Vegetative Growth Responses of Citrus Nursery Trees to Various Growth Retardants

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SUMMARY. As part of a larger study to improve rind color of citrus (*Citrus* spp.) fruit, this initial study was conducted to determine the concentration of various gibberellin-biosynthesis inhibitors required to elicit a biological response in citrus trees, as measured by vegetative growth. Paclobutrazol and GA₃ were included as control treatments at concentrations known to elicit growth-retarding or growth-promoting effects, respectively. Repeated (×4) foliar applications of GA₃ (at 64 ppm) increased growth of ‘Eureka’ lemon (*Citrus limon*) shoots by 63%, with no significant effect on rootstock and scion diameters. Repeated foliar applications of prohexadione-calcium (ProCa) at various concentrations (100, 200, 400, or 800 ppm) as well as uniconazole (at 500 or 1000 ppm) and paclobutrazol (at 0.25%) had no effect on rootstock or scion diameters 8 months after the first application. The high concentrations of ProCa (800 ppm) and uniconazole (1000 ppm), and the paclobutrazol treatment (0.25%) reduced shoot length compared with the control. Uniconazole at 1000 ppm resulted in the most growth retardation, which resulted in 34% shorter shoot length than the control. Although the number of nodes on the longest shoot did not differ from the untreated control, internode length differed significantly among treatments. ProCa at 400 and 800 ppm, uniconazole at 1000 ppm, and paclobutrazol at 0.25% significantly reduced internode length relative to the control by 31%, 56%, 50%, and 28%, respectively. Vegetative growth of ‘Eureka’ lemon nursery trees was retarded following the repeated (×4) foliar application of gibberellin-biosynthesis inhibitors. ProCa at 400 to 800 ppm and uniconazole at 1000 ppm were identified as prospective treatments for further field studies to test their effects on rind color enhancement of citrus fruit.

As part of a larger study to improve rind color of citrus fruit, this initial study was conducted to determine the concentration of various gibberellin-biosynthesis inhibitors required to elicit a biological response in citrus trees as measured by vegetative growth. Goldschmidt (1988) hypothesized that factors contributing to invigorating growing conditions are antagonistic to optimal rind color development.

The vegetative growth of citrus trees is stimulated by various exogenous factors, viz. high temperature,

high light intensity, nitrogen, and water, as well as endogenous hormones, viz. gibberellins and cytokinins. Young leaves and fruit are major sites of gibberellin biosynthesis (Salisbury and Ross, 1992; Spiegel-Roy and Goldschmidt, 1996). High endogenous gibberellin concentrations enhance stem elongation (Salisbury and Ross, 1992) and delay rind color development of citrus fruit (Garcia-Luis et al., 1985).

Growth retardants, some of which are gibberellin-biosynthesis inhibitors, reduce vegetative growth in plants by disrupting gibberellin biosynthesis (Smeirat and Qrunfleh, 1989). Aron et al. (1985) demonstrated that when paclobutrazol (Cultar®; Syngenta Crop Protection, Basel, Switzerland) was applied at 1

g·L⁻¹ on ‘Minneola’ tangelo (*Citrus reticulata* × *Citrus paradisi*) trees just before the onset of the summer flush, it reduced shoot length, internode length, and the number of shoots developed by 41%, 76%, and 44%, respectively. Similarly, Delgado et al. (1986) showed that paclobutrazol reduced internode length, and hence shoot length, of ‘Valencia’ sweet orange (*Citrus sinensis*) in Cuba. Uniconazole (Sunny®; Valent BioSciences, Chicago) reduced shoot length, number of lateral shoots per terminal, number of nodes per terminal, and internode length in ‘Wichita’ pecan (*Carya illinoensis*) (Graham and Storey, 2000) and ‘Cleopatra’ mandarin (*C. reticulata*) (Wheaton, 1989) trees. ProCa (Regalis® and Apogee®; BASF, Ludwigshafen, Germany) is used on apple (*Malus ×domestica*) and pear (*Pyrus communis*) fruit trees to reduce and control vegetative growth (Miller, 2002). Costa et al. (2001) reported that applications of 100 ppm ProCa significantly reduced shoot growth and increased fruit size in pears. ProCa acts primarily as a gibberellin-biosynthesis inhibitor, especially 3β-hydroxylation of GA₂₀ to GA₁ (Nakayama et al., 1992; Rademacher, 2001). Stover et al. (2004) found that two 500 ppm ProCa applications reduced the vegetative growth of six citrus genotypes tested by ≈40%.

In contrast to the affects of gibberellin-biosynthesis inhibitors on vegetative growth, their effects on rind color enhancement of citrus fruit are not well known. Monselise and coworkers (1976) reported that paclobutrazol contributed to the acceleration of chlorophyll degradation of sweet orange. Gilfillan and Lowe (1985) demonstrated that paclobutrazol increased ‘Satsuma’ mandarin (*Citrus unshiu*) rind color by 1 to 2 color rating units. This result was achieved when paclobutrazol was applied after physiological fruit drop (in November) at 1 g·L⁻¹, as well as in summer (January and February),

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Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
0.3048	ft	m	3.2808
2.54	inch(es)	cm	0.3937
25.4	inch(es)	mm	0.0394
0.001	ppm	g·L ⁻¹	1000
1	ppm	mg·L ⁻¹	1

and suggests that paclobutrazol suppressed the early summer growth flush (November–December), which might be more important for rind color development than the late summer flush (January–February). Monselise (1986) mentioned that paclobutrazol caused a more rapid change of rind color in ‘Topaz’ tangerine (*C. reticulata* × *C. sinensis*), an Israeli selection of ‘Ortanique’ tangerine. Preliminary results by Barry and Van Wyk (2004) showed that when ProCa was applied 2 weeks before anticipated harvest at 100 ppm to ‘Navelina Navel’ sweet orange, rind color was improved as a result of chlorophyll degradation and carotenoid biosynthesis. No other reports on the possible effect of gibberellin-biosynthesis inhibitors on rind color enhancement of citrus fruit were found.

The principal objective of this study was to determine the concentration of various gibberellin-biosynthesis inhibitors required to retard shoot growth in citrus nursery trees. This information could then be used in field studies to test the effects of gibberellin-biosynthesis inhibitors on rind color enhancement of citrus fruit.

Materials and methods

PLANT MATERIAL AND SITE. During the 2005–06 summer growing season, 108 potted nursery trees of ‘Eureka’ lemon budded on ‘X639’ rootstock [‘Cleopatra’ mandarin × trifoliolate orange (*Poncirus trifoliata*)] of similar size and with at least three strong primary branches were selected at Nucellar Nursery, Simondium, Western Cape province, South Africa (lat. 33°50’S, long. 18°58’E, 160 m altitude). These trees were 21 months old at the start of the experiment.

TREATMENTS APPLIED. Potted nursery trees were randomly allocated to treatments that were applied as foliar sprays, viz. untreated control; GA₃ (ProGibb®, Valent BioSciences) at 64 ppm a.i.; ProCa at 100, 200, 400, or 800 ppm; uniconazole at 500 or 100 ppm; and paclobutrazol at 0.25%. Paclobutrazol and GA₃ were included as control treatments at concentrations known to elicit growth-retarding and growth-promoting effects, respectively. Kaolin particle film (Surround® WP Crop Protectant; Engelhard, Iselin, NJ) at 2 ppm a.i. was applied together with all treatments to easily distinguish new

growth flushes throughout the assessment period. All treatments were applied four times on 15 Nov. 2005, 27 Dec. 2005, 16 Feb. 2006, and 31 Mar. 2006, and these application dates were planned to coincide with various growth flushes during the summer growing season.

DATA COLLECTION. Rootstock and scion diameters were measured 2 cm below and 3 cm above the bud union, at the start of the experiment (15 Nov. 2005), 6 weeks thereafter (27 Dec. 2005), and at the end of the experiment (20 July 2006). Three shoots per tree were selected, marked, and their length was measured at the start of the experiment. Thereafter, only the length of the new growth was measured and internodes were counted at each assessment date. The purpose of this study was not to quantify the optimal concentration of growth retardant required to achieve maximum growth retardation, but rather to determine at what concentration various gibberellin-biosynthesis inhibitors caused a vegetative growth response in citrus nursery trees. Therefore, and because all shoots did not flush and grow out, data analysis was done on the longest shoot to quantify the treatment effects on growth retardation.

STATISTICAL DESIGN AND ANALYSIS. Experimental layout was a completely randomized block design consisting of 12 single-tree replicates. Blocking was used to reduce the possible effect of experimental error due to within-site variation as a result of lighting and microclimate. Analysis of variance was conducted using the general linear model (GLM) procedure of SAS (version 9.1; SAS Institute, Cary, NC) and least significant difference values were used to separate treatment means. Due to initial differences in stem diameter among trees, analysis of covariance was conducted with initial stem diameter and shoot length as covariates.

Results and discussion

Rootstock diameter did not differ among treatments throughout the experiment (Table 1). Significant differences in scion diameter were measured at the onset of the trial and 6 weeks thereafter, but there were no significant differences among treatments at the final measurement (Table 1). When the initial rootstock and scion diameters were fixed by covariance analysis, there were no significant differences among treatments on the final rootstock and scion diameters. In this short-term study

Table 1. Mean rootstock and scion diameters of citrus nursery trees of ‘Eureka’ lemon budded onto ‘X639’ rootstock after treatment with GA₃, prohexadione-calcium (ProCa), uniconazole, and paclobutrazol to determine the concentration of various gibberellin-biosynthesis inhibitors required to retard shoot growth in citrus nursery trees. Measurements were made at the start of the experiment (15 Nov. 2005), 6 weeks thereafter (27 Dec. 2005), and at the end of the experiment (20 July 2006).

Treatment (a.i.) ^z	Rootstock diam (mm) ^z			Scion diam (mm)		
	15 Nov. 2005	27 Dec. 2005	20 July 2006	15 Nov. 2005	27 Dec. 2005	20 July 2006
Control	14.3 NS ^y	13.9 NS	14.5 NS	11.1 bc	11.0 bc	11.8 NS
GA ₃ 64 ppm	14.6	14.2	15.3	12.3 a	11.5 abc	12.1
ProCa 100 ppm	14.1	14.2	14.4	10.9 c	11.2 bc	11.2
ProCa 200 ppm	14.3	14.2	14.5	11.5 abc	11.0 bc	11.8
ProCa 400 ppm	15.4	15.3	15.7	11.8 abc	11.3 bc	11.6
ProCa 800 ppm	13.4	13.5	14.2	11.2 bc	10.7 c	11.3
Uniconazole 500 ppm	15.0	15.3	15.6	12.1 ab	11.9 ab	12.2
Uniconazole 1000 ppm	14.8	14.6	15.1	11.4 abc	11.0 bc	11.4
Paclobutrazol 0.25%	14.3	14.8	15.3	12.2 a	12.4 a	12.2
P value	0.2780	0.3159	0.3179	0.0430	0.0482	0.2207
LSD ^x	1.45	1.60	1.41	1.00	1.02	0.91

^z1 ppm = 1 mg·L⁻¹, 1 mm = 0.0394 inch.

^yMeans within columns followed by different letters are significantly different at $P \leq 0.05$; NS = nonsignificant.

^xLeast significant difference.

(i.e., 8 months), there was too little time for a treatment response in root-stock and scion diameters, although differences would be expected with a longer-term study (Smeirat and Qrunfleh, 1989).

Shoot length of the longest shoot was 63% longer for the GA₃ treatment than for the control (Fig. 1), which confirms previous reports

that GA₃ applied at 64 ppm stimulates citrus shoot growth (Mudzunga, 2000). This response is not unexpected given the role of gibberellins in enhancing stem elongation (Salisbury and Ross, 1992). In contrast, shoot length of the trees that received the high concentrations of ProCa (800 ppm) and uniconazole (1000 ppm), and the paclobutrazol treatment

(0.25%), were shorter than that of the control (Fig. 1). The 1000 ppm uniconazole treatment had 34% shorter shoot length than the control. Shoot length of the other treatments did not differ from that of the control (Fig. 1).

Although the number of nodes on the longest shoot did not differ in any of the treatments from the untreated control (Fig. 2), internode

Fig. 1. Shoot length of the longest shoot of 'Eureka' lemon nursery trees at the end of the experiment on 20 July 2006 after treatment with GA₃, prohexadione-calcium (ProCa), uniconazole (Unicon), and paclobutrazol (Pac) to determine the concentration of various gibberellin-biosynthesis inhibitors required to retard shoot growth in citrus nursery trees. Means followed by a different letter are significantly different at $P \leq 0.10$ (least significant difference = 76.7); 1 ppm = 1 mg·L⁻¹, 1 mm = 0.0394 inch.

Fig. 2. Number of nodes on the longest shoot of 'Eureka' lemon nursery trees the end of the experiment on 20 July 2006 after treatment with GA₃, prohexadione-calcium (ProCa), uniconazole (Unicon), and paclobutrazol (Pac) to determine the concentration of various gibberellin-biosynthesis inhibitors required to retard shoot growth in citrus nursery trees. Means followed by a different letter are significantly different at $P \leq 0.05$ (least significant difference = 6.96); 1 ppm = 1 mg·L⁻¹.

Fig. 3. Internode length of the longest shoot of 'Eureka' lemon nursery trees the end of the experiment on 20 July 2006 after treatment with GA₃, prohexadione-calcium (ProCa), uniconazole (Unicon), and paclobutrazol (Pac) to determine the concentration of various gibberellin-biosynthesis inhibitors required to retard shoot growth in citrus nursery trees. Means followed by a different letter are significantly different at $P \leq 0.05$ (least significant difference = 3.87); 1 ppm = 1 mg·L⁻¹, 1 mm = 0.0394 inch.

Fig. 4. Photographs of 'Eureka' lemon shoots that illustrate the effect of growth retardants on vegetative growth. (A) untreated control (internode length = 14.7 mm); (B) 400 ppm prohexadione-calcium (ProCa) (internode length = 10.1 mm). Note the shortening of internode length by >30% in the Pro-Ca treatment compared with the untreated control treatment; 1 mm = 0.0394 inch, 1 ppm = 1 mg·L⁻¹. (To view this figure in color, please view the paper online through the ASHS website: ashs.org.)

length differed significantly among treatments (Fig. 3). ProCa at 400 and 800 ppm, uniconazole at 1000 ppm, and paclobutrazol at 0.25% reduced internode length relative to the control by 31%, 56%, 50%, and 28%, respectively (Figs. 3 and 4). In this study, the cause of shorter shoot length was not due to fewer nodes,

but rather due to shorter internode length (Fig. 4).

These results compare favorably with those in previous reports on the effects of gibberellin-biosynthesis inhibitors on citrus growth. For example, vegetative growth retardation following paclobutrazol treatment was achieved with 'Mexican' lime (*Citrus aurantifolia*) (Medina-Urrutia and Buenrostro-Nava, 1995), lemon (Harty and van Staden, 1988; Smeirat and Qrunfleh, 1989), 'Valencia' sweet orange (Delgado et al., 1986), and 'Minneola' tangelo (Aron et al., 1985; Greenberg et al., 1993); uniconazole reduced shoot length of sour orange (*Citrus aurantium*) (Swietlik, 1986) and 'Cleopatra' mandarin seedlings (Wheaton, 1989); and Stover et al., (2004) showed that two 500 ppm ProCa applications reduced the vegetative growth of six citrus genotypes tested.

In conclusion, vegetative growth of 'Eureka' lemon nursery trees was retarded following the repeated foliar application of gibberellin-biosynthesis inhibitors. Because it is unlikely that paclobutrazol would be registered on citrus due to its persistence in the environment and the plant (Goulston and Shearing, 1985), ProCa at 400 to 800 ppm and uniconazole at 1000 ppm are prospective treatments for further field studies to test their effects on rind color enhancement of citrus fruit.

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