Developmental Stage of 'Snow White' Indian Hawthorn and 'Sky Pencil' Holly Affects Response to Cyclanilide[1]

Zoe Williamson, Gary J. Keever, J. Raymond Kessler, Jr., and John W. Olive[2]

Abstract

A study was conducted to determine the role that plant developmental stage (DS) has on the efficacy of cyclanilide (CYC), a plant growth regulator (PGR) with cytokinin-like properties. In 2007, single foliar applications of 200 ppm CYC were applied to 'Snow White' Indian hawthorn (*Rhaphiolepis indica* (L.) Lindl. Ex Ker Gawl.) and 'Sky Pencil' holly (*Ilex crenata* Thunb.) at progressively later stages of lateral shoot development: bud break (DS 1), active shoot elongation (DS 2), or to recently matured shoots (DS 3). The experiment was repeated in 2008 and included an additional application to Sky Pencil holly before bud break (DS 0). In 2007 and 2008, Snow White Indian hawthorn treated with CYC at all DS formed more shoots than untreated plants, except in summer 2008 (DS 3 only) and fall 2008 (DS 1 only). New shoot counts of CYC-treated Indian hawthorn were 53 to 67% and 46 to 65% higher than those of untreated plants in summer and fall, respectively, in 2007 and 26 to 39% and 29 to 48% higher in summer and fall, respectively, in 2008. In the fall of 2008, the only effect of DS on shoot counts of Indian hawthorn was that plants treated at DS 3 formed 29% more new shoots than plants treated at DS 1; DS had no effect on shoot counts in 2007. Symptoms of foliar injury to Indian hawthorn included reddening, chlorosis, and cupping of new growth that appeared between 15 and 30 days after treatment (DAT) and, although not quantified in 2007, were generally less severe and more transitory as DS increased. In 2007, foliar injury lasted until about 90 to 120 DAT in DS 2 and DS 3 plants, but was more persistent in DS 1 plants. In 2008, foliar injury was also transient, regardless of DS, but was highest when CYC was applied at DS 1 or DS 2. CYC-treated Sky Pencil holly had formed more shoots than untreated plants by summer and fall of 2007. Although Sky Pencil holly treated at DS 2 in 2007 formed fewer shoots than DS 1 plants in fall, quality ratings were higher due to a larger canopy that was dense and compact. In the fall of 2008, shoot counts of CYC-treated Sky Pencil holly were greater than those of untreated plants, regardless of DS, and there were no effects of DS on shoot counts. Quality ratings of Sky Pencil holly treated at DS 2 or DS 3, but not at DS 0 and DS 1, were higher than those of untreated plants. In both years of the study, symptoms of foliar injury on Sky Pencil holly were minimal, suggesting a relatively high tolerance to foliar-applied CYC, and all plants were considered marketable.

Index words: plant growth regulator, auxin transport inhibitor, lateral shoots, nursery production.

Chemicals used in this study: cyclanilide [1-(2,4-dichlorophenylaminocarbonyl)-cyclopropane carboxylic acid] (Tiberon SC).

Species used in this study: 'Snow White' Indian hawthorn [*Rhaphiolepis indica* (L.) Lindl. Ex Ker Gawl.], 'Sky Pencil' holly (*Ilex crenata* Thunb.).

Significance to the Horticulture Industry

In an earlier study, single foliar applications of 25 to 200 ppm cyclanilide (CYC), promoted shoot development in 12 of the 19 woody landscape species tested, and foliar injury on sensitive species was transient. Previous studies have indicated that application timing or plant developmental stage plays a role in determining plant response to plant growth regulators (PGRs). In the current study, foliar applications of 200 ppm CYC were made to 'Snow White' Indian hawthorn and 'Sky Pencil' holly at three stages of new shoot development: bud break [development stage 1(DS 1)], active shoot elongation (DS 2), and to recently matured shoots (DS 3). A repeat of the experiment in 2008 included an additional application to Sky Pencil holly before bud break (DS 0). Cyclanilide promoted branching of both species, regardless of the developmental stage at application. However, persistent foliar injury to Indian hawthorn treated at bud break reduced end-of-season plant quality compared to plants treated later and to untreated plants. Sky Pencil holly was tolerant to foliar applications of CYC and the minimal foliar injury that did occur would not have affected marketability. Plants of both species treated at DS 2 and DS 3 were dense, compact, and of higher quality at the end of the growing season than untreated plants. These results indicate a difference in sensitivity of the two species to foliar-applied CYC, that applications should be avoided to Indian hawthorn at bud break, and that the highest quality in Sky Pencil holly was achieved when CYC was applied at later stages of shoot development (DS 2 and DS 3).

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[2]Graduate Student, Professor, and Professor, Department of Horticulture, Auburn University, Auburn, AL 36849, and Director, Ornamental Horticulture Research Center, Mobile, AL 36689, respectively. Corresponding author Gary J. Keever, keevegj@auburn.edu.

Introduction

Apical dominance, the suppression of lateral bud outgrowth by auxins produced in shoot apices, is broken when terminal shoots are removed (Cline 1997, Cline and Harrington 2007). This process has practical implications during the production of most woody ornamental shrubs that usually require pruning multiple times to promote the dense canopies preferred by consumers (Banko and Stefani 2007, VanderWoude 2002). However, pruning is labor intensive and the tissue removed during pruning increases production time by at least 3 weeks per pruning (Larson 1983, VanderWoude 2002). Exogenous application of synthetic cytokinins such as benzyladenine (BA) or plant growth regulators (PGRs) with cytokinin-like activity also can reduce apical dominance by counteracting auxins (Cline 1997, Cline and Harrington 2007, Oates et al. 2004, 2005a, Pedersen et al. 2006). Cyclanilide (CYC), a PGR currently marketed in combination with ethephon as the cotton (*Gossypium* spp.) defoliant Finish and more recently as Tiberon SC for use as a branching agent on nursery crops, acts as an auxin transport inhibitor, temporarily interrupting apical dominance and allowing the outgrowth of latent lateral buds (Cline 1997, Cline and Harrington 2007, Pedersen 2006). In research conducted on

apple (*Malus* spp.) and sweet cherry (*Prunus* spp.), single foliar applications of 25 to 200 ppm CYC promoted lateral shoot development on current and previous season's wood, but long-term leaf or meristem morphology was unaffected (Elfving and Visser 2005, 2006a, 2006b).

The efficacy of the cytokinin benzyladenine (BA) was affected by numerous factors, including species (Keever and Foster 1990), plant stage of development (Oates et al. 2005b, Ohkawa 1979), season of application (Nauer and Boswell 1981), application rate and number (Keever and Foster 1990, Little 1985, Oates et al. 2004), and application interval (Oates et al. 2005a). While the prospect of promoting lateral shoot development without affecting long term morphology makes CYC an ideal candidate for application to ornamental species, much less is known about factors affecting efficacy of CYC and plant injury. Sternberg and Struve (2007) reported a rate-dependent increase in branching of container-grown tree whips of *Acer* ×*freemanii* 'Jeffsred', *Cercis canadensis* L., *Diospyros virginiana* L., *Malus* ×'Prairie Fire', *Malus* ×'Harvest Gold', and *Quercus rubra* L. following CYC application. In other work, single foliar applications of 25 to 200 ppm CYC, applied in spring while plants were actively growing, promoted terminal and lateral shoot development of 12 of 19 woody ornamental species tested (Holland et al. 2007a). Quality of treated plants was generally higher than that of untreated plants; however, single applications of 25 to 200 ppm CYC caused transient foliar injury that decreased plant quality of Olivia™ and Eleanor Taber™ Indian hawthorns. In a later study, 3 weekly or biweekly applications of 100 to 300 ppm CYC promoted greater shoot formation of Sky Pencil (*Ilex crenata*) and 'Foster' (*Ilex* ×*attenuata* Ashe) hollies and Olivia™ and Eleanor Taber™ Indian hawthorns than single applications (Holland et al. 2007b). Foliar injury occurred on all species tested; however, symptoms were transient and plant quality of CYC-treated plants was generally greater than that of untreated plants. Symptoms of foliar injury were generally minor in tolerant species like holly, but more severe in sensitive species like Indian hawthorn, which concurs with results from other studies (Holland et al. 2007a, Williamson et al. 2009a, 2009b).

Foliar applications were made during the growing season for most of the published CYC research with responsive species; however, the application timing and closely related plant stage of development often varied from study to study (Banko and Stefani 2007, Holland et al. 2007a, 2007b, 2008, Williamson et al. 2009a). Numerous studies using BA have indicated that application timing, plant stage of development or both play a role in PGR efficacy and sensitivity of a given species to foliar injury. Balsam fir [*Abies balsamea* (L.) Mill.] Christmas trees formed the most lateral shoots with the least injury when a single BA application was made towards the end of the bud elongation period. Foliar injury increased if the application was made prior to or after this physiological event and included shoot discoloration and needle chlorosis (Little 1985). Applying BA soon after the third leaf of African evergreen (*Syngonium podophyllum* Schott.) had fully expanded was more effective in promoting lateral shoot growth than applying BA at either the first or fifth leaf stage and was ineffective if basal branching had naturally begun prior to application (Wang and Boogher 1987). Indian hawthorn developed more new shoots and foliar injury following spring applications than after summer applications (Oates et al. 2004). When BA was applied to immature shoots of inkberry (*Ilex nigra* L.) holly and Indian hawthorn, foliar injury was more severe than when applied to plants at a more advanced stage of development (Oates et al. 2005b). In a study that evaluated the interactive effects of CYC and pruning, applications were made as lateral shoot length increased, either: immediately following pruning [developmental stage 1 (DS 1)], when shoots were 1.3–2.6 cm (0.5–1.0 in) in length (DS 2), or when shoots were 2.6–5.1 cm (1.0–2.0 in) in length (DS 3). At 60 DAT, the highest number of shoots were on unpruned Snow White Indian hawthorn and Sky Pencil holly when treated at DS 2 and DS 3, respectively; however, more injury was usually associated with the most effective treatment for promoting shoot formation. Fewer new shoots formed on plants pruned before CYC application than on those not pruned, possibly due to less leaf surface area to absorb CYC, but quality of pruned plants was usually higher (Holland et al. 2008). Effects of stage of plant development on CYC efficacy in non-pruned plants are unknown. Therefore, the objective of this study was to determine how stage of development affects plants' response to CYC. Our overall goal was to maximize new shoot development and plant quality, while minimizing or avoiding foliar injury in two species that differ in sensitivity to CYC.

Materials and Methods

2007. Uniform liners of Snow White Indian hawthorn in 32-cell flats and Sky Pencil holly in 50-cell flats were repotted into 0.95 liter (qt) pots on November 27, 2006, and January 23, 2007, respectively. Growth medium was a 7:1 pinebark:sand mix amended per m^3 (yd^3) with 9.5 kg (16 lb) 17N–2.6P–10K (PolyOn 17–6–12, Pursell Industries, Sylacauga, AL), 0.9 kg (1.5 lb) Micromax (The Scotts Company, Marysville, OH) and 3 kg (5 lb) dolomitic limestone. The liners were grown in a glass greenhouse located in Auburn, AL (USDA cold hardiness zone 8a; 32.6° N, 85.5° W) with a heat set point of 18 C (65 F) and a ventilation set point of 25 C (78 F). On March 30, 2007, plants were spaced outdoors on an unshaded nursery pad, with overhead irrigation applied twice daily, totaling about 2.5 cm (1.0 in). Plants were covered with perforated white polyethylene sheets when low temperatures were forecasted, which occurred on April 7, 8, and 9, when minimum temperatures were 0.0, 1.2, and 2.7 C (32, 34, and 37 F), respectively.

On April 6, May 1, and June 8, plants of each species were treated with single foliar applications of 200 ppm CYC at three stages of new shoot development during either: bud break [development stage 1(DS 1)], active shoot elongation (DS 2), or to recently matured shoots (DS 3), respectively. When treated at DS 1, DS 2, and DS 3 shoot lengths were approximately 1.0–3.0 cm (0.4–1.2 in), 3.0–5.0 cm (1.2–2.0 in), or 5.0–7.0 cm (2.0–2.8 in), respectively. When treated at DS 1, DS 2, and DS 3, plant height of Snow White Indian hawthorn averaged 10.6, 12.7, and 16.4 cm (4.2, 5.0, and 6.5 in), respectively, and plant height of Sky Pencil holly averaged 9.4, 12.9, and 31 cm (3.7, 5.1, and 12.2 in), respectively. The CYC solutions included a nonionic surfactant, Buffer-X (Kalo Agr. Chemicals, Overland, KS), at 0.2% by volume, and were applied using a CO_2 sprayer with a flat-spray nozzle (XR TeeJet 8003VK, TeeJet Technologies, Wheaton, IL) at 138 kPa (20 psi) in 0.2 liter·m^{-2} (equivalent to 2 qt·100 ft^{-2}). Treatments were applied in the shade to minimize phytotoxicity and maximize absorption. After a minimum of 6 hours, plants were returned to the nursery pad. An untreated

treatment was included for comparison. Dry and wet-bulb temperatures were recorded at the application times and relative humidity was determined. Temperature and relative humidity at the time of applications were 17 C (62 F) and 42%, 21 C (70 F) and 60%, and 26 C (78 F) and 75% on April 6, May 1, and June 8, respectively. Treatments were arranged within species in a completely randomized design and replicated with ten (Snow White Indian hawthorn) or eight (Sky Pencil holly) single plants.

Initial growth data were collected on plants in a given CYC treatment and on untreated plants prior to CYC application on April 6, May 1, and June 8, and included: counts of new lateral and terminal shoots [≥ 1 cm (0.4 in) in length]; and plant height and widths [mean width = (widest width + width 90° to widest width) ÷ 2].

Following CYC application, shoot counts, plant height and widths were taken over a period of time, but only once in summer and once in fall on a given plant, due to differences in timing of new shoot development that varied among and within treatments. Growth data were collected in summer (July 10–August 7) and fall (October 3–October 30). End-of-season plant quality was rated on November 4, approximately 210, 180, and 150 DAT after application at DS 1, DS 2, and DS 3, respectively. Quality was rated on a scale of 1 to 4 (1 = minimal branching, open, leggy, and unmarketable (due to foliar injury) to 4 = prolific branching, dense, compact, and highly marketable and was assessed by the same person on the same day.

2008. The experiment was repeated in 2008 using similar methodology unless otherwise noted. In March of 2008, uniform plants of Snow White Indian hawthorn in 3.8 liter (#1) containers were obtained from Moore and Davis Nursery in Shorter, AL. Sky Pencil holly in 3.8 liter (#1) containers, grown in the previously described substrate, were overwintered outdoors in full sun, and covered with perforated white polyethylene sheets when freezing temperatures were forecasted. On June 17, 2008, plants of both species were repotted into 7.2 liter (#3) containers and treated with pendimethalin (Pendulum 2G, BASF Corp., Research Triangle Park, NC), a preemergence herbicide, at 2.2 kg ai·ha^{-1} (2 lb ai·A^{-1}).

Initial plant height, widths and shoot counts were collected and foliar sprays applied using the same protocol previously described. CYC applications were made to Snow White Indian hawthorn on March 13 (DS 1), April 7 (DS 2), and May 6 (DS 3) when plant height averaged 17.1, 22.0, and 23.1 cm (6.7, 8.7, and 9.1 in), respectively. In 2008, an additional treatment was included with Sky Pencil holly; CYC was applied before bud break (DS 0). Applications were made on March 13 (DS 0), April 7 (DS 1), May 6 (DS 2), and June 11 (DS 3) when plant height of Sky Pencil holly averaged 57.8, 62.5, 63.4, and 65.9 cm (22.8, 24.6, 25.0, and 25.9 in), respectively. Temperature and relative humidity at the times of application at DS 1, DS 2, and DS 3 were 16 C (70 F) and 56%, 18 C (72 F) and 65%, 17 C (81 F), and 33% and 22 C (78 F) and 75%, respectively.

Growth data were collected over approximately a one-month period in summer (July 7–August 4) and fall (September 22–October 20), but only once in each period on a given plant. Summer plant injury was rated on August 4, approximately 150, 120, and 90 DAT of Indian hawthorn at DS 1, DS 2, and DS 3, respectively, and 150, 120, 90, and 60 DAT of Sky Pencil holly at DS 0, DS 1, DS 2, and DS 3, respectively. Injury was rated on a scale of 1 to 5 and the scale varied with species. The scale for Snow White Indian hawthorn was 1 = no injury; 2 = green leaves, mild chlorosis and/or reddening of new growth; 3 = green leaves, mild chlorosis and/or reddening and twisting or curling of new leaves; 4 = necrotic leaves with deformed foliage; and 5 = plant death. The scale for Sky Pencil holly was 1 = no injury; 2 = green leaves, mild chlorosis on old growth and slightly pointed leaves; 3 = green leaves, mild chlorosis, smaller and more pointed leaves; 4 = necrotic leaves with deformed foliage; and 5 = plant death. Fall plant injury and quality were rated on October 20, approximately 210, 180, and 150 DAT of Indian hawthorn at DS 1, DS 2, and DS 3, respectively, and 210, 180, 150, and 120 DAT of Sky Pencil holly at DS 0, DS 1, DS 2, and DS 3, respectively. Plant quality of both species was rated on a scale of 1 to 5 (1 = minimal branching, open and leggy to 5 = prolific branching, dense and compact). Injury was not considered in the quality rating unless it was so severe that it led to other changes in the appearance of the plants, such as severe stunting in 2007, but not in 2008.

An analysis of variance was performed on all responses using PROC GLIMMIX in SAS version 9.3 (SAS Institute, Cary, NC). Initial data were used in analysis of covariance when including the covariate improved the model outcome. Injury and quality ratings were analyzed using the multinomial probability distribution. Where residual plots and a significant COVTEST statement using the HOMOGENEITY option indicated heterogeneous variance, a RANDOM statement with the GROUP option was used to correct heterogeneity. Differences among treatments were determined using ESTIMATE statements for all responses. Data presented are medians for injury and quality ratings and least squared means for all other responses. All significant differences were calculated with $\alpha = 0.05$.

Results and Discussion

Snow White Indian hawthorn. In 2007, CYC promoted shoot initiation on Indian hawthorn (Table 1); however, foliar injury, including chlorosis, reddening, and slight cupping on new growth, was evident as early as 15 to 30 DAT. Injury adversely affected the plants' appearance and would have reduced marketability if the plants were of a marketable size when treated. Symptoms, although not quantified, were less severe and more transient as DS increased and were transitory, lasting until about 90 to 120 DAT on plants treated at DS 2 and DS 3. Similar foliar injury following CYC application to Indian hawthorn was previously reported (Holland et al. 2007a, 2007b, 2008, Williamson et al. 2009a). In summer growth measurements, Indian hawthorn treated at DS 1, DS 2, and DS 3 had formed 70% (90 to 120 DAT), 55% (60 to 90 DAT), and 72% (30 to 60 DAT), respectively, more new shoots than untreated plants and 44% (180 to 210 DAT), 64% (150 to 180 DAT), and 57% (120 to 150 DAT) more than untreated plants in fall (Table 1). DS did not impact shoot formation in summer or fall which contrasts with results from previous studies that evaluated the effect of DS on the response of several species to BA application (Oates et al. 2005b) and the response of Snow White Indian hawthorn and Sky Pencil holly to CYC application following pruning (Holland et al. 2008). In summer, height and width values of plants treated at DS 2 or DS 3 were similar to or higher than those of untreated plants, but plants treated at DS 1 were shorter and narrower than plants in all other treatments

Table 1. Effects of stage of development and cyclanilide on plant growth and quality of container-grown 'Snow White' Indian hawthorn, 2007.

Development stage[z]	Shoot counts[y]		Plant height (cm)		Plant width[x] (cm)		Quality rating[w]
	Summer[v]	Fall[u]	Summer	Fall	Summer	Fall	Fall
Untreated	15	26	19.3	24.7	29.4	39.7	2
DS 1	25	38	14.3	17.8	24.3	35.0	1
DS 2	23	43	19.7	22.4	33.1	44.5	4
DS 3	25	41	18.2	24.8	29.4	42.7	4
Untreated vs. DS 1	***[t]	***	***	***	**	**	*
Untreated vs. DS 2	**	***	ns	ns	*	**	***
Untreated vs. DS 3	***	***	ns	ns	ns	*	***
DS 1 vs. DS 2	ns	ns	***	***	***	***	***
DS 1 vs. DS 3	ns	ns	**	***	**	***	***
DS 2 vs. DS 3	ns	ns	ns	ns	ns	ns	ns

[z]Single foliar sprays of 200 ppm cyclanilide were applied at bud break (DS 1), at the beginning of active shoot elongation (DS 2), or to recently matured shoots (DS 3).

[y]Total number of actively growing terminal and lateral shoots.

[x]Average plant width = (widest width + width perpendicular to widest width) ÷ 2.

[w]Median quality rating: 1 = minimal branching, open and leggy to 4 = prolific branching, dense and compact.

[v]Summer data were collected between July 10 and August 7 at 90 to 120 days after treatment (DAT), 60 to 90 DAT, and 30 to 60 DAT of plants treated at DS 1, DS 2, and DS 3, respectively.

[u]Fall data were collected between October 3 and October 30 at 180 to 210 DAT, 150 to 180 DAT, and 120 to 150 DAT of plants treated at DS 1, DS 2, and DS 3, respectively.

[t]Treatment comparisons using estimate statements at α = 0.05 (*), 0.01 (**) or 0.001 (***), ns = not significant.

(Table 1). Similarly, in fall height and width were suppressed in only DS 1 plants. However, Snow White Indian hawthorn treated at DS2 or DS 3 were wider than untreated plants in fall, a response previously reported following CYC application (Holland et al. 2007a, 2007b, 2008) and speculated to result from the formation of numerous lateral shoots. End of season median quality ratings of plants treated at DS 2 and DS 3 were higher than those of untreated plants due to the formation of a wider canopy that appeared denser and more compact (Table 1). Plants treated at DS 1 were visibly lower in quality than plants in all other treatments due to the persistence of foliar injury and stunting at 210 DAT. In the spring of 2008, about 270 DAT, 30% of plants treated at DS 1 were dead and foliage of the remaining plants was misshapen, discolored, and exhibited symptoms of leaf spot caused by the fungus *Entomosporium maculatum*. No plants in any other treatment died over winter or were infected with fungal leaf spot.

In 2008, Snow White Indian hawthorn treated with a single application of 200 ppm CYC at DS 1 and DS 2 formed 39% (120 to 150 DAT) and 26% (90 to 120 DAT), respectively, more shoots than untreated plants by summer and 15% (180 to 210 DAT) and 29% (150 to 180 DAT) more shoots by fall (Table 2). In summer, DS 3-treated plants

Table 2. Effects of stage of development and cyclanilide on plant growth and quality of container-grown 'Snow White' Indian hawthorn, 2008.

Development stage[z]	Shoot counts[y]		Plant height (cm)		Injury rating[x]	Quality rating[w]
	Summer[v]	Fall[u]	Summer	Fall	Summer	Fall
Untreated	23	48	25.7	32.6	1	4.0
DS 1	32	55	23.9	36.8	3	4.0
DS 2	29	62	23.3	29.9	3	4.5
DS 3	27	71	23.6	28.7	2	5.0
Untreated vs. DS 1	**[t]	ns	*	*	***	ns
Untreated vs. DS 2	*	*	*	ns	***	*
Untreated vs. DS 3	ns	***	*	ns	***	**
DS 1 vs. DS 2	ns	ns	ns	*	ns	ns
DS 1 vs. DS 3	ns	**	ns	**	**	*
DS 2 vs. DS 3	ns	ns	ns	ns	*	ns

[z]Single foliar sprays of 200 ppm cyclanilide were applied at bud break (DS 1), at the beginning of active shoot elongation (DS 2), or to recently matured shoots (DS 3).

[y]Total number of actively growing terminal and lateral shoots.

[x]Median injury rating: 1 = no injury; 2 = green leaves, mild chlorosis and/or reddening of new growth; 3 = green leaves, mild chlorosis and/or reddening and twisting or curling of new leaves; 4 = necrotic leaves with deformed foliage; and 5 = plant death.

[w]Median quality rating: 1 = minimal branching, open and leggy to 4 = prolific branching, dense and compact.

[v]Summer data were collected between July 7 and August 4 at 120 to 150 days after treatment (DAT), 90 to 120 DAT, and 60 to 90 DAT of plants treated at DS 1, DS 2, and DS 3, respectively.

[u]Fall data were collected between September 22 and October 20, at 180 to 210 DAT, 150 to 180 DAT, or 120 to 150 DAT of plants treated at DS 1, DS 2, and DS 3, respectively.

[t]Treatment comparisons using estimate statements at α = 0.05 (*), 0.01 (**) or 0.001 (***), ns = not significant.

had formed a similar number of new shoot as untreated plants. In fall, DS 2 plants formed 29% more new shoots than untreated plants, while DS 3 plants formed 48% more new shoots than untreated plants and 29% more than DS 1 plants, which formed a similar number as untreated plants. Similarly, Holland et al. (2008) reported that CYC-treated Snow White Indian hawthorn formed more new shoots when CYC was applied to progressively longer shoots following pruning. All CYC-treated plants were shorter than untreated plants in the summer; however, in the fall, DS 1 plants were taller than plants in other treatments (Table 2). Plant width of CYC-treated Indian hawthorn was not affected by CYC application in the summer or fall (data not shown). Injury ratings of CYC-treated Indian hawthorn were higher than those of untreated plants in the summer, which concurs with results from a previous study that evaluated the effects of pruning and a 200 ppm CYC application on Indian hawthorn (Holland et al. 2008), although ratings were higher for plants treated at DS 1 or DS 2 than for those treated at DS 3. Injury was evident approximately 30 DAT and included minor reddening, chlorosis, and cupping or keeling of new foliage. These results are similar to those from the previous year when injury appeared to decrease as DS increased. Injury was transitory, lasting until about 120 to 150 DAT, and end-of-season median injury ratings were similar, regardless of CYC application or DS (data not shown). In contrast to the previous year, injury was transient in DS 1 plants, possibly because plants and leaf surface area were larger in 2008. Holland et al. (2007b, 2008) also noted transitory injury following CYC applications to Indian hawthorn. When compared to those of untreated plants, end-of-season median quality ratings of Indian hawthorn treated at DS 2 and DS 3 were higher, whereas ratings of plants treated at DS 1 were similar (Table 2). These results agree with those of previous studies and indicate Indian hawthorn readily forms new shoots in response to CYC application (Banko and Stefani 2007, Holland et al. 2007a, 2007b, 2008, Williamson et al. 2009a, 2009b). Generally, shoot counts and quality ratings were highest and foliar injury less severe and more transient when Snow White Indian hawthorn was treated with CYC at DS 2 or DS 3, a response seen in Olivia™ Indian hawthorn treated at different developmental stages with BA (Oates et al. 2005b). In 2007, application to Snow White Indian hawthorn at DS 1 decreased plant height and width and quality ratings, when compared to those of DS 2 and DS 3 plants and may have contributed to the loss of 30% of plants in the spring of 2008. Injury was less severe in 2008, possibly due to the use of larger plants with a larger surface area to absorb CYC and the absence of freezing temperature following CYC application.

Sky Pencil holly. In 2007, mild injury to new shoots of Sky Pencil holly that included chlorosis and slightly more pointed and smaller leaves was evident approximately 30 DAT. Symptoms, although not quantified, appeared to decrease at later developmental stages but were minor and would not have affected marketability of plants in any treatment. Sky Pencil holly treated at DS 1, DS 2, and DS 3 in 2007 had formed 52% (90 to 120 DAT), 33% (60 to 90 DAT), and 43% (30 to 60 DAT), respectively, more shoots than untreated plants by summer and 93% (180 to 210 DAT), 37% (150 to 180 DAT), and 74% (120 to 150 DAT) more shoots than untreated plants by fall (Table 3). In summer, DS 1 plants had formed 14% more new shoots than DS 2 plants, but there were no other differences in shoot counts among plants treated with CYC. In fall, DS 1 and DS 3 plants had formed 41 and 27% more shoots, respectively, than DS 2 plants. These results contrast with those of a previous CYC study that evaluated the effects of pruning and a 200 ppm CYC spray on Sky Pencil holly in which shoot counts increased linearly with increasing DS. In summer and fall, untreated plants were taller than plants treated at DS 1; whereas in fall, DS 2 and DS 3 plants were similar in height and shorter than untreated plants, respectively (Table 3). Effects of CYC on plant width

Table 3. Effects of stage of development and cyclanilide on plant growth and quality of container-grown 'Sky Pencil' holly, 2007.

Development stage[z]	Shoot counts[y]		Plant height (cm)		Plant width[x] (cm)		Quality rating[w]
	Summer[v]	Fall[u]	Summer	Fall	Summer	Fall	Fall
Untreated	21	27	37.4	62.4	9.9	14.1	2.0
DS 1	32	52	23.9	52.6	10.1	15.2	3.0
DS 2	28	37	43.3	61.0	10.9	14.6	4.0
DS 3	30	47	38.3	51.9	9.9	13.1	3.5
Untreated vs. DS 1	***[t]	***	***	**	ns	ns	*
Untreated vs. DS 2	***	**	*	ns	*	ns	**
Untreated vs. DS 3	***	***	ns	**	ns	ns	*
DS 1 vs. DS 2	*	***	***	**	ns	ns	*
DS 1 vs. DS 3	ns	ns	***	ns	ns	**	ns
DS 2 vs. DS 3	ns	**	*	**	*	*	ns

[z]Single foliar sprays of 200 ppm cyclanilide were applied at bud break (DS 1), at the beginning of active shoot elongation (DS 2), or to recently matured shoots (DS 3).

[y]Total number of actively growing terminal and lateral shoots.

[x]Average plant width = (widest width + width perpendicular to widest width) ÷ 2.

[w]Median quality rating: 1 = minimal branching, open and leggy to 4 = prolific branching, dense and compact.

[v]Summer data were collected between July 10 and August 7 at 90 to 120 days after treatment (DAT), 60 to 90 DAT, and 30 to 60 DAT of plants treated at DS 1, DS 2, and DS 3, respectively.

[u]Fall data were collected between October 3 and October 30 at 180 to 210 DAT, 150 to 180 DAT, and 120 to 150 DAT of plants treated at DS 1, DS 2, and DS 3, respectively.

[t]Treatment comparisons using estimate statements at $\alpha = 0.05$ (*), 0.01 (**) or 0.001 (***), ns = not significant.

were minimal. End of season median plant quality ratings of CYC-treated Sky Pencil holly were higher than those of untreated plants (Table 3), and treated plants were generally fuller and more compact. Quality ratings of plants treated at DS 2 were higher than those of plants treated at DS 1, while ratings for plants treated at DS 2 and DS 3 were similar.

In 2008, Sky Pencil holly treated with 200 ppm CYC at DS 0, DS 1, and DS 2 but not DS 3, formed 24% (120 to 150 DAT), 41% (90 to 120 DAT), and 35% (60 to 90 DAT), respectively, more shoots than untreated plants in summer (Table 4). Data collection occurred at 30 to 60 DAT for DS 3 plants, which may have been too short of a period for plants to fully respond to CYC. In fall, plants treated at DS 0, DS 1, DS 2, and DS 3 had formed similar numbers of new shoots and 65% (180 to 210 DAT), 71% (150 to 180 DAT), 64% (120 to 150 DAT), and 65% (90 to 120 DAT), respectively, more shoots than untreated plants. Median injury ratings in summer were higher in plants treated at DS 0, DS 1 and DS 2 than for untreated plants, while injury ratings for untreated plants and those treated at DS 3 were similar, possibly because DS 3 plants did not have sufficient time to react to CYC. Symptoms of injury, first evident about 30 DAT, included mild chlorosis on old growth and a slight elongation of developing leaves. Injury was transitory, lasting until about 90 to 120 DAT, and end of season median injury ratings were similar, regardless of CYC application or DS (data not shown). In summer height was not affected by CYC application or DS (data not shown); however, plants treated at DS 2 or DS 3 were shorter than plants in all other treatments in fall. In summer and fall, average width of CYC-treated plants was greater than that of untreated plants, which concurs with results from previous studies (Holland et al. 2008, Williamson 2009a, 2009b), but contrasts with 2007 results (Table 3). When compared to untreated ones, plants treated with CYC at DS 2 or DS 3 were shorter in the fall; however, width of CYC-treated plants was greater, which is considered desirable by nurserymen and consumers in a cultivar with a naturally narrow, upright columnar growth habit and could reduce the number of liners per pot needed to produce marketable plants. End of season median quality ratings were higher for plants treated at DS 2 and DS 3, but similar for untreated plants and plants treated at DS 0 and DS 1, which may reflect the shorter time between CYC applications and the end of the season and the transitory nature of plants' response to single applications of CYC (Holland et al. 2007a).

In 2007 and 2008, Sky Pencil holly treated with 200 ppm CYC formed more new shoots than untreated plants, except in summer of 2008 when treated at DS 0 and DS 3. In 2007 and 2008, the only visible abnormal effects of CYC treatment in Sky Pencil holly were slightly elongated leaves and mild chlorosis; however, the change in leaf shape did not detract from plant quality and the chlorosis was transitory. Treated plants were denser and more compact than untreated plants. In 2008, Sky Pencil holly treated with CYC were wider than untreated plants in summer and fall which would be beneficial during production where multiple liners are usually required per pot to produce plants of sufficient width to be marketable. Similarly, in previous studies an increase in plant width gave the appearance of a denser canopy that was higher in quality (Elfving and Visser 2006b, Holland et al. 2007a, 2007b, Williamson et al. 2009a, 2009b). In 2007 and 2008, end of season plant quality ratings were highest

Table 4. Effects of stage of development and cyclanilide on plant growth and quality of 'Sky Pencil' holly, 2008.

Development stage[z]	Shoot counts[y]		Plant height (cm)	Plant width[x] (cm)		Injury rating[w]	Quality rating[v]
	Summer[u]	Fall[t]	Fall	Summer	Fall	Summer	Fall
Untreated	136	55	115.6	14.1	17.2	1.0	4.0
DS 0	169	91	113.9	15.8	18.8	2.0	4.5
DS 1	192	94	114.9	17.7	19.3	1.5	4.5
DS 2	183	90	104.0	17.7	19.4	2.0	5.0
DS 3	144	91	95.4	17.8	20.4	1.0	5.0
Untreated vs. DS 0	*[s]	**	ns	**	*	***	ns
Untreated vs. DS 1	**	**	ns	***	**	**	ns
Untreated vs. DS 2	**	**	*	***	**	***	**
Untreated vs. DS 3	ns	**	***	***	***	ns	***
DS 0 vs. DS 1	ns	ns	ns	**	ns	ns	ns
DS 0 vs. DS 2	ns	ns	*	**	ns	ns	ns
DS 0 vs. DS 3	ns	ns	***	***	*	*	*
DS 1 vs. DS 2	ns	ns	*	ns	ns	ns	ns
DS 1 vs. DS 3	**	ns	***	ns	ns	ns	*
DS 2 vs. DS 3	*	ns	ns	ns	ns	**	ns

[z]Single foliar sprays of 200 ppm cyclanilide were applied before bud break (DS 0), at bud break (DS 1), at the beginning of active shoot elongation (DS 2), or to recently matured shoots (DS 3).

[y]Total number of actively growing terminal and lateral shoots.

[x]Average plant width = (widest width + width perpendicular to widest width) ÷ 2.

[w]Median injury rating: 1 = no injury; 2 = green leaves, mild chlorosis on old growth and slightly pointed leaves; 3 = green leaves, mild chlorosis, smaller and more pointed leaves; 4 = necrotic leaves with deformed foliage; and 5 = plant death.

[v]Median quality rating: 1 = minimal branching, open and leggy to 4 = prolific branching, dense and compact.

[u]Summer data were collected between July 7 and August 4 at 120 to 150 days after treatment (DAT), 90 to 120 DAT, and 60 to 90 DAT of plants treated at DS 1, DS 2, and DS 3, respectively.

[t]Fall data were collected between September 22 and October 20, at 180 to 210 DAT, 150 to 180 DAT, or 120 to 150 DAT of plants treated at DS 1, DS 2, and DS 3, respectively.

[s]Treatment comparisons using estimate statements at $\alpha = 0.05$ (*), 0.01 (**) or 0.001 (***), ns = not significant.

in plants treated at DS 2 and DS 3, when compared to those of untreated plants and DS 0 (only included in 2008) and DS 1 plants.

The results of this research give further evidence that foliar applications of CYC promote lateral shoot development of woody ornamentals. Plant quality ratings of CYC-treated plants were usually higher than those of untreated plants, due to an increase in plant density. Year-to-year inconsistencies in plant response to CYC may have been due to differences in initial plant size [liners in 2007 and plants in 3.8 liter (#1) containers in 2008]. Similarly, results from previous studies indicate that plant response varied when PGR applications were made to plants that differed in size (Banko and Stefani 2007, Holland et al. 2007a). Also the severity of injury to Indian hawthorn in 2007 may have been at least partially due to environmental factors. In the month of March, temperatures were elevated and were followed by record breaking low temperatures on April 7 and 8 of that year (AWIS Weather Services 2007, Wolfe and Angel 2008). The severity of the freeze in combination with applying CYC to DS 1 plants 1 to 3 days prior to the freeze may have contributed to injury that was severe enough to result in 30% plant death, even though all plants were covered during the period of freezing temperatures. Cyclanilide has the potential to substitute for or reduce mechanical pruning in the production of at least some woody ornamental shrubs. However, application timing based on physiological parameters (height, shoot length or stage of development) will affect shoot development and potential injury.

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