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Effect of daminozide and flurprimidol on growth, flowering and bulb yield of *Eucomis autumnalis* (Mill.) Chitt.

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ABSTRACT

Eucomis autumnalis (Mill.) Chitt., commonly known as pineapple lily, is a new ornamental pot plant with great marketing potential. This work evaluated the effects of two gibberellin synthesis inhibitors (daminozide and flurprimidol) applied as commercial plant growth regulators (PGRs) B-Nine and Topflor on the growth, flowering, and bulb yield in *E. autumnalis*. The PGRs were applied three times as substrate drenches or foliar sprays at the concentration of 15 mg dm^{-3} (flurprimidol) or 4250 mg dm^{-3} (daminozide). Plant growth was restricted only by flurprimidol, particularly when it was applied as substrate drenches. Plant height was reduced by 48% at anthesis and by 38% at flower senescence, compared to the untreated control. Regardless of the application method, flurprimidol increased the leaf greenness index (SPAD) and bulb weight. Daminozide treatments were ineffective in controlling plant height and negatively influenced bulb weight. Foliar sprays of daminozide increased the length of inflorescences and the number of flowers per inflorescence.

Key words: B-Nine, growth retardant, pineapple lily, Topflor

INTRODUCTION

The genus *Eucomis* L'Hér. consists of 12 species of bulbous plants (Asparagaceae family) endemic to the summer rainfall areas of South Africa (Zonneveld and Duncan 2010). The plants produce decorative raceme inflorescences composed of numerous star-shaped flowers that are transformed into decorative capsule fruits after pollination. A tuft of green bracts is located at the top of the inflorescence, which is why this plant is commonly known as pineapple lily or pineapple flower. *Eucomis autumnalis* (Mill.) Chitt. is a particularly interesting species with green-white, pleasantly fragrant flowers. This ornamental crop is grown mainly for cut flowers and flowering potted-plants for indoor display (Luria et al. 2011). *E. autumnalis* has long been used as a natural medicine due to its high biological activity (Bisi-Johnson et al. 2011, Masondo et al. 2014a, Salachna et al. 2015a). Unfortunately, acquiring bulbs from natural habitats poses a threat of extinction of the entire *E. autumnalis* population (Taylor and van Staden 2001, Ndhlala et al. 2012). Moreover, the natural propagation rate of this species through offsets is very low (Fitchet and van de Venter 1988). Therefore, the use of various compounds that stimulate and accelerate bulb production in *E. autumnalis* is important (Masondo et al. 2014b, Salachna et al. 2015b).

The main problem in pineapple lily cultivation under cover is excessive growth of flower stems that often bend or break (Filios and Miller 2013).

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Plant quality is also decreased by excessively long leaves growing from bulbs, especially under high temperatures or low light conditions (De Hertogh and Le Nard 1993). A solution to this is the use of plant growth regulators (PGRs) to control plant size (Whipker et al. 2011, Carlson et al. 2015). In addition, PGRs can also affect other processes, such as flowering and bulb reproduction (Pobudkiewicz and Treder 2006, Taha 2012). However, plant response to PGRs may differ even within the same species and depends on the retardant type, dose and application method, frequency of application, age of plant, nutritional status and environmental conditions (Basra 2000, Barett and Bartuska 2010, Salachna and Zawadzińska 2013, Sprzączka and Laskowska 2013, Ahmad et al. 2015). A literature search on the impact of retardants on the growth of pineapple lily returned reports for only three cultivars. Carlson et al. (2015) recommend the use of paclobutrazol at 0.5-2.0 mg/pot, uniconazole at 0.25-2.0 mg/pot or flurprimidol at 0.5-1.0 mg/pot for 'Leia' (hybrid of *E. zambesiaca* Baker and *E. vandermerwei* I.Verd.) forced in the greenhouse. Filios and Miller (2013) succeeded in limiting the growth of *E. comosa* 'Innocennce' and 'Tugela Ruby' grown under normal greenhouse conditions by applying paclobutrazol at 4.0 and 8.0 mg/pot and flurprimidol at 2.0, 4.0 and 8.0 mg/pot. So far, there have been no data on the use of growth retardants in the cultivation of *E. autumnalis* as potted plants. Thus, the present study aimed to investigate the effects of flurprimidol and daminozide on the growth, flowering and bulb yield of *E. autumnalis*.

MATERIAL AND METHODS

The study was conducted in two seasons, in an unheated plastic tunnel that belongs to the Department of Horticulture, West Pomeranian University of Technology in Szczecin (53°25' N, 14°32' E). The bulbs of *Eucomis autumnalis* used in the experiment were obtained every season from Holland. Bulbs with a circumference of 16-18 cm and average weight of 81-86 g were stored in the dark at 8-10ºC until planting. After treatment with fungicides (Topsin 0.7% and Kaptan 1%), the bulbs were planted 3 cm below the substrate surface in round plastic pots (18-cm diameter) in mid-April in both growing seasons (2013 and 2014). The growing medium used was a peat substrate of pH 6.5 that contained Hydrocomplex fertilizer (5% $N\text{-}NO_3$, 7% $N\text{-}NH_4$, 11% P_2O_5, 18% K_2O, 2.7% MgO, 8% S, 0.015% B, 0.2% Fe, 0.02% Mn, and 0.02% Zn) at a dose of 5 g dm^{-3}. The pots were placed on tables 60 cm above the ground. Two commercial retardants were tested: Topflor 015 SL (SePRO Corporation, USA) containing 1.5% flurprimidol [alpha-(1-methylethyl)-alpha-(4-(trifluoromethoxyphenyl)-5-pyrimidinemethanol] and B-Nine 85 SG (Chemtura, Netherlands) containing 85% daminozide (4-(2,2-dimethylhydrazinyl)-4-oxobutanoic acid). The PGRs at the concentration of 15 mg dm^{-3} (flurprimidol) or 4250 mg dm^{-3} (daminozide) were applied on 3, 10 and 17 June 2013 and on 2, 9 and 16 June 2014. Two methods of application were used: substrate drenches (100 ml solution per pot, i.e. flurprimidol at 1.5 mg per pot and daminozide at 425 mg per pot for each application) or foliar sprays (30 ml solution per plant, i.e. flurprimidol at 0.46 mg per plant and daminozide at 127 mg per plant for each application). Control plants were not treated with PGRs. The plants were grown under the natural photoperiod. Mean monthly air temperatures inside the tunnel were (2013/2014): April (11.7ºC/15.1ºC), May (17.9ºC/18.9ºC), June (20.3ºC/19.7ºC), July (22.4ºC/24.1°C), August (20.0ºC/19.6ºC), and September (16.7ºC/18.3ºC).

The number of days from planting the bulbs to anthesis was recorded. When the first row of flowers had opened on the inflorescence, the following parameters were measured: total plant height (from the substrate level to the top of the inflorescence), total number of leaves per plant, length of the central leaf in a rosette, and width of the central leaf between its extremities. Leaf greenness index was measured with a SPAD-502 Chlorophyll Meter (Minolta, Japan). Mean SPAD value was calculated based on three readings of three leaves from each plant. Percentage of marketable plants was determined as the number of potted plants with a high ornamental value (compact habit, no visible defects, presence of at least one inflorescence). When the flowering had finished and ornamental fruits had been produced (29-37 days after anthesis), total plant height, inflorescence length (without the scape), and diameter of the scape (under the inflorescence) were recorded. The number of flowers per inflorescence was counted according to the number of pedicels in the inflorescences. After bulb harvesting, the number and total weight of bulbs per plant were determined.

The study was designed as a fully randomized univariate experiment in four replications comprising five plants each. The measurements were statistically verified using the analysis of variance and Statistica 12 software (Statsoft, Poland). Due to insignificant differences between the years, data are presented as the mean of two growing seasons.

RESULTS AND DISCUSSION

The results of the study showed that only flurprimidol significantly reduced plant height (Tab. 1). The plants drenched or sprayed with flurprimidol were shorter by 48% and 21%, respectively, at the beginning of anthesis, and by 38% and 22% at flower senescence, compared with the control. Flurprimidol applied as substrate drenches was more effective than foliar sprays. Similar results had been obtained by Filios and Miller (2013), who proved that flurprimidol drenches (2.0 mg/pot) caused height reduction by 31% and 29% in *Eucomis comosa* 'Innocence' and *E. comosa* 'Tugela Ruby', respectively. Daminozide foliar sprays or substrate drenches were ineffective in controlling the height, neither at the beginning nor towards the end of the flowering stage (Tab. 1). This was consistent with the report by Pobudkiewicz and Treder (2006), who compared the effects of flurprimidol and daminozide on the oriental lily 'Mona Lisa' and found that plant growth was inhibited only by flurprimidol. These two retardants have different mechanisms of action. Flurprimidol belongs to inhibitory compounds with an N-containing heterocycle and inhibits gibberellin production (specifically GA_1) early in the isoprenoid pathway by blocking the oxidation of *ent*-kaurene to *ent*-kaurenoic acid (Grossman 1992, Sun and Kamiya 1994). Daminozide inhibits the late stages of GA biosynthesis. It blocks 3ß-hydroxylation, thereby inhibiting the formation of highly active GAs from inactive precursors (Rademacher 2000).

The use of the investigated retardants did not change the number of leaves (data not shown), but substantially affected leaf width and length (Tab. 1). The plants grown with flurprimidol drenching had narrower leaves compared with those sprayed with daminozide. Moreover, the leaves of the plants drenched or sprayed with flurprimidol were significantly shorter than in the control, by respectively 24% and 19%. Similar results had been reported by Kapczyńska and Malik (2016), who demonstrated that soaking bulbs of two *Lachenalia* cultivars in flurprimidol at 30 mg L^{-1} shortened the length of the first and second leaf by, respectively, 18% and 17% in 'Ronina' and by 23% and 23% in 'Rupert' Plants with shorter leaves are easier to pack and transport. Also, their density per square meter of cultivation space may be higher, thus making the production more cost-effective (Rademacher 2015). During the experiment, no symptoms of leaf phytotoxicity were observed after flurprimidol or daminozide application. The plants treated with flurprimidol had a significantly higher leaf greenness index than the control plants and plants treated with daminozide (Tab. 1). More intense coloration of the leaves after flurprimidol treatment was also observed in the oriental lily 'Mona Lisa' (Pobudkiewicz and Treder 2006) and Giant Chincherinchee (Salachna and Zawadzińska 2013). Flurprimidol treatment may increase the chlorophyll content and thus enhance leaf greenness (Gaussoin et al. 1997, Zawadzińska et al. 2013). The higher leaf greenness index of plants treated with flurprimidol (Tab. 1) means that the chlorophyll leaf content is higher, and this can improve plant quality and appearance.

The number of days from planting the bulbs to the beginning of anthesis was affected by the type of retardant and its application method (Tab. 2). The plants drenched with flurprimidol or sprayed with daminozide began flowering 3 days later than the control ones. However, there were no differences in flowering date between the plants sprayed with flurprimidol or drenched with daminozide and the control. Carlson et al. (2015) had proved

Table 1. Effects of flurprimidol and daminozide on height and leaf characteristics of *Eucomis autumnalis*

Treatment	Plant height at anthesis (cm)	Plant height at flower senescence (cm)	Leaf width (cm)	Leaf length (cm)	Greenness index of leaves (SPAD)
Control	30.7 a	34.3 a	5.30 ab	23.7 a	31.1 b
Flurprimidol drenches	16.1 c	21.3 c	4.68 b	18.1 b	34.6 a
Flurprimidol foliar sprays	24.1 b	26.9 b	4.94 ab	19.3 b	35.7 a
Daminozide drenches	31.0 a	34.6 a	5.05 ab	22.6 a	32.2 b
Daminozide foliar sprays	29.4 a	38.6 a	5.55 a	23.0 a	32.6 b
Significance	**	**	*	***	**

Explanations: means marked with the same letter in a column do not differ significantly at $p \leq 0.05$ according to Duncan's New Multiple Range Test; *,**: significant at $p \leq 0.05$, or 0.01, respectively

Table 2. Effects of flurprimidol and daminozide on the number of days to anthesis, inflorescence characteristics and percentage of marketable plants of *Eucomis autumnalis*

Treatment	Days to anthesis	Inflorescence length (cm)	Scape diameter (cm)	Number of flowers per inflorescence	Marketable plants (%)
Control	82 b	19.1 b	0.88 b	75 ab	88 b
Flurprimidol drenches	85 a	14.2 c	1.08 a	68 bc	90 b
Flurprimidol foliar sprays	80 b	16.1 c	0.81 b	65 c	95 a
Daminozide drenches	80 b	18.5 b	0.73 b	60 c	88 b
Daminozide foliar sprays	85 a	21.5 a	0.88 b	81 a	90 b
Significance	**	*	**	*	*

Explanations: means marked with the same letter in a column do not differ significantly at $p \leq 0.05$ according to Duncan's New Multiple Range Test; *,**: significant at $p \leq 0.05$, or 0.01, respectively

that retardants slightly delayed flowering in the pineapple lily 'Leia'. Delayed flowering has also been observed in other geophytes after the application of a retardant (Whipker et al. 2011, Pobudkiewicz and Treder 2006, Sprzączka and Laskowska 2013, Salachna and Zawadzińska 2013, Kapczyńska and Malik 2016).

The obtained results revealed that foliar sprays of flurprimidol increased the percentage of marketable plants (Tab. 2). The quality of inflorescence depended on the type of retardant and its application method. The plants drenched or sprayed with flurprimidol produced significantly shorter inflorescences (by 26% and 16%, respectively), compared with the control ones (Tab. 2). The scape of the plants drenched with flurprimidol also had an increased diameter. The effects of daminozide depended on its application method. The plants sprayed with daminozide had the longest inflorescences with the greatest number of flowers. Those drenched with daminozide produced significantly fewer flowers per inflorescence compared with the control, but the number of flowers was similar to that produced by the plants sprayed with flurprimidol. In some species, daminozide can increase the number of flowers (Kazaz et al. 2010, Hashemabadi et al. 2012) and its foliar application is more effective than substrate drenching (Grossman 1992).

The plants drenched with flurprimidol produced significantly more daughter bulbs than the plants in the other treatments (Fig. 1). Moreover, the bulbs produced by the plants drenched or sprayed with flurprimidol had a significantly higher weight compared with the control, by respectively 12% and 20%. A slight reduction in weight of the bulbs was observed after treatment with daminozide (Fig. 2). The effect of plant growth regulators on bulb yield in *E. autumnalis* was ambiguous. This might be due to the fact that PGRs affect the balance of plant hormones in the treated plants and

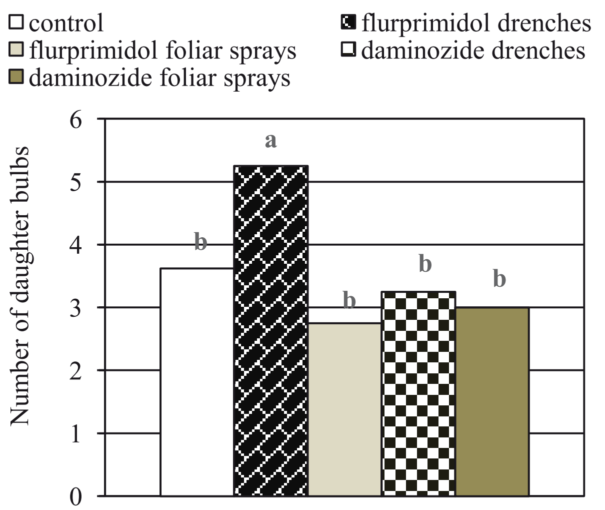

Figure 1. Effects of flurprimidol and daminozide on the number of daughter bulbs of *Eucomis autumnalis*. Means represented by columns marked with the same letter do not differ significantly at $p \leq 0.05$ according to Duncan's New Multiple Range Test

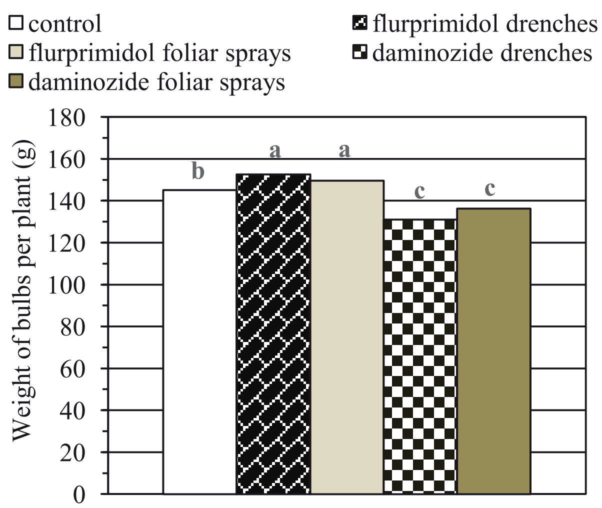

Figure 2. Effects of flurprimidol and daminozide on the weight of bulbs of *Eucomis autumnalis*. Means represented by columns marked with the same letter do not differ significantly at $p \leq 0.05$ according to Duncan's New Multiple Range Test

promote, inhibit or otherwise modify the plant's physiological processes (Rademacher 2015). Ilczuk et al. (2005) had proved that flurprimidol used as a supplement in an *in vitro* medium increased the number of new bulblets per original bulb and the size of newly developed bulblets in *Hippeastrum* × *chmielii*. Zheng et al. (2012) demonstrated that the lily 'Sorbonne' treated with PGRs had higher levels of starch, sucrose and indole-3-acetic acid in the bulbs, which may stimulate the formation of new scales and bulb growth. The greater bulb weight of the plants treated with flurprimidol might be due to the mechanism of sink direction change. Restricted synthesis of gibberellins after flurprimidol application reduced not only plant height but also the number of flowers in the inflorescence and their length (Tab. 2). The assimilates produced in the leaves were transported probably in greater amounts to the developing bulbs than to the flowers. The stimulating effect of flurprimidol may improve the growth of daughter bulbs and shorten the time of growing up to the commercial size to produce flowers. Further work is required to assess the mechanism of the effects of PGRs on the distribution and concentration of endogenous growth substances within the bulb of *E. autumnalis*.

In summary, the results of this study seem to indicate high suitability of flurprimdiol as a plant growth regulator in *E. autumnalis* production. To obtain plants with the quality required by the potted crop market, the most effective treatment appears to be the application of flurprimdiol in foliar sprays. Drenches of flurprimdiol may be used to increase the number of daughter bulbs in *E. autumnalis*.

CONCLUSIONS

1. Flurprimidol foliar sprays or substrate drenches effectively improve plant shape expressed as more compact growth, improved leaf greenness index and higher bulb yield of *E. autumnalis*.
2. Daminozide was ineffective in controlling the height of *Eucomis* plants.
3. Foliar sprays of daminozide increased inflorescence length and the number of flowers per inflorescence.

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AUTHOR CONTRIBUTIONS

All the authors contributed equally to this work. P.S. – designed and performed the experiments, analyzed the data and wrote the paper; A.Z. – analyzed the data and wrote the paper.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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