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INFLUENCE OF THE POSTHARVEST ENVIRONMENT ON THE STORAGE POTENTIAL AND PROPAGATION PERFORMANCE OF UNROOTED CUTTINGS OF HERBACEOUS ORNAMENTALS

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INFLUENCE OF THE POSTHARVEST ENVIRONMENT ON THE STORAGE POTENTIAL AND PROPAGATION PERFORMANCE OF UNROOTED CUTTINGS OF HERBACEOUS ORNAMENTALS

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ABSTRACT

Plants propagated from vegetative cuttings have become an increasingly important market in the United States. Significant economic losses occur annually due to poor performance, damage or death of cuttings. This occurs in large part due to the industry's lack of knowledge of the proper postharvest environments to provide to cuttings. This project was conducted to improve the understanding of the postharvest physiology of unrooted cuttings in order to optimize performance and longevity. Experiments were conducted to quantify the effect of temperature on respiration and ethylene production rates of unrooted cuttings. Additionally, the effect of the stock plant environment on the initial carbohydrate concentration of cuttings and the subsequent postharvest performance was also explored. Respiration rates of vegetative cuttings were greatest 2 h after harvest and decreased rapidly during the first 24 h. As postharvest temperature increased, the initial respiration rate increased. Regardless of how long poinsettia cuttings were stored at 10 °C, when they were transferred to 20 °C respiration rates increased. A decline in rooting quality was noticeable 2 to 4 d prior to a decline in shoot quality. Storage potential of poinsettia cuttings was maximal at 10 °C. Ethylene production in vegetative cuttings was directly correlated to storage temperature. As storage temperature increased, ethylene production increased. Poinsettia cuttings harvested in the evening produced more ethylene than cuttings harvested in the morning. Stock plant growing environment also impacts cutting postharvest performance. Adventitious root formation in propagation is related to the carbohydrate status of the unrooted cuttings. Carbohydrate levels were 2.5-times greater in plants that were grown

in high-light than plants grown in low-light conditions. Carbohydrate status was not significantly correlated with ethylene production; however, rooting performance was positively correlated with carbohydrate status, as carbohydrate concentrations increased, rooting increased.

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INTRODUCTION

Plant material propagated from unrooted vegetative cuttings has become increasingly important to the United States floriculture industry. Stock plant production occurs primarily offshore in Central and South America. Consequently, the success of U.S. growers has become increasingly dependent on their ability to receive high quality cuttings. In general, if growers receive cuttings within 48 h after harvest and the shipping container temperatures have been relatively cool, cuttings quality and performance is acceptable. However, any deviation from the ideal shipping situation results in significant losses in terms of poor cutting performance, plant damage, or death. Losses occur annually due to problems in transit. Most of these losses occur due to the floriculture's industry's lack of knowledge concerning the proper postharvest environment to provide to vegetative cuttings.

An enormous amount of research has been conducted on the postharvest physiology of horticultural commodities such as fruits, vegetables, and fresh herbs; however, very little research has been done on vegetative cuttings. Vegetative cuttings consist of leaves, stems, and sometimes flowers. They are most comparable to fresh herbs and leafy green vegetables. However, unlike herbs and leafy vegetables, cuttings are expected to continue to grow following the postharvest environment.

The goal of this research was to understand the postharvest physiology of vegetative cuttings in order to reduce losses and improve cutting performance for U.S. growers.

CHAPTER ONE

STORAGE TEMPERATURE AFFECTS THE RESPIRATION RATE OF UNROOTED CUTTINGS OF HERBECEOUS ORNAMENTALS

Abstract

Adventitious root formation in propagation is related to the carbohydrate status of the unrooted cuttings, so it is important to maintain carbohydrate levels during the postharvest environment that occurs between cutting harvest from stock plants and propagation. The objective of this project was to determine the effect of postharvest temperatures on the respiration rates of unrooted shoot-tip cuttings of four herbaceous ornamental species. Unrooted poinsettia (*Euphorbia pulcherrima* ex Klotsch Willd. cv. Prestige Red), New Guinea impatiens (*Impatiens hawkeri* Bull cv. Sonic White), double impatiens (*Impatiens walleriana* Hook cv. Double-up Violet), and petunia (*Petunia ×hybrida* Vilm. cv. Improved Charlie) cuttings were harvested from stock plants and placed in glass jars inside incubators that maintained postharvest temperatures of 10, 15, 20, or 25 °C. The respiration rate of the cuttings was measured 2, 6, 10, 24, 48, and 72 h after harvest. The magnitude of the respiration rates varied amongst species; however, the trends over time and the responses to temperature were similar. Respiration rates were greatest at 2 h after harvest then decreased rapidly during the first 24 h in the postharvest environment. Steady state respiration rates occurred after 24 h. As temperature increased, the initial respiration rates increased, the percentage reduction in

respiration rates during the first 24 h increased, and the steady state rates were also higher. Q_{10} values were calculated at 5 °C intervals from 10 to 25 °C. The Q_{10} values at 10-15 °C ranged from 2.0 to 3.4 amongst species. The Q_{10} values decreased as temperature increased, so that the values ranged from 1.3 to 2.1 at 20-25 °C.

Introduction

The production of vegetative shoot-tip cuttings for the U.S. floriculture industry primarily occurs in Mexico and Central America. In 2006, 878 million unrooted cuttings with an average wholesale value of $0.07 each were imported into the U.S. (Jerardo, 2007). Due to the shift to increased importation of plant material, high-quality cuttings have become a significant prerequisite for economic success in the floriculture industry. While most horticultural crops are consumed following the postharvest environment, unrooted cuttings are planted on a propagation bench and then must resume growth. Adventitious root formation requires carbohydrates in the basal stem and the basipetal transport of sugars from the leaves (Rapaka et al., 2005). If carbohydrate status of the cutting is low, then the pools of carbohydrates in the leaves are refilled before these leaves can export sugars to the basal stem. Postharvest carbohydrate depletion can also promote premature leaf senescence (Druege et al., 2004). Therefore, low carbohydrate status in the cutting will delay and/or reduce rooting in propagation or even jeopardize the survival. Consequently, it is important to maintain the carbohydrates in the cutting

tissues during the postharvest period in order to provide high quality cuttings that perform well for propagators.

Respiration is the oxidative breakdown of starches and sugars via glycolysis, the tricarboxilic acid cycle, and an electron transport system to release energy while forming water and CO_2. Respiration plays an essential role in the growth and survival of plants, e.g., it is the driving force for biosynthesis, cellular maintenance, and active transport in plants (Atkin and Tjoelker, 2003; Atkin et al., 2005). The postharvest respiration rate of perishable commodities, including fruits, vegetables, herbs, and cut flowers, is a major factor affecting product quality and storage potential. Postharvest respiration rates are affected by cultivar, plant age, growing conditions, and handling conditions (Kader, 1987; Peiris et al., 1997). Degree of perishability is directly related to respiration rate with a low rate of respiration often associated with a long storage life (Deshpande et al., 2002; Kader, 1987; Nei et al., 2006).

Temperature is the primary factor that impacts the postharvest storage potential of perishable commodities (Kader, 1987). Increased storage temperature results in increased respiration rates as demonstrated for many plants including arugula (Peiris et al., 1997), *Grevillea* cut flowers (Joyce et al., 2000), marjoram (Böttcher et al., 1999), matricaria flowers (Böttcher et al., 2001), and Saint-John's wort (Böttcher et al., 2003). The influence of temperature on respiration rate can be quantified using the Q_{10} value which is a unit-less quantity that expresses the effect of temperature on respiration rates. The Q_{10}

values range from 1.0 to 4.0 for the postharvest respiration rates of various horticultural crops (Fonseca et al., 2002a; Kadner, 1987).

Boxes of unrooted cuttings are generally held in coolers set at 10 °C to accommodate both chilling-sensitive and non-sensitive species prior to being shipped via air freight (Faust and Lewis, 2005). The shipping duration for boxes of unrooted cuttings typically requires 2 to 3 d to reach their destination in North America. Shipping boxes often contain foam insulation and frozen gel packs, however much of the shipping environment lacks precise temperature control which results in cuttings being exposed to non-optimal temperatures during transit.

Postharvest respiration rates have been determined for many fruits, vegetables, and herbs; however, limited data is available for unrooted cuttings of herbaceous ornamental plants. Therefore, the objective of this research was to determine the effect of constant postharvest temperatures on the respiration rates of unrooted shoot-tip cuttings of four vegetatively-propagated herbaceous ornamentals.

Materials and Methods

Cutting production. Poinsettia ‘Prestige Red’, New Guinea impatiens ‘Sonic White’, double impatiens ‘Double-up Violet’, and petunia ‘Improved Charlie’ rooted cuttings were transplanted into 3.8-L plastic pots containing a commercial peat-based growing medium (Middleweight Mix # 3-B, Fafard Inc., Anderson, S.C., U.S.A.) and grown as stock plants in the greenhouse (Clemson University, Clemson, S.C., U.S.A.).

The plants were fertilized at each irrigation with water-soluble fertilizer providing ($mg \cdot L^{-1}$) 250 N, 37 P, 208 K, 83 Ca, 33 Mg, plus micronutrients (Peters Excel; Scotts-Sierra Horticultural Products Company, Marysville, Ohio, U.S.A.). Heating/ventilation set points for temperature control were 18.3/22.2 °C, providing an average air temperature of 20 °C. A retractable 50% PPF-reduction shade cloth shaded the stock plants when the irradiance outside the greenhouse exceeded 1035 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Postharvest treatments. Thirty-six shoot-tip cuttings (6-cm stem length) of poinsettia and sixty shoot-tip cuttings (2.5-cm stem length) of New Guinea impatiens, double impatiens, and petunia were harvested from stock plants between 0830 HR and 0900 HR, weighed, and wrapped in wetted brown multifold hand towels (24.1 cm × 23.4 cm) to prevent desiccation. Three cuttings of poinsettia were placed in each 1.0-L glass container (Ball wide-mouth jar; Jarden Home Brands, Daleville, Ind., U.S.A.). Five cuttings of double impatiens, New Guinea impatiens, or petunia were placed in each 0.5-L glass container (Ball wide-mouth jar; Jarden Home Brands, Daleville, Ind., U.S.A.). Three glass containers per species plus one empty glass container were placed inside incubators set at 10, 15, 20, or 25 °C. The experiment was repeated three times per species in May and June 2004 (petunia), Dec. 2004 (New Guinea impatiens), Oct. 2005 (poinsettia), and Nov. 2005 (double impatiens).

Carbon dioxide measurements. The glass containers were left unsealed (lids off) and exposed to ambient air conditions while in the incubators in order to prevent cuttings from being exposed to modified O_2 or CO_2 atmospheres. Thirty minutes prior to each

headspace gas sampling the containers were sealed with lids fitted with rubber septums. After the containers had been sealed for thirty minutes, two headspace gas samples (1 mL) were removed from each jar through the rubber septums using tuberculin syringes. Sampling was performed at 2, 6, 10, 24, and 48 h after cutting harvest for all four species, while an additional measurement was recorded at 72 h for poinsettia and double impatiens. Samples were analyzed for CO_2 using a gas chromatograph with a thermal conductivity detector (Shimadzu GC-8A, Kyoto, Japan).

Respiration rates and Q_{10}. The difference in CO_2 levels in the containers with cuttings versus the control (empty) container was used along with the container volume, plant mass, and length of time the containers were sealed to determine the respiration rate (CO_2 evolution; $mg \cdot g^{-1} \cdot h^{-1}$) of each species at each temperature at each sample time. The respiration rates recorded 2 h after harvest were also used to determine Q_{10} values with the following equation:

$$Q_{10} = \left(\frac{R_2}{R_1} \right)^{\frac{10}{(T_2 - T_1)}} \qquad \text{[Eq. 1]}$$

where R_2 is the respiration rate at temperature T_2 (°C), and R_1 is the respiration rate at temperature T_1 (°C) (Platenius, 1942). Temperatures were compared at 5 °C intervals. Steady state respiration rates were calculated for the respiration data occurring after 24 h.

Statistical analysis. Data were analyzed with the analysis of variance (ANOVA) and general linear model (GLM) procedures in SAS v. 9.1 (SAS Institute, Inc., Cary, N.C., U.S.A.). Main effects were compared ($P \leq 0.05$) using Fisher's least significant

difference (LSD) test. Means of the time-temperature interactions were compared ($P \leq 0.05$) using the least-square means (LSMEANS) test. Changes in respiration rate with time were estimated for each temperature by non-linear regression analysis (SigmaPlot 8.0; Systat Software, Inc., San Jose, Calif., U.S.A).

Results and Discussion

Respiration rates. The respiration data obtained for each species were described by an exponential decay relationship between respiration rate and time after harvest (Fig. 1.1) using the following equation:

$$R = a_0 + a_1 e^{-a_2 t} \quad \text{[Eq. 2]}$$

where R = respiration rate ($mg \cdot g^{-1} \cdot h^{-1}$); t = time after harvest (hours); and a_0, a_1, and a_2 are expressed as a function of temperature with the following exponential equation:

$$a_0, a_1, \text{ or } a_2 = b_0 e^{b_1 T} \quad \text{[Eq. 3]}$$

where T = postharvest temperature (°C) while b_0 and b_1 are constants. Combining Eqns. 2 and 3 yields the respiration rate models for each species (Table 1.1). For all species, when the respiration rates were subjected to a factorial ANOVA, postharvest temperature, time after harvest, and their interaction were all significant at $P \leq 0.001$.

The respiration rates declined between 2 and 24 h after harvest and the percent reduction was related to temperature (Fig. 1.1). For example, the respiration rate of poinsettia cuttings decreased 12 % during the first 24 hours at 10 °C, while over the same

time period there were 25, 42, and 46 % reductions in respiration at 15, 20, and 25 °C, respectively. New Guinea impatiens cuttings had the same general trend: 30, 42, 54, and 65 % reduction in respiration at 10, 15, 20, and 25 °C, respectively; while double impatiens cuttings experienced a 25, 47, 62, and 57 % reduction in respiration rate during the first 24 h at 10, 15, 20, and 25 °C, respectively. Petunia cuttings had the highest percentage reduction across all temperatures: 59, 66, 69, and 72 % at 10, 15, 20, and 25 °C, respectively. After cuttings had been in the postharvest environment for 24 h, regardless of species and temperature, there were no further significant decreases in respiration rates.

The steady state respiration rates, measured from 24 to 72 h after harvest, were relatively similar across species (Fig. 1.1). For example, at 10 °C the steady state respiration rates for all four species ranged from 0.10 to 0.15 $mg \cdot g^{-1} \cdot h^{-1}$, while at 20 °C the range was 0.19 to 0.21 $mg \cdot g^{-1} \cdot h^{-1}$. Poinsettia, New Guinea impatiens and petunia cuttings displayed similar percentage increases in steady state respiration rates with increasing temperatures. For example, as temperature increased from 10 to 15 °C, the steady state respiration rate increased 21-24%. The steady state respiration rate increased 50-54 % as temperature increased from 10 to 20 °C. Finally, as temperature increased from 10 to 25 °C, the steady state respiration rate increased 91-129 %. Double impatiens displayed lower percentage increases in steady state respiration rates as temperature increased. Since the a_0, a_1, and a_2 terms of the exponential decay equation are temperature dependent, as postharvest temperature increases, so does steady-state respiration (Böttcher et al., 2003).

The respiration rates observed in this study of unrooted herbaceous ornamental cuttings were similar to those reported for other crops comprised primarily of vegetative tissues: alfalfa (Despande et al., 2002), asparagus (Lill et al., 1990; Platenius, 1942), Galega kale (Fonseca et al., 2002b), shredded cabbage (Nei et al., 2006), spinach (Platenius, 1942), rocket leaves (Koukounaras et al., 2007), mint, parsley, and watercress (Hruschka and Wang, 1979). Plant parts with vegetative meristems have relatively high respiration rates compared to other plant parts (e.g., fruits) (Kader, 1987). Lill et al. (1990) found that the highest respiration rates occurred in the meristematic tips of asparagus.

The exponential decay curves used to describe trends in the respiration rates of vegetative cuttings have also been used for other vegetative horticultural crops, such as marjoram (Böttcher et al., 1999), Saint-John's wort (Böttcher et al, 2003), and asparagus spears (Brash et al., 1995). As postharvest temperature increased, respiration rates declined more rapidly during the first 24 h after harvest. At lower postharvest temperatures, respiration rates declined more gradually (Fig. 1.1). Kader (1987) stated that respiration generally declines rapidly in vegetative tissues because substrates are quickly depleted. The rate of decline in the respiration rates of Saint-John's wort during the first 12 h after harvest increased with increasing storage temperature (Böttcher et al., 2003). Lill et al. (1990) found that the respiration rate of asparagus declined rapidly at 16 °C between 2 and 24 h after harvest, and rates approached values that were approximately 30 % of the respiratory peak. Similar reductions were found in poinsettia (25.8 %) and New Guinea impatiens cuttings (21.1 %) stored at 15 °C. However, double

impatiens and petunia cuttings had a much greater reduction in respiration rates, 49.6 % and 57.6 %, respectively. At 20 °C, the respiration rate of alfalfa declined more than 50 % in the first 24 h of storage (Brash et al., 1995). Over the first 24 h of storage, vegetative cuttings had similar decreases in respiration rate at 20 °C (41.4, 49.2, 48.3, and 62.0 % for poinsettia, New Guinea impatiens, double impatiens, and petunia, respectively).

Q_{10} values. The Q_{10} values determined 2 h after harvest varied with species and postharvest temperature (Table 1.2). In general, the Q_{10} values decreased with increasing postharvest temperature range; therefore, changes in respiration rates were greater at lower temperatures. Poinsettia, double impatiens, and New Guinea impatiens cuttings had the highest Q_{10} values at 10 to 15 °C (3.0 to 3.4) versus petunia cuttings (2.0). At the warmer temperatures (20-25 °C), the Q_{10} values ranged from 1.3 for double impatiens to 2.1 for poinsettia.

Platenius (1942) stated that in order for Q_{10} values to have physiological meaning, they must be applied to the initial rates since comparisons at any later stage would be made between products of different physiological ages due to differences in respiratory metabolism. Thus, in this experiment Q_{10} values were calculated for the respiration rates collected 2 h after harvest. Platenius (1942) also stated that Q_{10} values were lower at upper temperature ranges because the rate of acceleration of biological reactions diminishes as higher temperatures are approached. All four species of vegetative cuttings had Q_{10} values that decreased with increasing 5 °C temperature

increments (Table 1.2). The relationship between plant respiration and temperature typically follows the Van't Hoff rule with the rate of the reaction increasing two- to three-fold for every 10 °C rise in temperature (Atkin et al., 2005; Kader, 1987). However, Q_{10} values can vary among temperature ranges. The Q_{10} values observed for vegetative cuttings ranged between 2.0-3.5 at 10-15 °C, 1.5-3.0 at 15-20 °C, and 1.0-2.0 at 20-25 °C. These values are comparable to Q_{10} values observed for arugula (Peiris et al., 1997), Saint-John's wort (Böttcher et al., 2003), majoram (Böttcher et al., 1999) and the inflorescences of *Grevillea* cut flowers (Joyce et al., 2000).

Conclusions

This study has demonstrated that the respiration rates of unrooted vegetative cuttings are dependent on species and postharvest temperature. Knowledge of respiration rates has several postharvest implications. First, predicting respiration rates will help determine the feasibility of using modified atmosphere packaging (MAP) in the shipment of unrooted cuttings, since the success of MAP depends on the accuracy of respiration rate models (Fonseca et al., 2002a). Second, the depletion of non-structural carbohydrates affects cutting longevity and root formation in propagation following shipment (Druege and Kadner, 2008; Rapaka et al., 2005). Finally, respiration not only depletes carbohydrate levels but also releases heat (Böttcher et al., 1999, 2001, 2003; Fonseca et al., 2002a; Kader, 1987). Increased respiration rates increase the amount of heat

produced by the cuttings, which affects packaging design, the use of ice, and amount of refrigeration required to remove excess heat.

Table 1.1. Exponential equations for a_0, a_1, *and* a_2 (Eq. 3) used in the exponential decay equation (Eq. 2) for the respiration rates of unrooted cuttings of four species of herbaceous ornamentals and their corresponding R^2 values. The responses generated by these equations are displayed in Fig. 1.1.

Species	Exponential equations					
	a_0	R^2 value	a_1	R^2 value	a_2	R^2 value
Euphorbia pulcherrima 'Prestige Red'	$0.0228e^{0.0972T}$	0.98	$0.0375e^{0.0873T}$	0.94	$0.0019e^{0.1815T}$	0.99
Impatiens walleriana 'Double-up Violet'	$0.0305e^{0.0708T}$	0.98	$0.0466e^{0.0953T}$	0.99	$0.0027e^{0.1974T}$	0.99
Impatiens hawkeri 'Sonic White'	$0.0803e^{0.0340T}$	0.59	$0.0126e^{0.1523T}$	0.99	$0.0346e^{0.0650T}$	0.97
Petunia ×hybrida 'Improved Charlie'	$0.0754e^{0.0451T}$	0.92	$0.2389e^{0.0656T}$	0.52	$0.1154e^{0.0495T}$	0.21

Table 1.2. Calculated Q_{10} values (Eq. 1) for unrooted herbaceous ornamental cuttings held at constant postharvest temperatures. Q_{10} values were calculated with the mean respiration data ± 1 SE collected 2 h after the start of the experiment.

Species	Q_{10} values		
	10-15 °C	15-20 °C	20-25 °C
Euphorbia pulcherrima 'Prestige Red'	2.9-3.3	2.4-2.5	2.1
Impatiens walleriana 'Double-up Violet'	3.2-3.3	1.3-1.7	1.2-1.6
Impatiens hawkeri 'Sonic White'	3.3-3.5	2.7-3.2	2.0
Petunia ×*hybrida* 'Improved Charlie'	2.0	1.7-1.8	1.6-1.8

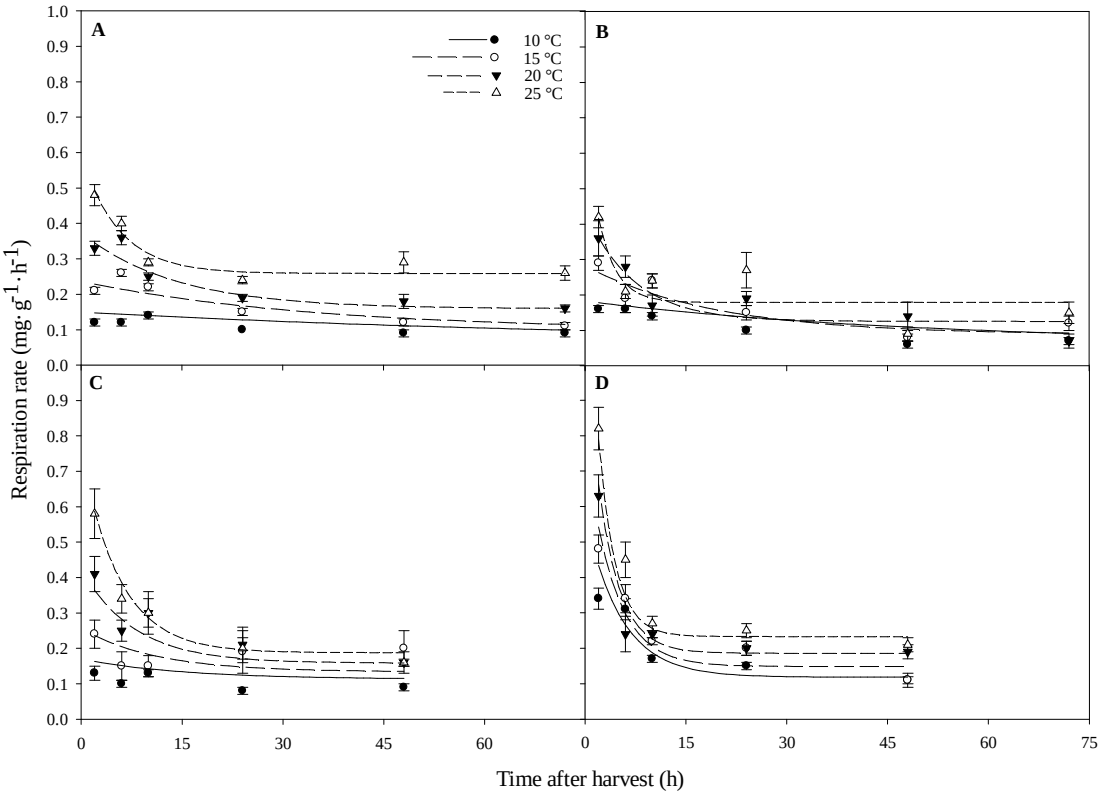

Figure 1.1. The change in respiration rates ($mg \cdot g^{-1} \cdot h^{-1}$) of (A) poinsettia (*Euphorbia pulcherrima* 'Prestige Red'), (B) double impatiens (*Impatiens walleriana* 'Double-Up Violet', (C) New Guinea impatiens (*Impatiens hawkeri* 'Sonic White'), and (D) petunia (*Petunia ×hybrida* 'Improved Charlie') cuttings at four different postharvest temperatures: 10 °C (●), 15 °C (○), 20 °C (▼), and 25 °C (△). Error bars represent ±1 SE. Lines represent the exponential decay rates (Table 1.1) at each postharvest temperature.

CHAPTER TWO

PRE-COOLING DURATION AFFECTS THE RESPIRATION RATE OF UNROOTED POINSETTIA CUTTINGS

Abstract

Adventitious root formation in propagation is related to the carbohydrate status of the unrooted cuttings, so it is important to maintain carbohydrate levels during the postharvest environment that occurs between cutting harvest from stock plants and propagation. The objective of this research was to determine if maintaining cuttings at 10 °C for a predetermined period of time (referred to as “pre-cooling”) was able to suppress respiration rates when cuttings were then transferred to warmer temperatures (20 °C). The respiration rate of unrooted poinsettia cuttings was measured at 20 °C following pre-cooling at 10 °C for 0, 3, 6, or 24 h prior to transfer to 20 °C. When cuttings were transferred to 20 °C, regardless of pre-cooling duration, respiration rates increased. Cuttings pre-cooled for 0 h had the highest average respiration rate. Calculated cumulative carbon dioxide (CO_2) evolution 72 h after harvest was also predicted. Cuttings maintained at constant 10 °C for 72 h respired the least amount of CO_2, whereas cuttings pre-cooled for 0 h respired the most.

Introduction

The production of vegetative shoot-tip cuttings for the United States floriculture industry occurs primarily in Mexico and Central America. Due to the shift to increased importation of plant material, high-quality products have become an increasingly significant prerequisite for economic success in the floriculture industry. Perishable commodities are produced and stored or transported for increasingly longer periods of time, and unfavorable environmental conditions potentially decrease quality and reduce postharvest life. The requirement for high vigor and viability is extremely important in regards to vegetative cuttings since the generation of adventitious roots and further growth in propagation are essential.

Respiration is the oxidative breakdown of starches and sugars via glycolysis, the tricarboxilic acid cycle, and an electron transport system to release energy while forming water and CO_2. Respiration plays an essential role in the growth and survival of plants. It is the driving force for biosynthesis, cellular maintenance, and active transport in plants (Atkin and Tjoelker, 2003; Atkin et al., 2005). Postharvest respiration rates are affected by cultivar, plant age, growing conditions, and handling conditions (Kader, 1987; Peiris et al., 1997). The postharvest respiration rate of perishable commodities is a major factor affecting product quality and storage potential. Increased respiration rates deplete available carbohydrates in unrooted cuttings which results in decreased adventitious root formation in propagation (Rapaka et al., 2005). Boxes of unrooted cuttings are generally stored in 10 °C coolers prior to shipping and ice packs are often placed inside each box to help maintain cool temperatures during shipping (Faust and Lewis, 2005). The boxes

usually reach their final destination in the U.S. 2 to 3 d after the cuttings are harvested. However, much of the shipping environment lacks temperature control, exposing cuttings to non-optimal temperatures during transit.

Since cuttings are held at 10 °C prior to shipping and ice packs are used in packaging to maintain 10 °C for as long as possible during transit, the objective of this research was to determine if maintaining cuttings at 10 °C for a predetermined period of time (referred to as "pre-cooling") was able to suppress respiration rates when cuttings were then transferred to warmer temperatures (20 °C).

Materials & Methods

Plant material. A single rooted poinsettia 'Prestige Red' cutting was transplanted into 3.8-L plastic pots containing a commercial peat moss mix (Middleweight Mix #3-B, Fafard Inc., Anderson, S.C., U.S.A.) and grown as stock plants in the greenhouse. They were fertilizer at every irrigation with water containing water-soluble fertilizer providing ($mg \cdot L^{-1}$) 250 N, 37 P, 208 K, 83 Ca, 33 Mg, plus micronutrients (Peters Excel; Scotts-Sierra Horticultural Products Company, Marysville, OH, U.S.A.). Heating/ventilation set points for temperature control were 18.3/22.2 °C, providing an average air temperature of 20 °C. Plants were shaded when the irradiance outside the greenhouse exceeded 1035 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Pre-cooling. Forty-five cuttings (6-cm stem length) were harvested from stock plants between 0830 and 0900 HR. Cuttings were weighed and wrapped in wetted brown

multifold hand towels (24.1 cm × 23.4 cm) to prevent desiccation. Three cuttings were placed in each 1.0-L glass container (Ball wide-mouth jar; Jarden Home Brands, Daleville, Ind., U.S.A.). Three glass containers containing cuttings plus one empty glass container were placed inside an incubator set at 10 °C. Additionally, 12 glass containers (three containers per pre-cooling duration) containing cuttings were placed at 10 °C for 0, 3, 6, or 24 h and then transferred to 20 °C for 72 h. The 20 °C incubator also contained an empty 1.0-L glass container. The experiment was repeated three times between Oct. and Nov. 2004.

Carbon dioxide measurements. In order to prevent cuttings from being exposed to anaerobic conditions, the containers were left unsealed (lids off) exposed to ambient air conditions while in the incubators. Thirty minutes prior to each headspace gas sampling the containers were sealed with lids fitted with rubber septums. Sampling was performed at 2, 6, 10, 24, 48, and 72 h after transfer to 20 °C for pre-cooled cuttings. Respiration rates were determined at 10 °C prior to containers being transferred to 20 °C. After the containers had been sealed for thirty minutes, two headspace gas samples (1 mL) were removed from each jar through the rubber septums using tuberculin syringes. Samples were analyzed for CO_2 using a gas chromatograph with a thermal conductivity detector (Shimadzu GC-8A, Kyoto, Japan).

Respiration rates. The difference in CO_2 levels in the containers with cuttings versus the control (empty) container was used along with the container volume, cuttings

weight, and length of time the containers were sealed to determine the respiration rate (CO_2 evolution; $mg \cdot g^{-1} \cdot h^{-1}$) of each species at each temperature at each sample time.

Statistical analysis. Data were analyzed with the analysis of variance (ANOVA) and general linear model (GLM) procedures in SAS v. 9.1 (SAS Institute, Inc., Cary, N.C., U.S.A.). Main effects were compared ($P \leq 0.05$) using Fisher's least significant difference (LSD) test. Changes in respiration rate with time were estimated for each pre-cooling treatment by non-linear regression analysis (SigmaPlot 12.0; Systat Software, Inc., San Jose, Calif., U.S.A.) using the following formula:

$$R = a + be^{-ct}$$

Where R = respiration rate (CO_2 evolution; $mg \cdot g^{-1} \cdot h^{-1}$); t = time after harvest (hours); and *a*, *b*, and *c* are constants.

Results

Prior to transfer to 20 °C, respiration rates of all pre-cooling treatments were similar to cuttings maintained at constant 10 °C (data not shown). After transfer to 20 °C, respiration rates of the cuttings increased regardless of pre-cooling duration (Fig. 2.1). Respiration rates after transfer to 20 °C were described by an exponential decay relationship across time (Table 2.1). Forty-eight hours after transfer to 20 °C, the decrease in respiration rate was no longer statistically significant regardless of pre-cooling treatment.

When the respiration rates were subjected to a factorial ANOVA, pre-cooling duration and time at 20 °C were both significant at $P \leq 0.001$. However, their interaction was not significant. Average respiration rates showed no clear trend with pre-cooling duration (Table 2.1). Cuttings pre-cooled for 0 h had the highest respiration rate at 20 °C, whiles cuttings pre-cooled for 3 or 24 h had the lowest rates.

Cumulative CO_2 evolution by the cuttings was determined for each pre-cooling treatment and cuttings held at constant 10 °C using the equations for the decay curves presented in Table 2.1 (Table 2.2). Since respiration rates determined for each pre-cooling treatment prior to transfer to 20 °C were similar to cuttings maintained at constant 10 °C, the 10 °C decay curve:

$$R = 0.0759 + 0.0548e^{-0.0227t}$$

was used to predict CO_2 evolution by cuttings during the pre-cooling period. Twenty-four hours after harvest, the decay curves predicted that cuttings maintained at constant 10 °C or pre-cooled for 24 h (cuttings transferred to 20 °C after this point) would respire the least amount of CO_2, whereas cuttings not pre-cooled (held at constant 20 °C) would respire the most. Seventy-two hours after harvest, the decay curves predicted that cuttings maintained at constant 10 °C would respire on average 38 % less CO_2 than cuttings that were not pre-cooled. Cuttings pre-cooled for 3, 6, or 24 h would respire more total CO_2 on average than cuttings at constant 10 °C (187 %, 220 %, and 196 %, respectively) but less than cuttings that were not pre-cooled (71 %, 84 %, and 75 % respectively).

Discussion

When pre-cooled cuttings were transferred to 20 °C, respiration rates increased. Uchino et al. (2004) and Nei el al. (2006) have developed models that determine the effect of temperature and time on respiration rate. Both observed when temperature was held constant, the respiration rate changed with time; however, when temperature changed, respiration rate was dependent on both time and temperature. If storage temperature was increased, respiration rate of the commodity increased. Zhu et al. (2006) found that temperature fluctuations between 3 and 8 °C during storage of common white mushroom (*Agaricus bisporus*) resulted in decreased shelf-life and increased respiration rates. When *Menta longifolia* stems were transferred from 1.5 °C to 20 °C, respiration rates of the stems increased to the level of cuttings held at constant 20 °C (Kenigsbuch et al., 2007).

When the amount of CO_2 evolved during the postharvest period was determined, the less CO_2 respired by the cuttings during the pre-cooling phase, the greater the rise in respiration rates when cuttings were transferred to 20 °C. The evolution of CO_2 by cuttings correlates with a decline in non-structural carbohydrates – oxidation of 1 mol of hexose sugar releases 6 mol of CO_2. In shredded cabbage, sugar concentrations decreased with time at both 5 and 20 °C with greater decreases observed at 20 °C (Nei et al., 2006). Thus, when storage temperature increased, respiration rate increased to a rate proportional to the availability of sugars. Pre-cooling cuttings for 3, 6, or 24 h reduced total CO_2 evolution versus cuttings that were not pre-cooled. However, as pre-cooling duration increased, the total amount of CO_2 respired also increased. Atkin and Tjoelker

(2003) stated that at high temperatures, respiration is limited by substrate availability. Therefore, the longer the cuttings were held at 10 °C, the more substrate there remained available for respiration at 20 °C. The decay curves also varied with pre-cooling duration. Respiration rates of cuttings pre-cooled for 3 and 6 h declined more rapidly when transferred to 20 °C than cuttings pre-cooled for 24 h. The more rapid the decline in respiration rates and the quicker the cuttings reach steady-state respiration the less total CO_2 respired over time.

Conclusion

Since respiration rates are dependent on temperature and non-structural carbohydrate availability, pre-cooling cuttings at 10 °C was not sufficient to suppress respiration rates when cuttings were transferred to 20 °C. Thus, the best way to decrease respiration rates and potentially increase storage potential is to store cuttings at constant low temperatures.

Table 2.1. Exponential decay curves at 20 °C, where *R* = respiration rate (CO_2 evolution; $mg \cdot g^{-1} \cdot h^{-1}$) and t = time at 20 °C (h), R^2 values, and average respiration rates for poinsettia cuttings exposed to five different pre-cooling durations.

Pre-cooling duration	Exponential decay equation	R^2 value	Avg. respiration rate ($mg \cdot g^{-1} \cdot h^{-1}$)
0 h at 10 °C	$R = 0.0598 + 0.4380e^{-0.0282t}$	0.97	0.31a[z]
3 h at 10 °C	$R = 0.0977 + 0.3600e^{-0.0644t}$	0.96	0.24c
6 h at 10 °C	$R = 0.1224 + 0.3850e^{-0.0583t}$	0.99	0.28b
24 h at 10 °C	$R = 0.0416 + 0.3274e^{-0.0253t}$	0.99	0.24c

[z]Mean separation within each species by Fisher's least significant difference test ($P \leq 0.05$). Numbers within the average respiration rate with the same letter are not statistically different.

Table 2.2. The effects of pre-cooling duration on calculated cumulative CO_2 evolution over time in unrooted poinsettia cuttings. CO_2 evolution is predicted based on decay equations ±1 SE determined for each treatment (Table 2.1).

Pre-cooling treatment	Calculated cumulative CO_2 evolution ($mg \cdot g^{-1}$)		
	Time after harvest (h)		
	24	48	72
Constant 10 °C	0.7-4.3	1.7-7.6	2.9-10.8
0 h at 10 °C	6.8-11.0	11.3-17.3	14.2-21.8
3 h at 10 °C	5.8-7.1	9.4-10.7	11.7-13.9
6 h at 10 °C	6.4-7.8	11.0-12.4	14.3-15.9
24 h at 10 °C	0.7-4.3	6.3-12.3	9.9-17.0

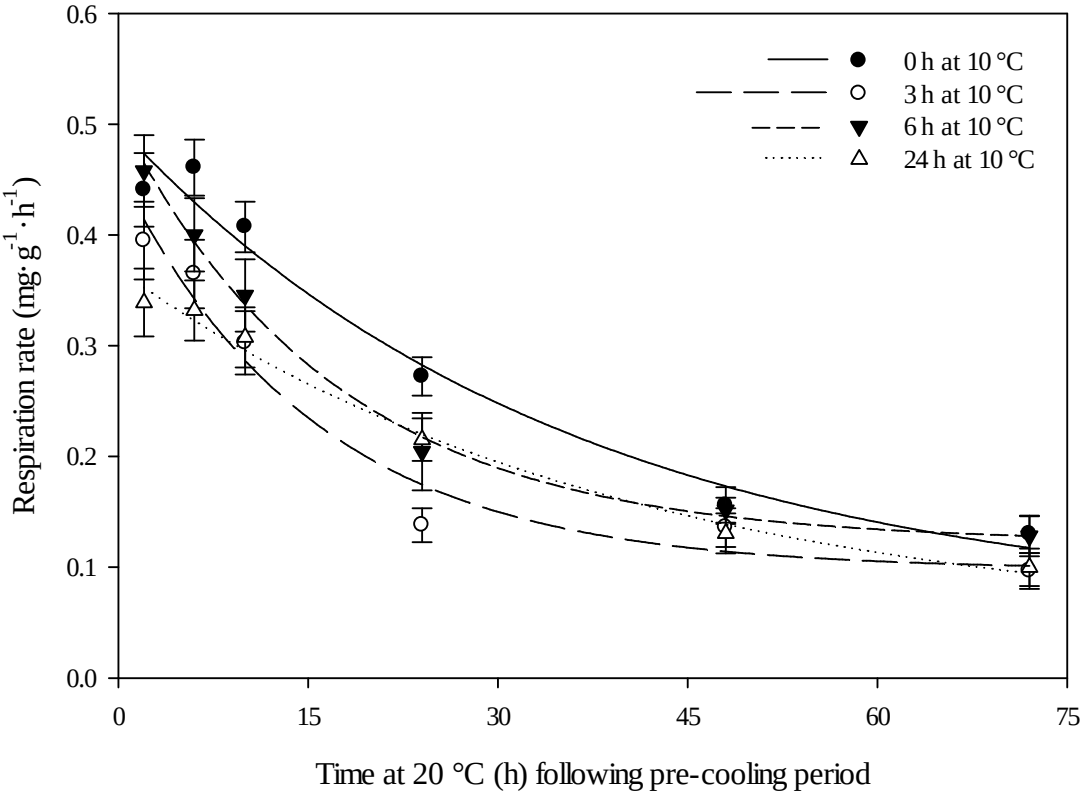

Figure 2.1. The effect of pre-cooling on the respiration rate (CO_2 evolution; $mg \cdot g^{-1} \cdot h^{-1}$) of unrooted *Euphorbia pulcherrima* 'Prestige Red' cuttings at four different pre-cooling (10 °C) durations: 0 h (●), 3 h (○), 6 h (▼), and 24 h (△). Error bars represent ±1 SE. Lines represent respiration decay rates for each pre-cooling duration (Table 2.1).

CHAPTER THREE

STORAGE TEMPERATURE AND DURATION AFFECT THE QUALITY AND ROOTING PERFORMANCE OF POINSETTIA CUTTINGS

Abstract

Unrooted poinsettia (*Euphorbia pulcherrima* ex Klotsch Willd. cv. Prestige Red) cuttings were placed in plastic bags at 0, 5, 10, 15, 20, or 25 °C for 2, 4, 6, 8, 10, 12, or 14 d and then placed on a propagation bench for 21 d. Shoot quality was given a visual rating (1-5 scale – 1: dead, 3: average, 5: excellent) immediately after storage, after one day in propagation, and at the end of propagation. Rooting quality was given a visual rating (1-5 scale) at the end of propagation. After 24 h in propagation, chlorophyll fluorescence (F_v/F_m) readings were recorded for each treatment. Regardless of storage temperature, all cuttings declined in quality as storage duration increased. The quality rating received immediately after storage was not indicative of the quality rating after 21 d in propagation. Trends in the chlorophyll fluorescence values at each storage temperature after 24 h in propagation were a strong indicator of final cutting evaluation at the end of propagation. A decline in rooting quality was noticeable two to four days prior to a decline in shoot quality. Storage potential was maximal at 10 °C where cuttings could be stored for 8 to 10 d without any detrimental effects on cutting quality, rooting performance, and cutting survival.

Introduction

The production of unrooted cuttings for the Unites States (U.S.) floriculture industry takes place primarily in Mexico and Central America. Boxes of unrooted cuttings are shipped via cargo planes and ground transportation and usually reach their final destination in the U.S. 2 to 3 d after the cuttings are harvested. The boxes of cuttings are generally stored in 10 °C coolers prior to shipping and ice packs are often placed inside each box; however, much of the shipping environment lacks temperature control, exposing cuttings to non-optimal temperatures affecting both cutting quality and performance (Faust and Lewis, 2005; Wang, 1987). Producers of tropical foliage cuttings attempt to maintain temperatures between 15.5 and 18.5 °C during shipment, but this only occurs when cuttings are transported using temperature controlled trucks since there is little temperature control in air shipments (Conover, 1976). Further problems also occur when boxes are delayed in transit. Doyle et al. (2003) noticed that the variability in the propagation performance of different batches of *Pelargonium* cuttings was due to delays in transit.

While shipping can be viewed as a form of storage (Hamilton et al., 2002), there are also several potential benefits to storing cuttings prior to shipping. The storage of cuttings could be beneficial to meeting demand if there was a shortage of stock plants, and it would allow producers to increase production capacity by storing surplus cuttings in anticipation of peak sales (Eisenberg et al., 1978; Hentig and Knösel, 1986). However, both storage time and temperature have a negative impact on plant quality and performance, with the quality of cuttings decreasing with increasing time in storage

(Eisenberg et al., 1978; Poole and Conover, 1993). Conover (1976) noted that for each additional day beyond four days in storage there was a 20-25 % reduction in the quality of tropical foliage cuttings.

Sub-optimal temperature storage can result in water-soaking of tissue, discoloration, accelerated senescence, increased susceptibility to disease, and loss of vigor (Friedman and Rot, 2006; Saltveit and Morris, 1990). Vegetative ornamental species of tropical origin are often sensitive to chilling. Symptoms can appear during exposure to chilling temperatures, but they more frequently occur after the plants have been returned to warmer temperatures (Friedman and Rot, 2006). The extent of injury is dependent on the interaction between time and temperature - occurrence of injury increases as temperature decreases (to the point where plant tissue freezes) and exposure time increases (Saltveit and Morris, 1990).

Chlorophyll fluorescence (F_v/F_m) is used to measure the ability of photosystem II to capture and convert light into energy (Lichtenthaler and Babani, 2004). As chilling injury damages the photosystems and tissue dehydration impairs photosynthesis, F_v/F_m values decline as more light is fluoresced by the tissue.

Poinsettia is the number one flowering potted plant produced in the U.S. with over 40 million plants with a total wholesale value of $169.2 million sold in 2006 (Jerardo, 2007). Being of tropical origin, poinsettias are sensitive to chilling temperatures. Nell and Barrett (1986) showed that poinsettia plants displayed leaf abscission when exposed to 3 °C storage temperatures for more than 4 d. Similar leaf abscission was observed when the plants were stored at 25 °C for more than 4 d (Nell and

Barrett, 1986). Hentig and Knösel (1986) stored unrooted poinsettia cuttings for a maximum of 10 d at 12-13 °C.

Because of its economic importance to the floriculture industry and the problems that occur at both low and high temperatures, the objective of this experiment was to determine the maximum storage potential of unrooted poinsettia cuttings. Shoot and root quality were evaluated across a range of temperatures both after storage and after propagation to determine the effect of storage temperature and duration on cutting performance.

Materials and Methods

Plant material. Rooted cuttings of poinsettia 'Prestige Red' were transplanted into 3.8-L plastic pots containing a commercial peat moss mix (Middleweight Mix #3-B, Fafard Inc., Anderson, S.C., U.S.A.) and grown as stock plants in the greenhouse. They were fertilized at every irrigation with water containing water-soluble fertilizer providing ($mg \cdot L^{-1}$) 250 N, 37 P, 208 K, 83 Ca, 33 Mg, plus micronutrients (Peters Excel; Scotts-Sierra Horticultural Products Company, Marysville, Ohio, U.S.A.). Heating/ventilation set points for temperature control were 18.3/22.2 °C, providing an average air temperature of 20 °C. Plants were shaded when the irradiance outside the greenhouse exceeded 1035 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Stock plants were maintained vegetative by providing day-extension lighting with high-pressure sodium lamps from 1700 to 2400 HR.

Storage. Three-hundred six cuttings (6-cm stem length) were harvested from stock plants between 0830-0900 HR. Three cuttings were placed in each of the 99 sealed,

0.95-L, 68.6 µm-thick, plastic freezer bags (Ziploc, S.C. Johnson & Son, Inc., Racine, Wis., U.S.A.). After packaging, 12, 21, 21, 21, 15, and 9 bags were placed in incubators set at 0, 5, 10, 15, 20, and 25 °C, respectively, and stored for up to 14 d. Bags of cuttings were removed from storage at 2-d intervals. Not all temperature × duration treatment combinations were evaluated due to cutting availability and preliminary experiments that indicated durations beyond 6 d would result in cutting mortality due to chilling injury at low storage temperatures and extreme dehydration at high storage temperatures (Table 3.1). Three replicates of three cuttings were also placed directly in propagation at the time of harvest as unstored controls. The experiment was repeated twice, in Mar. and Apr. 2006.

Propagation. After the appropriate storage duration, cuttings were removed from three bags at each storage temperature and placed in inert foam rooting medium (Oasis Wedge, Smithers-Oasis North America, Kent, Ohio, U.S.A.) on a mist propagation bench. No rooting hormones were applied. The mist system was operated during the daylight hours (6 s every 6 min) through the duration of the experiment. Greenhouse heating/ventilation temperature set points of 23/26 °C provided an average air temperature of 23 °C, and bench heating provided a rooting media temperature of 25 °C. The relative humidity in the greenhouse averaged 55 %. Cuttings were shaded when the outside irradiance exceeded 517 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Cutting evaluation. Cuttings were given a visual rating at the time of propagation, after 24 h in propagation, and after 21 d in propagation. After 21 d in propagation, cuttings were also given a visual evaluation for rooting. The rooting response and shoot

quality were visually determined from three replications of three cuttings per treatment using the following scoring systems. For the shoots at the time of propagation and after 24 h in propagation, 1: dead; 2: poor, extremely wilted; 3: average, slight wilting; 4: good, turgid; 5: excellent, fully turgid. For the shoots after 21 d in propagation, 1: dead; 2: poor, more than four abscised leaves; 3: average, three to four abscised leaves; 4: good, one to two leaves abscised; 5: excellent, no leaf abscission. For rooting, 1: no rooting; 2: poor, 1 or 2 roots visible on exterior of foam wedge; 3: average, foam wedge 50 % filled with visible roots; 4: good, foam wedge 75 % filled with visible roots; 5: excellent, foam wedge greater than 75 % filled with visible roots. The same person evaluated all of the treatments and recorded the visual ratings.

Chlorophyll fluorescence. After 24 h in propagation, one cutting per replicate (three cuttings per treatment) was evaluated for chlorophyll fluorescence (quantum yield efficiency, F_v/F_m) using a portable OS5-FL modulated fluorometer (Opti-Sciences Inc., Hudson, N.H., U.S.A.). Dark-adapted leaf clips were placed on the first fully expanded leaf of each cutting. The leaf area was dark adapted for 30 min before a fiber optic light-inducting probe was inserted into the cuvette of each clip. Tissue was first pulsed with a weak modulated light (<0.4 $\mu mol \cdot m^{-2} \cdot s^{-1}$) to monitor ground state fluorescence (F_o). A 35-W halogen lamp (8000 $\mu mol \cdot m^{-2} \cdot s^{-1}$) was then used to deliver a saturating pulse of light to determine maximum fluorescence (F_m). Variable fluorescence (F_v) was determined by subtracting F_o from F_m ($F_v = F_m - F_o$). Quantum yield efficiency was determined by dividing F_v by F_m (F_v/F_m).

Statistical analysis. Data were analyzed with the analysis of variance (ANOVA) and general linear model (GLM) procedures in SAS v. 9.1 (SAS Institute, Inc., Cary, N.C., U.S.A.). Means of the time-temperature interactions were compared using the least-square means (LSMEANS) test.

Results

Average quality ratings of the stored cuttings were compared to those of the unstored controls. Storage temperature affected the duration the cuttings could be stored before quality differed significantly from unstored cuttings. All cuttings, regardless of storage temperature, declined in shoot quality as storage duration increased (Table 3.2). The shoot quality rating received immediately following storage was not always indicative of cutting quality after 24 h or 21 d in propagation. At 0 and 5 °C, shoot quality declined from the start to the end of propagation. As storage duration increased at 10, 15, and 20 °C, shoot quality improved at the end of propagation versus the start.

At the time of propagation, stored cuttings were significantly different from unstored cuttings after 2 d at 20 and 25 °C, 4 d at 0 °C, 8 d at 15 °C, and 10 d at 5 and 10 °C. After 24 h in propagation cutting quality was significantly different from unstored cuttings after 6 d at 5 and 15 °C and 8 d at 10 °C, a 2 to 4 d reduction, while the maximum storage duration of cuttings stored at the other temperatures did not change. At the final evaluation, following 21 d in propagation, cuttings stored at 0, 20, and 25 °C significantly differed from unstored cuttings at 4 d of storage; cuttings stored at 5 °C

differed at 8 d; cuttings stored at 15 °C at 10 d; and cuttings stored at 10 °C did not differ significantly until 12 d of storage.

Data were pooled to determine the percentage of cutting survival at the end of propagation (Table 3.2). Cutting survival was greater than 90 % following 2 d of storage at 0, 20, and 25 °C, 4 d at 15 °C, 6 d at 5 °C, and 8 d at 10 °C. Percent survival dropped below 75 % after 4 d at 25 °C, 6 d at 0 and 20 °C, 8 d at 5 °C, 10 d at 15 °C, and 12 d at 10 °C. Cutting survival dropped below 50 % after 6 d at 0, 20, and 25 °C, 10 d at 15 °C, and 14 d at 15 °C. Cuttings stored at 10 °C had greater than 50 % survival after 14 d in storage. Therefore, although cuttings stored at 5 and 15 °C did not significantly differ qualitatively from unstored cuttings until 8 and 10 d of storage, respectively, less than 75 % of the cuttings survived. Cuttings stored at 10 °C were significantly different from unstored cuttings after 12 d of storage, but had 67 % survival, while storing cuttings at 10 °C for 10 d increased survival to 89 %.

Rooting quality was measured after 21 d in propagation (Fig. 3.1 B). None of the treatments, including the unstored controls, had an average root rating greater than 4 after 21 d in propagation. Storage impacted rooting performance 2 to 4 d sooner than shoot quality (Table 3.2). Unstored cuttings had a higher root rating than stored cuttings. Rooting quality differed significantly from unstored cuttings after 2 d of storage at 20 and 25 °C, 4 d at 0 °C, 6 d at 5 °C, and 8 d at 10 and 15 °C. Cutting survival at these storage durations was greater than 85 % across all storage temperatures.

Chlorophyll fluorescence was measured as an indicator of quality decline during storage (Fig. 3.1C). In healthy leaves, regardless of species, the F_v/F_m value should be

around 0.8 (Lichtenthaler and Babani, 2004). Cuttings stored at 0, 5, 20, and 25 °C all had chlorophyll fluorescence values below 0.8 after 6 d in storage. Cuttings stored at 10 and 15 °C had F_v/F_m values above 0.8 until after 12 d in storage. Trends in chlorophyll fluorescence were also important for predicting cutting performance in propagation. As cutting shoot quality declined with increasing storage duration (Fig. 3.1A), F_v/F_m values also declined (Fig. 3.1C). Cuttings stored at 0, 5, 20, and 25 °C had decreasing F_v/F_m values as storage duration increased. At 0 °C, F_v/F_m values dropped quickly between 4 and 6 d of storage as did final shoot quality ratings. On the other hand, cuttings stored at 10 and 15 °C maintained consistent F_v/F_m values above 0.8 until after 10 d in storage when values began to decline.

Discussion

The optimal temperature for storage varies with the species and organ being stored. For example, the optimum for *Grevillea* cut flowers is 0 °C (Joyce et al., 2000), while *Ficus benjamina* and *Ficus lyrata* have an optimum storage temperature at 20 °C (Buck and Blessington, 1982). The storage potential of many plants is optimal between 7.5 and 15 °C. For example, in the current experiment, poinsettia 'Prestige Red' cuttings had the greatest storage potential when cuttings were stored at 10 °C. When this storage temperature was maintained, unrooted cuttings could be stored for 10 d without shoot quality differing significantly from unstored cuttings. Similarly, Hentig and Knösel (1986) stored unrooted poinsettia 'Annette Hegg' and 'Angelika' cuttings for a maximum of 10 d at 12-13 °C. Many species of potted tropical foliage plants maintained good

quality ratings 60 d after storage when storage temperatures were maintained at 13 or 16 °C (Poole et al., 1984). Tulio et al. (2002) were able to maximize the storage of jute (*Corchorus olitorius* L.) leaves at 8 d when the storage temperature was 8 °C.

As storage temperatures increased, storage potential and post-storage performance of the poinsettia cuttings decreased. This has been seen in jute leaves (Tulio et al., 2002), *Grevillea* cut flowers (Joyce et al., 2000), *Leucocoryne* cut flowers (Elgar et al., 2003), *Ficus* plants (Buck and Blessington, 1982), and iceberg lettuce (Schofield et al., 2005). Storage temperatures between 20 and 25 °C decreased maximum storage duration of the poinsettia 'Prestige Red' cuttings to 4 d. Similarly, coleus cuttings could be stored for 4 to 6 d at 22 °C (Hamilton et al., 2002). Unlike unrooted cuttings, leaf and flower shoot emergence of bulbs and corms can be delayed or inhibited when storage temperatures drop below 20 °C (Kim et al., 1996; Matsuo and Van Tuyl, 1984).

Plants held in the dark generally experience leaf drop during storage. This has been documented in *Ficus* (Peterson and Blessington, 1981; Steintz et al., 1987), coleus (Hamilton et al., 2002), croton (Wang, 1987), and *Codiaeum* (Poole et al., 1984). However, regardless of storage temperature or duration in this experiment, the poinsettia cuttings experienced negligible leaf drop during storage. Leaf drop did occur during subsequent propagation (data not presented), and the extent of defoliation was reflected by a decrease in the final shoot quality rating.

A decrease in root quality of poinsettia cuttings was noticeable before shoot quality decreased. Root quality was impacted 2 to 4 d sooner than shoot quality, regardless of storage temperature. While storage potential of *Dracaena fragrans* cuttings

is a lot greater than poinsettia cuttings (weeks versus days), Conover (1994) found that root growth was comparable to unstored controls for up to six weeks of storage while shoot growth was comparable for up to eight weeks. Regardless of storage temperature, root growth of *Dracaena fragrans* cuttings was delayed when stored for more than six weeks (Conover and Poole, 1992). Wang (1987) showed that croton root number was unaffected by temperature for up to 10 d in shipping. *Pelargonium* cuttings stored at 10 °C for one week survived as well as cuttings stored at 2 or 4 °C, but if storage duration exceeded one week, rooting was severely reduced (Paton and Schwabe, 1987). The rooting percentage of *Pelargonium* cuttings also decreased with increasing storage duration and temperature (Enfield, unpublished data).

The unstored poinsettia cuttings had a higher root rating than stored cuttings. Similarly, unstored cuttings of *Rhododendron* (Davis and Potter, 1985), *Hibiscus,* and *Pelargonium* (Serek et al., 1998) rooted better than stored cuttings. The use of liquid auxin rooting hormone may be useful in overcoming the reduction in rooting quality of stored cuttings. Applying liquid auxin at rates between 1500 and 3000 $\mu L \cdot L^{-1}$ to unrooted, unstored cuttings of *Phlox paniculata* increased root mass and improved rooting uniformity (Enfield, 2002). Carnation cuttings stored for two weeks had increased rooting percentages when cuttings were treated with rooting hormone at the time of propagation (Garrido et al., 1996).

Poinsettia is a chilling sensitive species. Cuttings of the cultivar ‘Freedom Red’ developed symptoms of chilling injury after 48 h at 5 °C (Enfield, unpublished data). However, results from this experiment showed that cuttings from the cultivar ‘Prestige

Red' were able to withstand 2 d at 0 °C and 4 d at 5 °C without any negative effects on shoot and root quality. Nunes et al. (2006) showed that papaya fruit developed chilling injury symptoms after a 2-h exposure to 1 °C. Chilling injury symptoms developed on cut basil after 2 d of storage at 4 °C (Meir et al., 1997). Some chilling sensitive tropical foliage species, such as *Aglaonema* and *Codiaeum*, are damaged at storage temperatures of 10 °C (Poole et al., 1984). Poinsettia 'Prestige Red' cuttings, on the other hand, showed no signs of chilling damage above 5 °C, and the optimum storage temperature was 10 °C.

When cuttings were stored at 5 °C for longer than 4 d, they were of acceptable quality when removed from storage, but quality declined as soon as 24 h in propagation. Unrooted poinsettia 'Annette Hegg Dark Red' cuttings stored for one week at 5 °C were also of acceptable quality upon removal from storage but deteriorated by the end of propagation (Eisenberg et al., 1978). *Lantana camara* cuttings exposed to chilling temperatures did not show symptoms until they were returned to warmer temperatures (Friedman and Rot, 2006). Poole and Conover (1993) did not notice chilling injury on tropical foliage plants until they had been removed from storage for 2 to 4 d. On the other hand, *Heliotropium arborescens* cuttings express symptoms during storage at chilling temperatures (Friedman and Rot, 2006). As storage duration increased at 0 °C, chilling injury symptoms on poinsettia 'Prestige Red' cuttings became noticeable during storage. Cuttings turned an olive-green color and had a water-soaked appearance particularly on the younger leaves.

The chlorophyll fluorescence values measured for poinsettia 'Prestige Red' cuttings decreased rapidly at 0, 5, 20, and 25 °C as storage duration increased. The cuttings stored at 0 and 5 °C exhibited chilling injury whereas the cuttings stored at 20 and 25 °C became dehydrated during storage. In geranium cuttings, Doyle et al. (2003) found that the level of stress measured by chlorophyll fluorescence correlated with rooting performance – stressed cutting shipments had lower chlorophyll fluorescence values. In jute leaves (Tulio et al., 2002) and kangaroo paw cut flowers (Joyce and Shorter, 2000), chlorophyll fluorescence values decreased prior to the onset of chilling injury at low storage temperatures. In basil, F_v/F_m values dropped before visual symptoms were observed – as early as 1 d in storage at 4 and 8 °C (Meir et al., 1997)

Storage potential is limited at different temperatures by several factors including ethylene production, respiration rates, and carbohydrate depletion. Plants can be exposed to both endogenous and exogenous sources of ethylene during storage and/or transit. Since the synthesis and perception of ethylene are metabolic processes, the rate of ethylene production is temperature dependent and holding the tissue at the lowest possible temperature will reduce perception (Saltveit, 1999). The rate of ethylene production in fresh *Heliotropium arborescens* cuttings at 20 °C was higher than in cuttings stored at 2-8 °C (Friedman and Rot, 2006). Serek et al. (1998) attributed the loss of *Hibiscus* and *Pelargonium* cutting quality during storage to stress-induced ethylene. Increased storage temperature results in increased respiration rates. This has been demonstrated for many commodities including arugula (Peiris et al., 1997), asparagus spears (Brash et al., 1995), marjoram (Böttcher et al., 1999), and Saint-John's wort

(Böttcher et al., 2003). Increased respiration rates and long storage durations deplete available carbohydrates in unrooted cuttings. Davis and Potter (1985) showed that sucrose and glucose levels were higher in *Rhododendron* cuttings stored at 2 °C versus 21 °C. Reduction in the carbohydrate levels in cuttings resulted in decreased adventitious root formation in propagation (Rapaka et al., 2004).

Conclusions

The storage duration of poinsettia 'Prestige Red' was maximized when cuttings were stored at 10 °C. Cuttings were able to be stored at 10 °C for 8 to 10 d without any significant loss in shoot and root quality, and cutting survival was greater than 85 %. A decrease in rooting quality was noticeable 2 to 4 d prior to a decline in shoot quality. Chlorophyll fluorescence (F_v/F_m) was a helpful predictor of cutting performance in propagation. Since data presented are the maximum storage potential at each storage temperature, cuttings would likely be unable to survive the additional stress of 48 to 72 h in shipping. However, this information can be used to determine the feasibility of short-term storage or longer shipping durations using controlled temperature transit.

Table 3.1. Storage temperatures (°C) and durations (d) examined.

Temp (°C)	Duration (d)
0	2, 4, 6, 8
5	2, 4, 6, 8, 10, 12, 14
10	2, 4, 6, 8, 10, 12, 14
15	2, 4, 6, 8, 10, 12, 14
20	2, 4, 6, 8, 10
25	2, 4, 6

Table 3.2. The effect of storage temperature (°C) and duration (d) on the average shoot and root visual quality rating and survival (%) of poinsettia 'Prestige Red' cuttings. Shoot ratings were as follows – Propagation: immediately following storage; 24 h: one day after propagation; and Final: 21 d after propagation. Root ratings occurred 21 d after propagation. Shoot and root rating values represent average visual rating ±1 SE. Data were pooled to determine percent survival.

Temp.	Duration	Shoot Rating			Root	%
(°C)	(d)	Propagation	24 h	Final	Rating	Survival
--	0	4.9 ± 0.05	4.9 ± 0.05	5.0 ± 0.00	3.9 ± 0.23	100
0	2	5.0 ± 0.00	5.0 ± 0.02	4.7 ± 0.21	3.3 ± 0.22	94
	4	4.2 ± 0.17**	3.7 ± 0.14***	3.8 ± 0.31**	2.6 ± 0.18***	89
	6	3.7 ± 0.27***	2.1 ± 0.06***	1.3 ± 0.21***	1.2 ± 0.15***	6
	8	3.6 ± 0.21***	1.6 ± 0.20***	1.0 ± 0.00***	1.0 ± 0.00***	0
5	2	5.0 ± 0.00	5.0 ± 0.00	4.9 ± 0.07	3.3 ± 0.22	100
	4	5.0 ± 0.00	4.7 ± 0.17	4.7 ± 0.15	3.4 ± 0.16	100
	6	4.8 ± 0.17	4.2 ± 0.28**	4.3 ± 0.27	2.9 ± 0.30**	100
	8	5.0 ± 0.00	3.1 ± 0.20***	2.6 ± 0.53***	1.8 ± 0.35***	67
	10	3.3 ± 0.22***	2.3 ± 0.22***	1.6 ± 0.14***	1.5 ± 0.31***	50
	12	3.2 ± 0.12***	2.0 ± 0.07***	1.2 ± 0.22***	1.2 ± 0.13***	6
	14	1.9 ± 0.18***	2.1 ± 0.08***	1.0 ± 0.00***	1.0 ± 0.00***	0
10	2	5.0 ± 0.00	5.0 ± 0.00	5.0 ± 0.00	3.1 ± 0.25*	100
	4	4.5 ± 0.22	4.7 ± 0.18	4.9 ± 0.15	3.0 ± 0.24*	100
	6	4.6 ± 0.17	4.5 ± 0.22	5.0 ± 0.02	3.8 ± 0.55	100
	8	4.3 ± 0.20	3.6 ± 0.16***	4.5 ± 0.30	2.2 ± 0.20***	100
	10	3.2 ± 0.16***	3.5 ± 0.22***	4.1 ± 0.47	2.7 ± 0.45***	89
	12	2.3 ± 0.17***	2.1 ± 0.14***	1.8 ± 0.11***	1.3 ± 0.07***	61
	14	2.6 ± 0.17***	2.3 ± 0.33***	3.3 ± 0.33***	1.9 ± 0.19***	83
15	2	5.0 ± 0.00	5.0 ± 0.00	5.0 ± 0.00	3.1 ± 0.18*	100
	4	4.8 ± 0.16	4.7 ± 0.17	4.9 ± 0.05	2.3 ± 0.21***	100
	6	4.3 ± 0.27	4.1 ± 0.42**	4.1 ± 0.30	3.1 ± 0.32*	89
	8	4.1 ± 0.06**	3.6 ± 0.19***	3.9 ± 0.50*	2.3 ± 0.43***	89
	10	2.8 ± 0.38***	3.2 ± 0.12***	3.7 ± 0.53**	2.1 ± 0.46***	83
	12	2.1 ± 0.23***	2 ± 0.23***	2.6 ± 0.62***	1.5 ± 0.26***	61
	14	1.5 ± 0.22***	2.3 ± 0.33***	2.0 ± 0.4***	1.4 ± 0.22***	39

Table 3.2 (Continued)

20	2	3.5 ± 0.21***	3.7 ± 0.35***	4.3 ± 0.37	2.9 ± 0.27**	94
	4	2.2 ± 0.15***	3.0 ± 0.32***	3.1 ± 0.44***	1.5 ± 0.18***	78
	6	1.6 ± 0.27***	1.9 ± 0.30***	2.1 ± 0.49***	1.5 ± 0.23***	33
	8	1.5 ± 0.22***	1.3 ± 0.18***	1.5 ± 0.24***	1.1 ± 0.13***	6
	10	1.9 ± 0.10***	1.7 ± 0.23***	1.0 ± 0.00***	1.0 ± 0.00***	0
25	2	4.1 ± 0.20***	3.8 ± 0.18***	4.2 ± 0.41	2.6 ± 0.22***	94
	4	2.5 ± 0.22***	3.0 ± 0.08***	3.2 ± 0.67***	2.1 ± 0.30***	67
	6	2.3 ± 0.17***	2.3 ± 0.15***	2.1 ± 0.32***	1.3 ± 0.15***	50

*, **, *** are significantly different from the control cuttings (0 d duration) at $P \leq 0.05$, 0.01, and 0.001, respectively, as determined by the LSMEANS test.

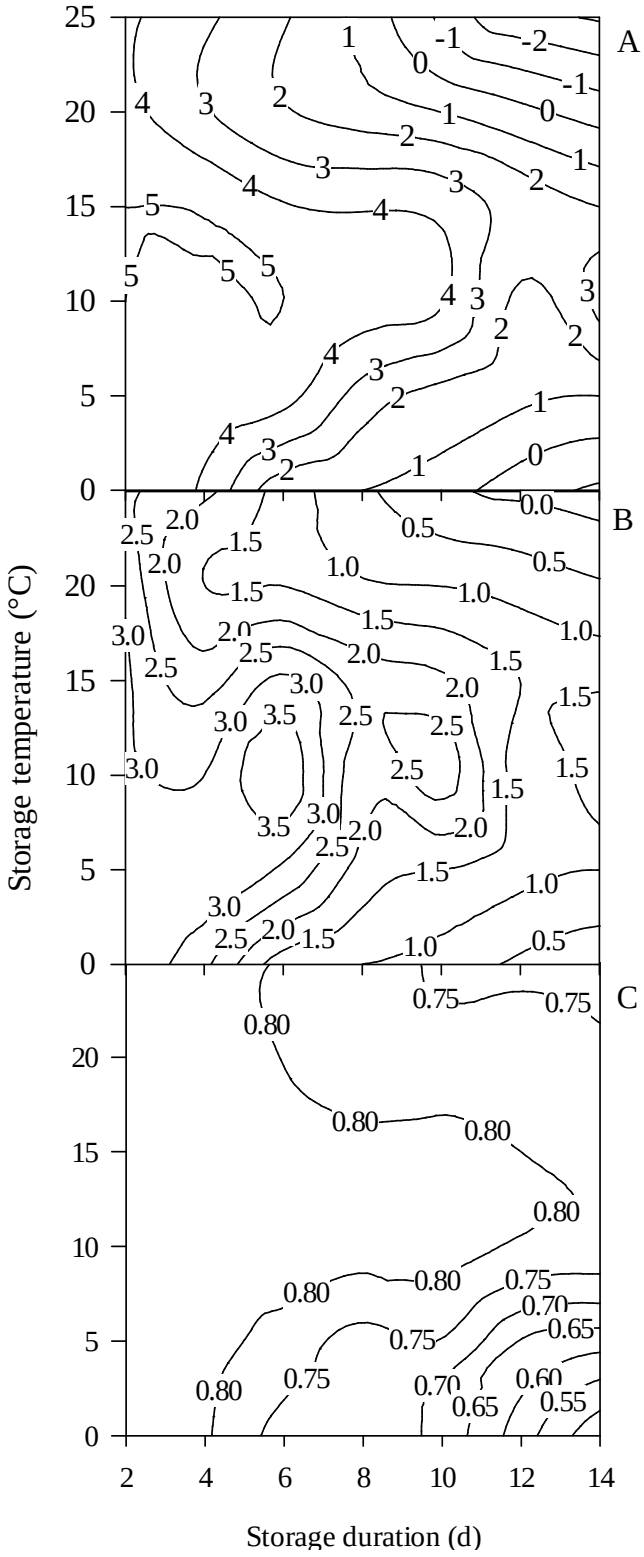
Storage temperature (°C)
Storage duration (d)
A
B
C
25
20
15
10
5
0
2
4
6
8
10
12
14

Figure 3.1. Contour plots of the effect of storage duration and storage temperature on (A) the average final shoot rating of cuttings after 21 d in propagation, (B) the average final root rating of cuttings after 21 d in propagation, and (C) chlorophyll fluorescence values (F_v/F_m) after 24 h in propagation. Shoot ratings were qualified visually using the following scale – 1: dead; 2: poor, more than four abscised leaves; 3: average, three to four abscised leaves; 4: good, one to two abscised leaves; 5: excellent, no leaf abscission. Root ratings were qualified visually using the following scale – 1: no rooting; 2: poor, 1 or 2 roots visible in foam wedge; 3: average, foam wedge 50 % filled with roots; 4: good, foam wedge 75 % filled with roots; 5: excellent, foam wedge greater than 75 % filled with roots.

CHAPTER FOUR

FACTORS AFFECTING ENDOGENOUS ETHYLENE PRODUCTION BY UNROOTED POINSETTIA CUTTINGS

Abstract

The effects of the postharvest environment on the production of endogenous ethylene by unrooted poinsettia (*Euphorbia pulcherrima* ex Klotsch Willd. cv. Prestige Red) cuttings were examined in four experiments. In the first experiment, cuttings were placed in sealed plastic bags (five cuttings per bag) at 20 and 25 °C. Air samples were collected from the plastic bags and analyzed for ethylene. Sample collection continued hourly for 6 hours until all bags had detectable levels of ethylene. Cuttings stored at 25 °C had detectable ethylene two hours after harvest, and 100 % of the bags had detectable ethylene four hours after harvest. Cuttings stored at 20 °C had detectable ethylene three hours after harvest, and 100% of the bags had detectable ethylene six hours after harvest. In the second experiment, cuttings were sealed in plastic bags (two cuttings per bag) with their leaves held vertically using paper sleeves (36.3-mm diameter) or horizontally. The bags of cuttings were then placed either vertically or horizontally in incubators set at 15, 20, or 25 °C. Air samples were collected 24, 48, and 72 hours after harvest and analyzed for ethylene. Neither leaf nor cutting orientation had a significant effect on ethylene production. However, as storage temperature increased, ethylene production increased. In the third experiment, cuttings were harvested at 0900 HR and 1500 HR, sealed in plastic bags (two cuttings per bag) and stored in incubators set at 15,

20 or 25 °C. Air samples were collected from each bag 24, 48 and 72 hours after harvest. Cuttings harvested at 1500 HR produced more ethylene than cuttings harvested at 0900 HR. As storage temperature increased, ethylene production increased. In the fourth experiment, cuttings were harvested and stored in unsealed plastic bags for 18 hours at 10 or 20 °C (pre-packaging phase). Following the pre-packaging phase, cuttings were placed in sealed plastic bags (two cuttings per bag) and placed back at the same temperature or transferred to the opposite temperature (i.e., 10 °C/10 °C, 10 °C/20 °C, 20 °C/10 °C, or 20 °C/20 °C), referred to as the post-packaging phase. Cuttings stored at 20 °C produced more ethylene than cuttings stored at 10 °C. Cuttings stored at 20 °C during the pre-packaging phase and then transferred to a post-packaging temperature of 10 °C produced more ethylene than cuttings maintained at a constant 10 °C. Sixty-six hours after harvest, cuttings stored at 20 °C during the post-packaging phase were not significantly different.

Introduction

In 2006, 878 million unrooted cuttings were imported into the United States (U.S.) for the floriculture industry (Jerardo, 2007). Boxes of unrooted cuttings are shipped via cargo planes and ground transportation and usually reach their final destination in the U.S. 2 to 3 d after the cuttings are harvested. The boxes of cuttings are generally stored in 10 °C coolers prior to shipping and ice packs are often placed inside each box; however, much of the shipping environment lacks temperature control, exposing cuttings to non-optimal temperatures affecting both cutting quality and performance (Faust and Lewis, 2005; Wang, 1987). High vigor and viability are

extremely important in regards to vegetative cuttings since the generation of adventitious roots and further growth in propagation are essential.

During the shipping process, plants are at risk of exposure to ethylene. Ethylene is a naturally occurring gaseous plant hormone that is typically produced endogenously by plants in response to a stressor. It is effective at part-per-million ($\mu L \cdot L^{-1}$) to part-per-billion ($nL \cdot L^{-1}$) concentrations (Salveit, 1999). Plants can be exposed to both endogenous and exogenous sources of ethylene during shipping. Exogenous sources include truck exhaust, cigarette smoke, and ripening fruit. Høyer (1995b) showed that ethylene concentrations $\geq 0.03\ \mu L \cdot L^{-1}$ can occur during transport of potted plants in refrigerated trucks with the majority of that ethylene not arising from the plant material.

Ethylene promotes the degradation of proteins and accumulation of amino acids (Jiang et al., 2002). Exposure to ethylene can stimulate flower wilting, shattering, failure to open or abscission (Nell and Leonard, 2005). In leaves, ethylene promotes chlorophyll degradation (yellowing), abscission and premature senescence (Saltveit, 1999). The presence of ethylene inhibits further ethylene synthesis in vegetative tissue, termed autoinhibition (Saltveit, 1999). Kadner and Druege (2004) showed that ethylene exposure during storage and transport was involved in the leaf senescence of geranium cuttings. The severity of damage is dependent on the age of the commodity, temperature, ethylene concentration, and duration of exposure (Kader, 1985).

“Stress ethylene” is the accelerated biosynthesis of ethylene in association with environmental or biological stresses, including wounding, chilling, and drought (Morgan and Drew, 1997; Yakimova and Woltering, 1997). When plant tissue is cut, pierced,

squeezed, bent, or touched, ethylene production is usually promoted. Mechanical stresses experienced by cuttings during normal packaging and shipment, which do not cut or pierce the tissue, can also induce ethylene production. Wound ethylene is a form of stress ethylene (Yu and Wang, 1980). Kadner et al. (2000) showed that harvesting geranium cuttings from stock plants induced the production of wound ethylene.

Poinsettia is the number one flowering potted plant produced in the U.S. with over 40 million flowering plants sold in 2006 (Jerardo, 2007). Poinsettia cuttings are subjected to mechanical and physical stress during harvest and packaging. The unrooted cuttings are densely packed in boxes with their leaves bent vertically upwards. Following shipment, leaf yellowing and abscission is often observed, and the presence of ethylene in the package is thought to be responsible (Faust and Lewis, 2005). According to Dole and Gibson (2006), poinsettia cuttings are not sensitive to 1 $\mu L \cdot L^{-1}$ ethylene for up to a 24-h exposure, but may be sensitive to higher doses or longer exposure times.

Because of their economic importance and the problems encountered during shipment attributed to the presence of ethylene, unrooted poinsettia cuttings were used to examine the effects of the postharvest environment on endogenous ethylene production. The first objective was to determine the time-course for wound ethylene production following cutting harvest. The second objective was to determine the effects of mechanical and physical stresses (leaf orientation and cutting orientation) on the production of ethylene. The third objective was to determine the effect of storage and shipping temperature on ethylene production. The final objective was to determine the

effect of time of harvest (morning versus afternoon) on the rate of endogenous ethylene production.

Materials and Methods

Stock plant management. Rooted cuttings of poinsettia 'Prestige Red' were transplanted into 3.8-L plastic pots (one cutting per pot) containing a commercial peat moss mix (Middleweight Mix #3-B, Fafard Inc., Anderson, SC, USA) and grown as stock plants in the greenhouse. They were fertilized at every irrigation with water containing water-soluble fertilizer providing ($mg \cdot L^{-1}$) 250 N, 37 P, 208 K, 83 Ca, 33 Mg, plus micronutrients (Peters Excel; Scotts-Sierra Horticultural Products Company, Marysville, Ohio, U.S.A.). Heating/ventilation set points for temperature control were 18.3/22.2 °C, providing an average air temperature of 20 °C. Plants were shaded when the irradiance outside the greenhouse exceeded 1035 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Wound ethylene. Fifty cuttings (6-cm stem length) were harvested from the stock plants between 0900-0930 HR. Five cuttings were placed in each of 10 sealed 0.95-L, 68.6 µm-thick, plastic freezer bags (Ziploc, S.C. Johnson & Son, Inc., Racine, WI, USA). Five bags were randomly placed inside incubators set at 20 or 25 °C. Air samples were taken from each bag hourly through 6 h after harvest, until all bags had detectable levels of ethylene. To ensure that the bags resealed after each sample, syringes were inserted through a silicon patch stuck to each bag. The experiment was replicated twice between May and June 2006.

Leaf and cutting orientation. One hundred twenty cuttings (6-cm stem length) were harvested from stock plants between 0900-0930 HR. One half of the cuttings (60) were fitted with paper tubes (diameter: 36.3 mm) to keep leaf orientation vertical during storage, while the leaves of the remaining cuttings were kept horizontal. The cuttings from leaf orientation treatments (vertical or horizontal) were placed (two cuttings each) in a wetted inert foam medium (Oasis Wedge, Smithers-Oasis North America, Kent, Ohio, USA), resulting in 60 sets of inert foam trays (30 with vertical oriented leaves and 30 with horizontal leaves). The inert foam trays containing the cuttings were placed in one of 60 sealed 0.95-L, 68.6 µm-thick, plastic freezer bags (Ziploc, S.C. Johnson & Son, Inc., Racine, WI, USA). Ten bags from each leaf orientation treatment (20 bags total) were placed on shelves inside incubators set at 15, 20 or 25 °C. One half of each leaf orientation treatment (five bags each) was placed on the shelves either vertically (standing upright) or horizontally (laying flat). Air samples were removed from the bags after 24, 48, and 72 h in storage. The experiment was replicated twice in May 2006.

Morning versus afternoon harvest. Sixty cuttings (6-cm stem length) were harvested from stock plants between 0900-0930 HR and another 60 cuttings were harvested between 1500-1530 HR. Two cuttings were placed in each of 30 (60 total) sealed 0.95-L, 68.6 µm-thick, plastic freezer bags (Ziploc, S.C. Johnson & Son, Inc., Racine, WI, USA). Ten bags from each harvest time were randomly placed on shelves inside incubators set at 15, 20, or 25 °C. Air samples were removed from the bags after 24, 48, and 72 h in storage. The experiment was replicated twice in June 2006.

Bulk storage. Between 1500-1530 HR, 40 cuttings (6-cm stem length) were harvested from stock plants. Cuttings were divided into groups of 20 and placed in plastic bags (30.3 L, 12.7 µm-thick). The tops of the bags remained unsealed to allow for air/gas movement and placed at 10 or 20 °C for 18 h (referred to as the "pre-packaging" phase). After 18 h, two cuttings per temperature treatment were placed into each of 20 (10 bags per pre-packaging temperature) sealed 0.95-L, 68.6 µm-thick, plastic freezer bags (Ziploc, S.C. Johnson & Son, Inc., Racine, WI, USA). For the "post-packaging" phase, one half of the bags (five bags per temperature) returned to the same temperature as during the 18-h pre-packaging treatment (i.e., 10 °C/10 °C and 20 °C/20 °C) and one half of the bags were transferred to the opposite temperature (i.e., 10 °C/20 °C and 20 °C/10 °C). Bags were sampled for ethylene 24, 48, and 72 h after packaging (42, 66, and 90 h after harvest). The experiment was replicated twice between July and Aug. 2006.

Ethylene measurements. Two headspace gas samples (1-mL) were removed from each bag through a silicon port using tuberculin syringes. Samples were analyzed for ethylene content using a gas chromatograph with a flame ionization detector (Shimadzu GC-9A, Kyoto, Japan).

Statistical analysis. Data were analyzed with the analysis of variance (ANOVA) and general linear model (GLM) procedures in SAS v. 9.1 (SAS Institute, Inc., Cary, NC, USA). Means of the main effects were compared ($P \leq 0.05$) using Fisher's least significant difference (LSD) test. When treatment interactions were significant, means of the interactions were compared ($P \leq 0.05$) using the least-square means (LSMEANS) test.

Results

Wound ethylene. All bags of cuttings, regardless of storage temperature, had detectable levels of ethylene by 6 h after harvest (Fig. 4.1). Ethylene was first detectable at 25 °C in 10 % of the bags 2 h after harvest and in 60 % of the bags at 20 °C 3 h after harvest (Fig. 4.1A). More than 50 % of the bags, regardless of storage temperature, had detectable levels of ethylene by 3 h. All bags of cuttings stored at 25 °C had detectable ethylene 4 h after harvest and bags at 20 °C after 6 h. The average ethylene concentration per bag increased faster in cuttings stored at 25 °C (Fig. 4.1B). Five hours after harvest, cuttings stored at 25 °C had approximately four-times more ethylene per bag than cuttings stored at 20 °C.

Leaf and cutting orientation. Neither leaf orientation (Fig. 4.2A) nor cutting orientation (Fig. 4.2B) had a significant effect on ethylene production. However, storage temperature did have a significant effect on ethylene production (Fig. 4.2C). As storage temperature increased, ethylene concentration inside the sealed bags increased. Regardless of storage temperature or time after harvest, ethylene levels inside the bags did not exceed 150 $nL \cdot L^{-1}$. Ethylene concentrations were highest at 25 °C; however, 48 h after harvest, concentrations at 20 °C were not significantly different from 25 °C. Ethylene concentrations at 15 and 20 °C were not significantly different at any sample time. Ethylene concentrations at 25 °C remained constant and decreased slightly at 72 h. At 15 and 20 °C, ethylene concentrations increased through 48 h and then remained constant at 15 °C and decreased at 20 °C.

Morning versus afternoon harvest. Cuttings harvested at 0900 HR (Fig. 4.3A) produced less ethylene than cuttings harvested at 1500 HR (Fig. 4.3B). Storage temperature had a significant effect on ethylene production across all sample times (storage durations). Time of harvest (morning versus afternoon) had a significant effect on ethylene production after 48 and 72 h of storage. The interaction between time of harvest and storage temperature was significant at 24 and 48 h of storage. Through 48 h of storage, cuttings harvested at 1500 HR and stored at 25 °C produced significantly more ethylene than the other treatments. Regardless of time of harvest, cuttings stored at 15 °C produced the least amount of ethylene. After 72 h of storage, cuttings harvested at 1500 HR and stored at 20 and 25 °C had the highest ethylene concentrations. The ethylene concentrations in the bags of cuttings stored at 15 °C increased throughout the 72 h of storage, whereas the ethylene concentrations in the bags of cuttings stored at 20 and 25 °C increased through 48 h of storage and the either decreased or remained constant.

Bulk storage. Cuttings stored at the 20 °C pre-packaging temperature produced more ethylene than cuttings stored at 10 °C (Fig. 4.4A). Similarly, cuttings stored at the 20 °C post-packaging temperature produced more ethylene than cuttings stored at the 10 °C post-packaging temperature (Fig. 4.4B). The effect of temperature (10 °C versus 20 °C) on ethylene production was more pronounced during the post-packaging storage phase of the experiment. Ethylene concentrations increased through 66 h after harvest and then decreased. All pre- and post-packaging temperature combinations were significantly different 42 h after harvest (Fig. 4.4C). Cuttings stored at 20 °C during the pre-packaging phase and then transferred to 10 °C for the post-packaging phase

(20 °C/10 °C) produced more ethylene than cuttings that were stored at 10 °C for both pre- and post-packaging (10 °C/10 °C). Sixty-six hours after harvest, cuttings stored at 20 °C during the post-packaging phase were not significantly different regardless of pre-packaging temperature. As time after harvest increased, the average daily temperature (ADT) changed depending on the treatment combination. Cuttings maintained at the same pre- and post-packaging temperature had a constant ADT, whereas cuttings transferred to the opposite temperature during the post-packaging phase had an increasing ADT. For the 10 °C/20 °C treatment, the ADT was 15.7, 17.3, and 18 °C at 42, 66, and 90 h after harvest, respectively. For the 20 °C/10 °C treatment, the ADT was 14.2, 12.7, and 12 °C at 42, 66, and 90 h after harvest, respectively.

Discussion

Perhaps the greatest effect that ethylene has on the quality of vegetative cuttings is the induction of chlorophyll degradation and leaf abscission (Serek et al., 1998). However, in all experiments, endogenous ethylene production was not high enough to promote leaf yellowing or abscission in poinsettia cuttings during storage (data not presented). Ethylene levels in the packages never exceeded 0.6 $\mu L \cdot L^{-1}$ (600 $nL \cdot L^{-1}$), and in most cases, levels ranged between 0.2 and 0.3 $\mu L \cdot L^{-1}$. Dole and Gibson (2006) stated that poinsettia cuttings do not exhibit symptoms of leaf yellowing until after exposure to 1 $\mu L \cdot L^{-1}$ for more than 24 h.

Sensitivity to ethylene varies with species and plant part. The quality of *Pelargonium* cuttings exposed to 0.5 $\mu L \cdot L^{-1}$ ethylene were not affected (Mutui et al.,

2005); whereas, kalanchoe, carnation, *Achillea*, *Celosia*, *Helianthus*, *Penstemon*, and *Weigla* flowers were sensitive to an ethylene concentration of 0.5 $\mu L \cdot L^{-1}$ (Kader, 1985; Redman et al, 2002; Serek and Reid, 2000). Flowering bulbs can be affected by ethylene levels as low at 0.1 $\mu L \cdot L^{-1}$ (Kader, 1985). The external application of ethylene can accelerate chlorophyll degradation and senescence, as is the case with many cut flowers (Elgar et al., 2003), *Pelargonium* cuttings (Mutui et al., 2005), and parsley (Ella et al., 2003). However, in our experiments, poinsettia cuttings were not exposed to exogenous ethylene. Only the effects of postharvest treatment on endogenous ethylene were examined. Had the cuttings been exposed to exogenous sources of ethylene, leaf yellowing may have been more prevalent.

In the processing of fruits and vegetables, wounding (i.e., slicing, shredding, and cutting) results in an increase in ethylene production (del Aguila et al., 2006; Lulai and Suttle, 2004; Mao et al., 2007; Marrero and Kader, 2006). Mutui et al. (2005) stated that storing cuttings shortly after harvest leads to the production of wound-derived ethylene that accumulates in the packing material. Although the biosynthesis of wound ethylene in poinsettia cuttings was most likely triggered immediately after harvest, detectable ethylene levels did not occur in the package until 2 h after harvest at 25 °C. However, the gas chromatograph used to analyze ethylene levels was not able to detect ethylene until concentrations reached approximately 10 $nL \cdot L^{-1}$ in the sealed bags. Using a flow-through method to detect ethylene concentrations, Kadner et al. (2000) were able to detect wound ethylene production in *Pelargonium* cuttings almost immediately following harvest at concentrations less than 2 $nL \cdot L^{-1}$. However, radish did not produce detectable levels of

ethylene until 9 h after processing (del Aguila et al., 2006). Saltveit and Dilley (1978) found in stem sections of etiolated pea seedlings that as temperature increased the length of the ethylene synthesis lag period decreased. The ethylene synthesis lag period for poinsettia cuttings was an hour longer at 20 °C than 25 °C.

Leaf bending has been implicated in the induction of ethylene production (Morgan and Drew, 1997). Mechanical stress has also been shown to induce ethylene production in flowering poinsettia plants (Kader, 1985). We hypothesized that mechanical stresses associated with packaging unrooted poinsettia cuttings, particularly leaf bending, would stimulate ethylene production. However, neither leaf nor cutting orientation stimulated excess ethylene production.

At 20 and 25 °C storage temperatures, the rate of ethylene production in poinsettia cuttings decreased or remained constant after 48 h. Faust and Lewis (2005) reported the same occurrence in poinsettia cuttings, and Yakimova and Woltering (1997) reported a similar occurrence in cut carnation flowers after 45 hours. Pak choy leaves showed a gradual decline in ethylene production as storage time increased (Able et al., 2005). The decline in ethylene after 48 h could be the result of feedback inhibition of ethylene synthesis, which occurs in vegetative tissues (autoinhibition) or when commodities are stored under high concentrations of CO_2 (Merodio and de la Plaza, 1989; Saltveit, 1999).

Although CO_2 levels in the packages were not measured, low rates of ethylene production by the poinsettia cuttings, particularly at warmer storage temperatures, could have been the result of high CO_2 levels in the sealed bags. In the storage of fennel,

ethylene production decreased when CO_2 levels were increased (Escalona et al., 2006). We have measured concentrations of 4 % CO_2 in sealed packages of vegetative cuttings (unpublished data).

Poinsettia cuttings harvested in the afternoon produced higher levels of ethylene. Ethylene production may be related to the carbohydrate status of the cuttings. Sugars could serve as the energy suppliers of ethylene synthesis (Philosoph-Hadas et al., 1985). Cuttings harvested in the afternoon have higher concentrations of non-structural carbohydrates due to photosynthesis. Philosoph-Hadas et al. (1985) found that when tobacco leaf discs were treated with glucose or sucrose ethylene production was stimulated. The addition of sucrose to broccoli florets increased ethylene production (Niskikawa et al., 2005). On the other hand, Rapaka et al. (2007a) found that time of harvest did not influence the amount of ethylene produced by *Portulaca* cuttings, and increased carbohydrate concentrations delayed ethylene production in many cut flowers (Pun and Ichimura, 2003; Verlinden and Garcia, 2004).

The cuttings harvested at 0900 HR for the morning versus afternoon harvest experiment produced approximately three-times more ethylene than the cuttings harvested at 0900 HR for the leaf and cutting orientation experiment even though both experiments had the same number of cuttings per bag and the same storage temperatures. Cutting mass was not accounted for in these experiments. Within an experiment, cuttings of the same size were harvested; however, cutting size varied between experiments. It is probable that cuttings harvested for the morning versus afternoon harvest experiment were larger and/or more mature than the cuttings harvested for the leaf and cutting

orientation experiment, influencing ethylene production. Macnish et al. (2004) noted that sensitivity of cut *Chamelaucium* Desf. flowers to ethylene varied with harvest.

The rate of ethylene production is temperature dependent. Since the perception of ethylene is a metabolic process, holding the tissue at the lowest possible temperature will reduce perception (Saltveit, 1999). As storage temperature increased, the rate of ethylene production in poinsettia cuttings also increased. As ADT increased during the bulk storage experiment, ethylene concentrations also increased. The rate of ethylene production in fresh *Heliotropium* cuttings at 20 °C was higher than in cuttings stored at 2-8 °C (Friedman and Rot, 2006). Storing fully developed leaves (used as cuttings) of *Radermachera sinica* at 10 °C blocked the plants response to ethylene (Høyer, 1995a). Fresh cut radish showed increasing ethylene production with increasing temperature from 1 to 10 °C (del Aguila et al., 2006). As temperature increased from 5 to 10 °C, ethylene production increased in pineapple (Marrero and Kader, 2006).

Kadner et al. (2000) demonstrated that low temperatures suppressed ethylene production in *Pelargonium* cuttings, and re-warming cuttings resulted in increased ethylene production. When poinsettia cuttings were transferred from 10 to 20 °C, ethylene production increased, and concentrations were not significantly different from cuttings held at constant 20 °C. Since ethylene biosynthesis is an enzymatic process, reaction rate is temperature dependent. The rate-controlling step of ethylene synthesis is the production of 1-aminocyclopropane-1-carboxylic acid (ACC) (Philosoph-Hadas et al., 1985). Wounding and other mechanical stresses promote the conversion of S-adenosyl methionine (SAM) to ACC by inducing the synthesis of ACC synthase (Saltveit, 1999;

Yu and Wang, 1980). When cuttings were transferred from 10 to 20 °C, ACC synthase activity would have increased, resulting in increased ethylene synthesis. When cuttings were stored at 20 °C before transfer to 10 °C, it is probable that the pool of ACC was large enough to maintain ethylene production even after cuttings were transferred to a cooler temperature.

Understanding how the postharvest environment affects ethylene production by unrooted cuttings is important in the development of improved methods for shipping and packaging. Modified atmosphere packaging (MAP) is an important technique for fruit and vegetable preservation during shipment and storage (Wang et al., 1998) and may be a feasible alternative in the packaging of vegetative cuttings. Removing ethylene from the package could enhance the storage quality of ethylene sensitive vegetative cuttings. Removing citrus leaf discs from an ethylene atmosphere reduced ethylene production by the leaf tissue (Riov and Yang, 1982). Selecting a film that allows for ample ethylene movement is key. Cameron and Yang (1982) demonstrated that 95 % of the resistance to diffusion of ethane, which has diffusion properties similar to ethylene, away from cucumber fruits was due to the plastic film. Low-density polyethylene (LDPE) films are commonly used in MAP. Of the polyethylene films available, LDPE has the greatest permeability to ethylene, and ethylene permeability is independent of film thickness (Wang et al., 1998). Ethylene permeability increases with temperature; however, the effect of increasing temperature is much greater at lower temperatures (Wang et al., 1998). Another packaging alternative could be the use of films impregnated with

compounds, such as potassium permanganate, that absorb ethylene from the air (Chairat and Kader, 1999). However, absorption capacity is a limitation with this type of film.

Conclusions

At 25 °C, poinsettia cuttings produce detectable levels of wound ethylene as soon as 2 h after harvest. Leaf bending did not induce excess endogenous ethylene production in poinsettia cuttings. Cuttings harvested in the morning produced less ethylene than cuttings harvested in the afternoon. Storage temperature had the most pronounced effect on ethylene production – as storage temperature increased, ethylene production increased. Thus, ethylene synthesis is suppressed by storing cuttings at the lowest, non-damaging temperature possible. Understanding the effects of the postharvest environment on ethylene production is important in implementing changes in package design that eliminates or reduces exposure of cuttings to ethylene during shipment.

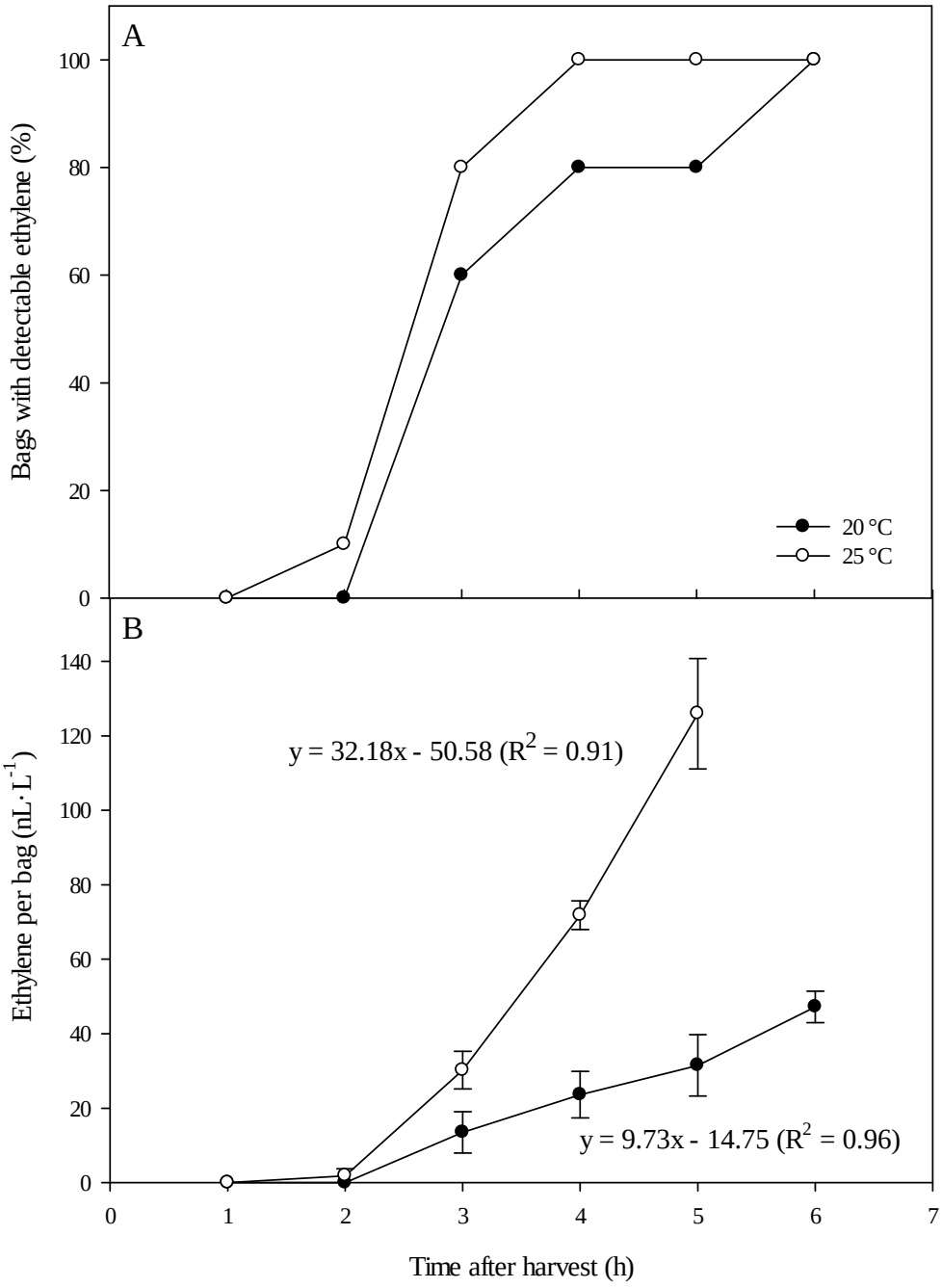

Figure 4.1. The effect of storage temperature (25 °C: ● and 20 °C: ○) on (A) the time-course of wound ethylene production of poinsettia cuttings in sealed 0.95-L plastic bags, and (B) the average amount of ethylene ($nL \cdot L^{-1}$) produced per bag. Error bars represent ±1 SE.

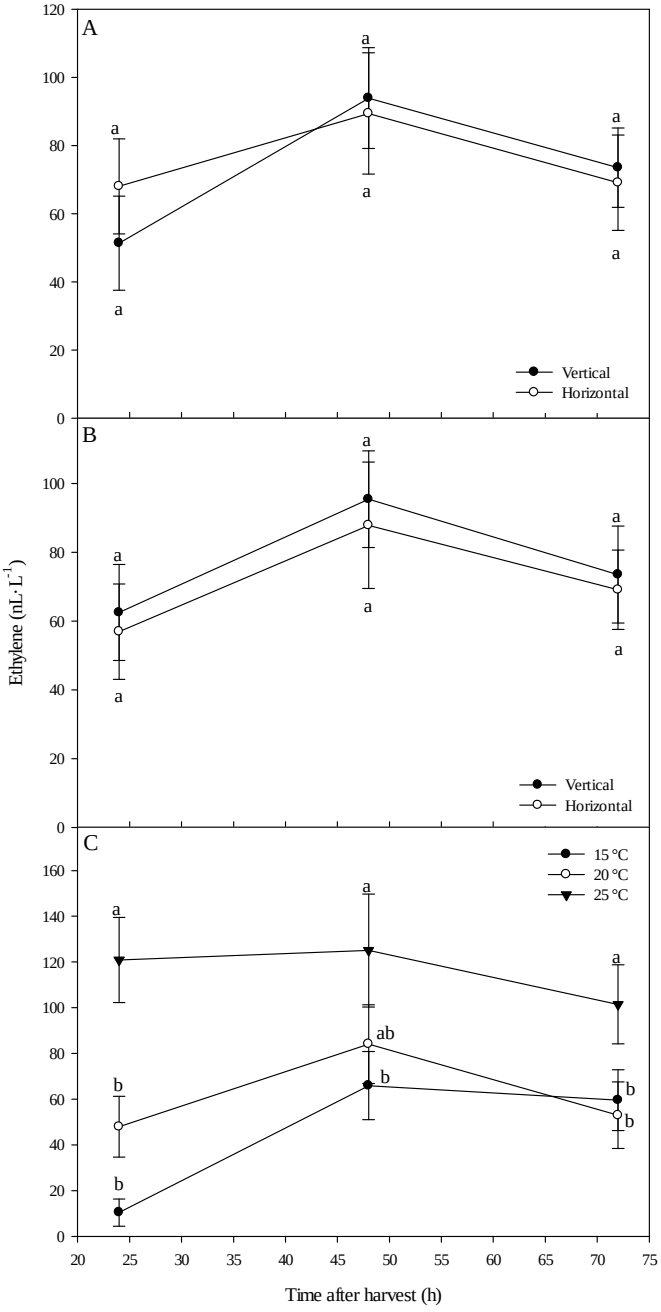

Figure 4.2. The effect of (A) leaf orientation (vertical: ● or horizontal:○), (B) cutting orientation (vertical: ● or horizontal:○), and (C) storage temperature (15 °C: ●, 20 °C: ○, or 25 °C: ▼) on the ethylene production of poinsettia cuttings stored in sealed 0.95-L plastic bags. Error bars represent ±1 SE. For each sub-figure (A, B, and C), treatments within a sample time (time after harvest) with the same letters are not significantly different ($P \leq 0.05$) via Fisher's LSD test.

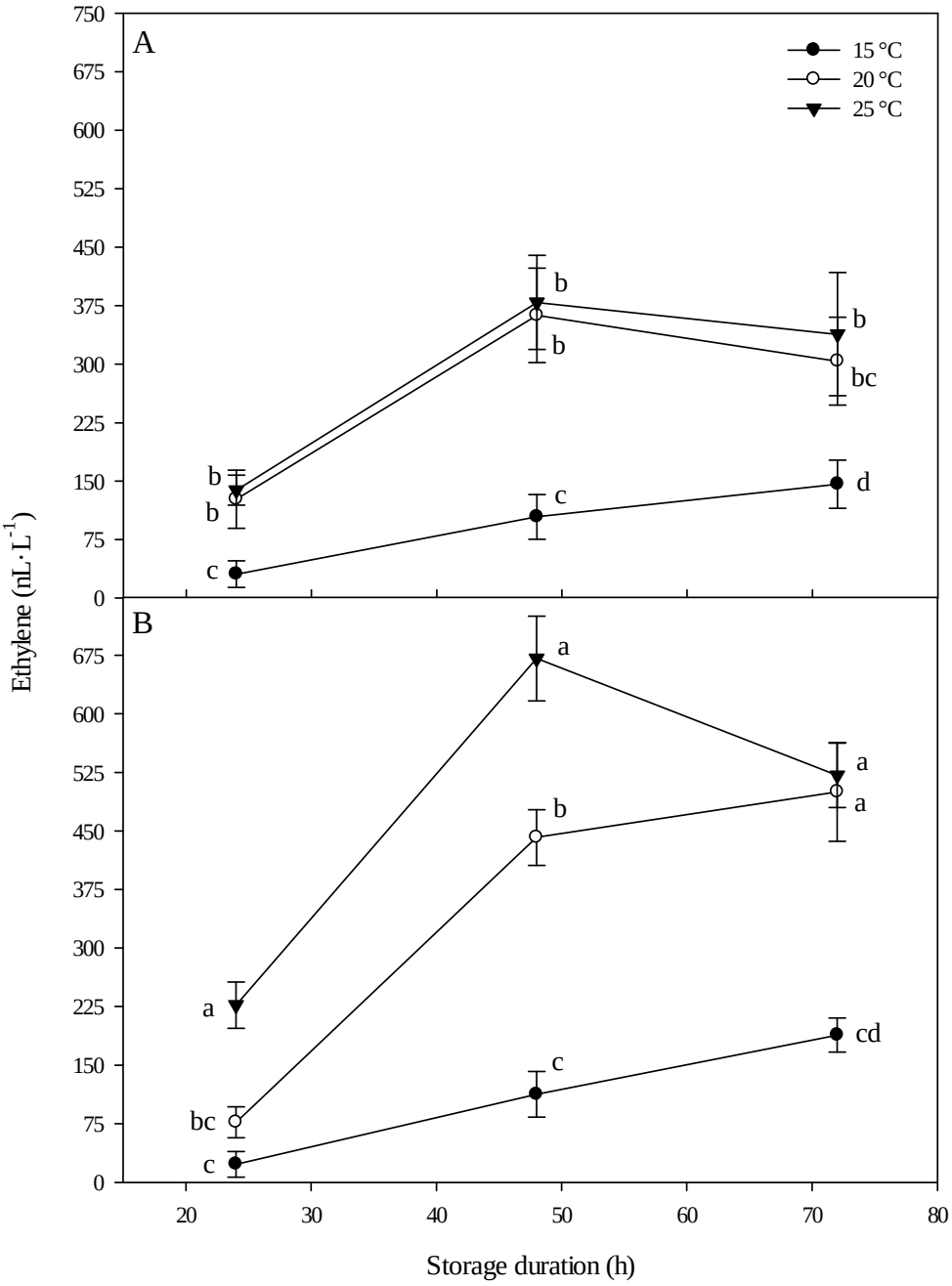

Figure 4.3. The effect of (A) a morning harvest (0900 HR) versus (B) an afternoon harvest (1500 HR) on the ethylene production of poinsettia cuttings stored in sealed 0.95-L plastic bags at 15 °C (●), 20 °C (○), or 25 °C (▼). Error bars represent ±1 SE. Across both harvest times, ethylene concentrations within a sample time (storage duration) with the same letters are not significantly different ($P \leq 0.05$) via Fisher's LSD test.

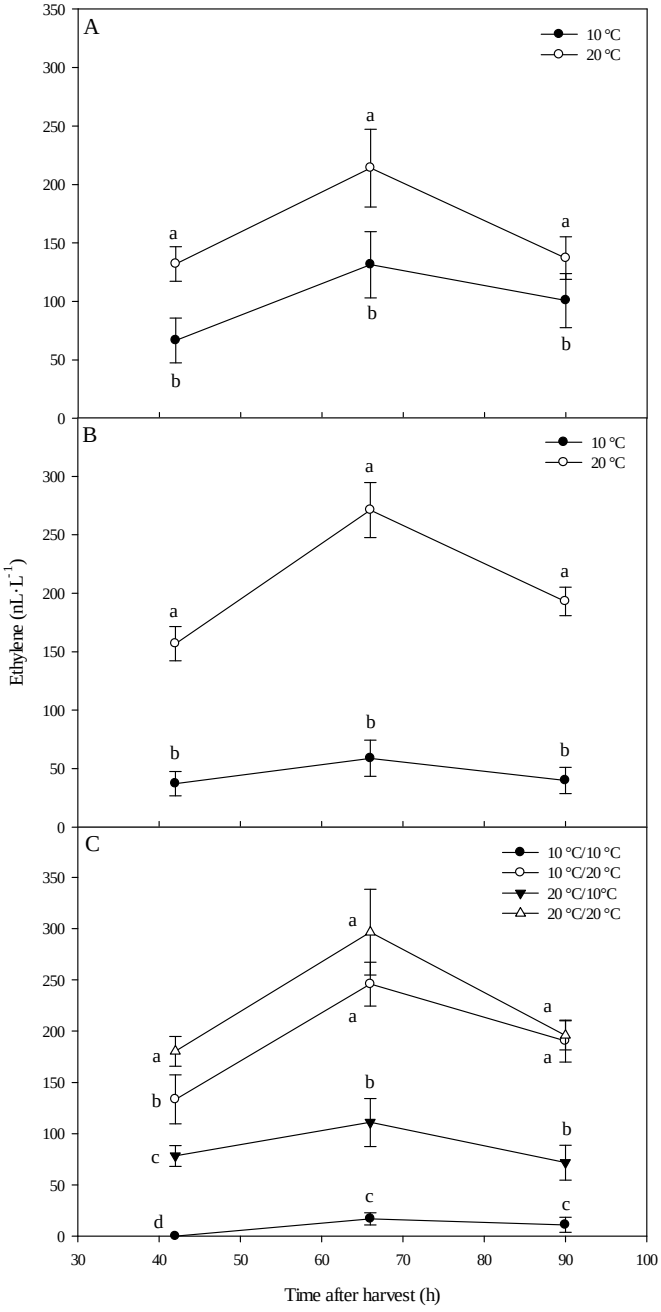

Figure 4.4. The effect of (A) pre-packaging temperature (10 °C: ● or 20 °C: ○), (B) post-packaging temperature (10 °C: ● or 20 °C: ○), and (C) their interaction (10 °C/10 °C: ●, 10 °C/20 °C: ○, 20 °C/10 °C: ▼, or 20 °C/20 °C: ▽) on the ethylene production of poinsettia cuttings stored in sealed 0.95-L plastic bags. Error bars represent ±1 SE. For each sub-figure (A, B, and C) treatments within a sample time (time after harvest) with the same letters are not significantly different ($P \leq 0.05$) via the Fisher's LSD test.

CHAPTER FIVE

STOCK PLANT DAILY LIGHT INTEGRAL AFFECTS THE CARBOHYDRATE STATUS AND PROPAGATION PERFORMANCE OF UNROOTED POINSETTIA CUTTINGS

Abstract

Poinsettia (*Euphorbia pulcherrima* ex Klotsch Willd. cv. Prestige Red) is an ornamental potted flowering plant that is commonly propagated from shoot-tip cuttings harvested from stock plants. In this study, we investigated the effect of daily light integral (DLI) delivered to the stock plants and time of harvest (0800 HR versus 1800 HR) on the carbohydrate dynamics, ethylene production, and propagation performance of unrooted poinsettia cuttings. Stock plants were delivered either a high-light (17.0 $mol \cdot m^{-2} \cdot d^{-1}$) or low-light (1.5 $mol \cdot m^{-2} \cdot d^{-1}$) environment for one week prior to removing cuttings. One hour prior to the first cutting harvest, stock plants were transferred to the opposite light environment. Eighteen cuttings were harvested from stock plants at each light environment twice daily. Nine of the cuttings were packaged in sealed 0.95-L plastic bags and placed through a simulated shipment prior to ethylene sampling and propagation. The shoot tips consisting of the meristem through first fully expanded leaf of the remaining nine cuttings were used for carbohydrate analysis. DLI had the most significant impact on cutting carbohydrate status. Carbohydrate levels were 2.5-times greater in plants that were transferred from low to high DLI conditions than plants transferred from high to low DLI conditions. Sugars accounted for a higher percentage of

total carbohydrates in low-light plants; however, starch never accounted for more than 20 % of the total carbohydrate content regardless of treatment. There was no significant correlation between cutting carbohydrate status and ethylene production; however, ethylene production during simulated shipment decreased as duration under low DLI increased. After 21 d in propagation, there was a significant effect of stock plant DLI on rooting performance. Cuttings harvested from low-light stock plants had lower root ratings, which declined as duration under low light increased. Furthermore, rooting performance was positively correlated with carbohydrate status, as carbohydrate concentrations increased, rooting increased.

Introduction

The production of vegetative shoot-tip cuttings for the U.S. floriculture industry occurs primarily in Mexico and Central America. Therefore, the success of U.S. growers has become increasingly dependent on the ability of offshore producers to ship high quality cuttings. Boxes of unrooted cuttings are shipped via cargo planes and ground transportation and usually reach their final destination in the U.S. 2 to 3 d after the cuttings are harvested. Much of the shipping environment lacks temperature control, exposing cuttings to non-optimal temperatures affecting both cutting quality and performance (Faust and Lewis, 2005; Wang, 1987). In 2006, more than 878 million unrooted cuttings with an average wholesale value of $0.07 each were imported into the U.S. (Jerardo, 2007). High vigor and quality are extremely important in regards to

vegetative cuttings since adventitious roots formation and further growth in propagation are essential.

Optimal light intensity varies between species and stage of production, for example light intensities during flowering or stock plant production are much higher than during propagation. A number of qualitative aspects of cuttings are improved by increased stock plant light intensity or DLI, such as vigor, resistance against fungal diseases, and rate of rooting (Spaargaren, 2001). Cut carnations produced during periods of low light intensity have a shorter vase life than those produced during periods of high light (Mayak and Dilley, 1976). However, lower irradiance levels during stock plant production increased rooting percentage of azalea (Economou and Read, 1986) and *Acer palmatum* (Behrens, 1988) cuttings. Thus, the environment provided to stock plants is important for rooting success.

DLI influences a number of plant characteristics including yield, flower size, flower number, branching, and plant size (Bredmore, 1993; Enfield, 2002; Niu et al., 2001; Serek, 1991). The rooting of cuttings is also influenced by DLI delivered in the propagation environment. Light levels below an optimum either reduce or eliminate rooting. This has been demonstrated in *Phlox paniculata* cuttings (Enfield, 2002), *Gypsophila paniculata* cuttings (Islam and Willumsen, 2001), *Rhododendron* cuttings (Davis and Potter, 1987), and petunia microshoots (Cabaleiro and Economou, 1992).

An increase in stock plant DLI results in increased photosynthesis. Carbohydrates are the direct products of plant photosynthetic activity. They constitute a source of energy

and metabolites as well as structural building blocks for the plant (Pun and Ichimura, 2003). Sufficient light for photosynthesis is required for plants to provide the needed dry matter for growth (Reid et al., 2002). Carbohydrate levels in plant tissues cycle both diurnally and seasonally (Clarkson et al., 2005; Rapaka et al., 2007b; Sivaci, 2006).

There are mixed results regarding the interaction between carbohydrate status and ethylene production (Philosoph-Hadas et al., 1985; Rapaka et al., 2007a). Ethylene is a naturally occurring gaseous plant hormone that is typically produced endogenously by plants in response to a stressor. Exposure to ethylene can stimulate flower wilting, shattering, failure to open or abscission (Nell and Leonard, 2005). In leaves, ethylene promotes chlorophyll degradation (yellowing), abscission and premature senescence (Saltveit, 1999). The severity of damage is dependent on the age of the commodity, temperature, ethylene concentration, and duration of exposure (Kader, 1985). Mechanical stresses experienced by cuttings during normal packaging and shipment, which do not cut or pierce the tissue, can induce ethylene production. Stress ethylene is the accelerated biosynthesis of ethylene in association with mechanical, environmental, or biological stresses (Morgan and Drew, 1997; Yakimova and Woltering, 1997). When plant tissue is cut, pierced, squeezed, bent, or touched, ethylene production is usually promoted.

Poinsettia is a common herbaceous ornamental that is propagated by shoot-tip cuttings. It is the number one flowering potted plant produced in the U.S. with over 40 million flowering plants sold in 2006 (Jerardo, 2007). The first objective of this study was to examine the number of days required for the carbohydrate status of stock plants

grown under low DLI conditions to recover under high DLI conditions and for the carbohydrate status of stock plants grown under high DLI conditions to be depleted under low DLI conditions. The second objective of this study was to evaluate the effect of carbohydrate status on ethylene production and subsequent propagation performance of poinsettia cuttings.

Materials and Methods

Plant material. Rooted poinsettia 'Prestige Red' cuttings were transplanted into 1.8-L plastic pots containing a commercial peat moss mix (Middleweight Mix #3-B, Fafard, Inc., Anderson, S.C., U.S.A.) and grown as stock plants in the greenhouse for one year prior to the experiment. They were fertilized at every irrigation with water containing water-soluble fertilizer providing ($mg \cdot L^{-1}$) 250 N, 37 P, 208 K, 82 Ca, 33 Mg, plus micronutrients (Peters Excel; Scotts-Sierra Horticultural Products Company, Marysville, Ohio, U.S.A.). Heating/ventilation set points for temperature control were 18.3/22.2 °C, providing an average air temperature of 20 °C. Plants were shaded when the irradiance outside the greenhouse exceeded 1035 $mol \cdot m^{-2} \cdot s^{-1}$.

Stock plant DLI. In January 2007, one week prior to cutting harvest, stock plants were placed in growth rooms under two different light levels. Fifty stock plants were placed in each room. Temperature was maintained between 21 and 23 °C, and artificial lighting was provided by metal halide lamps for 16 $h \cdot d^{-1}$ (0800-2400 HR). The metal halide lamps were the only source of light. The high-light growth room provided plants

with a PPF of 295 µmol·m^{-2}·s^{-1} resulting in a DLI of 17.0 mol·m^{-2}·d^{-1}. Plants in the low-light growth room were placed under shade cloth, which reduced light levels by 90 %. The PPF under the shade cloth was 26 µmol·m^{-2}·s^{-1} resulting in a DLI of 1.5 mol·m^{-2}·d^{-1}.

Cutting harvest. After one week growing in either a 1.5 or 17.0 mol·m^{-2}·d^{-1} DLI environment, stock plants were transferred to the opposite light environment between 0700-0800 HR. The first cutting harvest (0800 HR) occurred immediately after stock plant transfer. Stock plants were maintained in the transferred DLI environment for the remainder of the experiment. Eighteen cuttings (6-cm stem length) were harvested from each DLI environment twice daily (0800 and 1800 HR) for 4 d. Nine cuttings were used for propagation performance and ethylene production, and nine cuttings were used for carbohydrate analysis.

Cuttings used for evaluation of propagation performance and ethylene production were weighed, and three cuttings from each DLI treatment were placed in each of 6 (3 bags per DLI treatment) sealed 0.95-L, 68.6 µm-thick, plastic freezer bags (Ziploc, S.C. Johnson & Son, Inc., Racine, Wis., U.S.A.). The bags of cuttings from each harvest were then subjected to a 48-h simulated shipment: 10 h at 10 °C, 14 h at 15 °C, 10 h at 10 °C, and 14 h at 25 °C. Following simulated shipment, air samples were taken from each bag and analyzed for ethylene content, and the cuttings were propagated as described below. Cuttings used for carbohydrate analysis were prepared as described below.

Measurement of ethylene production. At the end of simulated shipping, two headspace gas samples (1-mL) were removed from each bag through a silicon port using

tuberculin syringes. Samples were analyzed for ethylene content using a gas chromatograph with a flame ionization detector (Shimadzu GC-9A, Kyoto, Japan). Ethylene concentrations were determined per gram of cutting fresh weight.

Propagation environment and determination of cutting quality. Following the 48-h postharvest treatment, the cuttings were inserted into an inert foam medium (Oasis Wedge, Smithers-Oasis North America, Kent, Ohio, U.S.A.) and placed on a mist propagation bench. No rooting hormones were applied. The mist system was operated during the daylight hours (6 s every 6 min) through the duration of the experiment. Greenhouse heating/ventilation temperature set points of 23/26 °C provided an average air temperature of 23 °C, and bench heating provided a rooting media temperature of 25 °C. The relative humidity in the greenhouse averaged 55 %. Cuttings were shaded when the outside irradiance exceeded 517 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

After 21 d in propagation, rooting response and shoot quality were visibly determined from three replications of three cuttings per treatment using the following scoring systems. For the shoots after 21 d in propagation, 1: dead; 2: poor, more than four abscised leaves; 3: average, three to four abscised leaves; 4: good, one to two leaves abscised; 5: excellent, no leaf abscission. For rooting, 1: no rooting; 2: poor, 1 or 2 roots visible on exterior of foam wedge; 3: average, foam wedge 50 % filled with visible roots; 4: good, foam wedge 75 % filled with visible roots; 5: excellent, foam wedge greater than 75 % filled with visible roots. The same person evaluated all of the treatments and recorded the visual ratings.

Carbohydrate analysis. Carbohydrate concentrations were analyzed in the tips (meristem through first fully-expanded leaf) of cuttings (3 replicates of 3 cutting tips per DLI and time of day treatment) immediately after harvest. Plant tissue was immediately frozen in liquid nitrogen and stored at -70 °C until lyophilization. Following lyophilization, plant material was ground and soluble sugars from 50 mg of tissue were extracted with 12 methanol:5 chloroform:3 water (MCW; by volume) as described by Miller and Langhans (1989). Mannitol (1 mg) was added as an internal standard. The extracts were evaporated to dryness in vacuo at 40 °C, and the residue was dissolved in 2 mL high-purity water (18.2 $M\Omega cm^{-1}$, NANOpure Diamond, Barnstead International, Dubuque, Iowa, U.S.A.). Glucose, fructose, and sucrose were separated using a Dionex DX-300 High Performance Liquid Chromatography system with a 4 mm × 250 mm CarboPac™ column and detected with an electrochemical detector (Dionex, Sunnyvale, Calif., U.S.A.). Quantification of the above sugars was based on the calibration curves obtained from their respective standards. Starch in dried residue following soluble sugar extraction was determined using enzymatic hydrolysis of starch using amyloglucosidase (from Rhizopus mold, EC 3.2.1.3; Sigma chemicals, St. Louis, Mo., U.S.A.) into glucose (Haissig and Dickson, 1979).

Statistical analysis. The experiment was conducted with three cuttings per replicate, and three replicates per treatment (n=3). Data were analyzed with the analysis of variance (ANOVA), general linear model (GLM), and correlation model (CORR) procedures in SAS v. 9.1 (SAS Institute, Inc., Cary, NC, USA). Main effects were compared ($P \leq 0.05$) using Fisher's least significant difference (LSD) test. When treatment

interactions were significant, means of the interactions were compared ($P \leq 0.05$) using the least-square means (LSMEANS) test.

Results

Carbohydrate analysis. The significance of treatment effects on glucose, fructose, reducing sugars (RS; glucose + fructose), sucrose, total sugars (TS; RS + sucrose), starch, and total non-structural carbohydrates (TNC; TS + starch) is presented in Table 5.1. Stock plant DLI independently and the interaction of the number of days in the DLI treatment and stock plant DLI had the most significant effect on the carbohydrate status of cuttings. Cuttings grown under high light had TNC concentrations 2.5-times greater than low-light cuttings (Fig. 5.1). TNC concentrations in high-light cuttings increased through the second day and then remained constant. Concentration in low-light cuttings decreased through the third day. Time of harvest had a significant effect on TNC concentration, increasing between 0800 HR and 1800 HR.

RS and sucrose concentrations each accounted for 50 % of the TS concentration. The ratio of TS to TNC was constant under low DLI conditions and decreased under high DLI conditions as day of harvest increased. TS accounted for 95-99 % of TNC concentrations in low DLI grown cuttings and 83-89 % of TNC concentrations in high DLI grown cuttings.

Ethylene production. As days in the DLI treatment increased, ethylene production increased in cuttings harvested from high DLI stock plants and decreased in cuttings

harvested from low DLI stock plants independent of time of day the cuttings were harvested (Fig. 5.2). On the first day of cutting harvest there was no difference in ethylene production across all treatments. Cuttings harvested in the morning from low DLI stock plants on the second day produced significantly more ethylene then the other treatments. During morning harvests, cuttings from low DLI stock plants produced more ethylene than high DLI cuttings until the third day, while during evening harvests, low DLI cuttings produced more ethylene than high DLI cuttings until the fourth day (Fig. 5.2).

When ethylene production was subjected to a factorial ANOVA, days in the DLI treatment (D) was significant at $P \leq 0.05$; time of harvest (T) was significant at $P \leq 0.001$; and stock plant DLI (L) was not significant. Two of the two-way interactions, D×T ($P \leq 0.05$) and D×L ($P \leq 0.01$), were significant as well as the three-way interaction, D×T×L ($P \leq 0.01$). To determine the validity of the interaction, the significant two-way interactions were sliced by main effects. The 0800 HR harvest on the second day was the only D×T interaction that was significant. When the D×L interaction was sliced by day, the second, third, and fourth harvest day were all significant, and when it was sliced by DLI, 1.5 $mol \cdot m^{-2} \cdot d^{-1}$ was significant. Therefore, at low DLI conditions, as days in the DLI treatment increased, ethylene production decreased. When correlations between ethylene and carbohydrate concentrations were calculated, no correlations were present.

Propagation performance. After 21 d in propagation, there was no significant difference in shoot quality (Table 5.2). Stock plant DLI had a significant effect ($P \leq 0.001$)

on root quality. Cuttings harvested from low DLI stock plants had lower root ratings, which declined as duration under low DLI increased. Cuttings harvested from low DLI plants at 1800 HR had lower root rating than cuttings from the 0800 HR harvest, but differences were not significant. Root quality was positively correlated to the carbohydrate status of the cutting. As total non-structural carbohydrate concentrations increased, root rating increased ($R^2 = 0.44$, $P \leq 0.01$).

Discussion

Stock plants were grown under the opposite DLI treatment for one week prior to cutting harvest to examine the number of days required for the carbohydrate status of cuttings to recover after low DLI stock plants were transferred to high DLI, and the number of days required to deplete the carbohydrate levels in cuttings after high DLI stock plants were transferred to low DLI. Upon transfer to the opposite DLI environment, carbohydrate levels in the cuttings changed rapidly. By the 1800 HR harvest on the first day, carbohydrate levels had been depleted under a low DLI environment and increased under a high DLI environment. Carbohydrate levels continued to increase at high DLI and decrease at low DLI through the third day before becoming constant.

Current commercial cutting production practices emphasize morning harvests in order to meet cutting demand for shipping and to reduce exposure to heat. Similarly, most vegetables are harvested in the morning when the temperature and respiration rates are low (Thompson, 1996). It has been suggested that harvesting leafy vegetables in the

afternoon would cause less damage during shipping (Phan, 1987), and delayed harvest of some species of vegetative cuttings would improve postharvest quality and performance (Rapaka et al., 2007b). In the case of poinsettia cuttings, while the cuttings harvested at 1800 HR from a low DLI had lower root ratings, there was no significant effect of time of harvest on propagation performance.

On the other hand, stock plant DLI influenced the postharvest storage quality of poinsettia cuttings. Rooting quality was higher for cuttings harvested from high DLI stock plants. As duration under low DLI growing conditions increased, rooting quality decreased. Stock plant DLI influences several quality aspects of vegetative cuttings including vigor and rate of rooting (Spaargaren, 2001). In this study, shoot quality at the end of propagation was not affected by stock plant DLI.

The rooting ability of cuttings is often discussed in relation to carbohydrate content (Rapaka et al., 2005). Sugars can increase or have no effect on rooting. In this study, rooting performance correlated with carbohydrate content. As carbohydrate concentration decreased, root rating decreased. Similarly, *Potentilla* microcutting establishment increased with the application of sucrose (Wainwright and Scrace, 1989). However, in azalea cuttings, there was no correlation between sugar level and rooting (Apine and Kondratovičs, 2005). The carbohydrate status of cuttings not only has implications for rooting success, but also storage potential. Increased shelf-life in cut flowers has been associated with high-light growing conditions resulting in increased carbohydrate contents in the flowers (Pun and Ichimura, 2003). High carbohydrate status has also been shown to increase the storage life of lettuce and cabbage (Lipton, 1987).

The leaf starch pool of tomato plants exposed to low-light conditions was depleted within 12 h of darkness, while it took 48-60 h of darkness for the starch pool to be depleted in high-light adapted plants (Gary et al., 2003).

Photosynthesis replenishes carbohydrates that are metabolized or mobilized overnight. In this study, carbohydrate concentrations accumulated in the meristematic tissues during the day, indicative of a diurnal pattern. Regardless of DLI, TNC concentrations were 21 % higher in cuttings harvested in the afternoon. This same diurnal trend has been documented for lantana cuttings (Rapaka et al., 2007b), portulaca cuttings (Rapaka et al., 2007a), and leafy green vegetables (Clarkson et al., 2005). On the other hand, the TS concentration in Chinese cabbage did not vary with harvest time (Klieber et al., 2002).

This study showed that stock plant DLI had a significant effect on cutting TNC concentrations. In low DLI stock plants, the TNC concentrations in the harvested cuttings were lower than in cuttings harvested from high DLI stock plants. Irradiance levels have been shown to be positively correlated to the carbohydrate status of several plants including Easter lily (Miller and Langhans, 1989), *Ficus* (Veneklass and den Ouden, 2005), *Pelargonium* cuttings (Rapaka et al., 2005), and 'TifEagle' bermudagrass (Bunnell et al., 2005). Gary et al. (2003) found that carbohydrate and starch concentrations were 30-50 % lower in tomato leaves following exposure to low irradiance.

Rapaka et al. (2007a, 2007b) found significantly higher starch levels in leaves when compared to TS. In this study, however, the TS levels were higher when compared to starch. Starch never accounted for more than 20 % of the TNC concentration at any

cutting harvest. Since the meristems of cuttings, which are sinks for photosynthates, were analyzed, it would be expected that sugar levels would be higher compared to starch, which is the primary storage form of carbohydrates. However, as the duration stock plants were under a high DLI increased, the amount of starch present in the meristematic tissue increased, whereas under low DLI, almost no starch was present, indicating that photosynthesis was not adequate to meet plant demand for photosynthates. Low light levels, particularly those found in interior environments, result in levels of photosynthesis that produce less than half of the carbohydrates needed for metabolic processes in plants (Reid et al., 2002).

The effect of cutting carbohydrate status on postharvest ethylene production was also investigated. The results indicate that carbohydrate status had no effect on the production of ethylene by poinsettia cuttings. Similarly, Rapaka et al. (2007a, 2007b) found no significant correlation between ethylene production and carbohydrate concentration in portulaca and lantana cuttings. In contrast, elevated carbohydrate levels can enhance or delay ethylene production. The application of glucose and sucrose to tobacco leaf discs (Philosoph-Hadas et al., 1985) and elevated carbohydrate levels in broccoli florets (Nishikawa et al., 2005) stimulated ethylene production, while harvested carnation flowers showed delayed ethylene production (Verlinden and Garcia, 2004). This suggests that the regulation of ethylene production by carbohydrates may be dependent on species, tissue, or developmental stage.

Furthermore, just as carbohydrate status did not influence ethylene production, time of harvest also had no effect on ethylene production. The same effect has been documented for portulaca and lantana cuttings (Rapaka et al., 2007a, 2007b). However Rapaka et al. (2007a, 2007b) demonstrated that although time of harvest did not affect ethylene production, it did affect ethylene sensitivity. In this study, unrooted poinsettia cuttings showed no changes in ethylene sensitivity, with little to no leaf abscission occurring after simulated shipment.

Stock plant DLI did not have a clear effect on ethylene production. However, as duration of exposure to low DLI conditions increased, ethylene production tended to decrease. Varying responses in ethylene production have been found in plants grown at different DLIs. For example, in *Delphinium* cut flowers, high light intensities during production decreased ethylene production (Pun and Ichimura, 2003), while *Campanula carpatica* plants grown under high irradiance produced more ethylene than plants grown under low light levels (Serek, 1991).

Conclusions

When poinsettia cuttings are harvested from stock plants exposed to low DLI conditions for several days prior to harvest, producers are likely to notice delayed rooting during propagation. The DLI stock plants are exposed to prior to harvest affects the carbohydrate status of the cuttings, which impacts propagation performance. As the number of days the stock plants were grown under low DLI conditions increased, the

TNC status of the cuttings decreased which correlated to a decrease in rooting. On the other hand, ethylene production during simulated shipment did not correlate with cutting carbohydrate status. Therefore, it is recommended that commercial producers should consider practices that optimize greenhouse light levels such as improved shade curtain management, the use of high-transmission glazing, or supplemental lighting in order to maintain the carbohydrate status of the cuttings. This would help improve the postharvest quality and propagation performance of unrooted poinsettia cuttings.

Table 5.1. ANOVA of treatment effects on the carbohydrate status of unrooted poinsettia 'Prestige Red' cuttings.

Treatment	Glu[z]	Fruc	RS	Suc	TS	Starch	TNC
R^2	0.82	0.83	0.83	0.82	0.86	0.90	0.90
Day[y] (D)	**	**	**	NS	NS	**	NS
Light (L)	***	***	***	***	***	***	***
Time (T)	NS	NS	NS	**	**	NS	**
D×L	***	***	***	***	***	***	***
D×T	NS	NS	NS	NS	NS	NS	NS
L×T	NS	NS	NS	**	**	NS	**
D×L×T	*	**	**	*	**	**	***

[z]Abbreviations: Glu = glucose, Fruc = fructose, RS = reducing sugars (glucose + fructose), Suc = sucrose, TS = total sugars (RS + sucrose), TNC = total non-structural carbohydrates (TS + starch)

[y]Day = days in DLI treatment, Light = stock plant daily light integral, Time = time of harvest (0800 HR versus 1800 HR)

NS, *, **, *** Non-significant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 5.2. The effect of days in DLI treatment, time of harvest, and stock plant daily light integral on average shoot and root quality of poinsettia 'Prestige Red' cuttings after 21 d in propagation. Values represent average rating ±1 SE.

Days in DLI treatment	Time of Harvest	Shoot Rating		Root Rating	
		DLI ($mol \cdot m^{-2} \cdot d^{-1}$)		DLI ($mol \cdot m^{-2} \cdot d^{-1}$)	
		1.5	17.0	1.5	17.0
1	0800 HR	5.0±0.0 a[z]	4.9±0.1 a	3.9±0.3 a	3.2±0.8 ab
	1800 HR	4.6±0.4 a	4.6±0.4 a	2.5±0.4 bc	3.7±0.7 a
2	0800 HR	5.0±0.0 a	4.9±0.1 a	3.0±0.0 ab	3.4±0.1 ab
	1800 HR	5.0±0.0 a	5.0±0.0 a	2.7±0.4 abc	3.5±0.2 a
3	0800 HR	4.8±0.2 a	5.0±0.0 a	2.8±0.7 abc	3.5±0.4 a
	1800 HR	4.6±0.4 a	5.0±0.0 a	2.2±0.2 bc	3.7±0.4 a
4	0800 HR	5.0±0.0 a	4.9±0.1 a	2.2±0.4 bc	3.1±0.6 ab
	1800 HR	4.9±0.1 a	5.0±0.0 a	1.6±0.1 c	3.2±0.5 ab

[z]Mean separation within each treatment by Fisher's LSD test ($P \leq 0.05$). Numbers within either shoot rating or root rating with the same letter are not statistically different.

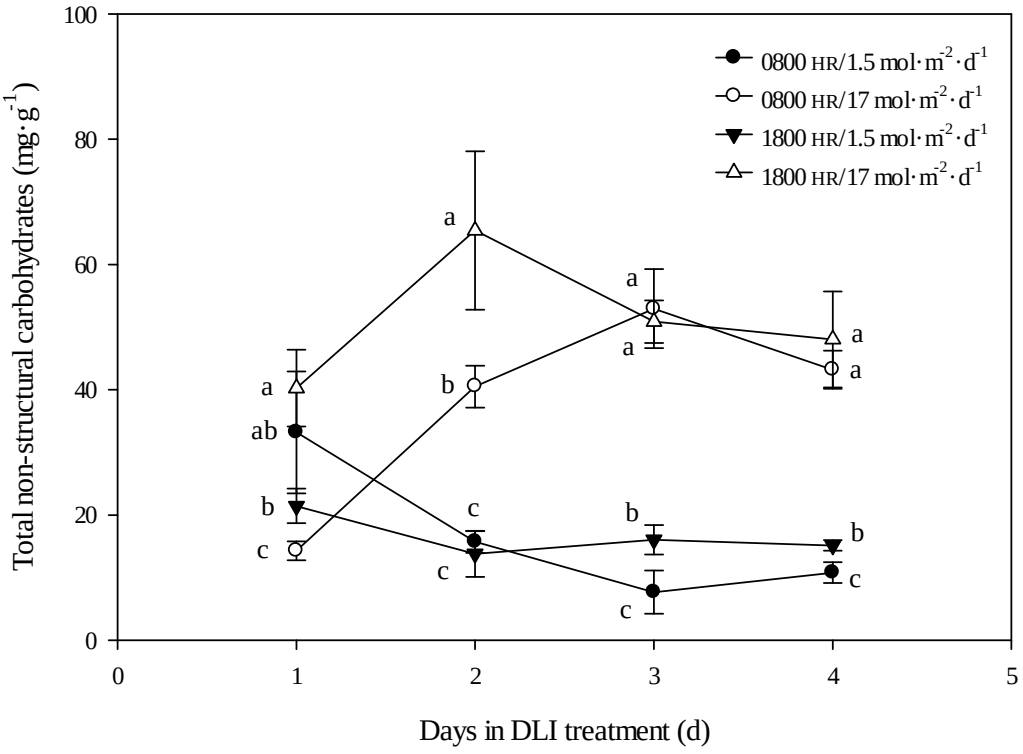

Figure 5.1. Effect of stock plant DLI and time of harvest on total non-structural carbohydrate concentrations of unrooted poinsettia 'Prestige Red' cuttings. Error bars represent ±1 SE. Values are expressed on a dry matter basis. Values with the same letter within a sample time (days in DLI treatment) are not significantly different ($P \leq 0.05$) via Fisher's LSD test.

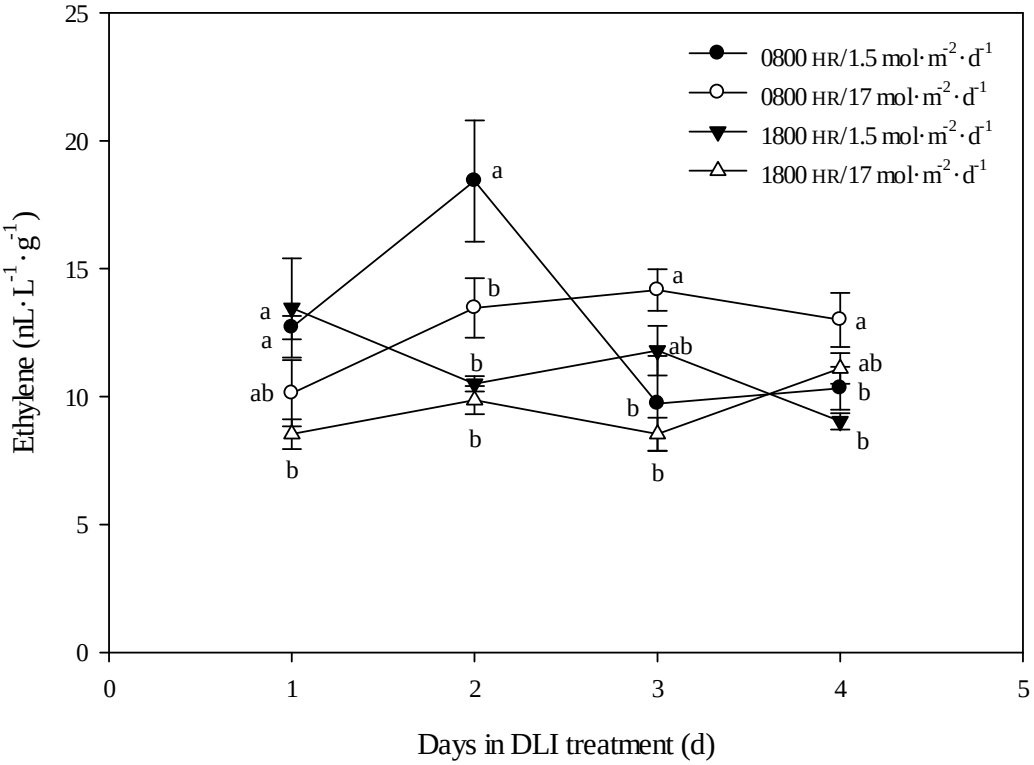

Figure 5.2. The effect of stock plant daily light integral, time of harvest, and days in the DLI treatment on the ethylene production in sealed 0.95-L sealed plastic bags by unrooted poinsettia cuttings harvested at 0800 HR or 1800 HR. Ethylene concentrations are presented on a gram fresh weight basis. Error bars represent ±1 SE. Ethylene concentrations within a sample time (days in DLI treatment) with the same letters are not significantly different ($P \leq 0.05$) via Fisher's LSD test.

CONCLUSIONS

Currently, the vegetative cuttings industry works within the framework that temperature is not controlled but merely modified for a limited number of hours after the boxes are shipped. However, this research project has determined that postharvest temperature is the primary factor affecting the physiology and performance of vegetative cuttings. Temperature controls the respiration rates of vegetative cutting which affects carbohydrate availability for root production in propagation post-shipping/storage. As postharvest temperature increases, respiration rates increase resulting in an accelerated depletion of carbohydrate reserves. The depletion of carbohydrate reserves is further exasperated when cuttings are harvested from stock plants grown under low light environmental conditions. Temperature also affects ethylene production in vegetative cuttings. As postharvest temperature increases, ethylene production increases which can result in leaf chlorosis and abscission. Finally, postharvest temperature affects the potential storage duration of vegetative cuttings. Under non-optimal storage temperatures, storage potential is reduced and propagation performance is also adversely affected.

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