Bulgarian Journal of Agricultural Science, 21 (No 6) 2015, 1198-1203
Agricultural Academy

THE EFFECT OF BENZYLADENINE AND GIBBERELLIC ACID ON THE GROWTH AND FLOWERING OF *HELLEBORUS ORIENTALIS* LAM.

M. HENSCHKE, P. K. CZUCHAJ and S. J. SZCZEPANIAK
Poznan University of Life Sciences, Department of Ornamental Plants, 60-594 Poznan, Poland

Abstract

HENSCHKE, M., P. K. CZUCHAJ and S. J. SZCZEPANIAK, 2015. The effect of benzyladenine and gibberellic acid on the growth and flowering of *Helleborus orientalis* Lam. *Bulg. J. Agric. Sci.*, 21: 1198–1203

The aim of the study was to treat *Helleborus orientalis* 'Red Hybrids' with benzyladenine and gibberellic acid in foliar and soil applications in order to obtain intensively growing and flowering plants. Vegetative growth and flowering of plants was observed over the period of three years. The type of growth regulator had a significant effect on plant height and the number of leaves starting from the second year of growing. Plants produced with a BA + GA_3 solution were shorter and had a larger number of leaves than those treated with a BA solution. The manner of growth regulator application had a significant effect primarily on the number of leaves, which was higher in the case of soil application of growth regulator solutions in comparison to foliar applications.

Key words: *Helleborus orientalis*, growth regulators, application, number of vegetative shoots, number of flowering shoots

Introduction

Hellebores are geophytes of the Ranunculaceae family. The genus comprises about 20 species, which can naturally be found chiefly in the Balkan Peninsula, in Western Europe and in China. Two groups of hybrid cultivars of the oriental hellebore are frequently used in horticulture practice. One group includes vegetative propagated clones. The other group includes cultivars reproduced from seeds, but they are produced by specific selection, thanks to which the plants share a large number of common traits. Such cultivars, created by individual gardening companies, are often referred to as 'hybrids' (Rice and Strangman, 1993).

Hellebores bloom in late winter or early spring and flowering is induced by the end of the cold period. This facilitates acceleration of flowering in winter months, e.g. in Western Europe hellebores are grown for the Christmas season. Due to their minimal temperature requirements hellebores are perfect alternatives to poinsettia (*Euphorbia pulcherrima*), very popular at that time of the year. Different species from this genus, primarily black hellebore and oriental hellebore, are grown in containers and for cut flowers (Ganslmeier and Henseler, 1985; Pogroszewska, 1996). Studies conducted to date concerned e.g. production of young plants, substrate quality, fertilisation, the effect of day/night temperatures on growth and photosynthesis, accelerated cultivation, etc. (Pogroszewska, 1995; Pogroszewska, 1996; Piskornik et al., 2000; Kraus and Warren, 2002; Piskornik, 2003 a,b; Henschke et al., 2009; Czuchaj et al., 2010; Lowder et al., 2010). On a large scale oriental hellebore is usually propagated from seeds. The in vitro method is still being developed and it is rarely applied, whereas rhizome division is not very efficient (Piskornik et al., 1999; Seyring, 2002; Dhooghe and Van Labeke, 2007).

Due to the slow growth of these long-lived perennials cultivation in containers takes a long time. In order to produce plants with a larger number of shoots in a shorter time they can be treated with growth regulators. Growth regulators were shown to have a positive effect on the formation of new shoots in decorative shrubs and perennials, or on yielding of *Allium karataviense* 'Ivory Queen' (Grzesik and Rudnicki, 1985; Bessler, 1995; Pogroszewka et al., 2007). The aim of this study was to treat hellebores with benzyladenine and gibberellic acid in foliar and soil applications in order to obtain intensively growing and flowering plants.

E-mail: mohen@up.poznan.pl

Materials and Methods

From 2008 to 2010 experiments on oriental hellebore 'Red Hybrids' were conducted at the Marcelin Experimental Station, the Poznań University of Life Sciences. Plants for the investigations were provided by Syngenta Seeds Sp. z o.o. They were supplied on 22 April 2008 in multitrays with 72 cells of 9 cm^3. Seedlings, on average with 5 leaves, were planted into pots of 1025 cm^3. The substrate was Klasmann highmoor peat, pH 3.91, supplemented with 0.5 $g \cdot dm^{-3}$ Peters Professional PL Special (15:11:29) fertiliser and 5 $g \cdot dm^{-3}$ calcium carbonate ($CaCO_3$). After liming substrate pH was 6.80. The plants were treated with growth regulators, i.e. either benzyladenine (BA) or a mixture of benzyladenine and gibberellic acid ($BA+GA_3$), or with water as the control - after two months of growing. The applications were performed twice at monthly intervals (on 22 June and on 22 July 2008). Gibberellic acid in a commercially available preparation Gibrescol 10 MG, was applied. Water solutions of growth regulators were applied by watering and spraying. Solutions of 500 $mg \cdot dm^{-3}$ benzyladenine and 150 $mg \cdot dm^{-3}$ gibberellic acid were used for watering at 50 ml per pot, while solutions of 3000 $mg \cdot dm^{-3}$ benzyladenine and 150 $mg \cdot dm^{-3}$ gibberellic acid at 20 ml per plant were used for spraying. Plants were grown in a greenhouse. Only in November plants were cooled for a month in a polytunnel, in which the average diurnal temperature was 5.4°C. Plants were returned to the greenhouse in December. During the acceleration period the average daily temperature in the greenhouse was 13.3°C during the day and 11.6°C at night. Cooling and acceleration were repeated during all the years of the study. During the cultivation every ten days the plants were supplemented with a 0.15% solution of a multinutrient Peters Professional PL Special (15:11:29) fertiliser at 100 ml per pot.

Plant growth was assessed in October 2008, 2009 and 2010, i.e. in the consecutive years of cultivation, based on plant height, the number of vegetative shoots and the number of leaves. Due to the low dynamics of changes in the number of shoots analyses were conducted on the results of measurements from 2008 and 2010. Plant flowering was evaluated only in December 2009 and 2010, because very few plants bloomed in the first year of cultivation. The number and length of flowering shoots were determined at anthesis. The results were subjected to three-way analysis of variance using the Statistica 10.0 software package. Factors differentiating the trait under analysis included the year of measurement, growth regulator and the method of its application. One combination comprised 12 plants, each grown in a separate pot. The significance of differences between the measurements of factors was examined by Tukey's test and presented in a graphic form to facilitate interpretation of the results.

Results and Discussion

The year of cultivation and the applied growth regulator had highly significant effects on vegetative growth of hellebore (Table 1). Regardless of the other factors, the number of vegetative shoots and the number of leaves significantly depended on the method of growth regulator application. The interaction of the application method and growth regulator or the year of cultivation was statistically confirmed only for the number of leaves. All the traits describing vegetative growth of plants had significantly higher values in the consecutive years of hellebore cultivation.

In 2008 the BA or $BA+GA_3$ solution, regardless of its application method, had a significant effect on the increase in the number of vegetative shoots, as compared with the control. In 2010 only watering with solutions of these growth regulators had a significant effect on the number of the shoots (Figure 1). In 2010 spraying with BA or $BA+GA_3$ solutions did not

Table 1
The F-test statistics and significance levels of three-way analysis of variance for vegetative growth traits, with the year of cultivation, growth regulator and the application method for *Helleborus orientalis* 'Red Hybrids' as differentiating factors (*α≤0.001; ** α≤0.01; *α≤0.5; ns – non-significant)**

Trait	Year of cultivation	Growth regulator	Application method	Year of cultivation x growth regulator	Year of cultivation x application method	Growth regulator x application method	Year of cultivation x growth regulator x application method
Number of vegetative shoots	491.9***	67.0***	12.0***	9.8***	3.7 ns	1.2 ns	3.4*
Height	1304.8***	144.3***	1.4 ns	31.5***	1.2 ns	15.0***	2.1 ns
Number of leaves	236.0***	662.8***	122.7***	10.3***	8.1***	40.1***	30.1*

result in a significant increase in the number of vegetative shoots in relation to the control, watered or sprayed with water. In turn, Lubell et al. (2005) showed that after a single spraying of benzyladenine solution at a similar concentration the number of propagules in *Helleborus* x *hybridus* in the second year of cultivation was 62% higher than in the control. Another study by Hetman and Witek (2008) showed that the BA+GA_3 mixture in the first year of cultivation had a positive effect on the number of shoots in *Kohleria amabilis* only in foliar applications. Moreover, the growth regulators were applied at a much lower concentration (50 mg·dm^{-3}).

In spite of the fact that in our experiment watering with water or with growth regulator solutions had a more favourable effect on the number of vegetative shoots in 2010, whereas the effect of watering with water or with the BA+GA_3 solution was more advantageous in 2008, a significant difference was found only for the use of BA in 2010. The highest number of shoots, i.e. 7.27, recorded in 2010 in plants watered with the BA solution, did not differ significantly from that in plants watered with the BA + GA_3 solution, i.e. 6.73. A more advantageous effect of spraying two hosta cultivars (*Hosta sp.*) with an Arbolin 038 preparation (containing 0.3% of BA and 0.05% of GA) was shown by Witomska et al. (2010). However, the effect depended on the applied dose, since at a lower dose the number of shoots in the second year was even higher. At the application of a larger dose in the beginning the number of shoots was the highest and in the second year it was identical to that in the control. As it results from the experiments conducted by Bessler (1995), the response of perennials to the application of cytokinins depends not only on their concentration, but also on the period of treatment. Application of a BAP solution at a low concentration of 10 mg·dm^{-3} for a longer period of time (10 weeks) has a more advantageous effect on the formation of a greater number of lateral shoots in *Coreopsis grandiflora* 'Early Sunrise'.

Height of the control plants, watered or sprayed with water, was significantly greater than that of plants treated with growth regulators regardless of the application method in 2008 and 2010, or that of plants treated with the BA + GA_3 solution in 2009 (Figure 2). The type of growth regulator and the application method affected plant height in our experiment in 2009 and 2010. Significantly taller plants were produced using the BA solution than the BA + GA_3 solution. Height of plants treated with the BA solution depended significantly on the application method in 2009 and 2010. Taller plants were produced when the BA solution was sprayed rather than used as a watering solution. By contrast, when the BA + GA_3 solution was used, plants watered with the solution of these growth regulators grew taller, which was confirmed by the statistical analysis of the results in 2010. A similar study was conducted by Hetman and Witek (2008). In their experiment they stimulated growth and flowering of *Kohleria amabilis* by watering or spraying a mixture of BA and GA_3, but the concentrations were lower, ranging from 50 to 200 mg·dm^{-3}. At the highest concentration of the solution plants grew taller than the water-treated control. In terms of the effect of mixture application method watering resulted in lower plants. The effect of gibberellic acid on plant height in *Impatiens hawkeri* from the Petticoat series was also investigated by Dobrowolska and Startek (2004). However, 4 and 8 weeks after spraying gibberellic acid at 10 mg·dm^{-3} plants they were of the same height as the water-sprayed control.

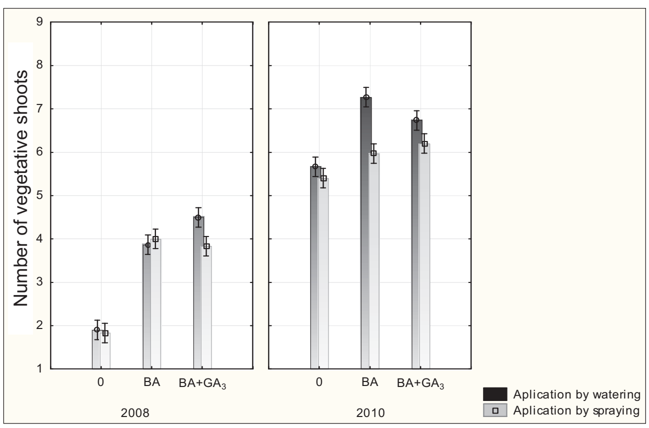

Fig. 1. The effect of growth regulators on the number of vegetative shoots of *Helleborus orientalis* 'Red Hybrids' during the two years of cultivation (standard deviation in boxplot diagrams denote 0.95 confidence intervals)

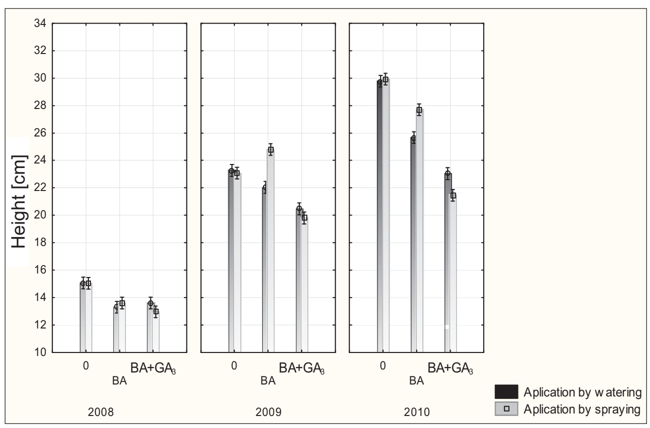

Fig. 2. The effect of growth regulators on the plant height of *Helleborus orientalis* 'Red Hybrids' during the three years of cultivation (standard deviation in boxplot diagrams denote 0.95 confidence intervals)

In relation to the control the number of leaves in all the years of the study was significantly higher in growth regulate-treated plants, regardless of the application method (Figure 3). Plants watered with growth regulator solutions produced more leaves than sprayed plants, which was confirmed by the statistical analysis for the number of leaves in the first and third years (2008 and 2010) for BA and BA+GA_3 and in the second year of cultivation (2009) for BA+GA_3. The use of the BA+GA_3 solution stimulated formation of a greater number of leaves, as it was confirmed statistically for both application methods in 2010 and for watering with growth regulator solutions in 2009. In an experiment by Lubell et al. (2005) in which *Helleborus* x *hybridus* was sprayed once with the same dose of BA as in this experiment, in the second year the number of leaves per plant was by approx. 13% higher. However, at the application of a higher BA solution concentration (4500 mg·dm^{-3}), the number of leaves increased by almost 40%. In contrast, Hetman and Witek (2008), when spraying and watering *Kohleria amabilis* with a BA + GA_3 mixture at concentrations of 50 – 200 mg·dm^{-3} showed that neither spraying nor watering had a positive effect on this trait.

Flowering of hellebores, which started in the second year of cultivation, depended first of all on the year, but also on plant treatment with a growth regulator and its application method (Table 2). In 2010 the number of flowering shoots was significantly higher in older plants, but the shoots were significantly shorter than in the first year of flowering, i.e. in 2009 (Figure 4). Plants sprayed with growth regulator solutions (BA or BA + GA_3) had a significantly greater number of flowering shoots than in the case of watering with these solutions. The number of flowering shoots was also higher in relation to that in the control, i.e. plants watered or sprayed with water. The greatest number of flowering shoots recorded in BA-sprayed plants did not differ significantly from that produced by plants sprayed with the BA + GA_3 solution. In the second year growth regulators and their application methods were shown to have no effect on the number of flowering shoots. Only plants watered with water or BA solution formed significantly higher numbers of flowering shoots than those sprayed with BA + GA_3. Similar results were recorded by Hetman and Witek (2008). As early as the first year of cultivation, the higher the concentrations of the BA + GA_3 mixture were applied when watering or spraying *Kohleria amabilis*, the lower the number of inflorescences was formed. In turn, Janowska (2013) soaked rhizomes of *Zantedeschia* Spring. in BA + GA_3 solutions in order to increase the abundance of flowering. Irrespective of the concentrations applied, the yield of flowers in the first year of cultivation was 2.5 - 3.5 times greater.

Regardless of the application method, in both years flowering shoots of plants treated with growth regulators were significantly shorter than those of the control (Figure 5). Irrespective of the application method, in 2009 BA-treated plants had significantly longer shoots than plants treated with the BA + GA_3 solution. In contrast, in 2010 plants watered with the BA solution produced significantly shorter flowering shoots than those watered with the BA + GA_3 solution. The effect of the application method on length of hellebore flowering shoots was found only for the application of BA solution. In both years BA-sprayed plants produced significantly longer shoots than those watered with the BA solution. In another study Zalewska et al. (2008) treated *Chrysanthemum gran-*

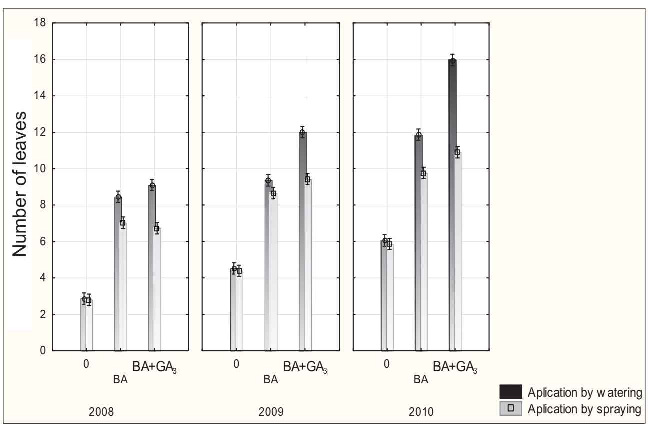

Fig. 3. The effect of growth regulators on the number of leaves of *Helleborus orientalis* 'Red Hybrids' during the three years of cultivation (standard deviation in boxplot diagrams denote 0.95 confidence intervals)

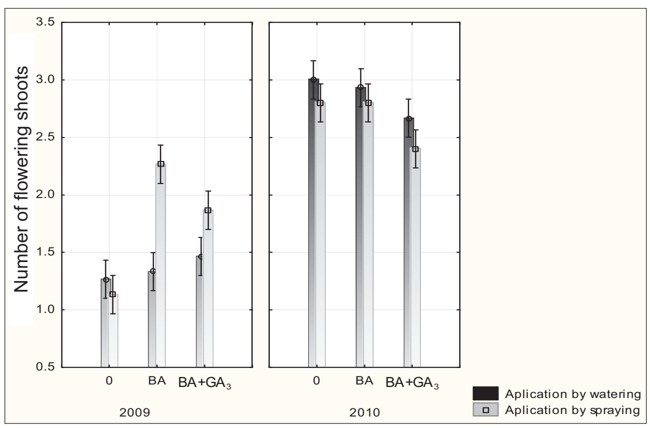

Fig. 4. The effect of growth regulators on the number of flowering shoots of *Helleborus orientalis* 'Red Hybrids' during two years of cultivation (standard deviation in boxplot diagrams denote 0.95 confidence intervals)

Table 2
The F-test statistics and significance levels of three-way analysis of variance for flowering traits, with the year of cultivation, growth regulator and the application method for *Helleborus orientalis* 'Red Hybrids' as differentiating factors (*α≤0.001; ** α≤0.01; *α≤0.5; ns – non-significant)**

Trait	Year of cultivation	Growth regulator	Application method	Year of cultivation x growth regulator	Year of cultivation x application method	Growth regulator x application method	Year of cultivation x growth regulator x application method
Number of flowering shoots	160.6***	3.3*	1.1 ns	6.9**	9.9**	3.0 ns	2.3 ns
Length of flowering shoots	28.3***	196.0***	18.6***	51.6***	2.5 ns	15.7***	1.1 ns

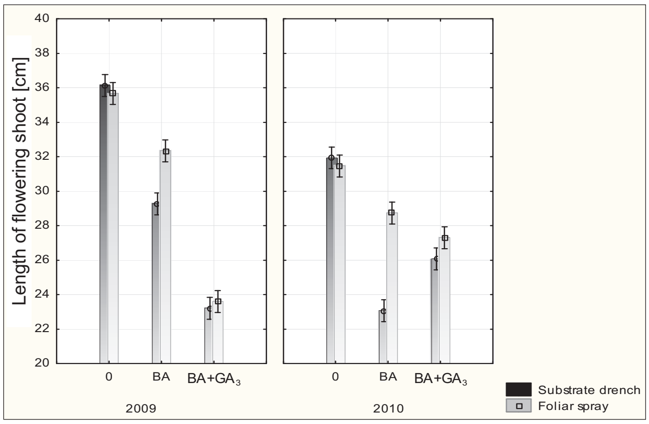

Fig. 5. The effect of growth regulators on the length of flowering shoots of *Helleborus orientalis* 'Red Hybrids' during two years of cultivation (standard deviation in boxplot diagrams denote 0.95 confidence intervals)

diflorum seedlings three times with gibberellic acid applied at 500 mg·dm^{-3}. The authors showed that in only one cultivar shoot length during flowering was greater than in the control, whereas it was similar in the other cultivar was similar.

Conclusions

The application of BA or BA + GA_3 solutions in relation to the control had a significant effect on the increase in the number of vegetative shoots in both years of analyses, whereas it increased the number of inflorescence shoots only in the first year. Spraying the above mentioned growth regulator solutions on plants had a more advantageous effect on the formation of inflorescence shoots, while in the third year of the study a more advantageous effect on vegetative shoots was observed for watering with solutions of those growth regulators. Regardless of the application method, plants treated with growth regulators, i.e. BA or BA + GA_3, were significantly lower and they had shorter inflorescence shoots than the control, watered or sprayed with water. The type of growth regulator had a significant effect on plant height and the number of leaves starting from the second year of cultivation. Lower plants with a greater number of leaves were produced using the BA + GA_3 solution than BA solution. The growth regulator application method had a significant effect primarily on the number of leaves, which was greater on plants watered with growth regulator solutions than those sprayed with them.

References

Bessler, B., 1995. Cytokinininduzierte Seintentriebbildung bei Stauden. *Gartenbauwissenschaft*, **60** (5): 218-223.

Czuchaj, P., M. Henschke and S. Szczepaniak, 2010. Growth and flowering of *Helleborus argutifolius* depending on the dose and type of Fertilizer. *Zesz. Prob. Post. Nauk Rol.*, **551**: 31-37.

Dhooghe, E. and M-Ch. Van Labeke, 2007. *In vitro* propagation of *Helleborus* species. *Plant Cell Tiss Organ Cult.,* **91**: 175-177.

Dobrowolska, A. and L. Startek, 2004. Effects of growth regulators on growth and Habit of *Impatiens hawkeri* W. Bull. to Petticoat group. *Folia Univ. Agric. Stetin. Agricultura*, **236** (94): 27-32.

Ganslmeier, H. and K. Hanseler, 1985. Schnittstauden. *Verlag Eugen Ulmer*, Stuttgart, pp. 278-289.

Grzesik, M. and R. M. Rudnicki, 1985. The use of growth regulators in nursery production of woody ornamental plants. *Acta Horticulturae*, **167**: 417-422.

Henschke, M., S. Szczepaniak, P. Czuchaj and E. Kozik, 2009. The effect of calcium carbonate and top dressing with Peters Professional Special on growth and flowering of *Helleborus lividus* Aiton. *Folia Hort.,* **21** (1): 105-117.

Hetman, J. and M. Witek, 2008. Influence of BA + GA_3 on the growth and flowering of *Kohleria amabilis* (Planchon & Linden) Fritsch. *Zesz. Probl. Post. Nauk Rol.*, **525:** 171-179.

Janowska, B., 2013. Effect of growth regulators on flower and leaf yield of the calla lily (*Zantedeschia* Spring.). *Hort. Sci.*, Prague, **40** (2): 78-82.

Kraus, H. T. and S. L. Warren, 2002. Nutrient and Ph management programs for nursery production of *Helleborus* x *hybridus*. *SNA* Research Conference Container-Grown Plant Production, **47:** 18-22.

Lofwder, A., H. T. Kraus, F. A. Blazich and S. L. Warren, 2010. Day/night temperatures influence growth and photosynthesis during containerized production of selected species of *Helleborus* (Hellebores). *J. Environ. Hort.*, **28** (3): 179-186.

Lubell, J. D., D. M. Thompson and M. H. Brand, 2005. Foliar sprays of benzyladenine increase bud and propagule production in *Epimedium* x *rubrum* Morren and *Helleborus* x *hybridus* L. *Propagation of Ornamental Plants*, **5** (1): 19-22.

Piskornik, M., 2003a. Production of Christmas Rose young plants from seedlings during one vegetation season. *EJPAU*, **6** (1). http://www.ejpau.media.pl/volume6/issue1/ horticulture/art-05.html

Piskornik, M., 2003b. Improving Christmas Rose (*Helleborus niger* L.) young plants production. *Acta Sci. Pol. Hortorum Cultus*, **2** (2): 69-74.

Piskornik, M., Z. Piskornik, A. Klimek and M. Mrugasz, 1999. Rozmnażanie ciemiernika białego (*Helleborus niger*) 'Praecox' Propagation of Christmas rose (*Helleborus niger*) 'Praecox', XII Ogólnopolski Naukowy Zjazd Kwiaciarzy, Skierniewice, 9-10 grudnia: 72-73.

Piskornik, M., A. Klimek, A. Lis-Krzyścin, A. Gąsior and A. Krzywda, 2000. Effect of slow-release fertilizers on growth of Christmas rose (*Helleborus niger* L.) seedlings. *Zeszyty Naukowe Instytutu Sadownictwa i Kwiaciarstwa*, **7:** 297-301.

Pogroszewska, E., 1995a. Accelerated cultivation of white hellebore (*Helleborus niger*). Nauka Praktyce Ogrodniczej. Materiały Ogólnopolskiej Konferencji Naukowej, Lublin, (7-8): 859-861.

Pogroszewska, E., 1995b. Accelerated cultivation of garden hellebore (*Helleborus* x *hybridus* Hort.) in a greenhouse. Nowe Rośliny i Technologie w Ogrodnictwie. 20 Ogólnopolskie Sympozjum w Roku Jubileuszu 40-lecia Wydziału Ogrodniczego AR w Poznaniu, (17-19): 397-400.

Pogroszewska, E., H. Laskowska and W. Durak, 2007. The effect of giberellic acid and benzyloadenine on the yield of (*Allium karataviense* Regel.) 'Ivory Queen'. *Acta Sci. Pol. Hortorum Cultus*, **6** (1): 15-19.

Rice, G. and E. Strangman, 2001. The Gardener's Guide to Growing Hellebores. *Timber Press Portland*, Oregon, pp. 42-43.

Seyring, M., 2002. *In vitro* cloning of *Helleborus niger*. *Plant Cell Rep.*, **20**: 895-900.

Witomska, M., A. Jaszczuk and A. Ilczuk, 2010. Branching stimulation in *Hosta* sp. *Horticult. and Landsc. Architect*., **31:** 35-41.

Zalewska, M., A. Babicka and I. Wojciechowska, 2008. The influence of gibberellic acid on the growth and flowering of cascade chrysanthemum cultivars in outsider glasshouse. *Zesz. Probl. Post. Nauk Rol.*, **525**: 525-533.

Received June, 1, 2015; accepted for printing October, 5, 2015