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# Effects of Growth Regulators and Sowing Densities in American Ginseng (*Panax quinquefolius* L.) Culture

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**Summary**

Plant growth regulators as well as sowing density are one of the most important factors affecting yield and metabolite formation of medicinal plants. A three-year field experiment examining the effects of two sowing densities (15 x 3 cm and 15 x 5 cm) and foliar application of growth regulators on root yield and ginsenoside content at American ginseng (*Panax quinuefolius* L.*)* was conducted. In mid-June of the 1st and 2nd year of growth, various growth regulators were evaluated against a control spray of water only: B-Nine 85 SP, Pommit Extra 110 SL and Arbostim 10 SL. The growth regulators tested significantly affected plant population, root weight and accumulation of root ginsenosides. Foliar applications of Pommit Extra and B-Nine during the first and second year, especially at lower densities, increased root weight, whereas Arbostim decreased them. Foliar application of all growth regulators tested (particularly of Pommit Extra and Arbostim) increased total root ginsenoside content after three year growth. Plant grown at higher densities (15 x 3 cm) contained relatively higher amounts of ginsenosides. Spraying American ginseng plants with Pommit Extra or B-Nine significantly increase yield of roots and ginsenosides content.

**Key words.** American ginseng – plant growth regulators – plant density – ginsenoside content

## Introduction

*Panax quinquefolius* L., commonly called American ginseng (belonging to the *Araliaceae* family) is a slow growing, long-lived herbaceous perennial plant, that has been harvested from its native state and cultivated in Canada and the USA for over 100 years. Nowadays, its cultivation has expanded from North America to Asia, Australia and Europe (Holland, France and Poland). The root is the most valued part of the plant that can reduce stress, increase physical stamina, stimulate the immune system and glandular functions, slow the degeneration of cells and increase life expectancy. Its roots are rich in organic components, whereas saponin glycosides, known as ginsenosides are recognized for their healthful properties (Park et al. 2005). American ginseng culture is not an easy task; the plants have specific environmental and cultural requirements (shading, mulching) and are very susceptible to fungal diseases (Li 1995; Anonymous 2001). Moreover, ginseng has a normal production cycle from three to six years (Savage 1991). Although impact of cultural factors on the content of individual and total ginsenosides (Court et al. 1996; Reynolds 1998) has been reported, relatively little progress has been made in developing new production practices that significantly affect the manner in which the crop is grown. The objective of this study is to report on the growth and active substances content and yields in American ginseng roots growing in two densities and treated with different commercially available growth regulators.

## Materials and Methods

An experiment lasting three years was conducted on experimental fields at the University of Agriculture in Lublin (N 51 09' 06.81", E 22 28' 23.42"), located on luvisols. Seeds of *Panax quinquefolius* used for plantation were supplied by Prof B.F. Zilkey (Delhi Research Station, Ontario, Canada), where this plant was identified. Stratified seeds of American ginseng were hand-seeded at the beginning of September on conventional raised beds (30–40 cm, 1.2 m. wide) and afterwards the soil was mulched with oat straw (about 5-cm-high layer) kept on the beds for three years. During vegetation, shading with plastic fabric (produced by Panax Limited, Wisconsin, USA) designed for an 82 % reduction in light penetration as well as hand-weeding were ensured. Prior to sowing, plots were fertilized with 80 kg P $ha^{-1}$, 150 kg K $ha^{-1}$ and every spring with 40 kg N $ha^{-1}$ and 20 kg Mg $ha^{-1}$. During vegetation, fungicides recommended on commercial plantations according to Anonymous (2001) were applied. American ginseng was sown on plots (with 2.4$m^2$ area) in two sowing densities: 15 x 3 cm and 15 x 5 cm. In mid-June, when in the first year of vegetation plants produced single trifoliate leaf and in the second year – an aerial shoot with a whorl of two leaves with 3 leaflets, each plot was sprayed with 200 ml of solution per plot (on control plots spraying was carried out with water only) with a hand-held sprayer. Plants were sprayed with following commercially available preparations containing different growth regulators: B-Nine 85 SP (active sub-

stance: daminozide, 85 %; produced by Uniroyal Ltd. USA) in a form of single spraying (2.5 ml per 1 lof water), Arbostim 10 SL (gibberellic acid, 10 %; Varichem T. Ostrowski) in a form of single (5 ml per 1 lof water) or double (2.5 ml per 1 lof water) spraying at two weeks interval and Pommit Extra 110 SL (8 % potassium salt of α-naphthalene acetic acid and 3 % urea; Varichem T. Ostrowski) in the form of a single spraying with solution containing 0.45 ml of preparation in 1 lof water. Stand counts were made at the end of July of each year for three consecutive growing seasons. In the autumn (September) of the first and second year of growth, roots of 5 randomly chosen plants from each plot were measured and dried at 38 °C. After the 3rd year, all roots from each plot were dug up, washed, measured and then dried at 38 °C in a drying chamber. Chemical analyses (by HPLC method described in KOŁODZIEJ et al. 2006) for particular ginsenosides content (obtained as a mean of the four replicates) were performed in the Department of Biology and Pharmaceutical Biotechnology at the Medical University in Łódź. The experimental design was a randomized complete block with four replications. Data were analyzed with the SAS general linear model procedure (version 8.2 SAS Institute, Cary, N.C.). Testing for significance of mean effects and interactions on all variables was calculated using ANOVA analysis of variance.

## Results and Discussion

The seeds were planted in September and germinated in spring (April–May). In the first growth period, a reduction of the number of plants, especially on control plots, was observed (one of the causal agents being fungal diseases), whereas the highest plant density was observed on plots with Pommit Extra application at narrow plant spacing. The reduction of plant population in following years was smaller, but caused further significant decrease in plant density. After 3 years of growth, a relatively higher percentage of plants surviving (in comparison to the first year) was noted on the plots where Pommit Extra and B-Nine were applied, while the lowest survival rate was recorded after spraying with Arbostim. In the experiment, a significant effect of sowing density was recorded and, beginning from the second year of growth, growth regulator application on plants population (Fig. 1, Table 1). In the experiment on the plots with Arbostim application, during the second growth period, the aboveground parts did not appear and ginseng plants remained for one year in a form of a root with a small rhizome (plant density, counted as a number of stems visible, dramatically lowered). This phenomenon, sometimes observed on commercial plantations, called "sleeping roots", which could cause a significant decrease in yields, usually appears after a cold spring or when an apical bud has been mechanically damaged. It occurs in *Delphinium* as well as terrestrial orchids (MCMANUS 1979). After such a period, in spring of the third year of cultivation, ginseng produced stems with two or three compound leaves again; what is more, plant population increased. However, the plants themselves were weaker, with significantly lighter roots.

Independently from growth regulator application, stand decline in following three years of cultivation was generally slightly higher (on average by 53 %) on the

Table 1. Influence of sowing density and growth regulators on American ginseng plant population in the first, second and third year of vegetation as shown in Fig.1.

| Source of variation | 1st year | 2nd year | 3rd year |
|---|---|---|---|
| growth regulator | NS | *** | *** |
| Sowing density | *** | *** | *** |
| growth regulator x row spacing | NS | *** | *** |

NS, ***, **, *: Nonsignificant or significant at at P≤0.01, 0.05 or 0.1

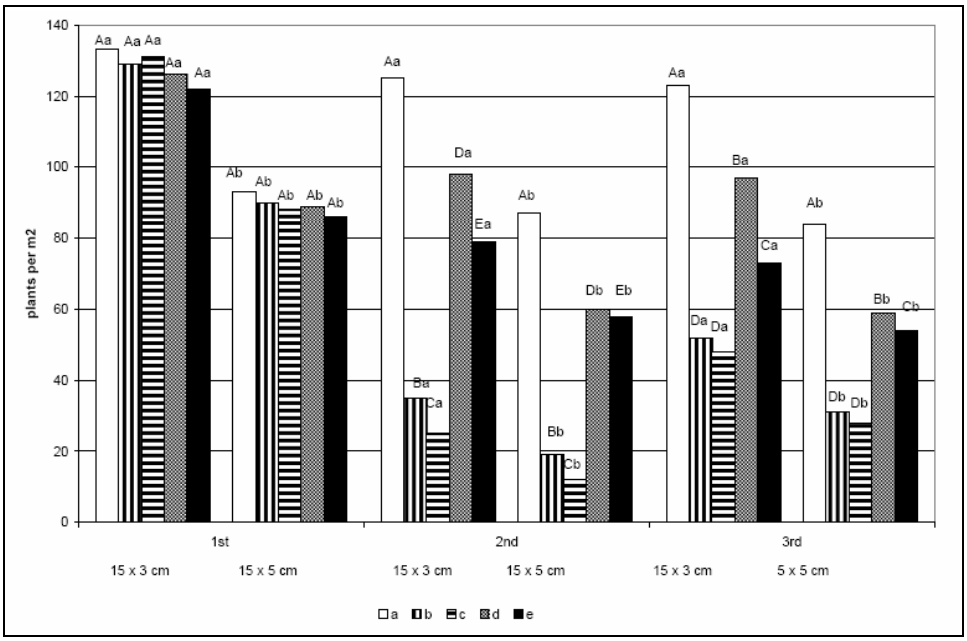



Fig. 1. Influence of sowing density (15 x 3 cm and 15 x 5 cm) and growth regulators [(a) Pommit Extra 110 SL, (b) 1x Arbostim 10 SL, (c) 2x Arbostim 10 SL, (d) B-Nine 85 SP and (e) control] on American ginseng plant population in the first, second and third year of vegetation. Different letters indicate significant differences between the treatments – upper case letters separate means calculated for different growth regulators, lower case letters separate means calculated for sowing densities (P≤0.05)

Table 2. Influence of sowing density (15 x 3 cm and 15 x 5 cm) and growth regulators (B-Nine 85 SP, Arbostim 10 SL, Pommit Extra 110 SL and control-water) on total and particular ginsenosides content (mg $g^{-1}$) in American ginseng roots as well as ginsenosides yield (g $m^{-2}$) and air dry weight of single root in following three years of vegetation.

| Preparation | Sowing density (cm) | Air dry weight of root (g $plant^{-1}$) | | | Ginsenosides yield (g $m^{-2}$) |
|---|---|---|---|---|---|
| | | 1st | 2nd | 3rd | |
| Pommit Extra | 15 x 3 | 0.189 ± 0.010[z] | 1.683 ± 0.11 | 6.011 ± 0.09 | 69.0 ± 3.18 |
| | 15 x 5 | 0.202 ± 0.008 | 1.730 ± 0.09 | 7.903 ± 0.12 | 52.7 ± 2.07 |
| Arbostim (1x) | 15 x 3 | 0.148 ± 0.006 | 1.223 ± 0.10 | 1.090 ± 0.07 | 6.5 ± 0.67 |
| | 15 x 5 | 0.153 ± 0.020 | 1.320 ± 0.06 | 1.944 ± 0.12 | 4.7 ± 0.35 |
| Arbostim (2x) | 15 x 3 | 0.109 ± 0.010 | 1.650 ± 0.06 | 1.132 ± 0.02 | 4.8 ± 0.08 |
| | 15 x 5 | 0.198 ± 0.003 | 0.450 ± 0.06 | 2.224 ± 0.14 | 3.8 ± 0.41 |
| B-Nine | 15 x 3 | 0.137 ± 0.001 | 1.647 ± 0.02 | 5.443 ± 0.47 | 41.2 ± 4.46 |
| | 15 x 5 | 0.179 ± 0.010 | 1.970 ± 0.04 | 7.896 ± 0.33 | 34.2 ± 2.98 |
| Control | 15 x 3 | 0.139 ± 0.002 | 1.208 ± 0.03 | 4.759 ± 0.09 | 24.1 ± 2.65 |
| | 15 x 5 | 0.193 ± 0.008 | 1.551 ± 0.11 | 5.020 ± 0.02 | 15.8 ± 0.24 |
| Source of variation | | | | | |
| growth regulator | | ** | *** | *** | *** |
| sowing density | | *** | NS | *** | *** |
| interaction | | ** | *** | *** | ** |

| Preparation | Sowing density (cm) | Ginsenosides content (mg $g^{-1}$) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | $R_{b1}$ | $R_{b2}$ | $R_c$ | $R_d$ | $R_e$ | $R_{g1}$ | Total |
| Pommit Extra | 15 x 3 | 60.32 | 0.51 | 5.54 | 8.21 | 12.69 | 6.09 | 93.36 ± 0.27 |
| | 15 x 5 | 47.52 | 0.53 | 3.66 | 8.01 | 14.52 | 5.21 | 79.44 ± 1.21 |
| Arbostim (1x) | 15 x 3 | 68.41 | 1.07 | 7.25 | 6.32 | 27.06 | 4.30 | 114.41 ± 2.28 |
| | 15 x 5 | 50.06 | 0.39 | 5.28 | 11.16 | 6.12 | 5.25 | 78.26 ± 1.79 |
| Arbostim (2x) | 15 x 3 | 59.71 | 0.55 | 5.18 | 7.37 | 10.07 | 5.69 | 88.58 ± 0.64 |
| | 15 x 5 | 41.41 | 0.95 | 3.09 | 5.35 | 6.57 | 3.58 | 60.96 ± 0.98 |
| B-Nine | 15 x 3 | 42.97 | 1.33 | 7.57 | 11.19 | 11.10 | 3.87 | 78.03 ± 5.35 |
| | 15 x 5 | 43.59 | 1.28 | 4.33 | 11.10 | 9.31 | 3.73 | 73.34 ± 2.41 |
| Control | 15 x 3 | 30.65 | 0.57 | 2.25 | 4.68 | 25.45 | 5.84 | 69.44 ± 1.19 |
| | 15 x 5 | 30.96 | 0.18 | 3.78 | 6.08 | 12.33 | 4.94 | 58.27 ± 1.69 |
| Source of variation | | | | | | | | |
| growth regulator | | *** | ** | ** | *** | *** | * | *** |
| sowing density | | ** | ** | *** | ** | *** | *** | *** |
| interaction | | ** | * | ** | ** | *** | ** | *** |

[z]Data are means ± SE; NS, ***, **, *: nonsignificant or significant at P≤0.01, 0.05 or 0.1

plots with higher seed density in comparison to the lower density. This is similar to the findings of Reeleder et al. (2000), who observed a significant increase in plant population on plots with higher seed density treatments.

Ginseng plants produced significantly heavier roots after Pommit Extra treatment, while Arbostim (for both application treatments) negatively affected the root weight, especially in the second and third year (Table 2). Roots from plots with Pommit Extra and B-Nine application were heavier (by 2.883 and 2.876 g respectively in the last year) than the control, whereas the smallest roots (three times as light as the control on average) were obtained from plots sprayed with Arbostim. This result is similar to the significant decrease in root yield observed by Nowak and Ciećko (1991) after gibberellic acid applied to the Swedish turnip as well as in the case of ginseng (Kołodziej et al. 2006). In the third year of the experiment, control roots (from plots without growth regulators application) had lower air dry weight per single root (by about one third in comparison with Pommit Extra and B-Nine application).

Independently from the growth regulators tested, roots obtained from the plots with denser plant population were lighter (by about 30 %) than those grown in lower density. Similar results were obtained by Konsler and Shelton (1984) and Reeleder et al. (2000). They

found that seeding rates increased and root size decreased whereas their yields increased. There was found significant interactions between treatments and row spacing in the case of air dry weight of single root. Thus, using lower row spacing and growth regulators, root weight can be improved.

Our examination of six saponin glycosides (ginsenosides) resulted in a wide variation in content depending on the kind of growth regulator used, plants density as well as their interaction (Table 2). The total amount of ginsenosides varied from 58.03 to 114.41 mg $g^{-1}$ of dry matter and it was higher than that found on commercial plantations in Canada and the USA (Li 1995; Court et al. 1996; Reynolds 1998; Reeleder et al. 2000; Li and Wardle 2002). In general, foliar application of chosen growth regulators resulted in higher ginsenosides accumulation, which agrees with our earlier study (Kołodziej et al. 2006). Similarly, addition of plant growth regulators increased the saponin content (8.7–10.9 % by dry weight) in the cell culture of American ginseng (Zhong et al. 1996). Raw material from plots sprayed with Pommit Extra and B-Nine was characterized by significantly higher content of ginsenosides as well as ginsenosides yield in comparison to the untreated control. Zhang et al. (1999) also observed positive effect of auxins (NAA and IAA) on ginsenosides synthesis. As far as Arbostim is concerned, a significant (almost two-fold) increase in the sum of ginsenosides tested in comparison to the control object was observed (twice a year application at wider plant spacing being the exception), which corresponded to our previous study, where a mixture of Pommit Extra and Arbostim also caused an almost two-fold increase in the sum of ginsenosides. At the same time, however, ginsenoside yield per square meter was significantly lower [mainly due to significantly lower plant population (Table 1 and 2, Fig. 1)].

It is worth emphasizing that plants grown in lower densities accumulated a lower amount of determined ginsenosides, which was similar to the results obtained by Reeleder et al. (2000). In the experiment, a significant interaction between growth regulators applied and row spacing used was found, thus sowing ginseng seeds in higher densities and spraying plants with Pommit Extra, Arbostim (once a year) and B-Nine significantly increased total ginsenosides accumulation and yield. Regarding to the quantitative analysis of particular ginsenosides, growth regulators application caused the largest decrease in $R_e$ content relative to the control. Roots from the control object were higher in $R_{b1}$ and $R_e$ (about 80 % of the total determined compounds), than in $R_{b2}$ and $R_c$. We recorded that the amount of particular ginsenosides in control roots was similar to that reported by Court et al. (1996), Reynolds (1998), Wills and Stuart (2001) and Li and Wardle (2002) in roots of three year-old American ginseng. These authors, as well as Wang et al. (1999) recorded clear domination of $R_{b1}$ and $R_e$ or $R_d$, as we did. The lowest abundance measured in our experiment (among determined panaxadiols) was for $R_{b2}$ saponin, which agrees with previously published data (Court et al. 1996; Reeleder et al. 2000; Wills and Stuart 2001). In the experiment, significant increases in $R_{b1}$, $R_d$ and panaxosides from $R_{g1}$ group in the roots from the plots where Pommit Extra was applied were observed. It agrees with our previous study, where a mixture of gibberellic acid with potassium salt of α- naphthalene acetic acid was tested (Kołodziej et al. 2006). B-Nine application led to an increase in the $R_{b1}$, $R_c$, $R_{b2}$ and $R_d$ level and to a decrease in $R_e$ and $R_{g1}$ in comparison to the control, whereas Arbostim resulted in almost two-fold increase in $R_{b1}$ content. Independently from growth regulators application, similarly as in the results of Reeleder et al. (2000), $R_{b2}$, $R_e$ and $R_{g1}$ levels increased with increasing the sowing density. Therefore, using different sowing densities (and at the same time applying specific growth regulators) in American ginseng culture may be very important in obtaining precisely determined content of active substances in its raw material. In general, spraying plants (especially sown in higher densities) with Pommit Extra or B-Nine significantly increase not only weight of roots but also ginsenosides content and yield.

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