Benzyladenine (BA) Promotes Ramet Formation in *Hemerocallis*[1]

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Abstract

In 2000 and 2004, cultivars of *Hemerocallis* were treated with foliar applications of 2500 or 5000 ppm benzyladenine (BA) for 1, 2, 3, 4 or 5 consecutive weeks. In both years, BA increased ramet production of all cultivars although treatment response was cultivar dependent. In 2000, an increasing number of BA applications resulted in 'Lavinia Love' and 'Beguine' forming 1.6 to 3.2 and 0.7 to 1.1 more ramets, respectively, than control plants at 9 weeks after initial treatment (WAT), regardless of concentration. Increasing BA concentration increased ramet formation by up to one ramet in 'Beguine' and up to 3 ramets in 'Lavinia Love' at 9 WAT, regardless of the number of applications. At 9 WAT in 2004, increasing weekly applications of BA increased ramet formation in 'Dainty Deb' from a mean of 1.3 ramets with one application to a mean of 3.0 ramets with five applications, and in 'Sarah Sikes hybrid' from 0.5 ramets with one application to 2.1 ramets with five applications, regardless of concentration. Compared to control plants, 'Dainty Deb' increased ramet production by 1.9 ramets and 2.3 ramets at 9 WAT in 2004 when treated with 2500 ppm and 5000 ppm BA, respectively, regardless of the number of applications. Compared to untreated controls, 'Sarah Sikes hybrid' formed 1.1 and 1.3 more ramets when treated with 2500 ppm and 5000 ppm BA, respectively.

Index words: plant growth regulator, daylily, cytokinin, propagation, herbaceous perennial.

Growth regulator used in this study: BAP-10, benzyladenine (BA), 6-benzylaminopurine, *N*-(phenylmethyl)-1*H*-purin-6-amine.

Species used in this study: daylily, *Hemerocallis* x 'Dainty Deb', unnamed hybrid of 'Second Thoughts' x 'Neal Berrey' (referred to as 'Sarah Sikes hybrid'), 'Beguine', 'Lavinia Love'.

Significance to the Nursery Industry

Daylily production has traditionally centered on producing new plantlets (ramets) from established plants and more recently from tissue culture. For growers or hybridizers without the specialized equipment to perform tissue culture, producing more ramets from existing plants has been the only way to increase numbers. A crucial limit to this method of propagation is the slow production of ramets by many popular cultivars. The number of ramets produced on newly potted, single-shoot divisions of daylilies can be significantly increased by 1 to 3 consecutive weekly applications of benzyladenine (BA) at 2500 ppm, depending on cultivar. Increasing the number of consecutive applications or the concentration of BA to 5000 ppm will likely produce only slight further increases. BA as BAP-10 currently remains unlabelled for use on daylilies, but projected application costs of 2500 and 5000 ppm solutions is about 2.2 and 4.4¢, respectively, per 3.8 liter (#1) container, assuming four pots per square foot.

Introduction

Hemerocallis is one of the most popular genera in ornamental production today with the American Hemerocallis Society listing more than 56,000 named and 40,000 registered cultivars (1). Daylilies are usually propagated asexually, either by tissue culture or removal of ramets from older, established plants. Daylily growers without specialized equipment necessary for tissue culture production must rely on harvesting ramets from established plants. Harvesting ramets from established plants, when left to nature, can be a slow, erratic process with some cultivars producing more ramets than others (3).

Ramets in daylilies develop from lateral or rhizomic buds, but outgrowth of these buds appears to be suppressed by apical dominance. Hormonal interaction between auxins and cytokinins is a primary mechanism of apical dominance (2), and exogenous applications of cytokinins can stimulate lateral bud growth. Application of the synthetic cytokinin benzyladenine (BA) induced the outgrowth of lateral buds in hosta (7), and offsets formed from BA-induced buds were removed from the mother plant within 30 days of BA application and rooted under intermittent mist (9). These findings suggest that BA might facilitate conventional propagation methods by increasing the number of ramets available for harvest from daylilies.

A single application of up to 2500 ppm BA had minimal effect on shoot formation in either Indian hawthorn or nandina, but two applications 12 weeks apart increased shoot formation in 'Harbour dwarf' nandina (10). Furthermore, up to five weekly applications of 2500 or 5000 ppm BA were more effective in promoting shoot formation in 'Harbour Dwarf' nandina (11) and Olivia™ and Eleanor Taber™ Indian hawthorn (12) than a single application. Studies with hosta (5), *Dieffenbachia* (6), and Mugo pine (14) have reported similar greater effectiveness with multiple applications of BA. The objective of our study was to determine the effects of multiple foliar applications of BA on ramet formation in daylilies when applied at 2500 ppm or 5000 ppm.

Materials and Methods

This study consisted of two experiments, one in 2000 and a second in 2004. On July 14, 2000, 3.8-liter (#1) plants of *Hemerocallis* x 'Beguine' and 'Lavinia Love' grown in full sun were separated into uniform single shoot divisions and potted into 3.8-liter (#1) pots using a pine bark:sand sub-

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strate (6:1, by vol) amended per m^3 with 7.1 kg (12 lb/yd^3) of 18N–2.6P–10K (Osmocote 18–6–12, 9-month formulation, The Scotts Co., Marysville, OH), 3 kg (5 lb/yd^3) of dolomitic limestone, and 0.9 kg (1.5 lb/yd^3) of Micromax (The Scotts Co.). Immediately after potting, plants were placed in full sun and irrigated by overhead impact sprinklers twice daily, applying 3.8 cm (1.5 in) per day. On August 3, plants with one shoot per pot and evidence of surface root development were selected for uniformity and assigned to treatments. On August 4, plants began receiving weekly foliar sprays of 2500 or 5000 ppm BA (BAP-10, Plant-Wise Biostimulant Co., Louisville, KY) for 1, 2, 3, 4 or 5 consecutive weeks. Treatments were applied with a CO_2 sprayer equipped with a TeeJet 8001 nozzle (Spraying Systems Co., Wheaton, IL) at 138 kPa (20 psi) outlet pressure and in a volume of 0.2 liters/m^2 (equivalent to 2 qt/100 ft^2). A non-ionic surfactant (Buffer-X, Kalo Agr. Chemicals, Overland, KS) was added to spray treatments at 0.2%. Plants were removed from irrigation for treatment and were hand watered for 12 hours after treatment without wetting the foliage. Temperature and relative humidity at treatment were 30.0C (86F) and 77% (August 4), 32.8C (91F) and 70% (August 11), 37.8C (100F) and 50% (August 18), 34.5C (94F) and 60% (August 25) and 30.6C (87F) and 88% (September 5). Ramets were counted at 3, 6, and 9 weeks after initial treatment (WAT) and after shoots had emerged the following spring (March 13, 2001, or 27 WAT). Treatments in this 2 × 5 factorial experiment plus an untreated control were completely randomized within cultivar and replicated with 8 single plants.

On April 2, 2004, plants of *Hemerocallis* x 'Dainty Deb' and 'Sarah Sikes hybrid' were separated into single shoot divisions and subjected to similar methodology as plants were in 2000 except as noted below. The substrate was amended per m^3 with 9.5 kg (16 lb/yd^3) 17N–2.6P–10K (Polyon 17–6–12, 9-month formulation, Pursell Industries, Sylacauga, AL). On May 10, plants were graded for uniformity and blocked by number of initial ramets, ranging from 0 to 2. Treatments were arranged in a randomized complete block design within cultivar and replicated with 10 single plants. Temperature and relative humidity at treatments were 29.5C (85F) and 66% (May 11), 31.7C (89F) and 68% (May 18), 31.1C (88F) and 62% (May 25), 22.8C (73F) and 97% (June 1) and 30.6C (87F) and 70% (June 8). On all dates, except June 1, plants were removed from irrigation, treated outdoors and not irrigated until the following day. On June 1, plants were removed from irrigation and placed inside a greenhouse for treatment due to inclement weather. New ramets not present at initial treatment were counted at 3, 6, 9 and 12 WAT.

Data from both experiments were analyzed using one-way analysis of variance (ANOVA) and two-way ANOVA to test the interaction between BA concentration and number of consecutive applications of BA. Trend analyses were determined by the general linear models procedure using orthogonal contrasts (SAS Institute, Cary, NC).

Results and Discussion

There were no interactions between concentration and number of applications of BA for ramet counts in any cultivar in either experiment, hence only main effects are reported. Neither were there any adverse effects on plant appearance due to concentration or number of consecutive applications of BA.

2000. At 3 weeks after initial treatment (WAT) ramet counts in 'Lavinia Love' changed quadratically in response to increasing BA applications, regardless of concentration (Table 1). A single BA application more than doubled ramet production compared to control plants. Maximum ramet production at 3 WAT occurred following two applications of BA, with ramet production more than three times that of the controls. At 6 WAT, ramet counts increased linearly in response to increasing BA applications with 3.1 and 2.9 more ramets forming in plants receiving 4 and 5 applications, respectively, than in controls. Similar linear increases in ramet counts per pot with increasing application number occurred at 9 WAT and after dormancy (27 WAT). This large early increase followed by a leveling off in ramet production concurs with earlier work showing the short-lived properties of BA (5, 7, 11, 12, 13).

Ramets on 'Lavinia Love' increased linearly in response to increasing concentration, regardless of BA application number. Compared to controls, ramets increased by 162, 164, 171, and 150% at 3, 6, 9 and 27 WAT, respectively, when treated with 2500 ppm BA. Increasing BA concentration from 2500 ppm to 5000 ppm increased new ramet production to 192, 207, 214 and 167% more than that of controls at 3, 6, 9 and 27 WAT, respectively. Greater effectiveness of 5000 ppm BA compared to 2500 ppm agrees with a previous study with 'Harbour Dwarf' nandina (11).

Plants of 'Beguine' increased ramet production linearly at all collection dates in response to increasing application number except 6 WAT, when the response was quadratic (Table 2). When plants were treated with five BA applications, new ramets increased up to 37, 37, 32 and 41% at 3, 6, 9, and 27 WAT, respectively, regardless of BA concentration. Responses to BA application number were less pronounced in 'Beguine' than in 'Lavinia Love' due to much higher ramet formation

Table 1. Mean number of ramets formed in 'Lavinia Love' daylily as affected by consecutive weekly BA applications and concentration. Treatments were made in 2000 on newly potted and established single-shoot divisions. n = 10.

Consecutive weekly applications	Weeks after initial treatment			
	3	6	9	27
0	1.3	1.4	1.4	1.8
1	2.8	3.1	3.0	3.3
2	4.1	4.2	4.4	5.1
3	3.7	4.0	4.3	4.8
4	—[z]	4.5	4.6	4.8
5	—[z]	4.3	4.4	5.1
Significance[y]	Q***	L***	L***	L***
Concentration (ppm)	**Weeks after initial treatment**			
	3	**6**	**9**	**27**
0	1.3	1.4	1.4	1.8
2500	3.4	3.7	3.8	4.5
5000	3.8	4.3	4.4	4.8
Significance	L***	L***	L***	L***

[z]At 3 WAT, applications 4 and 5 had not been applied; data from plants receiving 3, 4, or 5 applications were averaged.

[y]Application number × concentration not significant, hence means are averaged over either concentration or application number; trend analyses linear (L) or quadratic (Q) at $P = 0.001$ (***).

Table 2. **Mean number of ramets formed in 'Beguine' daylily as affected by consecutive weekly BA applications and concentration. Treatments were made in 2000 on newly potted and established single-shoot divisions. n = 10.**

Consecutive weekly applications	Weeks after initial treatment 3	6	9	27
0	3.0	3.5	3.4	3.9
1	4.3	4.3	4.1	4.9
2	3.7	4.3	4.3	4.9
3	4.1	4.8	4.5	5.1
4	—[z]	4.5	4.4	5.4
5	—[z]	4.1	4.5	5.5
Significance[y]	L*	Q*	L*	L**
Concentration (ppm)	**Weeks after initial treatment 3**	**6**	**9**	**27**
0	3.0	3.5	3.4	3.9
2500	4.0	4.4	4.3	5.1
5000	4.1	4.4	4.4	5.2
Significance	L*	NS	NS	L*

[z]At 3 WAT, applications 4 and 5 had not been applied; data from plants receiving 3, 4, or 5 applications were averaged.

[y]Application number × concentration not significant, hence means are averaged over either concentration or application number; trend analyses nonsignificant (NS), linear (L), or quadratic (Q) at *P* = 0.05 (*) or 0.01 (**).

Table 3. **Mean number of ramets formed in 'Dainty Deb' daylily as affected by consecutive weekly BA applications and concentration. Treatments were made in 2004 on newly potted and established divisions. n = 10.**

Consecutive weekly applications	Weeks after initial treatment 3	6	9	12
0	0.0	0.5	1.7	2.6
1	0.2	1.9	3.0	4.4
2	0.3	2.1	3.2	4.2
3	0.3	2.9	4.2	5.7
4	—[z]	3.2	4.1	5.0
5	—[z]	3.0	4.7	5.2
Significance[y]	L*	L***	L***	Q***
Concentration (ppm)	**Weeks after initial treatment 3**	**6**	**9**	**12**
0	0.0	0.5	1.7	2.6
2500	0.2	2.1	3.6	4.7
5000	0.4	3.0	4.0	5.0
Significance[x]	NS	L***	L***	L***

[z]At 3 WAT, applications 4 and 5 had not been applied; data from plants receiving 3, 4, or 5 applications were averaged.

[y]Application number × concentration not significant, hence means are averaged over either concentration or application number; trend analyses nonsignificant (NS), linear (L), or quadratic (Q) at *P* = 0.05 (*) or 0.001 (***).

in untreated 'Beguine' control plants, but similar ramet counts for the two cultivars when treated with BA. Untreated plants of 'Beguine' averaged 3.0 new ramets per pot. The maximum number of ramets per pot after dormancy was 5.5 new ramets per pot in plants treated with five BA applications, an average increase of 1.6 ramets per pot compared to controls. In 'Lavinia Love' the difference between maximum ramet counts and controls was 3.3 ramets per pot, indicating cultivar differences in *Hemerocallis* when treated with BA. Cultivar differences in response to BA have been previously reported (4).

Ramet counts in 'Beguine' increased linearly in response to increasing concentration of BA at 3 and 27 WAT, but were unaffected by BA concentration at 6 and 9 WAT. At 3 WAT plants treated with 5000 ppm BA showed an average increase of 1.1 ramet compared to controls. This difference remained nearly constant throughout data collection. After dormancy, plants treated with 5000 ppm BA produced 5.2 ramets per plant on average compared to 3.9 by untreated control plants.

2004. Ramet formation in 'Dainty Deb' increased linearly at all sampling dates in response to increasing BA application number (Table 3). Increases at 3 WAT averaged only 0.2 to 0.3 ramets and may reflect treatment of plants less established than in 2000 (13) or cultivar differences (4). At 6 WAT, increases in ramet formation relative to untreated control plants ranged from 280% to 500% as applications increased. At 9 WAT, plants that had received a single BA application had nearly twice the number of ramets as did the controls. Maximum ramet production at 9 WAT was 4.7 in plants that received five BA applications, 176% more ramets per pot than plants receiving five BA applications at 6 WAT. A quadratic response was observed at 12 WAT, when plants treated with a single BA application increased ramet production 69% relative to untreated controls. Numeric maximal ramet formation was reached at 12 WAT in plants that received three BA applications, a 119% increase relative to untreated controls. Ramet formation in 'Dainty Deb' increased linearly in response to increasing BA concentration, except at 3 WAT

Table 4. **Mean number of ramets formed in 'Sarah Sikes hybrid' daylily as affected by consecutive weekly BA applications and concentration. Treatments were made in 2004 on newly potted and established divisions. n = 10.**

Consecutive weekly applications	Weeks after initial treatment 3	6	9	12
0	0.0	1.1	2.9	3.5
1	0.2	2.1	3.4	4.1
2	0.1	2.7	4.0	4.7
3	0.2	2.5	3.8	4.7
4	—[z]	2.8	4.3	4.8
5	—[z]	3.0	5.0	5.1
Significance[y]	NS	L***	L***	L**
Concentration (ppm)	**Weeks after initial treatment 3**	**6**	**9**	**12**
0	0.0	1.1	2.9	3.5
2500	0.1	2.5	4.0	4.7
5000	0.2	2.7	4.2	4.6
Significance[x]	NS	L***	L*	NS

[z]At 3 WAT, applications 4 and 5 had not been applied; data from plants receiving 3, 4, or 5 applications were averaged.

[y]Application number × concentration not significant, hence means are averaged over either concentration or application number; trend analyses nonsignificant (NS) or linear (L) at *P* = 0.05 (*), 0.01 (**), or 0.001 (***).

when there was no response, regardless of number of applications. Increases were 500, 135, and 92% at 6, 9, and 12 WAT, respectively. The decrease in the percent difference over time reflects the natural formation of ramets in control plants.

Plants of ‘Sarah Sikes hybrid’ increased ramet production linearly with increasing number of BA applications, except at 3 WAT, when there was no response (Table 4). Relative to ramet counts in control plants, increases in ramets from five BA applications ranged from 173% at 6 WAT, to 72% at 9 WAT, and 46% at 12 WAT. These trends highlight the relatively rapid increase in ramet formation in daylily following BA applications, followed by a more gradual increase. Ramet production in ‘Sarah Sikes hybrid increased linearly at 6 and 9 WAT with increasing BA concentration, but was not affected by concentration at 3 or 12 WAT. Increases in ramet formation relative to those of control plants were 145% at 6 WAT and 45% at 9 WAT. All cultivars in these two experiments increased ramet formation in response to a single foliar application of BA, regardless of concentration. This response concurs with results of Swann (15) who used various application methods of BA to increase ramet formation in ‘Stella D’Oro’ daylilies. However, our results show a clear benefit to using multiple foliar applications of BA to increase ramet formation of daylily cultivars. The number of ramets produced on newly potted, single-shoot divisions of daylilies were significantly increased by 1 to 3 consecutive weekly of BA at 2500 ppm, depending on cultivar. Increasing the number of consecutive applications or the concentration of BA to 5000 ppm produced only slight further increases. These responses to BA agree with those of others showing multiple BA applications to be more effective than a single BA application (5, 6, 14).

Ramet formation increased linearly in response to increased BA concentration or was unaffected by concentration. Similar increases in offset formation in hostas with increased BA concentration have previously been reported (4, 8), although the response was cultivar dependent in hostas (4).

Plants of all daylily cultivars formed more ramets when treated with multiple applications of BA, thus providing plantlets for propagation. Ramet formation was rapid following multiple BA applications, potentially allowing removal of plantlets as early as 3 to 6 WAT. Treated plants were fuller than untreated controls, and if the ramets stimulated to form by BA were not removed for propagation, plants may be more marketable or marketable sooner.

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