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BA-Induced Shoot Formation in Indian Hawthorn[1]

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Abstract

A study was conducted to determine effects of multiple weekly applications of different concentrations of a synthetic cytokinin, benzyladenine (BA), on two Indian hawthorn [*Rhaphiolepis* Lindl. Cor. Poir. *indica* (L.) Lindl. (*Crataegus indica* L.)] cultivars, 'Olivia' and 'Eleanor Taber'. In a greenhouse in winter 2000–2001, 'Olivia' Indian hawthorn received four weekly applications of 2500 ppm or 5000 ppm BA. At 45 days after last treatment (DALT) untreated plants had formed no new shoots, while plants treated with 2500 and 5000 ppm BA produced 6.8 and 5.4 new shoots, respectively. New leaves of plants treated with 5000 ppm BA were abnormally mottled, twisted, and cupped. In an outdoor nursery in summer 2001, 'Olivia' in 3.8 liter (#1) and 26.5 liter (#7) containers received three and two applications, respectively, at concentrations from 0 to 5000 ppm. Untreated plants in 26.5 liter (#7) containers formed 4.7 new shoots at 30 days after initial treatment (DAIT), and new shoots increased from 83 with 1250 ppm BA to 126 with 5000 ppm BA. Plants in 26.5 liter (#7) containers were retreated in September, at which time shoots were inactive. At 30 DAIT controls averaged less than one new shoot per plant, while plants averaged 18 new shoots when treated with 1250 ppm BA and 105 new shoots when treated with 5000 ppm BA. In spring 2002, plants in 3.8 liter (#1) containers were retreated following the same protocol as in 2001, in conjunction with a new set of 'Olivia' and the Indian hawthorn cultivar, 'Eleanor Taber'. At 30 DALT new shoot formation increased three fold in 'Olivia' and five fold in 'Eleanor Taber' after three applications of up to 5000 ppm BA. Injury increased with increasing BA rates in 'Olivia' at 30 DALT, with leaf cupping and discoloration apparent on plants treated with concentrations above 1250 ppm. No injury was observed in 'Eleanor Taber'. By 90 DALT new shoots had matured normally and exhibited minimal phytotoxic symptoms.

Index words: lateral branching, cytokinin, plant growth regulator, chemical pruning.

Species used in this study: 'Olivia' and 'Eleanor Taber' Indian hawthorn [*Rhaphiolepis* Lindl. Cor. Poir. *indica* (L.) Lindl. (*Crataegus indica* L.)].

Chemicals used in this study: N^6-benzylaminopurine (benzyladenine, BA, BAP-10).

Significance to the Nursery Industry

Indian hawthorn cultivars typically require multiple prunings during production for the development of well-branched, compact plants. With three weekly applications of 1250 to 2500 ppm benzyladenine (BA), lateral bud break was stimulated, requiring little or no mechanical pruning to produce well-branched marketable plants, and foliar injury was minimal. BAP-10 (BA) currently remains unlabelled for use on Indian hawthorn, but projected application cost of 2500 and 5000 ppm solutions is about 2.2 and 4.4¢ per 3.8 liter (#1) container, assuming four pots per square foot. Abnormal leaf curling and discoloration generally occurred when BA was applied at higher concentrations, particularly when applied to immature foliage. The symptoms were transitory, but severe enough to warrant avoiding concentrations above 2500 ppm BA on new growth.

Introduction

Indian hawthorns (*Rhaphiolepis indica*) are dense, mound forming, evergreen shrubs that grow 1 to 2 m (3–6 ft) tall and form dark glossy green leaves borne in terminal clusters. White to pink fragrant flowers are borne in dense, upright tomentose racemes or panicles from mid-April to early May. Hardy in USDA Cold Hardiness Zones 7b to 10, Indian hawthorns are widely utilized for textural effect in containers, groupings, or mass plantings (1).

Indian hawthorn displays little natural branching during commercial nursery production, and without pruning, plants are sparsely branched, poorly shaped, and unmarketable. Plants in 3.8 liter (#1) containers require at least one pruning during the 18 to 20 months from liners to a marketable stage, while those in 11.4 liter (#3) containers require two additional prunings and an additional 9 to 12 months of production time (Tom Dodd Nurseries, Semmes, AL, pers. comm). A minimum of three weeks of active growing time is lost with each pruning (9). Vegetative growth in Indian hawthorn is most pronounced in early spring, prior to flowering and subsequent fruit development, and is greatly reduced thereafter. Pruning to remove fruit and stimulate new growth is labor intensive and time consuming, but considered necessary to produce marketable plants of Indian hawthorn efficiently.

Cytokinins are plant growth regulators that function in overcoming apical dominance and promoting lateral shoot initiation and cell division (2, 6). Exogenous application of cytokinins, including benzyladenine (BA), promotes axillary bud growth and branching in woody landscape plants (3, 5, 7, 10). Keever and Foster (3) found that response to BA was species dependent. A single application of up to 2500 ppm BA had minimal effect on shoot formation in both Indian hawthorn and nandina, but two applications over the course of three months increased shoot formation in 'Harbour Dwarf' nandina grown in a greenhouse under night-break lighting. Furthermore, five applications of 2500 ppm and 5000 ppm BA applied weekly to 'Harbour Dwarf' nandina were much more effective in promoting shoot formation than a single application (4).

The objective of our study was to investigate the effects of BA concentration when applied multiple times on growth and development of Indian hawthorn.

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Materials and Methods

Greenhouse screening. A preliminary trial using multiple applications of BA (BAP-10, Plant-Wise Biostimulant Co., Louisville, KY) on 'Olivia' Indian hawthorn (*Rhaphiolepis indica*) was conducted during the winter of 2000–2001. In fall 2000, unbranched liners in 10 cm (4 in) pots were repotted into 3.8 liter (#1) containers of a pine bark:sand (7:1 by vol) medium amended per cubic meter with 3 kg (5 lb/yd^3) dolomitic limestone, 0.9 kg (1.5 lb/yd^3) Micromax (The Scotts Co., Marysville, OH), and 7.2 kg (12 lb/yd^3) Osmocote 17N–3.1P–10K (17–7–12, The Scotts Co.). Plants were placed in an unshaded polyethylene greenhouse with ventilation and heat set points of 26.5C (78F) and 21C (70F) under incandescent night-break lighting from 10:00 pm–2:00 am, and were watered when the media appeared dry. Plants with terminal buds and no immature foliage at the initiation of treatments received four weekly applications of 0, 2500, or 5000 ppm BA at 0.2 liters/m^2 (2 qt/100 ft^2) on February 23, March 2, March 9, and March 16, 2001. Treatments were completely randomized and replicated using five single plants. Foliar sprays included 0.2% (v/v) Buffer X (Kalo Agr. Chemicals, Overland, KS), a nonionic surfactant, and were applied with a compressed air sprayer equipped with a flat spray nozzle (Tee Jet 8001VS, Bellspray, Inc., Opeloussa, LA) at 138 kPa/cm^2 (20 psi). Treatments were halted prior to the fifth scheduled application due to the development of marginal necrosis and abnormal leaf twisting in plants treated with 5000 ppm BA. Temperature and relative humidity at each time of application were 31C (88F) and 61%. At 45 days after last treatment (DALT), the total number of new shoots longer than 2.5 cm (1 in) with leaves unfurling, and the lengths of the three longest shoots per plant were quantified. Based on positive results with the range of concentrations used in the greenhouse, additional tests were conducted outdoors beginning in spring 2001. In all tests, BA applications were halted at the first signs of phytotoxicity in any treatment.

Multiple BA applications, 2001. Uniform plants of 'Olivia' in 10 cm (4 in) and 3.8 liter (#1) pots were transplanted on April 12, 2001, into 3.8 liter (#1) and 26.5 liter (#7) pots, respectively, using the same amended substrate used in the greenhouse screening, and placed outdoors in full sun. Overhead irrigation was applied twice daily at a rate of approximately 1.3 cm (0.5 in) at each irrigation. At the initiation of treatments in spring 2001, spring growth had matured and there were few immature shoots on any of the plants. Plants in 3.8 liter (#1) containers received three applications on June 22, June 29, and July 6. Plants in 26.5 liter (#7) received two applications on June 22 and June 29. Plants were treated with 0, 1250, 2500, 3750, or 5000 ppm BA. Temperature and relative humidity at the times of application were 27C (80F) and 83%, 27C (80F) and 88%, and 35C (95F) and 60%, respectively. Treatments were completely randomized within container size, and each treatment was replicated using ten single plants. At 30 days after initial treatment (DAIT) new shoots longer than 2.5 cm (1 in) were quantified.

To determine effectiveness of BA at a time of year when vegetative growth in Indian hawthorn is minimal, plants in 26.5 liter (#7) containers received a second series of two applications on September 19 and September 26, following previously described procedures. Applications were halted due to expected colder temperatures prior to the maturation of any flush stimulated by treatment; injury was not apparent at the time of the second application. At 30 DAIT, number of new shoots per plant were quantified.

Multiple BA applications, 2002. 'Olivia' in 3.8 liter (#1) pots previously used in 2001 were retreated on May 6, May 13, and May 20, 2002, following the same methodology. At the time of initial application, plants were actively growing with immature soft shoots. Temperature and relative humidity at the three times of application were 25C (78F) and 50%, 20C (68F) and 50%, and 25C (77F) and 75%, respectively. Data collected at 30, 60, and 120 DALT included the number of new shoots at least 2.5 cm (1 in) long with unfurling first leaves, an injury rating of new foliage (1 = healthy; 2 = chlorotic/distorted; 3 = extensive distorted foliage/marginal necrosis; 4 = extensive necrosis; 5 = dead), and a growth index [GI = (height + widest width + width 90°) / 3].

Additional uniform plants of 'Olivia' and 'Eleanor Taber' Indian hawthorn in 3.8 liter (#1) containers were obtained from a commercial nursery at the end of March 2002. Plants were treated on May 20, May 27, and June 3, 2002, using the same weekly BA treatments previously described. At the time of initial application plants exhibited actively growing, immature tender growth. Temperature and relative humidity at the three times of application were 20C (68F) and 71%, 28C (83F) and 65%, and 33C (91F) and 56%, respectively. Data collected at 30, 60, and 90 DALT included the number of new shoots formed, an injury rating, and growth index, all as previously described.

Data were subjected to analysis of variance using SAS statistical software (8). Response to BA rate was determined by orthogonal polynomial analyses.

Results and Discussion

Greenhouse screening. New shoot numbers and shoot length in 'Olivia' Indian hawthorn changed quadratically ($P < 0.001$) at 45 DALT (data not shown). Controls exhibited no new growth, while plants treated with 2500 ppm BA formed an average of 6.8 new shoots with an average length of 9.5 cm (3.8 in). Plants treated with 5000 ppm BA formed an average of 5.4 new shoots with an average length of 7.1 cm (2.8 in). Stimulation of new shoot formation with multiple weekly applications of BA contrasts with the negligible response to single applications of up to 2500 ppm BA in Indian hawthorn previously reported (3), but supports the results of Keever and Morrison (4) in which multiple applications increased new shoot formation in 'Harbour Dwarf' nandina. New leaves on plants treated with 5000 ppm BA, but not 2500 ppm BA, were mottled, abnormally twisted, and cupped.

Multiple BA applications, Summer 2001. At 30 days after initial treatment (DAIT) 'Olivia' in 3.8 liter (#1) containers exhibited a linear increase in new shoot numbers in response to increasing BA concentration (Table 1). There were over 2.5 times more new shoots at 1250 ppm BA and over 4 times more at 3750 ppm and 5000 ppm BA compared to untreated plants. New shoot numbers increased linearly in 'Olivia' in 26.5 liter (#7) containers in response to the first series of two BA applications (Table 1). With BA applications, there were over 18 times more new shoots with 1250 ppm BA, and 27 times more with 5000 ppm BA compared to untreated plants. Following a pronounced mid-season response to BA, there was little or no new growth on treated or untreated plants by

Table 1. New shoot formation in 'Olivia' Indian hawthorn grown in 3.8 liter (#1) and 26.5 liter (#7) containers following multiple applications of BA applied in 2001 and 2002[z].

	3.8 liter (#1)			26.5 liter (#7)	
	Summer 2001	Spring 2002		Summer 2001	Fall 2001
BA concn. (ppm)	New shoot counts[y]	New shoot counts	Injury rating[x]	New shoot counts	New shoot counts
0	1.6	3.6	1.2	4.7	0.2
1250	4.3	10.4	1.9	82.5	17.7
2500	5.5	11.3	2.5	81.5	46.8
3750	6.9	13.7	2.8	87.7	75.7
5000	6.9	13.0	2.3	125.5	105.2
Significance[w]	L***	Q**	Q***	L***	L***

[z]Benzyladenine (BA) was applied to plants in 3.8 liter containers on June 22, June 29, and July 6, 2001. Plants in 26.5 liter containers were treated on June 22 and June 29. A second series of treatments were applied to plants in 26.5 liter container on September 19 and September 26. Plants in 3.8 liter containers were retreated on May 6, May 13, and May 20, 2002. Data were collected 30 days after inital treatment (DAIT) for plants in 3.8 liter containers in 2001 and both application series for plants in 26.5 liter containers, and 30 days after last treatment in 2002.

[y]New shoots measured at least 2.5 cm (1 in) long and leaves were unfurling.

[x]New foliage injury rating: 1 = healthy; 2 = chlorotic/distorted; 3 = extensive distortion, marginal necrosis; 4 = extensive necrosis; 5 = dead. Ratings ≤ 2.0 were considered commercially acceptable.

[w]Response linear (L) or quadratic (Q) at $P = 0.001$ (**) or $P = 0.001$ (***), based on orthogonal polynomial analyses.

late summer. The second series of applications also resulted in a linear increase in new shoot number as BA concentration increased (Table 1). Controls formed an average of less than one new shoot. With BA applications, the number of new shoots increased from 18 with 1250 ppm BA to 105 new shoots with 5000 ppm BA. Treated plants appeared denser than untreated plants, but appeared otherwise similar in size, shape, and leaf formation. Plants showed no signs of phytotoxic response in any treatment at times of data collection, possibly due to the lack of immature foliage at times of BA application.

Multiple BA applications, Spring 2002. At 30 DALT new shoot number in retreated 'Olivia' responded quadratically to increasing BA rates following three applications (Table 1). The number of new shoots increased over 2.5 times with 1250 ppm BA and 3.6 times with 5000 ppm BA. The injury rating also responded quadratically, with controls rated 1.2 and plants receiving 5000 ppm BA rated 2.3. Plants receiving 3750 ppm BA produced new leaves that were abnormally mottled, twisted, and cupped. Plants receiving 5000 ppm BA exhibited similar symptoms, with some plants displaying marginal necrosis. Necrotic leaves abscised shortly after final application, but had no effect on injury rating conducted at 30 DALT Growth index was unaffected by treatment at 30 DALT ($\bar{X} = 8.25$, SE = 0.39). At 60 DALT no additional new shoots had formed ($\bar{X} = 10.6$, SE = 0.72) and phytotoxic response remained unchanged (data not shown). At this time, growth index was not significantly affected by treatments. Although no larger than controls, treated plants were visibly denser. By 120 DALT new shoots formed in response to treatment had matured. The number of new shoots present was minimal ($\bar{X} = 1.1$, SE = 0.21), and plants displayed no phytotoxic symptoms (data not shown). Growth index remained unaffected by treatments ($\bar{X} = 30.93$, SE = 0.55).

At 30 DALT in the second experiment of spring 2002, new shoot numbers in 'Olivia' increased linearly with increasing BA rates after three weekly applications (Table 2). With BA applications, the number of new shoots of 'Olivia' increased 133% with 1250 ppm BA and 374% with 5000 ppm BA, compared to untreated plants. With BA applications, the number of new shoots of 'Eleanor Taber' responded quadratically, increasing 59% with 1250 ppm BA and 128% with 5000 ppm BA (Table 3). The injury rating increased in 'Olivia' with increasing BA concentrations at 30 DALT (Table 2). Controls averaged 1.3, and with BA applications, ratings increased from 1.8 at 1250 ppm BA to 2.3 at 5000 ppm BA. Injury symptoms were similar to those observed in previous experiments with 'Olivia' Indian hawthorn. 'Eleanor Tabor' showed no significant phytotoxic response due to treatment (data not shown). Growth index was not significantly affected by BA applications in either cultivar at 30 DALT ($\bar{X} = 15.47$, SE = 0.61).

By 90 DALT shoots counted at 30 DALT had matured, and only new immature shoots were quantified. At this time,

Table 2. Response of 'Olivia' Indian hawthorn grown in 3.8 liter (#1) containers to multiple applications of BA applied in 2002[z].

	30 DALT		90 DALT	
BA concn. (ppm)	New shoot counts[y]	Injury rating[x]	Injury rating	GI[w]
0	3.9	1.3	1.0	27.5
1250	9.1	1.8	1.0	30.8
2500	13.5	2.0	1.0	33.0
3750	15.3	2.0	1.8	31.4
5000	18.5	2.3	2.0	33.0
Significance[v]	L***	L**	L**	L*

[z]Benzyladenine (BA) was applied on May 20, May 27, and June 3, 2002. Data were collected 30 and 90 days after last treatment (DALT).

[y]New shoots measured at least 2.5 cm (1 in) long and leaves were unfurling.

[x]New foliage injury rating: 1 = healthy; 2 = chlorotic/distorted; 3 = extensive distortion, marginal necrosis; 4 = extensive necrosis; 5 = dead. Ratings ≤ 2.0 were considered commercially acceptable.

[w]GI= (height + widest width + width 90°) / 3.

[v]Response linear (L) at $P = 0.05$ (*), $P = 0.01$ (**), or $P = 0.001$ (***), based on orthogonal polynomial analyses.

Table 3. Response of 'Eleanor Taber' Indian hawthorn grown in 3.8 liter (#1) containers to multiple applications of BA applied in 2002[z].

	30 DALT	90 DALT	
BA concn. (ppm)	New shoots counts[y]	New shoot counts	Injury rating[x]
0	18.9	8.9	1.0
1250	30.0	14.1	1.0
2500	42.3	12.8	1.5
3750	48.1	14.2	1.7
5000	43.1	10.8	1.4
Significance[w]	Q**	Q*	L*

[z]Benzyladenine (BA) was applied on May 20, May 27, and June 3, 2002. Data were collected 30 and 90 days after last treatment (DALT).

[y]New shoots measured at least 2.5 cm (1 in) long and leaves were unfurling.

[x]New foliage injury rating: 1 = healthy; 2 = chlorotic/distorted; 3 = extensive distortion, marginal necrosis; 4 = extensive necrosis; 5 = dead. Ratings ≤ 2.0 were considered commercially acceptable.

[w]Response linear (L) or quadratic (Q) at $P = 0.05$ (*) or P = 0.01 (**), based on orthogonal polynomial analyses.

treatment had no effect on new shoot formation in 'Olivia' ($\bar{X}$ = 1.9, SE = 0.23). 'Eleanor Taber' showed a quadratic change in new shoot formation (Table 3), with plants receiving 1250 ppm BA or 3750 ppm BA having 57% more new shoots than untreated controls. Plants treated with 2500 ppm BA displayed a 44% increase in new shoot numbers, and plants treated with 5000 ppm BA showed a 21% increase in new shoot numbers compared to untreated plants. However, relative to the total shoot counts at 30 DALT, new shoot counts in control plants at 90 DALT probably represent a higher percentage of buds to break than in plants treated with BA. At 90 DALT 'Olivia' showed a linear increase in injury rating with increasing BA concentrations (Table 2). Injury rating for 'Eleanor Taber' increased up to 3750 ppm BA, before decreasing at 5000 ppm BA (Table 3). The decrease in injury rating was due to leaf abscission. Growth index at 90 DALT increased linearly in 'Olivia' (Table 2), but not 'Eleanor Taber' ($\bar{X}$ = 29.73, SE = 0.52). Growth index of 'Olivia' increased 12% with 1250 ppm BA and 20% with 5000 ppm BA.

Multiple exogenous BA applications stimulated new shoot formation in Indian hawthorn cultivars with increasing BA concentrations, regardless of the time of year or stage of shoot development when applied. These results agree with those previously reported with nandina cultivars (4). Plant size, as measured by growth index, either was not affected or increased with BA application. Plants treated with BA were visibly more dense and compact than control plants, which were sparsely branched and open in appearance.

Phytotoxic effects of BA increased with increasing BA concentrations. New foliage was more cupped and twisted, and foliar color more mottled with increasing BA concentrations. Necrosis was evident at the highest concentration. Presence or absence of new growth at the time of application affected the severity of injury. Plants in the greenhouse screening were dormant when applications were initialized. Minimal phytotoxic symptoms were evident at the highest concentration at the completion of the application series. Plants treated in early May had soft new growth, which was severely injured upon application of the higher BA concentrations. New growth of plants treated in June and September had hardened and injury was minimal. These results suggest BA applications should be avoided when plants are flushing to minimize injury. By 120 DALT of plants treated in 2002, shoots present at times of application were fully matured, and subsequent shoots were unaffected by treatments. Therefore, BA can be used to stimulate dormant bud growth and branching in Indian hawthorn cultivars 'Olivia' and 'Eleanor Taber' during the growing season without the biomass losses associated with manual pruning.

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