



**FOLIAR SPRAYS OF BENZYLADENINE INCREASE BUD AND PROPAGULE PRODUCTION IN
EPIMEDIUM X RUBRUM MORREN AND *HELLEBORUS X HYBRIDUS* L.**

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Abstract

Epimediums and hellebores are herbaceous perennials that are slow to increase and command high prices. This research examined the influence of N⁶-Benzyladenine (BA) foliar sprays on bud development, propagule production and plant performance of *Epimedium x rubrum* Morren and *Helleborus x hybridus* L. To determine an appropriate BA concentration, plants were sprayed with 0, 1500, 3000, 4500 or 6000 mg l⁻¹ BA once in the spring. In a separate study, epimediums and hellebores were sprayed in May, June, July and August with 0, 20, 40, 60 or 80 ml of 3000 mg l⁻¹ BA. Single spring sprays resulted in few significant differences for *Epimedium* and no differences for *Helleborus*. Multiple sprays at 40 and 60 ml for *Epimedium* and at 60 and 80 ml for *Helleborus* doubled the number of buds in comparison to controls. Optimum BA sprays allowed for one or two additional propagules to be derived per epimedium plant. The number of propagules that could be made per hellebore plant was higher than the control for any volume of BA applied. BA caused foliar phytotoxicity in the form of severe burn and necrosis in *Epimedium*. For *Helleborus*, end of the season BA applications stimulated bud outgrowth in the late summer and fall, however this growth was sensitive to injury during overwintering. Hellebores treated with BA produced subsequent foliage with altered leaf morphology.

Key words: Cytokinin, division, herbaceous perennial, rhizome.

INTRODUCTION

Epimediums and hellebores are shade-tolerant herbaceous perennials. There is building interest in these plants because of their garden versatility, diverse ornamental attributes and potential economic value. Epimediums have evergreen, semi-evergreen or deciduous leaves that emerge in bright colors and elegant bell-shaped and often spurred flowers in spring. Hellebores have attractive deciduous or evergreen foliage and large cup-shaped flowers from January to April. Epimediums are commonly propagated by rhizome division to preserve cultivar identity and because of low seed viability (Nau 1996, Probst 1998). Hellebores can be produced from seed but this method of propagation results in progeny of variable quality, with some offspring resembling the parents and others with limited ornamental value. Variability in seed progeny and the rising demand for hellebore cultivars with striking flower arrangement and color make rhizome division the preferred method of hellebore production for many growers.

Unlike many other herbaceous perennials, epimediums and hellebores are slow growing. As a result, few propagules (divisions) may be taken from individual

stock plants. In addition, it may take up to two growing seasons for nurseries to produce a salable plant from these divisions. This slow generation time makes it difficult for growers to produce these plants inexpensively and has resulted in a high wholesale cost per plant, which must be passed along to consumers. Epimediums and hellebores typically cost 40% more than other shade tolerant perennials (The Plant Group Inc. 2003 Catalog, Sunny Border Inc. 2003 Catalog). Less common epimedium and hellebore species and cultivars can retail for \$25 to \$100 for small pot liners (Probst 2004).

Vegetative buds of epimedium and hellebore grow from rhizomes, but lateral bud development is suppressed by apical dominance of the rhizomic apex. The mechanism of apical dominance is regulated by the hormonal interaction between auxins and cytokinins (Cline 1988). N⁶-Benzyladenine (BA), a synthetic cytokinin, has been used to stimulate outgrowth of suppressed rhizomic buds in hosta (Keever 1994). Garner et al. (1998) have shown that sequential BA applications maximize bud development and propagule production in hosta. BA has also been used to facilitate shoot multiplication in three epiphytic species of *Tillandsia* (Bessler 1997).

Our research focused on applying foliar sprays of BA to *Epimedium* and *Helleborus* in order to increase bud production. Consequently, more propagules could be made and the time and cost of plant propagation could be reduced. To determine a concentration of BA that is effective in promoting rhizomic bud development in epimedium and hellebore we sprayed plants with increasing concentrations of BA once in the spring. In addition, we conducted a volume study to evaluate the influence of sequential applications of increasing quantities of BA solution on bud and propagule production for these species.

MATERIALS AND METHODS

Epimedium

To produce uniform liners of epimedium for this research, 4 l container grown stock plants of *Epimedium x rubrum* Morren were divided into three to six bud divisions (propagules) and potted in 1.4 l nursery containers in March 2001 and 2002. Dormant stock plants were held in a minimally heated greenhouse (setpoint 0°C) until needed for experimentation. Divisions were made by washing the potting medium from the crown, trimming roots to 10-12 cm, then cutting through the crown with a knife. Divisions were potted in Metro Mix 510 Growing Medium (Scotts Co., Marysville, Ohio). To facilitate the establishment of divisions they were grown in a greenhouse with set points of 21°C day and 17°C night and natural lighting for eight weeks following division, before being moved to cold frames. Cold frames were equipped with sand and weed barrier floors, concrete walls and 50% lath shading.

BA solutions were prepared by diluting concentrated BAP-10 (100 g l⁻¹ BA) solution (Plant-Wise Biostimulant Co., Louisville, KY) in distilled water. Tween 20 (Fisher Scientific, Fair Lawn, NJ) at 1 ml l⁻¹ was added to all BA solutions as a surfactant before foliar application. Application was made with a 3-gallon capacity Solo 457 Pressure Sprayer (Solo Inc., Newport News, Virginia). For the concentration study, plants were randomly assigned to receive a single foliar application of BA at 0, 1500, 3000, 4500 or 6000 mg l⁻¹ on May 15 of both years. BA sprays were applied until foliar runoff with each plant receiving approximately 20-ml of solution. For the volume study, plants were randomly assigned to receive 0, 20, 40, 60 or 80-ml of 3000 mg l⁻¹ BA once in May, June, July and August. For both studies, control treatments without BA were sprayed with distilled water and Tween 20. Plants were grown in cold frames until the end of the growing season. Plants were hand-weeded, irrigated as needed and provided a soluble 20N-8.74P-116.6K fertilizer (Peters 20-10-20 Fertilizer, Scotts Co., Marysville, Ohio) at 150 mg l⁻¹ nitrogen every 14 days. Plant harvest and quantification took place in October of both years.

Helleborus

For this research 2 l container grown seedling progeny of *Helleborus x hybridus* were acquired from a local grower in winter 2002. Plants were held in a minimally heated greenhouse (setpoint 0°C) until needed for experimentation. In May 2003, hellebores were moved to cold frames similar to the ones used for *Epimedium*, where they remained until the end of the growing season. The concentration and volume studies were accomplished as outlined for *Epimedium*. Plants were irrigated, fertilized and weeded as described for *Epimedium*. Plant harvest and quantification was accomplished in October 2003.

Data Collection

For both epimedium and hellebore, plant harvest was accomplished by un-potting the plants, shaking the potting media from the roots and washing the crowns. During plant harvest the number of leaves and buds were counted. To estimate propagation potential, the number of two to three bud propagules that could be made from each plant was determined. Leaf fresh and dry weight was recorded. Leaf area was measured using a LI-COR model LI-3100 leaf area meter (LI-COR, Lincoln, Nebr.).

Experimental Design and Statistical Analysis

Epimediums and hellebores for both the concentration and volume studies were arranged separately in the cold frames as a randomized complete block design with 5 treatments. For *Epimedium*, both the concentration and volume studies were repeated for two years and the data from both years was combined for statistical analysis resulting in 20 replications per treatment. For *Helleborus*, there were 10 replications per treatment since this study occurred in 2003 only. Analysis of variance (ANOVA) was performed on data using statistical analysis systems (SAS Institute, 1999) and the ANOVA (PROC ANOVA) procedure. When effects were significant at $P \leq 0.05$, treatment means were separated using Dunnett's test.

RESULTS AND DISCUSSION

Epimedium

For the single spring sprays of BA there were very few significant differences (Table 1). BA was observed to cause symptoms of foliar toxicity in the form of severe marginal necrosis to full necrosis for *E. x rubrum*. This is shown in the data as significantly smaller leaf area at 4500 and 6000 mg l⁻¹ in comparison to the control. Although not statistically significant, there was also a clear negative trend in leaf weight as BA concentration increased.

As with single BA applications, multiple applications caused substantial foliar damage in terms of re-

Table 1. The growth response of *Epimedium x rubrum* to increasing concentration of BA applied as a single foliar spray in spring.

BA concentration mg l ⁻¹	Means ^z					Leaf area (cm ²)
	No. of leaves	No. of buds	No. of propagules ^y	Leaf weight (g)		
				Fresh	Dry	
0	14.1	20.3	3.8	7.7	2.8	373.8
1500	12.0	24.5	4.3	7.6	2.7	338.3
3000	13.0	20.3	3.7	6.5	2.5	291.2
4500	12.3	23.1	4.2	6.5	2.4	217.4*
6000	19.0*	20.6	3.9	6.1	2.3	237.3*

^zMeans within columns followed by an * are significantly different from the control (0 BA) according to Dunnett's Test $P \leq 0.05$, n=20.

^yNumber of possible two to three bud propagules (divisions) that could be made per plant.

Table 2. The growth response of *Epimedium x rubrum* to increasing volume of BA at 3000 mg l⁻¹ applied in May, June, July and August.

Volume of BA (ml) ^y applied/month	Means ^z					Leaf area (cm ²)
	No. of leaves	No. of buds	No. of propagules ^x	Leaf weight (g)		
				Fresh	Dry	
0	9.2	23.0	4.2	4.4	1.8	245.0
20	6.8	31.5	4.9	2.3*	1.2	170.4*
40	5.0*	42.3*	6.0*	1.6*	0.9*	117.4*
60	3.1*	40.6*	5.9*	1.1*	0.6*	82.3*
80	2.9*	37.4*	4.9	0.6*	0.3*	61.7*

^zMeans within columns followed by an * are significantly different from the control (0 BA) according to Dunnett's test $P \leq 0.05$, n=20.

^yVolume of BA at 3000 mg l⁻¹ applied in May, June, July and August.

^xNumber of possible two to three bud propagules (divisions) that could be made per plant.

ductions in leaf area, leaf fresh and dry weight, and number of leaves (Table 2). In addition, increasing volumes of BA lead to increasing reductions in leaf number, leaf fresh and dry weight, and leaf area due to severe burn and necrosis. Plants receiving the highest volume (80 ml) had only one quarter the leaf area as controls. Despite the loss in photosynthetic area, BA volume of 40 ml resulted in development of significantly more buds per plant and BA volumes of 40 or 60 ml nearly doubled the number of buds in comparison to controls (Table 2). The increased number of buds at 40 or 60 ml of applied BA can be translated into the ability to make more division propagules. Optimum BA sprays of 40 or 60 ml allowed for one or two additional propagules to be derived per plant.

In preliminary trials with BA applied to *E. x versicolor* 'Sulphureum' Morren and *E. pinnatum* ssp. *colchicum* Boiss, two semievergreen to evergreen species with thicker foliage than *E. x rubrum*, these species exhibited better tolerance of BA as far as phytotoxicity was concerned. Without the loss of photosynthetic area due to phytotoxicity, these species may show a stronger response to and benefit more from BA appli-

cations than *E. x rubrum*. Additional research examining BA use on a broad range of *Epimedium* species should be pursued.

Helleborus

Single spring sprays did not result in significant differences for the plant growth parameters measured for *Helleborus* (Table 3). Although not statistically different due to a relatively small number of replications (n=10), BA sprays, especially at higher concentrations, may have resulted in production of additional buds and potential propagules when compared to 0 BA controls. When different volumes of BA were applied to hellebores once a month, bud number was significantly greater than the control for both the 60 and 80 ml applications (Table 4). Plants receiving these higher volumes developed at least two times as many buds as controls. In addition, the number of propagules that could be made was significantly higher than the control for any volume of BA applied. Application of higher volumes of BA also appeared to increase leaf fresh weight, but the increase was not statistically significant, again due to the relatively small number of replications (n=10).

Table 3. The growth response of *Helleborus x hybridus* to increasing BA concentration applied as a single foliar spray in spring.

BA concentration mg l ⁻¹	Means ^z					Leaf area (cm ²)
	No. of leaves	No. of buds	No. of propagules ^y	Leaf weight (g)		
				Fresh	Dry	
0	11.3	3.4	2.6	40.1	9.0	464.4
1500	13.0	4.9	3.2	43.3	9.7	478.6
3000	12.8	5.5	3.4	36.4	8.5	436.1
4500	15.9	5.0	3.8	49.6	11.4	535.9
6000	15.6	5.0	3.8	42.9	9.7	472.5

^zMeans within columns followed by an * are significantly different from the control (0 BA) according to Dunnett's Test $P \leq 0.05$, n=10.

^yNumber of possible two to three bud propagules (divisions) that could be made per plant.

Table 4. The growth response of *Helleborus x hybridus* to increasing volume of BA at 3000 mg l⁻¹ applied in May, June, July and August.

Volume of BA (ml) ^y applied/month	Means ^z					Leaf area (cm ²)
	No. of leaves	No. of buds	No. of propagules ^x	Leaf weight (g)		
				Fresh	Dry	
0	14.8	3.5	3.2	38.7	8.5	467.2
20	19.9	5.5	4.6*	62.1	11.6	573.5
40	23.3*	5.4	5.0*	72.0*	13.4	643.1
60	21.1	7.5*	5.3*	65.4*	12.0	613.6
80	19.4	8.9	5.1*	71.0*	18.1*	619.0

^zMeans within columns followed by an * are significantly different from the control (0 BA) according to Dunnett's test $P \leq 0.05$, n=10.

^yVolume of BA at 3000 mg l⁻¹ applied in May, June, July and August.

^xNumber of possible two to three bud propagules (divisions) that could be made per plant.

BA applications made at the end of the season tended to stimulate bud outgrowth in late summer and fall, a time of year when hellebores are typically dormant. In additional studies not reported here, we found that the fall-stimulated growth was sensitive to injury during overwintering. Furthermore, some BA treated plants that were overwintered and forced in the spring, produced foliage with altered morphology. The affected foliage was somewhat stunted, lighter green in color and was frequently more dissected than normal.

When deciding whether to incorporate BA foliar sprays during propagation of *Helleborus*, the unfavorable foliar effects that can result need to be considered along with the advantages of more buds. Plants showing the altered leaf morphology may require a longer three-year propagation cycle to produce a salable plant with normal appearance. Additional studies should be conducted to determine if proper timing of BA applications can reduce or prevent abnormal foliar development following dormancy.

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REFERENCES

- BESSLER B. (1997). The use of 6-benzylaminopurine for rapid multiplication of tillandsias. *HortScience*, 32: 256-258.
- CLINE M. G. (1988). Apical dominance. *Botanical Review*, 57: 318-358.
- GARNER J. M., KEEVER G. J., EAKES D. J., KESSLER J. R. (1998). Sequential BA applications enhance offset formation in hosta. *HortScience*, 33: 707-709.
- KEEVER G. J. (1994). BA-induced offset formation in hosta. *Journal of Environmental Horticulture*, 12: 36-69.
- NAU J. (1996). *Ball Perennial Manual: Propagation and Production*. Ball Publishing, 487 pp.
- PROBST D. R. (1998). *Garden Elegance*. American Nurseryman, 187 (5): 34-40.
- PROBST D. (2004). *Garden Vision Catalog*. Probst D. (Ed.). Hubbardston, MA, USA, 20 pp.
- SAS INSTITUTE (1999). *Software release 8.02*. SAS Inst., Cary, N.C.
- SUNNY BORDER INCORPORATED. (2003). *Sunny Border Catalog*. Bennerup P. (Ed.). Kensington, CT, USA, 70 pp.
- THE PLANT GROUP INCORPORATED. (2003). *Plant Group Catalog*. Frinberg I. (Ed.), 52 pp.