. CRC Press, Boca Raton, Florida.

Beadle, N.C.W. 1981. The vegetation of Australia. Cambridge: Cambridge University Press.

Clark, D.G, E.K. Gubrium, J.E. Barrett, T.A. Nell, and H.J. Klee. 1999. Root formation in ethylene-insensitive plants. Plant Physiol. 121:53–59.

Copes, W. 2000. Crop profiles for bedding plants in Washington. Washington State University Publisher. pp. 1-13.

El Ahmadi, A.B., Stevens, M.A., 1979. Reproductive responses of heat tolerant tomatoes to high temperatures. J. Am. Soc. Horticultural Sci. 104-5: 686–691.

Fossler, G.M. 1993. First data reported from AFE consumer tracking study. Illinois State Florist Association Bulletin 468:5-8.

Guilioni, L., J. Wery and F. Tardieu. 1997. Heat stress-induced abortion of buds and flowers in pea: Is sensitivity linked to organ age or to relations between reproductive organs? Ann Bot. 80: 159-168.

Gusta, L.V., B.J. O'Connor and R.S. Bhatty. 1997. Flax (*Linum usitatissimum* L.) responses to chilling and heat stress on flowering and seed yield. Can. J. Plant Sci. 77:97-99.

Harding, S.A., J.A. Guikema, and G.M. Paulsen. 1990. Photosynthetic decline from high temperature stress during maturation of wheat. Plant Physiol. 92:654-658.

Huang, B., and Q.Xu. 2000. Root growth and nutrient element status of creeping bentgrass cultivars differing in heat tolerance as influenced by supraoptimal shoot and root temperatures. J Plant Nutr. 23: 979-990.

Hunt, R. 1990. Basic growth analysis. Unwin Hyman Publisher Ltd. pp:25-29.

Ismail, A. M, A. E. Hall and J.D. Ehlers. 2000. Delayed-leaf-senescence and heat tolerance traits mainly are independently expressed in cowpea. Crop Sci. 40: 1049-1055.

Kelly, R.O., Z.N. Deng, and B.K. Harbaugh. 2007. Evaluation of 125 petunia cultivars as bedding plants and establishment of class standards. HortTechnology 17: 386-396.

Kuroyanagi, T. and G.M. Paulsen. 1988. Mediation of high-temperature injury by roots and shoots during reproductive growth of wheat. Plant Cell Environ. 11:517-523.

Lichtenthaler, H.K., 1996. Vegetation stress: an introduction to the stress concept in plants. J Plant Physiol. 148, 4–14.

Liu, M. 2009. Development of a rapid and effective screening method for basal stress tolerance of *Petunia x hybrida.* Thesis. Louisiana State University, Baton Rouge.

Medina, C, and L. Cardemil. 1993. *Prosopis chilensis* is a plant highly tolerant to heat shock. Plant, Cell and Environment 16: 305–310.

Natarajan S. 2005. High temperatures stress responses of *Salvia splendens* and *Viola x wittrockiana.* Ph.D Diss. Louisiana State University, Baton Rouge.

Natarajan S. and J.S.Kuehny. 2008. Morphological, physiological, and anatomical characteristics associated with heat preconditioning and heat tolerance in *Salvia Splendens.* Journal of the American society for horticultural science. 133(4): 527-534.

Ortiz, C. and L. Cardemil. 2001. Heat-shock responses in two leguminous plants: a comparative study. Journal of Experimental Botany 52: 1711-1719.

Park, S.Y., R.Shivaji, J.V.Krans and D.S.Luthe.1996. Heat-Shock response in heat tolerant and nontolerant variants of Agrostis palustris Huds. Plant Physiol. 111: 515-524.

Pollock, C.J., C.F. Eagles, C.J. Howarth, P.H.D. Schunmann, and J.L. Stoddart. 1993. Temperature Stress, pp. 109-127 in Plant Adaptation to Environmental Stress. L. Fowden, T. Mansfield, and J. Stoddart, editors. Chapman & Hall, London.

Sung, D., F. Kaplan, K. Lee and C. L. Guy. 2003. Acquired tolerance to temperature extremes. *TRENDS* in Plant Sci. vol.8 (4):179-187.

United States Department of Agriculture. 2008. Floriculture crops 2007 summary. Sp Cr 6-1 (08) pp. 9-29.

Vierling, E. 1991 The roles of heat-shock proteins in plants. Annu Rev Plant Physiol Plant Mol Biol. 42: 579-620.

Yeh, D.M and P.Y. Hsu. 2004. Heat tolerance in English ivy as measured by an electrolyte leakage technique. J Hortic Sci Biotech. 79: 298-302.

CHAPTER 6. SUMMARY AND CONCLUSIONS

Heat stress is one of the greatest challenges for growth and development of bedding plants during greenhouse production and postproduction in the landscape, particularly in the southern United States or during the summer months. Inducing acquired thermotolerance during production by exposing plants to supraoptimal temperatures for specific durations in the greenhouse would be most beneficial to growers in heat stressed environments. However, little research has been done to investigate the morphological effects of bedding plants during heat shock or enduring heat stress preconditioning in the greenhouse or the resulting performance in the landscape.

Preliminary studies indicated that petunia grown at enduring heat stress 35/25 or 40/30ºC induced some desirable traits such as compact vegetative growth for improved shipping and landscape performance but also resulted in detrimental effects to petunia flowering habit during greenhouse production. Although petunia grown at these temperatures were able to adapt in the landscape, growing plants at enduring 35/25 or 40/30ºC in the greenhouse did not result in marketable plants and would therefore not be a recommended growth temperature to growers. Preliminary heat shock studies revealed that exposure to 35 or 40ºC for 2 h once per week did not have a significant effect on petunia growth and development.

Further research of growth under enduring heat stress and heat shock treatments with increased temperatures, durations and frequency at every 3 d during production were attempted and investigated with subsequent acquired thermotolerance test. Results indicated that the effect of heat shock or enduring heat stress temperature for inducing acquired thermotolerance appeared to be most critical at 45ºC for petunia Dreams ‘Midnight’, although the critical duration and frequency necessary for a heat tolerant marketable plant at this temperature was not fully elucidated within the treatments used. Longer durations at critical temperatures (enduring heat stress) appeared to promote better heat tolerance but did not result in a marketable plant. Therefore heat shock using shorter durations at higher frequencies than every 3 d during production should be considered for future research.

The critical acquired thermotolerance temperature of 45ºC observed in petunia Dreams 'Midnight', was further investigated on nineteen cultivars of petunia that were separated into 3 different plant classes; floribunda, grandiflora and spreading. Heat shock at 45ºC for 4 h every 3 d during greenhouse production did not have a significant effect on growth and development of petunia cultivars or classes compared to petunia grown at control temperature (30/25ºC). Petunia cultivars exposed to heat shock did not appear to have increased heat tolerance or better performance in the landscape. However, certain cultivars did perform differently in the greenhouse and the landscape indicating that cultivars may differ in heat tolerance or have different critical temperatures for inducing acquired thermotolerance. The study also revealed that the decreased overall landscape performance in cultivar 'Wave Pink' contradicted previous landscape evaluations where 'Wave Pink' was observed as having better performance.

This research was the first to quantify morphological and physiological responses to heat shock and enduring heat stress during bedding plant production for developing a greenhouse protocol for inducing acquired thermotolerance for improved landscape survivability. While the critical temperatures used in this study are effective for promoting heat tolerance in petunia, specific exposure durations or frequency of exposure during production should be further investigated in order to define an effective acquired thermotolerance protocol to improve landscape survivability.

VITA

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