

ABSTRACT

LEATHERWOOD, WILLIAM ROLAND. Managing the Postharvest Physiology of Unrooted Cuttings to Enhance Shipping and Postharvest Quality. (Under the Direction of John M. Dole).

United States ornamental plant producers imported approximately \$61 million worth of unrooted cuttings in 2006. The top three greenhouse production crops geraniums (*Pelargonium* L'Hér. ex Ait.), poinsettias (*Euphorbia pulcherrima* Willd. ex Klotzsch) and New Guinea impatiens (*Impatiens hawkeri* W. Bull) had a wholesale value of \$330 million and were produced from approximately 138 million cuttings. Understandably, improving cutting quality by reducing losses induced by ethylene exposure and shipping damage is an area of active research. To investigate possible areas for improvement in cutting production and shipping modified atmosphere (MA) storage of unrooted cuttings, the use of 1-methylcyclopropene (1-MCP) use as an ethylene inhibitor of unrooted cuttings and ethephon, [(2-chloroethyl) phosphonic acid] use in stock plant management was studied.

Modified atmosphere storage of impatiens 'Sonic Red', geranium 'Kardino' and poinsettia 'Visions of Grandeur' cuttings showed that cuttings held in 1:20 oxygen:carbon dioxide generated higher ethylene concentrations than any other treatment including atmospheric control. Cuttings stored in ratios of 10:10, 10:5, 5:10, 5:5 oxygen to carbon dioxide generated less ethylene compared to atmospheric control. Cuttings stored in 10:5 performed best during propagation, with less leaf yellowing or abscission than any other treatment.

Application of $700 \mu\text{L} \cdot \text{L}^{-1}$ 1-MCP prior to ethylene treatment prevented ethylene damage to *Begonia hybrida* 'Anita Louise', *Portulaca oleracea* L. 'Sleeping Beauty' and *Lantana camara* L. 'Patriot Sunbeam'. Also, $700 \mu\text{L} \cdot \text{L}^{-1}$ 1-MCP application to poinsettia 'Visions of

Grandeur', impatiens 'Sonic Red', geranium 'Rocky Mountain White', *Pelargonium peltatum* (L.) L'Hérit. 'Mandarin', *Petunia* × *hybrida* 'Suncatcher coral prism' cuttings caused significant ethylene generation. 1-MCP application reduced geranium 'Kardino' root numbers and delayed adventitious root formation of *Angelonia angustifolia* Benth. 'Carita Lavender', *Calibrachoa* × *hybrida* Llave & Lex. 'Terra Cotta', *I. hawkeri* 'Sonic Red', *Portulaca oleracea* L. 'Fairytale', *Sutera cordata* Kuntze 'Abunda Blue Improved' and *Verbena* × *hybrida* Groenl. & Ruempl. 'Aztec Wild Rose', though 1-MCP rooting effects were overcome by subsequent immediate exposure to ethylene.

Ethephon is used to increase stock plant branching and sink tissues abscission. Ethylene evolution from cuttings harvested from recently treated stock plants is suspected to cause leaf abscission of unrooted cuttings during shipping. Impatiens 'Sonic Red' and 'Sonic White' cuttings harvested from stock plants treated with higher ethephon doses resulted in greater ethylene concentrations during storage. Cuttings harvested 24 hours after treatment with 0, 250, 500 or 1000 mg · L⁻¹ ethephon produced 0.07, 1.3, 1.7 or 5.8 mg · L⁻¹ · g⁻¹ (fresh weight) ethylene in the first 24 hours of storage at 20 °C, respectively. Cuttings harvested 24 hours after treatment with 500 mg · L⁻¹ ethephon stored at 10, 15, 20, and 25° C for 24 hours produced 0.37, 0.81, 2.03 and 3.55 mg · L⁻¹ · g⁻¹ (fresh weight) ethylene. Ethephon treatment effects were measurable on harvested cuttings up to 3 weeks post application.

Managing the Postharvest Physiology of Unrooted Cuttings to Enhance Shipping and
Postharvest Quality

by
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DEDICATION

When I am among the trees,
especially the willows and the honey locust,
equally the beech, the oaks and the pines,
they give off such hints of gladness.
I would almost say that they save me, and daily.

I am so distant from the hope of myself,
in which I have goodness, and discernment,
and never hurry through the world
but walk slowly, and bow often.

Around me the trees stir in their leaves
and call out, "Stay awhile."
The light flows from their branches.

And they call again, "It's simple," they say,
"and you too have come
into the world to do this, to go easy, to be filled
with light, and to shine."

- Mary Oliver

BIOGRAPHY

William Roland Leatherwood was born November 7, 1969 to Richard Keith and Heidi Impertro Leatherwood. Roland's university education began in August 1988 at the University of North Carolina Asheville where he received a Bachelor of Arts degree in German and Bachelor of Science degrees in Economics and Management. After working in public mental health for several years, Roland elected to attend North Carolina State University to pursue an advanced degree in Horticulture. While taking preparatory post-baccalaureate classes, Roland worked closely with Dr. Eric Davies isolating polysomes from gravistimulated corn roots. In May of 2005, Roland completed his Masters of Science in the Department of Horticultural Science at North Carolina State University under the direction of Dr. John Williamson. In August of 2005, Roland began work towards his Doctorate in the same department under the direction of Dr. John Dole.

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LITERATURE REVIEW

Possible strategies for managing the postharvest physiology of unrooted herbaceous cuttings.

The Economic Value of Unrooted Cuttings

Ornamental plant producers use cuttings shipped from around the world in finished plant production. The value of unrooted cutting imports into the United States totaled over \$61 million in 2006 with the bulk coming from Central America, South America, Mexico, and Asia (Foreign Agricultural Service, 2007). In the same year finished flats, hanging baskets, and pots for just three cutting propagated species, geraniums (*Pelargonium* L'Hér. ex Ait.), New Guinea impatiens (*Impatiens hawkeri* W. Bull) and poinsettias (*Euphorbia pulcherrima* Willd. ex Klotzsch) from the top 15 producing states, had a wholesale value of \$330 million and were produced from approximately 138 million cuttings (National Agriculture Statistics Service, 2007). In this case, the loss of a single cutting is \$2.38 lost in potential revenue.

Understandably, reducing losses of cuttings during shipping is of critical research interest to cutting producers, rooting stations, and finished plant producers. Postharvest quality of cuttings may be increased with improved stock plant culture, by slowing general postharvest metabolism, reducing postharvest ethylene exposure, and inhibiting ethylene response. Application of these techniques singly or in combination may allow growers to schedule labor more efficiently, tailor shipping schedules and deliver a higher quality product.

Modified Atmosphere Storage

The energy required for maintaining homeostasis during postharvest handling comes from extant cutting carbohydrate, lipid and protein reserves. The abiotic stresses imposed by harvest and shipping tend to up regulate respiration hastening the loss of carbohydrate reserves (Sturm and Tang, 1999). Quality of subsequent rooting is also directly proportional to carbohydrate content (Rapaka et al., 2005). Whether sugars regulate senescence is still an open question (Wingler et al., 2006) and their role in regulating postharvest senescence is even more unknown. However the positive correlation between carbohydrate content and postharvest viability is well established (Kader and Watkins, 2000; Rapaka et al., 2005) and senescence processes may also be hastened by low carbohydrate reserves (van Doorn, 2004). Thus, methods of slowing postharvest carbohydrate use in cuttings are of great interest to growers.

Modified atmosphere packaging (MAP) and controlled atmosphere (CA) storage are broadly used to increase shelf life of many agricultural products from seafood to cut flowers. The adjusted atmosphere is achieved by altering O₂ and CO₂ partial pressures using a predetermined gas mixture (active modified atmosphere) or by specialized packaging films that selectively limit the diffusion of gasses (passive modified atmosphere).

Responses of tubers, fruits, leaves, stems and flowers to modified atmosphere storage has been widely studied and documented (Beaudry, 1999; Beaudry, 2000; Farber and Dodds, 1995; Kader and Watkins, 2000; Watkins, 2000). Green beans stored under increased CO₂ concentrations have reduced titratable acidity and an increase in soluble proteins (Buescher and Adams, 1983). Additionally, green beans stored in 3% oxygen 3% carbon dioxide (3:3)

maintained higher soluble sugars and overall quality compared to those stored in air (Sanchez-Mata et al., 2003).

Low oxygen environments (~1.5%) have been used to extend the shelf life of basil to 45 from 18 days, yet any increase in CO₂ concentrations reduced storage time for this crop (Lange and Cameron, 1998). To the contrary, investigators have shown that respiration is reduced in lettuce stored in super-atmospheric CO₂ concentrations alone (Escalona et al., 2006). Modified atmosphere storage (5:10) extended broccoli shelf life, by reducing water loss, chlorophyll loss, and soluble sugar consumption. Additionally, increased soluble sugar concentrations also correlate with reduced expression carbohydrate transport proteins. Down regulation of citrate synthase in modified atmosphere treated broccoli suggests reduced lipid metabolism (Eason et al., 2007).

These studies make clear that responses to MA vary among species, organ types, and developmental stages for the same modified atmosphere treatment. The technology can extend shelf life in one instance or cause destructive anaerobic metabolism in another. For example, low O₂ concentrations increase lettuce shelf life (Beaudry, 1999), but activates fermentation metabolism in apples (Beaudry, 2000). Additionally, different plant tissues appear to have different tolerances for changes in atmospheric oxygen and carbon dioxide concentrations (Beaudry, 1999; Izumi et al., 1996) resulting in a broad range of results for the same plant.

Metabolic Responses

Changes in modified atmosphere gas composition, specifically lowering O₂ content, are generally aimed at slowing respiration thus increasing available carbohydrates via metabolic rationing and increasing subsequent storage time. Kidd and West (1927; 1945) established the general principle indirectly by measuring carbohydrate content of controlled atmosphere (CA) stored seeds and fruits. Slowing primary metabolism by decreasing O₂ concentration is successfully applied in a range of crops and the positive relationship between carbohydrate content and postharvest performance of produce, cut flowers and cuttings has been demonstrated (Beaudry, 1999; Rapaka et al., 2005; van Doorn, 2004). However, in the intervening years a greater understanding of postharvest physiological responses to hypoxia has evolved.

Beneficial secondary metabolic responses to low O₂ include reduced ethylene biosynthesis and sensitivity, decreased chlorophyll degradation, reduced cell wall degradation, and decreased phenolic oxidation (Beaudry, 2000). Undesirable metabolic changes under hypoxia include decreased aroma biosynthesis for climacteric fruits such as banana and strawberry. Generation of off flavors during low oxygen storage in fruits rich in anthocyanins, carotenoids, phenolics, and volatile compounds, has also been reported (Mattheis and Fellman, 2000). In many crops, hypoxia slows respiration which is partly attributed to reduced polyphenol-oxidase, ascorbate-oxidase and glycolic-oxidase activity (Kader, 1986; Solomos, 1982).

The assertion that low O₂ treatments can be applied to a range of plant materials cannot be made since in many crops a decrease in O₂ is accompanied by a concomitant

induction of fermentative metabolism. Determining the lowest O₂ concentration at which respiration is slowed without inducing fermentation is fraught with technical challenges. Two terminal oxidases cytochrome c oxidase (CytOx) and alternative oxidase (AltOx) operate in the final stages of respiration. CytOx has a high affinity for O₂ (K_m of 0.1% to 0.15% O₂) and is typically present and functional in all plant tissues. AltOx has a lower O₂ affinity (K_m of 1% to 3% O₂) and is not always present in plant tissues (Mapson and Burton, 1962; Solomos, 1977; Vanlerberghe and McIntosh, 1997). Additionally AltOx activity is regulated by several allosteric effectors including mitochondrial redox state, electron transport, carbohydrate status and gene expression (Vanlerberghe and McIntosh, 1997). Thus any effort establishing a consistent K_{1/2} must take into account how total K_m may vary due to concentration and level of activity for these two oxidases. Additionally, epidermal and tissue resistance to oxygen diffusion will alter the point at which respiration is decreased but fermentation remains inactive. For example, K_{1/2} will be much higher for whole apples than peeled packaged apple sections.

Reduced respiration generally allows for an increased shelf life by rationing carbohydrate reserves. However, as described above, reducing oxygen concentrations activates fermentation metabolism in many crops resulting in visual damage and off flavors. Many undesirable metabolic responses to hypoxia change under increased CO₂ concentrations (Watkins, 2000; Yang and Chinnan, 1988).

By reducing O₂ and increasing CO₂ concentrations, respiration slows without concurrent fermentation pathway activation (Costa et al., 1994; Farber and Dodds, 1995; Henderson and Buescher, 1977; Kader, 1986). Additionally, low O₂ and high CO₂ inhibits

ethylene biosynthesis (Mathooko, 1996) by inhibiting the 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase mediated conversion of ACC to ethylene (de Wild et al., 2003).

Importantly, increasing CO₂ concentrations appears to increase cellular pH and further slow chlorophyll degradation (Buescher and Brown, 1979).

High CO₂ concentrations also increase flux through glycolysis aiding in maintaining ATP pools. Increased flux through glycolysis appears not to involve the same enzymes affected by low O₂ (Kerbel et al., 1990). Increased carbon flux through the fermentative pathway under increased CO₂ has also been reported yet fermentation is not as active as in hypoxic conditions (Ke et al., 1995). Interestingly, AltOx activity is increased in high CO₂ concentrations, while CytOx activity is repressed. Though this response does vary depending on a temperature, time of year and CO₂ concentration (Lang and Kader, 1997).

It is clear that MAP or CA can be used to modulate central and secondary metabolism of a variety of crops, usually with the goal of rationing carbohydrate reserves and slowing senescence responses. Since carbohydrate concentration also has a positive correlation to cutting postharvest performance (Rapaka et al., 2008), modified atmosphere storage is an attractive technique for improving cutting postharvest quality.

An important research question will be what reduction in respiration will enhance shelf life enough such that the cost of materials and handling imposed by MA are offset or exceeded by the improvement in quality and/or reduction in losses. Hundreds of species propagated from cuttings are used in floriculture production. An important question to investigate is if cuttings from other species will exhibit the same variability in response to MA that produce exhibits, or will a restricted range of treatments prove beneficial.

Additionally MA may act as a fumigant that eradicates insects before or during shipping (Conyers and Bell, 2007). Certainly further research of MA uses for improving postharvest quality of unrooted herbaceous cuttings is needed.

Ethylene

Ethylene is the primary plant hormone induced by abiotic stress and it regulates many cellular and developmental processes. Ethylene promotes leaf yellowing, abscission and general senescence of croton (*Codiaeum variegatum* Blume.), lantana (*Lantana camara* L.) , and zonal geranium (*Pelargonium hortorum* Bailey (pro sp.) [*inquinans* × *zonale*]) unrooted cuttings (Kadner et al., 2000; Müller et al., 1998). For successful rooting ethylene must be carefully managed during handling, shipping and propagation.

Though a potential problem during shipping and propagation, ethylene plays key roles in physiological responses to drought stress, shade avoidance, apoptosis, and adventitious root formation (Clark et al., 1999; Mergemann and Sauter, 2000; Sharp and LeNoble, 2002; Stepanova and Alonso, 2005). Pierik et al. (2006) proposed a biphasic model of ethylene action dependent upon ethylene concentration to describe plant responses. At lower concentrations ethylene promotes growth in plants such as tobacco, while higher concentrations slow growth or induce senescence. Other researchers have also published on the growth promoting effect of ethylene.

Growth Responses

Plant meristems provide the undifferentiated cells which become new roots, shoots, leaves and flowers. In root meristems, the quiescent center contains a few of these undifferentiated cells. Arabidopsis plants that constitutively synthesize ethylene had a greater number of cell divisions in the quiescent center. Exogenous ethylene also had the same effect while plants deficient in ethylene synthesis had far fewer stem cell divisions (Ortega-Martínez et al., 2007). As adventitious roots arise from the undifferentiated tissue of the pericycle, it is reasonable to speculate that similar interactions may be observed between ethylene and pericycle cells.

Stress Responses

Immediately after removal from the stock plant, cuttings will begin generating wound induced ethylene (O'Donnell et al., 1996). Jasmonate and ethylene together activate genes involved in suppressing herbivory and repairing wounds. However, both compounds also activate exclusive sets of genes related to different types of biotic and abiotic stresses (Devoto and Turner, 2005). Typical amounts of ethylene generated are between 6 to 8 nL · g⁻¹ · hr⁻¹ decreasing to zero over a 6 hour period (O'Donnell et al., 1996).

Within a few minutes after excision from the stock plant, cuttings of most species are already experiencing water stress. Abiotic stresses increase ethylene biosynthesis (Morgan and Drew, 1997) as a means of activating stress response genes. Water deficit stress in detached wheat (*Triticum aestivum* L. cv. Anza) leaves promotes 1-aminocyclopropane 1-carboxylic acid synthase (ACCase) and thus ethylene synthesis (Apelbaum and Yang, 1981).

Also, detached bean (*Phaseolus vulgaris* L.) and cotton (*Gossypium hirsutum* L.) leaves generated more ethylene during water stress than whole plants given similar treatment (Morgan et al., 1990). Reported amounts of ethylene generated by excised plant parts under water stress range from 30 to 63 $\text{nL} \cdot \text{g}^{-1} \cdot \text{hr}^{-1}$ (Apelbaum and Yang, 1981; Morgan et al., 1990). Since ethylene biosynthesis prompted by water stress is approximately 800% greater than wound related ethylene, water stress ethylene is going to be of greater concern to unrooted cutting producers than wound ethylene.

Transpiration can be a large source of cutting postharvest water loss. Since water loss is a significant stress resulting in excessive ethylene generation from detached plant parts, reducing stomatal conductance prior to cutting harvest may reduce ethylene generation and maintain cutting quality (Apelbaum and Yang, 1981; Morgan et al., 1990; Sharp and LeNoble, 2002).

Stomatal conductance is negatively correlated to xylem sap ABA concentration (Srivastava, 2002). Corn seedlings inhibited in ABA synthesis generate higher amounts of ethylene under water stress than similarly treated control seedlings. The ABA deficient seedlings return to normal levels of ethylene synthesis under water stress when exogenous ABA is applied (Sharp and LeNoble, 2002). ABA up-regulates genes in *Arabidopsis thaliana* (L.) Heynh. guard cells involved with dehydration, cold, signal transduction (phosphatases), and transcription factors. Also, ABA binding protein has been localized to the guard cell protoplast surface in *Vicia faba* L. (Fan et al., 2004). The involvement of ABA in stomatal conductance and water stress response, and the presence of guard cell ABA

binding proteins suggests the possibility of using exogenous ABA as a tool for reducing stomatal conductance.

Temperature will also have a strong effect on postharvest ethylene biosynthesis of cuttings. Naturally, as temperatures increase biological activity increases, and presumably so does ethylene biosynthesis. Other effects of temperature on ethylene biosynthesis could occur. Cut roses stored at 2° C generate far higher concentrations of ethylene when moved to 22° C than un-stored roses (Faragher and Mayak, 1984). It could be possible that cuttings that normally do not generate much ethylene will do so after having been chilled, or refrigerated during transport.

1-methylcyclopropene as an Ethylene Inhibitor

Ethylene presents postharvest problems for important floricultural species. For those sensitive species, research into preventing damage from excess ethylene has involved silver thiosulfate, silver nitrate and 1-methylcyclopropene (1-MCP) pretreatments as well as packaging design and 1-MCP sustained release mechanisms (Kadner and Druege, 2004; Kadner et al., 2000; Macnish et al., 2004).

1-MCP irreversibly binds to ethylene binding sites yet does not activate subsequent downstream ethylene mediated developmental signaling such as growth, chlorophyll degradation and senescence processes. It is used widely in the produce and floricultural industries and is increasingly used on unrooted cuttings (Blankenship and Dole, 2003). In zonal geranium, 1-MCP prevents leaf yellowing of unrooted stored cuttings and reportedly improves rooting percentage (Kadner and Druege, 2004; Serek et al., 1998). However, other

researchers have reported fewer adventitious roots on 1-MCP treated geranium cuttings compared to untreated cuttings (Rapaka et al., 2008).

Interestingly, 1-MCP treatment stimulated excessive ethylene biosynthesis in unrooted zonal geranium cuttings (Kadner and Druege, 2004; Rapaka et al., 2008) a response similar to specific arabidopsis mutants. The rate limiting step of ethylene biosynthesis is the conversion of *S*-adenosyl-L-methionine to aminocyclopropane by 1-aminocyclopropane-1-carboxylic acid synthase (ACS). An enzyme called ETO1 that binds to and dephosphorylates ACS negatively regulates ACS activity and turnover (Wang et al., 2004). True *eto1* arabidopsis mutants overproduce ethylene because of a defect in ETO1 and lack of ACS regulation. Since 1-MCP is a strong ethylene signalling antagonist, the observation that 1-MCP treatment increases ethylene biosynthesis in unrooted zonal geranium cuttings indicates that zonal geranium ethylene biosynthesis is regulated by an auto-inhibitory feedback mechanism.

Carbohydrate Reserves

Cuttings of many species tolerate initial wounding and water stress well but ultimately fail due to carbohydrate exhaustion. The abiotic stresses imposed by harvest and shipping tend to increase respiration hastening the loss of carbohydrate reserves (Sturm and Tang, 1999) and the failure of homeostasis. The energy required for maintaining homeostasis during storage comes from extant cutting carbohydrate, lipid and protein reserves and quality of subsequent rooting is directly proportional to carbohydrate content

(Rapaka et al., 2005). Senescence processes may also be activated by low carbohydrate reserves (van Doorn, 2004).

Altering the carbohydrate distribution in the parent plant through the abscission of sink tissues, such as flowers, is commonly accomplished by the application of 2-Chloroethylphosphonic acid (ethephon). Ethephon is lipophilic and upon entering the relatively more basic apoplastic environment it undergoes a degradation reaction releasing ethylene, chloride and a phosphate ion (Warner and Leopold, 1969; Yang, 1969). Ethylene inhibits IAA synthesis (Chadwick and Burg, 1970; Weber and Osborn, 1969) and promotes ABA synthesis (Hansen and Grossmann, 2000).

Application of compounds containing ethephon, typically Florel™, results in the loss of flower buds and flowers, thus reducing sink tissue carbohydrate demands. Ethephon is also used to increase branching and thus cutting yields of stock plants (Faust and Lewis, 2005; Hayashi et al., 2001). However, since ethylene can induce leaf senescence in shipping and packaging, cuttings still evolving ethephon-induced ethylene are a problem. Since ethephon treated plants do not rapidly replace the abscised sink tissues, it may be possible to delay harvest until ethylene levels drop to a safer concentration. Additionally, ethylene slows carbohydrate use (Roitsch, 1999) possibly increasing cutting carbohydrate content as a result.

Lamb's lettuce (*Valerianella locusta*) stored at 30° C more rapidly lost sucrose content than at 20° C, yet fructose and glucose content remained the same. While at 10° C sucrose content was unchanged, but glucose and sucrose content decreased (Enninghorst and Lippert, 2003). Kiwifruit also exhibit changes in carbohydrate composition based on storage

temperature. Low temperature decreases amylase activity thus increasing starch content and also decreases sucrose phosphate synthase activity (Zhang Y et al., 2004). Lowering the postharvest temperature of cuttings may initiate similar responses in addition to slowing general respiration.

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CHAPTER 2

Modified atmosphere use in extending unrooted cutting quality and viability

This chapter was prepared in the style of the Journal of the American Society for Horticultural Science and will be submitted for review

Abstract. Methods to reduce cutting losses during shipping are of critical interest for cutting producers, rooting stations, and finished plant producers. Cuttings of New Guinea Impatiens (*Impatiens hawkeri* W. Bull ex Gard.) ‘Sonic Red’, geranium (*Pelargonium ×hortorum* L.H. Bailey (pro sp.) [*inquinans* × *zonale*]) ‘Kardino’ and poinsettia, (*Euphorbia pulcherrima* Willd. ex Klotzsch) ‘Visions of Grandeur’ were harvested and stored in gas concentrations ranging from atmospheric air (21% oxygen:0.03% carbon dioxide) to 1%:20% oxygen:carbon dioxide (1:20) for 7 days at 20 °C. Cuttings held in 1:20 oxygen:carbon dioxide generated higher concentrations of ethylene than any other treatment including the atmospheric control. Poinsettia and geranium cuttings stored in this treatment were severely damaged at the end of storage. Cuttings of all species stored in ratios of 10:10, 10:5, 5:10, 5:5 oxygen to carbon dioxide generated less ethylene compared to the atmospheric control. Cuttings stored in 10:5 performed best during propagation, with less leaf yellowing or abscission than any other treatment.

Ornamental plant producers use cuttings shipped from around the world in finished plant production. The value of unrooted cutting imports into the United States totaled over \$61 million in 2006 with the bulk coming from Central America, South America, Mexico, and Asia (Foreign Agricultural Service, 2007). From the top 15 producing states, for the three most important cutting propagated species, as gauged by volume of imports, geraniums (*Pelargonium* L'Hér. ex Ait.), poinsettias (*Euphorbia pulcherrima* Willd. ex Klotzsch) and New Guinea impatiens (*Impatiens hawkeri* W. Bull) finished flats, hanging baskets, and pots had a wholesale value of \$330 million and were produced from approximately 138 million

cuttings (National Agriculture Statistics Service, 2007). In this case, the loss of a single cutting is \$2.38 lost in potential revenue. Understandably, reducing losses of cuttings during shipping is a critical research interest for cutting producers, rooting starters, and finished plant producers.

Modified atmosphere packaging (MAP) and controlled atmosphere (CA) storage are broadly used to increase shelf life many agricultural products from seafood to cut flowers. The adjusted atmosphere is achieved by altering O₂ and CO₂ partial pressures using a predetermined mixture (active modified atmosphere) or by specialized packaging films that limit the diffusion of gasses (passive modified atmosphere).

Responses of tubers, fruits, leaves, stems and flowers to modified atmosphere storage has been widely studied and documented (Beaudry, 1999; Beaudry, 2000; Farber and Dodds, 1995; Watkins, 2000). These studies make clear that modified atmosphere response varies between species, organ types, and developmental stages. The technology can extend shelf life in one instance or cause destructive anaerobic metabolism in another. For example, low O₂ concentrations increase lettuce shelf life (Beaudry, 1999), but activates fermentation metabolism in apples (Beaudry, 2000). Additionally, different plant tissues appear to have different tolerances for changes in atmospheric oxygen and carbon dioxide concentrations (Beaudry, 1999; Izumi et al., 1996) resulting in a broad range of results for apparently similar products.

Increased CO₂ and reduced O₂ concentrations depress respiration (Mathooko, 1996b) and production of ethylene (Watkins, 2000). Interestingly, similar conditions reportedly increases ethylene biosynthesis in pear fruit (de Wild et al., 2003) while reducing it in others

though the latter appears related to physiological development (Beaudry, 2000). Glycolysis, starch catabolism, fermentation and cell wall metabolism are also impacted by modified atmosphere storage (Beaudry, 1999). In commodities with a high degree of pigment metabolism, phenolics, and volatile compounds, modified atmospheres can degrade some qualities influenced by color, aroma and flavor (Mattheis and Fellman, 2000).

In many vegetable products, low O₂ concentrations prompt decreased respiration activity which is partly attributed to reduced polyphenol-oxidase, ascorbate-oxidase and glycolic-oxidase activity (Kader, 1986; Solomos, 1982). Reduced respiration allows for an increased shelf life as general metabolism is also decreased. However, simply reducing oxygen concentrations frequently activates fermentation metabolism in many species resulting in damage and off flavors. Reducing O₂ and increasing CO₂ concentrations can reduce respiration without concurrent fermentation pathway activation (Costa et al., 1994; Farber and Dodds, 1995; Henderson and Buescher, 1977; Kader, 1986).

Importantly, increasing CO₂ concentrations appears to increase cellular pH and slow chlorophyll degradation (Buescher and Brown, 1979). Green beans stored under increased CO₂ concentrations have reduced titratable acidity and an increase in soluble proteins (Buescher and Adams, 1983). Additionally, green beans stored in 3% oxygen 3% carbon dioxide (3:3) maintained higher soluble sugars and overall quality compared to those stored in air (Sanchez-Mata et al., 2003).

Low oxygen environments (~1.5%) have been used to extend the shelf life of basil to 45 from 18 days, yet any increase in CO₂ concentrations reduced storage time for this crop (Lange and Cameron, 1998). Modified atmosphere storage (5:10) extended broccoli shelf

life, by reducing water loss, chlorophyll loss, and soluble sugar consumption (Eason et al., 2007). However, the increased soluble sugar concentrations also correlate with reduced expression carbohydrate transport proteins. Additionally, down regulation of citrate synthase in modified atmosphere treated broccoli suggests a reduction in lipid metabolism. Other investigators have shown that respiration is reduced in lettuce stored super-atmospheric CO₂ concentrations alone (Escalona et al., 2006).

Changes in modified atmosphere gas composition are generally aimed at slowing respiration thus increasing available carbohydrates via metabolic rationing. Specific effective O₂ to CO₂ ratios do vary widely between species and tissue types. Increased available carbohydrates result in a more nutritive product, but also one that lasts longer as cell homeostasis activities are extended.

Since carbohydrate concentration also has a positive correlation to cutting postharvest performance (Rapaka et al., 2008), modified atmosphere storage is an attractive area of research to improve storage time and postharvest quality of unrooted herbaceous cuttings. To investigate the usefulness of modified atmosphere storage in extending unrooted cutting postharvest quality, we conducted experiments with cuttings of New Guinea Impatiens (*Impatiens hawkeri* W. Bull ex Gard.) ‘Sonic Red’, poinsettia (*Euphorbia pulcherrima* Willd. ex Klotzsch) ‘Visions of Grandeur’, and geranium (*Pelargonium ×hortorum* L.H. Bailey (pro sp.) [*inquinans* × *zonale*]) ‘Kardino’ stored in a range of atmospheric conditions. The cuttings were assessed for carbohydrate content and quality after storage, and quality after propagation with the objective of determining if modified atmospheres would improve the production process.

Materials and Methods

Plant material. Unrooted cuttings were propagated into 30 cm, 13.85 L, plastic pots containing a soilless growing substrate (Fafard P3, Conrad Fafard, Inc., Agawam, Mass.). Once rooted, these plants were maintained as stock plants and irrigated manually with clear water, $600 \text{ mg} \cdot \text{L}^{-1}$ N liquid fertilizer (20N-4.37P-16.6K, Ultrasol, SQM North America, Atlanta), or flowable lime (Limestone F, Cleary Chemical Inc., Dayton, N.J.) to maintain a target electrical conductivity reading (E.C.) of 2.0 to 3.5 $\text{dS} \cdot \text{m}^{-2}$ and a pH of 5.8 to 6.2 for poinsettia and geranium, and a target E.C. of 1.0 to 1.5 $\text{dS} \cdot \text{m}^{-2}$ and a pH of 5.8 to 6.2 for impatiens. Nutritional status was monitored using the pour through method. Temperature set points were 24/18° C (day/night), average daily light integral (DLI) was $6.1 \text{ mol} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$.

Treatments. Two node geranium cuttings with at least two fully expanded leaves, two node New Guinea impatiens cuttings with at least three fully expanded leaves and three node poinsettia cuttings with at least two fully expanded leaves, of similar size and age were arbitrarily harvested from all parts of the stock plants. For each treatment six cuttings were placed into a clean 0.95 L glass jar. The jar lids were fitted with a neoprene sampling septum, and tubing appropriate for applying the treatment gas. The jars were sealed and flushed for 10 minutes with nitrogen at $200 \text{ ml} \cdot \text{min}^{-1}$. Subsequently, the jars were flushed with varying combinations of O_2 and CO_2 (Fig. 1) for 10 minutes at $200 \text{ ml} \cdot \text{min}^{-1}$. The gas supply tubes were tightly clipped after the gas treatment was introduced. The cuttings were

stored in the jars at 20 °C for 7 days in darkness. After storage, the cuttings were propagated into flats containing a soilless growing substrate and kept under mist for the duration of the experiment.

Sampling. Air samples were collected immediately after sealing each jar and every 48 hours thereafter and analyzed for ethylene, O₂, and CO₂ concentration using gas chromatography. For ethylene, a gas chromatograph (GC, Varian 3400; Varian Inc. Walnut Creek, Calif.) fitted with a glass column (Porapak Q, 80-100 mesh, 183 cm x 2 mm) running at 120° injector, 120° column, and 130° C detector (flame ionization) temperatures was used to measure ethylene concentration during storage. Flow rates for the He carrier, H₂ and O₂ were 30, 16 and 90 mL · min⁻¹, respectively. Ethylene quantification was based a response factor generated using a 1 µL · L⁻¹ ethylene standard. Injection volume was 1 mL of headspace gas drawn via a neoprene port on the jar lid. For O₂, and CO₂ a gas chromatograph (GC, Shimadzu Scientific Instruments, Inc. Columbia, Md.) fitted with a stainless steel column (Carboxen, 60-80 mesh, 457 cm x 2 mm) running at 250° injector, 250° C detector (TCD-T) temperatures. The column program ran for 6 minutes at 30 mL · min⁻¹ helium carrier with an initial column temperature of 35° and final temperature of 200° C.

Analysis of soluble sugars and organic acids. Carbohydrates were extracted from freeze dried, powdered cuttings using an extraction solvent of 60% methanol, 25% chloroform and 15% water and an internal standard containing 800 and 400 mg · L⁻¹ lactose and cellobiose, respectively (Pattee et al., 2000). A 10 mL solution containing approximately 50 mg of

sample was vortexed for 2 minutes, centrifuged and then decanted to evaporate overnight. The remaining residue was re-suspended in 2 mL of deionized distilled water. Carbohydrate content of the samples was determined using an HPLC equipped with a CarboPac PA-1 column (Dionex, Sunnyvale, CA), 250 mm long, 4 mm i.d., fitted with a CarboPac PA-1 guard column (Dionex). The column oven was set at 30 °C. The mobile phase used 200 mM NaOH sparged with helium for 2 h. Ten μL of sample was injected using water as the carrier solvent. Flow was set at $1.0 \text{ mL} \cdot \text{min}^{-1}$ (isocratic) using a Dionex GS50 gradient pump. The detector was a Dionex pulsed amperometric (PAD) set to 100 nC (nanocoulombs). Samples were filtered through Dionex OnGuard II H columns to remove free amino acid interference. The samples were run with dilutions of standards to construct five-point curves fitted to zero.

Organic acid content of the samples was determined using a Thermo Separations System consisting of a P 2000 pump, AS 3000 autosampler, a UV 6000LP Diode Array detector and a SCM 1000 mobile phase degasser. The operating system was ChromQuest v. 4.1 (Thermo Scientific, Inc., Waltham, Mass.). The HPLC was equipped with a BioRad HPX 87-H, 300 mm length, 7.8 mm interior diameter column, and a BioRad cation H cartridge guard column. The column oven temperature was set to 60° C, the flow rate was $0.8 \text{ mL} \cdot \text{min}^{-1}$ using a 0.03 N sulfuric acid mobile phase.

Data collection. During storage the cuttings were examined every 48 hours for evidence of phytotoxicity. Overall quality of the cuttings was rated on a scale of 5 to 1. A 5 cutting showed 0% chlorosis, necrosis, or leaf abscission. A 4 cutting showed a maximum 25% combined damage from the symptoms described previously, a 3 cutting 50% damage, a 2

cutting 75% damage and a 1 cutting was 100% damaged. Additionally, the number of abscised and yellow leaves was recorded. The same scales were used to evaluate the cuttings during and after propagation.

Statistical analysis. Within the study each experiment was designed as a randomized complete block design of three blocks with 12 treatments in an incomplete factorial of 5 O₂ concentrations by 5 CO₂ concentrations, by 7 days of treatment. All statistical analyses were conducted using SAS 9.1 (SAS Institute, Cary, NC). Differences between the means of ethylene concentrations and storage quality for a given day were tested using Fisher's Protected LSD at $\alpha = 0.05$. Further, orthogonal contrasts were used to partition treatment effects into main effects and interactions.

Differences between concentrations of various soluble sugars and organic acids after storage were analyzed using Fisher's Protected LSD at $\alpha = 0.05$. Further, orthogonal contrasts were used to partition treatment effects into main effects and interactions.

Additionally, differences between the means of quality ratings and abscised leaves after propagation were tested using Fisher's Protected LSD at $\alpha = 0.05$. Similar to the analyses above, orthogonal contrasts were used to partition treatment effects into main effects and interactions.

Results

Ratios of increased oxygen to carbon dioxide (10:10, 10:5) during storage resulted in less ethylene production from poinsettia cuttings compared to control (Fig. 2). However, no

treatment resulted in statistically significant differences in ethylene biosynthesis by geranium cuttings. During storage poinsettia and New Guinea impatiens cuttings held in 1:20 oxygen:carbon dioxide generated higher concentrations of ethylene than any other treatment including control, though geranium cuttings did not. Treatments with ratios far from 1 (21:0.03, 1:20) and those with very low oxygen (0:0 and 1:0) generally decreased subsequent propagation performance for poinsettia and geranium (Fig. 3) relative to the best single overall treatment (10:5). Four treatments (10:0, 5:0, 1:10, 1:5) yielded intermediate results and were dropped from the study.

There was no significant change in oxygen concentration for New Guinea impatiens cuttings held in 21:0.03 over the 7 d storage period yet carbon dioxide concentrations significantly increased over the same period (Fig. 3). Oxygen concentrations for all other impatiens cutting storage treatments significantly increased over the storage period from the initial treatment concentration. No significant changes were observed in carbon dioxide concentrations for these same treatments. Treatments of poinsettia and geranium cuttings exhibited similar trends (data not shown).

During storage, impatiens cuttings stored in 1:20 generated significantly more ethylene than all the other treatments, $0.16 \mu\text{L} \cdot \text{L}^{-1} \cdot \text{g}^{-1}$ compared to a mean of $0.05 \mu\text{L} \cdot \text{L}^{-1} \cdot \text{g}^{-1}$ ethylene for all other treatments which were statistically similar ($P < 0.05$) to control. Surprisingly, impatiens cuttings from all treatments exhibited no outward differences during storage, appearing healthy and undamaged after storage (data not shown). However, cuttings held in 10:0, 21:0.03 or 1:20 declined rapidly in the propagation environment (Fig. 1). Only 25 and 72% of the cuttings treated with 1:20 and 21:0.03 during storage survived to root,

respectively. Alternatively, 100% of cuttings stored at 5:5, 10:5 and 0:1 rooted within 11 days of propagation and showed no chlorosis or necrosis.

Geranium cuttings produced surprisingly little ethylene during storage compared to poinsettia with no significant differences observed between treatments (Fig. 5). However, ethylene did accumulate in all treatments during storage. Quality during storage was best for geranium cuttings treated with 10:5 and 5:10. Geranium cuttings held in 1:20 declined rapidly during storage between 5 and 7 d (Fig. 5). Cuttings with higher quality after storage, those treated with 10:5 and 5:10, performed best in propagation with best overall quality and fewer abscised leaves compared to those stored in 21:0.03. However, post propagation quality overall was poor for all geranium cutting treatments.

Total soluble carbohydrate content of control geranium cuttings frozen immediately after harvest ($43 \text{ mg} \cdot \text{g}^{-1}$) did not significantly differ from cuttings stored for 7 days in 5:10 ($48 \text{ mg} \cdot \text{g}^{-1}$) or 5:5 ($42 \text{ mg} \cdot \text{g}^{-1}$) treatments (Fig. 6). However, all other treatments, including those stored in atmospheric air, had significantly less soluble carbohydrate content compared to control cuttings, i.e. those harvested from the plant and immediately frozen. Geranium cuttings stored for 7 days in 21:0.03, 10:5, 1:20, and 0:0 had 22, 32, 31 and 23 $\text{mg} \cdot \text{g}^{-1}$, respectively, soluble carbohydrate content.

Total organic acid content of control geranium cuttings ($199 \text{ mM} \cdot \text{L}^{-1}$) did not significantly differ among cuttings stored for 7 days in 5:10 ($172 \text{ mM} \cdot \text{L}^{-1}$) or 5:5 ($181 \text{ mM} \cdot \text{L}^{-1}$) treatments (Fig. 7). Geranium cuttings stored in 21:03, 10:5, 1:20 and 0:0, however had significantly less ($150, 143, 160$ and $138 \text{ mM} \cdot \text{L}^{-1}$) total organic acids compared to the

control cuttings. Additionally, cuttings stored in oxygen rich treatments ($> 1\%$) contained propionate, while those stored in low oxygen ($\leq 1\%$) did not.

Poinsettia cuttings stored in 1:20 oxygen:carbon dioxide generated high concentrations ($0.41 \mu\text{L} \cdot \text{L}^{-1} \cdot \text{g}^{-1}$) of ethylene compared to those stored in 10:10, 10:5 or 21:0.03 which generated 0.07, 0.07 and $0.1 \mu\text{L} \cdot \text{L}^{-1} \cdot \text{g}^{-1}$ ethylene, respectively (Fig. 2). No significant differences in quality after storage were observed between 10:10, 10:5 or 21:0.03 treatments. However cuttings stored in 1:20, 1:0 and 0:0 suffered significant damage during storage (Fig. 2), which subsequently performed poorly during propagation (Fig. 3). Poinsettia cuttings stored in 10:5 had significantly higher quality ratings after propagation compared to those stored in 21:0.03 (Fig. 3).

Total soluble carbohydrate content ($136 \text{ mg} \cdot \text{g}^{-1}$) of untreated, un-stored poinsettia cuttings differed significantly from cuttings stored for 7 days in all treatments (Fig. 6). Cuttings stored in 21:0.03, 10:5, 5:10 and 5:5 had significantly more (77, 78, 75 and $76 \text{ mg} \cdot \text{g}^{-1}$ respectively) soluble carbohydrate content compared to cuttings stored in 1:20 ($47 \text{ mg} \cdot \text{g}^{-1}$).

Total organic acid content of control poinsettia cuttings ($66 \text{ mM} \cdot \text{L}^{-1}$) did not significantly differ from cuttings stored for 7 days in any treatment (Fig. 7). Poinsettia cuttings stored in 10:5, 5:10 and 5:5 had significantly more ($78, 81$ and $74 \text{ mM} \cdot \text{L}^{-1}$) total organic acids compared to the cuttings stored in 0:0. Additionally, cuttings stored in any treatment with oxygen synthesized propionate, while those stored in $< 21\%$ oxygen synthesized lactate. Neither of these organic acids was detected in un-stored control cuttings.

Discussion

The use of modified atmosphere storage for preserving plant tissues has been successful with a broad range of plant parts and species. The significant differences in the *impatiens* cutting quality ratings after propagation (Fig. 1) show clearly that MAP is a promising tool for extending cutting storage time and improving postharvest quality.

The technique used to treat the cuttings, though not gas tight is a reasonable approximation of what is economically and practically feasible for cutting producers. Changes in atmospheric composition during storage reflect well-established models of respiration in plant tissue. For example, those cuttings held in atmospheric air, steadily increased carbon dioxide concentrations due to respiration. Yet, these cuttings did not use oxygen at a rate that could not be replaced by diffusion through the jar seal thus maintaining a steady slightly hypoxic concentration (Fig. 5). Oxygen diffused into the hypoxic treatment jars and concentrations increased steadily. Increased carbon dioxide concentrations appeared to diffuse out of the jars and concentrations dropped over the storage period. Cutting producers will likely use a treatment system that will behave similarly, starting the storage period in a prescribed treatment condition, which diffuses steadily over time. Additionally, growers will likely store cuttings no longer than 2 to 3 days which will allow for less time for diffusion of the storage atmosphere.

New Guinea impatiens. New Guinea impatiens cuttings had the best propagation performance when stored in atmospheres of reduced O₂ and increased CO₂ (Table 1) similar to citrus fruit, pineapple fruit, parsley and spinach (Beaudry, 1999). Surprisingly, *impatiens* cuttings

exhibited no visible change during dark storage, yet in propagation some treatments (1:20, 21:0.03, 0:0) developed complete leaf necrosis within 36 hours suggesting that the cutting's ability to handle high-energy photosynthetic light reactions had been compromised.

Increased ethylene biosynthesis of impatien cuttings stored in high concentrations of carbon dioxide is counter to MA research involving other crops. Increases in CO₂ up to 60% of volume decreases ACC activity in tomato, winter squash and cucumber fruits (Mathooko, 1996a) and subsequently, reduces ethylene accumulation in storage. Yet, New Guinea impatiens cuttings treated with 1:20 generated 0.16 $\mu\text{l} \cdot \text{L}^{-1} \cdot \text{g}^{-1}$ ethylene compared to 0.05 $\mu\text{l} \cdot \text{L}^{-1} \cdot \text{g}^{-1}$ ethylene for cuttings stored in 21:0.03 over a 7 d storage period. Additionally, no significantly different results in ethylene accumulation were observed between 21:0.03 and the other MA treatments. The response of impatien cuttings to increased carbon dioxide concentration may be due to significant physiological stress, such as pH change, imposed by the unusual storage condition, or a broader range of carbon dioxide treatment should be explored.

Poinsettia. Poinsettia cutting quality during propagation was best preserved in 10:10 and 10:5, which are similar to optimum MA treatments for spinach and parsley (Beaudry, 1999). Low oxygen environments < 10% damaged these cuttings and caused significant ethylene generation (Table 1). Soluble carbohydrate content of cuttings from all treatments differed significantly from untreated, un-stored cuttings. Those cuttings stored in 21:0.03 had significantly less soluble carbohydrate than untreated, un-stored cuttings, likely due to normal respiration and a decreased demand for carbohydrate substrates for growth.

Interestingly, there were no significant total soluble carbohydrate content differences between treatments yet propagation performance was significantly different between treatments. The observation suggests that additional factors other than carbohydrate content are important to postharvest performance.

Hypoxia and increased CO₂ concentrations stimulated lactic acid accumulation in poinsettia cuttings, suggesting fermentative pathway activation. Similar results have been reported by several researchers for a broad range of commodities (Beaudry, 2000; Kader, 1986; Watkins, 2000). Propionate was found in poinsettia cuttings from all treatments except 0:0 and untreated, un-stored cuttings. Possible sources of propionate include odd-chain β -oxidation, catabolism of amino acids or as the final product of metabolism of phytanic acid, derived from the degradation of chlorophyll (Lucas et al., 2007). Given the known degradation of chlorophyll in zonal geranium cuttings during storage (Serek et al., 1998), the latter is likely the foremost source of propionate. Cuttings from the 0:0 treatment were so rapidly damaged during storage, it is unlikely the chlorophyll degradation pathways were active.

The results indicate that poinsettia cuttings require high concentrations of oxygen for respiration during storage, yet soluble carbohydrate content was statistically ($P > 0.05$) similar across treatments that preserved quality during storage (10:5) and those that did not (21:0.03, 5:10, 5:5). The top two treatments in terms of storage quality, 21:0.03 and 10:5, both had significantly ($P > 0.05$) similar total soluble carbohydrate just after storage yet cuttings stored in 21:0.03 had significantly ($P \leq 0.05$) lower quality ratings than 10:5 after

propagation. The observation suggests that in addition to carbohydrate content, another factor for improved propagation performance is maintained by the 10:5 treatment.

Rapaka et al. (2005) demonstrated that rooting performance of geranium cuttings stored for 7 days in the dark is directly dependent upon the recovery of non-photochemical quenching (qN) of chlorophyll fluorescence (CF) as well as pre-severance photosynthetic photon flux density (PPFD). It is possible that for poinsettia cuttings storage in 10:5 delays degradation of the photosynthetic apparatus. Thus, these cuttings are able to rapidly synthesize carbohydrates and ATP in the high light propagation environment whereas cuttings stored in 21:0.03 are less able to recover.

As with New Guinea impatiens cuttings, poinsettia cuttings stored in 1:20 generated high concentrations of ethylene. Additionally, those cuttings stored in 10:10 or 10:5 generated significantly less ethylene than cuttings stored in 21:0.03. These results in combination with the ethylene results for New Guinea impatiens cuttings and Mathooko's work (1996a) point towards a new hypothesis of cutting ethylene generation during MA storage. As with some fruits, such as tomato, and winter squash increased CO₂ may reduce postharvest ethylene generation in unrooted cuttings but concentrations exceeding 10% appear to cause ethylene generation. Thus a narrow range of MA treatments, such as those between 10:5 and 5:10, are feasible for reducing ethylene, improving storage quality and postharvest performance of unrooted poinsettia and New Guinea impatiens cuttings.

Geranium. Geranium cuttings, unlike poinsettia and New Guinea impatiens cuttings, generated very little ethylene during storage. The highest cumulative mean concentration

after 7 d storage was 0.02 compared to $0.4 \text{ ml} \cdot \text{L}^{-1} \cdot \text{g}^{-1}$ for poinsettia cuttings. There was little significant difference in ethylene concentration between treatments, except for 10:05 which generated significantly less ethylene than the other treatments (Table 1). However, the overall trend was an increase in ethylene concentration over the storage period, likely due to stress induced ethylene production (Devoto and Turner, 2005).

Unrooted zonal geranium cuttings stored in 10:5 and 5:10 had similar quality ratings during storage and subsequent propagation and both were significantly different from 21:0.03 treated cuttings (Table 1). Yet cuttings held in 10:5 had significantly less soluble carbohydrate and organic acid content than cuttings treated with 5:10. Geranium cutting performance during propagation was disappointing with the best treatments falling just short of being commercially acceptable, scoring only a 3.4 on a 5 point scale, which may have been due to the 20°C storage temperature, 10°C higher than recommended for this species (Dole and Gibson, 2006). In addition, storage duration was 3 to 4 days longer than typical shipping times of 2 to 3 days.

Other researchers (Rapaka et al., 2007; Rapaka et al., 2005) demonstrated a positive relationship between carbohydrate content and geranium cutting propagation performance. Our results expand this conclusion by uncovering two possibilities. A range of carbohydrate content can support satisfactory postharvest performance. Also, factors other than carbohydrate content play a role in cutting postharvest quality.

Lactate was found in both untreated un-stored cuttings, and in cuttings from treatments (21:0.03, 5:5, and 1:20) that performed poorly in propagation. Lactate was not present in the two treatments (10:5 and 5:10) that had the highest quality ratings after

propagation. Taking propagation performance as an indicator of stress during storage, we suggest that lactate dehydrogenase (LDH) activity is up-regulated in zonal geranium cuttings in response to the stresses of MA treatment and storage time. In low stress storage conditions LDH is not up-regulated and lactate is not found. No lactate was found in the 0:0 cuttings; however, these cuttings were so damaged by the MA treatment, a normal physiological response was improbable. Also, lactate was found in cuttings treated under normal atmospheric and hypoxic conditions, suggesting that LDH plays physiological roles in zonal geranium in addition to fermentative metabolism.

Propionate was found in zonal geranium cuttings from all treatments except 1:20, 0:0 and untreated, un-stored cuttings. Given the known degradation of chlorophyll in zonal geranium cuttings during storage (Serek et al., 1998), it is likely chlorophyll degradation is the foremost source of propionate.

Intuitively, one would expect stressed cuttings to have high rates of leaf abscission. Examination of the data suggests that treatments with poor storage and propagation quality results (1:0, 5:5, 1:20, 0:0), had unexpectedly low leaf abscission. These anomalous responses, should not be interpreted as a positive result, rather, most of these cuttings were so damaged by treatment they could not complete the physiologically active process of abscission.

Conclusions. The worst treatment for all species in this study was 1:20, which typically resulted in severe damage during storage or immediately after propagation. The cutting responses are likely due to cellular acidosis. Researchers have observed *in vivo* using nuclear

magnetic resonance measurements (Siriphanich and Kader, 1986) and γ -aminobutyrate accumulation (Ke et al., 1993) that high CO₂ concentrations lower cellular pH. However, buffering capacity differs markedly between tissues and species (Watkins, 2000) so some variation may be seen. For all species, the cuttings subjected to the most stressful treatments (1:20 and 0:0) also contained the least soluble carbohydrate content suggesting extraordinary demands on central metabolism.

Clearly, 10:5 was the best single overall treatment for all species tested in terms of best cutting quality at the end of propagation (Table 1). Typically high soluble carbohydrate content and titratable acidity are correlated to good MA application and postharvest produce quality (Beaudry, 1999). Additionally, fermentation and accumulation of stress compounds are avoided in MA to minimize ‘off flavors’ in produce (Kader and Watkins, 2000). In this study, there was no apparent relationship carbohydrate content and cutting performance. Also, the presence of lactate and propionate, which would ordinarily create off flavors, was immaterial as flavor is not a consideration in cutting propagation. In fact, the opportunity to take advantage of the fermentative pathway may aid cutting carbohydrate rationing.

These observations suggest a broader range of biochemical responses can be tolerated in MA cuttings. Yet, unlike produce, cuttings must remain photosynthetically competent and able to withstand light reaction and propagation stresses, and thus must be physiologically robust after MA storage. The MA environment, while slowing general metabolism, must support cutting homeostasis to a degree not required for produce. Slowing general metabolism too dramatically results in impaired homeostasis and hidden damage. Such was apparent with the poinsettia cuttings stored in 21:0.03 or 5:10, which had high quality ratings

after storage and had high carbohydrate and organic acid content, yet had lower quality ratings and high leaf abscission during propagation.

The best MA conditions for storing produce vary widely. However, very similar treatments (10:5, 5:10) appear to be best for cuttings, at least among the species tested thus far. Happily, these treatment ranges can all be achieved using available MA packaging (Beaudry, 1999). However, responses to MA will vary by species and growing conditions of the stock plants from which cuttings are produced. Additionally, impacts on rooting performance should be assessed given the positive relationship between adventitious root formation and carbohydrate content. Certainly further research into MA storage of other species, MAP packaging and rooting effects is warranted.

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		Oxygen (%)				
		0	1	5	10	21
Carbon Dioxide (%)	0	3.91 ± 0.14	5.00 ± 0.00^Y	4.09 ± 0.14	3.13 ± 0.25^Y	-- ^Z
	0.03	--	--	--	--	3.02 ± 0.18
	5	--	4.11 ± 0.14^Y	5.00 ± 0.00	5.00 ± 0.00	--
	10	--	4.72 ± 0.11^Y	4.87 ± 0.07	4.68 ± 0.10	--
	20	--	1.22 ± 0.43	--	--	--

^Z -- indicates the combination was not used as a treatment.

^Y These treatments dropped from subsequent experiments.

Fig 1. New Guinea Impatien cutting quality after 11 days in propagation preceded by 7 days storage in a range of modified atmospheric conditions. Values shows are treatment means (n=3) $\pm$ one standard error.

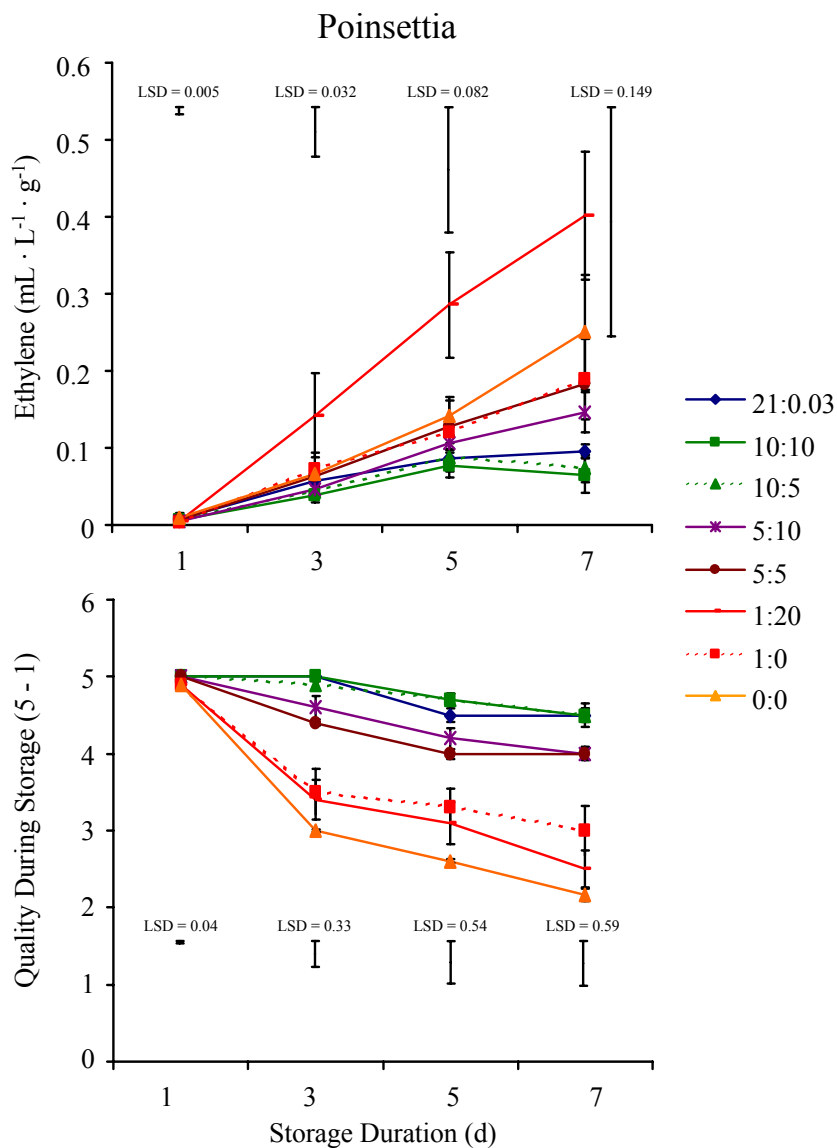


Fig 2. Effect of modified atmosphere storage on ethylene biosynthesis and quality during storage of poinsettia cuttings. Treatment means ($n=4$) for quality (5 = best, 1 = worst) and ethylene concentration ($\text{mL} \cdot \text{L}^{-1} \cdot \text{g}^{-1}$) during storage are plotted. Error bars through data points represent ± 1 standard error. Standard error for all treatments for a given day is represented by a single bar including the LSD value for that set of data points.

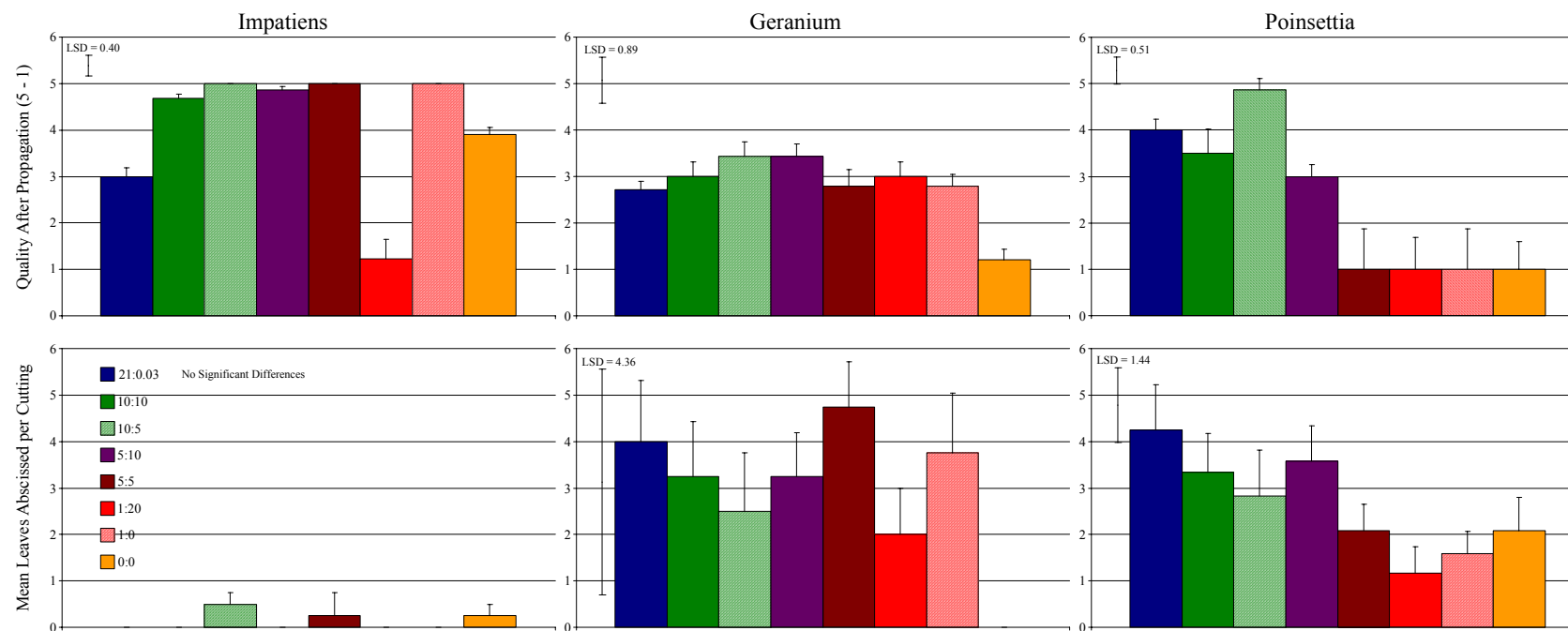
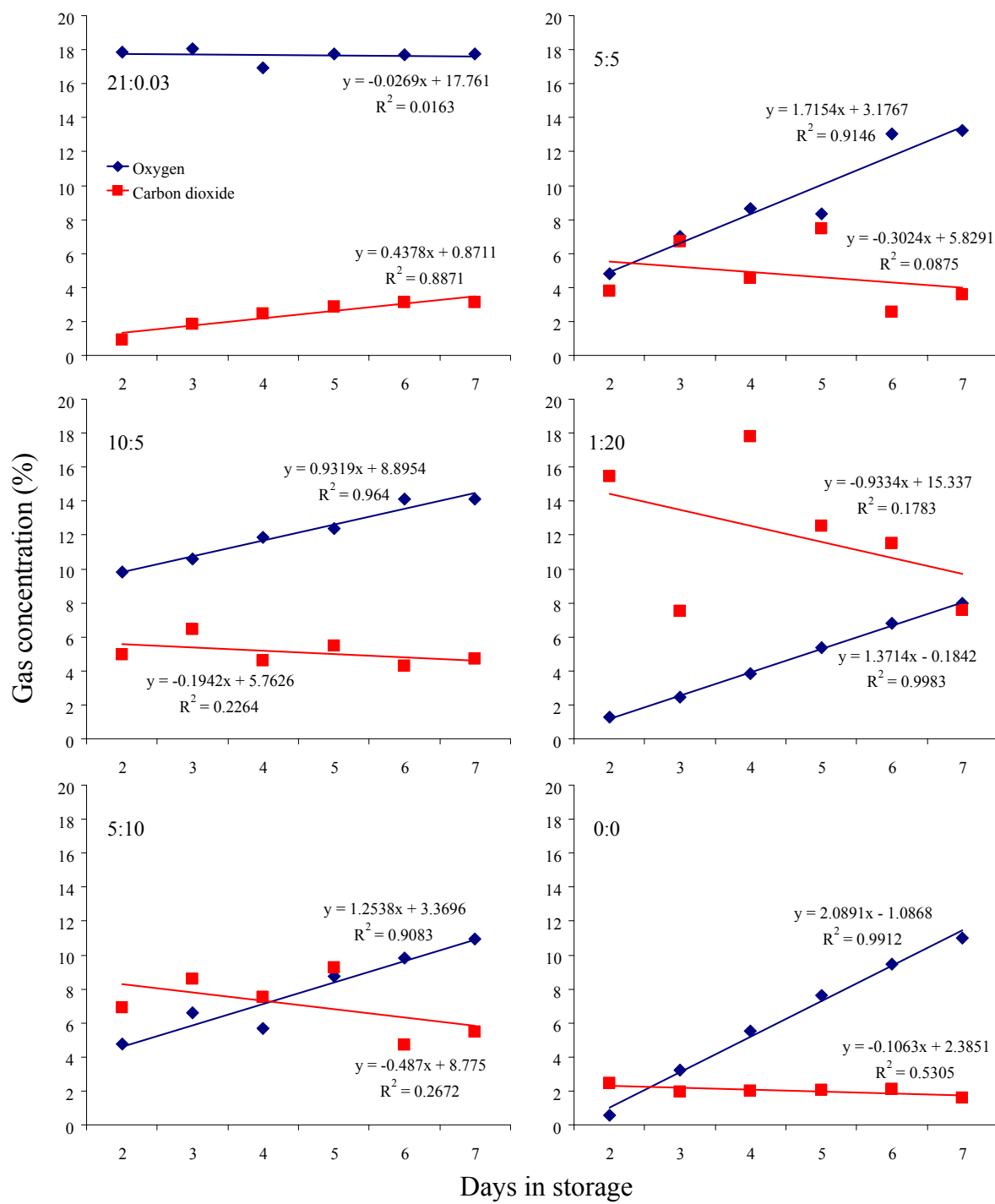


Fig 3. Effect of modified atmosphere storage on leaf abscission during propagation and post propagation quality of poinsettia, geranium and New Guinea impatiens cuttings. Treatment means (n=4) for quality (5 = best, 1 = worst) and numbers of leaves abscised per cutting during propagation are shown. Error bars represent ± 1 standard error. Additional statistical differences analyzed by one degree of freedom contrasts and are summarized in table 1.

Fig 4. Changes in oxygen and carbon dioxide concentration in sealed jars of New Guinea impatiens cuttings expressed as a percentage of total. Treatment means (n=3) during a 7 d dark storage period are shown. Treatments are represented as percentage ratios i.e. 21:0.03 is 21% oxygen and 0.03% carbon dioxide in the upper left of each figure.



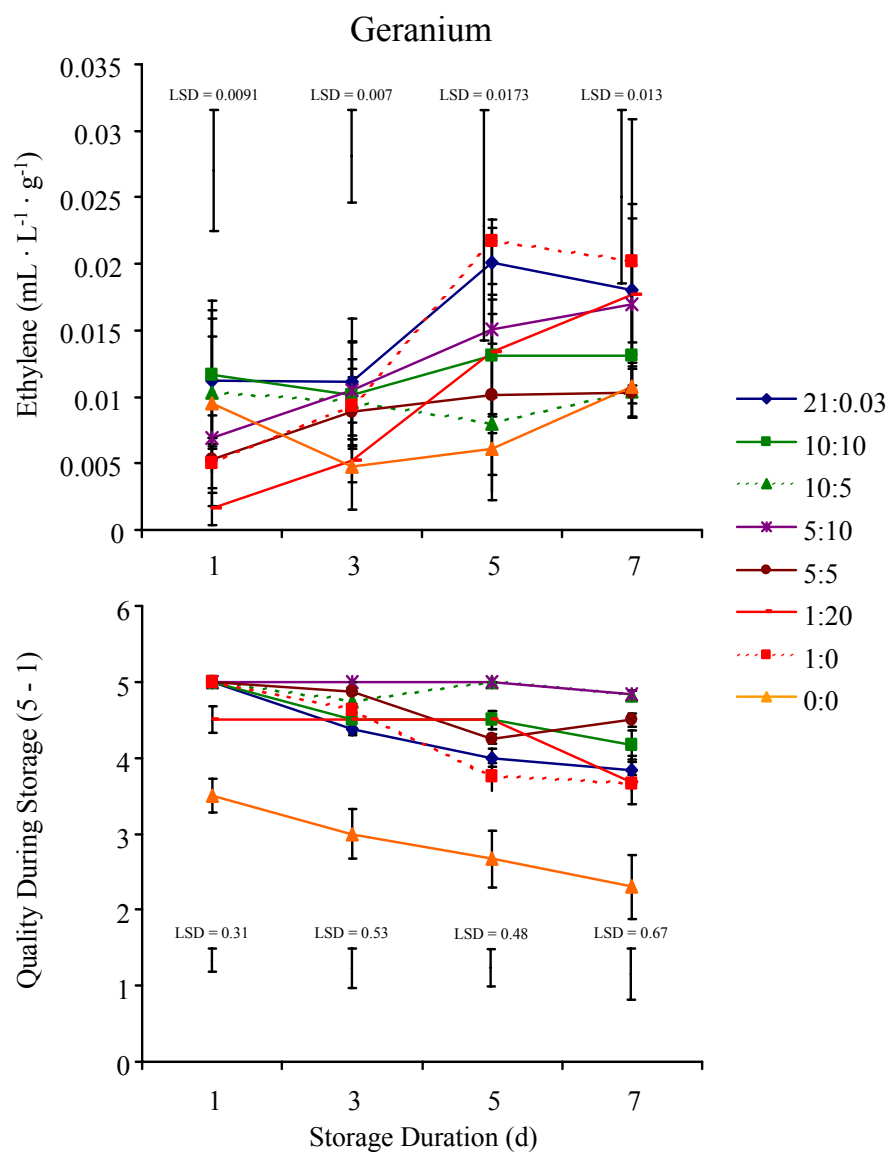
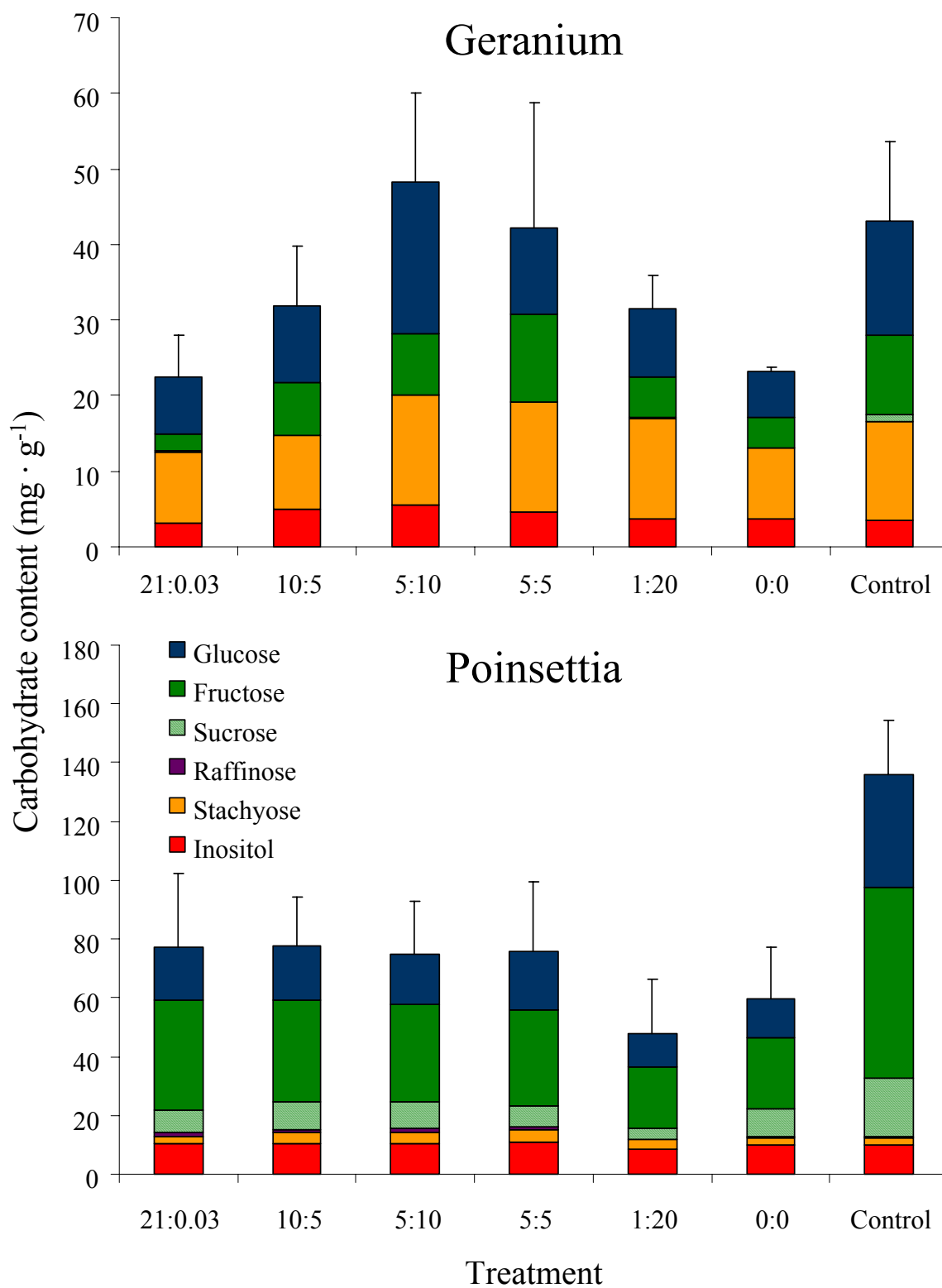


Fig 5. Effect of modified atmosphere storage on ethylene biosynthesis and quality during storage of geranium cuttings. Treatment means ($n=4$) for quality (5 = best, 1 = worst) and ethylene concentration ($\text{mL} \cdot \text{L}^{-1} \cdot \text{g}^{-1}$) during storage are plotted. Error bars through data points represent ± 1 standard error. Standard error for all treatments for a given day is represented by a single bar including the LSD value for that set of data points.

Fig 6. Effect of modified atmosphere storage on soluble carbohydrate biosynthesis and content during storage of poinsettia and geranium cuttings. Treatment means (n=4) for soluble carbohydrate content ($\text{mg} \cdot \text{g}^{-1}$) after a 7 d dark storage period are shown. Bar height represents total soluble carbohydrate content while bands represent the proportion of specific carbohydrate species. Treatments such as 10% oxygen, 5% carbon dioxide are represented as 10:5. The control treatment represents cuttings frozen immediately after harvest. Error bars represent ± 1 standard error for total soluble carbohydrate.



.Fig 7. Effect of modified atmosphere storage on organic acid biosynthesis and content during storage of poinsettia and geranium cuttings. Treatment means (n=4) for organic acid content ($\text{mg} \cdot \text{g}^{-1}$) after a 7 d dark storage period are shown. Bar height represents total organic acid content while bands represent the proportion of a specific organic acid. Treatments such as 10% oxygen, 5% carbon dioxide are represented as 10:5. The control treatment represents cuttings frozen immediately after harvest. Error bars represent ± 1 standard error for total organic acid.

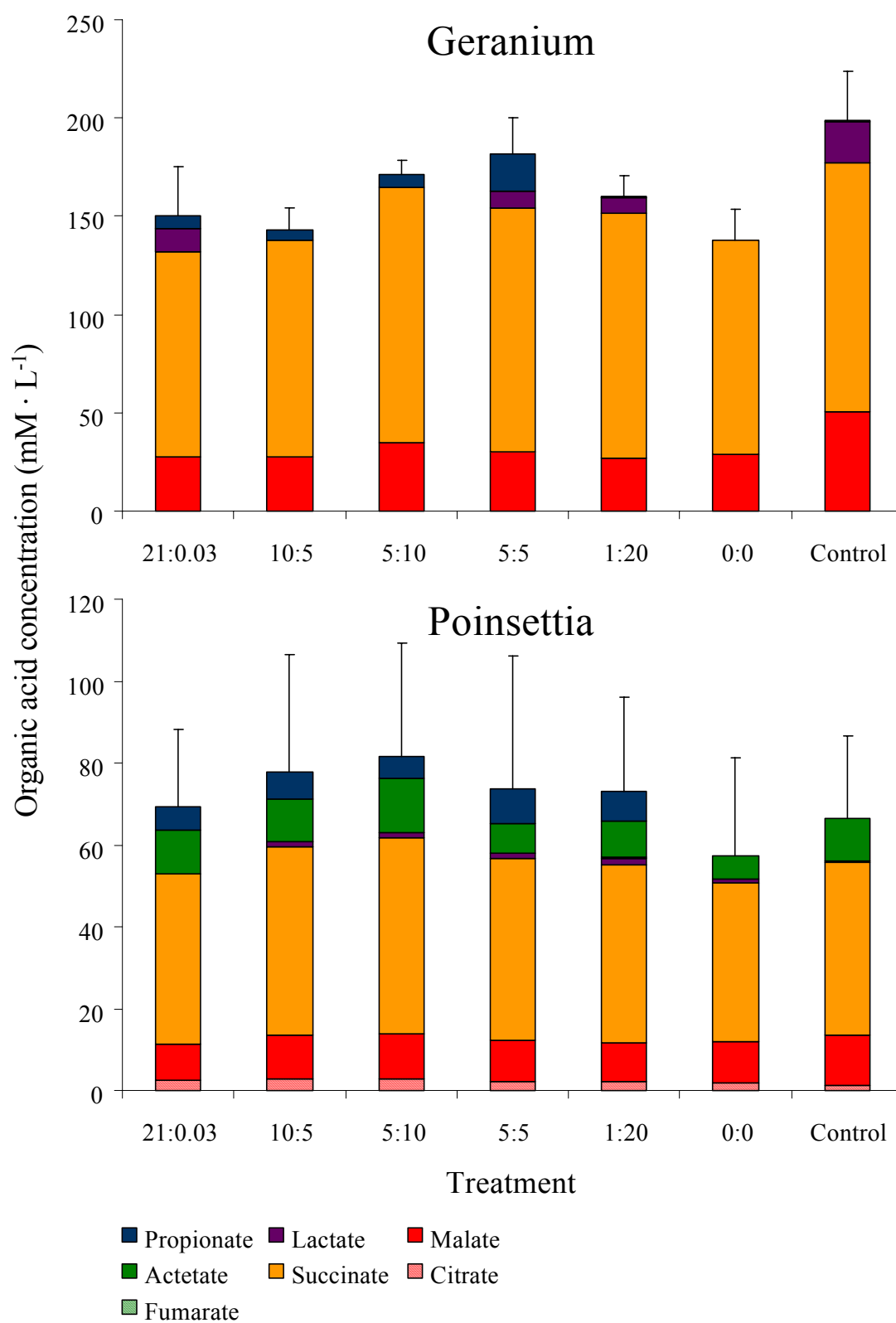


Table 1. Significant differences between selected modified atmosphere treatments. Comparisons of ethylene generated during storage, quality after storage, leaf abscission during propagation, and quality after propagation are made. Differences between means (n=5) were analyzed using single degree of freedom orthogonal contrasts as follows below.

Single degree of freedom contrasts	Geranium				New Guinea Impatiens				Poinsettia			
	Storage Quality	Propagation Quality	Ethylene	Leaf Absc	Storage Quality	Propagation Quality	Ethylene	Leaf Absc	Storage Quality	Propagation Quality	Ethylene	Leaf Absc
High O ₂ ($\geq 10\%$) vs. Low O ₂ ($< 10\%$)	*	NS	NS	NS	NS	NS	*	NS	*	*	*	*
Balanced (10:10, 10:5, 5:10, 5:5) vs. Imbalanced (21:0, 1:20, 1:0, 0:0)	*	NS	NS	NS	NS	*	NS	NS	NS	*	NS	NS
High CO ₂ (> 0.03) vs. Low CO ₂ (≤ 0.03)	*	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS
Control (21:0) vs. Balanced	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Control vs. Extremes (1:20, 0:0)	*	*	NS	NS	NS	NS	*	NS	*	*	*	NS
Control vs. 10:5	*	*	NS	NS	NS	*	*	NS	*	*	*	NS
All Others vs. 10:5	*	*	*	*	NS	NS	*	NS	*	*	*	NS

NS, * Nonsignificant, significant at $P \leq 0.05$, respectively.

CHAPTER 3

1-methylcyclopropene improves ethylene tolerance of unrooted herbaceous cuttings but affects adventitious root development.

This chapter was prepared in the style of the Journal of the American Society for Horticultural Science and will be submitted for review

Abstract. Careful ethylene management during shipping is critical for maintaining the quality of unrooted cuttings of many herbaceous species. 1-methylcyclopropene (1-MCP) is an effective mitigant against ethylene induced abscission and yellowing and may affect adventitious root formation. Of sixty taxa propagated from herbaceous cuttings, only *Begonia hybrida* ‘Anita Louise’, *Portulaca oleracea* L. ‘Sleeping Beauty’ and *Lantana camara* L. ‘Patriot Sunbeam’ were sensitive to the exogenous application of $1 \mu\text{L} \cdot \text{L}^{-1}$ ethylene, as demonstrated by rapid leaf abscission within 24 h of treatment. Application of $700 \mu\text{L} \cdot \text{L}^{-1}$ 1-MCP prior to ethylene treatment prevented ethylene damage. 1-MCP application ($700 \mu\text{L} \cdot \text{L}^{-1}$) to *Euphorbia pulcherrima* Willd. ex Klotzsch ‘Visions of Grandeur’, *Impatiens hawkeri* W. Bull ‘Sonic Red’, *Pelargonium hortorum* ‘Rocky Mountain White’ *Pelargonium peltatum* (L.) L’Hérit. ‘Mandarin’, *Petunia* $\times$ *hybrida* ‘Suncatcher coral prism’ cuttings caused significant ethylene generation. Ethylene at $1 \mu\text{L} \cdot \text{L}^{-1}$ improved adventitious rooting of *Fuchsia triphylla* L. ‘Honeysuckle’ and *Pelargonium hortorum* Bailey (pro sp.) [*inquinans* $\times$ *zonale*] ‘Rocky Mountain White’. Subsequent tests showed that 1-MCP application reduced root number of *Pelargonium hortorum* ‘Kardino’ and delayed adventitious root formation of *Angelonia angustifolia* Benth. ‘Carita Lavender’, *Calibrachoa* $\times$ *hybrida* Llave & Lex. ‘Terra Cotta’, *I. hawkeri* ‘Sonic Red’, *Portulaca oleracea* L. ‘Fairytale’, *Sutera cordata* Kuntze ‘Abunda Blue Improved’ and *Verbena* $\times$ *hybrida* Groenl. & Ruempl. ‘Aztec Wild Rose’. These rooting effects were partially overcome by subsequent immediate exposure to ethylene.

Ethylene promotes leaf yellowing, abscission and general senescence in unrooted cuttings of croton (*Codiaeum variegatum* Blume.), lantana (*Lantana camara* L.) , and zonal geranium (*Pelargonium hortorum* Bailey (pro sp.) [*inquinans* × *zonale*]) (Kadner et al., 2000; Müller et al., 1998). For successful rooting, ethylene must be carefully managed during handling, shipping and propagation. Though a problem during shipping, ethylene, along with abscisic acid (ABA), coordinates growth under water stress and aids adventitious root formation (Clark et al., 1999; Mergemann and Sauter, 2000; Sharp and LeNoble, 2002; Stepanova and Alonso, 2005).

Ethylene presents postharvest problems for some species but not all. For those sensitive species, research into preventing damage from excess ethylene has involved silver thiosulfate, silver nitrate and 1-methylcyclopropene (1-MCP) pretreatments as well as packaging perforation to allow ethylene dissipation and 1-MCP sustained release mechanisms (Kadner and Druege, 2004; Kadner et al., 2000; Macnish et al., 2004).

1-MCP acts as an ethylene binding site antagonist so disrupts subsequent downstream ethylene mediated developmental signaling. It is used widely in the produce and floricultural industries and is increasingly used on unrooted cuttings (Blankenship and Dole, 2003). In zonal geranium, 1-MCP prevents leaf yellowing of unrooted cuttings and reportedly improves rooting percentage (Kadner and Druege, 2004; Serek et al., 1998). However, other researchers have reported fewer adventitious roots on 1-MCP treated geranium cuttings compared to untreated cuttings (Rapaka et al., 2008). Interestingly, 1-MCP treatment reportedly stimulated excessive ethylene biosynthesis in unrooted geranium cuttings (Kadner and Druege, 2004; Rapaka et al., 2008). Since 1-MCP is a strong agonist of ethylene binding

sites, the observation indicates that geranium ethylene biosynthesis may be regulated by an autoinhibitory feedback mechanism (Kadner and Druege, 2004).

To better characterize the effect of 1-MCP treatment on a variety of cuttings, we sampled a broad range of vegetatively propagated herbaceous taxa for sensitivity to exogenous ethylene application, 1-MCP efficacy in preventing ethylene damage and 1-MCP effects on adventitious root development. Further studies with angleonia (*Angelonia angustifolia* Benth.) ‘Carita Lavender’, calibrachoa (*Calibrachoa* × *hybrida* Llave & Lex.) ‘Terra Cotta’, poinsettia (*Euphorbia pulcherrima* Willd. ex Klotzsch) ‘Visions of Grandeur’, zonal geranium ‘Kardino’, New Guinea impatiens (*Impatiens hawkeri* W. Bull) ‘Sonic Red’, ivy geranium (*Pelargonium peltatum* (L.) L’Hérit.) ‘Mandarin’, petunia (*Petunia* × *hybrida*) ‘Suncatcher coral prism’, portulaca (*Portulaca oleracea* L.) ‘Fairytale Sleeping Beauty’, sutera (*Sutera cordata* Kuntze) ‘Abunda Blue Improved’ and verbena (*Verbena* × *hybrida* Groenl. & Ruempl.) ‘Aztec Wild Rose’ examined the degree of delayed adventitious root development and excessive ethylene synthesis due to 1-MCP application to unrooted cuttings.

Material and Methods

Plant materials – taxa survey. Unrooted cuttings were propagated during October 2004 into 25 cm plastic pots containing a soilless growing substrate (Fafard P3, Conrad Fafard, Inc. Agawam, Mass.) and propagated under intermittent mist. Once rooted, these plants were maintained as stock plants and irrigated manually with clear water, 600 mg · L⁻¹ N liquid fertilizer (20N-4.37P-16.6K, Ultrasol, SQM North America, Atlanta), or flowable lime

(Limestone F, Cleary Chemical Inc., Dayton, N.J.) to maintain a target pour though E.C. of 2.0 to 3.5 dS · m⁻² and a pH of 5.8 to 6.2. Plants were thoroughly watered before cutting harvest. Temperature set points were 24/18°C, average daily light integral (DLI) was 11 mol · m⁻² · d⁻¹ during the experiment.

Plant materials – early rooting and root development experiments. Unrooted cuttings of angelonia ‘Carita Lavender’, calibrachoa ‘Terra Cotta’, ivy geranium ‘Mandarin’, New Guinea impatiens ‘Sonic Red’, petunia ‘Suncatcher Coral Prism’, poinsettia ‘Visions of Grandeur’, portulaca ‘Fairytale Sleeping Beauty’, sutera ‘Abunda Blue Improved’, verbena ‘Aztec Wild Rose’ and zonal geranium ‘Kardino’ were propagated on 12 September 2006 into 25 cm, 6.85 L, plastic pots containing a soilless growing substrate (Fafard P3, Conrad Fafard, Inc.) and kept under intermittent mist during propagation. Once rooted, these plants were maintained as stock plants and irrigated manually with clear water, 600 mg · L⁻¹ N liquid fertilizer (20N-4.37P-16.6K, Ultrasol, SQM North America, Atlanta), or flowable lime (Limestone F, Cleary Chemical Inc., Dayton, N.J.) to maintain a target pour though E.C. of 2.0 to 3.5 dS · m⁻² and a pH of 5.8 to 6.2. Plants were thoroughly watered before cutting harvest. Temperature set points were 24/18°C, average DLI was 11 mol · m⁻² · d⁻¹ during the experiment.

Ethylene and 1-MCP treatment. Single node cuttings, or larger as appropriate, of similar size and age were arbitrarily harvested from all parts of the stock plant. Cuttings were placed into clean glass jars covered by a damp paper towel moistened with DI water. Jars were placed

into 210 L gas tight chambers and treated with 0 or 700 nL · L⁻¹ 1-MCP (EthylBloc, Floralife Inc., Walterboro, S.C.) for 4 hours. After 1-MCP treatment was completed, the jars were divided randomly and equally among three chambers and the chamber atmospheres were adjusted to 0, 0.1 or 1.0 µL · L⁻¹ ethylene. Cuttings remained in the chambers overnight (at least 20 hrs). The 0 ppm chamber contained activated charcoal to absorb extraneous ethylene. Before propagation, leaf turgidity (1 = all turgid, 2 = one or two wilted, 3 = all wilted), number of abscised leaves, and overall cutting quality (excellent, good, fair, poor, dead on a scale from 5 to 1 respectively) was recorded.

Data collection – taxa survey. After 1-MCP and ethylene treatment cuttings were propagated into bedding plant flats filled with soilless growing substrate (Fafard P3, Conrad Fafard, Inc.) and placed under mist. Cuttings were observed daily for symptoms of ethylene damage. Root development was recorded at three weeks after propagation on the following scale: 3 = presence of more than five roots longer than 2 cm. 2 = presence of more than five roots shorter than 2 cm, 1 = callus development or presence of one to five roots, 0 = no callus or root development.

Data collection – early rooting experiment. After treatment cuttings were propagated into flats containing a soilless growing substrate (Fafard P3, Conrad Fafard, Inc.) and placed under mist operating at 6 seconds every 6 minutes for the first 48 hours then, 6 seconds every 10 minutes for 10 d. Data were recorded daily for the number of abscised leaves, yellowing of leaves at least 1/3 of full size, and cutting quality using the scales described in the ethylene

and 1-MCP treatment section above. Additionally the number of cuttings showing initials or callus formation, and the number of cuttings showing roots > 1 mm was recorded daily for 10 days.

Data collection – root development experiment. After treatment cuttings were propagated into flats of perlite and placed under mist operating at 6 seconds every 6 minutes for the first 48 hours then, 6 seconds every 10 minutes for 21 d. Data were recorded daily for the number of abscised leaves, yellowing of leaves at least 1/3 of full size, and cutting quality using the scales described in the ethylene and 1-MCP treatment section above. At the end of propagation cuttings were evaluated for root number (defined as a root at least 1 mm long), 5 longest roots, root area and dry weight.

Statistical analysis. The taxa survey was conducted as a completely randomized design (CRD) with 12 individual cutting replications and a factorial treatment design of two 1-MCP concentrations (0 and $700 \text{ nL} \cdot \text{L}^{-1}$) by three ethylene concentrations (0, 0.1 and $1 \mu\text{L} \cdot \text{L}^{-1}$). Statistical analysis was conducted using PROC GLM with a hypothesis test statement and an error term of replicates within treatment.

The remaining experiments were conducted as randomized complete block designs (RCBD) of three blocks and a factorial treatment design of two 1-MCP concentrations (0 and $700 \text{ nL} \cdot \text{L}^{-1}$) by three ethylene concentrations (0, 0.1 and $1 \mu\text{L} \cdot \text{L}^{-1}$). Statistical analysis of the early rooting data was conducted using analysis of variance (ANOVA) in combination with several methods of means testing and regression (SAS Institute, Cary, N.C.). Early

rooting experimental main effects were analyzed using PROC MIXED at $P \leq 0.05$. Weibull model (Equation 1) fitting was tested using PROC NLIN at $P \leq 0.05$. Model terms were evaluated for significance by comparison of F values at $\alpha = 0.05$. The Weibull model is the best choice when modeling time to event data such as root emergence, flowering, or germination (Dias, 2001).

Data from the root development experiment were also tested for main effects using analysis of variance (ANOVA) using a general linear model (SAS Institute, Cary, N.C.). Subsequent comparisons between treatments or means of combined treatments were made using orthogonal contrasts at the $P \leq 0.05$ comparison level.

Results.

Taxa survey. Cuttings of lantana ‘Patriot Sunbeam’, portulaca, and begonia ‘Snowcap’ showed leaf abscission when treated with 1 but not $0.1 \mu\text{L} \cdot \text{L}^{-1}$ ethylene (Table 1). However, begonia and portulaca cuttings defoliated by ethylene still rooted rapidly. Ethylene defoliated lantana cuttings rooted very poorly. Adventitious root formation on cuttings of *Fuchsia triphylla* L. ‘Honeysuckle’ was improved by $1 \mu\text{L} \cdot \text{L}^{-1}$ ethylene treatment and the same treatment improved rooting of zonal geranium ‘Rocky Mountain White’ cuttings. Treatment with 1-MCP prevented ethylene induced defoliation in all species. The rooting of begonia ‘Miss Murry’ and *Calibrachoa hybrida* ‘Terra cotta’ cuttings was reduced by 1-MCP treatment (Table 1). However, the rooting of *Lantana camara* ‘Sunbeam Patriot’ and *Pelargonium peltatum* ‘Amethyst’ were improved by 1-MCP application. Adventitious

rooting appeared unaffected by ethylene or 1-MCP exposure for the majority of taxa tested (Table 2).

Ethylene generation. After being treated with 1-MCP, petunia ‘Suncatcher Coral Prism’ cuttings generated $1.05 \mu\text{L} \cdot \text{L}^{-1} \cdot \text{g}^{-1}$ fresh weight ethylene, while impatiens ‘Sonic Red’ cuttings produced $0.13 \mu\text{L} \cdot \text{L}^{-1} \cdot \text{g}^{-1}$ fresh weight ethylene by the end of a 7 day storage period (Fig. 1). Control cuttings for these two taxa produced 0.08 and $0.04 \mu\text{L} \cdot \text{L}^{-1} \cdot \text{g}^{-1}$ fresh weight ethylene over the same period, respectively. Similarly, zonal geranium ‘Kardino’ and ivy geranium ‘Mandarin’ cuttings produced 0.54 and $0.4 \mu\text{L} \cdot \text{L}^{-1} \cdot \text{g}^{-1}$ fresh weight ethylene respectively and poinsettia ‘Visions of Grandeur’ cuttings produced $0.22 \mu\text{L} \cdot \text{L}^{-1} \cdot \text{g}^{-1}$ fresh weight ethylene. Untreated cuttings for these taxa produced 0.02 , 0.01 , and $0.06 \mu\text{L} \cdot \text{L}^{-1} \cdot \text{g}^{-1}$ fresh weight ethylene over the same period, respectively.

Early rooting experiment. Treatment with 1-MCP caused significant rooting delays in angelonia, calibrachoa, impatiens, portulaca, sutera and verbena (Fig 2). Time to 100% of cuttings showing root emergence was delayed by 0.39, 0.73, 1, 0.59, 0.31 and 0.60 days for angelonia, calibrachoa, impatiens, portulaca, sutera and verbena, respectively.

1-MCP delayed both the initial onset of adventitious root development and subsequent emergence. For example, first root emergence of 1-MCP treated impatiens cuttings took an additional 1.7 days. Though no delay in initial root emergence was observed in 1-MCP treated verbena cuttings, an additional 0.6 days was required for all treated cuttings to show roots compared to untreated. Those species, which normally took longer to develop

adventitious roots, were more delayed by 1-MCP treatment than species, which normally took less time to develop adventitious roots. For example for portulaca cuttings, a rapidly rooting species, 1-MCP treatment delayed initial root emergence 0.36 d, whereas with impatiens cuttings, a slower rooting species, 1-MCP delayed initial root emergence 1.70 d. However, 100% of cuttings from all species showed some adventitious rooting by 10 d.

Root development experiment. Geranium cuttings treated with 1-MCP had significant differences in root length and number (Fig 3), area (Fig 4), dry weight (Fig 5) and abscission (Fig 6) compared to untreated cuttings. Subsequent exposure to ethylene partially reversed adventitious root inhibition. For example, the root numbers and lengths of 1-MCP treated cuttings exposed to $1 \mu\text{L} \cdot \text{L}^{-1}$ ethylene were not significantly ($P > 0.05$) different compared to control cuttings treated with $1 \mu\text{L} \cdot \text{L}^{-1}$ ethylene. 1-MCP treated cuttings unexposed to ethylene had significantly ($P \leq 0.05$) fewer roots per cutting 15.79 compared to 25.53 for control. Roots on these cuttings were also shorter, 22.12 cm compared to 32.08 cm for control. Among cuttings not treated with 1-MCP, there were no significant ($P > 0.05$) differences in root length or number between ethylene treatments.

During propagation, 1-MCP treated geranium cuttings had less leaf yellowing than untreated cuttings. However, 1-MCP treated cuttings abscised a greater number of leaves than did untreated cuttings (Fig. 6).

Discussion

In the taxa survey adventitious rooting of most species appeared unaffected by 1-MCP. Previous work has shown that 1-MCP may slow rooting of hibiscus and chrysanthemum (Kadner and Druege, 2004). However, there are incidences of both increased and decreased adventitious rooting with 1-MCP treatment in our study and in other studies (Kadner and Druege, 2004; Serek et al., 1998). In our study, 1-MCP treatment significantly reduced rooting of begonia ‘Miss Murry’ and calibrachoa ‘Terra Cotta’ cuttings. Yet appeared to increase rooting of ivy geranium ‘Amethyst’ and lantana ‘Patriot Sunbeam’ cuttings. As the taxa survey rooting evaluations were conducted at three weeks after propagation, it is possible that some differences in rooting response were overcome by subsequent root development, especially with rapidly rooting taxa.

Our results, and others, show that treatment with 1-MCP decreased leaf abscission in ethylene sensitive species and delayed leaf yellowing in others (Serek et al., 1998). No significant difference in leaf yellowing of zonal geranium cuttings after any 1-MCP and ethylene treatment was observed. Postharvest leaf yellowing and senescence of unrooted zonal geranium cuttings is inversely related to the combined effect of carbohydrate content and ethylene exposure (Rapaka et al., 2008). The cuttings used in this study were not stored long enough to deplete carbohydrate reserves to a point where senescence would be triggered, approximately 72 hours (Rapaka et al., 2008). However, there was a significant increase in abscised leaves of zonal geranium cuttings treated with 1-MCP and subsequently exposed to $0.1 \mu\text{L} \cdot \text{L}^{-1}$ ethylene, and an observed but non-significant increase in leaf yellowing for this treatment. The cause may be a dosage dependent biphasic response to

ethylene as observed by other researchers (Pierik et al., 2006). Lower concentrations ($0.1 \mu\text{L} \cdot \text{L}^{-1}$) result in a specific set of physiological responses, while the higher doses ($0.1 \mu\text{L} \cdot \text{L}^{-1}$) do not.

Clearly 1-MCP delayed the onset of rooting in many taxa and decreased root number in zonal geranium. For rapidly rooting species such as portulaca, the delay was hardly perceptible and may not be commercially important. With slower to root taxa, such as New Guinea impatiens, the delay was more easily observed and could become significant enough to delay production cycles. For example, geranium cuttings with fewer and shorter roots due to 1-MCP use, would result in weaker, slower growing plants after propagation. The decrease in root number may also be a problem for rapidly rooting species and more work should be done on this matter.

Interestingly, subsequent treatment with ethylene appears to partially reverse the inhibitory adventitious rooting effect caused by 1-MCP. Zonal geranium cuttings treated with 1-MCP, and then exposed to $1 \mu\text{L} \cdot \text{L}^{-1}$ ethylene had similar root numbers and lengths compared to control cuttings untreated with 1-MCP. Additionally, 1-MCP treated zonal geranium cuttings subsequently exposed to ethylene from the root development and taxa survey experiments had significantly greater root numbers and lengths compared to 1-MCP treated cuttings that were not exposed to ethylene. These observations support the results of Kadner and Druege (2004) and Serek et al. (1998) and help explain the inconsistent results reported regarding 1-MCP effects on adventitious rooting.

Serek et al. (1998) treated zonal geranium cuttings with 1-MCP and propagated one set immediately and stored another set in perforated polyethylene bags. The stored cuttings

had a significantly higher rooting percentage compared to untreated stored cuttings. Kadner and Druege (2004) treated zonal geranium cuttings with 1-MCP and placed them into polypropylene boxes. These cuttings had similar numbers of roots compared to untreated stored zonal geranium cuttings. Cuttings in our study were placed into 210 L steel drums with plexiglass tops after 1-MCP treatment and then exposed to exogenous ethylene.

It has been established that 1-MCP treatment can cause significant ethylene biosynthesis in some species. Ethylene biosynthesis is likely regulated by feedback inhibition triggered by ethylene binding (Alonso and Stepanova, 2004; Wang et al., 2004). 1-MCP acts as an antagonist of ethylene binding and signalling, disrupting the feedback inhibition mechanism resulting in increased ethylene biosynthesis.

The differences in storage headspace among these studies mean that the cuttings involved received varying doses of ethylene during storage after 1-MCP exposure. Our own results establish that subsequent ethylene exposure partially reversed 1-MCP's adventitious root inhibiting effect. Thus it is not surprising that cuttings stored after 1-MCP treatment exhibited little or no inhibition of adventitious root formation. The observation is certainly worthy of further investigation.

The decrease in zonal geranium root length of 1-MCP treated cuttings is possibly attributed to a delay in the onset of rooting, a reduction in root growth rate or root cell elongation, or interference with root emergence. Roots from treated and untreated cuttings appeared to grow at the same rate once emerged, but differed in length because they grew for a different number of days. However, this explanation fails to account for the drop in root number. A more encompassing explanation of these results is that 1-MCPs irreversible

attachment to ethylene binding sites interfered with root initiation at very early stages of adventitious root formation.

Studies involving ethylene's role in rice adventitious root formation offer possible interpretations of our results. The adventitious rootless 1 (*arl1*) mutant in rice does not form adventitious roots due to the failure to initiate periclinal divisions of the pericycle. ARL1 is an auxin and ethylene response factor that is expressed in a pattern that mirrors that of auxin distribution. Additionally, ARL1 is involved in auxin and ethylene mediated cell dedifferentiation and it is active in the pericycle and adjacent peripheral vascular tissues in the rice stem cylinder (Liu et al., 2005). Since ARL1 is active in ethylene mediated cell dedifferentiation, 1-MCP is likely to disrupt the expression of homologous ARL1 genes in other species. If true, 1-MCP treatment would result in phenotypic responses similar to *arl1* rice.

Ethylene plays another key role later in rice adventitious root formation. Prior to root emergence in submerged rice, localized epidermal cell death is observed on the node external to the emerging root tip. Eventually a crack forms through which the root emerges. Apoptosis is confined only to those cells covering the emerging root tip. Cell death is inducible by submersion in water and application of 1-aminocyclopropane-1-carboxylic acid (1-ACC) but is prevented by the application of 2,5-norbornadiene (bicyclohepta-2,5-diene) (Mergemann and Sauter, 2000), a non-competitive inhibitor of ethylene receptors similar to 1-MCP.

Thus, the reduction in root numbers caused by 1-MCP treatment may be attributed to interference with the up-regulation of genes orthologous to ARL1 in the species examined

here. In our work 1-MCP and ethylene treatments were carried out soon after harvest and the cuttings were placed soon thereafter into propagation. So the 1-MCP and ethylene effects observed here are the result of those very early treatments. Since ARL1 is responsible for inducing periclinal divisions within the pericycle, the very earliest step of adventitious root formation, 1-MCP may interfere most importantly with a similar protein in these species.

Though clearly necessary for adventitious root emergence in rice, it is less clear what role ethylene may have in adventitious root emergence in herbaceous cuttings.

The shorter roots of 1-MCP treated cuttings suggests delayed emergence or reduced root growth rate or elongation. However, one must also consider the fact that 1-MCP treated cuttings subsequently exposed to ethylene, had roots as long as control cuttings, suggesting that the problem is primarily delayed dedifferentiation rather than emergence or growth. Research investigating arabidopsis ethylene biosynthesis regulation may offer an explanation of these observations (Chae and Kieber, 2005).

Studies using arabidopsis have shown that a calcium-dependent protein kinase (CDPK) phosphorylation of 1-aminocyclopropane-1-carboxylate synthase (ACS) proteins in response to unknown stimuli prevents ubiquitination and subsequent 26S proteasome degradation allowing further ACS activity. Still other stimuli result in the dephosphorylation of ACS which is then ubiquitinated by the ETO1-CUL3 (ethylene overproducer – cullulin ubiquitin ligase) complex, and proteasome degradation thus limiting ethylene biosynthesis (Chae and Kieber, 2005; Wang et al., 2004). Additionally, transcriptional regulation of ethylene response genes is regulated by feedback inhibition via the EBF1,2 (ethylene insensitive-3 f-box binding protein) complex (Alonso and Stepanova, 2004). If the enzyme

responsible for the phosphorylation of ACS is among those down-regulated by EBF1,2, 1-MCP binding would result in increased expression of this enzyme and thus, increased ACS activity and ethylene biosynthesis.

Typically, the root tip of wild type arabidopsis seedlings contains two to three cells in the meristem or quiescent center. Arabidopsis seedlings mutated such that they produce excessive ethylene in the roots, exhibit supernumerary divisions in the quiescent center cells. Exogenous ethylene exposure also induces supernumerary quiescent center divisions in wild type seedlings (Ortega-Martínez et al., 2007). Adventitious roots typically arise from the pericycle, a type of meristem. If exogenous ethylene stimulates excessive cell division in arabidopsis root tip meristems, it is possible that the same affect occurs in the pericycle, resulting in increased adventitious root numbers. Similarly, decreased ethylene concentration or signalling could depress pericyclic meristem divisions, resulting in fewer adventitious roots.

The results presented here help characterize the effects of 1-MCP use on unrooted herbaceous cuttings. However, several new questions are raised. The very earliest events of cell differentiation and root initial formation of 1-MCP treated cuttings should be investigated. Anatomical studies of the pericycle focused on cell differentiation of 1-MCP treated and untreated tissues would be most useful. Additionally, information concerning root growth rates and elongation would give some direction to these efforts. It is also clear that 1-MCP may make an appealing adjunct to molecular studies investigating ethylene-signalling pathways.

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$$Y = a \left(1 - e^{-\left\{ \left[\frac{(X-l)^c}{k} \right] \right\}} \right)$$

Equation 1. The Weibull function used to analyze adventitious rooting response. In this case the model terms may be interpreted as follows: Y is the percentage of cuttings with roots on day X , a is the total percent of rooted cuttings on a given day, l is the number of days to the onset of rooting, k represents the number of days from onset to maximal rooting. The dimensionless parameter c estimates the symmetry of the germination distribution around the normal curve ($c > 3.60$ = negative asymmetry, $c < 3.26$ = positive asymmetry, and $3.26 \leq c \leq 3.60$ = symmetrical).

Table 1. Effect of ethylene and 1-MCP on rooting of cuttings from 14 taxa of vegetatively propagated taxa. Root ratings: 3 = more than 5 roots > 2 cm, 2 = more than 5 roots < 2 cm, 1 = callus development or presence of 1 to 5 roots, and 0 = no callus or root development. Means are an average of 12 single cutting replications.

Species	Cultivar	Defoliation by							Significance		
		1 nL · L ⁻¹	0 nL · L ⁻¹ 1- MCP			700 nL · L ⁻¹ 1- MCP					
		Ethylene	0	0.1	1	0	0.1	1	1-MCP	Ethylene	Interaction
<i>Angelonia angustifolia</i> Benth.	Carita Lavender	N	1.9	1.6	1.8	1.8	1.8	1	NS	NS	*
<i>Begonia hybrida</i>	Anita Louise	N	2.8	3	3	2.9	3	3	NS	*	NS
	Miss Murry	N	2.3	2.1	2	2	1.9	2	*	NS	NS
	Snowcap	Y	3	3	2.4	2.7	2.8	2.8	NS	NS	**
	Cultivar unknown	N	2	1.9	1.7	1.8	1.7	2	NS	NS	*
<i>Calibrachoa hybrida</i>	Terra Cotta	N	3	3	3	2.9	2.9	2.7	*	NS	NS
<i>Fuchsia triphylla</i> L.	Honeysuckle	N	1.8	2.5	2.8	2	2.2	2.8	NS	**	NS
<i>Pelargonium hortorum</i> Bailey	Rocky Mountain										
(pro sp.) [<i>inquinans</i> × <i>zonale</i>]	White	N	3	2.7	2.7	2.8	2.8	2.4	NS	*	NS

Table 1 (continued).

Species	Cultivar	Defoliation by							Significance		
		1 nL · L ⁻¹	0 nL · L ⁻¹ 1- MCP			700 nL · L ⁻¹ 1- MCP					
		Ethylene	0	0.1	1	0	0.1	1	1-MCP	Ethylene	Interaction
<i>Pelargonium peltatum</i> (L.)	Amethyst										
L'Hérit.		N	2.5	3	2.2	3	2.9	2.8	*	NS	NS
<i>Lantana camara</i> L.	Patriot Sunbeam	Y	2	2	0.2	2	2	2	***	***	***
<i>Euphorbia pulcherrima</i> Willd.	Christmas Star										
ex Klotzsch		N	0.7	1	0.4	1	0.2	0.7	NS	NS	*
<i>Portulaca oleracea</i> L.	Fairytales										
	Sleeping Beauty	Y	3	3	3	3	3	3	NS	NS	NS

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.005, 0.0005, respectively.

Table 2. Species unaffected by 1-MCP or ethylene after 21 days in propagation.

Species	Cultivar
<i>Abutilon megapotamicum</i> (A. Spreng.) A. St.-Hil. & Naudin	none
<i>Alternanthera dentata</i> (Moench) Scheygr.	New burgundy
	Party Time
	Red
<i>Begonia hybrida</i>	Frosty
	Tom Ment
	Cultivar unknown
	Cultivar unknown
<i>Centradenia hybrida</i>	Purple Showers
<i>Solenostemon scutellarioides</i> (L.) Codd	Aurora
	Cultivar unknown
	Dark Copper
	Rustic Orange
<i>Impatiens walleriana</i> Hook. f.	Fiesta Pink Ruffle
<i>Pelargonium hortorum</i> Bailey	Blues
	Charleston
	Tango
<i>Graptophyllum pictum</i> Griff.	none
<i>Hemigraphis alternata</i> T. Anderson	none
<i>Iresine herbstii</i> Hook.	Purple Lady
<i>Pelargonium peltatum</i> (L.) L'Hérit.	Beach
	Lambda
	Mandarin

Table 2 (continued).

Species	Cultivar
<i>Impatiens hawkeri</i>	White Blizzard
	Fanfare Orange
	Sonic Red
	Sonic White
	Super Sonic Peach
	Super Sonic Red
	Super Sonic White
<i>Perilla frutescens</i> L. ex B.D.Jacks. var. <i>crispa</i> (Benth.) H.W.Li	Magilla
<i>Petunia</i> × <i>hybrida</i>	Suncatcher Coral Prism
<i>Plectranthus ciliatus</i> E.Mey.	Gold Coin
<i>Plectranthus ecklonii</i> Benth.	Mona Lavender
<i>Plectranthus tomentosus</i> Benth. ex E.Mey.	Nicolletta
<i>Plectranthus amboinicus</i> (Lour.) Spreng.	none
<i>Plectranthus madagascariensis</i> Benth.	none
<i>Salvia officinalis</i> L.	Tricolor
<i>Scaevola aemula</i> R.Br.	Blue Wonder
<i>Streptocarpus</i> × <i>hybridus</i> Voss	none
<i>Strobilanthes dyeriana</i> Mast.	none
<i>Sutera cordata</i> Kuntze	Abunda Blue Improved
<i>Torenia fournieri</i> Linden ex Fourn.	Summer Wave Blue
<i>Tradescantia zebrina</i> G.Don	Zebrina
<i>Verbena</i> × <i>hybrida</i>	Aztec Wild Rose

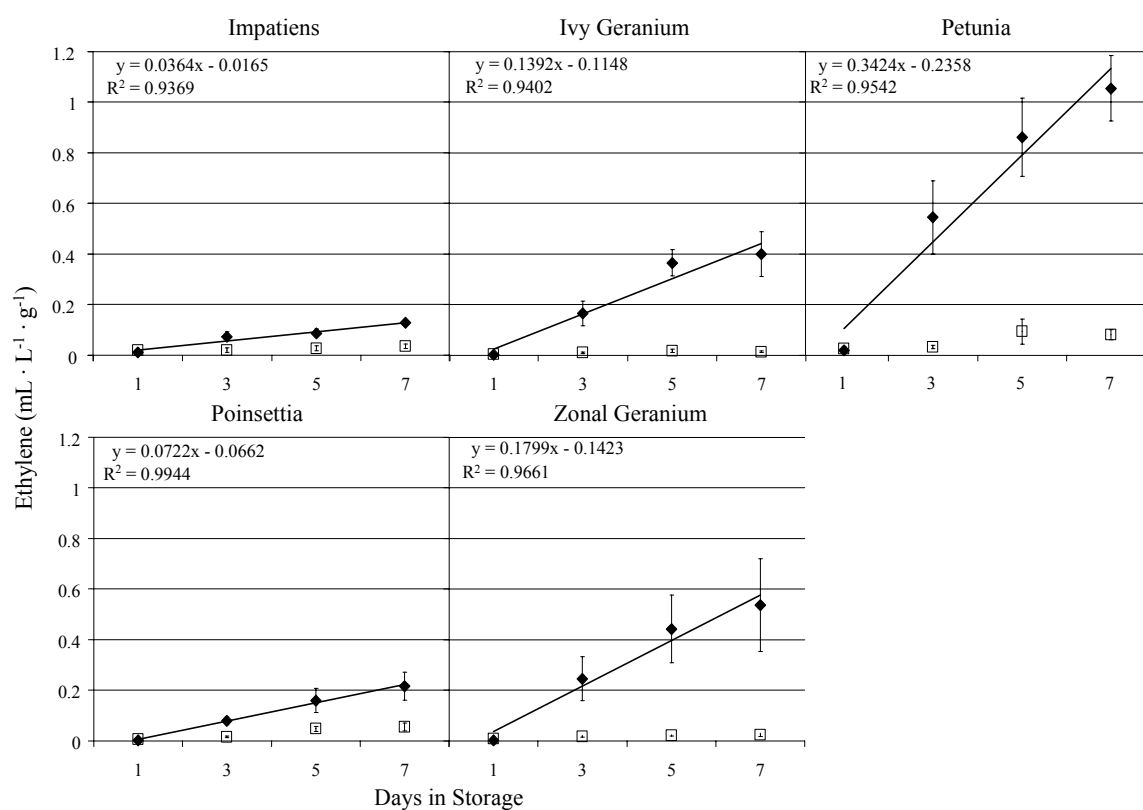


Fig. 1. Means (n=3) of cumulative ethylene concentrations over a 7 d period from 700 nL · L⁻¹

¹ 1-MCP (◆) and untreated (□) cuttings were plotted with error bars showing ± 1 SE.

Linear equations were generated on the entire dataset and superimposed on the means.

Equations and fit were given above each curve.

Fig. 2. Effect of 1-MCP treatment on adventitious root development of six vegetatively propagated species. Means ($n=3$) of percentage of cuttings with adventitious roots is plotted against days after propagation. Solid lines are Weibull predicted percentage rooting and ▲ and ■ are observed mean percentage rooting. Shaded areas represent a 95% confidence interval. Parameter estimates l , k and c are followed by the respective standard error in parenthesis. The parameter a (in this case total percentage rooted) is not included as it was universally estimated as 1 with no standard error.

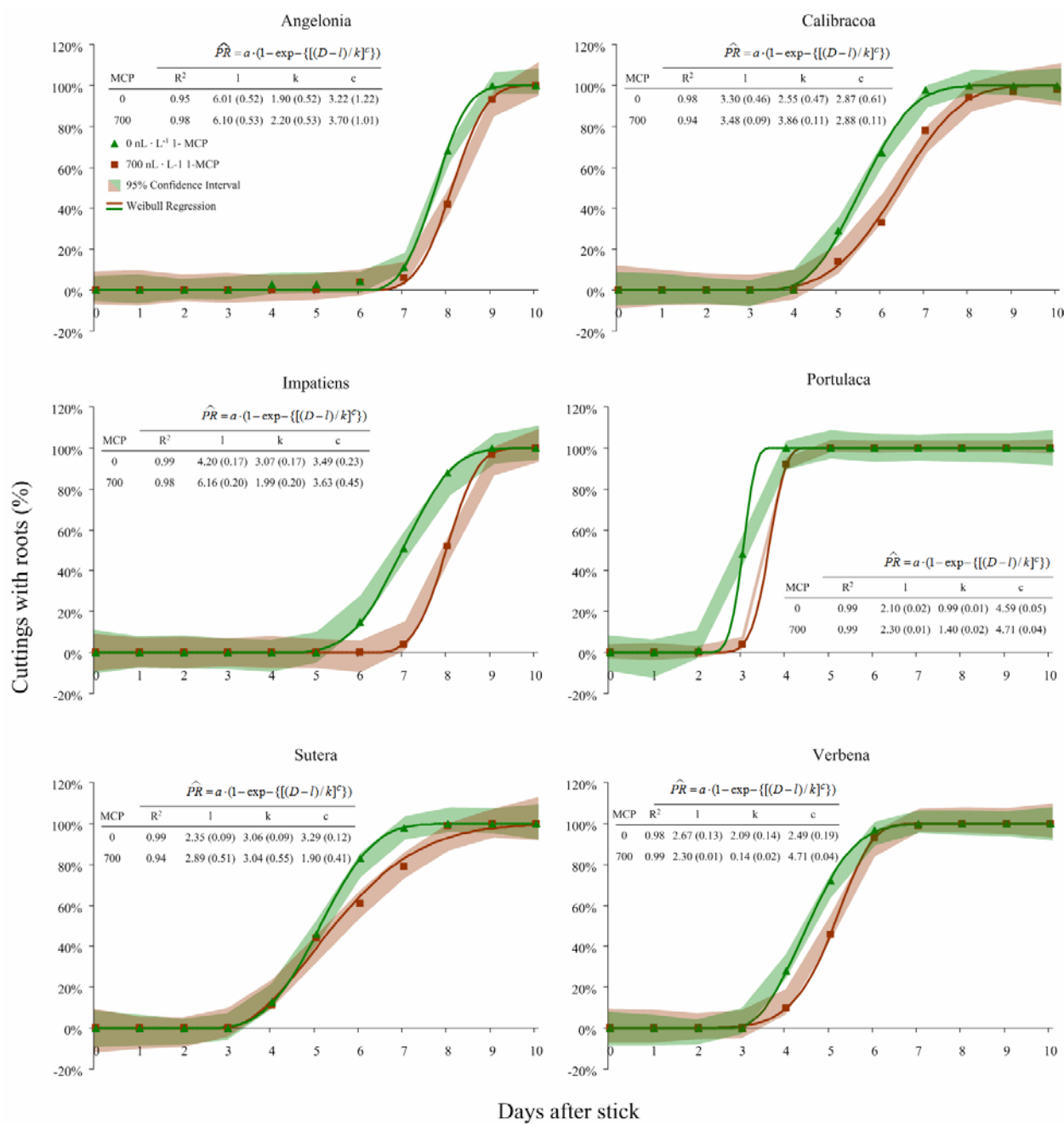
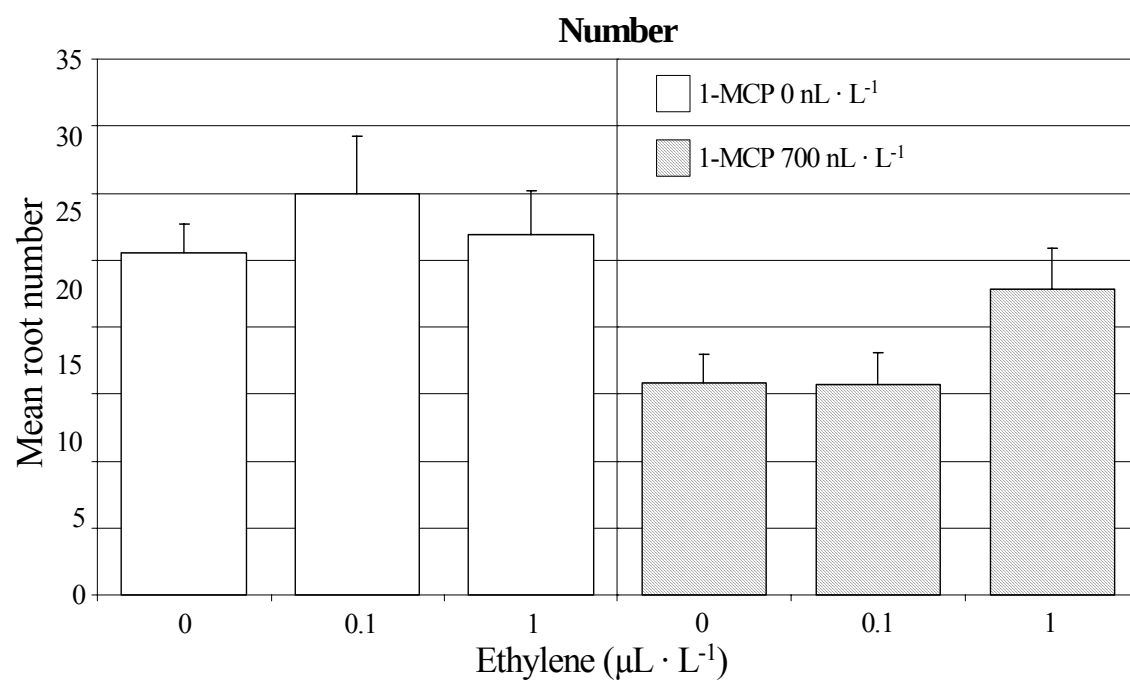
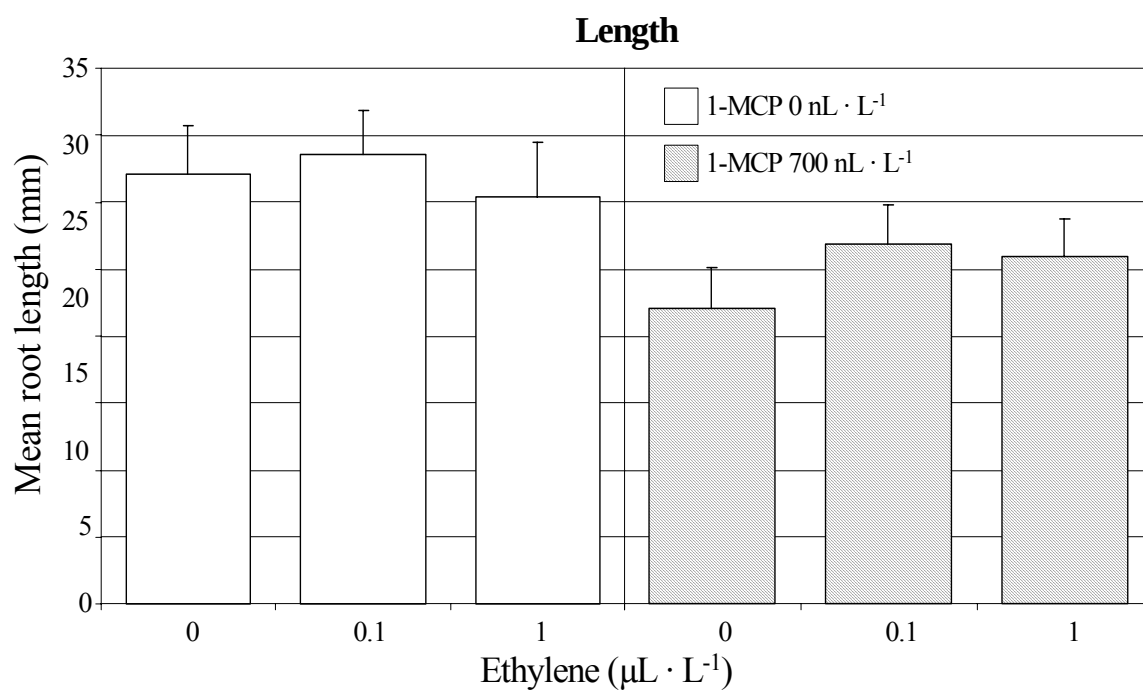


Fig. 3. Differences in zonal geranium ‘Kardino’ cutting root numbers and lengths 21 days after propagation. Error bars represent $\pm$ one standard error. Statistical differences analyzed by one degree of freedom contrasts and are summarized in table 3.



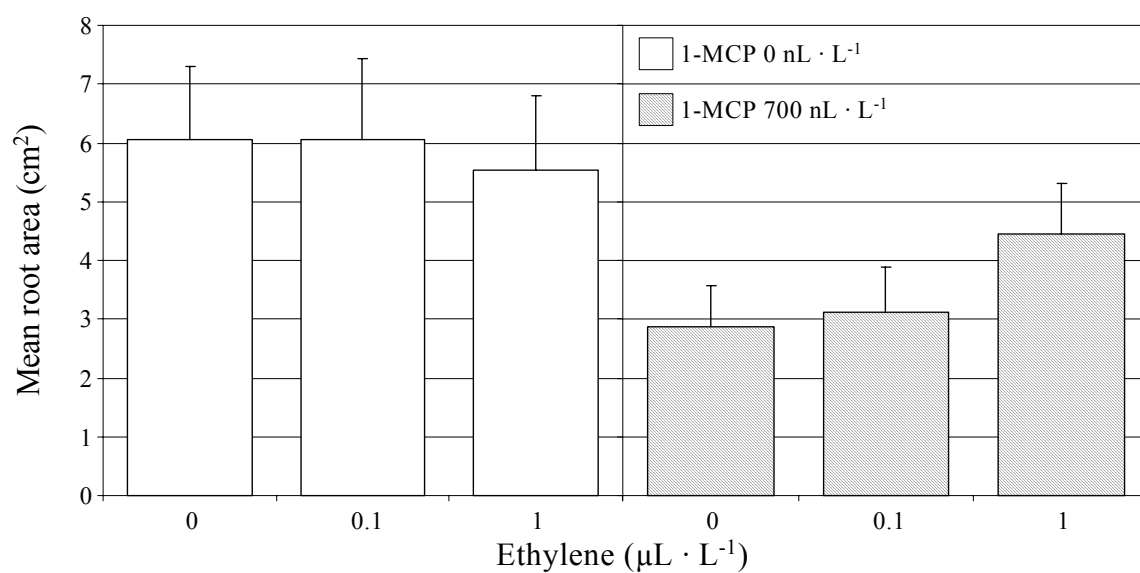


Fig. 4. Differences in zonal geranium ‘Kardino’ cutting root area 21 days after propagation. Error bars represent $\pm$ one standard error. Statistical differences analyzed by one degree of freedom contrasts and are summarized in table 3.

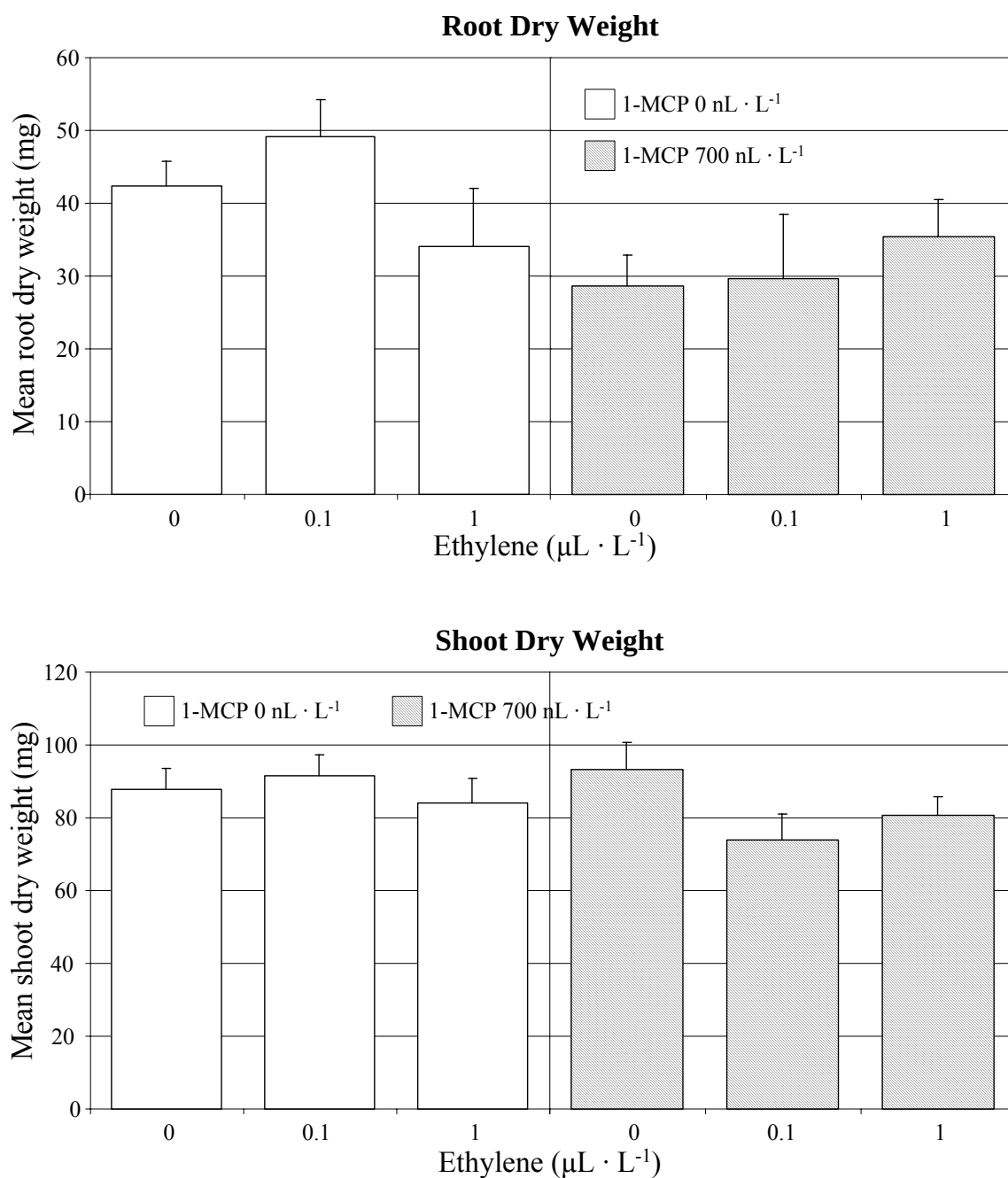


Fig. 5. Differences in zonal geranium ‘Kardino’ cutting root and shoot dry weights 21 days after propagation. Error bars represent $\pm$ one standard error. Statistical differences analyzed by one degree of freedom contrasts and are summarized in table 3.

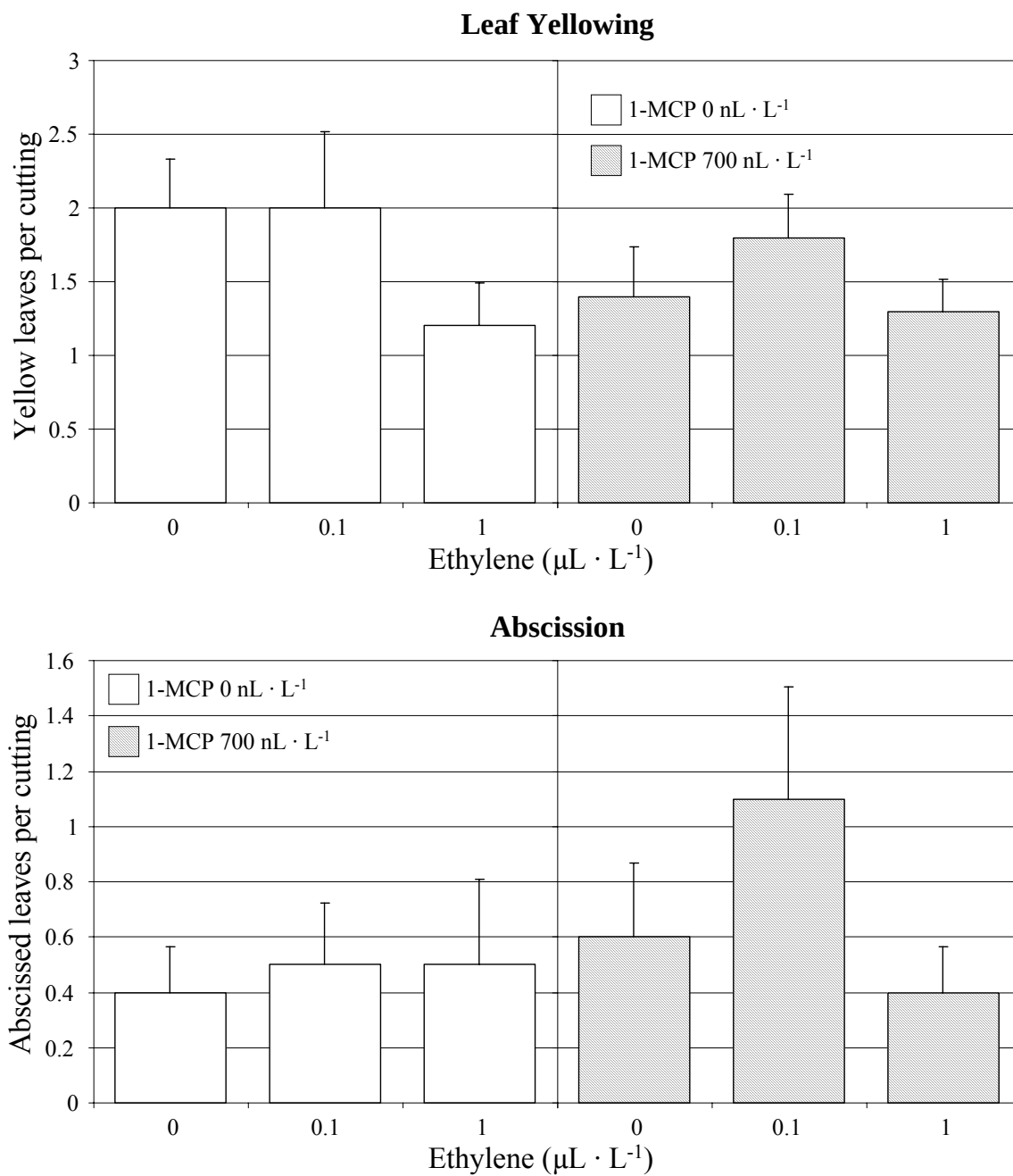


Fig. 6. Differences in leaf yellowing and abscission of geranium 'Kardino' cuttings 21 d after propagation. Error bars represent $\pm$ one standard error. Statistical differences analyzed by one degree of freedom contrasts and are summarized in table 3.

Table 3. Changes in adventitious root length, number, dry weight, shoot dry weight, leaf yellowing and abscission after 1-MCP and ethylene treatments. Differences between means (n=5) were analyzed using orthogonal contrasts. Data are shown in Figs. 1 – 4.

	Root Number	Root Length	Root Area	Root Dry Weight	Shoot Dry Weight	Leaf Yellowing	Leaf Abscission
Single degree of freedom contrasts.							
Untreated vs. 1-MCP	*	*	*	*	NS	NS	*
Untreated vs. 1-MCP 1 $\mu\text{L} \cdot \text{L}^{-1}$ ethylene	*	*	*	*	NS	NS	NS
Untreated 0 vs. 1 $\mu\text{L} \cdot \text{L}^{-1}$ ethylene	NS	NS	NS	NS	NS	NS	NS
Untreated 0.1 vs. 1 $\mu\text{L} \cdot \text{L}^{-1}$ ethylene	NS	NS	NS	NS	NS	NS	NS
Untreated 0 $\mu\text{L} \cdot \text{L}^{-1}$ ethylene vs. 1-MCP 1 $\mu\text{L} \cdot \text{L}^{-1}$ ethylene	*	*	*	*	NS	NS	NS
Untreated 0.1 $\mu\text{L} \cdot \text{L}^{-1}$ ethylene vs. 1-MCP 0.1 $\mu\text{L} \cdot \text{L}^{-1}$ ethylene	*	NS	NS	NS	*	NS	*
Untreated 1 $\mu\text{L} \cdot \text{L}^{-1}$ ethylene vs. 1-MCP 1 $\mu\text{L} \cdot \text{L}^{-1}$ ethylene	NS	NS	NS	NS	NS	NS	NS
1-MCP 0 $\mu\text{L} \cdot \text{L}^{-1}$ ethylene vs. 1-MCP 0.1 $\mu\text{L} \cdot \text{L}^{-1}$ ethylene	NS	NS	NS	NS	*	NS	NS
1-MCP 0 $\mu\text{L} \cdot \text{L}^{-1}$ ethylene vs. 1-MCP 1 $\mu\text{L} \cdot \text{L}^{-1}$ ethylene	NS	NS	NS	NS	NS	NS	NS
1-MCP 0.1 $\mu\text{L} \cdot \text{L}^{-1}$ ethylene vs. 1-MCP 1 $\mu\text{L} \cdot \text{L}^{-1}$ ethylene	NS	NS	NS	NS	NS	NS	NS

NS, * Nonsignificant, significant at $P \leq 0.05$.

CHAPTER 4

Ethephon residual catalysis on unrooted *Impatiens hawkeri* cuttings and stock plants

This chapter was prepared in the style of the Journal of the American Society for Horticultural Science and will be submitted for review

Abstract. Ethephon, [(2-chloroethyl) phosphonic acid] is used in stock plant management to increased branching and abscise sink tissues. However, ethylene production resulting from ethephon application is suspected to cause leaf abscission of unrooted cuttings during shipping. *Impatiens hawkeri* W. Bull ‘Sonic Red’ and ‘Sonic White’ stock plants were treated with 0, 250, 500 or 1000 mg· L⁻¹ ethephon. One to 21 days later cuttings were harvested and stored at 20° C in sealed jars for 24 hours prior to ethylene measurement. Higher ethephon doses resulted in greater ethylene generation. Cuttings harvested 1 day after treatment with 0, 250, 500 or 1000 mg· L⁻¹ ethephon produced 0.07, 1.3, 1.7 or 5.8 mg· L⁻¹ · g⁻¹ (fresh weight) ethylene in the first 24 hours of storage at 20 °C, respectively. Twenty-one days after treatment, cuttings from the same plants produced 0.05, 0.05, 0.15 or 0.14 mg· L⁻¹ · g⁻¹ (fresh weight) ethylene in the first 24 hours of storage at 20 °C, respectively. As cuttings were harvested from day one to day 21, ethylene concentration produced within the first 24 hours of storage decreased quadratically. Rinsing cuttings, treated 24 hours earlier with 500 mg· L⁻¹ ethephon, by gently agitating for 10 s in deionized water reduced ethylene production to 0.7 mg· L⁻¹ · g⁻¹ (fresh weight) as compared to 1.7 for un-rinsed cuttings.

Cuttings harvested 24 hours after treatment with 500 mg· L⁻¹ ethephon stored at 10, 15, 20, and 25° C for 24 hours produced 0.37, 0.81, 2.03 and 3.55 mg· L⁻¹ · g⁻¹ (fresh weight) ethylene. The resulting temperature coefficient (Q₁₀) calculated from all replications is 5.15.

Ornamental plant producers use cuttings shipped from around the world for finished plant production. The value of unrooted cutting imports into the United States totaled over \$61 million in 2006 with the bulk coming from Central America, South America, Mexico, and

Asia (Foreign Agricultural Service, 2007). In the top 15 producing states, flats, hanging baskets, and pots of geraniums (*Pelargonium* L'Hér. ex Ait.), poinsettias (*Euphorbia pulcherrima* Willd. ex Klotzsch), and New Guinea impatiens (*Impatiens hawkeri* W. Bull) produced from approximately 138 million cuttings had a wholesale value of \$330 million. (National Agriculture Statistics Service, 2007). In this case, the loss of a single cutting is \$2.38 lost in potential revenue. Understandably, reducing losses of cuttings during shipping is a critical research interest for cutting producers, rooting starters, and finished plant producers.

Ethephon, [(2-chloroethyl) phosphonic acid] is used in stock plant management to increase branching and abscise sink tissues. Ethephon is lipophilic and upon entering the relatively more basic apoplastic environment it undergoes a degradation reaction releasing ethylene, Cl^- and $\text{H}_2\text{PO}_4^{2-}$ (Warner and Leopold, 1969; Yang, 1969). Application of ethephon results in the abscission of flower buds and flowers, likely due to ethylene induced promotion of ABA synthesis (Hansen and Grossmann, 2000), thus reducing sink tissue carbohydrate demands. Additionally, ethylene's inhibition of IAA synthesis (Chadwick and Burg, 1970; Weber and Osborn, 1969) is a likely cause of increased branching in both young liners and stock plants (Faust and Lewis, 2005; Hayashi et al., 2001) treated with ethephon. However, excess ethylene can damage unrooted cuttings by promoting apical meristem necrosis, leaf yellowing and abscission. Thus, cuttings harvested from stock plants still producing ethephon-induced ethylene may evolve damaging concentrations of ethylene during packaging and shipping. As ethephon treated plants do not rapidly replace the abscised sink tissues, it may be possible to postpone harvest until ethylene concentrations

return to normal after ethephon application. The objective of this study is to investigate how long residual ethephon activity persisted on cutting stock plants. We used a range of ethephon dosages and subsequent storage treatments to show how temperature and rinsing of cuttings may affect ethylene evolution.

Materials and Methods

Plant material. Five unrooted cuttings of each *Impatiens hawkeri* W. Bull ‘Sonic Red’ and ‘Sonic White’ were propagated into 30 cm, 13.85 L, plastic pots containing a soilless growing substrate (Fafard P3, Conrad Fafard, Inc., Agawam, Mass.). Once rooted, these plants were maintained as stock plants and irrigated manually with water alone, 600 mg · L⁻¹ N liquid fertilizer (20N-4.37P-16.6K, Ultrasol, SQM North America, Atlanta), or flowable lime (Limestone F, Cleary Chemical Inc., Dayton, N.J.) to maintain a target E.C. of 1.0 to 1.5 dS · m⁻² and a pH of 5.8 to 6.2 using the pour through monitoring method. Temperature set points were 24/18° C (day/night), and average daily light integral (DLI) was 6.1 mol · m⁻² · d⁻¹.

Ethylene generation. Ethephon (Florel, Monterey Lawn and Garden Products, Inc., Fresno, Calif.) treatments were applied at 4 pm with a 2 L, compression hand sprayer (Hudson Manufacturing Company, Chicago) at 0, 250, 500, or 1000 mg · L⁻¹ a.i. to four separate groups of 3 plants each. Cuttings were harvested at 9 am the next day and every 48 h thereafter for 21 d, for a total of 11 harvests. Two node cuttings of similar size and age with 3 to 4 fully expanded leaves were arbitrarily selected from all parts of the stock plant. Fresh

weights were recorded before the cuttings were placed in sealed 0.95 L glass jars, six cuttings per jar. The jars remained unopened for the duration of the storage time. An additional group of $500 \text{ mg} \cdot \text{L}^{-1}$ treated cuttings was gently rinsed for 10 s in 100 mL of deionized water and gently blotted dry before storage. Ethylene evolved from the cuttings was calculated on a fresh weight basis. The jars were stored in the dark at $20 \pm 1^\circ \text{C}$ for 7 d. Four additional sets of $500 \text{ mg} \cdot \text{L}^{-1}$ ethephon treated cuttings were harvested 24 hours after treatment and stored at 10, 15, 20 or 25°C , respectively, in sealed 0.95 L glass jars for 7 d. Ethylene concentrations in the jars were measured by gas chromatography every other day commencing 24 hours after each harvest.

Data collected. Evidence of phytotoxicity was recorded daily as the number of yellow or necrotic leaves, the percentage of leaf area affected, the number of abscised leaves and turgidity, which was ranked on the following scale: 1-all leaves turgid, 2-one or two leaves wilting, or 3-all leaves wilted.

Ethylene determination. A gas chromatograph (GC, Varian 3400; Varian Inc., Walnut Creek, Calif.) fitted with a glass column (Porapak Q, 80-100 mesh, 183 cm x 2 mm) running at 120° injector, 120° column, and 130°C detector (flame ionization) temperatures was used to measure ethylene concentration during storage. Flow rates for the He carrier, H_2 and O_2 were 30, 16 and $90 \text{ mL} \cdot \text{min}^{-1}$, respectively. Ethylene quantification was based a response factor generated using a $1 \text{ } \mu\text{L} \cdot \text{L}^{-1}$ ethylene standard. Injection volume was 1 ml of headspace gas drawn via a neoprene port on the jar lid.

Data analysis. Within the study each of the three experiments was designed as a randomized complete block design of three blocks with treatments of ethephon concentration, storage temperature, and rinsing of residual ethephon. Experimental results (i.e. ethylene evolved due to ethephon dosage, storage temperature, or rinsing) were tested for significance and best-fit linear, quadratic or higher order models using PROC GLM, then regressed using PROC REG (SAS Institute, Cary, N.C.). Model terms were evaluated for significance by comparison of F values at $\alpha = 0.05$. Eq. 1 was used to calculate Q_{10} values for every possible temperature combination in each replication. From these results a mean Q_{10} for each temperature combination was calculated. The resulting data was regressed using PROC GLM to determine the best-fit model.

Results

During storage, the quality and appearance of cuttings from all treatments were similar. Higher ethephon doses resulted in greater ethylene generation (Fig. 1). Cuttings harvested 24 h after treatment with 500 or 1000 $\text{mg} \cdot \text{L}^{-1}$ ethephon produced 1.7 or 5.8 $\mu\text{L} \cdot \text{L}^{-1} \cdot \text{g}^{-1}$ ethylene, respectively, within the first 24 h after harvest. Control cuttings produced 0.06 $\mu\text{L} \cdot \text{L}^{-1} \cdot \text{g}^{-1}$ ethylene in the same period.

Rinsing 500 $\text{mg} \cdot \text{L}^{-1}$ treated cuttings did not reduce evolved ethylene concentrations to that of the untreated control in the first 13 days after treatment (Fig. 2A). Cuttings harvested 24 hours after treatment with 500 $\text{mg} \cdot \text{L}^{-1}$ ethephon and rinsed in distilled water

produced $0.7 \mu\text{L} \cdot \text{L}^{-1} \cdot \text{g}^{-1}$ ethylene in the first 24 hours of storage compared to $1.7 \mu\text{L} \cdot \text{L}^{-1} \cdot \text{g}^{-1}$ ethylene from un-rinsed cuttings and $0.05 \mu\text{L} \cdot \text{L}^{-1} \cdot \text{g}^{-1}$ for untreated cuttings.

Ethylene was released in biologically significant amounts for at least an additional 21 days after treatment depending on application rate and temperature (Fig. 2B). Cuttings harvested 1 day after treatment with 0, 250, 500 or $1000 \text{ mg} \cdot \text{L}^{-1}$ ethephon produced 0.07, 1.3, 1.7 or $5.8 \mu\text{L} \cdot \text{L}^{-1} \cdot \text{g}^{-1}$ ethylene in the first 24 hours of storage, respectively. As subsequent sets of cuttings were harvested, ethylene produced within the first 24 hours of storage decreased quadratically d 1 through 21. Cuttings harvested 21 days after being treated by the same ethephon concentrations produced 0.05, 0.05, 0.15 or $0.14 \mu\text{L} \cdot \text{L}^{-1} \cdot \text{g}^{-1}$ ethylene in the first 24 hours of storage, respectively.

Lower temperatures resulted in less ethylene generation from ethephon treated cuttings (Fig. 3). Cuttings harvested 24 hours after treatment with $500 \text{ mg} \cdot \text{L}^{-1}$ ethephon and stored at 10, 15, 20, and 25°C evolved 0.37, 0.85, 2.59 and $3.56 \mu\text{L} \cdot \text{L}^{-1} \cdot \text{g}^{-1}$ of ethylene, respectively. Additionally, these cuttings had a highest temperature coefficient (Q_{10}) of 10.25 between 15° and 20°C , and 4.70 and 2.18 between 10° and 15°C , and 20°C and 25°C , respectively.

Discussion

Our findings support those of Woodrow et al. (1988) who using $[^{14}\text{C}]$ ethephon, showed that the ethylene released from ethephon activity continues for many days. Additionally, Riov and Yang (1982) demonstrated that after exposure to exogenous $12 \mu\text{L} \cdot \text{L}^{-1}$ ethylene, ethylene autocatalysis decreases to normal within 12 h of removing the

exogenous ethylene source. Thus, it appears that ethephon can have residual activity on impatiens stock plants for up to three weeks.

Decreasing ethephon concentration results in lower ethylene concentrations evolved from treated cuttings. Also, lower storage temperatures reduced the amount of ethylene generated by treated cuttings within 24 h of harvest. Although rinsing cuttings treated with $500 \text{ mg} \cdot \text{L}^{-1}$ ethephon reduced the amount of ethylene generated from ethephon application, ethylene concentrations were similar to amounts generated by unrinsed cuttings harvested from stock plants treated with $250 \text{ mg} \cdot \text{L}^{-1}$ ethephon.

In shipping, problems may occur when cuttings of ethylene sensitive species are packed alongside ethephon-treated cuttings. Species susceptible to low concentrations of ethylene such as *Lantana camera* L. or *Portulaca oleracea* L. could suffer leaf abscission when packaged alongside cuttings still releasing ethylene. However, the differences between the experimental protocol described here and commercial package conditions is significant and more work remains to be done to fully characterize the possible risks. For example a follow up experiment should be conducted combining various plant materials sensitive to ethylene with materials treated by ethephon.

Proper stock plant management has been critical in lowering cutting losses due to carbohydrate depletion during shipping. Rapaka et al. (2007) demonstrated an inverse relationship between cutting carbohydrate content at harvest and subsequent postharvest leaf senescence. Quality of subsequent rooting is also directly proportional to carbohydrate content (Rapaka et al., 2005). Other authors have suggested a correlation between cut flower petal senescence and sugar depletion (van Doorn, 2004).

Additionally, the energy required for maintaining homeostasis of unrooted cuttings during shipping comes from extant carbohydrate, lipid and protein reserves. The abiotic stresses imposed by harvest and shipping tend to increase respiration hastening the loss of carbohydrate reserves (Sturm and Tang, 1999). Whether sugars or carbohydrates regulate senescence is still an unanswered question (Wingler et al., 2006); their role in regulating postharvest senescence is even less understood.

Regardless, altering the carbohydrate distribution in the parent plant via abscission of sink tissues such as flowers and flower buds, increases carbohydrate availability to the shoots (Woodrow et al., 1988) and increases carbohydrate content of unrooted cuttings, thus increasing adventitious root formation (Rapaka et al., 2005) and propagation performance.

In addition to managing packaging risks and carbohydrate status, awareness of the ethylene status of unrooted cuttings will help producers and growers manage the cold chain and propagation environment more precisely. When harvested from recently treated stock plants, cuttings should be shipped and stored as cold as reasonable for the species to reduce residual ethylene production. Additionally, cuttings from stock plants recently treated with ethephon that are used in liner production may not require a subsequent ethephon treatment until later in propagation, as the ethephon applied by the cutting producer may still be active.

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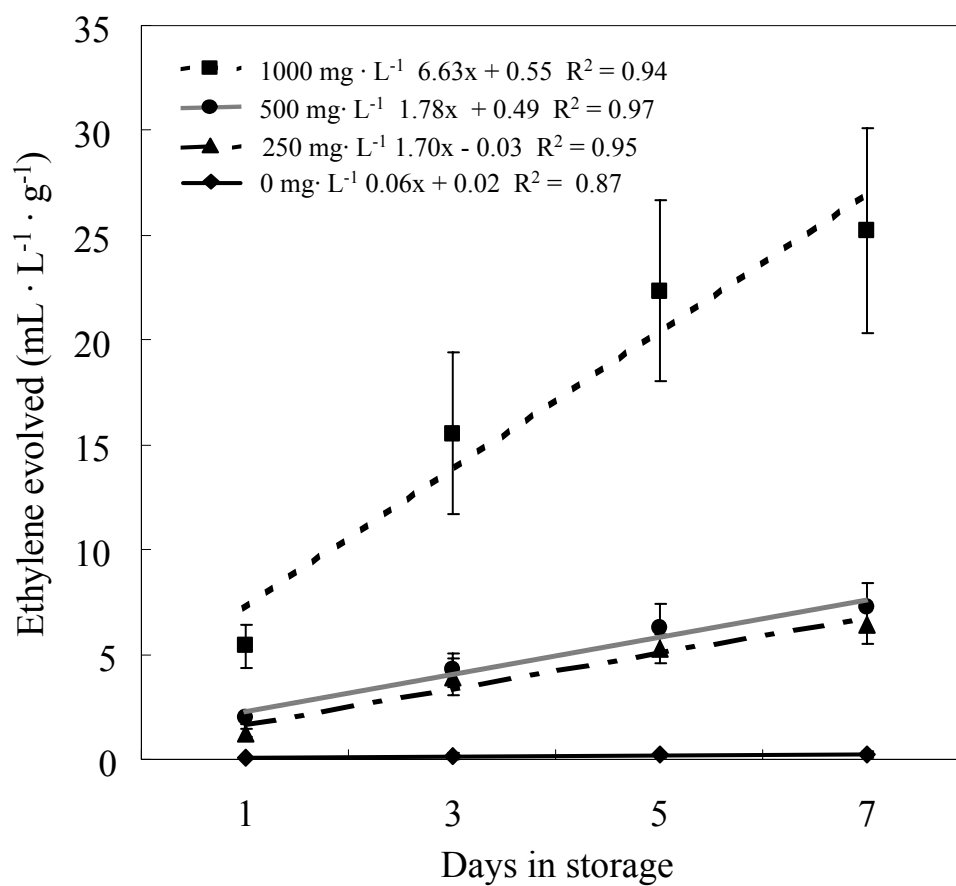
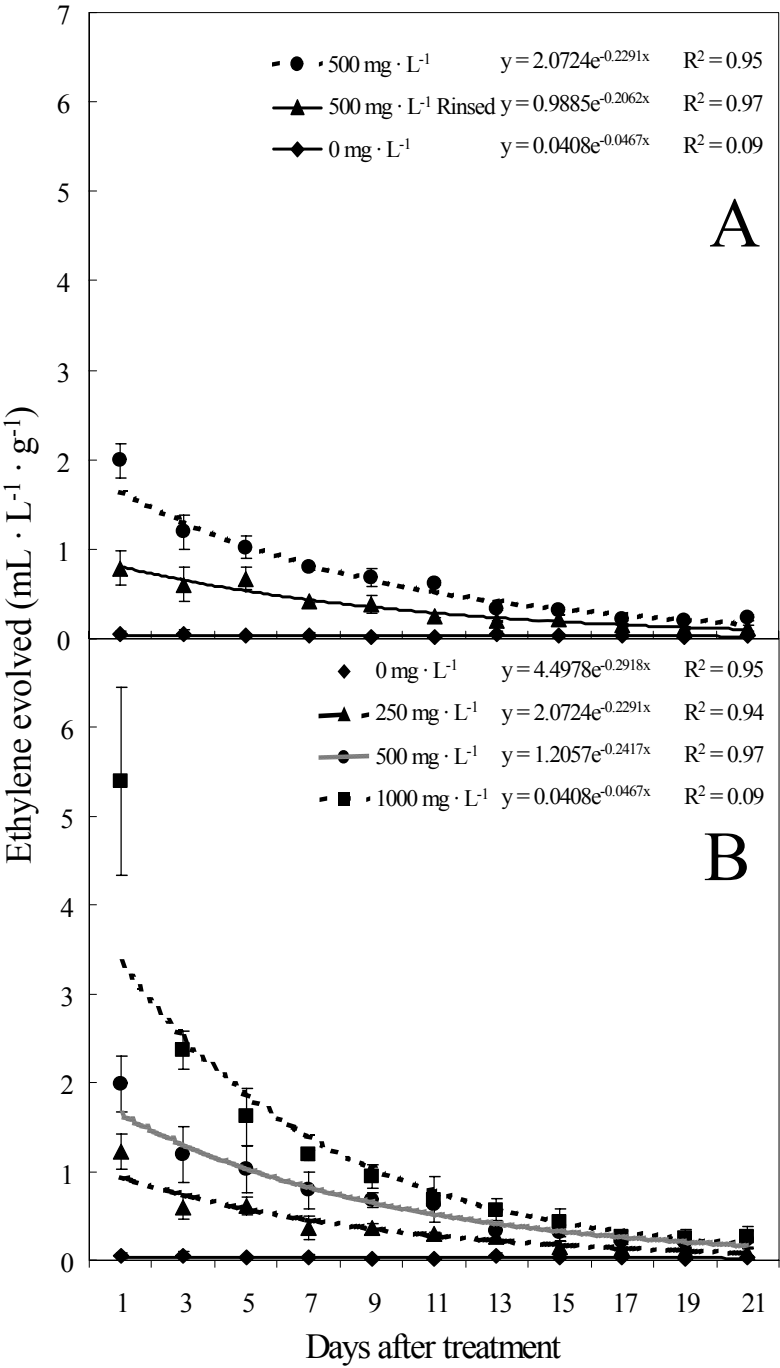


Fig 1. Cumulative ethylene evolved from unrooted *Impatiens* ‘Sonic Red’ and ‘Sonic White’ cuttings during storage at $20 \pm 1^\circ \text{C}$ commencing 24 hours after ethephon application to stock plants. Mean values of 3 replications ($n=3$) are presented. Error bars indicate ± 1 standard error.

Fig 2. Ethylene evolved during the first 24 hours after harvest for unrooted *Impatiens* 'Sonic Red' and 'Sonic White' cuttings harvested for 1 to 21 days after ethephon application to stock plants. Ethylene evolution after 24 hours of storage for rinsed cuttings (A) and a range of ethephon treatment concentrations (B) are shown. Mean values of 3 replications (n=3) are presented. Error bars indicate ± 1 standard error.



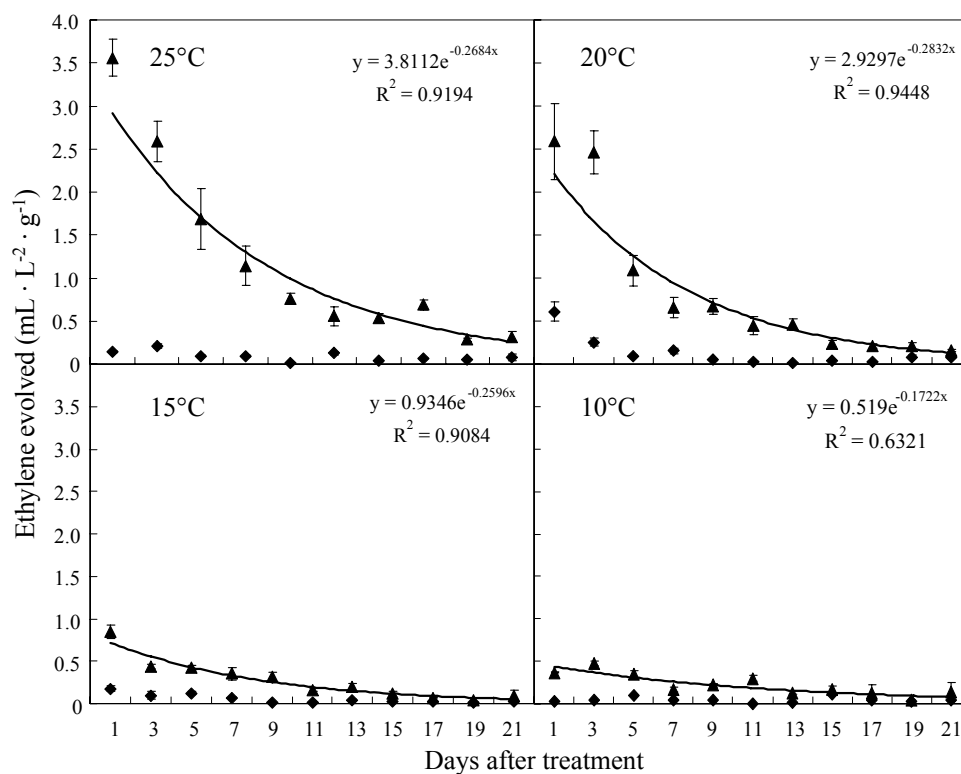


Fig 3. Ethylene accumulation during the first 24 hours after harvest for unrooted Impatiens ‘Sonic Red’ and ‘Sonic White’ cuttings stored at four different temperature conditions. Cuttings were harvested for 1 to 21 days after 500 mg · L⁻¹ (▲) and 0 mg · L⁻¹ (◆) ethephon application to stock plants. Mean ethylene concentrations from 3 replications (n=3) are reported as concentration per fresh weight. Error bars indicate ± 1 standard deviation.

$$Q_{10} = \left(\frac{R_2}{R_1} \right)^{\left(\frac{10}{T_2 - T_1} \right)}$$

Equation 1. The model used to calculate ethephon Q_{10} values for a range of temperatures. T_2 and T_1 represent the two temperatures of the range while R_2 and R_1 represent the respective rates of ethylene evolution for those temperatures.