Multiple Benzyladenine Applications Increase Shoot Formation in Nandina[1]

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Abstract

A study conducted from 2000 to 2002 determined the effects of multiple applications of benzyladenine (BA) applied at different concentrations on shoot formation, whole plant size, and phytotoxicity in *Nandina domestica* Thunb. In 2000, shoot counts increased in 'Harbour Dwarf' by up to 107% and 480% as the number of weekly BA applications at 2500 and 5000 ppm, respectively, increased to five. Likewise, the number of shoots per plant increased as BA rate increased. In response to five weekly foliar BA applications in 2001 and 2002, the number of new shoots on 'Harbour Dwarf' increased linearly 10-fold over the control as BA rate increased to 5000 ppm in 1250 ppm increments. In 2002, shoot formation increased linearly by up to six additional shoots per plant with increasing BA rate in 'Harbour Dwarf' and quadratically by up to two additional shoots per plant in 'Firepower' by 90 days after initial treatment (DAT) with five applications of up to 5000 ppm BA. Shoot formation in the tall, upright cultivars 'Royal Princess' and 'Moyer's Red' also increased linearly with increasing BA rate, by two and <1 shoot per plant, respectively, in 2002 only. Five foliar applications of 2500, 3750, and 5000 ppm BA injured upper, immature foliage, but did not adversely affect plant appearance by 90 DAT. Treatment effects on plant size were minimal.

Index words: plant growth regulator, branching, phytotoxicity, apical dominance, cytokinin.

Growth regulator used in this study: benzylamino purine or benzyladenine (BA), N-(phenylmethyl)-1*H*-purine-6-amine.

Species used in this study: nandina (*Nandina domestica* Thunb. 'Harbour Dwarf', 'Royal Princess', 'Moyer's Red', and 'Firepower').

Significance to the Nursery Industry

Although nandina is an exceptionally popular landscape plant in the southeastern United States, it forms new shoots slowly during container production, thus limiting the availability of cuttings for propagation. Even with multiple prunings, the development of well-branched, compact plants is slow, increasing nursery production time. In this study, increasing the number of weekly foliar applications of benzyladenine (BA) from 0 to 5 increased shoot formation in 'Harbour Dwarf' nandina. Additionally, when applied in five weekly applications, increasing the BA rate from 0 to 5000 ppm strongly stimulated new shoot development to decreasing degrees in 'Harbour Dwarf' (up to 305%), 'Royal Princess' (up to 153%), 'Firepower' (up to 36%), and 'Moyer's Red' (less than 1 new shoot per plant). Any phytotoxicity was temporary, and effects on plant size were minimal. These results show that multiple foliar applications of BA strongly promote new shoot development in nandina, thus potentially providing cuttings for propagation and shortening the time necessary to produce well-branched, compact nandinas. BAP-10, the commercial source of BA used in this study, is not EPA registered. The material cost of applying a single application of 2500 ppm and 5000 ppm BA to pot-to-pot spaced plants in 3.8 liter (#1) containers is $0.02 and $0.04 per plant, respectively.

Introduction

Nandina domestica and its cultivars are one of the most widely planted groups of broadleaf evergreen shrubs in southeastern U.S. gardens (1). 'Royal Princess' and 'Moyer's Red' have distinctly upright forms which reach 1.8–2.4 m (6–8 ft) in height with few lateral shoots. 'Harbour Dwarf' and 'Firepower' form compact, dense mounds of finer textured foliage, the latter requiring partial shade for optimal landscape effect. Rhizomatous in nature and forming cane-type shoots from the ground, nandinas develop few basal or lateral shoots and do not readily respond to mechanical pruning with multiple shoot formation during production. These characteristics often limit cutting for propagation and extend production time.

Exogenously applied cytokinins, including the adenine cytokinins, benzyladenine (BA) and PBA, promote axillary bud growth and branching of woody and herbaceous plants (2, 5, 6). Branching in 'Harbour Dwarf' nandina increased in response to single foliar applications of 1000 to 2500 ppm BA and to 2000 to 5000 ppm Promalin, a mixture of equal parts by weight of BA and GA_{4+7} (5); however, in a subsequent unpublished study, results were inconsistent.

Maene and DeBergh (7) reported that eight weekly applications of BA increased axillary shoot production in *Cordyline terminalis* 'Celestine Queen', as BA rates increased from 0 to 500 ppm. However, research conducted with frequent, multiple applications of BA has not been reported in other herbaceous or woody ornamental plants. Sequentially repeated (every 30 days) applications of BA, up to four times per plant, promoted continued offset formation in *Hosta* (4), and two applications of BA, well-spaced temporally (one in November, one in February) increased shoot counts in 'Harbour Dwarf' nandina grown in a greenhouse under night-break lighting (6).

The objective of this study was to determine the effects of multiple, weekly foliar BA applications, at 1250 to 5000 ppm, on shoot formation, plant size and appearance of 'Harbour Dwarf', 'Royal Princess', 'Moyer's Red', and 'Firepower' nandina.

Materials and Methods

Three experiments were conducted between 2000 and 2002 using similar methodology unless otherwise noted. The

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growth medium was a pinebark:sand substrate (6:1, by vol) amended per cu m (cu yd) with 8.3 kg (14 lb) 18N–2.6P–10K (PolyOn 18–6–12, Pursell Industries, Sylacauga, AL), 0.9 kg (1.5 lb) Micromax (The Scotts Company, Marysville, OH) and 3 kg (5 lb) dolomitic limestone. Plants were spaced outdoors in full sun under twice-daily overhead irrigation, receiving 1.8 cm (0.7 in) of water per day. Plants were re-spaced as they grew.

In each study, foliar sprays of BA (BAP-10, Plantwise Biostimulant Co., Louisville, KY; BAP-10 is not registered with the EPA) were applied in a volume of 0.2 liter/sq m (2 qt/100 sq ft) using a CO_2 sprayer with a flat spray nozzle (TeeJet 8004VS, Bellspray, Inc., Opelousas, LA) at 138 kPa/cu cm (20 psi). Buffer X (Kalo Agr. Chemicals, Overland, KS), a non-ionic surfactant, was added to the spray solutions at 0.2% (v/v). Foliar applications were made under tree shade to reduce the possibility of foliar burn by sun-drying of solutions or wetting of foliage by overhead irrigation. Applications were made as early as 8:30 a.m and as late as 4:30 p.m, with no discernible difference in plant response due to time of application. Dry and wet-bulb temperatures were recorded at application of each BA treatment, from which relative humidity (RH) was determined. Treated plants were allowed to dry overnight before being returned to irrigated growing area.

Data collected in all experiments included counts of new basal and lateral shoots. Additionally, in 2001 and 2002, growth index (GI) [(height + widest width + width 90° to widest width) ÷ 3], and ratings of phytotoxicity were made using the following scale: 1 = healthy; 2 = immature foliage puckering; 3 = immature foliage puckering, chlorosis and/or red margins; 4 = necrosis plus extensive puckering/discoloration; 5 = dead.

Table 1. Effect of BA concentration and application number on shoot counts of 'Harbour Dwarf' nandina, 2000.

BA rate (ppm)	Number of applications	Shoot counts[z]			
		6 WK[y]	9 WK	12 WK	15 WK
Control	0	0.6	0.9	1.2	1.3
2500	1	0.5	0.5	0.5	0.4
	2	0.3	0.3	0.3	0.4
	3	1.7	1.8	1.8	2.0
	4	1.3	1.6	1.7	1.8
	5	3.1	3.2	3.4	2.7
Significance[x]		L***	L***	L***	L**
5000	1	0.4	0.6	0.7	0.7
	2	1.1	1.2	1.6	1.3
	3	1.6	1.7	1.7	1.8
	4	2.7	2.8	2.9	2.2
	5	5.9	6.0	6.1	5.5
Significance		Q***	Q***	Q***	Q***
Contrast statements					
Rate response within 1 application		NS	NS	NS	NS
Rate response within 2 applications		NS	NS	NS	NS
Rate response within 3 applications		NS	NS	NS	NS
Rate response within 4 applications		L**	L**	L**	NS
Rate response within 5 applications		L***	L***	L***	L***

[z]Total number of lateral and basal shoots, excluding the initial shoot.

[y]WK = weeks after initial treatment.

[x]Significant application number × rate interaction at all times — linear (L) or quadratic (Q) regression response at $P = 0.01$ (**) or 0.001 (***); control included in analysis.

An analysis of variance of the data was made using the SAS General Linear Model (8) procedure to test the significance of main and interactive effects. Contrast statements were included to test linear and quadratic orthogonal response trends to BA rate and application number.

2000. Uniform, unbranched 'Harbour Dwarf' nandina in 7.6 liter (#2) containers, received 1, 2, 3, 4, or 5 foliar spray applications of BA at 2500 or 5000 ppm at weekly intervals beginning July 7, 2000. Temperature and RH at treatment applications ranged from 28C (82F) to 38C (100F) and 31% to 62%, respectively. A non-treated control was also included. Treatments in this 2 × 5 factorial experiment plus a control, were completely randomized, with 10 single plant replications. The total number of axillary and rhizomic shoots were counted at 6, 9, 12, and 15 weeks after the first BA treatment (WK); counts were cumulative.

2001. 'Harbour Dwarf' plants in 11.4 liter (#3) containers that were uniform in size with a single, unbranched shoot, each received five weekly foliar spray applications of BA at 1250, 2500, 3750, or 5000 ppm beginning on June 22, 2001. Temperature and RH at treatment applications ranged from 26C (80F) to 38C (100F) and 38% to 83%, respectively. BA treatments plus an untreated control were completely randomized, with 10 single plant replications. At 90 days after initial treatment (DAT) the number of new shoots were counted, GI was calculated, and plants were rated for phytotoxicity symptoms.

2002. In April, 2002 'Harbour Dwarf', 'Royal Princess', 'Moyer's Red', and 'Firepower' were stepped-up from 10 cm (4 in) pots into 7.6 liter (#2) containers of amended 6:1 pinebark:sand medium and spaced outdoors in full sun under overhead irrigation. Uniform plants with single shoots each received five weekly foliar spray applications of BA at 1250, 2500, 3750, or 5000 ppm beginning on June 6, 2002. Temperature and RH at treatment applications ranged from 28.3C (83F) to 36C (98F) and 36% to 53%, respectively. BA treatments plus an untreated control were completely randomized within cultivar, with 10 single plant replications. At 30 DAT new shoots were counted and phytotoxicity was rated (1 = healthy; 2 = red margins on upper, immature leaves; 3 = red margins and puckering/distortion on upper, immature leaves; 4 = necrosis plus red margins, puckering/distortion on upper, immature/mature leaves; 5 = dead). At 90 DAT all shoots (excluding the original) were counted and GI calculated.

Results and Discussion

2000. BA rate, number of applications, and the interaction between the two significantly affected shoot counts of 'Harbour Dwarf' at 6, 9, 12, and 15 weeks after the first BA application (Table 1). The number of new shoots increased linearly with increasing number of applications at a BA rate of 2500 ppm, whereas the number of shoots increased quadratically with increasing number of applications at a BA rate of 5000 ppm. Most shoots had formed by 6 WK, with little further development by 15 WK. In response to five applications, 3.1 and 5.9 new shoots per plant were formed on 'Harbour Dwarf' plants receiving 2500 and 5000 ppm BA,

Table 2. **Shoot counts, growth index, and phytotoxicity rating of 'Harbour Dwarf' nandina treated with 5 weekly foliar applications of BA at 4 rates, 2001.**

BA rate (ppm)	Shoot counts[z]	Growth index[y]	Phytotoxicity rating[x]
0	0.5	39.6	0.0
1250	2.3	37.2	0.0
2500	3.2	37.3	0.0
3750	1.5	39.7	0.0
5000	5.5	38.5	0.2
Significance[w]	L***	NS	NS

[z]Total number of lateral and basal shoots, evaluated 90 days after initial treatment.

[y]Growth index = (height + width$_1$ + width 90° to width$_1$) ÷ 3, in cm.

[x]Phytotoxicity rating: 1 = healthy; 2 = immature foliage puckering; 3 = immature foliage puckering, chlorosis and/or red margins; 4 = necrosis plus extensive puckering/discoloration; 5 = dead.

[w]Nonsignificant (NS) or significant linear (L) regression response at P = 0.001 (***).

respectively, whereas control plants had <1 new shoot at 6 WK. In some treatments, new shoot counts decreased between 12 WK and 15 WK, reflecting a random abortion of new shoots. A similar abortion was previously reported in hosta (5).

Increasing rates of BA had no effect on plants receiving ≤ three weekly applications, demonstrating rate ineffectiveness. In response to four and five applications, shoot counts rose linearly as BA rate increased, except at 15 WK for four applications (Table 1). By 15 WK plants treated five times with 2500 and 5000 ppm BA averaged 2.7 and 5.5 new shoots per plant, respectively, versus 1.3 new shoots for control plants. Based on the results of this experiment, shoot formation in 'Harbour Dwarf' nandina was stimulated most by five weekly applications of 5000 ppm BA. No adverse symptoms were observed in any treatment.

2001. At 90 DAT new shoots of 'Harbour Dwarf' nandina increased linearly with increasing rate of BA (Table 2). Shoot counts increased from 0.5 shoot per plant for the control to 5.5 shoots per plant for those receiving 5000 ppm BA, a 10-fold increase. Plant growth index and phytotoxicity symptoms were unaffected by BA at 90 DAT.

2002. At both 30 and 90 DAT, the number of emerging shoots for 'Harbour Dwarf' increased linearly as BA rate increased (Table 3). By 90 DAT, new shoot counts had increased from 0.2 for controls to 2.2, 4.2, 8.8, and 6.3 shoots per plant at 1250, 2500, 3750, and 5000 ppm BA applications, respectively. Emerging 'Royal Princess' shoots also increased linearly with increasing BA rate, up to 525% at 30 DAT and 153% at 90 DAT. Even though 'Moyer's Red' showed a linear response to BA, there was <1 new shoot per plant by 90 DAT. 'Firepower' responded to five applications of 5,000 ppm BA with 7.6 additional shoots per plant by 90 DAT; two more than control plants. Pronounced cultivar differences in response to BA application support results reported by Garner et al. (2) with *Hosta*, wherein only five of ten BA-treated *Hosta* cultivars responded with increases in offset counts.

In the 2002 experiment, all four nandina cultivars responded to increasing rates of multiple BA applications with increased shoot numbers. Significant trends were evident in 'Harbour Dwarf' and 'Royal Princess' within 30 DAT, and in all cultivars at 90 DAT. Other ornamentals have displayed a similar time lag after BA application. Garner et al. (3) found that eight of ten *Hosta* Tratt (*Funkia* K. Spreng; *Niobe* Salisb.) cultivars responded with increased offset numbers within 30 days of BA application, whereas all ten cultivars had increased offset formation by 60 DAT. Offset performance was generally higher with time since application. This type of trend was also noted in 'Harbour Dwarf' nandina grown in the greenhouse under night-break lighting. Keever and Foster (6) found these plants produced 52 to 67% of total new axillary shoots by January following an initial BA application in November, and the remaining 33 to 48% by April following a second BA application in February.

In this study, at 30 DAT 'Harbour Dwarf' and 'Moyer's Red' nandinas exhibited quadratic increases in phytotoxicity rating as BA rate increased, whereas 'Royal Princess' displayed a linear increase (Table 4). None of the cultivars displayed leaf necrosis, even at higher BA concentrations. By 90 DAT all cultivars had outgrown leaf discoloration and puckering, similar to 'Harbour Dwarf' at 90 DAT in 2001.

By 90 DAT, significant quadratic GI responses were present in all cultivars; however, none was considered of practical importance. Each cultivar responded differently to BA in height and width development, the components of GI (data not shown). 'Moyer's Red' responded to increasing BA rate

Table 3. **Shoot counts for 'Harbour Dwarf', 'Royal Princess', 'Moyer's Red', and 'Firepower' nandina treated with 5 weekly foliar applications of BA at 4 rates, 2002.**

BA rate (ppm)	'Harbour Dwarf'		'Royal Princess'		'Moyer's Red'		'Firepower'	
	30[z]	90	30	90	30	90	30	90
0	0.2[y]	0.2	0.4	1.5	0	0	—[w]	5.6
1250	0.4	2.2	0.6	2.3	0	0	—	5.3
2500	1.2	4.2	1.4	3.2	0	0	—	3.6
3750	3.9	8.8	1.5	2.6	0	0.2	—	5.0
5000	5.4	6.3	2.5	3.8	0.1	0.7	—	7.6
Significance[x]	L***	L***	L**	L*	NS	L**		Q*

[z]30 or 90 days after initial BA treatment.

[y]Total number of lateral and basal shoots.

[x]Nonsignificant (NS) or significant linear (L) and quadratic (Q) regression response at P = 0.05 (*), 0.01 (**), or 0.001 (***).

[w]Data not collected.

Table 4. Phytotoxicity rating and growth index for 'Harbour Dwarf', 'Royal Princess', 'Moyer's Red', and 'Firepower' nandina treated with 5 weekly foliar applications of BA at 4 rates, 2002.

Cultivar	BA rate (ppm)	Phytotoxicity rating[z] 30 DAT[x]	Growth index[y] 30 DAT	Growth index[y] 90 DAT
'Harbour Dwarf	0	1.0	—[w]	35.9
	1250	1.4	—	30.6
	2500	3.1	—	26.0
	3750	2.9	—	30.7
	5000	3.0	—	32.6
Significance[v]		Q***	—	Q***
'Royal Princess'	0	1.0	47.4	54.8
	1250	2.0	48.2	57.8
	2500	2.0	47.6	62.1
	3750	1.9	46.4	58.4
	5000	2.6	47.8	56.2
Significance		L***	NS	Q*
'Moyer's Red'	0	1.0	51.6	69.2
	1250	2.1	55.8	69.7
	2500	3.1	59.3	74.0
	3750	3.3	57.9	73.6
	5000	3.3	56.0	70.9
Significance		Q***	Q***	Q*
'Firepower'	0	—	40.3	44.5
	1250	—	39.1	40.1
	2500	—	39.1	33.2
	3750	—	42.6	31.8
	5000	—	36.9	38.8
Significance		—	NS	Q***

[z]Phytotoxicity rating: 1 = healthy; 2 = red margins on upper, immature leaves; 3 = red margins and puckering/distortion on upper, immature leaves; 4 = necrosis plus red margins, puckering/distortion on upper, immature/mature leaves; 5 = dead.

[y]Growth index = (height + width$_1$ + width 90° to width$_1$) ÷ 3, in cm.

[x]DAT = days after initial BA treatment.

[w]Data not collected.

[v]Nonsignificant (NS) or significant linear (L) and quadratic (Q) regression response at $P = 0.05$ (*) or 0.001 (***).

with a greater change in height relative to width, whereas, "Harbour Dwarf', 'Royal Princess', and 'Firepower' responded more in width. Generally, plants were more compact and fuller in appearance when treated with BA.

In conclusion, shoot formation in 'Harbour Dwarf' (2000 experiment) increased as application number increased with both 2500 and 5000 ppm BA. With five weekly applications, three to six additional shoots per plant emerged in response to 2500 and 5000 ppm BA, respectively. In 2001, in response to five weekly applications to 'Harbour Dwarf', linear increases in shoot counts were observed as BA rate increased from 1250 to 5000 ppm. Using the same treatments in 2002, shoot counts in 'Harbour Dwarf', 'Royal Princess', 'Moyer's Red', and 'Firepower' increased with increasing BA concentrations. Initially significant, but not severe, leaf discoloration and puckering were outgrown by plants within 90 days of application. Differences in GI responses, though significantly and variably influenced by BA application, depending on cultivar, were not of practical importance.

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