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EFFECT OF PRE-HARVEST APPLICATION OF GIBBERELLIC ACID ON THE CONTENTS OF PIGMENTS IN CUT LEAVES OF *Asarum europaeum* L.

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Abstract

The experiment determined the effect of gibberellic acid applied prior to harvest on the contents of plant pigments in cut leaves of wild ginger (*Asarum europaeum* L.), cultivated in an unheated plastic tunnel and in the field. Foliar application of GA_3 at a concentration of 100, 200, 400, 600 mg x dm^{-3} was repeated four times every two weeks. It has been proven that pre-harvest spraying of plants with gibberellic acid at a concentration of 100 mg x dm^{-3} has a positive effect on the content of photosynthetically active pigments in the leaves of *A. europaeum* cultivated in an unheated plastic tunnel. Application of GA_3 at a concentration of 600 mg x dm^{-3} led to the accumulation of the greatest amount of anthocyanins in the leaves of *Asarum europaeum* cultivated both in the unheated plastic tunnel and in the field. The response of plants to GA_3 application, expressed in the amount of flavonoids, depended on conditions related to the cultivation site. Pre-harvest treatment of *A. europaeum* plants with gibberellic acid at concentrations of 100-600 mg x dm^{-3} reduced the production of flavonoids in tunnel-grown wild ginger, but enhanced their accumulation in plants cultivated in the field. Pre-harvest application of gibberellic acid did not affect the fresh weight or dry mass content in plant material.

Key words: *Asarum europaeum*, florist greens, gibberellin, chlorophyll, anthocyanins, flavonoids

INTRODUCTION

Recently, an increased need for florist greenery has been noted. In the search for new species of plants that can be used as a source of greens for bouquets, more and more attention is devoted to perennial plants which are typically used in garden arrangements. European wild ginger (*Asarum europaeum* L.) is a perfect example. It is a native species that reaches a height of up to 30 cm, growing in shaded parts of leafy and mixed forests [1]. Its leaves are evergreen, shiny, dark green and kidney-shaped. The species is used as a covering plant for shaded spots under trees and in pots.

Requirements towards florist greenery are very high. It is expected that leaves will maintain their characteristic, decorative color at least as long as the other elements of a composition. For many years, research has mainly focused on maximizing the vase life during the post-harvest period. However, the physiological and anatomical characteristics that ultimately determine the vase life potential of cut flowers and leaves are formed during the pre-harvest period, as combination between genotype and growth environment [2,3]. A variety of abiotic and biotic factors, such as light, temperature, nutrient status and pest infection, can directly affect the biosynthesis of phytochemicals in plants and have a large impact on the quality of leafy crops [4]. High unheated and passively ventilated tunnels have been widely used in Asia, Europe and the USA [4–6]. High tunnels are known to produce higher yields and better quality of crops by extending their growth period and by providing protection from various adverse factors [7]. Leaf senescence, just like senescence of flowers, is controlled by plant hormones. The most important ones, next to cytokinins, are gibberellins, including gibberellic acid (GA_3). These substances can be used to treat plants during vegetation [8] or after harvesting in the form of conditioning [9–11]. Gibberellic acid affects the growth and development of a plant via regulation of DNA and RNA levels, increased intensity of cell division, biosynthesis of enzymes, proteins, carbohydrates and photosynthetic pigments [12]. The positive effect of exogenous application of GA_3 was noted by Shedeed et al. [13] in the study of *Codiaeum variegatum*, by Eraki [14] on roses, cv. 'Queen Elizabeth', and by Bedour et al. [15] on *Ocimum basilicum*.

The aim of this study was to determine whether cultivation site and gibberellic acid (GA_3), application in the form of plant spraying during vegetation, affect the content of plant pigments in cut leaves of *Asarum europaeum*, which is related to their decorative value and vase life.

MATERIALS AND METHODS

The experiment was based on 3-year-old plants of *Asarum europaeum* cultivated on beds, in an unheated foil tunnel and in the field with plant spacing of 40 x 25 cm. It was set up in random blocks in three replicates, where a plot with 6 plants served as a replicate.

Gibberellic acid was applied in the form of foliar spraying, four times every two weeks, at a concentration of 100, 200, 400 and 600 mg x dm^{-3}. Plants sprayed with distilled water were treated as control plants. The first round of spraying with phytohormone was conducted when plants had six fully developed leaves (20^{th} April in case of plants cultivated in the tunnel and 12^{th} May in case of wild ginger cultivated in the field). During growth, plants were watered and fertilized systematically every 2 weeks with the solution of Azofoska at a concentration of 0.3%.

The content of assimilation pigments, flavonoids and anthocyanins was analyzed. Leaves of *A. europaeum* were harvested two weeks after finishing the last phytohormone application (mid-June in case of plants in the tunnel and the beginning of July in case of plants cultivated in the field) and taken as material for analysis. The material was cut into small pieces and well mixed to obtain a representative assay. Weighed portions were taken from that material in order to determine the content of plant pigments. The leaf content of assimilation pigments and chlorophyll *a* and *b* was determined through their extraction in 80% acetone. An absorbance measurement was taken at two wavelengths (λ): 645 nm (chlorophyll *b*) and 663 nm (chlorophyll *a*). Next, the leaf pigment content was calculated according to Lichtenthaler and Wellburn's method [16]. Dry mass was determined after drying the material at a temperature of 105°C until permanent weight was reached. The material was weighed with utmost precision (up to 0.01g) on electronic scales. The contents of other plant pigments – anthocyanins and flavonoids – were determined in dry leaf matter. The content of flavonoids was determined by the spectrophotometric method by Christ and Muller (1960) after Strzelecka et al. [17]. The content of anthocyanins was determined by the spectrophotometric method following Miłkowska and Strzelecka [18]. The ground dry plant material was extracted for 24 hours in the mixture of ethanol and HCl. Next, an absorbance measurement was taken at a wavelength of (λ): 535 nm.

All analyses and measurements were done in 5 replicates.

Vase life of *A. europaeum* leaves was determined by recording the number of days until the time when the symptoms of loss of decorative quality appeared (30% of the leaf blade was wilted or had yellow or brown spots). The leaves were placed in distilled water under controlled conditions: temperature 23°C, relative humidity 60%, 16/8h photoperiod with a quantum irradiance of 35 μmol × m^{-2} s^{-1}. There were 3 replicates with 5 leaves in each.

The results were analyzed statistically by two-way analysis of variance, and the significance of differences was evaluated with multiple Tukey's confidence intervals at a level of significance of α=0.05 %.

RESULTS

The analysis of postharvest longevity of cut *A. europaeum* leaves showed a positive effect of tunnel cultivation on the measured value (Table 1). The leaves of tunnel-grown plants of the examined species preserved the decorative value longer by 34.9% in comparison to those harvested from field cultivation. The analysis of postharvest longevity of leaves of tunnel-grown *A. europaeum* and sprayed with gibberellic acid proved that there were significant differences between the examined treatments. GA_3 applied at 100 mg x dm^{-3}, 200 mg x dm^{-3} and 400 mg x dm^{-3} improved leaf longevity by more than 36%. In the case of field cultivation, gibberellic acid at all concentrations significantly affected the vase life of cut leaves. The greatest leaf longevity was found in the treatment where GA_3 was applied at a concentration of 600 mg x dm^{-3} – 70% higher in relation to the control.

Table 1
Effect of gibberellic acid on vase life of cut *Asarum europaeum* leaves obtained from an unheated plastic tunnel and from the field

Cultivation site	GA_3 concentration [mg x dm^{-3}]	Vase life [days]
Tunnel	0	61.3c
	100	83.7a
	200	75.0b
	400	70.7b
	600	60.5c
Field	0	36.3e
	100	50.2d
	200	52.3d
	400	59.8c
	600	61.7c
Mean for tunnel		**70.2A**
Mean for field		**52.1B**

*Means in columns marked with the same letter do not differ significantly at α= 0.05

Hormone concentration and cultivation place have a significant effect on the content of chlorophyll *a*, chlorophyll *b* and the total amount of analyzed pigments in the leaves of *A. europaeum*. The highest content of chlorophyll *a*, *b* and the highest sum of the contents of analyzed pigments (*a*+*b*) were noted in leaves of tunnel-grown plants treated with gibberellic acid at a concentration of 100 mg x dm^{-3} (Table 2). In case of field cultivation, the highest content of chlorophyll *a* and the highest sum (*a*+*b*) were found in the treatment where GA_3 was applied at a concentration of 200 mg x dm^{-3}. The most chlorophyll *b* in leaves harvested from the field was noted in control plants.

Table 2
Effect of gibberellic acid on chlorophyll contents in cut leaves of *Asarum europaeum* obtained from the unheated plastic tunnel and from the field

Cultivation site	GA_3 concentration [mg x dm^{-3}]	Examined feature		
		Chlorophyll *a* [mg x g^{-1}f.w.]	Chlorophyll *b* [mg x g^{-1} f. w.]	Sum (*a*+*b*) [mg x g^{-1} f.w.]
Tunnel	0	0.943*i	0.544d	1.487h
	100	1.684a	1.091a	2.775a
	200	1.232d	0.935b	2.167b
	400	1.091f	0.519f	1.610f
	600	0.826j	0.418i	1.244h
Field	0	1.056g	0.736c	1.792d
	100	1.008h	0.336j	1.344i
	200	1.325b	0.514g	1.839c
	400	1.220e	0.534e	1.754e
	600	1.291c	0.497h	1.788g
Mean for tunnel		**1.155B**	**0.701A**	**1.856A**
Mean for field		**1.180A**	**0.523B**	**1.704B**

*Means in columns marked with the same letter do not differ significantly at α=0.05

Higher contents of anthocyanins and flavonoids were observed in leaves obtained from cultivation in the plastic tunnel, in comparison to cultivation in the open field (Table 3).

The highest contents of anthocyanins in leaf tissues of plants cultivated in the unheated plastic tunnel were found when plants were treated with GA_3 at a concentration of 600 mg x dm^{-3}. The amount of the pigment examined was five times higher in comparison to its content in the leaves of plants sprayed with distilled water. The highest concentration of flavonoids was observed in the control. The treatment with gibberellin led to a decrease in the amount of flavonoids in the leaves of plants grown in the tunnel. The lowest level of flavonoids was found in the treatment where the highest concentration of hormone was applied (a decrease by approx. 32% in comparison to the control).

The analysis of anthocyanin and flavonoid contents in the leaves of field-grown plants revealed the highest concentration of the investigated pigments when plants were treated with GA_3 at the highest concentration. The amounts of anthocyanins and flavonoids were higher by 31.4% and 29.0%, respectively, in comparison to their contents in the leaves of plants sprayed with distilled water.

Table 3
Effect of gibberellic acid on anthocyanin and flavonoid contents in cut leaves of *Asarum europaeum* obtained from the unheated plastic tunnel and from the field

Cultivation site	GA_3 concentration [mg x dm^{-3}]	Examined feature	
		Anthocyanins [mg x g^{-1}d.w.]	Flavonoids [mg x g^{-1}d.w.]
Tunnel	0	0.65h	6.51a
	100	0.97d	5.22c
	200	1.78c	5.34b
	400	2.59b	4.67e
	600	3.25a	4.43f
Field	0	0.64i	3.93h
	100	0.65h	3.97h
	200	0.66g	4.62e
	400	0.73f	4.33g
	600	0,84e	5.07d
Mean for tunnel		**1.85A**	**5.23A**
Mean for field		**0.70B**	**4.38B**

*Means in columns marked with the same letter do not differ significantly at α= 0.05

The analysis of fresh weight and content of dry mass and water in the leaves of *A. europaeum* led to a conclusion that pre-harvest application of gibberellic acid did not affect the discussed parameters, regardless of cultivation site and concentration of the applied plant hormone (Table 4).

Table 4
Effect of gibberellic acid on fresh and dry weight per leaf and water content in cut leaves of *Asarum europaeum* obtained from the unheated plastic tunnel and from the field

Cultivation site	GA_3 concentration [mg x dm^{-3}]	Examined feature				
		Fresh weight per leaf [g]	Dry weight per leaf		Water content	
			[g]	%	[g]	%
Tunnel	0	2.1660a*	0.5200a	23.98a	1.6460a	76.02a
	100	2.1970a	0.5640a	25.68a	1.6330a	74.32a
	200	2.2630a	0.6270a	27.71a	1.6360a	72.29a
	400	2.2223a	0.5180a	23.31a	1.7040a	76.69a
	600	2.3290a	0.5350a	22.96a	1.7940a	77.04a
Field	0	2.0220a	0.5710a	28.23a	1.4510a	71.77a
	100	2.0220a	0.6260a	30.97a	1.3960a	69.03a
	200	2.0210a	0.6560a	32.44a	1.3650a	67.56a
	400	2.0420a	0.6170a	30.22a	1.425a	69.78a
	600	2.0090a	0.5640a	28.09a	1.4450a	71.91a
Mean for tunnel		**2.2350A**	**0.5530A**	**27.73A**	**1.682A**	**72.27A**
Mean for field		**2.0230A**	**0.6068A**	**29.99A**	**1.4162A**	**70.01A**

*Means in columns marked with the same letter do not differ significantly at α= 0.05

DISCUSSION

The analysis of postharvest longevity of cut *A. europaeum* leaves showed a positive effect of tunnel cultivation on the measured value (Table 1). The results obtained from this study suggest that tunnel production offers several benefits over field production when growing high-quality florist greens. The high tunnel frame and polyethylene glazing film reduced light transmission by ~ 23%. Crops in high tunnels receive a lower daily light integral, reduced air movement and altered air temperature compared with crops in the field [19–21]. *A. europaeum* is a shade-loving plant which requires shade at all time to produce good leaf yield. The vase life of leaves was also longer when plants were grown in the unheated tunnel. These observations confirm those obtained by Arumugan and Jawahalal [22] as well as Rao et al. [23] who found an increase in longevity of *Dendrobium* flowers when growing this plant under shading conditions. It is known that higher yield and better quality crops are obtained in unheated tunnels due to an extended growth period and protection from various adverse factors [7,24], which may improve the quality of leaves and their longevity.

The experiment showed that four applications of gibberellic acid by spraying plants with a solution at a concentration of 100 and 200 mg x dm^{-3} had a positive effect on the amount of chlorophyll *a* and chlorophyll *b* in the leaves of *A.europaeum* cultivated in the unheated plastic tunnel. Similarly, an increase in assimilation pigments caused by triple application of GA_3 was observed by Shah et al. [25] in tunnel-grown *Nigella sativa* plants. A positive effect of gibberellic acid solution treatment on chlorophyll *a+b* content was also proved by Yu et al. [26] who found an increase in these pigments during cultivation of *Paris polyphylla* in an unheated plastic tunnel. The above-mentioned authors suggest an effect of exogenous GA_3 applied in the form of plant spraying on the accumulation of endogenous gibberellic acid, which stimulates the production of assimilation pigments. Ouzounidou and Illias [27] explain the growth of chlorophyll content by the influence that the phytohormone has on the transformation of etioplasts into chloroplasts. Gibberellic acid also inhibits degradation of the discussed pigments by controlling the process of starch and sucrose hydrolysis into fructose and glucose, which indirectly affects the content of chlorophyll *a* and *b* in leaves [28,29].

Flavonoids are biologically active pigments that are commonly found in plants. Anthocyanins are the most common among them and they are responsible for the color of flower petals and fruits, less frequently of leaves and stems [30]. Flavonoids are like a filter that protects a plant from UV light. They act as anti-oxidants and reveal anti-virus, anti-fungal and anti-bacterial traits [31]. Excessive accumulation of anthocyanins in mature leaves is perceived as a consequence of the activity of stress factors, both biotic and abiotic, such as damage, pathogen attack, negative environmental conditions or shortage of nutrients [32]. The experiment showed a higher content of anthocyanins and flavonoids in the leaves of plants cultivated in the plastic tunnel compared to those growing in the open field, which indicates the effect of cultivation conditions on the production of the abovementioned pigments. Possibly, higher temperature and humidity of a tunnel are stressful conditions to perennials inhabiting shady forests. Oh et al. [4] found that lettuce plants accumulated a higher amount of flavonoids and anthocyanins in open field than in high tunnels. These authors explain that with reduced light intensity in a high tunnel, which may play a negative role in the production of secondary metabolites. Kleinhenz et al. [33] noted that shading did not increase the anthocyanin content in leaves of several lettuce varieties.

Spraying of plants with gibberellic acid in increasing concentration led to a greater amount of anthocyanins in the leaves of *Asarum europaeum* grown both in the unheated plastic tunnel and in the field. Plant reaction to GA_3 application, expressed in the amount of flavonoids produced, depended on cultivation site. Plants growing in the tunnel treated with gibberellic acid produced fewer flavonoids than those growing in the field.

The effect of plant hormones on the content of anthocyanins and flavonoids in plant leaves was presented in research by Kwack et al. [34] and Chung et al. [35]. They found an increase in the content of these pigments after treatment of plants with gibberellic acid. The authors explained that phenomenon with the effect that gibberellins had on the synthesis of phenylalanine ammonia-lyase (PAL) and tyrosine ammonia-lyase (TAL), the key enzymes of flavonoid and anthocyanin synthesis. Phenylalanine ammonia-lyase catalyzes spontaneous, non-oxidative deamination of L-phenylalanine to trans-cinnamic acid which, in the process of further metabolic changes, can be transformed into, for example, flavonoids. The thesis was confirmed by Montero et al. [36] who, after treatment of *Fragaria ananassa* fruit with a solution of gibberellic acid, observed an increased amount of anthocyanins as well as increased activity of PAL and TAL enzymes. After foliar application of GA_3 solutions, an increased amount of anthocyanins was also found in the leaves of *Hibiscus sabdariffa* [37] and *Ajuga reptans* [34].

In our experiment, growing conditions had no effect on biomass accumulation in *A. europaeum* leaves. Oh et al. [4] found that lettuce plants in open field did not fare well in biomass accumulation as those under high tunnels, which was probably due to unfavorable outdoor conditions, such as inadequate soil water content, too high light and strong wind. Spraying plants with gibberellic acid four times did not affect the fresh weight or dry mass content in the leaves of *A. europaeum*. No effect of foliar GA_3 application on dry mass content in the leaves of *Iris nigricans* was found by Al-Khassawneh et al. [8]. However, Soad et al. [38] observed that gibberellic acid applied by spraying affected both fresh weight and dry mass content of leaves, stems and roots of *Codiaeum variegatum.*

CONCLUSIONS

1. Cultivation of *A. europaeum* in an unheated tunnel beneficially influenced postharvest quality of leaves, which is proved by their high longevity and the sum of chlorophylls *a* and *b* as well as the higher content of anthocyanins and flavonoids.
2. Pre-harvest spraying of plants with gibberellic acid at a concentration of 100 mg x dm^{-3} has a positive effect on the content of photosynthetically active pigments in the leaves of *A. europaeum* cultivated in an unheated plastic tunnel.
3. GA_3 application at a concentration of 600 mg x dm^{-3} led to the accumulation of the greatest amount of anthocyanins in the leaves of *A. europaeum* cultivated both in an unheated plastic tunnel and in the field.
4. The response of plants to GA_3 application, expressed in the amount of flavonoids, depended on conditions related to the cultivation site. Pre-harvest treatment of *A. europaeum* plants with gibberellic acid at concentrations of 100-600 mg x dm^{-3} reduced the production of flavonoids in tunnel-grown wild ginger, but enhanced their accumulation in plants cultivated in the field.
5. Pre-harvest application of gibberellic acid did not affect the fresh weight or dry mass contents in plant material.

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Authors' contributions

The following declarations about authors' contributions to the research have been made: designing the experiments: EP, MJ; field research: MJ; biochemical analyses: AN; data analyses: KR; writing the manuscript: EP, KR, MJ.

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Wpływ przedzbiorczej aplikacji kwasu giberelinowego na zawartość barwników roślinnych w ciętych liściach *Asarum europaeum* L.

Streszczenie

W doświadczeniu badano wpływ kwasu giberelinowego zastosowanego przed zbiorem, na zawartość barwników roślinnych w ciętych liściach kopytnika pospolitego (*Asarum europaeum* L.), uprawianego w nieogrzewanym tunelu foliowym i w polu. GA_3 w stężeniu 100, 200, 400, 600 mg × dm^{-3} zastosowano dolistnie, czterokrotnie, w odstępach dwutygodniowych. Wykazano, że przedzbiorcze opryskiwanie roślin kwasem giberelinowym w stężeniu 100 mg × dm^{-3} korzystnie wpływa na zawartość barwników aktywnych fotosyntetycznie w liściach *A. europaeum* uprawianego w nieogrzewanym tunelu foliowym. Aplikacja GA_3 w stężeniu 600 mg × dm^{-3} spowodowała nagromadzenie największej ilości antocyjanów w liściach *Asarum europaeum* uprawianego zarówno w nieogrzewanym tunelu foliowym, jak i w polu.

Reakcja roślin na aplikację GA_3, wyrażona ilością nagromadzonych flawonoidów, zależała od warunków związanych z miejscem uprawy. Traktowanie przed zbiorem roślin *A. europaeum* kwasem giberelinowym w stężeniu 100–600 mg × dm^{-3} ograniczało wytwarzanie flawonoidów u kopytnika uprawianego w tunelu, ale sprzyjało ich gromadzeniu u roślin w polu. Przedzbiorcza aplikacja kwasu giberelinowego nie miała wpływu na świeżą masę i zawartość suchej masy w materiale roślinnym.

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