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Benzyladenine-Induced Shoot Formation in Indian Hawthorn (*Rhaphiolepis indica*)[®]

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In an outdoor nursery experiment conducted in summer 2001, benzyladenine (BA) was applied at concentrations up to 5000 ppm in 1250 ppm increments to plants (*Rhaphiolepis indica*) in 3.8-L (#1) and 26.5-L (#7) containers. By 30 days after last treatment (DALT) plants in 3.8-L (#1) containers treated with three weekly applications of 1250 ppm BA formed 169% more new shoots than controls and up to 331% more with 5000 ppm BA. Controls in 26.5-L (#7) containers formed 4.7 new shoots, and increased from 83 to 126 new shoots with two weekly applications of 1250 and 5000 ppm. Plants in 26.5-L (#7) containers were retreated in September, at which time shoots were inactive. By 30 DALT controls formed 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 an outdoor experiment conducted in Spring 2002 new shoot formation increased up to 374% at 30 DALT after three BA applications. Injury rating increased with increasing BA rates at 30 DALT. By 90 DALT new shoots had matured normally and exhibited minimal phytotoxic symptoms.

INTRODUCTION

Indian hawthorn [*Rhaphiolepis* Lindl. Cor. *Poir indica* (L.) Lindl. (syn. *Crataegus indica* L.)] is a dense, mound-forming evergreen shrub growing 1 to 2 m (3 to 6 ft) tall and forming dark glossy green leaves. White to pink fragrant flowers are borne in dense upright tomentose racemes or panicles from mid April to early May. Hardy in U.S.D.A. Cold Hardiness Zones 7b-10, Indian hawthorn is widely utilized for textural effect in containers, groupings, or mass plantings (Dirr, 1998).

Indian hawthorn displays very little natural branching during commercial production, and without pruning, plants are sparsely branched, misshapen, and

unmarketable. Plants in 3.8-L (#1) containers require at least one pruning and 18 to 20 months to reach a marketable stage, while those in 11.4-L (#3) containers require two additional prunings and an additional 9 to 12 months of production time and three or more weeks of active growing time may be lost with each pruning (Holt, pers. comm., Ryan, 1985). 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 as efficiently as possible.

Cytokinins are naturally occurring and synthetic plant growth regulators that function in overcoming apical dominance and promoting lateral shoot initiation and cell division (Kaminek et al., 1992; Mok and Mok, 1994). Exogenous application of cytokinins, including benzyladenine (BA), has been shown to promote axillary bud growth and branching in various woody landscape plants (Keever, 1990; Lewnes, 1976; Ryan, 1985; Wright, 1976). Keever and Foster (1990) found that response to BA was species dependent, with a single application of up to 2500 ppm BA having minimal effect on shoot formation in Indian hawthorn, but two applications over the course of 3 months were found to increase shoot formation in *Nandina domestica* 'Harbor Dwarf' grown in a greenhouse under night-break lighting. Furthermore, multiple applications of 2500 and 5000 ppm BA applied weekly were much more effective in promoting shoot formation than a single application on 'Harbour Dwarf' nandina (Keever and Morrison, 2003). Therefore, the objective of our study was to investigate the effects of BA concentrations when applied multiple times to Indian hawthorn.

MATERIALS AND METHODS

Multiple BA Applications, 2001. 'Olivia' in liner and 3.8-L (#1) pots were transplanted in April 2001 into 3.8-L (#1) and 26.5-L (#7) pots, respectively, containing a 7 pinebark : 1 sand medium (v/v) amended per m³ (yd³) with 3 kg (5 lb) dolomitic limestone, 0.9 kg (1.5 lb) Micromax (The Scotts Company, Marysville, Ohio) and 7.2 kg (12 lb) Osmocote 17N-3.1P-10K (17-7-12, The Scotts Company), and placed outdoors in full sun under overhead irrigation. Benzyladenine was applied weekly beginning in late June to plants in both container sizes. At the initiation of treatments, spring growth had matured, and there were few immature shoots on any of the plants. Foliar sprays included 0.2% (v/v) Buffer X (Kalo Agr. Chemicals, Overland, Kansas), a nonionic surfactant, and were applied with a compressed air sprayer equipped with a flat spray nozzle (Tee Jet 8002VS, Bellspray, Inc., Opelousa, Louisiana) at 138 kPa (20 psi). Plants in 3.8-L (#1) pots received three applications and plants in 26.5-L (#1) containers received two applications each of 0, 1250, 2500, 3750, or 5000 ppm BA. Treatments were completely randomized within pot size, and each treatment was replicated with ten single plants. At 30 days after initial treatment (DAIT) new shoots longer than 2.5 cm (1 inch) were quantified.

Following a pronounced mid-season response to BA, there was little or no new growth on treated or control plants by late summer. To determine the effectiveness of BA at a time of year when vegetative growth in Indian hawthorn is minimal, plants in 26.5-L (#7) containers received a second series of two applications beginning in mid-September following previously given procedures. At 30 DAIT the number of new shoots per plant were quantified.

Multiple BA Applications 2002. Based on positive results with the range of BA concentrations and number of applications used in 2001, 'Olivia' in 3.8-L (#1) pots used in 2001 were retreated beginning in May 2002 following the same methodology. At the time of initial application, plants were actively growing with immature shoots. Data collected at 30, 90, and 120 DALT included the number of new shoots at least 2.5 cm (1 inch) long with unfurling first leaves, an injury rating (1= healthy; 2= chlorotic/distorted immature foliage; 3= extensive distorted immature foliage/marginal necrosis; 4= extensive necrosis; 5= dead), and a growth index [GI= (height + widest width + width 90 °)/3].

Additional plants of 'Oliva' Indian hawthorn in 3.8-L (#1) containers were obtained from a commercial nursery at the end of March 2002. At the time of initial application in May, plants exhibited actively growing immature shoots. Data collected at 30, 60, and 90 DALT included the number of new shoots formed, and an injury rating with the same range as previously described.

RESULTS AND DISCUSSION

Multiple BA Applications, Summer 2001. At 30 DAIT 'Olivia' in 3.8-L (#1) containers exhibited a linear increase in new shoot formation in response to increasing BA concentration (Table 1). Stimulation of new shoot formation with multiple weekly applications of BA contrasts with the minimal response of single applications of up to 2500 ppm BA in Indian hawthorn previously reported (Keever and Foster, 1990), but supports the findings of Keever and Morrison (2003), in which multiple applications were made to nandina. Controls averaged 1.6 new shoots. New shoot formation increased 169% with 1250 ppm BA to 331% with 5000 ppm BA compared to the control. New shoots increased linearly in 'Olivia' in 26.5-L (#7) containers in response to the first series of two BA applications (Table 1). Controls exhibited an average of 4.7 new shoots. With BA applications, the number of new shoots increased from 1665% with 1250 ppm to 2570% with 5000 ppm BA. The second series of applications also resulted in a linear increase in new shoots 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 17.7 with 1250 ppm BA to 105.2 new shoots with 5000 ppm BA. Treated plants appeared denser than control plants, but were otherwise similar. Plants showed no signs of phytotoxic response in any treatment.

Multiple BA Applications, Spring 2002. At 30 DALT new shoot formation and phytotoxicity in retreated 'Olivia' increased with increasing BA rates following three applications (Table 1). Controls formed an average of 3.6 new shoots. The number of new shoots increased from 189% with 1250 ppm BA to 275% with 5000 ppm BA. Injury ratings increased with increasing BA rate, with a 1.2 rating for controls and 2.3 for plants receiving 5000 ppm BA. Plants receiving 2500 ppm BA produced new leaves that were abnormally mottled, twisted, and cupped. Plants receiving 5000 ppm BA displayed similar symptoms, with some plants displaying additional marginal, necrotic leaf tissue. Necrotic leaves abscised shortly after final application. Growth index was unaffected by treatment. At 90 DALT no additional new shoots had formed and injury rating remained unchanged (data not shown). At this time growth index was not significantly affected by treatments, however, treated plants appeared denser than controls. By 120 DALT new shoots formed in response

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

BA (ppm)	3.8-L (#1) container			26.5-L (#7) container	
	Summer 2001	Spring 2002		Summer 2001	Fall 2001
	New shoots ^y	New shoots	Injury rating ^x	New shoots	New shoots
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-L containers on 22 June, 29 June, and 6 July 2001. Plants in 26.5-L containers were treated on June 27 and July 6. A second series of treatments were applied to plants in 26.5-L containers on 19 September and 26 September.

Data were collected 30 days after initial treatment (DAIT) for plants in 3.8-L containers and both application series for plants in 26.5-L containers.

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

^x Injury rating : 1= healthy; 2= chlorotic/distorted immature foliage; 3= extensive distorted immature foliage; marginal necrosis 4= extensive necrosis; 5= dead.

^w Regression response linear (L) or quadratic (Q) at $P=0.01$ (**), $P=0.001$ (***).

to treatment had matured. The number of new shoots present was minimal and plants displayed no phytotoxic symptoms (data not shown).

At 30 DALT new shoot formation in previously untreated 'Olivia' increased with increasing BA rate after three weekly applications (Table 2). Controls of 'Olivia' formed 3.9 new shoots. With BA applications, the number of new shoots increased from 233% at 1250 ppm BA to 474% at 5000 ppm BA. Injury ratings increased with increasing BA concentrations (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. Symptoms were similar to those observed in previous experiments with Indian hawthorn. Growth index was not significantly affected by BA applications at 30 DALT (data not shown).

By 90 DALT shoots counted at 30 DALT had matured, and only new immature shoots were quantified. At this time, treatment had no effect on new shoot formation in 'Olivia' (data not shown). There was an increase in injury rating with increasing BA rate. 'Olivia' receiving 0 ppm, 1250 ppm, or 2500 ppm BA expressed no symptoms of injury. Symptoms on plants treated with higher concentrations were similar to those previously reported (Table 2). Growth index at 90 DALT increased linearly in 'Olivia', from 112% with 1250 to 120% with 5000 ppm BA (Table 2).

Multiple exogenous BA applications stimulated new shoot formation in Indian hawthorn with increasing BA concentrations, regardless of the time of year when applied or stage of shoot development. These results are in agreement with those

Table 2. Response of 'Olivia' Indian hawthorn grown in 3.8-L (#1) containers to multiple applications of benzyladenine (BA) applied in 2002^z.

BA (ppm)	30 DALT		90 DALT	
	New shoots ^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 once a week for three consecutive weeks beginning 20 May 2002. Data were collected 30 and 90 days after last treatment (DALT).

^y New shoots measured at least 2.5 cm (1 inch) long and leaves were unfurling

^x Injury rating: 1= healthy, 2= chlorotic/distorted immature foliage; 3= extensive distorted immature foliage, marginal necrosis; 4= extensive necrosis; 5= dead.

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

^v Regression response linear (L) at $P=0.05$ (*), $P=0.01$ (**), and $P=0.001$ (***)).

previously reported with nandina (Keever and Morrison, 2003). 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. With increasing BA concentrations, new foliage became more cupped and twisted, and foliar color more mottled. Necrosis was evident at the highest concentration. The physiological stage of plant development at the time of application appeared to affect the severity of injury. Plants treated in early May had soft, immature new growth, which was severely injured by 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. BA can be employed to stimulate new growth and branching of dormant buds in Indian hawthorn 'Olivia' without the loss of biomass associated with manual pruning.

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