

Effect of Plant Growth Regulators on Propagule Formation in *Hemerocallis* spp. and *Hosta* spp.

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Abstract. Field experiments were conducted in 2002 and 2003 to evaluate the effects of selected plant growth regulators on propagule production in *Hemerocallis* 'Happy Returns' and *Hosta* 'Gold Standard'. Benzyladenine (BA), chlormequat chloride (Cycocel), ethephon (Ethrel), prohexadione calcium (Apogee), and an experimental preparation of commercial seaweed extract (Acadian Seaplants Limited Liquid Seaweed Concentrate) amended with BA and IBA were tested at two times of application and three rates of application. Results with *Hemerocallis* showed that the application of the seaweed/PGR mixture at 3000 mg·L⁻¹, Cycocel at 3000 mg·L⁻¹ or BA at 2500 mg·L⁻¹ applied at flowering, increased the number of plants producing two divisions compared to control plants. In *Hosta*, no increase in divisions under any treatments was observed.

In fast-track field production of bare-root perennials, plants are grown for only one season. They are transplanted into the field in the spring, harvested and divided in the autumn of the same year. In this system, the objective is to produce as many reproductive divisions, or propagules, as possible from the plant harvested. *Hemerocallis* and *Hosta* are conventionally propagated by crown division but the number of propagules obtained through this technique is relatively low, only a few plants per mother plant (Keever et al., 1995). The crown is the part of a plant at the surface of the ground from which new shoots and roots are produced (UIUC, 1999). To be viable, a crown division must be composed of shoots, crown and roots. The exogenous application of BA to the mother plant of *Hosta* has been shown to stimulate offset formation resulting in a more rapid multiplication (Keever et al., 1995). BA also induces differentiation of adventitious buds, breaks bud dormancy, releases lateral buds from apical dominance and stimulates elongation of axillary buds (Keever, 1994). Other plant growth regulators are also used in ornamental horticulture: e.g., ethephon increases lateral branching in ornamental pepper (Khademi and Khosh-Khui, 1977); prohexadione-calcium reduces stem elongation in pear and apple (Costa et al., 2001; Guak et al., 2001); and chlormequat chloride produces plants with multiple buds per shoot (Gent and McAvoy, 2000). Prohexadione-calcium and chlormequat chloride are inhibitors of gibberellin biosynthesis, while ethephon is an ethylene-releasing compound. It was postulated that these products might also have an effect on increasing the number of divisions in *Hemerocallis* and *Hosta* under the fast-track system.

In the past few decades, interest in using

seaweed extracts in horticultural and agricultural crops has increased considerably (Norrie and Hiltz, 1999). Their beneficial effects are attributed to the presence of natural plant growth substances, especially cytokinins and substances mimicking cytokinin activity. Cytokinins, as well as other plant biostimulants (e.g., betaines), can improve plant resistance and tolerance to environmental stresses, diseases and insects (Norrie and Hiltz, 1999). Seaweed extracts have also been reported to improve growth, seed germination, flower production, fruit set, crop yield and frost hardiness (Abetz and Young, 1983; Lizzi et al., 1998). Lin and Chuen (1995) demonstrated that seaweed extract, in combination with BA and IBA, increased the number of tillers in *Zizania latifolia*, a perennial monocotyledon. This suggests that there may be potential for using this treatment combination to increase propagule production in bare-root perennials as well.

With little information available on the species under study, the current research attempted to enhance our knowledge on the effects of selected plant growth regulators on propagule production of *Hemerocallis* spp. and *Hosta* spp.

Materials and Methods

Field experiments were conducted at Balamore Farm Ltd, Great Village, Nova Scotia, Canada (45°, 25'N; 63°, 34'W) in 2002 and 2003. Replicated small plot field trials tested the effects of several plant growth regulators on *Hemerocallis* 'Happy Returns' and *Hosta* 'Gold Standard'. In 2002 and 2003, the two crops were planted on 22 and 28 May, respectively, using a modified Mechanical Transplanter model Holland 1500. One perennial bed consisted of three rows, 45 cm apart, with a spacing of 20 cm between plants within rows. Irrigation was provided every 3 to 4 d, starting at the end of June. Fields were fertilized before planting with

560 kg·ha⁻¹ of 12N–10P–20K, at the beginning of June with 392 kg·ha⁻¹ of 9N–4P–7K and at the beginning of August with 336 kg·ha⁻¹ of 9N–4P–7K. Plots measured 1.5 × 1 m in 2002; plot size in 2003 was 1 × 1 m. Each plot contained 15 to 20 plants; 5 plants per plot were randomly selected for measurements.

In 2002, plant growth regulators were applied using a CO₂ backpack sprayer with a 2-m boom and 8002 XR Tee-jet nozzle tips. One nozzle was blocked to allow coverage of 1.5 m of ground surface. The pressure was set at 207 KPa. In 2003, the plots being smaller, one 8003 LP Tee-jet nozzle tip was used and the pressure was set at 138 KPa. In both cases, the sprayer was calibrated to deliver 300 L·ha⁻¹ of spray solution.

Plant growth regulators

Five different formulations of plant growth regulators were used: 1) liquid formulation of BA, N-6-benzyladenine solution (BAP-10) (Plant-Wise Biostimulant Co., Ky.); 2) Cycocel Extra, chlormequat chloride at 460 g·L⁻¹ active ingredient (a.i.) (BASF Canada Inc., Ont., Canada); 3) Ethrel, ethephon at 240 g·L⁻¹ (a.i.) (Bayer CropScience Inc., AB, Canada); 4) Apogee, prohexadione calcium at 27.5 g·L⁻¹ (a.i.) (BASF Canada Inc., Ont., Canada); 5) seaweed/PGR mixture: liquid seaweed concentrate (Acadian Seaplants Ltd. liquid seaweed concentrate) (Acadian Seaplants Ltd., NS, Canada), indole-3-butyric acid (4-[3-Indolyl] butanoic acid, potassium salt) (Sigma Aldrich Co.), and N-6-benzyladenine solution (BAP-10) (Plant-Wise Biostimulant Co., Ky.). Commercial names will be used for the remainder of the manuscript.

Statistical design

Season 1 (2002). A separate three-factor factorial experiment was designed for each crop; the three factors were 1) plant growth regulator type, 2) rate of application, and 3) timing of application. Plant growth regulators used were BA, Cycocel, Ethrel, Apogee, and seaweed extract + 0.10% IBA + 0.05% BA. Rates used were 1×, 2×, and 3×, which represented different concentration for each product: BA = 1250, 2500, or 3750 mg·L⁻¹, Cycocel = 1000, 2000, or 3000 mg·L⁻¹, Ethrel = 300, 600 or 900 mg·L⁻¹, Apogee = 75, 150 or 225 mg·L⁻¹, seaweed extract + 0.10% IBA + 0.05% BA: 1000, 2000, or 3000 mg·L⁻¹ of seaweed extract. There were two timings of application: 1 July and 1 Aug. 2002.

Each experiment was replicated three times and two untreated control plots were included in each replication. Plants were transplanted into the field on 22 May and harvested on 22 Aug. 2002, due to the need for an early commercial harvest that year (this was an earlier date than planned).

Season 2 (2003). The three plant growth regulators which presented the best results from the previous field season were used for the second season, i.e., BA, Cycocel, and the seaweed/PGR mixture. The two crops were transplanted into the field on 28 May and harvested on 3 Oct. 2003. Factorial design experiments were performed with the same

factors as for season 1 with the exception that three PGRs instead of five were applied, four rates were tested: 0 (control), 1×, 2×, and 3×, and the two timings of application were 9 July and 12 Aug. 2003. Each experiment was replicated three times.

Stages of plant development and data collection

Season 1 (2002). The first PGR application (1 July) corresponded to the preflowering stage for the two species. Plant height ranged from 10 to 20 cm for *Hemerocallis* and growth index ranged from 5 to 15 cm for *Hosta*. At the time of the second application (1 Aug.), *Hemerocallis* plants were flowering and height ranged from 30 to 40 cm. For *Hosta*, 25% of plants were also flowering but most remained at a preflowering stage, with a growth index ranging from 10 to 20 cm. At harvest (22 Aug.), *Hemerocallis* was at the flowering stage and *Hosta* was at the preflowering stage.

Plant height (measured from the ground to the tallest extended leaf) for *Hemerocallis* and a growth index [GI = (height + width at widest point + width 90° to first width)/3] (Keever, 1994) for *Hosta* were measured on 10 July and 14 Aug.. On 22 Aug., five randomly selected plants were harvested from each plot using a garden fork and the number of divisions per plant was recorded.

Season 2 (2003). Stages of development for both species, at first and second PGR application were the same as in season 1. The first and second PGR applications were performed on 9 July and 12 Aug. 2003, respectively. At harvest (3 Oct.), both species were at the end of the flowering process.

There were few statistically significant differences in variables measured during the early part of the 2002 season; therefore, variables were measured only at harvest in 2003. Plant height for *Hemerocallis* and a growth index for *Hosta* were measured. The number of shoots or fans and the number of divisions per plant were recorded, in addition to shoot and root fresh weights.

Statistical analysis

For each experiment, data were compiled and analyzed using the statistical software package SAS (SAS Institute Inc., 1999). An analysis of variance at levels $\alpha = 0.05$ and $\alpha = 0.10$ was conducted on all data sets using the PROC GLM procedure. When means showed significant differences at these levels, a least square means (lsmeans) comparison test was performed. For some data sets, a group comparison using Minitab two-sample *t* test (Minitab Inc., 2000) was performed to specifically compare untreated plants with plants receiving specific treatments.

Results and Discussion

Hemerocallis

The summary of ANOVA for the effect of PGR type, PGR rate and timing of application on the variables measured during the experiments is presented in Table 1.

In 2002, the application of Cycocel at 3000 mg·L⁻¹ was the only treatment that produced a significantly ($\alpha = 0.10$) higher number of divisions compared to the controls. In the controls, 83% of plants produced one division per plant and 17% produced two divisions per plant, while a third of the plants that received Cycocel at 3000 mg·L⁻¹ produced one division and the remaining 66% produced two divisions per plants (data not shown). This represented a significant increase in economic yield compared to the controls.

In 2003, root fresh weight and the number of divisions per plant were higher for plants treated with the seaweed/PGR mixture and Cycocel at 3000 mg·L⁻¹, both applied at the flowering stage (timing 2), compared to control plants (Table 2). The application of BA at 2500 mg·L⁻¹ also produced more divisions per plant than the controls. The application of the seaweed/PGR mixture at 3000 mg·L⁻¹ produced the highest number of fans per plant (Table 2). For the control plants, 72% produced two divisions per plant while this proportion increased to 100% following the application of the seaweed/PGR mixture and Cycocel at 3000 mg·L⁻¹ at flowering (timing 2), and after the application of BA at 2500 mg·L⁻¹ also at this time (Table 3).

Due to the complexity of its composition, the specific mode of action of the seaweed/PGR mixture is not clear. The cytokinin-like substances (Sanderson and Jameson, 1986), by their action in releasing apical dominance, might be acting on the redistribution of resources toward crown development, producing a higher number of divisions. The presence of other plant biostimulants such as betaines (Whapham et al., 1993), or the presence of macro and micronutrients (Zodape, 2001) may improve the overall growth of the plant allowing for the production of more divisions.

Reallocation of resources may also be observed after the application of Cycocel. By its action as a gibberellin synthesis inhibitor, Cycocel has been shown to reduce shoot growth in many species; consequently, more carbohydrates may preferentially be translocated to crown or root development (Green et al., 1986).

The increase in divisions after BA treatment is consistent with previous studies where BA was shown to induce offset formation in *Hosta* (Keever, 1994).

Hosta

In 2002, there was no significant difference due to PGR treatments on the number of divisions per plant of *Hosta*. In 2003, however, there was a significant interactive effect of PGR type and timing of application for plant height ($P = 0.0001$), growth index ($P = 0.049$), and the number of vegetative buds per plant ($P = 0.029$) (Table 4). There was no significant effect on shoot and root fresh weight, on the number of shoots per plant or on the number of divisions per plant (data not shown).

These results are inconsistent with previous studies that have evaluated the effect of the exogenous application of BA on *Hosta* and have resulted in increased offset formation (Keever, 1994). In Keever's experiments, single eye divisions (no offset) were more likely to develop new offsets following the application of BA than were the multiple offset plants. In the current study, since the plants originated from a commercial operation, it is possible that multiple offset plants were initially transplanted in the field and this could have contributed to the lack of response observed. In addition, Keever's (2004) experiments were performed under more controlled conditions offering less impact of environment factors on final results; plants were grown in containers with artificial

Table 1. Summary of ANOVA for the effect of PGR type, PGR rate and timing of application on the number of divisions per plant in 2002 (harvested 22 Sept. 2002); height (cm), shoot and root fresh weight (g), the number of fans, and number of divisions per plant of *Hemerocallis* 'Happy Returns' in 2003 (harvested 3 Oct. 2003).

Source of variation	df	P values for variables shown					
		Divisions/ plant 2002	Ht (cm) 2003	Shoot fresh wt (g) 2003	Root fresh wt (g) 2003	Fans/ plant 2003	Divisions/ plant 2003
Replication	2	0.373	0.297	0.607	0.002	0.166	0.052
PGR	4	0.003	0.575	0.527	0.307	0.474	0.464
Rate	2	0.561	0.382	0.313	0.029	0.452	0.044
Timing	1	0.528	0.999	0.970	0.825	0.335	0.651
PGR × rate	8	0.039	0.877	0.337	0.022	0.125	0.181
PGR × timing	4	0.162	0.622	0.637	0.109	0.685	0.914
Rate × timing	2	0.585	0.740	0.463	0.002	0.047	0.020
PGR × rate × timing	8	0.667	0.228	0.429	0.098	0.375	0.443
Grand mean		1.2	42.2	40.5	112.5	4.4	2.6

Table 2 Effect of plant growth regulator treatments applied at flowering stage (timing 2) on root fresh weight (g), the number of fans per plant, and the number of divisions per plant recorded at harvest (3 Oct. 2003) for *Hemerocallis* 'Happy Returns'.

PGR treatments	Root fresh wt (g)	Fans/ plant	Divisions/ plant
Control	103.7 b ^z	4.1 b ^y	2.4 b ^z
Sw/PGR mix, 3000 mg·L ⁻¹	141.1 a	6.7 a	3.2 a
Cycocel, 3000 mg·L ⁻¹	129.8 a	4.3 b	3.0 a
BA, 2500 mg·L ⁻¹	99.3 b	4.4 b	3.0 a

^zMeans within columns followed by the same letters are not significantly different at $\alpha = 0.05$.

^yMeans within columns followed by the same letters are not significantly different at $\alpha = 0.10$.

Table 3 Percentage of plants of *Hemerocallis* 'Happy Returns' producing one or two divisions per plant under different PGR treatments in 2003.

PGR treatments	n	1 Division/ plant (%)	2 Divisions/ plant (%)
Controls	18	28	72
Sw/PGR mix, 3000 mg·L ⁻¹ , timing 2 ^z	3	0	100
BA, 2500 mg·L ⁻¹ , timing 2	3	0	100
Cycocel, 3000 mg·L ⁻¹ timing 2	3	0	100

^zTiming 1 = 9 July; Timing 2 = 12 Aug. 2003.

Table 4 Effect of plant growth regulators on plant height (cm), growth index (cm), and the number of vegetative buds per plant for *Hosta* 'Gold Standard' recorded at harvest (3 Oct. 2003).

PGRs	Ht (cm)		Growth index (cm)		Buds/plant	
	Timing 1 ^z	Timing 2	Timing 1	Timing 2	Timing 1	Timing 2
Sw/PGR mix	14.4 a ^y	12.4 c	24.1 a	22.2 b	2.4 b	3.5 a
BA	13.3 b	13.6 b	23.1 ab	22.9 ab	3.3 ab	3.2 ab
Cycocel	12.8 bc	13.7 ab	22.1 b	22.9 ab	3.3 a	2.6 ab

^zTiming 1 = 9 July; Timing 2 = 12 Aug. 2003.

^yMeans within 2 columns followed by the same letter are not significantly different at $\alpha = 0.05$. (n = 12).

substrate and they were placed in a greenhouse with ventilation and heat set points.

In 2002, due the need for an early commercial harvest, plants of *Hemerocallis* were still in the flowering process when harvested. As suggested by Zhou et al. (1995), floral buds are very competitive for photosynthates and it is at a later nutrient-storage stage that the assimilates are transported to the crown and root system. This may also explain the lack of response observed in 2002. Plants may need the extra time to show increase in divisions.

Results for *Hemerocallis* showed that application of the seaweed/PGR mixture, Cycocel, or BA increased the number of plants producing two divisions compared to control plants. These results have economic implications for bare-root production, which need to be verified on a larger field scale. In a commercial field, plant density is about 150 000 plants/ha. In 2003–04, *Hemerocallis* 'Happy Returns' divisions sold for \$1.60 each (Balamore Farm Ltd, catalogue 2003–04). If the proportion of plants producing two divisions can be increased from 70% to 100%, as seen after the application of the seaweed/PGR mixture, Cycocel or BA, this can potentially generate \$480,000/ha: an increase of \$72,000/ha as compared to a system without

plant growth regulators application. The cost to apply plant growth regulators is about \$100/ha, low compared to the possible financial returns due to these treatments.

No conclusive results were observed with *Hosta* in the course of this study. To show differences in growth and development, it may be necessary to start experiments with *Hosta* using uniform single offset divisions. This appears to be more important in *Hosta* than *Hemerocallis*. Therefore, further investigation with *Hosta* is needed.

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