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Full Length Research Paper

Suppression of stem growth in pot kalanchoe 'Gold Strike' by recycled subirrigational supply of plant growth retardants

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Effect of concentration of paclobutrazol and uniconazole supplied to the recycling nutrient solution in an ebb and flow system on the growth and flowering of kalanchoe cultivar 'Gold Strike' was examined. Plants potted in 10 cm (370 mL) pots were supplied with a nutrient solution containing 80 mL per pot of 0.5, 1.0, or 2.0 mg·L^{-1} paclobutrazol or of 0.125, 0.250, or 0.50 mg·L^{-1} uniconazole. Plants were irrigated five times during the reproductive short day treatment period. During each irrigation, the nutrition solution was filled to a depth of 2 cm above the pot base and was left for about 15 min before drainage to reservoir. The control treatment, a foliar spray of 240 mL·m^{-2} of 10 mg L^{-1} placlobutrazol or 2.5 mg·L^{-1} uniconazole was applied twice after pinching at one week interval. At all applied concentrations, plant size, flower stem length and stem diameter decreased as compared to the control. However, the number of florets increased in the treated plants. Results indicate that the subirrigational optimum concentration was 0.5 mg·L^{-1} paclobutrazol and 0.125 mg·L^{-1} uniconazole is comparable to foliar spray. Therefore, subirrigational application of growth retardants at lower concentrations could be used as an effective mean for suppressing stem growth. More uniform and environmentally sound application of growth retardants with the same effectiveness could be achieved.

Key words: Cuttings, *Kalanchoe blosfeldiana*, paclobtrazol, uniconazole.

INTRODUCTION

Kalanchoe (*Kalanchoe blossfeldiana*) is a genus of about 125 species of tropical, succulent flowering plants of the family, Crassulaceae. These plants are cultivated as ornamental houseplants and rock or "succulent" garden plants. They are popular because of their ease of propagation, low water requirements, and wide variety of flower colors typically borne in clusters well above the vegetative growth. However, over-growing of the flower stem often causes problems in shipping and handling and consequently deteriorates marketable plant quality.

The application of plant growth retardants (PGRs) is the most broadly used method for growth control as foliar spray or soil drop irrigation by commercial growers (Lee and Rho, 2000). The effectiveness of triazole PGRs on growth control varies depending on the plant cultivars, PGR concentration, and application method (Banko and Stefani, 1988; Lee et al., 1998; Schuch, 1994). A problem with many growth retardants has been finding an efficient application method that produces consistent results. Foliar spray applications are most commonly used in commercial practice, but can result in nonuniform plant

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Table 1. Formulation of the nutrient solutions used.

Growth stage	Ca^{2+}	Mg^{2+}	K^{+}	NH_4^{+}	NO_3^{-}	SO_4^{2-}	$H_2PO_4^{-}$	HCO_3^{-}	pH	EC
	mmoL·L^{-1}									(dS·m^{-1})
Prior to pinching	3.0	2.0	1.0	0.1	3.1	2.0	1.0	0.0	6.7	0.8
During the short day treatment	5.0	2.0	2.0	0.1	6.1	2.0	0.5	0.5	6.4	1.3
After short day treatment	4.4	2.0	2.0	0.1	5.0	2.0	1.0	0.5	6.5	1.2

Table 2. Plant growth retardants, treatment methods, and concentrations used for kalanchoe.

Plant growth retardant	Treatment method	Concentration (mg·L^{-1})
Control		0
Paclobutrazol	Foliar spray (Two sprays)	10.0
	Subirrigational supply (Five feedings)	0.5 1.0 2.0
Uniconazole	Foliar spray (Two sprays)	2.5
	Subirrigational supply (Five feedings)	0.125 0.25 0.5

size if proper techniques are not used, especially when applying the very active triazoles (Barrett and Nell, 1990; Barrett et al., 1994). On the other hand, soil drop irrigation uses lower concentration of PGRs and obtains greater efficacy, but is labor-incentive and often results in excessive suppression of growth because of the residual PGR chemicals in the pot media.

According to Million et al. (1999, 2002), recycled subirrigational supply of PGRs at lower concentrations significantly improved the effect on reduction of flower stem length and, at the same time, tentatively minimized the toxic residual chemicals in several floricultural crops. In the present study, we evaluated the efficacy of the recycled subirrigational supply on growth control of a kalanchoe cultivar, 'Gold Strike' using two popular PGRs, paclobutrazol and uniconazole. We also determined the appropriate concentrations of these PGRs for practical use and monitored the morphological changes of the flower stem after the treatment.

MATERIALS AND METHODS

Plant materials and culture

Three node cuttings of *K. blossfeldiana* 'Gold Strike' were taken from stock plants and rooted. After a month, rooted plants were transplanted into the rooting medium of peatmoss + perlite (1:1, v/v) and cultured under a short day condition of not more than an 11 h photoperiod during the first six weeks after pinching. The nutrient solution (Table 1) formulated separately for each of three growth stages were supplied through a mat subirrigation system.

Treatment of PGRs

PGRs were treated at 16th day after pinching, when the axillary branches reached 1.0~1.5 cm in length (Table 2). For recycled subirrigational supply, PGRs were mixed with nutrient solution and treated five times at every five days during the short-day condition. During the subirrigational treatment, 80 mL of nutrient solution containing growth retardants were applied per pot, so that the pots were submerged up to 2 cm from the bottom for 15 min. The remaining solution was collected in a nutrient solution tank and recycled. Foliar spray was conducted as control for evaluation of the efficacy of subirrigational supply. Eight mL (1 m^2/240 mL) paclobutrazol 10 mg·L^{-1} or uniconazole 2.5 mg·L^{-1}, which showed 30% reduction in plant size in the preliminary tests, were sprayed two times per pot with seven days interval (Lee et al., 2003).

Uniconazole: (E)-(+)-(S)-1(4-chlorophenyl)-4,4-Dimethyl-2(1,2,4-trialzol-1-yl)pent-1-ene-3-ol; paclobutrazol: (±)-(R*,R*)-beta-((4-chlorophenyl)methyl)-alpha-(1,1,-dimethyleth yl)-1H-1,2,4-triazole-1-ethanol.

Growth measurement and statistical analysis

Three replicates per treatment and eight plants per each replicate were used. Plant size was calculated as (height + average width)/2 (Million et al., 1999). Average width was determined as the mean of two widths measured at 90ºC from one another. Flower stem length was measured from the proximal to the distal end of the flower including pedicel. Number of florets was the number of flower cluster per stem. Internode length and stem diameter were measured from the third and fourth node from the shoot tip. For the measurements of chlorophyll concentration, leaf disks from vigorous leaves on second node from the shoot tip were sampled and were extracted with an 80% (v/v) acetone for 24 h. Chlorophyll concentration was determined by measuring absorbance with a

Table 3. Concentrations used to obtain a standard growth curve of *Raphanus sativus* L. 'Cheongwoon'.

Plant growth retardant	Concentration ($mg \cdot L^{-1}$)	Plant growth retardant	Concentration ($mg \cdot L^{-1}$)
Control	0	Control	0
Paclobutrazol	0.05	Uniconazole	0.5
	0.25		1.0
	1.25		2.0
	6.25		4.0
	31.25		8.0

spectrophotometer (Uvikon 922, Kotron Instrument, Italy) at 645 and 633 nm, and calculated as described by Arnon (1949). Dry weight at harvest was measured after drying at 80% for 72 h.

Data collected were analyzed for statistical significance by the SAS (Statistical Analysis System, V. 6.12, Cary, NC, USA) program. The experimental results were subjected to an analysis of variance (ANOVA) and Duncan multiple range test.

Microscopic examination

Ultrastructural analysis using scanning electron microscopy (SEM) was conducted to examine the structure of second node from flower stem top. Flower stem samples were fixed in 3% (v/v) glutaraldehyde overnight. Samples were then washed three times with phosphate buffered saline solution (137 mM NaCl + 2.7 mM KCl + 43 mM Na_2HPO_4 + 1.4 mM KH_2PO_4). Flower stem samples were dried with critical point dryer (CPD2, Pelcl, CA., USA) and fragments were positioned on stubs prior to gold coating in a sputter coater (SC 7640, Polaron, Sussex, England). Following coating with a thin layer of gold the specimens were observed and were micrographed using SEM (LEO-435VP, Zeiss, Germany) (Kim and Lee, 2002).

Bioassay of residual PGR

The amount of residual PGR in the subirrigated nutrient solution was observed. The investigation was conducted five times during the short-day treatment using *Raphanus sativus* L. 'Cheongwoon', which shows highly sensitive reaction to PGR. To obtain a standard growth curve depending on the concentrations of PGR, the plants seeds were sawed in 288-cell plug tray and subirrigated with nutrient solution mixed by six different concentrations of paclobutrazol or uniconazole. After five days, the length of hypocotyle was measured and the standard growth curve based on the PGR concentrations was drawn (Table 3). Recycled nutrient solutions from the kalanchoe subirrigational treatment with paclobutrazol 6.25 $mg \cdot L^{-1}$ or uniconazole 4 $mg \cdot L^{-1}$ was collected from each five times of recirculation and used to culture the radish plants. After five days, the length of hypocotyle was measured and compared to the standard growth curve to determine the amount of residual PGR in the solution.

RESULTS AND DISCUSSION

Quality traits

The changes in plant size and flower stem length of kalanchoe 'Gold Strike' is shown in Figure 1. Approximately 30% of reduction in plant size was resulted from both foliar spray and recycled subirrigational supply. Subirrigational treatment of paclobutrazol showed the better effect on the reduction of flower stem length at all concentrations, compared to foliar spray. Subirrigational treatment of uniconazole at 0.25 and 0.5 $mg \cdot L^{-1}$ decreased flower stem length, but the treatment of 0.125 $mg \cdot L^{-1}$ had no effect, compared to foliar spray.

The number of florets increased in all PGR treatments (Figure 2). Significantly higher number of florets was observed from subirrigational supply in both PGRs, compared to foliar spray, and the subirrigational treatment of paclobutrazol 0.5 $mg \cdot L^{-1}$ and uniconazole 0.5 $mg \cdot L^{-1}$ showed the highest effect. Starman and Williams (2000) reported that soil drench of uniconazole in *Scaevola aemula*, 'New Wonder' reduced plant width and flower stem length and increased the number of flowers and florets per stem regardless of the flower. They indicated that the reduction in flower stem length made the stem node thicker and denser, which supports the results in the present study. In addition, the higher increase in the number of florets from recycled subirrigational supply, compared to foliar spray, may be due to the high concentration of PGR used for foliar spray at early growth stage and adverse influences on overall growth of the plants.

Days to flower increased in all treatments, and it increased higher in subirrigational treatment of paclobutrazol 2 $mg \cdot L^{-1}$and uniconazole 0.5 $mg \cdot L^{-1}$ than in foliar spray treatment (Table 4). The number of flowering plants indicates the number of flowered plants among the six individuals per replication. Flowering rate significantly decreased in the subirrigational supply of paclobutrazol 2.0 $mg \cdot L^{-1}$ and uniconazole 0.5 $mg \cdot L^{-1}$. The reduction in flowering rate could be due to the downside effect of continuous treatment of PGR until the differentiation of flower bud, although PGR was applied at low concentrations.

The reduction in stem diameter was observed in all treatments, and the reduction effect was significantly greater in recycled subirrigational supply than in foliar spray (Table 4). The number of leaves increased by sub-

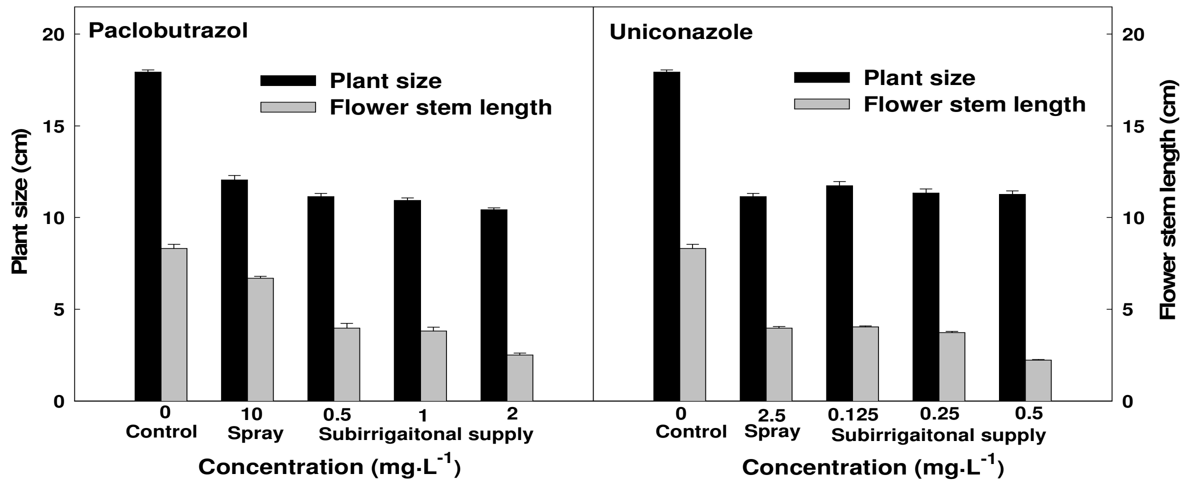

Figure 1. Plant size and flower stem length of kalanchoe 'Gold strike' as affected by concentration after subirrigational supply of plant growth retardants. Vertical bars represent standard errors of means.

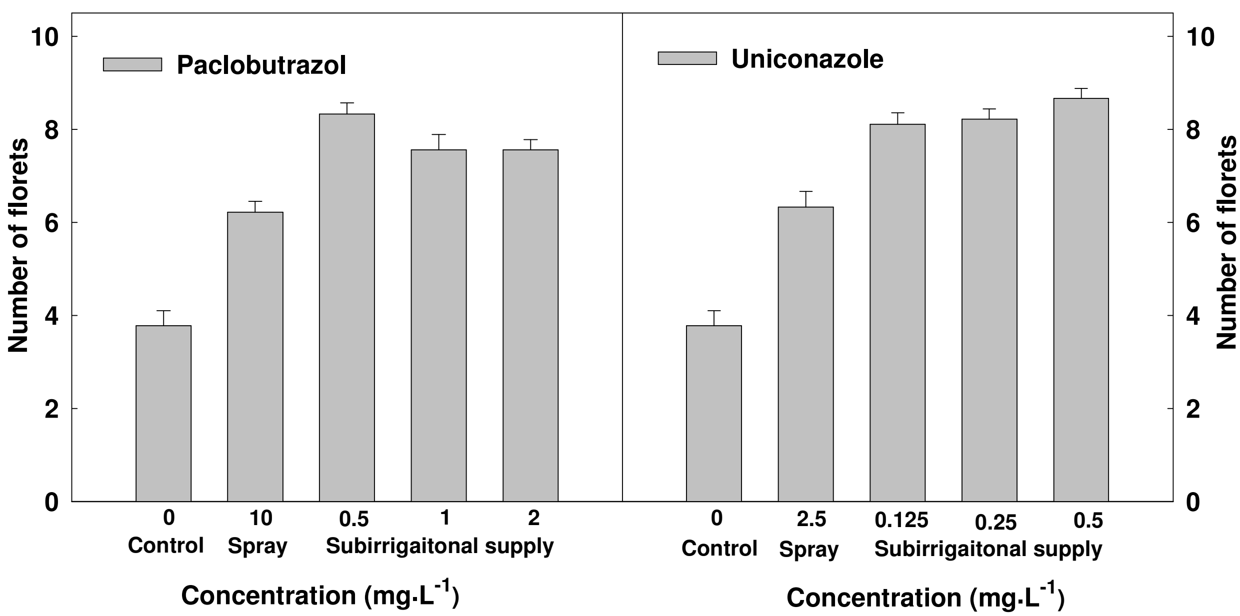

Figure 2. Effect of concentration of paclobutrazol and uniconazole on number of florets after subirrigational supply to kalanchoe 'Gold Strike'. Vertical bars represent standard errors of means.

irrigational supply of paclobutrazol 0.5, 1.0 mg·L^{-1} and uniconazole 0.125, and 0.25 mg·L^{-1} compared to the untreated control (Table 4).

Growth traits

Leaf area was affected by PGR and decreased significantly in all treatments, compared to the control. Leaf area was reduced less in subirrigational supply than in foliar spray (Table 5). It is considered that foliar spray of a high PGR concentration at early growth stage delayed overall plant growth, while repeated applications of low concentration in recycling subirrigation assured enough leaf area by gradually suppressing the plant growth. The level of leaf area reduction increased as the concentration increased.

Fresh and dry weights were reduced significantly in all

Table 4. Growth of kalanchoe 'Gold Strike' measured at 11 weeks after treatment of paclobutrazol and uniconazole.

Treatment	Conc. (mg·L^{-1})	Days to flower	No. of flowering plants	Stem diameter (cm)	No. of leaves
Control	0	101 c^{z}	6 a	0.39 a	51 e
Paclobutrazol					
Foliar spray	10	104 b	6 a	0.33 b	63 cd
Subirrigational supply	0.5	105 b	6 a	0.26 d	70 a-d
	1	105 b	6 a	0.25 d	73 a-c
	2	107 b	3 b	0.25 d	59 de
Uniconazole					
Foliar spray	2.5	104 b	6 a	0.29 c	59 de
Subirrigational supply	0.125	104 b	6 a	0.25 d	75 ab
	0.25	104 b	6 a	0.26 d	81 a
	0.5	107 a	1 c	0.25 d	67 b-d

^{z}Mean separation within columns by DMRT, P≤0.05.

Table 5. Growth of kalanchoe 'Gold Strike' measured at 11 weeks after subirrigational supply of paclobutrazol and uniconazole.

Treatment	Conc.(mg·L^{1})	Leaf area (cm^{2})	Fresh wt. (g)	Dry wt. (g)	Chlorophyll (µg·mg^{-1}fw)
Control	0	406.84 a	72.78 a	3.76 a	0.50 a
Paclobutrazol					
Foliar spray	10	228.26 de	46.30 de	2.40 cd	0.49 a
Subirrigational supply	0.5	294.40 bc	53.35 c	2.64 c	0.46 ab
	1	309.47 b	49.18 cd	2.47 cd	0.41 a-c
	2	224.76 c-e	46.58 de	2.34 de	0.35 bc
Uniconazole					
Foliar spray	2.5	189.28 e	42.32 e	2.13 e	0.41 a-c
Subirrigational supply	0.125	318.23 b	63.00 b	3.04 b	0.40 a-c
	0.25	305.64 bc	66.39 b	3.21 b	0.35 bc
	0.5	270.13 b-d	67.70 ab	3.06 b	0.33 c

^{z}Mean separation within columns by DMRT, P≤0.05.

treatments, compared to the control (Table 5). For paclobutrazol, fresh and dry weights were significantly higher in subirrigational supply only at 0.5 mg·L^{-1} than in foliar supply, while, for uniconazole, they were significantly higher at all concentrations in subirrigational supply. Chlorophyll concentration was lower in all PGR treatments, and decreased by increasing concentrations of both PGRs in subirrigational supply (Table 5).

Changes in plant cell tissue after PGR treatment

Figure 3 shows microscopic examination of transversal and longitudinal sections of flower stems at the second node treated with uniconazole 0.125 and paclobutrazol 0.5 mg·L^{-1}, which were considered to be the best concentrations for subirrigational supply in this study. PGR-treated plants carried denser vascular cambium (VC) cells which were suppressed in size, as compared to the control plants. The level of reduction in cell size was higher in xylem (X) than in phloem (P).

Thetford et al. (1995a,b) speculated that the effects of uniconazole foliar spray on stem diameter reduction of a forsythia, 'Spectabilis' was related to PGR transported through xylem tissues. They suggested that, when applied to roots, young stems, and the youngest leaves, the triazole PGR compounds quickly entered the plant and were translocated acropetally via the xylem to the leaves and resulted in the preferential suppression of xylem tissues, as observed from our present study. In addition, transversal section of cortex from the second node showed that the PGR-treated plants had very short and stocky cells, while the control plants had thin and long cells. This indicates that the suppression of flower

Table 6. The amounts of residual paclobutrazol and uniconazole in the nutrient solutions as affected by number of recyclings of the solution.

No. of recyclings of the solution	Paclobutrazol (mg·L^{-1})	Uniconazole (mg·L^{-1})
0	6.25 ab^{z}	4.00 b
1	5.02 b	3. 85 b
2	5.02 b	5. 30 a
3	6.25 ab	4. 78 ab
4	7.09 a	4.78 ab
5	3.10 c	2.88 c

^{z}Mean separation within columns by DMRT, P≤0.05.

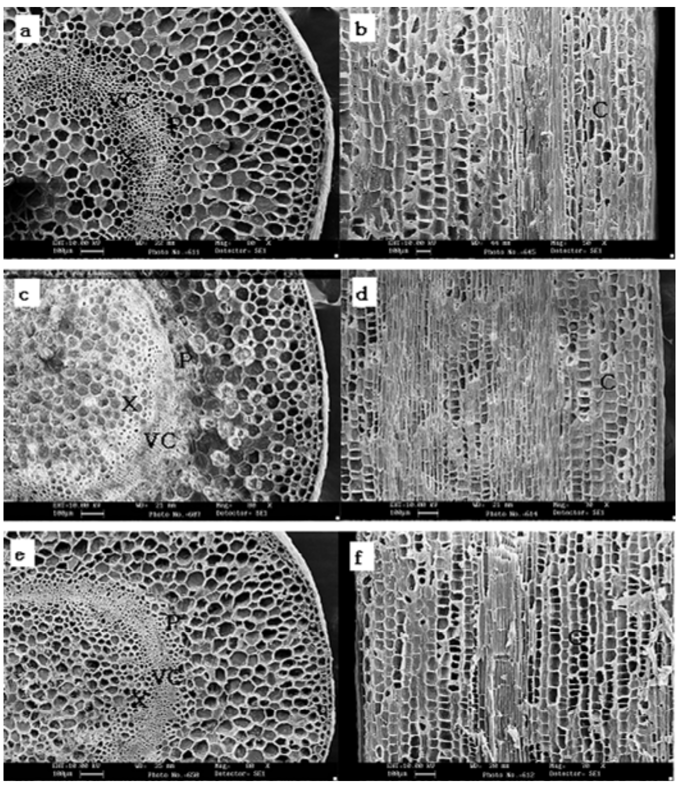

Figure 3. Scanning electron micrographs of transversal and longitudinal sections of flower stems of kalanchoe 'Gold Strike' taken from the second node at 11 weeks after subirrigational supply of 0 (a, b), 0.125 mg·L^{-1} uniconazole (c, d) or 0.5 mg·L^{-1} paclobutrazol (e, f). X, xylem; P, phloem; VC, vascular cambium; and C, cortex.

stem length after PGR treatment was actually due to the cell units shorten in length.

The amount of residual PGR

The amount of residual paclobutrazol in the recycled nutrient solution did not changed significantly until the 4th recirculation, as estimated based on the standard growth curve. However, when recycled five times, the paclobutrazol concentration dropped by 50% from its original concentration of 6.25 mg·L^{-1} (Table 6). A very similar result was observed from the treatment of uniconazole, which showed 30% reduction from its original concentration of 4 mg·L^{-1} when recycled five times.

Interestingly, the residue concentrations of both PGRs had a tendency to slightly increase until the 4th recirculation and this can be simply explained by the evaporation of the water from the nutrient solution in a relatively small scale. Adriansen and Odgaard (1997) indicated that the amount of residual PGR differed depending on the PGR treatment method, amount, and frequency. They reported that residual amount was not significantly reduced when paclobutrazol and uniconazole 2 mg·L^{-1} were mixed in the nutrient solution and stored in the dark for a week, but it dropped by 25-30% after four weeks. Therefore, it is inferred that similar results to the simulated tests using radish plants would be also obtained for the subirrigational treatment of kalanchoe, and the suppression of stem growth resulting from the accumulated effects of PGR treatment during the five times of recirculation.

Conclusion

Subirrigational supply of PGRs reduced kalanchoe plant size and increased the number of florets and leaf area more effectively, compared to foliar spray. However, the treatment with paclobutrazol 2 mg·L^{-1} or uniconazole 0.5 mg·L^{-1} tended to drop the flowering rate, and thereby was inappropriate for practical use. In the present study, subirrigational supply of paclobutrazol at 0.5 mg·L^{-1} and uniconazole at 0.125 mg·L^{-1} provided the best condition for effectively suppressing stem growth without deteriorating other important horticultural values in kalanchoe 'Gold Strike'.

Unlike subirrigation system, foliar spray often results in excessive growth suppression and toxicity due to the use of highly concentrated PGRs. Subirrigational supply alleviated these damages and enhanced the production of kalanchoe with higher uniformity and morphological balance. Eco-friendly subirrigational supply will also save grower's labor needed for PGR application.

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REFERENCES

Adriansen E, Odgaard P (1997). Residues of paclobutrazol and uniconazole in nutrient solutions from ebb and flood irrigation of pot plants. Sci. Hortic., 69: 73-83.

Arnon DI (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol., 24: 1-1.

Banko TJ, Stefani MA (1988). Growth response of selected container-grown bedding plants to paclobutrazol, uniconazole and daminozide. J. Environ. Hort., 6: 124-129.

Barrett JE, Bartuska CA, Nell TA (1994). Application techniques alter uniconazole efficacy on chrysanthemums. HortScience, 29: 893-895.

Barrett JE, Nell TA (1990). Factors affecting efficacy of paclobutrazol and uniconazole on petunia and chrysanthemum. Acta Hortic., 272: 229-234.

Kim YA, Lee JS (2002). Anatomical difference of neck tissue of cut roses as affected by bent neck and preservative solution. J. Kor. Soc. Hort. Sci., 43: 221-225.

Lee EK, Chung SK, Lee SW, Choi GW (1998). Effects of plant growth retardants on the growth and flowering in poinsettia. RDA. J. Hort. Sci., 40: 102-106.

Lee MY, Nam HC, Jeong BR (2003). Growth and flowering of Kalanchoe 'Rako' as affected by concentration of paclobutrazol and uniconazole. Acta Hortic., 624: 287-296.

Lee SW, Rho KH (2000). Growth control in 'New Guinea' impatiens (*Impatiens hawkeri hybrida*) by treatments of plant growth retardants and triazole fungicides. Kor. J. Hort. Sci. Technol., 18: 827-833.

Million JB, Barrett JE, Nell TA, Clark DG (1999). Inhibiting growth of flowering crops with ancymidol and paclobutrazol in subirrigation water. HortScience, 34: 1103-1105.

Million JB, Barrett JE, Nell TA, Clark DG (2002). One-time vs. continuous application of paclobutrazol in subirrigation water for the production of bedding plants. HortScience, 37: 345-347.

Schuch UK (1994). Response of chrysanthemum to uniconazole and daminozide applied as dip to cuttings or as foliar spray. J. Plant Growth Regul., 13: 115-121.

Starman TW, Williams MS (2000). Growth retardants affect growth and flowering of scaevola. HortScience, 35: 36-38.

Thetford M, Warren SL, Blazich FA (1995a). Response of *Forsythia* x *intermedia* 'Spectabilis' to uniconazole. I. Growth; Dry-matter distribution; and mineral nutrient content, concentration, and partitioning. J. Am. Soc. Hort. Sci., 120: 977-982.

Thetford M, Warren SL, Blazich FA, Thomas JF (1995b). Response of *Forsythia* x *intermedia* 'Spectabilis' to uniconazole. Ⅱ. Leaf and stem anatomy, chlorophyll, and photosynthesis. J. Am. Soc. Hort. Sci., 120: 983-988.