Exogenous Applications of Benzyladenine and Gibberellic Acid Inhibit Lower-leaf Senescence of Geraniums during Propagation

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Abstract. After postharvest shipping, the lower leaves of zonal geranium (*Pelargonium × hortorum*) cuttings often turn chlorotic and necrotic during rooting in a propagation environment. Our objective was to quantify the efficacy of spray applications of the plant growth regulators (PGRs) benzyladenine (BA) and/or gibberellic acid (GA) at various stages in propagation to reduce lower-leaf senescence and evaluate effects on subsequent rooting. In Expt. 1, cuttings of ‘Patriot White’ geraniums were harvested and treated with BA (2.5 or 5.0 mg·L⁻¹), BA + GA₄₊₇ (2.5 or 5.0 mg·L⁻¹ each), or GA₃ (0.5 or 2.0 mg·L⁻¹) either before or after a 2-day storage period simulating commercial shipping. Post-shipment application of all PGRs eliminated leaf yellowing compared with cuttings treated pre-shipment, but rooting was inhibited. In Expt. 2, the promotion of rooting from a rooting hormone preceding treatment with BA (1.25 to 5.0 mg·L⁻¹), BA+GA₄₊₇ (1.25 to 5.0 mg·L⁻¹ each), or GA₃ (0.25 to 2.0 mg·L⁻¹) was evaluated on ‘Patriot White’ geranium cuttings after a 2-day simulated shipping. Applying rooting hormones increased the percentage of fully rooted cuttings treated with BA and/or GA from 16.4% to 51.8%. In Expt. 3, cuttings of different geranium cultivars from a commercial producer varied in susceptibility and suppression of leaf yellowing after BA + GA₄₊₇ applications. We conclude that foliar applications of BA + GA₄₊₇ can suppress lower-leaf senescence and rooting during propagation of some geranium cultivars, and the inhibition of rooting can be at least partially overcome with an application of rooting hormone.

Potted flowering geraniums are one of the most popular floriculture crops worldwide. In the United States, geraniums had a wholesale value of ≈\$116 million dollars for the top 15 producing states in 2011 (U.S. Department of

Agriculture, 2012). Although seed-propagated geraniums are more frequently used for production of bedding plant flats (Armitage, 1994; Armitage and Kaczperski, 1992), vegetatively propagated geraniums account for 73% of the total value of geraniums (U.S. Department of Agriculture, 2012). The majority of vegetatively propagated geranium producers purchase unrooted cuttings originating from stock plant facilities primarily in Mexico, Central America, and Africa, necessitating shipping of cuttings by air freight (Serek et al., 1998; Swanson et al., 2007).

Unrooted geranium cuttings have a short postharvest life and low tolerance to high postharvest temperatures (Dole and Gibson, 2006; Faust and Lewis, 2005; Rapaka et al., 2008). Undesirable shipping conditions increased respiration, reduced carbohydrate concentration, and increased ethylene generation in geranium cuttings, which caused lower-leaf senescence during propagation (Mutui et al., 2005; Rapaka et al., 2008). Loss of lower leaves during propagation can reduce the marketability of rooted

cuttings or flowering plants after transplanting. Additionally, abscised leaves can host the pathogen *Botrytis cinerea* (Dreistadt, 2001; Powell and Lindquist, 1997) and cause considerable losses during propagation (Rogers, 1993). Therefore, fungicides are often applied during propagation and infected leaves are usually manually removed to reduce pathogen problems (Hausbeck et al., 1995; Purer and Mayak, 1988; Vollmer, 1999).

In addition to geranium cuttings, lower-leaf senescence is a common postharvest problem with some potted ornamental bulb crops and cut flowers. Exogenous applications of PGRs such as cytokinins and/or gibberellins suppress lower-leaf senescence of potted *Lilium* species and cultivars, including *L. longiflorum* (Han, 1997), *L. auratum* × *L. speciosum* (Ranwala and Miller, 1998), and *L. longiflorum* × asiatic *Lilium* sp. (Funnell and Heins, 1998). Additionally, PGR applications reduced leaf senescence of cut stems of *Alstroemeria hybrida* (Hicklenton, 1991), *Dendranthema × grandiflora* (Ferrante et al., 2003), *Lilium* (Han, 2001), *Mathiola incana* (Ferrante et al., 2009), *Solidago canadensis* (Philosoph-Hadas et al., 1996), and *Tulipa gesneriana* (Ferrante et al., 2003). Application of GA (Purer and Mayak, 1988) or cytokinins including thidiazuron (TDZ) or meta-toplin (Mutui et al., 2005, 2012) inhibited lower-leaf senescence of geranium cuttings, but to our knowledge, comprehensive evaluations of commercially available PGRs to suppress lower-leaf senescence in geranium have not been published. Therefore, our objectives were to: 1) quantify the efficacy of exogenous applications of BA and/or GA either before or after shipping to reduce lower-leaf senescence in geranium cuttings during propagation; 2) evaluate whether rooting hormones could overcome an inhibition of rooting from PGR applications; and 3) to quantify the variation in efficacy of exogenous BA + GA applications among geranium cultivars.

Materials and Methods

Expt. 1: Application timing. Rooted cuttings of ‘Patriot White’ geranium, a variety that is widely known to be susceptible to lower-leaf senescence, were obtained from a commercial producer (Ecke Ranch, Connellsville, PA). The cuttings were transplanted on 20 Oct. 2006 into 72-cm-diameter (6 L) round containers filled with soilless substrate (Fafard 3B; Conrad Fafard, Inc., Agawam, MA). A constant liquid fertilizer (15N–2.2P–12.5K Peters Excel© Cal-Mag; Everris, Marysville, OH) provided 250 mg·L⁻¹ nitrogen at each watering. Stock plants were maintained in a vegetative state by regularly pinching shoots and applying foliar spray solutions containing 250 mg·L⁻¹ ethephon (Florel; Rhône-Poulenc Ag Company, Research Triangle Park, NC) every 2 weeks. Ethephon sprays were discontinued at least 3 weeks preceding cutting harvest. The plants were grown in a glass-glazed greenhouse in Clemson, SC (lat. 35° N) with natural photoperiods and an average daily temperature of 21.7 ± 1.4 °C

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The use of trade names in this publication does not imply endorsement by Purdue, Michigan State, and Clemson University of products named nor criticism of similar ones not mentioned.

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Table 1. Effect of pre- and post-shipment spray application of three plant growth regulator (PGR) solutions containing BA, BA + GA₄₊₇, or GA₃ or surfactant only on the visual quality rating and number of senesced leaves of 'Patriot White' geranium cuttings (Expt. 1).^z

Application time	No PGR		Concn (mg·L ⁻¹)					
	No shipment control	Stored (simulated shipping)	BA		BA + GA ₄₊₇		GA ₃	
			2.5	5.0	2.5	5.0	0.5	2.0
Pre-shipment	3.1 ^y A ^x	2.2 AB	3.0 b ^w A	2.4 bAB	2.9 bAB	1.9 bB	2.8 bAB	2.1 bAB
Post-shipment	— ^v	—	5.0 aA	5.0 aA	5.0 aA	4.9 aA	5.0 aA	5.0 aA
PGR	*** ^u							
Application time	***							
PGR × application time	**							
<i>Senesced leaves (no./cutting)</i>								
Pre-shipment	0.8A	0.9A	0.5 aA	1.3 aA	0.8 aA	1.4 aA	0.6 aA	0.4 aA
Post-shipment	—	—	0.8 aA	0.0 bB	0.2 bB	0.1 bB	0.1 aB	0.0 bB
PGR	NS ^u							
Application time	***							
PGR × application time	***							

^zThe visual rating was recorded after 7 d in propagation, and the number of senesced leaves per cutting was recorded after 20 d in propagation.

^yA numerical visual rating on a scale from 1 to 5 (1 = poor, 2 = inferior, 3 = acceptable, 4 = good, and 5 = excellent) based on overall leaf color and senescence.

^xDifferent upper-case letters within a row are significantly different by Tukey's honestly significant difference (HSD) test at $P \leq 0.05$.

^wDifferent lower-case letters within a column indicate a significant effect of application time based on an analysis of variance at $P \leq 0.05$.

^vIndicates treatment not included.

^uNS, **, or *** indicates nonsignificant or significant at $P \leq 0.01$ or 0.001, respectively.

BA = benzyladenine; GA = gibberellic acid.

(mean ± SD). The photosynthetic daily light integral (DLI) was continuously measured inside the greenhouse using a Greenhouse Weather Tracker (Spectrum Technologies, Inc., East Plainfield, IL) and averaged 13.8 mol·m⁻²·d⁻¹.

Four hundred twenty uniform 2.5-cm shoot-tip cuttings with four leaves, of which at least one was fully developed, were harvested from stock plants beginning at 0800 HR on 22 Jan. 2007. Cuttings were randomly divided into three replicated lots of 10 cuttings each and were sprayed until runoff with a solution containing 300 mg·L⁻¹ non-ionic surfactant (CapSil; Aquatrols, Paulsboro, NJ) or solutions containing the surfactant and 2.5 or 5 mg·L⁻¹ BA (MaxCel; Valent BioSciences Corporation, Libertyville, IL), 0.5 or 2.0 mg·L⁻¹ GA₃ (Pro-Gibb; Valent BioSciences Corporation), or 2.5 or 5.0 mg·L⁻¹ each BA + GA₄₊₇ (Fascination; Valent BioSciences Corporation). Cuttings were placed in a plastic tray and applications were made in a climate-controlled room at 20 °C before or after simulated shipping (pre-shipment or post-shipment, respectively). The simulated shipping treatment consisted of placing all cuttings in zip-seal polyethylene bags (volume 0.946 L) that were packed in a shipping box and then placed in a dark refrigerated chamber. To simulate the temperatures in the shipping supply chain, air temperature was 10 °C from 1000 to 2000 HR and 15 °C from 2000 to 1000 HR on Day 1 followed by 10 °C from 1000 to 2000 HR and 25 °C from 2000 to 1000 HR on Day 2. The time from cutting harvest until placement into simulated shipping or directly into propagation was ≈1 h.

After simulated shipping, cuttings were placed in 10-cell white propagation strips (42-mL individual cell volume) filled with a propagation substrate comprised of a 2:1 ratio of soilless substrate (Fafard 3B) and coarse perlite. Twelve days after cuttings were stuck in the propagation medium, they were fertilized every 4 d with 15N–2.2P–12.5K (Peters

Table 2. Effect of pre-shipment and post-shipment application of three plant growth regulator (PGR) solutions containing BA, BA + GA₄₊₇, or GA₃ or surfactant only (untreated) on chlorophyll fluorescence (F_v/F_m), chlorophyll content (SPAD), percentage of cuttings that were well rooted (pullable), and root dry mass of 'Patriot White' geranium cuttings (Expt. 1).^z

PGR	Concn (mg·L ⁻¹)	F_v/F_m	SPAD	Pullable cutting (%)	Root dry mass (mg)
Untreated, no shipment	— ^y	0.76 a ^x	27.2 b	53.3 a	35.1 a
Untreated, shipment	—	0.62 b	27.2 b	46.7 a	34.2 a
BA	2.5	0.83 a	48.5 a	16.7 b	12.1 b
BA	5.0	0.84 a	48.3 a	3.3 b	5.4 b
BA + GA ₄₊₇	2.5	0.85 a	49.1 a	10.0 b	7.6 b
BA + GA ₄₊₇	5.0	0.85 a	49.6 a	0.0 b	4.8 b
GA ₃	0.5	0.84 a	46.2 a	0.0 b	4.5 b
GA ₃	2.0	0.84 a	47.0 a	0.0 b	2.7 b
PGR		*** ^w	***	***	***
Shipment		NS	NS	*	**
PGR × shipment		NS	NS	NS	NS

^zChlorophyll fluorescence and relative chlorophyll content were recorded after 7 d of propagation, whereas percentage of pullable cuttings and root dry mass were recorded after 20 d of propagation. Data presented are pooled across application timing.

^yNot applicable.

^xDifferent lower-case letters within a column are significantly different by Tukey's honestly significant difference (HSD) test at $P \leq 0.05$.

^wNS, *, **, or *** indicates nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

BA = benzyladenine; GA = gibberellic acid.

Excel® Cal-Mag) to provide 150 mg·L⁻¹ nitrogen at each watering. Air temperature and substrate temperature were maintained at 23 °C and mean DLI was 4 mol·m⁻²·d⁻¹.

Seven days after cuttings were stuck, each cutting was subjectively evaluated using a scale from 1 to 5 (1 = poor, 2 = inferior, 3 = acceptable, 4 = good, and 5 = excellent) based on overall leaf color and senescence. Chlorophyll fluorescence of 10 cuttings per treatment was measured on the adaxial epidermis of the most fully expanded leaf using a portable chlorophyll fluorescence system (Plant Efficiency Analyzer; Hansatech Instruments Ltd., Norfolk, U.K.). Leaves were dark-acclimated for 20 min within the manufacturer's plastic and foam clips before measurements were recorded. Fluorescence was measured by opening the shutter clip and exposing the leaf for 5 s to red light (peak wavelength = 650 nm) at 3000 μmol·m⁻²·s⁻¹ to saturate PS II. Chlorophyll fluorescence

was expressed as F_v/F_m . Relative chlorophyll content was measured by taking the average of three readings of the most fully expanded leaf using a chlorophyll meter (SPAD-502, Soil-Plant Analysis Development; Konica Minolta, Tokyo, Japan).

Twenty days after cuttings were stuck, the total number of senesced leaves was recorded and whether it was "pullable" (fully rooted and all media removed with roots; Donnell, 2005) or not when removed from the propagation tray. Cuttings were removed from propagation trays and substrate was gently rinsed off the roots. Roots were excised from the cutting and roots and shoots were dried separately in an oven at 70 °C for 3 d and then weighed. The experiment used a randomized complete block design in a factorial arrangement. Analyses of variance (ANOVA) and mean separation by Tukey's honestly significant difference test at $P \leq 0.05$ were performed using SPSS 17.0 (IBM Corp., Armonk, NY).

Expt. 2: Rooting hormone application. Stock plants of 'Patriot White' geranium were grown as described in Expt. 1. Cuttings were harvested and placed into simulated shipping as described in Expt. 1 except for the non-stored cuttings. After simulated shipping, cuttings were removed and the basal end of each cutting was either briefly placed in a solution containing 1000 mg·L⁻¹ indole-3-butyric acid (IBA) + 500 mg·L⁻¹ 1-naphthaleneacetic acid (NAA; Dip'N Grow Liquid Rooting Concentrate; Dip'N Grow, Clackamas, OR) or received no rooting hormone. Foliar spray applications (volume 0.20 L·m⁻²) of solutions containing 300 mg·L⁻¹ non-ionic surfactant (CapSil) or solutions containing the surfactant and 1.25, 2.5, or 5.0 mg·L⁻¹ BA (MaxCel); 1.25, 2.5, or 5.0 mg·L⁻¹ each of BA + GA₄₊₇ (Fascination); or 0.25, 0.5, or 2.0 mg·L⁻¹ GA₃ (ProGibb) were applied to cuttings. The propagation environment and culture as well as data collection were as described in Expt. 1. The experiment used a randomized complete block design in a factorial arrangement and the same statistical procedures were used as previously described.

Expt. 3: Cultivar screen. Cuttings of 'Designer Salmon', 'Fantasia Purple Sizzle', 'Fantasia Pink Shell', and 'Presto Dark Red' zonal geraniums were received from a commercial supplier (Ball Horticulture Co., West Chicago, IL) in the same box at Purdue University on 28 Feb. 2012. Cuttings were immediately placed in a dark refrigerated chamber with an air temperature of 10 °C. The next morning, the basal end of each cutting was dipped in a solution containing 1000 mg·L⁻¹ IBA + 500 mg·L⁻¹ NAA (Dip'N Grow Liquid Rooting Concentrate; Dip'N Grow). Cuttings were then placed in 72-cell (44-mL cell volume) black propagation trays filled with a propagation substrate comprised of a 2:1 ratio of soilless substrate (Fafard 1P; Conrad Fafard, Inc.) and coarse perlite. Foliar spray applications (volume 0.20 L·m⁻²) containing 300 mg·L⁻¹ non-ionic surfactant (CapSil; Aquatrols) and 0.0, 1.0, 2.0, 3.0, or 4.0 mg·L⁻¹ of BA + GA₄₊₇ each (Fresco; Fine Americas, Walnut Creek, CA) were applied immediately preceding placement under mist.

The plants were grown in a glass-glazed greenhouse under natural photoperiods in West Lafayette, IN (lat. 40° N) with day and night air temperature set points of 23 °C. Beginning at sticking, 2 s of mist consisting of tap water acidified with 93% sulfuric acid (Brenntag, Reading, PA) at 0.08 mL·L⁻¹ to reduce alkalinity to 100 mg·L⁻¹ and a complete water-soluble fertilizer (Jack's LX 16N-0.94P-12.3K Plug Formula for High Alkalinity Water; J.R. Peters, Inc., Allentown, PA) providing 50 mg·L⁻¹ nitrogen with each misting event was applied at 10 min for the first 7 d and at 20 min for 3 more days. Ten days after cuttings were stuck, mist was discontinued and cuttings were hand-irrigated with acidified water (as described previously) supplemented with a combination of two water-soluble fertilizers (3:1 mixture of 15N-2.2P-12.5K and 21N-2.2P-16.6K, respectively; Everris) to provide 200 mg·L⁻¹ nitrogen with each watering.

Table 3. Effect of post-shipment application of three plant growth regulator (PGR) solutions containing BA, BA + GA₄₊₇, or GA₃ or surfactant only (untreated) and rooting hormone on the visual rating, chlorophyll fluorescence (F_v/F_m), number of senesced leaves, and percentage of cuttings that were well rooted (pullable) of 'Patriot White' geranium cuttings (Expt. 2).²

PGR	Concn (mg·L ⁻¹)	Visual rating	F_v/F_m	Senesced leaves (no./cutting)	Pullable cutting (%)
Untreated, no shipment	— ^y	4.4 ^a a ^w	0.83 a	0.6 ab	75.0 a
Untreated, stored	—	2.4 d	0.69 b	1.0 a	60.0 ab
BA	1.25	4.4 ab	0.83 a	0.3 bc	60.0 ab
BA	2.5	4.5 a	0.83 a	0.2 bc	60.0 ab
BA	5.0	3.3 c	0.84 a	0.5 bc	25.0 bc
BA + GA ₄₊₇	1.25	5.0 a	0.85 a	0.0 c	15.0 bc
BA + GA ₄₊₇	2.5	5.0 a	0.84 a	0.0 c	25.0 bc
BA + GA ₄₊₇	5.0	3.4 c	0.85 a	0.2 bc	0.0 c
GA ₃	0.25	5.0 a	0.84 a	0.1 bc	40.0 a-c
GA ₃	0.5	5.0 a	0.84 a	0.0 c	10.0 c
GA ₃	2.0	3.8 bc	0.84 a	0.3 bc	5.0 c
PGR		*** ^v	***	***	***
Rooting hormone		NS	NS	NS	***
PGR × rooting hormone		NS	NS	NS	NS

²The visual quality rating and chlorophyll fluorescence were recorded 7 d after initiating propagation and the number of senesced leaves per cutting and the percentage of pullable cuttings were recorded after 20 d of propagation. Data presented are pooled across rooting hormone treatments.

^yNot applicable.

^aA numerical visual rating on a scale from 1 to 5 (1 = poor, 2 = inferior, 3 = acceptable, 4 = good, and 5 = excellent) based on overall leaf color and senescence.

^wDifferent lower-case letters within a column are significantly different by Tukey's honestly significant difference (HSD) test at $P \leq 0.05$.

^vNS or *** indicates nonsignificant or significant at $P \leq 0.001$, respectively.

BA = benzyladenine; GA = gibberellic acid.

Fig. 1. Effect of post-shipment application of three plant growth regulator (PGR) solutions containing BA, BA + GA₄₊₇, or GA₃, or surfactant only (untreated) and rooting hormone (RH) on the relative chlorophyll content (SPAD) and root dry mass of 'Patriot White' geranium cuttings (Expt. 2). The relative chlorophyll content was recorded after 7 d of propagation and root dry mass was recorded after 20 d of propagation. Each symbol represents the mean of 10 plants, and error bars represent ses of the mean. BA = benzyladenine; GA = gibberellic acid.

Seven days after sticking cuttings, SPAD and chlorophyll fluorescence measurements were made as previously described. After 28 d, the number of senesced leaves was

recorded and cuttings were removed from trays and substrate was gently washed off roots. Roots were excised from the stem and dried separately in an oven at 70 °C for 3 d

and then weighed. The experiment used a completely randomized design for each cultivar. ANOVA, regression analyses, and mean separation by Tukey's honestly significant difference test at $P \leq 0.05$ were performed using SPSS 17.0 (IBM Corp.).

Results

Expt. 1: Application timing. In general, a PGR application after shipment increased the visual quality rating of 'Patriot White' geraniums, but the effect depended on the PGR (Table 1). The visual ratings were 1.9 to 3.0 or 4.9 to 5.0 for cuttings treated with PGR solutions pre- or post-shipment, respectively. Chlorophyll fluorescence increased for stored cuttings with PGR application but was unaffected by application timing (Table 2). For example, although F_v/F_m of untreated geranium cuttings was 0.76 (non-stored) or 0.62 (stored), F_v/F_m of cuttings treated with PGRs had similar values ranging from 0.83 to 0.85. The SPAD value of geraniums treated with BA, BA + GA₄₊₇, or GA₃ increased by 70% to 82% compared with untreated cuttings, regardless of application time (Table 2). Post-shipment PGR applications were generally more effective at inhibiting leaf senescence than pre-shipment applications, but the effects varied among PGR treatments (Table 1). For example, application timing had no effect on the number of senesced leaves for cuttings treated with 2.5 mg·L⁻¹ BA, but cuttings treated post-shipment with 5.0 mg·L⁻¹ of either BA or BA + GA₄₊₇ had 1.3 fewer senesced leaves than cuttings that received the same treatments pre-shipment. The percentage of cuttings that were pullable after 20 d in propagation was diminished with pre-shipment PGR treatments (Table 2). For example, 46.7% of untreated, stored cuttings were considered pullable, but those treated with PGRs were 16.7% or less. Additionally, there were fewer pullable cuttings (15.7%) for cuttings receiving pre-shipment PGR applications compared with cuttings treated post-shipment (25.6%; data not shown). The highest concentrations of BA, BA + GA₄₊₇, and GA₃ decreased root dry mass substantially (by 84% or greater) compared with stored, untreated cuttings (Table 2). Root dry mass was 8.2 mg and 16.8 mg for cuttings treated pre- and post-shipment, respectively (data not shown).

Expt. 2: Rooting hormone application. Plant growth regulator applications improved the visual ratings of 'Patriot White' geranium cuttings 7 d after the beginning of propagation (Table 3). Visual rating increased from 2.4 for untreated stored cuttings to 5.0 for cuttings treated with 1.25 or 2.5 mg·L⁻¹ BA + GA₄₊₇ and 0.25 or 0.5 mg·L⁻¹ GA₃. Although rooting hormone had no effect on chlorophyll fluorescence of geranium cuttings (data not shown), F_v/F_m increased by 20% to 23% with PGR applications (Table 3). Rooting hormone application and PGR treatments interacted to affect the SPAD values of 'Patriot White' geraniums 7 d after application (Fig. 1). For example, SPAD values increased by 13%

with rooting hormone for non-stored and untreated cuttings but were similar across hormone applications for all stored and treated cuttings (Fig. 1). Rooting hormone had no effect on leaf senescence, whereas PGRs reduced leaf senescence by 50% to 100% compared with untreated cuttings (Table 3). The percentage of pullable cuttings increased from 16.4% to 51.8% for cuttings with rooting hormone compared with cuttings not receiving rooting hormone. Furthermore, the percentage of pullable cuttings was significantly reduced for cuttings treated with 5.0 mg·L⁻¹ BA + GA₄₊₇ and 0.5 and 2.0 mg·L⁻¹ GA₃ compared with untreated, stored cuttings (Table 3). Rooting hormone applications generally increased root dry mass but only at the lower PGR application concentrations tested (Fig. 1). For example, although root mass of cuttings treated with 0.25 mg·L⁻¹ GA₃ increased by 32.6 mg with the addition of rooting hormone, rooting hormone had no significant effect when the concentration of GA₃ increased to 2.0 mg·L⁻¹.

Expt. 3: Cultivar screen. Cuttings of 'Fantasia Pink Shell' and 'Presto Dark Red' geranium did not exhibit any leaf yellowing or phytotoxicity from PGR treatments and

appeared to be cultivars that were resistant to lower-leaf yellowing in propagation; therefore, data for these cultivars are not presented. When measured 7 d after PGR treatments were applied, SPAD increased in 'Fantasia Purple Sizzle' and 'Designer Salmon' as BA + GA₄₊₇ increased from 0.0 to 4.0 mg·L⁻¹ (Fig. 2). The PGR also decreased the number of senesced leaves by 0.5 (data not shown). In contrast, there was no significant PGR application trend for F_v/F_m on either geranium cultivar. The total number of senesced leaves 28 d after propagation decreased with increasing PGR concentration for 'Fantasia Purple Sizzle', although only cuttings treated with 4.0 mg·L⁻¹ BA + GA₄₊₇ had significantly fewer senesced leaves (Fig. 3). 'Designer Salmon' cuttings treated with 1.0 mg·L⁻¹ BA + GA₄₊₇ or greater had 1.0 to 1.4 fewer senesced leaves compared with untreated cuttings. The shoot dry mass for 'Designer Salmon' and 'Fantasia Purple Sizzle' geraniums was 469.9 and 393.3 mg, respectively, and was not influenced by PGR applications. Similarly, PGR application had no effect on root dry mass and was 24.8 and 28.2 mg for 'Designer Salmon' and 'Fantasia Purple Sizzle' geraniums, respectively.

Fig. 2. Effect of post-shipment application of BA + GA₄₊₇ on the relative chlorophyll content (SPAD) and chlorophyll fluorescence (F_v/F_m) of 'Fantasia Purple Sizzle' and 'Designer Salmon' zonal geraniums 7 d after applying solutions to cuttings (Expt. 3). Each symbol represents the mean of 12 plants, and error bars represent SEs of the mean. Regression lines are presented for significant correlations only with corresponding R^2 given. ***Significant at $P \leq 0.001$. BA = benzyladenine; GA = gibberellic acid.

Fig. 3. Effect of post-shipment application of BA + GA₄₊₇ on the number of senesced leaves and shoot and root dry mass of 'Fantasia Purple Sizzle' and 'Designer Salmon' geraniums 28 d after application (Expt. 3). Each symbol represents the mean of 12 plants, and error bars represent SEM of the mean. Different upper- or lower-case letters are significantly different by Tukey's honestly significant difference (HSD) test at $P \leq 0.05$ within 'Fantasia Purple Sizzle' or 'Designer Salmon' geraniums, respectively. BA = benzyladenine; GA = gibberellic acid.

Discussion

We quantified the efficacy of BA and GA on suppression of leaf senescence of geranium cuttings in propagation and documented how responses varied by PGR, application timing, rooting hormone, and cultivar. Previous unpublished research indicated that applications of BA + GA₄₊₇ to geranium stock plants suppressed subsequent lower-leaf senescence during propagation, but stem elongation also increased, resulting in cuttings with excessively long internodes. Therefore, BA + GA₄₊₇ applications are required after cutting severance from stock plants, and our data indicate that applying solutions post-shipment maximizes suppression of lower-leaf senescence. Additionally, although BA and GA inhibited adventitious root initiation and development in Expt. 1 (Tables 1 and 2),

application of rooting hormones partially overcame the suppression of rooting (Table 3; Fig. 1). Geranium cultivars vary in their susceptibility to lower-leaf senescence (Expt. 3), and there was no phytotoxicity or reduction in growth for non-susceptible cultivars in response to BA + GA₄₊₇ applications (data not shown). This could allow commercial growers to apply BA + GA₄₊₇ to cuttings of all geranium cultivars in propagation, thus simplifying management decisions. Additionally, current labels for PGRs containing BA + GA₄₊₇ include directions for use in preventing lower-leaf senescence of lilies. Along with what appeared to be a slightly more efficacious suppression of lower-leaf senescence, products containing BA + GA₄₊₇ are most likely the best candidate PGRs for commercial use in geranium propagation.

Our results compliment other studies that reported that BA and GA applications inhibited lower-leaf senescence for potted plants and cut flowers (Emino et al., 2002; Ferrante et al., 2003, 2009; Funnell and Heins, 1998; Han, 1997, 2001; Hicklenton, 1991; Philosoph-Hadas et al., 1996; Ranwala and Miller, 1998). However, our data are most relevant when considered with previous work by Mutui et al. (2005, 2012) and Purer and Mayak (1988). Purer and Mayak (1988) reported that the optical density of 'Red Bruni' geranium cuttings treated with 0.1 ppm GA₃ or silverthiosulfate (STS) preceding simulated shipping and propagation increased by 18% and 24%, respectively, compared with cuttings treated with water, indicating retardation of chlorophyll degradation and leaf senescence. The authors also reported fewer roots and reduced root dry mass for STS- and GA₃-treated cuttings compared with untreated cuttings; however, application of paclobutrazol or IBA suppressed the inhibitory action of GA₃ and STS on rooting. In a later study, leaf chroma and chlorophyll content of leaves treated with 5 to 20 μM TDZ were significantly higher and lower than untreated cuttings, respectively, in three geranium cultivars that were stored (Mutui et al., 2005). Additionally, rooting cuttings treated with 5 μM TDZ in solutions containing 4 $\mu\text{L}\cdot\text{L}^{-1}$ IBA increased the percentage of rooted cuttings by 17% and 40% for 'Katinka' and 'Fire', respectively, compared with untreated cuttings. Similarly, Mutui et al. (2012) reported that the chlorophyll a + b content of 'Katinka' geranium cuttings treated with 0.1 mM metoprolin (mT) or 5 μM TDZ were similar, but both significantly higher, than untreated cuttings. Interestingly, the percentage of rooted cuttings compared with untreated cuttings was not influenced by mT compared with a 77% reduction for TDZ-treated cuttings. Although TDZ and mT appear to be viable PGRs for suppression of lower-leaf senescence of geraniums, there are no commercially available products containing the active ingredients. A similar study (Emino et al., 2002) reported that 1-methylcyclopropene suppressed leaf yellowing but also rooting, whereas aminoethoxyvinylglycine suppressed leaf yellowing but resulted in phytotoxicity that was exacerbated as time in propagation increased. The authors also reported that foliar sprays containing 0.125 to 0.25 $\text{mg}\cdot\text{L}^{-1}$ increased the visual rating from 1.0 to 3.0 and from 2.5 to 4.5 for cuttings of 'Moonlight Red' and 'Sunrise Light Salmon' geraniums, respectively, when compared with untreated cuttings.

Although the goals of this and other studies were to identify effective PGR applications to inhibit leaf senescence in geranium cuttings during propagation, rapid and uniform adventitious root initiation and development is requisite during cutting propagation. PGRs that inhibit lower-leaf senescence of geranium have no use if they also inhibit rooting unless the suppression of rooting can be overcome. Our data, when taken together with Mutui et al. (2005, 2012), Purer and Mayak (1988), and Rapaka et al. (2008), demonstrate the

inverse relationship between antiethylene action agents, cytokinins, and gibberellins used for suppressing leaf yellowing on geranium and adventitious rooting of treated cuttings. Adventitious root primordia differentiation can be interrupted at an early stage in development in response to elevated cytokinin levels (Bollmark and Eliasson, 1986), whereas excess gibberellins can suppress intraprimordium cell division and reduce the number of cells per primordium (Haissig, 1972; Hartmann et al., 2011). Furthermore, inhibiting ethylene perception can reduce adventitious root formation by inhibiting ethylene-induced auxin biosynthesis and accumulation (Stepanova et al., 2005). However, the use of rooting hormones or GA biosynthesis inhibitors can alleviate the suppression of adventitious root initiation and development for cuttings treated with cytokinins and/or gibberellins.

The onset of lower-leaf senescence of geranium cuttings in propagation induced by suboptimal shipping conditions is difficult for propagators to predict. Therefore, preventive applications of BA + GA₄₊₇ could be warranted to avoid leaf yellowing as a result of undesirable shipping conditions. We estimated the PGR cost for a foliar application of solution containing 2.5 to 5.0 mg·L⁻¹ at a rate of 0.20 L·m⁻² covering 1000 m² of bench space using PGRCALC (Krug and Whipker, 2010). Using the average wholesale price for name-brand PGRs, it would cost \$4.74 to \$9.48 per 1000 m² to apply 2.5 to 5.0 mg·L⁻¹ BA + GA₄₊₇ for the PGR used in a spray application. Based on our calculations, we believe that the PGR and labor application costs to prevent lower-leaf senescence are minimal when compared with the potential labor costs to manually remove leaves and losses incurred from *Botrytis*-infected cuttings.

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