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Heat Stress Inhibits Chloroplast Development in Ivy Geranium

Anna McLaurin Horton

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By

Anna McLaurin Horton

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By

Anna McLaurin Horton

Approved:

_____
Richard L. Harkess
(Major Professor)

_____
Guihong Bi
(Committee Member)

_____
Shaun Broderick
(Committee Member)

_____
Michael S. Cox
(Committee Member/Graduate Coordinator)

_____
J. Mike Phillips
Department Head

_____
George M. Hopper
Dean
College of Agriculture and Life Sciences

Name: Anna McLaurin Horton

Date of Degree: May 4, 2018

Institution: Mississippi State University

Major Field: Horticulture

Major Professor: Richard L. Harkess

Title of Study: Heat stress inhibits chloroplast development in ivy geranium

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Candidate for Degree of Master of Science

Pelargonium peltatum, ivy geranium, experiences foliar bleaching at temperatures exceeding 30° C. Contessa™ Red (heat tolerant) and Temprano™ Lavender (heat susceptible) were compared. Established plants underwent temperature treatments of 15/20° C or 25/30° C night/day with moisture treatments of 80% or 30% substrate volumetric water content (VWC). Photosynthesis, leaf greenness and growth data were collected at days 0, 7 and 11. No differences in photosynthetic rate nor a decrease in greenness in developed leaves occured in either cultivar due to high temperature or drought. Contessa™ Red had overall greater growth and leaf greenness than Temprano™ Lavender. Greenness and growth increased similarly for both cultivars at 80% VWC. Any decrease in foliar bleaching due to drought was likely due to a decrease in growth. A second study using Temprano™ Lavender indicated foliar bleaching occurs in newly emerging, developing leaves.

DEDICATION

To my parents, David and Robin Horton, who have always provided me with the encouragement and support I needed to pursue my dreams.

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This work would not have been possible without the support I received from my committee members and the Department of Plant and Soil Sciences. Thank you for your time and the knowledge you shared that allowed me to develop and discover a passion for research.

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CHAPTER I

INTRODUCTION

In 2014, annual bedding and garden plants contributed $2.6 trillion to the United States' economy from total sales (USDA, 2014). Production of garden annuals and bedding plants is geographically diverse with the five major producing states being: Michigan, California, North Carolina, Texas and Virginia (USDA, 2014). Production in southern states, such as Mississippi, is minimal. Current production of annual bedding and garden plants in Mississippi contributes approximately $4.5 million to the total U.S. floriculture income (USDA, 2014). Expanding production of floriculture crops in states like Mississippi will require improvements in cultivation and cultivars as well as a greater understanding of heat stress and the physiological mechanisms contributing to heat tolerance.

In 2014, the wholesale value of geraniums was $155.3 million (USDA, 2014). Geraniums sold are from six different species or hybrids. These range from the traditional zonal geraniums (*Pelargonium* x*hortorum*) to the cascading and uniquely leaved ivy geraniums (*P. peltatum*). Other species include *P. domesticum, P. floribunda* and the various species of scented geraniums (Dole and Wilkins, 2005). While *P.* x*hortorum* has shown significant heat tolerance, *P. peltatum* exhibits devastating foliar bleaching or leaf whitening due to heat.

Plants encounter a number of stresses during production and retail. An abiotic stress of great interest in recent years is heat stress. Heat stress causes foliar bleaching in *P. peltatum*. Foliar bleaching not only reduces the salability of the crop at the retail level, but also can cause damage so severe it leads to the crop's complete inability to photosynthesize, leading to plant death (Dhir 2008; Dhir, et al., 2013).

Foliar bleaching due to heat stress may be caused by an accumulation of reactive oxygen species (ROS). Plant responses to ROS can vary. While the plant is thriving and not experiencing stress, ROS occur naturally and have no negative effect on plant function. However, under stressful conditions, an accumulation of ROS may signal the plant to induce protection against harmful environmental stresses. As the stress continues, the balance of ROS accumulation and use becomes imbalanced which in turn can cause severe damage to the plant cell. Plants have developed defenses against ROS, but the relationship and activity is not fully understood. An abundance of interrupted physiological processes may be the cause of inefficient heat tolerance in ivy geranium (Karuppanapandian et al, 2011).

The objectives of this research were to 1) compare the physiological effects of heat stress and drought in a heat tolerant and intolerant cultivar of *P. peltatum* and 2) to identify the specific location and time of initiation of foliar bleaching in relation to a heat event in a heat sensitive cultivar of *P. peltatum.*

CHAPTER II

LITERATURE REVIEW

Plant Material

The genus *Pelargonium* is significant to the floriculture industry and contains over 270 different species divided into sixteen sections. Most of the species are native to the Cape of South Africa (James et al, 2004). During cultivation, the genus has undergone significant changes to its DNA make up due to recombination from interspecific crosses. This recombinance is believed to be the cause of an extremely large chloroplast genome. The chloroplast genome in *Pelargonium* is the largest of all land plants and differs significantly in its make-up. Where most land plants share a genome very similar, the *Pelargonium* chloroplast genome has an incredible number of genetic repeats and inversions. For example, the genome has duplicate sequences present only once in other plants that code for 10 different proteins (Palmer et al, 1987). Palmer et al (1987) also states, over half of the chloroplast genome is duplicated in *Pelargonium*. Interspecific crosses responsible for this unique genome are also responsible for the generation of new species such as *P. peltatum*.

P. peltatum is believed to be the result of an interspecific cross between *P. zonale* and *P.* x*hortorum.* These species are in the taxonomic section Ciconium of *Pelargonium* (James

et. al, 2004). Because *P. zonale* and *P.* x*hortorum* could be ancestors of *P. peltatum* it is possible they may be sources to introduce heat tolerance back into *P. peltatum*. Optimum growing conditions for *P. peltatum* require temperatures between 20° C and 23° C (Murray et. al., 2012). Production of this crop generally begins in the early months of January and February so they are ready for retail sale in April and May. For hanging baskets in warm climates, total crop time should take only 8-9 weeks. During production, soil must be pasteurized as pathogens can cause significant damage. *P. peltatum* also encounters a physiological issue called edema. Edema causes the stomatal cells to rupture and form corky blisters on the underside of the leaf. This is believed to be caused by excessive turgor pressure during the night when the stomatal cells are closed (Dole and Wilkins, 2005). *P. peltatum* requires much cooler temperatures compared to its related taxa. Determining the physiological effects of heat stress on *P. peltatum* and the location and timing of foliar bleaching development in response to heat stress may illuminate methods to enhance heat tolerance expanding the potential retail market.

Heat Stress

In *P. peltatum*, foliar bleaching is devastating to the aesthetic value and shelf life of what could be a beautiful and valuable addition to floriculture production in southern states. Excessive temperature, heat stress, causes foliar bleaching in *P. peltatum*. Heat stress can be defined as a rise in temperature beyond a tolerated threshold for a period of time sufficient to cause irreversible damage to plant growth and development (Wahid et al., 2007). A rise in temperature above the tolerated threshold causes damage in a multitude of areas and has a negative cascading effect on plant survival. Damage may

occur within minutes of overexposure to heat. This damage begins on a cellular level causing disorganization and even cell death. Damage within the plant cell begins a multitude of negative reactions that can include the denaturation of proteins, reduced membrane stability, prohibiting the synthesis of proteins and inactivating enzymes within chloroplasts.

Another negative effect heat stress has on plant survival is the production of ROS (Karuppanapandian et al, 2011). Ultimately, an accumulation of ROS will lead to morphological damages in the stressed crop. Scorching of leaves and stems, leaf and flower abscission and discoloration of fruit are some of the most harmful morphological damages, especially considering ornamental crops are sold for their aesthetic value. Santarius and Muller (1979) state high temperatures negatively affect the photosynthetic apparatus in green tissue, supporting the idea foliar bleaching of young green leaves is caused by exposure to heat stress.

Heat Stress and Photosynthesis

Both physiological and developmental processes in plants can be affected by heat stress. Photosynthesis is imperative to the life and survival of plant species. Therefore, any interruption in this process is devastating to plant life. Photosynthesis is also the most sensitive of plant functions to increased temperatures (Xu et al., 2010). This process involves a series of reductive and oxidative reactions to convert light energy into sugars for usable energy. A natural byproduct of photosynthesis is the production of O_2, a ROS (Taiz and Zeiger, 2006). During photosynthesis, in photosystem one (PSI) and photosystem two (PSII), the flow of electrons can be affected by heat stress. Specifically,

it has been shown that after a heat event, reduction of the $P700^+$ in PSI was greatly increased. However, as reduction and electron flow increased, the half-life of $P700^+$ fell from over 500 ms to less than 50 ms (Sharkey, 2005). Because the half-life of the $P700^+$ falls so dramatically, photosynthesis is not able to completely reduce NADP to $NADPH^+$. Although the reduction of NADP to $NADPH^+$ is not complete, light harvesting and PSII is still functioning, leading to an abundance of free radicals and accumulation of O_2.

Reactive Oxygen Species (ROS)

Reactive oxygen species occur naturally as a byproduct of plant metabolism and photosynthesis, but as photosynthesis is inhibited in PSI one can assume the ROS O_2 begins to accumulate in the chloroplast. As the plant undergoes a heat event, it is believed ROS play a vital role in signaling for protection mechanisms. However, it has also been shown if the heat stress continues, accumulation of ROS also continues where it can cause devastating damage to the plant cell. The amount of ROS produced in the cell is a very delicate balance. Normal cell functions reduce O_2 into water and other ROS such as O_2, H_2O_2, HO_2 and $^1O_2$, the singlet oxygen. Production of ROS occurs not only in the chloroplast, but also in other cell organelles (Karuppanapandian et al, 2011). ROS produced in other cell organelles such as the mitochondria, peroxisomes and endoplasmic reticulum occur naturally, but are enhanced when under stress.

According to Karuppanapandian et al. (2011), the singlet oxygen is produced under ultraviolet (UV) stress in the chloroplast. The structural integrity of the chloroplast is essential for maintaining photosynthesis and active growth as it is the main location of all photosynthetic processes. Therefore, overproduction of ROS in the chloroplast,

especially the singlet oxygen, is very concerning. The singlet oxygen is believed to have negative effects on membrane proteins and lipids. During heat stress, movement of molecules across membranes is accelerated, loosening chemical bonds. As the bonds are loosened, membranes become more permeable and therefore more vulnerable to organisms within the cytoplasm of the cell that can damage chloroplast DNA which could code for certain protective proteins or enzymes (Wahid, et al., 2007). The singlet oxygen is also known to damage DNA directly (Viljanen, et al., 2002).

Heat Stress and Plant Development

The singlet oxygen responsible for damaging DNA could cause mutations and thus interfere with the development of the photosynthetic apparatus. Organelle development begins in the meristems of plants. All cell organs necessary for photosynthesis are developed before the leaf or stem emerges from the meristem. After reaching a certain point in leaf development, all meristematic activity ceases and any additional growth is the result of cell enlargement. Within the meristems of plants, proplastids exist which are the precursors for chloroplasts as well as amyloplasts, leucoplasts, chromoplasts and etioplasts. Amyloplasts are important for starch storage in roots, leucoplasts store lipids and chromoplasts accumulate plant pigment (Chiang et al, 2012). Etioplasts in shoots, however, develop in response to a lack of light. Etioplasts do have some photosynthetic organelles such as thylakoids and grana, but they can cause the shoots and leaves to appear bleached. In comparison, chloroplasts are produced in shoots under light conditions and produce functional photosynthetic organelles and green leaves (Armstrong et al., 1995). The phytohormone cytokinin plays an important role in the

differentiation of proplastids into chloroplasts and exogenously applied cytokinins have induced regreening in etiolated seedlings (Chiang et al., 2012).

The biosynthesis of chloroplasts begins with the reduction of photochlorophyllide to chlorophyllide (Armstrong et al., 1995). This first step in chloroplast biosynthesis is light dependent and requires light for photochlorophyllide to be reduced. An enzyme called protochlorophyllide oxidoreductase (POR) regulates this reduction. The two genes that code for the POR enzyme are identified as *PORa* and *PORb* and are members of the phytochrome gene family. Because foliar bleaching occurs in mature plants of *P. peltatum, PORb* would be the most vital gene to investigate because it has been identified in both adult plants and seedlings. *PORa*, however, is only present in young seedlings. *PORb* is very important in the greening of the stems and leaves and should be studied in *P. peltatum* to gain a greater understanding of its fate under heat stress (Armstrong et al., 1995).

Protection Mechanisms Against Heat Stress Induced Photooxidation

Recently, a novel protein, Chloroplast Enhancing Stress Tolerance (CEST), has been discovered within the chloroplast DNA (cpDNA) that could have positive effects on heat tolerance in certain crops. Foliar bleaching is a primary concern in production of *P. peltatum*. CEST has been proven to provide significant protection against photooxidation when overexpressed in transgenic *Arabidopsis*. This protein has been found in all photosynthetic organelles and is believed to be involved in the protection and development of chloroplasts (Yokotani et al., 2011). Plants containing CEST were treated with Paraquat, a photooxidative herbicide. Paraquat produces superoxide, a ROS that

causes photooxidation leading to plant death. *Arabidopsis* overexpressing for the CEST protein showed tolerance to Paraquat. These plants also showed significant tolerance to photooxidation caused by heat, drought and salinity (Yokotani et al., 2011). Several other proteins have been discovered in the genome of the chloroplast, suggesting the chloroplast should be an important focus in breeding for heat tolerance and other environmental hazards (Yokotani et al., 2011).

Research has also proven carotenoids play a specific role in protecting against ROS by acting as antioxidants. In particular, carotenoids may act against the singlet oxygen produced in response to stress. Carotenoids will react with the singlet oxygen in two different ways: 1) by provoking a chemical reaction or 2) by provoking an energy transfer reaction. Either of these may lead to the deactivation of the singlet oxygen (Viljanen et al., 2002). Studies have proven the presence of different carotenoids, such as β-carotene, lutein and lycopene, have different effects on antioxidant activity at different concentrations. For example, at 40 ppm β-carotene was the most efficient antioxidant. Lycopene was more efficient at 10 ppm. Overall, each carotenoid performed well as an antioxidant to the singlet oxygen. The carotenoid transfers an excited electron from the singlet oxygen to the carotenoid to deactivate the ROS. Once the excited state of the singlet oxygen is transferred to the carotenoid it is referred to as the carotenoid triplet where its energy is rapidly used within the cell (Viljanen et al., 2002). Norflurazon, an herbicide, also demonstrates the importance of carotenoids as an antioxidant. This herbicide acts by blocking carotenoid synthesis, causing extreme photooxidation and causing the chloroplast to lose all photosynthetic capabilities (Tamada et al., 2003). A

previous study conducted by Dhir (2008; 2013) found there was a greater degradation of chlorophyll than carotenoids after heat stress was induced in *P. peltatum* 'Beach' and 'Butterfly.' In 'Butterfly,' a heat sensitive cultivar, the amount of carotenoids present was greatly reduced indicating carotenoids play an important role in foliar bleaching in *P. peltatum*.

Plants are also known to produce other mechanisms of defense against heat stress such as heat shock proteins (HSP) (Wahid et al., 2007). HSPs are responsible for adapting the crop to increased temperatures and are identified by three different characteristics: a) their induction coincides with the organism under stress; b) their biosynthesis is extremely fast and intense; and c) they are induced in a wide variety of cells and organisms (Wahid et al., 2007). HSPs are classified into three groups, according to their molecular weight: HSP90, HSP70 and low molecular weight HSPs (LMW-HSP). These proteins are normally found in the organelles they protect; for example, the cell wall, chloroplasts and mitochondria. The specific function of HSPs is to aid in the synthesis and unfolding of proteins under heat stress. They are also known to be chaperones for other proteins (Wahid et al., 2007). While HSP's function is protecting the plant cell from damage induced by heat stress, each individual protein may have a different effect metabolically. The mechanisms by which some proteins contribute to heat tolerance are still unknown while others such as HSP70 have been established.

HSP70 is believed to have multiple functions. These functions include assisting in transcription and translation of proteins. It is also hypothesized that HSP70 has a major function in constructing ATP proteins as well as preventing denaturation (Wahid et al.,

2007). Thermotolerance after a heat event has been directly connected to HSP70. Information collected on this important HSP is from the study of mutant plants missing HSP70 where those mutant plants showed sensitivity to heat stress. LMW-proteins are expected to aid in membrane stability and its presence can be used as an indication of heat tolerance (Wahid et al., 2007).

Other proteins not characterized as HSP are also important to heat tolerance. There are a multitude of proteins responsible for protecting plant cells against high temperatures. The chlorophyll a/b-binding protein, located within the light-harvesting complex of the chloroplast, captures excited energy which then delivers the excited electrons to PSII and PSI. During heat stress, carbon fixation is limited causing an over-abundance of excited electrons, which will cause oxidative damage. To prevent this over-abundance of excited electrons, the synthesis of the chlorophyll a/b-binding protein responsible for light harvesting is reduced. This prevents the accumulation of excited electrons in the cell thus reducing or preventing oxidation (Xu et al., 2010).

Also of interest are the dehydrin proteins. The dehydrin proteins are very stable under high temperatures (Arora et al., 1998). These proteins were shown to increase in drought and heat stressed geraniums, indicating a possible relationship between the defense mechanisms of the two stresses (Wahid et al., 2007). Arora et al. (1998) found water stress induced heat tolerance in geraniums. They also state dehydrin may cause partially unfolded proteins to revert back to a folded state or inhibit their denaturation any further (Arora et al., 1998). Further insight into the importance of proteins such as the HSPs, dehydrins and carotenoids is needed. A better insight into the physiology behind

heat stress in *P. peltatum* could improve breeding techniques to develop a more heat tolerant variety of *P. peltatum*.

Breeding for Heat Tolerance

Conventional breeding methods can be problematic for several reasons including the difficulty in identifying viable heat tolerance sources. Limited germplasm and genetic diversity make it difficult for heat tolerant genotypes to be identified and incorporated into existing heat intolerant varieties. Genetic diversity is particularly difficult to identify in a plant such as *P. peltatum* because it is self-pollinated. Another difficulty in conventional breeding is determining if the offspring from the heat tolerant genotype is indeed heat tolerant or merely a plant with a greater growth potential. Plants with a greater growth potential often perform well regardless the stress. Finally, a third difficulty is finding a heat tolerant genotype that performs well under normal conditions as well as under heat stress. Often, heat tolerance is paired with an undesirable trait (Wahid et al., 2007). In *P. peltatum*, while the cultivar Contessa™ Red with dark red flowers performs well under high temperatures, Temprano™ Lavender' with light purple flowers does not tolerate temperatures above 30° C. Although red flowers are popular, studies have shown that purple or pink geraniums are the most desirable (Behe et al., 1999). In ivy geranium, heat tolerance may be linked to a less desirable flower color.

A conventional method, such as the backcross breeding method, would be an efficient method to introduce heat tolerance into a pink or lavender cultivar of *P. peltatum* such as Temprano™ Lavender. This method involves an initial hybridization event between two parents. It is also important to note the heat tolerance source should be

the mother plant. Heat tolerance has been directly related to cytoplasmic DNA, which is only inherited maternally (James et al., 2004). The progeny resulting from the initial cross should be heterozygous because *P. peltatum* is a self-pollinated crop. However, it is possible heterozygous alleles exist in each parent because the plant is vegetatively propagated. The F_1 progeny should be allowed to self and further segregate. In the F_2, the first selections can be made based on the plant's level of heat tolerance using Cell Membrane Thermostability (CMT). By using methods, such as CMT, to test for heat tolerance, the amount of heat tolerance between two cultivars such as Contessa™ Red and Temprano™ Lavender can be established. Comparing CMT results of each progeny can help identify the type of gene action (Naveed, et al., 2016).

Selections should also be based on traits typical of *P. peltatum*. This would include ivy shaped leaves, a cascading habit and flower color. It is important that only plants showing significant heat tolerance be selected. Heat tolerance is a quantitative trait controlled by many different genes. This means the degree of tolerance can vary drastically from plant to plant. Any plants selected containing minimal tolerances would create genetic drag in the improved population. Additional crosses should be made until a plant exists with significant heat tolerance with phenotypic characteristics typical of *P. peltatum*. The desired progeny would then be crossed with a *P. peltatum* of desirable color to introduce heat tolerance and maintain the important phenotypic traits of *P. peltatum*. Because this plant is primarily vegetatively propagated, seed production is not important and only one plant would be necessary to create a new marketable cultivar.

Before beginning these breeding techniques it is imperative to understand the taxonomy and relationships between all geranium species. With this knowledge one could establish a viable heat tolerance source to introduce into *P. peltatum*.

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CHAPTER III

HEAT AND DROUGHT STRESS ON PHOTOSYNTHESIS AND FOLIAR BLEACHING

Pelargonium peltatum (ivy geranium), contains a wide variation of heat tolerant and intolerant cultivars. A study was conducted to determine the effects of heat stress on photosynthesis and foliar bleaching in *P. peltatum*. The study used a heat tolerant cultivar, Contessa™ Red, and an intolerant cultivar, Temprano™ Lavender. The two cultivars underwent temperature treatments of 15/20° C and 30/35° C night/day for a seven day period. The plants also underwent moisture treatments of 30 or 80% volumetric water content (VWC) to observe each cultivar's ability to adapt to heat stress under a decreased watering regimen. Photosynthesis measurements and SPAD were measured before, during and after the heat treatments. Plant height and width were measured and a growth index calculated to determine the effects of increased temperatures on growth. This study found no differences in photosynthesis or SPAD between the heat tolerant and intolerant cultivars and no foliar bleaching was observed in mature leaves during the temperature treatments. Heat tolerant Contessa™ Red had a greater growth index than the heat intolerant Temprano™ Lavender regardless of temperature treatment. VWC had significant effects on SPAD, growth index and photosynthesis, but these results did not indicate drought stress would improve *Pelargonium peltatum*'s physiological response to heat stress. Changes in growth due to

drought may explain some grower's experiences with decreased bleaching in a drought stressed crop.

Introduction

Pelargonium peltatum (ivy geranium) is an ornamental crop produced for its beautifully colored flowers, ivy shaped leaves and cascading habit. While it is very popular, production in the southeastern United States is difficult due to the crop's inability to perform well under temperatures exceeding 30° C (Dhir, 2008; Dhir et al., 2013). Devastating foliar bleaching due to heat stress occurs in the leaves leaving them unable to photosynthesize. Abscission of the leaf from the stem occurs under prolonged heat stress eventually leading to an unsalable crop or plant death. Heat tolerance has been noted in cultivars such as Contessa™ Red (formerly 'Beach') while the more lightly colored Temprano™ Lavender (formerly 'Butterfly') suffers greatly from heat stress and foliar bleaching (Dhir, 2008; Dhir et al., 2013). By identifying the mechanism inferring heat tolerance we can gain a greater understanding of heat stress and identify key characteristics in selecting for a more heat tolerant ivy geranium for production in warmer climates.

Heat stress can be defined as a rise in temperature beyond a tolerated threshold for a period of time sufficient to cause irreversible damage to plant growth and development (Wahid, et al., 2007). A rise in temperature above the tolerated threshold has a negative cascading effect on plant survival. Damage within the plant cell begins a multitude of reactions that can include the denaturation of proteins, reduced membrane stability, prohibition of protein synthesis and inactivation of enzymes within chloroplasts.

In green tissue, high temperatures negatively affect the photosynthetic apparatus (Santarius and Muller, 1979). Degradation of the photosynthetic apparatus occurs as membranes of these essential organelles are destroyed by reactive oxygen species (ROS). ROS occur naturally as a byproduct of plant metabolism and photosynthesis, but as photosynthesis is inhibited in photosystem one (PSI), the ROS O_2 begins to accumulate in the chloroplast (Cho and Seo, 2005). As the plant undergoes a heat event, it is believed ROS play a vital role in signaling for protection mechanisms. However, if the heat stress continues, accumulation of ROS also continues where it can cause devastating damage to the plant cell. It appears the amount of ROS produced in the cell is a very delicate balance. Normal cell functions reduce O_2 into water and other ROS such as O_2, H_2O_2, HO_2, and $^1O_2$, the singlet oxygen.

According to Karuppanapandian et al. (2011), the singlet oxygen is produced under UV stress in the chloroplast. The structural integrity of the chloroplast is essential for maintaining photosynthesis and active growth as it is the main location of all photosynthetic processes. Therefore, overproduction of ROS in the chloroplast, especially the singlet oxygen, is toxic. The singlet oxygen is believed to have negative effects on membrane proteins and lipids. During heat stress, movement of molecules across membranes is accelerated, loosening chemical bonds. As the bonds are loosened, membranes become more permeable and therefore more vulnerable to organisms within the cell cytoplasm that can damage chloroplast DNA which may code for certain protective proteins or enzymes (Wahid, et al., 2007). The singlet oxygen is also known to damage DNA directly (Viljanen, et al., 2002).

Photosynthesis is the most sensitive plant function to increased temperatures and is greatly affected by the production of ROS (Xu, et al. 2010). Photosynthesis involves a series of reductive and oxidative reactions to convert particles of light into sugars for usable energy. A natural byproduct of photosynthesis is the production of O_2, a ROS (Taiz and Zeiger, 2006). During photosynthesis, in PSI and photosystem two (PSII), the flow of electrons can be affected by heat stress. Specifically, it has been shown that after a heat event, reduction of the $P700^+$, the primary electron donor in PSI, was greatly increased. However, as reduction of the $P700^+$ and electron flow increased, the half-life of $P700^+$ fell from over 500 ms to less than 50 ms (Sharkey, 2005). Because the half-life of the $P700^+$ falls so dramatically, photosynthesis is not able to completely reduce NADP to $NADPH^+$. Although the reduction of NADP to $NADPH^+$ is not complete, light harvesting and PSII is still functioning, leading to an abundance of free radicals and an accumulation of O_2.

A similar cascade, called photooxidation, occurs in response to excess light. Photooxidation results in an increase in ROS, negatively effecting plant growth and photosynthesis. Water stress during photooxidation is important in signaling for protection mechanisms which can protect photosynthesis and important plant cell structures (Osakabe, et. al., 2014). The catalyst, 9-cis-epoxycarotenoid dioxygenase 3 (NCED3), is important in the synthesis of abscisic acid (ABA). ABA during drought stress signals for stomatal closure and other physiological responses. In mutations in *Arabidopsis* where NCED3 was reduced or overexpressed, water use efficiency decreased or increased respectively in drought stressed plants (Iuchi, et al., 2001).

According to Kimura, et al. (2002), over 70% of genes induced by photooxidation are also induced by drought. Growers experience a decrease in foliar bleaching when heat susceptible cultivars of *P. peltatum* are slightly drought stressed (Dhir, 2008; Dhir et al., 2013).

Two cultivars of *P. peltatum* were compared under heat and drought stress to determine differences in photosynthetic response, leaf greenness and growth index between a heat tolerant and an intolerant cultivar and to determine if drought increased heat tolerance.

Materials and Methods

A physiological comparison of heat tolerance was conducted using a heat tolerant cultivar, Contessa™ Red, and a heat intolerant cultivar, Temprano™ Lavender, of *P. peltatum* (Syngenta Flowers, Inc., Boulder, CO) (Dhir, 2008; Dhir et al., 2011; 2013). On February 13, 2012 96 rooted cuttings of each cultivar were potted into 1L (15.24 cm diam.) pots in a conventional peat based substrate, ~ 70% peat:30% perlite v/v (Sunshine Mix 1, Sun Gro Horticulture, Agawam, MA). Plants were fertilized at every watering with 200 mg $N \cdot L^{-1}$ from 20N-4.4P-16.6K (Peter's Peat Lite 20-10-20, The Scott's Company, Marysville, OH) until plants had a substantial root system; about 6 weeks. Plants were then moved into growth chambers (Conviron CMP3000, Controlled environments Ltd., Winnipeg, Manitoba, Canada) and acclimated to 15/20° C night/day for three days. Using a randomized complete block design within a split plot design, split by temperature treatments, treatments were 15/20° C or 25/30° C night/day with moisture treatments of 30 or 80% volumetric water content (VWC) for 7 days. Each treatment

contained 3 subsamples for a total of 24 plants per replication. The experiment was replicated 3 times over time.

Soil moisture was measured daily using a Spectrum Technologies Watermark Sensor (WaterScout SM 100 Soil Moisture Sensor; FieldScout Soil Sensor Reader; Spectrum Technologies, Inc., Aurora, IL) during the course of the study to maintain the appropriate VWC for each treatment. The equation (((0.626×Desired VWC Percentage)×1200) – (Moisture Reading/100×1200) = ml water applied to the soil) was used to construct a table to determine the milliliters water necessary to return the water content of the soil to the required moisture percentage (Cochran, 2012).

Growth was measured by taking the initial and final height and width of each plant in the study. A growth index was calculated using the geometric formula for a cone: $\frac{1}{3}\pi \times r^2 \times h$. Height was measured from the top of the soil surface to the tallest point of active growth. Radius was calculated by averaging the width at the widest point and at 90° from the original width measurement and dividing the average width by 2. Photosynthetic rate ($\mu mol \cdot m^{-2} \cdot s^{-1}$) was measured using the Ciras-2 Portable Photosynthesis System (PP Systems International, Inc., Amesbury, MA) and leaf greenness, an indication of chlorophyll content, was measured with a Minolta SPAD-502 chlorophyll meter (Konica Minolta, Inc., Tokyo, Japan). Photosynthetic rate was measured on the third most developed leaf from the plant shoot. SPAD readings were averaged from three readings of the same plant from similarly developed mature leaves. Photosynthetic rate and SPAD measurements were taken in the growth chamber at days

0, 7 and 3 days after treatments ended, day 11, to determine response to and recovery from the stress treatments.

Growth indices, photosynthetic rate and SPAD measurements were measured at day 0. After baseline measurements were collected, the plants were moved into their respective treatments. On the final day of temperature treatments (day 7), photosynthetic rate and SPAD readings were again collected. On day 11, after the plants had experienced 3 full days of 15/20° C night/day, photosynthetic rate and SPAD readings were again collected. Final growth data were also collected at this time. The data were analyzed using the GLIMMIX Procedure (SAS 9.3; SAS Institute, Cary, NC).

Results

Main Effects and Interactions

Data were collected to determine the effects of temperature and drought over time on SPAD readings, photosynthesis, height, width and growth index. SPAD readings and photosynthesis measurements were collected to determine if changes in leaf greenness or photosynthetic function occurred in mature leaves in response to temperature and drought treatments. There were differences between cultivars in SPAD readings, height, width and growth index in mature leaves of *P. peltatum* (Table 3.1). Temperature had no effect on leaf greenness (Table 3.2), photosynthesis (Table 3.4), height (Table 3.1), width (Table 3.1) or growth index (Table 3.1) in any cultivar. Moisture by day influenced leaf greenness (Table 3.3 in both cultivars. Moisture by temperature also affected photosynthesis in both cultivars of geranium (Table 3.4).

SPAD Readings

Contessa™ Red had a greater SPAD reading (greener leaves) than Temprano™ Lavender regardless of treatment (Table 3.1). Changes in leaf greenness occurred by moisture and day for both cultivars of geraniums (Table 3.2). The 80% VWC treatment had an interaction effect on leaf greenness by cultivar and day (Table 3.2). No changes in SPAD occurred under the 30% VWC treatment from day 0 to 11 (Table 3.2). Under the 80% VWC treatment both cultivars had increased SPAD readings by the end of the study. Contessa™ Red had an increase in leaf greenness from day 0 to 7 whereas leaf greenness in Temprano™ Lavender did not increase until day 11 (Table 3.3). While increases in leaf greenness occurred at different rates for both Contessa™ Red and Temprano™ Lavender, Contessa™ Red had a greater leaf greenness throughout the course of the study (Table 3.3) Contessa™ Red also had a greater leaf greenness than Temprano™ Lavender under the 30% VWC treatment (Table 3.2).

Photosynthesis

Photosynthetic rate measurements were taken to determine each cultivar's response to 7 days of heat and drought stress. Cultivar did not have an effect on photosynthetic rate and no differences occurred in response to cultivar by day, moisture or temperature (Table 3.1). No changes between cultivar across days indicates the more heat tolerant cultivar did not recover or respond differently to stressful treatments than the intolerant cultivar. Temperature did not affect photosynthetic rate, leaf greenness, width or growth index alone, but photosynthetic rate did respond to temperature by VWC across days (Table 3.5). The 30% VWC treatment did not have any changes in

photosynthetic rate across days (Table 3.5). Photosynthetic rate increased from day 0 to 7 under the 80% VWC treatment (Table 3.5).

Growth: Height, Width and Growth Index

Cultivar had the greatest effect on size and growth index. Differences in growth index by cultivar indicate Contessa™ Red was overall larger than Temprano™ Lavender (Table 3.1). No differences in growth (height, width or growth index) between cultivars occurred by day or moisture indicating both cultivars had similar responses under drought stress (Table 3.1). Changes in response to moisture across days demonstrates VWC affected width (Table 3.1) and growth index (Table 3.1), but not height over time (Table 3.1). There were no changes in height from day 0 to 11 in the 30 or 80% VWC treatment (Table 3.1). Changes in width and growth index occurred over time in response to the VWC treatments (Table 3.6). There were no changes in width or growth index under the 30% VWC treatment from day 0 to 11 (Table 3.6). Differences between days occurred for both width and growth index under the 80% VWC treatment (Table 3.6).

Discussion

SPAD measures leaf greenness and is an indirect indicator of chlorophyll content. The SPAD readings from this study were taken from 3 similarly developed leaves from each plant. Unlike photooxidation, temperature had no effect on leaf greenness for either cultivar. Leaf greenness did not decrease in the heat treatment indicating chlorophyll content did not decrease in developed leaves in response to temperature stress (Li, et al., 2010). Moisture treatments affected leaf greenness in both cultivars. The drought stress treatment, 30% VWC, did not affect chlorophyll content in the leaves while 80% VWC,

the non-stressed treatment, had an increase in leaf greenness in both cultivars. However, Temprano™ Lavender did not respond as quickly as Contessa™ Red to the higher moisture treatment. Studies performed by Fanizza and others on grape plants indicated drought stressed plants had a decrease in leaf greenness or SPAD (Fanizza, G., et al., 2004).

As temperature increased, photosynthetic rate decreased in well-watered plants. There was no difference in photosynthetic rate in drought stressed plants, but it was noted the drought stressed plants were respiring rather than photosynthesizing. Similar effects were seen across days with drought stressed treatments. In well-watered plants photosynthetic rate increased with days. The initial increase in photosynthetic rate may have arisen from the plants adjusting to a more consistent environment within the growth chambers. There were no differences in photosynthetic rate between Contessa™ Red and Temprano™ Lavender. If foliar bleaching was due to photosynthetic inhibition in mature leaves, one would expect to see a greater decrease in photosynthetic rate in the heat susceptible cultivar. This is also an indicator that degradation of the photosynthetic apparatus resulting in foliar bleaching was not occurring in mature leaves of the heat susceptible cultivar. While a difference in VWC treatments affected photosynthetic rate, there were no cultivar differences in response to VWC. Thus, moisture alone was not responsible for increasing heat tolerance in *P. peltatum*. The 30% VWC treatment did not influence photosynthetic rate during the study; however the 80% VWC treatment showed significant increases in photosynthetic rate from day 0 to 7 in both cultivars.

Contessa™ Red had an overall greater growth index than Temprano™ Lavender regardless of treatment. Growth index increased in both cultivars from day 0 to 11 with Contessa™ Red maintaining a greater growth index across days. Temperature had no effect on growth, while moisture was responsible for increased growth in both cultivars from day 0 to 11 under the 80% VWC treatment. 30% VWC showed no changes in growth index over the course of the study.

During the course of the study it was observed mature leaves did not experience foliar bleaching in response to heat treatments; however, when the plants were removed from the growth chamber and allowed to develop further, bleaching was noted in the apical and axillary meristems. As the plants continued to grow after the heat treatment, patterns of bleaching developed from the apical and axillary meristems (Fig. 3.1). The patterns of bleaching may be due to the stage of leaf development during heat stress. The entirely white leaves initiated and developed entirely under stressful temperatures, while partially green leaves developed under some normal and some stressful conditions. This indicates foliar bleaching initiates during leaf initiation and early development. If damage occurs only in the apical and axillary meristems, decreased growth by drought treatments would explain the increased heat tolerance experienced by growers who slightly drought stress their crop to prevent foliar bleaching of the leaves.

Conclusion

Moisture did not affect the physiological response to heat stress in either cultivar. Any decrease in foliar bleaching due to drought stress was most likely due to a decrease in growth. Foliar bleaching was not due to inhibition of photosynthetic rate or

degradation of chlorophyll content. Temperature did not affect photosynthetic rate or leaf greenness in mature leaves of either cultivar. It is more evident foliar bleaching is due to compromised chloroplast development during leaf primordia initiation and early leaf development.

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Table 3.1 Effects of moisture, temperature, day and cultivar on leaf greenness, photosynthetic rate (Pn), height, width and growth index (GI cm^3) in ivy geraniums (*Pelargonium peltatum*) grown under two temperature (15/20° C or 25/30° C n/d) and two moisture (volumetric water content (VWC) at 30 and 80%) treatments. SPAD and Pn were measured in mature leaves on days 0, 7 and 11 of the study where heat treatments were stopped after 7 days.

		Leaf Greenness (SPAD)	Pn ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Height (cm)	Width (cm)	GI (cm^3)[z]
VWC (%)	30	-[y]	-	16.26 a[y]	-[x]	-
	80	-	-	16.54 a	-	-
Temperature (°C)	15/20	52.29 a[y]	-	16.19 a	32.46 a	7,253 a
	25/30	52.23 a	-	16.61 a	31.45 a	7,273 a
Day	0	-	-	16.23 a	-	-
	7	-	-	NA[w]	NA	NA
	11	-	-	16.57 a	-	-
Cultivar	Contessa™ Red	56.23 a	0.325 a	17.79 a	37.53 a	10,218 a
	Temprano™ Lavender	48.29 b	0.435 a	15.01 b	26.38 b	4,273 b

[z] Growth Index (GI) = $\frac{1}{3}\pi \times r^2 \times h$.
[y] Means separated within columns by treatment
[x] Indicates an interaction between treatments
[w] NA indicates data was not collected for height, width and GI on day 7

Table 3.2 Effects of temperature, day, cultivar and moisture (30 and 80% volumetric water content (VWC)) on leaf greenness (SPAD) in ivy geraniums (*Pelargonium peltatum*) grown under two temperature (15/20° C or 25/30° C n/d) and two moisture (VWC at 30 and 80%) treatments. Leaf greenness (SPAD) was measured in mature leaves of ivy geranium on day 0 before treatments began, day 7 while the plants were undergoing their perspective treatments and 3 days following their return to normal conditions on day 11.

	Temperature (C°, n/d)		Day			Cultivar	
VWC (%)	15/20	25/30	0	7	11	Contessa™ Red	Temprano™ Lavender
30	50 a	51 a	50 a	50 a	52 a	54 a	47 b
80	55 a[z]	53 a	-[y]	-	-	-	-

[z] Means separated within rows by treatment using Least Square Means test (P= 0.05).
[y] Indicates an interaction between treatments and further statistical analysis.

Table 3.3 Effects of cultivar and day at 80% volumetric moisture content (VWC) on leaf greenness (SPAD) in ivy geranium (*Pelargonium peltatum*) grown under two temperature (15/20° C or 25/30° C n/d) and two moisture (30 or 80% VWC) treatments. Leaf greenness was measured in mature leaves on days 0, 7 and 11 of the study.

	Day		
Cultivar	0	7	11
Contessa™ Red	51 b	63 a	60 a
Temprano™ Lavender	48 b[z]	48 b	54 a

[z]Means separated within rows using Least Square Means test, P = 0.05.

Table 3.4 Effects of temperature and moisture on photosynthetic rate ($\mu mol \cdot m^{-2} \cdot s^{-1}$) in ivy geraniums (*Pelargonium peltatum*) grown under two temperature (15/20° C or 25/30° C n/d) and two volumetric water content (VWC) (30 or 80%) treatments. Photosynthetic rate measurements were taken from the third most mature leaf on days 0, 7 and 11 of the study using a Ciras Photosynthesis Machine.

	Temperature (°C)	
VWC (%)	15/20	25/30
30	-1.22 a	-1.67 a
80	3.54 a[z]	0.86 a

[z] Means separated within rows by temperature using Least Square Means test, $P = 0.05$.

Table 3.5 Effects of day and moisture (30 and 80% volumetric water content (VWC)) on Photosynthetic rate ($\mu mol \cdot m^{-2} \cdot s^{-1}$) in ivy geraniums (*Pelargonium peltatum*) grown under two volumetric water content (VWC) (30 and 80%) treatments. Photosynthetic rate measurements were measured on the third most mature leaf on days 0, 7 and 11 of the study using a Ciras Photosynthesis Machine.

	VWC (%)	
Day	30	80
0	-1.18 a	0.381 a
7	-1.20 a	3.43 b
11	-1.95 a[z]	2.80 b

[z] Means separated within column by day using Least Square Means test, $P = 0.05$.

Table 3.6 Effects of volumetric water content (VWC) by day for width and growth index (GI) in ivy geraniums (*Pelargonium peltatum*) grown under two VWC (30 or 80%) treatments. Width was measured across the widest portion of the plant and again at 90 degrees from the original measurement to obtain an average width for each plant. Growth index was measured by calculating the volume of a cone using the average width and height of each plant on days 0 and 11 of the study.

	Width (cm)		Growth Index[y] (cm^3)	
	VWC (%)		VWC (%)	
Day	30	80	30	80
0	30.83 a	31.85 b	6,673 a	7,142 b
11	30.63 a[z]	34.5 a	6,401 a	8,765 a

[z] Means separated within column by day using Least Square Means test, $P = 0.05$.
[y] Growth index = $\frac{1}{3}\pi \times r^2 \times h$

Figure 3.1 Foliar bleaching in *Pelargonium peltatum* resulting from heat stress temperatures exceeding 30° C during leaf development.

CHAPTER IV

DEVELOPMENT OF FOLIAR BLEACHING IN IVY GERANIUM

Foliar bleaching caused by heat stress is devastating to the aesthetic value and shelf life of ivy geranium (*Pelargonium peltatum*). Previous studies have indicated foliar bleaching in this ornamental crop is not due to photosynthesis inhibition or degradation of the photosynthetic apparatus, but instead may be caused by a lack of proper photosynthetic organelle development. Temprano™ Lavender ivy geranium, a heat intolerant cultivar, was grown in growth chambers and acclimated to 15/20° C night/day until plants showed no evidence of foliar bleaching. Plants then underwent two temperature treatments; 15/20° C or 30/35° C night/day. Growth measurements and a visual rating of foliar bleaching from zero to five were taken daily for seven days for each plant. Under the 30/35° C treatment, plants showed some bleaching after three days and by day seven the newly emerging leaves were almost entirely bleached. Bleaching occurred only in the newly emerging leaves from the meristem. The percentage of bleaching on each leaf remained the same as the leaf developed. There was no foliar bleaching in the control treatment. Foliar bleaching is a result of incomplete photosynthetic organelle development in developing leaves under heat stress which begins to occur as early as 3 days after exposure to heat stress.

Introduction

Ivy geranium (*Pelargonium peltatum*) has a cascading growth habit with peltate leaves and delicate flowers. Production of this species is a challenge in warm climates due to its low tolerance of temperatures exceeding 30° C. The genus *Pelargonium* is significant to the floriculture industry and contains over 270 different species divided into sixteen sections. Commercially important species are the product of interspecific crosses, where *P. peltatum* is believed to be the result of a cross between *P. zonale* and *P.* x*hortorum*. These species are in the taxonomic section *Ciconium* of *Pelargonium* and are native to the Cape of South Africa (James, et al, 2004).

Temprano™ Lavender ivy geranium is highly susceptible to heat stress resulting in devastating foliar bleaching (Dhir 2008; Dhir, et al., 2013). This bleaching eventually leads to an unsalable crop or plant death. Heat stress is identified as a rise in temperature beyond the tolerated threshold for a period of time long enough to cause irreversible damage to plant growth and development (Wahid, et al., 2007). Temprano™ Lavender and other susceptible ivy geranium cultivars experience heat stress above 30° C (Dhir et al. 2013). Foliar bleaching due to heat stress may be caused by an inhibition of photosynthesis, degradation of the photosynthetic apparatus or improper photosynthetic organelle development. Previous studies have excluded photosynthesis inhibition as the cause of foliar bleaching and have indicated a loss of green pigment does not occur in developed, mature leaves during a heat stress event (Horton, Anna M.S. Thesis chapter III). However, observations in those studies have led to the belief improper development of the photosynthetic apparatus during leaf initiation and early development may be the

cause of foliar bleaching in heat stressed ivy geranium. Heat stress can begin a multitude of problems such as the production of reactive oxygen species (ROS) (Wahid et al., 2007). ROS can be beneficial at the initiation of the stress by signaling for protection mechanisms, but as the stress continues increases in ROS can be detrimental to plant life. ROS signaling is a delicate balance between production and scavenging (Bailey-Serres, 2006). One ROS produced during heat stress is the singlet oxygen, $^1O_2$ (Jajic, 2015; Tripathy, 2012). The singlet oxygen damages DNA and could cause mutations interfering with the development of the photosynthetic plant organs. Organelle development begins in the meristems of plants. All cell organs necessary for photosynthesis are developed before the leaf emerges from the meristem (Chiang, et al., 2012). After reaching a certain point in leaf development, all meristematic activity ceases and any additional growth is the result of cell enlargement. The objective of this study was to identify the timing of initiation of foliar bleaching in relation to a heat event in a heat sensitive cultivar of ivy geranium.

Materials and Methods

Optimum growing conditions for ivy geranium require temperatures between 20° and 23° C (Murray, et al., 2012). For this study, a heat susceptible cultivar, Temprano™ Lavender (formerly named 'Butterfly'; Syngenta Flowers, Inc., Boulder, CO), was selected (Dhir, 2008). Sixteen rooted cuttings of Temprano™ Lavender were potted into 1L (15.24 cm diam.) pots in a conventional peat-based substrate, ~ 70% peat: 30% perlite v/v (Sunshine Mix 1, SunGro Horticulture, Agawam, MA). Plants were fertilized at each irrigation with 200 mg $N \cdot L^{-1}$ from 20N-4.4P-16.6K (Peter's Peat Lite 20-10-20 (The

Scott's Company, Marysville, OH). Before beginning the study, plants were grown in growth chambers (Conviron CMP3000, Controlled environments Ltd., Winnipeg, Manitoba, Canada) and acclimated to 15/20° C night/day until each plant showed no evidence of foliar bleaching. On July 24, 2013 (day 0), 8 biological replicates of Temprano™ Lavender underwent temperature treatments of 15/20° C or 30/35° C night/day using a split plot arrangement of treatments.

The temperature treatments continued for seven days and the data collected included growth and a visual rating of foliar bleaching. Growth was measured by taking the initial and final height and width of each plant in the study. A growth index (GI) was calculated using the geometric formula for a cone, $\frac{1}{3}\pi \times r^2 \times h$, since the plant shape is roughly conical. Height was measured from the top of the soil surface to the tallest point of active growth. Radius was calculated by averaging the width at the widest point and at 90° from the original width measurement and dividing by 2. Foliar bleaching visual rating was based on a scale of 0 to 5 where 0 represented no evidence of foliar bleaching on any leaves and 5 indicated the newest unfolded leaf was entirely white (Table 4.1). Data were analyzed using the MIXED and REG procedures of SAS (version SAS 9.4; SAS Institute, Cary, NC).

Results

Temperature had no effect on the final height, average width or growth index of Temprano™ Lavender (Table 4.2). Changes in height, average width, and growth index occurred across days (Table 4.3). Foliar bleaching increased by day for plants growing at

30/35° C. Plants grown at the 15/20° C temperature treatment had no foliar bleaching (Fig. 4.1).

Height for both temperature treatments did not change from the pretreatment measurement taken on day 0 until day 3. While day 2 did not differ from day 0, there was an increase in height from day 1 to day 3. Height did not increase from day 4 through day 7 of the treatments (Table 4.3).

No changes in average width occurred between the first two days of the study. An increase in average width occurred between day 1 and day 2 and average width did not further increase through the end of the study, day 7 (Table 4.3)

Growth index did not increase between day 0 and day 1. An increase in GI occurred between day 1 and day 2. However, there was no further increase in GI between day 2 and day 7 (Table 4.3).

Analysis of foliar bleaching ratings by temperature treatment shows a change in rating for plants undergoing the 30/35° C temperature treatments across days. There was no foliar bleaching across days for plants grown at 15/20° C (Table 4.4). There was an increase in foliar bleaching under the 30/35° C treatment beginning as soon as 3 days after start of the heat treatment. As heat treatment continued, foliar bleaching reached 77 to 99% (rating 4.5) after 7 days (Table 4.4).

Discussion

Foliar bleaching was first observed in the newest unfolding leaf after 3 days of 30/35° C temperatures. At this stage only a very small portion of the leaf was white and

plants had an average rating of 0.4 (Table 4.4) on the foliar bleaching scale. As the study progressed, foliar bleaching increased, but only in the newly emerging leaves. At day seven the newest unfolding leaves had an average foliar bleaching rating of 4.5 (Table 4.4). It is important to note that once the leaf emerged from the apical meristem and began to expand, the percent of foliar bleaching did not increase. Foliar bleaching occurred during leaf primordia initiation and early development, likely due to a lack of proper chloroplast development in the leaf primordia as it developed from the apical meristem. It is likely this damage occurred before meristematic activity ceased and cells began to enlarge.

Within the meristems of plants, proplastids exist which are the precursors for chloroplasts as well as amyloplasts, leucoplasts, chromoplasts and etioplasts (Chiang et al., 2012). Amyloplasts are important for starch storage in roots, leucoplasts store lipids and chromoplasts accumulate plant pigment. Etioplasts in shoots, however, develop in response to a lack of light. Etioplasts do have some photosynthetic organelles such as thylakoids and grana, but they lack chlorophyll, resulting in white stems and leaves. In comparison, chloroplasts develop in shoots under light conditions and produce functional photosynthetic organelles and green leaves (Armstrong et al., 1995).

The biosynthesis of chloroplasts begins with the reduction of photochlorophyllide to chlorophyllide (Armstrong, et al., 1995). This first step in chloroplast biosynthesis is light dependent and requires illumination for photochlorophyllide to be reduced. An enzyme called protochlorophyllide oxidoreductase (POR) regulates this reduction. The two genes coding for the POR enzyme are identified as *PORa* and *PORb* and are

members of the phytochrome gene family (Armstrong, et al., 1995). *PORa* and *PORb* mutant seedlings etiolated in darkness show bleaching when exposed to light (Tripathy and Oelmuller, 2012). Because foliar bleaching can occur in mature plants of ivy geranium, *PORb* would be the most likely gene to investigate because it has been identified in both seedlings and adult plants. *PORa*, however, is only present in young seedlings. *PORb* is very important in the greening of stems and leaves (Armstrong, et al., 1995) and should be studied in ivy geranium to gain a greater understanding of its role under heat stress.

Height, average width and growth index increased over the course of the study, but these increases did not appear to follow the same pattern as the increase in foliar bleaching. This was most likely due the short amount of time growth was monitored in the study, 7 days.

Conclusion

Previous studies have shown foliar bleaching in ivy geranium was not due to photosynthesis inhibition or degradation of the photosynthetic apparatus (Horton, Anna, M.S. Thesis chapter III). This study was intended to determine the stage of development at which bleaching occurred and to provide a time frame for when bleaching occurs. The data revealed bleaching is visible in small amounts in newly emerging leaves three days after initiating heat stress. Bleaching was only observed in newly emerged leaves and, as those leaves expanded, the percent bleaching did not increase. Mature leaves did not show any sign of foliar bleaching under heat stress. The heat stress damage occurs during the meristematic activity of leaf initiation and development. As new leaves initiated and

emerged under heat stress conditions, they became increasingly bleached until day seven when most emerging leaves of Temprano™ Lavender ivy geranium were completely bleached.

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Table 4.1 Foliar bleaching visual rating scale for ivy geraniums (*Pelargonium peltatum*) grown under two temperatures (15/20 and 30/35 °C n/d) to determine the level of foliar bleaching in heat stressed plants.

Rating	Description
0	No evidence of foliar bleaching on any leaves
1	< 25% of the newest unfolding leaf is white
2	25-49% of the newest unfolding leaf is white
3	50-74% of the newest unfolding leaf is white
4	75-99% of the newest unfolding leaf is white.
5	100% of the newest unfolding leaf is white

Table 4.2 Effects of temperature (C°) on height (cm), average width (cm), growth index (cm^3) and foliar bleaching rating in Temprano™ Lavender ivy geraniums (*Pelargonium peltatum*) grown under two temperature (15/20° C or 30/35° C n/d) treatments.

Temperature	Height (cm)	Average Width (cm)	Growth Index (cm^3)	Foliar Bleaching (visual rating)[z]
15/20	11.39 a[y]	17.23 a	17.23 a	0.08 a
30/35	11.77 a	16.58 a	16.58 a	1.95 b

[z] Foliar bleaching was rated on a scale of 0 to 5 where 0 = 0%; 1 = <25%; 2 = 25-49%; 3 = 50-74%; 4 = 75-99%; 5 = 100% bleached.

[y] Means separated within columns using Least Square Means test, P= 0.05.

Table 4.3 Changes in height (cm), width (cm) and Growth Index (cm^3) across days in Temprano™ Lavender ivy geranium (*Pelargonium peltatum*) grown under two temperature (15/20° C or 30/35° C n/d) treatments from July 24 to July 31 (day 0 to 7).

	Day							
	0	1	2	3	4	5	6	7
Avg. Height	10.45 ab[z]	9.44 a	11.69 ac	12.50 c	11.75 ac	11.94 ac	12.50 c	12.31 c
Avg. Width	15.88 ab	14.53 a	17.22 c	17.59 c	17.81 c	17.44 c	17.22 c	17.56 c
GI	2,812 ab	2,101 a	3,749 bc	4,150 c	3,956 c	3,858 c	4,029 c	4,086 c

[z] Means separation within rows using Least Square Means test, P= 0.05.

Table 4.4 Foliar bleaching of the newest unfolding leaves of Temprano™ Lavender ivy geranium (*Pelargonium peltatum*) was visually rated on a scale of 0 to 5 where 0 = 0%, 1 = <25%, 2 = 25-49%, 3 = 50-74%, 4 = 75-99% and 5 = 100% bleached. Foliar bleaching was measured daily from July 24 to July 31 (day 0 to 7) under 15/20 or 30/35° C (night/day) temperature treatments.

Temperature	Day							
(° C)	0	1	2	3	4	5	6	7
15/20	0.0 a[z]	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
30/35	0.0 a	0.0 a	0.4 ab	1.5 bc	2.6 c	3.1 c	3.5 cd	4.5 d

[z] Means separated within rows using Least Square Means test, P =0.05.

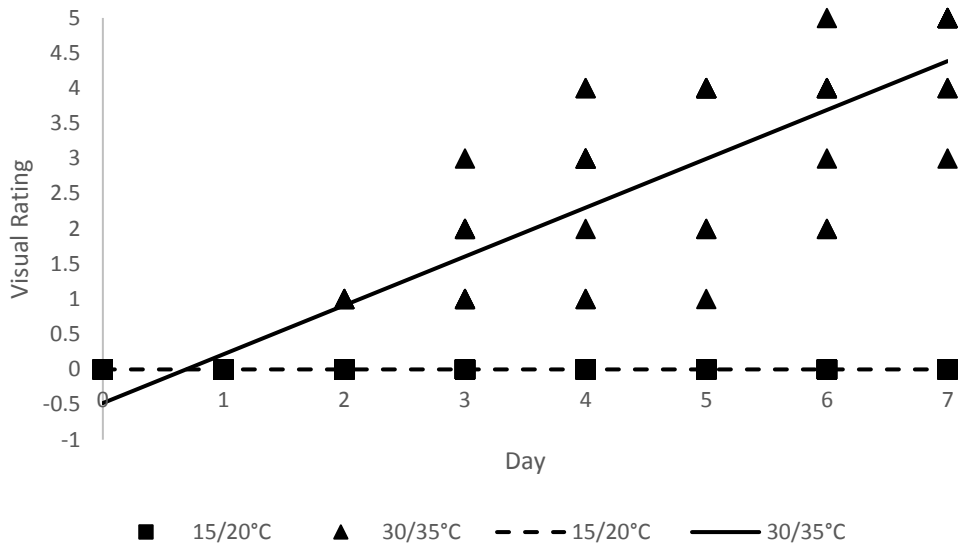

Figure 4.1 Regression analysis of foliar bleaching rating and day. Foliar bleaching of the newest unfolding leaves of Temprano™ Lavender ivy geranium (*Pelargonium peltatum*) was visually rated on a scale of 0 to 5 where 0 = 0%, 1 = <25%, 2 = 25-49%, 3 = 50-74%, 4 = 75-99% and 5 = 100% bleached. Foliar bleaching was measured daily from July 24 to July 31 (day 0 to 7) under 15/20 or 30/35° C (night/day) temperature treatments. 15/20° C ($y = 0x$; $R^2 = 1.0$) or 30/35° C ($y = 0.4877x - 1.17$; $R^2 = 0.78$).

CHAPTER V

CHLOROPLAST DEVELOPMENT OF IVY GERANIUM UNDER HEAT STRESS

Introduction

Foliar bleaching caused by heat stress is devastating to the aesthetic value and shelf life of ivy geranium (*Pelargonium peltatum*). Previous studies have indicated foliar bleaching in this ornamental crop is not due to photosynthesis inhibition or degradation of the photosynthetic apparatus, but instead may be caused by a lack of proper photosynthetic organelle development. Heat intolerant Temprano™ Lavender ivy geranium plants were exposed to temperatures 30 to 35° C in a controlled greenhouse setting. As a result of heat stress, Temprano™ Lavender fails to produce new leaves with functioning chloroplasts, resulting in white leaves and stems. Leaf tissue samples were collected of these white leaves and prepared for transmission electron microscopy (TEM) imaging to determine extent of chloroplast organelle development.

Materials and Methods

Mature plants of Temprano™ Lavender ivy geranium were grown in a glass greenhouse during summer months and watered daily. During this time, day temperatures in the greenhouse ranged from 30 to 35° C, resulting in bleached, white leaves. Samples were collected of mature green and mature white leaves and prepared for TEM imaging. The leaf samples were fixed in Karnovsky's Fixative (Schneider, 2014) and rinsed with a series of buffer washes (Schneider, 2014). Samples underwent six washes with a 0.1M phosphate buffer, 10 minutes each. Following the last phosphate buffer wash, samples were submerged in a 2% OsO_4 and 0.1M phosphate buffer solution on ice for 1.5 hours. Samples were then removed from ice and left at room temperature for 30 minutes before washing with distilled water 6 times, allowing them to soak for 10 minutes each wash. Next, the samples were soaked in a series of washes of 35%, 50%, 70% and 95% ETOH. After 15 minutes of soaking in each solution, samples were removed and placed in fresh solution for another 15 minutes per concentration. The last wash in 100% ETOH solution required 4 solution changes at 15 minutes each. Following the last ETOH wash, samples were moved into a 50:50 acetone and ETOH solution for 15 minutes. Samples were removed and placed into a fresh solution of acetone and ETOH for another 15 minutes before being submerged in 100% acetone, 2 times for 15 minutes each. Leaf samples were then placed in a 50:50 solution of acetone and Spurr's Resin for three to four hours on a rotator shaker. At the completion of this step, leaf samples were moved to a 25% Acetone and 75% Spurr's resin mixture and left overnight on the rotator. Samples were then transferred to a 100% Spurr's resin for another 24 hours on a rotator changing the

solution twice throughout the day. Samples were then moved to fresh resin for an additional six hours. Finally resin and leaf samples were placed in embedding containers and moved to an oven at 68° C overnight to harden. After resin embedded samples were removed from the oven, samples were thin sectioned and mounted to a grid for TEM imaging. Tissue sample were then examined using a transmission electron microscope (TEM) (JEM 1230, JEOL 120kV; JEOL USA, Inc., Peabody, MA). Micrographs were taken while examining the specimens and were then visually analyzed.

Results

TEM images of Temprano™ Lavender leaves indicated the chloroplasts of white leaves were not fully developed (Fig. 5.1A), while green leaves of the same plant had fully developed, normal chloroplasts (Fig. 5.1B). In abnormal chloroplasts, thylakoids were disjointed with very few grana stacks. It is also important to note an abnormally large starch granule was discovered in the chloroplasts of white leaves (Fig. 5.1A). Chloroplasts from green, normal leaves have fully formed thylakoids and grana with very small starch granules.

Discussion

TEM images of heat stressed Temprano™ Lavender ivy geranium reveal the chloroplasts of bleached leaves were underdeveloped, while mature green leaves of the same plant did not have any damage to their chloroplasts. Mature green and mature white leaves existed on the same plant (Fig. 5.2). Green leaves did not bleach white under the heat stress conditions. This indicates foliar bleaching and compromised chloroplast

function may be due to a lack of proper chloroplast initiation and development during leaf development under heat stress. This theory is supported by other findings in another study (Dhir et al., 2013; Horton, Anna M.S. Thesis Chapter III), where a pattern of white leaves was noted from the apical meristem of Temprano™ Lavender ivy geraniums after plants experienced fluctuating temperatures while entering and leaving heat treatments of 30° C.

Chloroplast initiation and organelle development begins in the meristems of plants. All cell organs necessary for photosynthesis are developed before the leaf or stem emerges from the meristem. After reaching a certain point in leaf development, all meristematic activity ceases and any additional growth is the result of cell enlargement. Within the meristems of plants, proplastids exist which are the precursors for chloroplasts as well as amyloplasts, leucoplasts, chromoplasts and etioplasts. Amyloplasts are important for starch storage in roots, leucoplasts store lipids and chromoplasts accumulate plant pigment (Chiang et al., 2012).

The starch in Figure 5.1A may be an indication proplastids in heat stressed meristems are differentiating to another structure besides a fully functioning chloroplast. Starches are normal in chloroplasts, but they tend to be small and only a secondary function when sugars are in abundance. Hummel, et al. (2010) provides evidence large accumulations of starch in chloroplasts could be caused by a blockage or disassembly of the Golgi apparatus. Understanding the alternate structure or dysfunctional chloroplast the proplastid of ivy geranium differentiates into under heat stress will be vital in identifying the physiological process being interrupted.

Etioplasts are the precursors to chloroplasts and will make plant leaves and stems white in the absence of light. The biosynthesis of etioplasts to chloroplasts is light dependent. The enzyme responsible for this transition is protochlorophyllide oxidoreductase (POR) (Plosher, et. al., 2011). The gene responsible for the POR enzyme in mature plants is *PORb* (Armstrong, et al., 1995). Additional research may need to be conducted to determine the presence of *PORb* during heat stress in ivy geranium. If *PORb* is compromised due to heat stress, the plant would lose the ability to properly synthesize an etioplast to a functioning chloroplast. Thus, making *PORb* an important part of further studies involving foliar bleaching in ivy geranium. The phytohormone cytokinin may also be an important aspect of future ivy geranium studies under heat stress. Cytokinins play an important role in the differentiation of proplastids into chloroplasts and exogenously applied cytokinins have induced regreening in etiolated seedlings (Chiang, et al., 2012).

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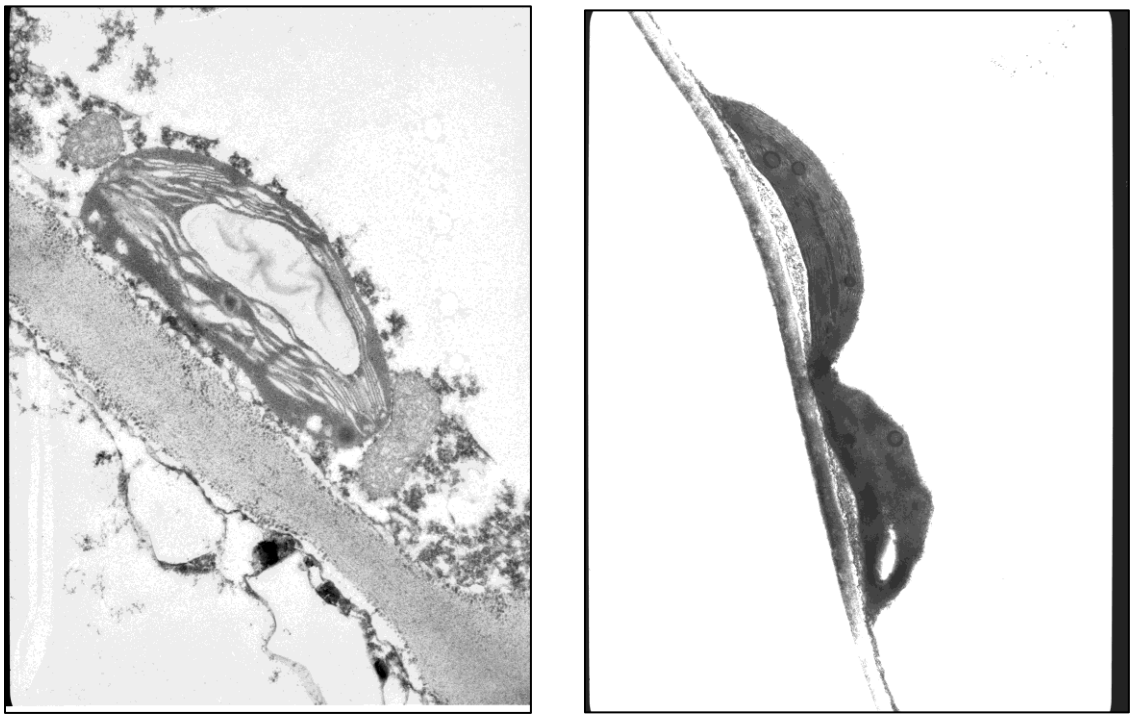

A B

Figure 5.1 Transmission electron micrograph of (A) an abnormal chloroplast from a mature, white (bleached) Temprano™ Lavender ivy geranium leaf grown under temperatures of 30° C and (B) a normal chloroplast taken from a green Temprano™ Lavender ivy geranium leaf grown at 20° C.

Figure 5.2 Mature green leaves and mature white leaves in *Pelargonium peltatum*. White leaves resulted from heat stress during leaf development with green leaves developing again upon return to non-stress temperatures.

CHAPTER VI

CONCLUSIONS

Ivy geranium, *Pelargonium peltatum*, has a cascading growth habit with peltate leaves and delicate flowers. Production of this species is a challenge in warm climates due to its low tolerance of temperatures exceeding 30° C. An understanding of the heat tolerance mechanism in ivy geranium would assist plant breeders and increase market penetration.

A heat tolerant, Contessa™ Red, and a heat sensitive cultivar, Temprano™ Lavender, of ivy geranium were used to determine whether drought stress had a physiological effect promoting heat tolerance and to determine if a heat stress event inhibited photosynthesis and causes foliar bleaching. Drought stress did not affect either the heat tolerant or sensitive cultivar's physiological response to heat stress. Any decrease in foliar bleaching of ivy geranium due to drought stress was most likely due to a decrease in growth. Heat stress did not affect photosynthetic rate or leaf greenness in expanded leaves of either cultivar. The stage of leaf development when foliar bleaching occurs under heat stress was found to occur in newly emerging leaves. Partial leaf bleaching was evident after three days of heat stress. As these leaves expanded, the percent bleaching did not increase. Mature leaves did not exhibit foliar bleaching. Heat stress damage most likely occurs during early leaf initiation and development in the

meristem. Leaves were increasingly bleached as new leaves emerged under heat stress conditions. After seven days of heat stress, most emerging leaves of were completely bleached. Evidence was found to support foliar bleaching is due to compromised chloroplast development during leaf primordia initiation and early leaf development.