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CONTENT

The production potential of the new Slovak red vine cultivars (*Vitis vinifera* L.)

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Abstract
Use of suitable vine cultivars in cool-climate regions is necessary for production of grapes and subsequently wines of high quality. Sugars and acids are major parameters of grape and wine quality. Assortment of red grape cultivars in Slovakia was enriched with 7 new cultivars with high exceptional quality, which open wide scope for production of red wines with high quality. The studies were conducted on the 7 new red cultivars - Hron, Váh, Nitria, Rimava, Rudava, Torysa and Rosa registered in 2011. In the period of experiment (2010-2014) were evaluated: yield (kg per vine bush and $t.ha^{-1}$), sugar content (in kg per 100 litres of juice, i.e. ^{o}NM) and content of total acids ($g.l^{-1}$). The highest yield was determined for cultivar Váh (average yield 4.48 kg/vine bush) - together with Nitria (4.36 kg/vine) formed one homogenous group. The lowest average yield was determined for cultivar Torysa (1.62 kg/vine bush). The highest average sugar content in juice was observed for Rosa (24.96 ^{o}NM) and Rudava (24.70 ^{o}NM) and the lowest for Rimava (22.80 ^{o}NM) and Nitria (23.04 ^{o}NM). The highest average total acid content was observed for Váh (8.04 $g.l^{-1}$), Nitria (8.00 $g.l^{-1}$) and Hron (7.9 $g.l^{-1}$) and the lowest for Torysa (6.02 $g.l^{-1}$).

Keywords: *Vitis vinifera* L., red cultivar, fermentable sugars, total acidity, yield

Introduction

The actual viticultural assortment, although allowing the obtention of different types of wines of great diversity, requires continuous improvement by creating new cultivars combining superior features of production and quality (Bian et al., 2004).
Assortment of red grape cultivars in Slovakia was enriched with seven new cultivars with high exceptional quality, which open wide scope for production of red wines with high quality. New red cultivars Hron, Váh, Nitria, Rimava, Rudava, Torysa and Rosa were registered in 2011 and supplemented the first Slovak red cultivar Dunaj registered in 1997. Cultivars Hron, Nitria, Rimava and Váh have a common origin. They were created by crossing of two French cultivars - Castets and Abouriou noir - in the Research Institute of Viticulture and Enology in Bratislava in 1976. These varieties were part of the world collection of cultivars which has been collected from all over the world and assessed for their suitability for environmental conditions of Slovakia. Castets is fertile cultivar, and gives dark wines with Cabernet flavour. Abouriou noir has middle fertility and provides a soft, quality wines with intensive colour without a distinctive aroma (type of Pinot Noir). Rudava and Torysa are result of breeding of French cultivars Castets with triple crossing of of Soviet Union origin I-35-9. Genotype I-35-9 is a result of crossing of cultivars Tenturier, Aleatico and Puchljakovskij Černyj. Tenturier is a French cultivar with low fertility, teinturier. Aleatico is an Italian cultivar, very fertile with muscatel aroma. Puchljakovskij Černyj is a type with functional female flowers, grapes ripen relatively late and is more of a table type. Cultivar Rosa is result of the crossing of the French teinturier cultivar Picpoul Noir and Frankovka modrá (Blaufrankisch) conducted in 1951. About 10 years later was made crossing with cultivar Traminer. Breeder of all cultivars is Ing. Dorota Pospíšilová, PhD. (Anonym, 2016).

The production of quality wines requires a judicious balance between the sugar, acid and flavour components of wine. Acids play a crucial role in the winemaking process, including the organoleptic quality and the physical, biochemical and microbial stability of wine (Volschenk, van Vuuren and Viljoen-Bloom, 2006).
The objective of the study was to evaluate of grape yield, content of fermentable sugars and total acid in seven new Slovak red wine cultivars in vineyard in Dolné Orešany village (Small Carpathian vine-growing region).

Material and Methods

Description of the experimental site

Vineyard is located in the Small Carpathian vine-growing region, vine-growing district Orešany, in cadastre of village Dolné Orešany, on the site Špintrále. Altitude of site is about 225 m a.s.l. Vineyard is on slope with south-east inclination.
The subregion is warm (with 50 or more summer days), dry, with mild winter. The sum of average daily temperatures over 10 ^{o}C is 2500-3000. The length of the period with the air temperature above 5 ^{o}C is over 231 days. The average air temperature in January is -1-3 ^{o}C. The average air temperature during vegatation period is 15-16 ^{o}C. According to regionalization and long-term data is the average annual temperature 10 ^{o}C and the average temperature during vegetation period 16.5 ^{o}C at an altitude 200 m a.s.l. The annual precipitation is 810 mm and during vegetation period 450 mm. Hydrometeorological observations and measurements show that in recent years the average temperature increases and rainfall decreases.
Site is a part of the mountain Small Carpathians, which significantly affected the composition and evolution of soils on the south-eastern slopes and foothills. Vineyard is planted on the moderate soil (soil parent material is schist – slate which protrude from the soil). Soil reaction (pH) is 6.2.
Vineyard was planted in 2004. Space is 3 m x 1.2 m. Used rootstock is *Vitis berlandieri* x *Vitis riparia* Kober 5BB. Training system is simple curtain with trunk height 1.6 m. Vine bushes have two semi-long canes with 7 buds and three spurs with 2 buds. Total number of buds per vine is 20 (5.55 buds per m^{2}). Grass is grown in every second inter row as cover crop and during vegetation period was used mowing and mulching as required. In other inter rows was used shallow cultivation of soil. In vine rows was used application of herbicides. Summer pruning was made manual. Pest management was performed according to forecasts and monitoring. Grape harvest was manual. Evaluated cultivars were Hron, Nitria, Rimava, Rudava, Torysa, Vah and Rosa.

Evaluated parameters were:

- Average grape yield (in kilograms per vine bush);
- Soluble solids (Sugar content) in juice in ^{o}NM (kilograms per hectolitre);
- Total acids content in juice (grams per litre).

Grape yield was measured after grape harvest from individual vine bushes. Sugar content was measured by normalised hydrometer with temperature correction. Total acid content in juice was measured by titration method after juice separation from must.

Characteristics of evaluated cultivars

Hron

It is cultivar for small-scale production. Produces wines with excellent quality – they are among the best in this group of hybrids. Wines are full and expressive. They have a pleasant cabernet character and a beautiful dark red colour. Grape ripens mid-late. Fertility is medium - 7-14 t ha^{-1}, the sugar content in juice is 20-24 kg.hl^{-1} and the acid content is 8-11 g.l^{-1}. The

average weight of the bunch is 118 grams. Recommended is cane pruning (for top bush shapes) and with a load to 10-12 buds per m^2.

Nitria
It provides wines with good quality in the most vintages. Wines are harmonious, full, beautiful dark colour. Appreciated is their gentle but expressive cabernet flavour. Cultivar is very fertile and has reliable fertility – grape yields are 11-19 t ha^{-1}, with a sugar content in juice 18-23 kg.hl^{-1} and acid content 7,5- 9 g.l^{-1} (Pospíšilová et al., 2005). The average weight of the bunch is 127 grams. Recommended is cane pruning with 7-9 buds per m^2.

Rimava
It provides high-quality wines with cabernet character. Wines are in majority of vintages evaluated as full, harmonious, pleasant colour. Yields are high (9-15 t.ha^{-1}), with sugar content in juice 18.5-24 kg.hl^{-1} and acid content 8-11 g.l^{-1}. Term of grape harvest is in mid-October (Pospíšilová et al., 2005). The average weight of the bunch is 150 grams. Maximum load is 8-10 buds per m^2.

Rudava
It is characterized by good fertility and quality of wines. It provides wines with full red ruby colour. Wines have fine cabernet flavour in some years. Grape yields are 9-11 t ha^{-1}. Sugar content in juice varies between 19-23 kg.hl^{-1} and acid content is 9-12 g.l^{-1} (Pospíšilová et al., 2005). The average weight of the bunch is 111 grams. Recommended is cane pruning and 8-10 buds per m^2.

Torysa
Offers exceptional wines that are full-bodied, rich in tannins and red colour. The wine has a soft, fine, cabernet flavour (but is lost at higher yields). Fertility is proportional to the soil where it is grown. In poorer soils yields reach 6-7 t.ha^{-1}, in the deep, fertile soils 8-12 t.ha^{-1}. Generally has higher sugar content in juice – according to vintage - 19-25 kg.hl^{-1} and acid content is 8-10 g.l^{-1} and higher (Pospíšilová et al., 2005). The average weight of the bunch is 160 g. Recommended number of buds after pruning is 10 buds per m^2.

Váh
It provides wines with very high quality, especially in warmer vine-growing areas. The wine is characterized by a beautiful dark red colour and distinctive cabernet taste. Yields are 10-15 t.ha^{-1}, with a sugar content of juice 19-25 kg.hl^{-1} and acid content 7-9 g.l^{-1} (Pospíšilová et al., 2005). The average weight of the bunch is 90 grams. Recommended is cane pruning and 10 buds per m^2.

Rosa
Rosa has an extremely interesting character of the wine. It provides a dark red wine, which has unusual flavour. Aroma reminds the smell of roses especially in older wines. Yields are lower – from 5 to 8 t ha^{-1}, sugar content in juice is 18-25 kg.hl^{-1} and content of acids 8-12 g.l^{-1} (Pospíšilová et al., 2005). The average weight of the bunch is 83 grams. Recommended is long cane pruning and 10-12 buds per m^2.

Statistical analysis
The significance of differences (at $P \leq 0.05$) in yield (kg/vine bush), content of acids (g.l^{-1}) and fermentable sugars (kg.hl^{-1} = ^{o}NM) was checked by the statistical method (Analysis of variance – ANOVA) using the Tukey test.

Results and Discussion

Evaluation of the average yield of grapes per vine bush and per hectare

Highly significant differences were present between years (F =49,599, p < 0.01) and between cultivars (F = 16.553, p < 0.01) for average crop level - average yield of grapes per vine bush. The highest yield was determined for cultivar Váh (together with cultivar Nitria formed one homogenous group) and cultivar the lowest for cultivar Torysa (Table 1).
The statistically highest yield was in 2011 and the lowest in 2014 (Table 2).

Table 1 The average grape yield of evaluated cultivars

statistical difference between cultivars	Cultivar	average yield (kg/vine bush)	Homogenous Groups
++ (highly significant)	Torysa	1.62	a
	Hron	2.32	ab
	Rosa	2.48	b
	Rudava	3.54	c
	Rimava	4.18	cd
	Nitria	4.36	d
	Váh	4.48	d

Table 2 The average grape yield during experimental period

statistical difference between years	Year	average yield (kg/vine bush)	Homogenous Groups
++ (highly significant)	2014	2.50	a
	2010	2.99	ab
	2013	3.33	bc
	2012	3.77	c
	2011	3.83	c

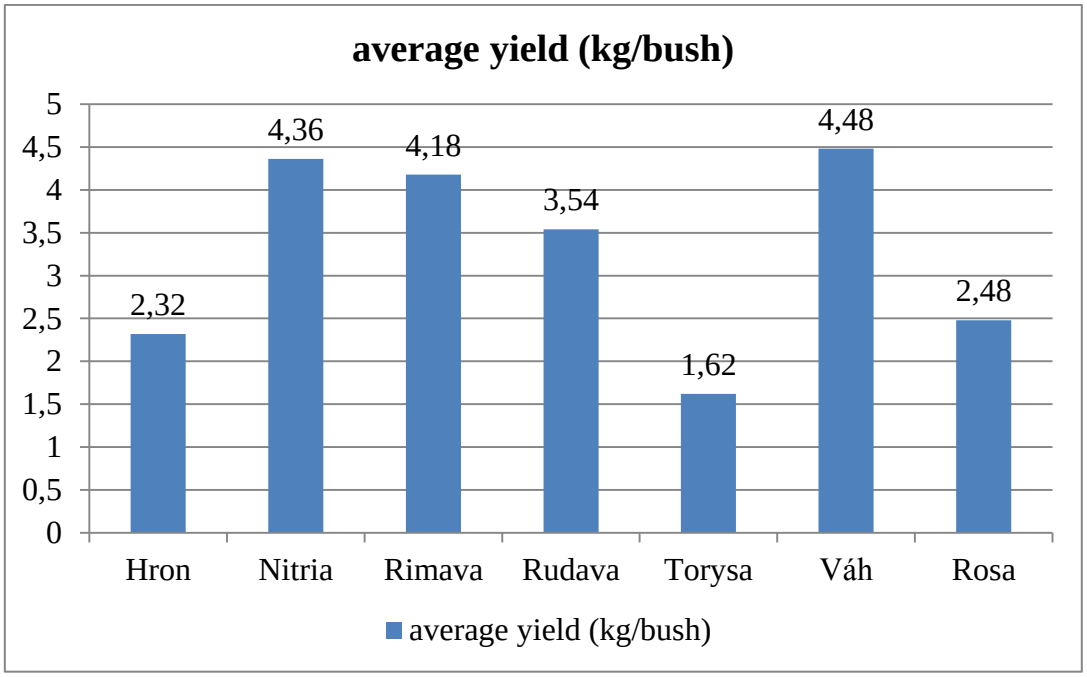

Figure 1 The average yield (kg) per bush of evaluated cultivars in the years 2010-2014

Evaluation of the average yield of grapes per hectare

The five-year observation evaluation of the observed cultivars provides opportunity of objective evaluation of their production potential. Unfavorable conditions were in years 2010 and 2014, very good vintages were 2011 and 2012 and average vintage was 2013.

Pospíšilová and Korpás (1998) characterized fertility of cultivar Hron as middle and according to soil fertility ranges from 7 to 14 $t.ha^{-1}$ and our results correspond with these authors because average yield for experimental period was 7.9 $t.ha^{-1}$. Mimimum yield (6.0 $t.ha^{-1}$) was in 2012 and maximum (10.3 $t.ha^{-1}$) in 2010.

Pospíšilová et al. (2005) found than fertility of cultivar Torysa depends on soil characteristics. Grape yields are 6-7 $t.ha^{-1}$ in less fertile soils and 8-12 $t.ha^{-1}$ in deep, fertile soils. Average yield in experiment was 6.02 $t.ha^{-1}$ (minimum yield 5.2 and maximum 6.9 $t.ha^{-1}$) and in our opinion it is result of soil characteristics of site (slate soil in vineyard).

Pospíšilová (2005) described Rosa as cultivar with lower fertility (5-8 $t.ha^{-1}$) and results of our experiment (average yield 7.5 $t.ha^{-1}$) confirm their results.

For other cultivars results of our experiment do not match the information of Pospíšilová et al. (2005) and Pospíšilová and Korpás (1998). Pospíšilová et al. (2005) published information that cultivar Nitria provides yields 11-19 $t.ha^{-1}$ but in our experiment we found lower average yield (8.0 $t.ha^{-1}$).

Pospíšilová and Korpás (1998) described cultivar Rimava as regularly fruiting with yields 9-16 $t.ha^{-1}$. Average yield of Rimava in experiment was 6.98 $t.ha^{-1}$ and this results do not correspond with cited authors.

Pospíšilová et al. (2005) evaluated Rudava as a cultivar with average yields 9-11 t ha^{-1} but average yield in our experiment was 7.32 $t.ha^{-1}$.

Pospíšilová and Korpás (1998) characterized Váh as cultivar with small berries but regular fertility 10-13,5 $t.ha^{-1}$. Our results do not confirm declared fertility because average yield was 8.04 $t.ha^{-1}$.

From the point of view of yield quantity obtained in experiment was possible use of grape for production of the highest quality category of wines – quality wine with the attribute and wine with protected designation of origin (Hronský et al. 2006; Hronský and Pintér, 2009).

Evaluation of the content of fermentable sugars in the juice

Highly significant differences were present between years ($F = 46.008$; $p < 0.01$) and between cultivars ($F = 4.898$, $p < 0.01$) for average sugar content in juice (in $kg.hl^{-1}$).

The highest sugar content was determined for cultivar Rosa (together with cultivar Rudava formed one homogenous group) and the lowest for cultivar Rimava (Table 3).

The absolutely the highest sugar content was determined for cultivar Rudava (28 ^{o}NM) in 2012 and the lowest (20.5 ^{o}NM) for cultivars Nitria and Váh in 2014. The statistically highest sugar content in juice was in 2012 and the lowest in 2014 (Table 4).

Table 3 The average sugar content in juice of evaluated cultivars

statistical difference between cultivars	Cultivar	average sugar content ($kg.hl^{-1}$)	Homogenous Groups
++ (highly significant)	Rimava	22.88	a
	Nitria	23.04	a
	Váh	23.34	ab
	Hron	23.68	ab
	Torysa	23.88	ab
	Rudava	24.70	b
	Rosa	24.96	b

Table 4 The average sugar content in juice during experimental period

statistical difference between years	Year	average sugar content (kg/hl)	Homogenous Groups
++ (highly significant)	2014	21.43	a
	2010	22.50	ab
	2013	23.13	b
	2011	25.60	c
	2012	26.26	c

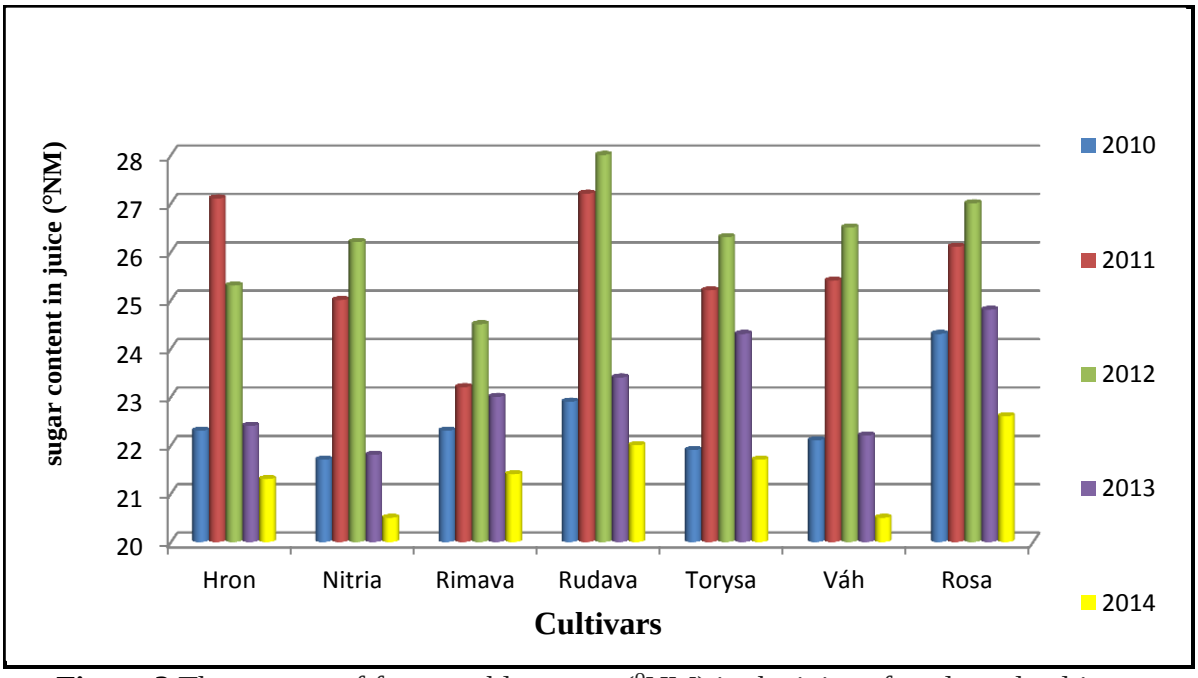

Figure 2 The content of fermentable sugars (^{o}NM) in the juice of evaluated cultivars

Content of fermentable sugars in 2010 did not reach high values due to unfavorable weather conditions. The highest sugar content (24.3 ^{o}NM) was determined in cultivar Rosa and the lowest (21.7 ^{o}NM) was measured in cultivar Nitria.

The more suitable conditions were in years 2011 and 2012 and sugar content in juice was higher. The highest sugar content in juice in 2011 was found in cultivar Hron (27.1 °NM) and the lowest (23.2 °NM) in Rimava. The highest sugar content (28.0 ^{o}NM) in 2012 was observed in Rudava, thanks to a later term of harvest also. Relatively high content of sugar in juice but among cultivars in this year the lowest was found in Rimava (24.5 °NM). Year 2013 can be considered as average vintage. The highest sugar content reached cultivar Rosa (24.8 °NM) and the lowest Nitria (21.8 °NM). Year 2014 was the worst from the point of view of fermentable sugars content in juice. Relatively low content of fermentable sugars was observed in juice of all studied cultivars. The highest value was recorded again in the cultivar Rosa (22.6 °NM) and the lowest (20.5 ^{o}NM) cultivars Nitria and Vah, because of the strong pressure of diseases and pest and therefore were harvested at an earlier term.

Pospíšilová and Korpás (1998) reported the sugar content in juice of cultivar Hron - according to vintages - in range between 20-24.5 kg.hl^{-1}. Our results correspondent with mentioned authors because average sugar content in our experiment was 23.68 ^{o}NM and in year 2011 even 27.1 ^{o}NM. Lower content of fermentable sugars (and higher content of acids) in 2014 was result of unsuitable weather conditions and widespread occurrence of diseases and pests.

Pospíšilová et al. (2005) published information that sugar content in juice of cultivar Nitria is 18-23 kg.hl^{-1}. Our results correspond with them because average value was 23.04 ^{o}NM and in years 2011 and 2012 was higher - up to 2-3 ^{o}NM.
Pospíšilová and Korpás (1998) reported sugar content in juice of cultivar Rimava 18.5-25.5 ^{o}NM. Our results concerning sugar content correspond with cited authors.
Pospíšilová (2005) evaluated Rudava as a cultivar with sugar content in juice between 19-23 kg.hl^{-1}. Average sugar content was 24.7 ^{o}NM and in years 2011 and 2012 much higher (up to 27 ^{o}NM).
Pospíšilová et al. (2005) found than sugar content in juice is generally higher - 19-25 kg.hl^{-1} (depends on year). Sugar content in juice found in experiment coincides with information by Pospíšilová et al. (2005) – average value was 23.88 ^{o}NM and in year 2012 was higher (26.3 ^{o}NM).
Pospíšilová and Korpás (1998) characterized Váh as cultivar with sugar content 18.5-25.5 kg.hl^{-1} (depends on year and quantity of grape) and results of our experiment are similar and in good year 2012 higher (26.5 ^{o}NM).
Pospíšilová et al. (2005) described Rosa as cultivar with sugar content in juice 18-25 kg.hl^{-1}. Our results confirm their results - average value was 24.96 ^{o}NM and in suitable years 2011 and 2012 was sugar content in juice (26.1 resp. 27 ^{o}NM) higher in compare to their results.
From the point of view of content of fermentable sugars in juice found in experiment were grapes suitable for production quality wine with the attribute – from kabinetné (cabinet – minimum required sugar content 19 ^{o}NM) for Váh in 2014 to hrozienkový výber (raisin selection minimum required sugar content 28 ^{o}NM) for Rudava in 2012. Supposed content of natural alcohol in wine after total fermentation of fermentable sugars is from 11.3 vol.% vol. to 16.7 vol.%. For stability of red wine and it´s appropriate sensory properties is required content of natural alcohol 12-13 vol. % (Hronský et al., 2006).

Table 3 The average total acid content in juice of evaluated cultivars

statistical difference between cultivars	Cultivar	average total acid content (g.l^{-1})	Homogenous Groups
++ (highly significant)	Torysa	6.02	a
	Rimava	6.98	ab
	Rudava	7.32	ab
	Rosa	7.50	ab
	Hron	7.90	b
	Nitria	8.00	b
	Váh	8.04	b

Table 4 The average total acid content in juice during experimental period

statistical difference between years	Year	average total acid content (g.l^{-1})	Homogenous Groups
++ (highly significant)	2012	6.44	a
	2011	6.72	a
	2013	7.19	ab
	2010	8.10	bc
	2014	8.53	c

Evaluation of the total acid content in juice
Highly significant differences were present between years ($F = 8.862$; $p < 0.01$) and between cultivars ($F = 4.115$, $p < 0.01$) for average acid content in juice (in g.l^{-1}).

The highest total acid content was determined for cultivar Váh (together with cultivars Nitria and Hron formed one homogenous group) and the lowest for cultivar Torysa (Table 5). The absolutely lowest content of acids in year 2010 was found in cultivar Torysa (5.2 $g.l^{-1}$) and the highest in cultivar Hron (10.3 $g.l^{-1}$). The statistically highest acid content in juice was in 2014 and the lowest in 2012 (Table 6).

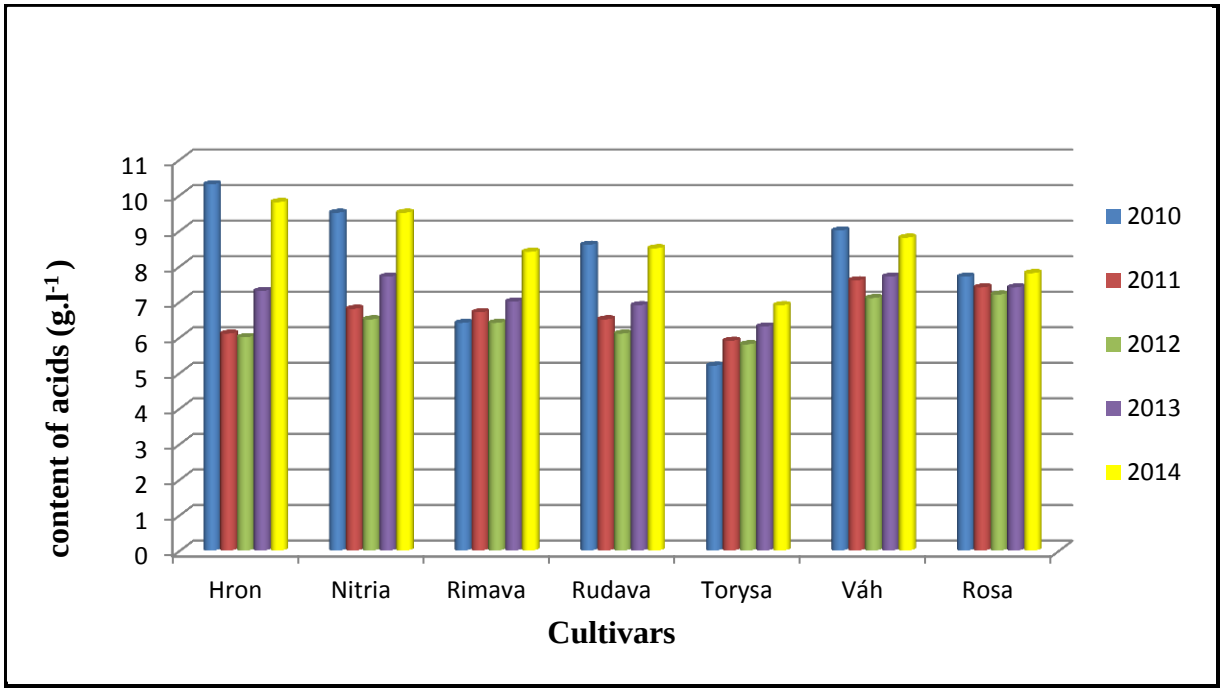

Figure 3 Total acid content in juice of evaluated cultivars

Conclusion

Highly significant differences were determined between years of experimental period for average yield of grapes (kg/vine bush), average sugar content in juice (in $kg.hl^{-1}$) and average total acid content ($g.l^{-1}$).

The highest average yields of grapes were determined for cultivars Váh (4.48 kg per vine bush) and Nitria (4.36) – these cultivars together formed one homogenous group). The lowest average yield of grapes was observed for cultivar Torysa (1.62 kg per vine bush).

The statistically highest yield was in 2011 and the lowest in 2014.

The highest average sugar content was determined for cultivar Rosa (24.9 $kg.hl^{-1}$, i.e. 24.9 ^{o}NM) - together with cultivar Rudava (24.7 ^{o}NM) formed one homogenous group. The lowest sugar content was observed for cultivar Rimava (22.8 ^{o}NM) – together with cultivar Nitria (23 ^{o}NM) formed one homogenous group. The statistically highest sugar content was determined in 2012 and the lowest in 2014.

The absolutely the highest sugar content was determined for cultivar Rudava (28 ^{o}NM) in 2012 and the lowest (20.5 ^{o}NM) for cultivars Nitria and Váh in 2014. The statistically highest sugar content in juice was in 2012 and the lowest in 2014 (Table 4).

The highest total acid content was determined for cultivar Váh (8 $g.l^{-1}$) - together with cultivars Nitria (8 $g.l^{-1}$) and Hron (7.9 $g.l^{-1}$) formed one homogenous group. The lowest average total acid content was determined for cultivar Torysa (6 $g.l^{-1}$). The statistically the highest average total acid content in juice was in 2014 and the lowest in 2012.

The absolutely lowest content of acids in year 2010 was found in cultivar Torysa (5.2 $g.l^{-1}$) and the highest in cultivar Hron (10.3 $g.l^{-1}$).

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Effect of chlorocholine chloride (CCC) on the height of the plants, its selected stem cells size changes and bulb's inulin content of Jerusalem artichoke (*Helianthus tuberosus* L.)

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Abstract
In 2015, the experiment was carried out in the field on using the growth retardant - chlorocholine chloride (CCC) - to reduce the plants' height of Jerusalem artichoke (*Helianthus tuberosus* L.). The plants were sprayed with the solutions in 3 concentrations: 0.07%, 0.14% and 0.21% at the stage of 40 cm height. Almost 3 months later, the stem tissue pieces of the 5th internode were taken and put into 70% alcohol and then cut longitudinally on the sledge microtome. The lengths of the stem, cortical near vascular bundle and pithial parenchyma cells were measured. At the end of vegetation bulbs form sprayed and unsprayed plants were evaluated for inulin content. The used growth retardant - CCC - was found to be only partly useful to lower the height of the Jerusalem artichoke. A Few weeks after the spray, the plants caught up with the growth and significant effect of lowering the plants' height disappeared. Lowering the height of Jerusalem artichoke plants after spraying them with CCC was a result of shortening of lengths of cortical parenchyma and tracheid cells. Use of CCC to spray the plants resulted in increasing in their bulb the content of inulin.

Keywords: chlorocholine chloride, Jerusalem artichoke plant, growth retardant, inulin

Introduction

In the past, Jerusalem artichoke (*Helianthus tuberosus* L.) was used to feed animals and poor people (van Loo et al., 1995). Nowadays, the plant is used in the industry and in some rich countries also in a diet of overweight people. In this aspect, the importance of its main bi-carbohydrate – inulin - in the world has been increasing (Ramnani et al., 2010, Xiao et al., 2011). In the industry, the plant is used to manufacture cellulose (Gunnarsson *et al.*, 2014). In the production, it has been found to be tolerant to various climatic conditions and resistant to frost, draught, salinity, most of diseases and pests. Moreover, it has small soil requirements (Xiao et al., 2011). The plant is high and grows in the field up to 3 meters. It is good in terms of bio-mass production but so high plants can also easily lodge and the bulbs collecting may be, thereby, difficult. One of the ways to solve this problem could be use of growth retardants - a group of chemicals commercially used to limit the growth of the cultivated plants (Rademacher., 2000).The main purposes of the experiment were to see, if a growth retardant could be used to lower the height of Jerusalem artichoke, to find out, which tissues of the plant's stem are actually affected by it and to show the effect of the used growth retardant on the inulin content in the bulbs.

Material and methods

Field experiment

The bulbs of Jerusalem artichoke (*Helianthus tuberosus* L.) used in the experiment were of the cultivar Albik and were bought in 2014 from a private Polish trade company in Sławno

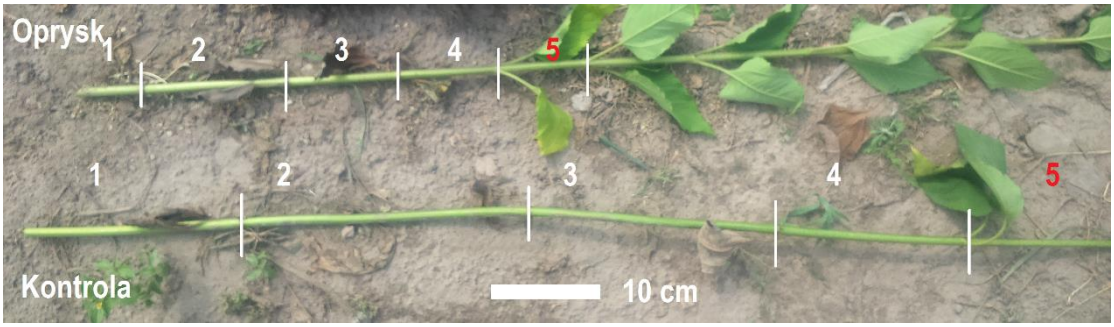
near Poznań. The planting material was of a very high quality. The used bulbs were of medium size, without any symptoms of rotting, in cross section, they were white. They had little elongated shape and sharp ending. The soil in the field used for the experiment was podsolic, rich in basic elements (N, P, K) with a proper acidity (pH) and salinity (EC).

Spraying plants with CCC and measuring plant's height

When the plants reached the height of about 40 cm, on May 16th, 2015, out of 6 rows with the plants, 20 plants of the exactly the same height - 40 cm, with differences in their plant height less than 1 cm, on each row, were selected. These plants were marked with ribbons and they were given numbers. The spraying with the retardant commercial name: Antywylegacz płynny 675 SL with an active compound - chlorocholine choride (CCC) - was done on May 18th, 2015. There were 3 different concentrations of the growth retardant tested: 0.07% used on the 1 row from the left, 0.14% - used on the 3 row from the left 0.21% - used on the 5th row from the left. The spraying was done with the use of hand sprayer of 10 liters volume. The working air pressure was 2-3 atmospheres. The plants form the 3 rows: 2nd, 4th and 6th were covered with plastic folia to protect them against the chemical. They were the check ones (they were not sprayed). The measurements of the plant's height were done in the following weeks, starting from May 18th 2015. Every week, at 2 pm, the plants were measured with a ruler from the bottom (soil level) to the highest point on the plant.

Histological measurements

There were done in order to show changes in the selected stem cells during the activity of the used growth retardant. As no changes were reported on the stems cells of seed stalks cross sections of onion and carrot (Hołubowicz, 1998), in this experiment only cell measurements on the longitudinal stem sections were done. On August 10th, 2015, when clear and permanent differences between sprayed (lower) and unsprayed (higher) plants were already seen, the 2 cm long samples of the stem exactly in the middle between 5th and 6th pair of leaves, i.e. in the 5th internode (Photo 1) counting from ground, were taken. Altogether, 20 samples from the row 3rd (sprayed with 0.14 % concentration of the growth retardant) and 20 samples from the row 4th (unsprayed) were taken. The tissue samples were placed into 70% ethanol solution and placed into the refrigerator for storing. They were then cut longitudinally directly (without putting them into the paraffin) on the sledge microtome, and placed on the microscope glass in a drop of glycerol. In the prepared this way longitudinal stem tissues, the histological measurements were done. The following cells characters were measured: length of 10 individual top epidermal cells in a roll, length 10 individual cortical parenchyma cells in a roll from the layer just under the epiderma cells, length of 10 individual tracheid cells the most internal and length of 10 individual pithal parenchyma cells on the third row below xylem (Photo 2). They were 4 replications of 5 sections for each treatment. Sections with the biggest differences were also photographed after colouring with the stain tissue - safranine - to improve the contrast for the pictures.



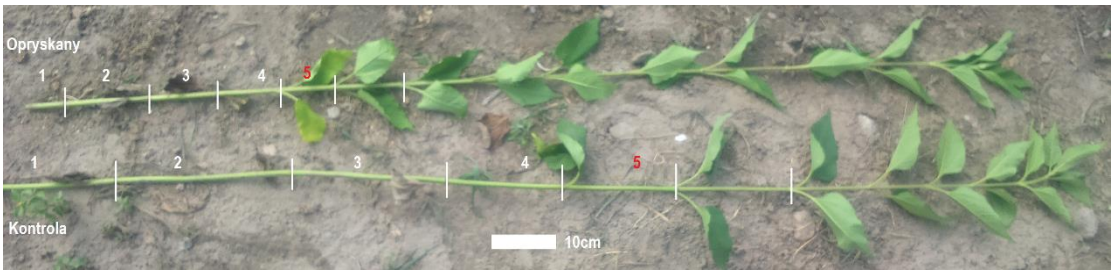


Photo 1 The check (unsprayed) and sprayed with 0.14 % CCC plants of the Jerusalem artichoke with the marked 5th internode (red colour), from which stem sample for histological longitudinal sections and measurements of cells were taken

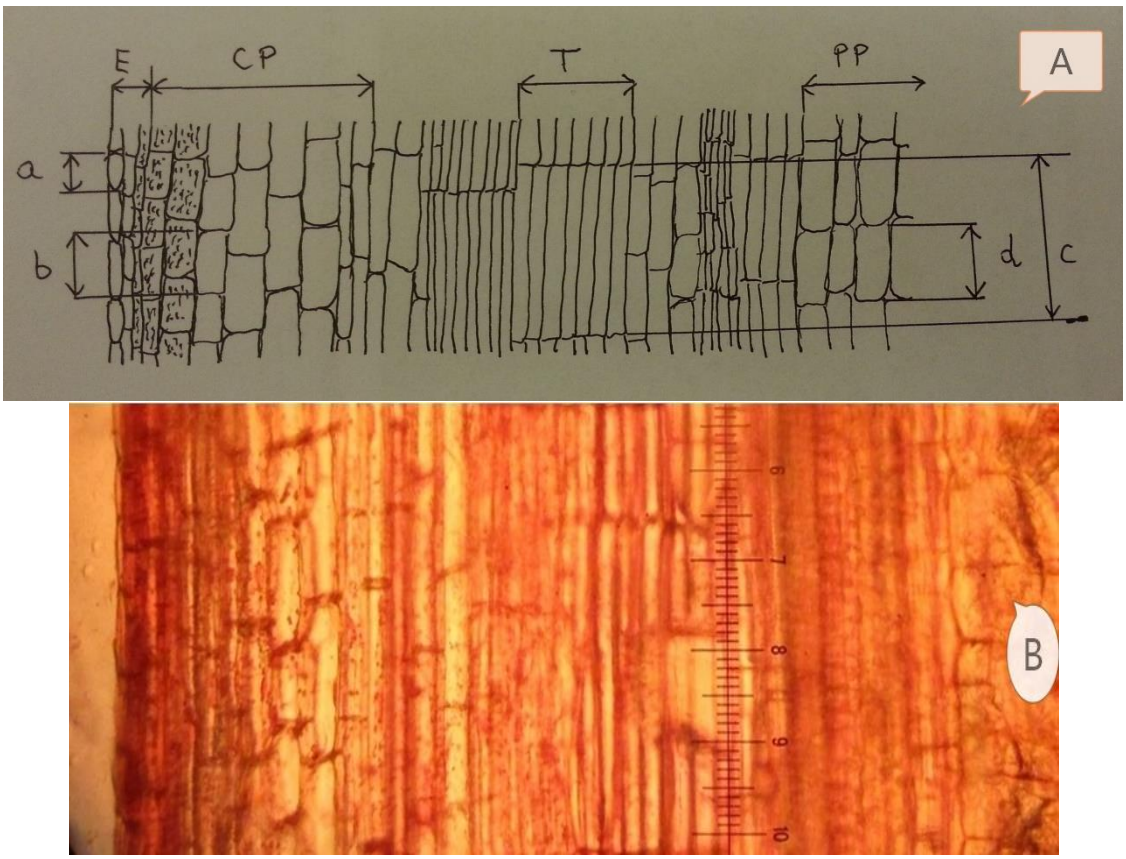


Photo 2 Drawing (A) and photograph (B) - magnification 100 x, part of the longitudinal section of the stem of Jerusalem artichoke cv. Albik. stained with 1% safranine. with the marked tissues and cells. Explanations: E- epidermal, CP - cortical parenchyma, T - tracheids, PP - pithal parenchyma, a - length of epidermal cell, b - length of cortical parenchyma cell, c - length of tracheid cell, d - length of pithal parenchyma cell

Checking the inulin content in the bulbs

The bulbs of the Jerusalem artichoke were collected on October 20th 2015. It was done when all the plants completed blooming. Using the spade, the bottom of the plant was lifted, and the bulbs were taken out. They were then packed in plastic bags. For each of the 4 treatments, there were 1 set of 3 bulbs collected, i.e. all together 12 bulbs were collected. The inulin content was evaluated using the gas chromatography SEC (former GPC). The inulin was extracted from the bulbs by preparing 1 g of mashed tissue, then 10 g of water was added and the extraction took place at 85℃ for 3 hours. Then, the sample was gradually mixed. The inulin content was evaluated using High Pressure Size Exclusion Chromatography (HPSEC). The 3 columns of SEC for analyses in water conditions were used in the water conditions

(Shodex OHpak SB-800HQ) as well as a protecting column SB-G (Showa Denko, Japan). The moving fraction (eluent) was 0.1 M water solution of sodium nitrate ($NaNO_3$). The used fluency speed was 0.3 ml/minute. To clean the inulin, the equipment was used produced by the companies Malvern, TX, USA and Wiscotek 305 TDA. The used calibration was done on pullulan as a standard. The inulin was analyzed in $H_2O/NaNO_3$ the coefficient of breaking the solvent was 1.3340, and the sample - 0.149. The mean mol mass, poli- dispersion was calculated by the programme Omi SEC 4.7 of the company Malvern, TX, USA (Gaafar et al., 2010). The results were expressed in g of inulin per 100g of fresh weight.

Statistical analyses of the data

The variance for all the received results was calculated. The smallest significant differences were calculated based on the Duncan's test for α - 0.05 and marked with different letters. For the chemical analyses results of the inulin content in the bulbs, standard deviations (SD) were also calculated.

Results and discussion

Effect of the CCC on the plants' height

The response of plants to spraying with CCC depended on the used concentration of the growth retardant. All the tested CCC concentrations, in different way affected the plants. If the CCC growth retardant was used in the lowest concentration. i.e. in 0.07%, the lowering of the plants was 17 and 18 weeks after spraying and again 21 and 22 weeks after spraying. If the growth retardant was used in the concentration 0.14%, the lowering of the plants was seen in the field already after 5 weeks but did not differ significantly. After 8 weeks, the plants sprayed with CCC grew faster and the height of the plants was the same. If the growth retardant was used in the concentration 0.21%, the lowering effect on the plant was the biggest (Fig.1). They were smaller already 5 weeks after spraying and the effect was seen for 5 next weeks. It was also seen 14 and 20 weeks after spraying the plants again.

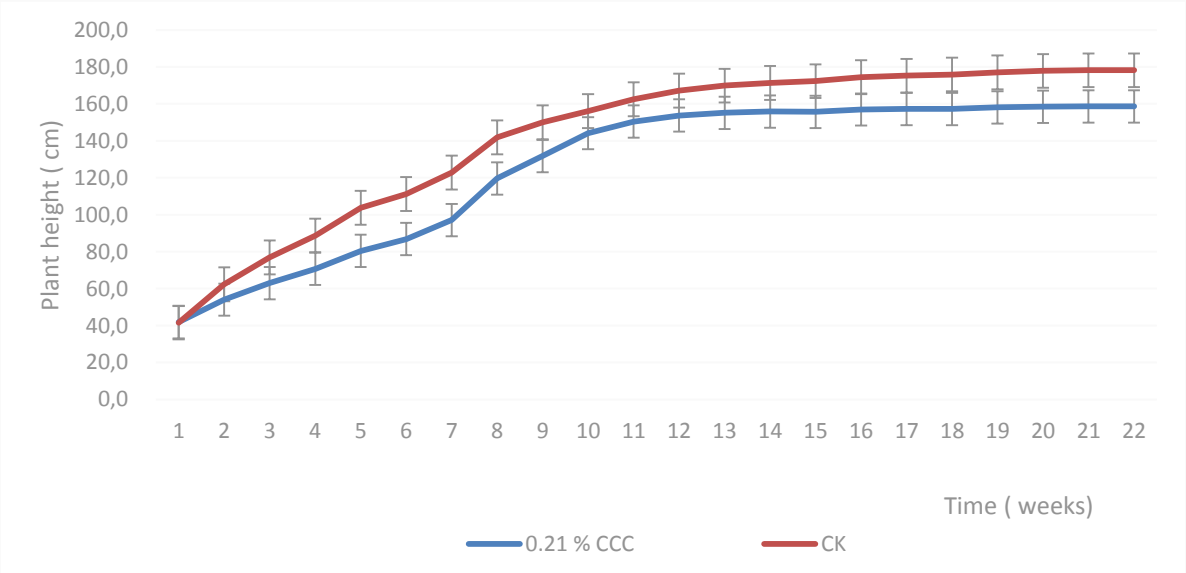


Figure 1 Effect of spraying Jerusalem artichoke cv. Albik plants with 0.21% CCC on the plants' height (cm). SD - standard deviation - vertical bars, CK - Check

Effect of the CCC on the stem tissue histological changes

The carried out measurements of the selected cells of the stems of Jerusalem artichoke sprayed with 0.14% CCC showed that it had effect on length of some of them when plants

were sprayed with the growth retardant, it did not affect the lengths of epiderma cells and pithal parenchyma cells but it did affect lengths of cortical parenchyma cells and tracheid cells (Tab.1). In some specimens, these differences were easily seen (Photo 3).

Table 1 Effect of spraying Jerusalem artichoke cv. Albik plants with 0.14 CCC on the length (µm) of their epidermal (E), cortical parenchyma (CP), tracheid (T) and pithal parenchma (PP) cells in the 5th internode of the stem, CK- check plants.

Plants	E		CP		T		PP	
number	CK	CCC	CK	CCC	CK	CCC	CK	CCC
1	8.1	7.8	15.1	10.9	19.0	11.2	11.8	10.0
2	8.6	8.9	15.1	12.9	19.9	13.2	13.7	12.4
3	8.6	8.3	14.9	14.8	19.7	12.6	11.9	13.4
4	8.3	7.7	15.3	12.0	19.9	13.2	12.2	11.5
5	8.2	7.9	15.1	15.2	22.2	12.9	11.7	12.5
6	8.6	7.9	16.3	15.2	17.6	12.9	11.4	12.5
7	8.8	7.6	17.9	14.2	16.1	14.3	11.3	13.2
8	10.8	8.6	15.6	14.4	21.8	14.3	14.8	11.4
9	8.7	8.4	17.3	12.8	21.5	11.3	10.0	11.4
10	8.4	8.6	15.2	14.1	22.7	11.8	13.3	11.0
	8.71a*	8.17a	15.78 b	13.65a	20.04b	12.77a	12.21a*	11.93a

*means followed by the same letters for a given tissue are not significantly different according to the Duncan's test for α=0.05

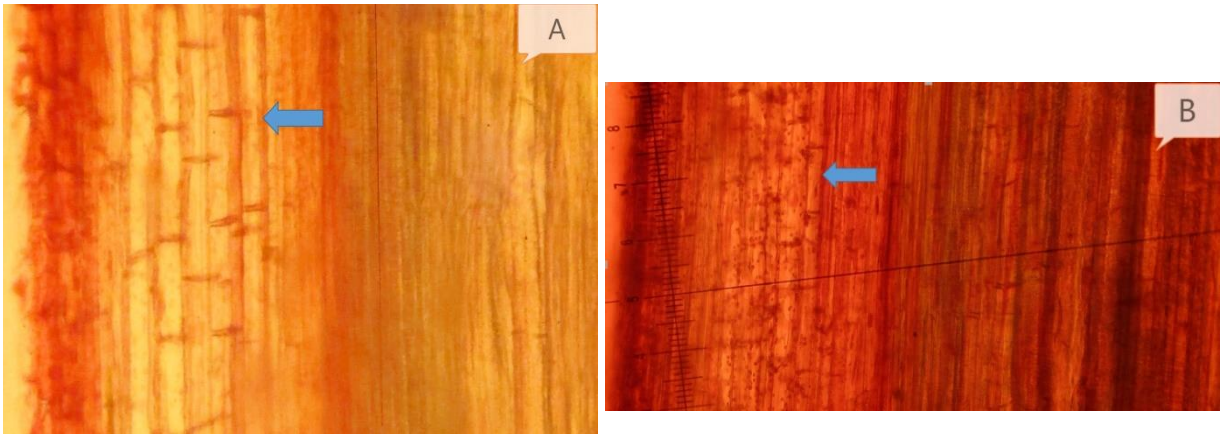


Photo 3 Photograph (magn. 100 x, stained with 1% safranine) of the part the longitudinal sections of the 5th internode of Jerusalem artichok cv. Albike. A-Plants unsprayed. B-Plants sprayed with 0.14 % CCC. One could see dicrease in cortical parenchyma cells length (marked with the blue arrow).

Effect of the CCC on the inulin content in the bulbs

The carried out chemical analyses proved that the use of the growth retardant CCC had an effect on inulin content in tables of Jerusalem artichoke when the plants were sprayed with it in the concentration 0.14%, the content of the inulin was the highest, and when then it was check, it was the lowest (Tab. 2). The inulin level in all investigated bulbs was very stable.

Table 2 Effect of spraying Jerusalem artichoke cv. Albik plants with the growth retardant CCC on the inulin content in their bulbs (g/100g fresh weight)

Treatment	Replications			
	1	2	3	Mean
0.07% CCC	20.08	20.89	20.60	20.52b*
0.14% CCC	22.12	22.31	22.12	22.18c
0.21% CCC	20.84	20.57	20.42	20.61b
Check	19.74	19.67	19.69	19.70a

*- means followed by the same letters are not significantly different according to the Duncan's test for α=0.05

Discussion

The used growth retardant - CCC - was found to be only partly useful to lower the height of the Jerusalem artichoke. Its effectiveness depended on the used concentration. As a rule , higher the concentration used, sooner and stronger lowering effect was seen. This, in case of CCC, is in agreement with many other results of previous experiments on linseed (Freer, 1993), common bean (Rafique-Uddin, 1984) or petunia (Shi and Li, 1987). However, a few weeks after the spray, the plants caught up with the growth and significant effect of lowering the plants' height disappeared. Similar effect was observed for the seedlings of *Zinnia* (Latimer, 1991), *Tagetes erecta* (Keever and Cox, 1989) and *Oenothera biennis* (Collins and Scarisbrick, 1987).The most effective CCC used to lower the plants' height of Jerusalem artichoke was observed with the concentration 0.21%. The lowering effect was seen already 5 weeks after spraying for 4 following weeks. Much better results with CCC on Jerusalem artichoke were received by Wawrzyniak in 2015. He used CCC in much higher concentration, i.e. 0.75%, and lowered Jerusalem artichoke plants already after 1 week. Moreover, the received result was permanent for as long as 12 weeks. Based on the results of changes in length of cortical ant near vascular bundles parenchyma cells on the longitudinal sections of stems of the plants, an explanation could be proposed of the mechanism of changes in them after spraying with CCC. Lowering of the stems length was a result of shortening of the parenchyma cells in both cortical and near vascular bundles areas. This is in agreement with the observations about the mechanism of lowering onion and carrot seed stalks sprayed with ethephon reported by Hołubowicz (1998). Changes in cell sizes, as a result of growth retardant use, were also reported for *Chrysanthemum morifolium* (Sachs and Kofranek, 1963). The used growth retardant had an effect on the inulin content in the Jerusalem artichoke bulbs, When the plants were sprayed with 0.14% CCC, it was the highest and it was the check in which treatment bulbs was the lowest. This result was different than in the studies carried out by Wawrzyniak (2015) with the same cultivar of Jerusalem artichoke but higher concentration (0.75 %) of CCC. In this case, the used concentration had no effect on the inulin content in the bulbs.

Conclusions

1. The used growth retardant - CCC - was found to be only partly useful to lower the height of the Jerusalem artichoke. A few weeks after the spray, the plants caught up with the growth and significant effect of lowering the plants' height disappeared.
2. Lowering the height of Jerusalem artichoke plants after spraying them with CCC, was result of shortening of lengths of cortical parenchyma and tracheid cells in the 5th internode.
3. Use of CCC to spray the plants resulted in increasing in their bulbs the content of inulin.

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Diversity of indigenous and emerging viral pathogens of cherry and grapevine in Slovakia and their effective detection

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Abstract
Cherries and grapevines are among the attractive fruits suitable for direct consumption as well as for industrial processing, however, the profitability of their production may be adversely affected by a number of viral pathogens. In our work, we have performed the full-length or partial genome characterization of selected viruses occurring on cherry and grapevine in Slovakia, in order to obtain original data on their genetic variability, relationships and the evolutionary forces shaping this diversity using standard and next-generation sequencing tools. Besides the first report of Little cherry virus-1 in Slovakia, an indigenous virus, Prune dwarf virus, was analysed and its molecular variability assessed. In grapevine, two newly recognized pathogens were characterised (Grapevine Pinot gris virus, Grapevine Syrah virus-1). The ability to provide a tool for a reliable detection is a key parameter in an effort to fight and control the viral pathogens. As the absence or scarcity of pathogen molecular data may complicate the development of diagnostic tests, we have optimized the updated RT-PCR tools for the polyvalent and effective detection of studied viruses.

Keywords: virus, variability, genome, RT-PCR, sequencing

Introduction

As most of the vegetatively propagated crops, cherries and grapevines are exposed to the attacks of a variety of pests and pathogens (including viruses) causing heavy losses and shortening their productive life. Depending on environmental conditions, the virus isolate and host, viruses can induce a variety of symptoms on leaves (chlorosis, yellowing, mosaic, ringspot, necrosis, malformations) and fruits (reduction) or, alternatively, the infection can remain asymptomatic (Hadidi et al., 2011; Martelli et al., 2014).

Emerging viruses are causal agents of infectious diseases whose incidence is increasing following its first introduction into a new host population/new geographical area or whose incidence is increasing in an existing host population as a result of long-term changes in its underlying epidemiology and can often be accompanied by a significant increase in symptom severity (Elena et al., 2014). An increased risk of introduction/export of new pathogenic viruses or emergence of new forms of existing viruses due to the changing agro-climatic conditions (global warming, increase of the vector spectrum, change of the plant communities) and social-economic conditions (market globalization, opening of the boundaries, migration) presents a serious problem for phytopathology management.

One of the most important tools of preventive control measures against plant viruses is fast and reliable diagnostics (Lopez et al., 2003), which requires a high degree of polyvalence. In many cases, however, effective detection can be adversely affected by high viral genetic variability. Mutation, positive and negative selection, and genetic drift are the main evolutionary forces generating such variability (García-Arenal et al., 2001).

Until recently, the extreme intra- and inter-specific diversity characterising of viral agents has effectively prevented a full ab initio assessment of the viruses present in a plant sample. However, the recent developments of Next Generation Sequencing (NGS) technologies and bioinformatics have drastically changed research on viral pathogens (Massart et al., 2014). NGS can provide tools allowing the detection and characterization, without prior knowledge, of all the viruses and virus-like agents infecting a sample of the target species.

Material and Methods

Viral isolates were collected from naturally infected cherry and grapevine plants from distinct locations and agro-ecological contexts in Slovakia. The presence of viruses in the samples (leaves, flowers, fruits, seeds) was checked by double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) using commercially available (Bioreba, Loewe) or in-house specific antibodies or by polymerase chain reaction (PCR) under various arrangement using published or newly designed primers. Partial or complete genome data were obtained by standard dideoxy sequencing. Obtained data were analysed by bioinformatic programs (Clustal, MEGA, DnaSP…) and compared with sequences available in international databases. Samples for the metagenomic analysis, either total RNA, or small interfering (si)RNA were subjected to the NGS analysis (Illumina) as an external service.

Results and Discussion

The recent works using NGS technologies clearly showed that the "virome" of the perennial plants is larger than initially thought, with a number of novel and potentially important viruses identified in the last years (Barba et al., 2013). The analyses of siRNA pool from a grapevine plant from western Slovakia and mapping of obtained contigs revealed a mixed infection of this single plant with several known viruses: Arabis mosaic virus (ArMV), Grapevine fanleaf virus (GFLV), Grapevine rupestris stem pitting-associated virus (GRSPaV), Grapevine fleck virus (GFkV), Grapevine leafroll-associated virus 1 and 3 (GLRaV-1 and GLRaV-3). Interestingly, analysis of siRNA reads revealed also the presence of two recently described viruses, i.e. Grapevine Pinot gris virus (GPGV) and Grapevine Syrah virus-1 (GSyV-1), both previously undetected in Slovakia or in any other country in Central Europe (Glasa et al., 2014; Glasa et al., 2015).

This discovery prompted further study to evaluate the occurrence and incidence of GPGV and GSyV-1 in Slovakia by testing a total of ca 100 additional samples. To this purpose, new pairs of primers were designed, allowing the development and optimization of highly polyvalent RT-PCR detection of these viruses and assess, for the first time in the central European region, their genetic variability.

Surprisingly, a high incidence (ca 30%) of GPGV and GSyV-1 was detected in a range of white- and red-berried cultivars. However, no particular symptom could be attributed to the GPGV and GSyV-1, as most of the grapevine plants analysed in this work were simultaneously infected by other viruses, as determined by specific RT-PCRs targeting ArMV, GFkV, GLRaV-1, GPGV, GVA. This is not unusual, as co-infections of few viruses were often found in single grapevines.

Fruit tree viruses can evolve as complex viral populations due to the perennial character of their natural host (Jridi et al., 2006). Besides mixed infection of a single grapevine plant with 2 or more viral pathogens, a high within-isolate genetic variability and mixed populations of variants belonging to several groups/strains of the same virus can be observed. Indeed, as ascertained by cloning and sequence analysis of haplotypes, several Grapevine virus A (GVA) samples were detected to harbour a population of different sequence variants (Predajňa and Glasa, 2016). Such dynamic genetic structure of virus populations has a significant role in the epidemiology of the viruses as they constitute the basis of their adaptation to the environment.

In an effort to assess the health status of cherry plantations in Slovakia, a survey was carried out in 11 localities known in the past for cherry production for the presence of common pathogens: Prunus necrotic ringspot virus (PNRSV), Prune dwarf virus (PDV), Apple chlorotic leaf spot virus (ACLSV), Cherry virus A (CVA).
As a typical indigenous virus, PDV showed a high degree of nucleotide sequence diversity over a small geographical region (the highest direct distance between localities tested in this work was 250 kilometers). Phylogenetic analyses grouped the Slovak cherry PDV isolates into different clades, which suggests that PDV may have been introduced from several origins in the sampled area, rather than a diversification of the isolates from a single ancestor.
The results reported here suggest that even for such a long studied and relatively well-known virus such as PDV, a part of its variability and the events in its evolutionary history may still be incompletely known or understood, with consequence that its diagnostic using molecular assays may still show some limitations due to the inability of existing assays to cope with part of the viral diversity. Further efforts, taking into account the broadest possible fraction of the viral diversity are therefore warranted, in order to further improve the efficiency and the reproducibility of diagnostic assays.
Besides common viruses, the attention has been paid to reveal the presence of new or emerging viral pathogens. Until now, no evidence of natural infection of cherries with Plum pox virus (Glasa et al., 2013, García et al., 2014) was obtained from Slovakia. However, from about 60 cherry samples, 5 were tested positive for the Little cherry virus-1 (LChV-1) presence using the RT-PCR (Glasa et al., 2015). The partial LChV-1 CP sequences obtained in this work and those retrieved from GenBank showed a substantial variability. Although five Slovak isolates were genetically homogenous, they formed a distinct phylogenetic cluster, divergent from the previously characterised European LChV-1 isolates (nucleotide identities reached 82.3 – 84.2%). The detection of LChV-1 isolates in old trees of local cherry genotypes and a degree of their molecular variability suggests a long-term establishment of this virus in the country.
The results obtained have provided the original epidemiological data needed for the establishment and optimisation of phytosanitary measures to control plant viruses.

Conclusion

In our work, we have performed the full-length or partial genome characterization of selected viruses occurring on cherry and grapevine in order to obtain original data on their genetic variability, relationships and the evolutionary forces shaping this diversity using standard and next-generation sequencing tools. Available genomic data were further used for the development and optimization of molecular tools for their detection. Our study confirms a need for a continual assessment of the virus molecular variability (also at the regional level) as a prerequisite to develop polyvalent detection tools, applicable in the national risk assessment of pathogens and an efficient management and control of the viral diseases.

Acknowledgements

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The survey of Quercus species and varieties in Buda Arboretum

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Abstract
The Buda Arboretum has a rich botanical collection. Our work has aimed the registration, dendrometric measurements and assessment of tree development and health status of the *Quercus* species and varieties. The 25 *Quercus* trees were measured, assessed between 16th February 2014 and 29th September 2014. Further on calculations to determine the value of trees are based on the method of the Hungarian Tree Care Association. According to the results, uncertainties of visual assessment can be amended with instrumental assessment, to have reliable conclusions. The age of trees ranged between 23 and 126 years. Oldest trees are found in the Upper Garden: *Q. robur* (Nr. 25) and *Q. robur* 'Umbraculifera' (Nr. 22). Although the average value of trees is good (4.25), according to the results of the visual assessment and calculations, the trees in the worst condition are: *Q. cerris* (Nr. 19) and *Q. robur* 'Fastigiata' (Nr. 11). The life expectancy of the trees with proper tree care can be extended. The theoretical value of the trees was calculated according to the following factors: the price of nursery trees, age, protection and situation of the tree in the settlement, crown quality, tree care and viability, dendrology value. The calculated theoretical value of the 25 trees together is 563 674 036 HUF (appr. 1 880 000 EUR).

Keywords: tree assessment, dendrometric measurements, theoretical value, tree care

Introduction

The Buda Arboretum located on the south slope of Gellert Hill in Budapest, is one of the richest dendrological collections with horticultural and urban forestry focus of Hungary. The area was covered by vineyards until 1861. The structure and the collection were formed by the works of Károly Räde, Béla Rerrich and Gyula Magyar. The Buda Arboretum is the university campus of Szent István University, used for education and research, but is open for recreation of public visitors. The latest development project was carried out between 2010 and 2012, thanks to European Union's funds (KMOP – 3.2.1/09-B). Parallel to the development work, the survey and registration of the botanical files became necessary. The presented work has aimed the assessment and registration of one of the most important genus, *Quercus* species and subspecies. The 25 *Quercus* trees were measured and assessed between 16th February 2014 and 29th September 2014.
The relevant *Quercus* species and subspecies, Bean (1981), Krüssmann (1984), Gencsi and Vancsura (1992), Schmidt and Tóth (2008) reviewed. The assessment method is based on the work of the Hungarian Tree Care Association – Szaller et al. (2013). The method considers the trunk and canopy measurements, as well as by visual assessment evaluates the tree by rating from 1 to 5. The aim of the work was also to give tree care suggestions based on the work of and Schmidt and Varga (2007), and to estimate the value of the trees with the help of evaluation guide of the Hungarian Tree Care Association published by Szaller et al. (2013).

Material and Methods

Tree identification and tree age

25 *Quercus* trees were identified for measurements on the map, drawn by Schmidt (2013): *Quercus cerris* – 2pc., *Quercus coccifera* – 1pc., *Quercus frainetto* – 1pc., *Quercus ilex* –

1pc., *Quercus libani* – 1pc., *Quercus petraea* – 2pc., *Quercus pontica* – 2pc., *Quercus pubescens* – 2pc., *Quercus robur* – 5pc., *Quercus robur* 'Fastigiata' – 4pc., *Quercus robur* 'Pectinata' – 1pc., *Quercus robur* 'Umbraculifera' – 1pc., *Quercus trojana* – 1pc., *Quercus* × *turneri* 'Pseudoturneri' – 1pc.
The year of planting was identified by the registry of the Department Floriculture and Dendrology of Szent István University, and by further note of the department. No exact planting year was registered for *Q. cerris, Q. petraea, Q. pubescens, Q. robur, Q. robur* 'Fastigiata' and *Q. robur* 'Umbraculifera'. In these cases the tree age was estimated by the guideline of the Hungarian Tree Care Association (2013). The method supposes that the trunk diameter grows 1 cm every year, influenced by the trees productivity and ecological features of the habitat. For low productivity species, like *Q. pubescens* 0.85 ratio, for medium productivity species, like *Q. petraea*, *Q. robur* 1 ratio and for fast productivity species, like *Q. cerris* 1.15 ratio was used in the following formula:
Age of tree = Trunk diameter (cm) × Trunk diameter and age ratio.
According to the age of tree, the year of planting can be calculated.

Trunk and canopy measurements
Caliper was used to measure the trunk diameter 1 m above the surface or under the branching where it was occurred. The average crown diameter was calculated from four canopy radius. Four meters long slide rule was used to the measure of trees with bush habits, while on larger trees the 4 m long slide rule was used to measure of height as reference point. The evaluation of root, trunk, crown, tree care and viability the method of Radó (1999) was applied by rating (1 to 5). The results of the evaluation can be described with a single number, that is the arithmetic mean of evaluation values (1-5).

Visual tree assessment and further calculations
The following tree assessment data were compiled in table:

- The name of the tree (species, subspecies)
- The number of map sheet Schmidt (2013), where the tree is situated
- Year of planting
- Age of tree
- Trunk diameter (cm)
- Crown diameter (m)
- Height of the tree (m)
- Evaluation of root (1-5; 1, as the negative pole of the scale 5, as the positive pole of the scale)
- Evaluation of trunk (1-5)
- Evaluation of crown (1-5)
- Evaluation of tree care or arboriculture (1-5)
- Evaluation of viability (1-5)

During the assessment further instruments were used: Sony DSC-W310 digital camera, an for the evaluation Microsoft Excel programme.
The measurements and the rating of tree's data took place in the Buda Arboretum, on 16th February, 1st May, 24th May, 5th April, 1st July, 27th September in 2014.

Estimation of theoretical financial value of trees
The estimation was calculated according to the evaluation guide of the Hungarian Tree Care Association – Szaller et al. (2013). The necessary data, the price of the saplings were

collected according to Hungarian tree nurseries. The price of special subspecies was calculated from German, Italian or British prices (like *Q. robur* 'Umbraculifera'). The age of trees was calculated according to literature, the dependent multiplier was determined with linear interpolation. All the trees are protected at the area of Buda Arboretum so the multiplier is 10 and it increases the value of the trees. The further data came from the results of Radó (1999) assessment. The *Quercus* species represent high dendrology value (Schmidt, 2011 in Szaller, 2013), which also increases the final theoretical value assessment. At the end the final theoretical value was given according to the following formula (Szaller et al., 2013):

Value of the tree = A × B × C × D × E × M
A = Tree nursery's basic price
B = Age multiplier
C = Protection and situation of the tree in the settlement
D = The evaluation of the crown
E = The evaluation of the tree care or arboriculture and viability
M = The multiplier according to dendrology value.

Results and Discussion

The age of trees based on the registry is shown in Fig 1. The tree age of some *Quercus* trees were calculated according to trunk diameter, and productivity according to the guideline of the Hungarian Tree Care Association (2013). The oldest trees were found in the Upper Garden of Buda Arboretum. The age of *Q. robur* (Nr. 16, 17, 18), *Q. cerris* (Nr. 19), *Q. petraea* (Nr. 20), *Q. pubescens* (Nr. 21) is 89, they were planted together. The *Q. libani* (Nr. 24) is 79 years old. The oldest, 126 years old trees are *Q. robur* (Nr.25*)* and *Q. robur* 'Umbraculifera' (Nr. 22), they were probably planted by Károly RÄDE as representatives of *Fagaceae* taxons. The youngest tree was planted in 1995. (After the assessment in 2014 some new *Quercus* species were planted, not involved in the survey.)

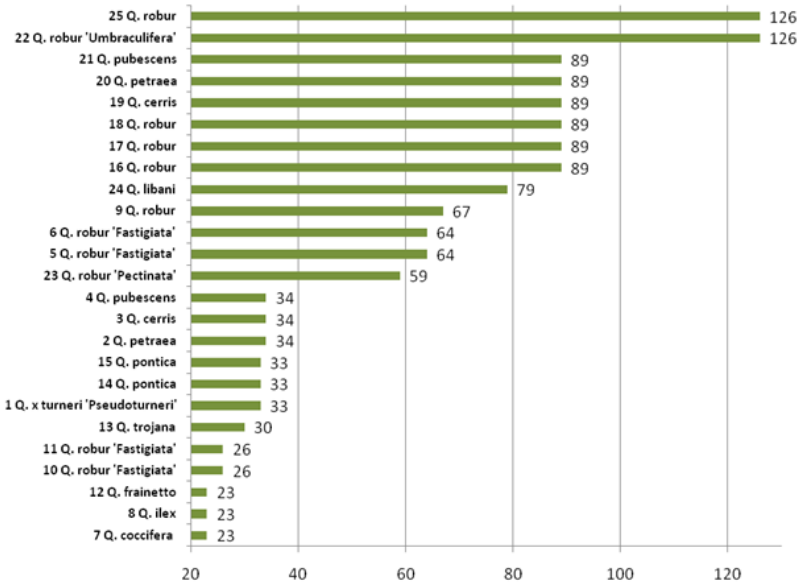

Figure 1 The age of *Quercu*s trees in Buda Arboretum (according to registry of the Department of Floriculture and Dendrology and own calculations)

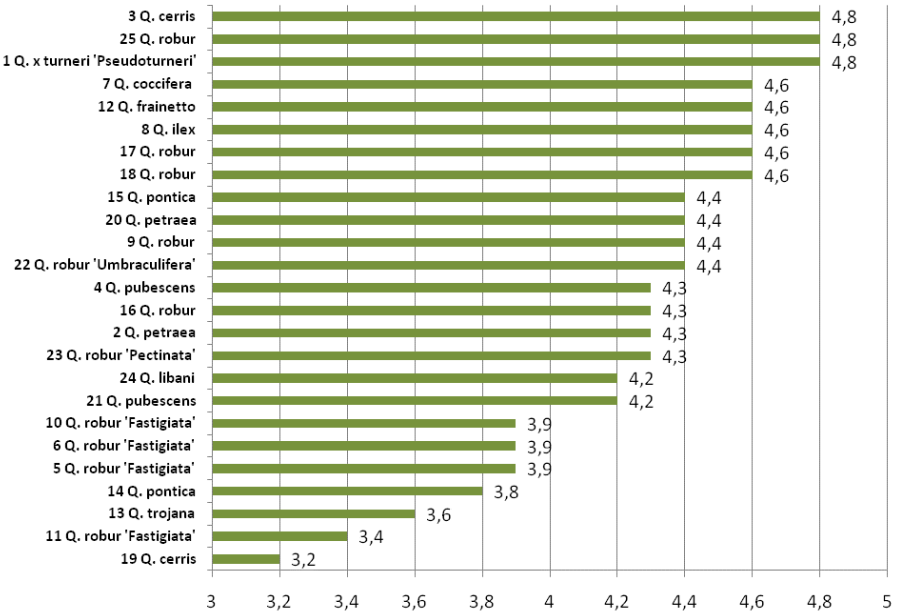

Figure 2 The aggregated value of *Quercus* trees in Buda Arboretum by Radó (1999) assessment (own calculation)

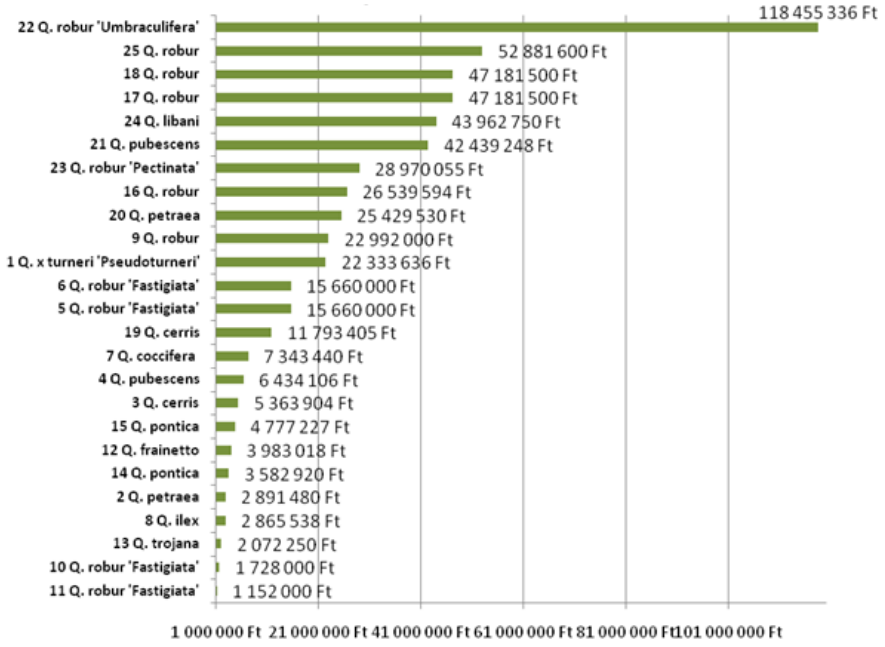

Figure 3 The theoretical financial values (given in HUF) of the *Quercus* trees of Buda Arboretum (own calculations)

The data of the tree measurements and the assessment of the trees' value are displayed in Table 1 (see in appendix).

The arithmetic means of the ratings of tree values are summarised in the Figure 2. The average value is 4.25, which qualifies as good. The *Q. cerris* (Nr. 19) show the worst condition with the value 3.2. On the trunk of the tree, there is a hole. The tree could be sustained with proper treatment. The other tree in critical condition is *Q. robur* 'Fastigiata' (Nr. 11) because of bad immediate environment. Proper landscaping is proposed. The *Q. pontica* (Nr. 14) suffers from decays on the branch that should be cared as following: elimination of decayed branches, caring of injuries, removal of wild branches of rootstock.

The proper care and increase of direct habitat could increase the condition and life expectancy of the *Q. robur* 'Fastigiata' species (Nr. 5, 6, 10). The *Quercus* trees with 4 or better values are in good condition, although continuous tree care is suggested, especially care of injuries and decays.
The theoretical financial value of the trees was calculated according to the following factors: tree nursery's basic price, age, protection and situation of the tree in the settlement, crown, tree care and viability, dendrology value. The calculated cumulative theoretical value (Figure 3) is 563 674 036 HUF (appr. 1 880 000 EUR). The most valuable is *Q. robur* 'Umbraculifera' (Nr. 22), worth 118 455 366 HUF (appr. 395 000 EUR). The least valuable is *Q. robur* 'Fastigiata' (Nr. 11), worth 1 152 000 HUF (appr. 3840 EUR).

Conclusion

Among the 25 *Quercus* trees of Buda Arboretum several exceptional can be found. The enormous size of *Q. robur* 'Umbraculifera' is suitable for solitaire tree in huge parks, that is why it is rare. The example of the Buda Arboretum has characteristic growth habit. Here is the oldest *Q. libani* of Hungary that is also the pride of the Garden. The trees, suitable for milder climate are also in a good condition. The oldest trees were found in the Upper Garden of Buda Arboretum. The age of Q. robur (Nr. 16, 17, 18), Q. cerris (Nr. 19), Q. petraea (Nr. 20), Q. pubescens (Nr. 21) is 89, the Q. libani (Nr. 24) is 79 years old. The very oldest, 126 years old trees are *Q. robur* (Nr. 25) and *Q. robur* 'Umbraculifera' (Nr. 22) were probably planted by Károly Räde as representatives of *Fagaceae* taxons. The youngest tree was planted in 1995. (After the assessment in 2014 new Quercus species were planted.)
In the case of Q. libani, further assessment would be needed to investigate the inner decay, by the results of visual assessment the trunk disorder ("bottle trunk") can be supposed. That is why reliable assessment is expected only with visual and later instrumental measures.
The average value of the assessed trees is 4.25, which qualifies as good. The *Q. cerris* (Nr. 19) and *Q. robur* 'Fastigiata' (Nr. 11) have the worst condition with the value 3.2; bad results were calculated for the *Q. pontica* (Nr. 14). The value could be corrected with proper tree care (landscaping care of tree injuries, elimination of decayed parts, increase of direct habitat). The *Quercus* trees with 4 or better values are in good condition, although continuous tree care is suggested, especially care of injuries and decays.
The theoretical financial value of the trees is 563 674 036 HUF (appr. 1 880 000 EUR). The most valuable is *Q. robur* 'Umbraculifera' (Nr. 22), worth 118 455 366 HUF (appr. 395 000 EUR). The least valuable is *Q. robur* 'Fastigiata' (Nr. 11), worth 1 152 000 HUF (appr. 3840 EUR).

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Appendix
Table 1 The results of the assessment of *Quercus* trees in Buda Arboretum

Nr.	The name of the tree (species, subspecies)	The Nr. of map sheet	Age of tree	Year of planting	Trunk diameter (cm)	Trunk circumference (cm)	Trunk height (cm)	Crown diameter (m)	Height of tree (m))	Root ev. (1-5)	Trunk ev. (1-5)	Crown ev. (1-5)	Tree care. (1-5)	Viability (1-5)	Average value
1	*Q. x turneri* 'Pseudoturneri'	19C1	33	1986	55	160	40	10	9.5	5	5	5	4	5	4.8
2	*Q. petraea*	15C2	34	1985	30	100	340	8.5	10	5	4	4	4	4.5	4.3
3	*Q. cerris*	15C2	34	1985	19	64	290	3.5	12	5	5	5	4	5	4.8
4	*Q. pubescens*	15C2	34	1985	34	108	135	9.1	9	4	5	5	3.5	4	4.3
5	*Q. robur* 'Fastigiata'	15C2	64	1955	59	171	25	6.5	15	4	5	4	3	3.5	3.9
6	*Q. robur* 'Fastigiata'	15C2	64	1955	51	160	50	6.5	14.7	4	5	4	3	3.5	3.9
7	*Q. coccifera*	14B1	23	1996	40 30	130 95	10 57	7.4	7	4	5	5	4	5	4.6
8	*Q. ilex*	11B2	23	1996	49	170	8	8.3	7	4	5	5	4	5	4.6
9	*Q. robur*	11B2	67	1952	62	193	200	16.7	19	3	5	5	4	5	4.4
10	*Q. robur* 'Fastigiata'	12C3	26	1993	46	140	5	5.8	10	3.5	5	4	3	4	3.9
11	*Q. robur* 'Fastigiata'	12C3	26	1993	21	73	50	3.5	8.4	3	5	4	2	3	3.4
12	Q. frainetto	16B1	23	1996	33	99	91	10.3	8.5	5	4	5	4	5	4.6
13	*Q. trojana*	16A1	30	1989	2	8	6	0.9	0.9	5	4	4	2	3	3.6
14	*Q. pontica*	16C3	33	1986	56	170	20	7	5.5	5	4	4	3	3	3.8
15	*Q. pontica*	16C3	33	1986	27	90	10	2.4	4.5	5	4	5	4	4	4.4
16	*Q. robur*	6B2	89	1930	47	155	210	13	15	3.5	5	4	5	4	4.3
17	*Q. robur*	6B2	89	1930	60	198	300	12.5	17	5	5	5	4	4	4.6
18	*Q. robur*	6B2	89	1930	74	253	270	18.5	17	5	4.5	4.5	5	4	4.6
19	*Q. cerris*	6C1	89	1930	71	225	370	18.5	16.5	4	2	3	4	3	3.2
20	*Q. petraea*	6A1	89	1930	84	240	100	14.5	16	5	5	4	4	4	4.4
21	*Q. pubescens*	6B1	89	1930	64	205	100	17	15	4	5	4	4	4	4.2
22	*Q. robur* 'Umbraculifera'	5C2	126	1893	69	226	90	19	16	4	5	5	4	4	4.4
23	*Q. robur* 'Pectinata'	5B2	59	1960	38	116	90	5	12	5	5	4	4	3.5	4.3
24	*Q. libani*	5B2	79	1940	57	177	350	14.5	14	4	3	4	5	5	4.2
25	*Q. robur*	5A3	126	1893	97	298	170	13.5	16	5	5	4	5	5	4.8
										4.36	4.58	4.38	3.82	4.12	4.252

Study of the influence of growth regulators on growth and vegetative behaviours of seedlings of certain species of Linden (*Tillia* ssp.)

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Abstract
In present study *Tilia* ssp. seeds were stratified at 4°C for different durations (0, 3, 6, 9 or 12, 15 weeks), treated with gibberellic acid (GA_3) (0, 200, 400, 600, 800 and 1000 mg L ') to break dormancy and allow germination. The pretreated seeds were germinated at 20°C under 12 h light. One additional parameter, the effect of light on seed germination was also studied and the seeds treated with 400, 600 and 800 mg L ' GA_3 solution or stratified for 6, 9 and 12 weeks were germinated at 20°C in the dark or 12 h light. Stratification of seeds at 4°C for 9, 12 and 15 weeks, or treatment of seeds with 600 or 800 mg L^{-1} GA_3 was successfully overcome dormancy in Tilia ssp. seeds. Treatment of seeds with light or dark did not play a main role in the germination.

Key words: seed pretreatment, dormancy, germination, light

Introduction

Species of the genus ***Tilia*** are among the most widely used decorative trees in the ornamental gardening. According Magherini and Nin (1994) production of planting material is difficult for the following reasons: first - in vegetative method of propagation by shoots the obtained plants retain the ability to form shoots and creating problems in maintaining the landscape objects, second - in the propagation by seeds the seeds are in deep dormancy and need specific presowing treatment. Vanstone and Rose developed a way of improving the germination of the seed and obtaining a greater number of seedlings by treated with growth regulators. Regeneration from seeds is the most often used and cheapest method of propagation in many species. The ***Tilia*** species has dormancy and the various methods are used to overcome dormancy. Stratification, scarification and gibberellins have a promotive effect on the germination of many species of angiosperms and gymnosperms. Nevertheless, these methods vary from one species to the other, accentuating the need for formulating species-specific treatments. In naturally dispersed seeds, the chilling requirement is obtained during the winter season; in the nursery the same result may be achieved by keeping seeds at low temperature, generally within a range of between 1 and 5°C, mixed with a moist peat or sand (cold stratification). Dormancy in which exposure of seeds to chilling or light is required for its termination is often overcome by gibberellins. The role of gibberellic acid in promoting seed germination in some species with dormant seeds has been described by various authors. Since species differ in their level of dormancy, determining the optimal level of GA_3 concentration is paramount.
Physiological dormancy is the primary dormancy present in the genus ***Tilia***. Stratification and GA_3 treatment of seeds were shown the effectively to break dormancy and to increase the seed germination in some ***Tilia*** species. The aims of the present study was to examine the influence of stratification and GA_3 on seed germination of ***Tilia*** - T. cordata Mill., T. platyphyllos Scop., T. tomentosa Moench. to enhance germination, as well as to evaluate the effects of light on germination.

Material and methods

Mature ripened fruits were collected in November from Plovdiv, Bulgaria. Seeds were cleaned and stored at 4°C until used and the seeds were randomly sampled for all experiments described.

Several experiments were conducted to determine the effects of different methods on seed dormancy breaking and germination. For cold moist stratification, seeds were soaked in water for 24 h before being mixed with moist sand. The seeds were stratified for 0, 3, 6, 9, 12 and 15 weeks at 4°C. Seeds were germinated in plastic boxes, placed in a germination chamber at 20°C, under a 12 h light photopenod.

In order to test the effects of gibberellic acid (GA_3) on the germination of the seeds, seeds were placed in solution with 200 mg L^{-1} GA_3, 400 mg $L^{-1}GA_3$, 600 mg $L^{-1}GA_3$, 800 mg $L^{-1}GA_3$, 1000 mg $L^{-1}GA_3$ or, for the control, distilled water for 24 hours at 23°C.

One additional parameter, the effect of light on seed germination was studied using a seed germination chamber. Seeds treated with 400, 600 and 800 mg L~' GA_3 solution or stratified for 6, 9 or 12 weeks at 4°C were germinated at 20°C in dark and 12 h light.

All the experiments were conducted in Completely Randomized Design using fifty seeds each in four replicated for all treatments. Seeds showing radicle emergence were recorded as germinated and removed from plastic boxes, for a period of 30 days and after cultivated in individual pots. Supported had high air humidity (60-80%) and temperature of 25 ° C. The results were reported three months after the experiment. Were examinated following characteristics: percentage of germinated seeds %; average root length (cm); average root number; average leaf number; average leaf area (cm^2). Results were subjected to ANOVA and means were compared by Duncan's Multiple Range Test. Germination percentages were transformed by arcsin prior to analysis.

Table 1 Influence of GA_3 on germination characteristics of species of genus *Tilia*

№	Vari-ants	*Tilia cordata*					*Tilia platyphyllos*					*Tilia tomentosa*				
		GP%	Avg. root length	Avg. root number	Avg. leaf number	Avg. leaf area (cm^2)	GP%	Avg. root length	Avg. root number	Avg. leaf number	Avg. leaf area (cm^2)	GP%	Avg. root length	Avg. root number	Avg. leaf number	Avg. leaf area (cm^2)
1	0	6,7	1,3	3,4	0,7	0,8	3,2	1,2	3,2	0,3	0,4	12,8	2,4	4,1	1,1	1,2
2	200	69,2	3,4	5,6	1,3	1,9	43,8	3,0	3,7	1,1	1,2	73,4	4,5	5,6	1,7	1,9
3	400	84,7	3,7	6,4	2,7	3,1	67,5	3,5	4,3	2,0	3,0	75,9	5,6	5,8	3,1	2,7
4	600	89,3	4,3	6,0	3,8	2,9	73,7	3,8	5,8	3,1	3,1	88,5	5,8	6,7	4,3	3,4
5	800	82,4	4,2	3,2	3,0	1,6	77,8	4,0	5,9	3,4	3,3	63,7	4,2	4,3	4,6	2,9
6	1000	32,4	2,8	3.2	1,7	0,9	53,4	1,9	4,3	1,2	2,4	60,0	4,3	2,1	2,5	2,6

Table 2 Influence of cold stratification on germination characteristics of species of genus *Tilia*

№	Varia nts	*Tilia cordata*					*Tilia platyphyllos*					*Tilia tomentosa*				
		GP%	Avg. root length	Avg. root number	Avg. leaf number	Avg. leaf area (cm^2)	GP%	Avg. root length	Avg. root number	Avg. leaf number	Avg. leaf area (cm^2)	GP%	Avg. root length	Avg. root number	Avg. leaf number	Avg. leaf area (cm^2)
1	0	6,7	1,2	0,7	0,9	0,7	5,1	0,9	1,3	1,2	0,9	10,1	1,4	1,0	1,3	1,2
2	3	36,5	1,4	0,9	0,9	0,8	20,6	1,1	2,7	1,6	0,7	29,4	2,7	1,3	2,1	1,7
3	6	50,4	2,5	1,2	1,4	0,9	38,7	1,7	2,9	1,5	0,9	58,8	2,3	2,7	2,7	1,5
4	9	86,3	3,1	2,1	2,3	1,3	50,8	2,4	3,4	2,1	1,3	78,9	3,8	3,9	3,5	2,7
5	12	84,8	2,7	2,7	1,7	1,7	72,0	1,9	3,7	1,7	1,5	83,7	3,9	3,9	4,6	2,9
6	15	80,9	2,3	2,1	1,6	0,9	70,3	1,7	2,5	1,8	1,3	80,3	2,7	2,4	3,7	1,7

Results and discussion

Only 6.7%; 3.2% and 12.8% of non-treated seeds germinated (Table 1). The stratification of 9 and 12 weeks significantly increased germination (Table 2). Increasing the duration of stratification resulted in a significant increase in germination

percentage (GP%) with 9 or 12 weeks allowing for 86.3, 72.0 and 83.7% germination, respectively.
All GA_3 treatments improved germination. Treatment of seeds with 600 mg L^{-1} GA_3 solution was successful in breaking dormancy resulting in 89.3% germination of Tilia cordata and 88.5 % of Tilia tomentosa; with 800 L^{-1} GA_3 solution – 77.8 % of Tilia platyphyllos. Additional concentration of GA_3 did not increase or decrease germination percentage significantly.
Germination percentage of the seeds stratified for 6, 9 and 12 weeks Table 3. or treated with 400, 600and 800 mg L^{-1} GA_3 solution Table 4. did not show significant differences between light and dark treatment at 20°C.

Table 3 Influence of light condition and GA_3 concentration on germination characteristics of species of genus Tilia

№	Variants		*Tilia cordata*					*Tilia platyphyllos*					*Tilia tomentosa*				
			GP%	Avg. root length	Avg. root number	Avg. leaf number	Avg. leaf area (cm^2)	GP%	Avg. root length	Avg. root number	Avg. leaf number	Avg. leaf area (cm^2)	GP%	Avg. root length	Avg. root number	Avg. leaf number	Avg. leaf area (cm^2)
1	400	light	84,7	3,7	5,6	2,7	3,1	67,7	3,5	4,3	2,0	3,0	75,9	5,6	5,8	3,1	2,7
		dark	80,3	2,4	4,9	2,5	3,0	60,3	3,0	4,1	1,7	2,5	70,3	4,3	4,2	2,1	1,3
2	600	light	89,5	4,3	6,4	3,8	2,9	73,8	3,8	5,3	3,1	3,1	88,7	5,8	6,7	4,3	3,4
		dark	80,3	3,1	5,3	2,9	1,0	70,4	2,3	4,3	2,8	2,1	80,3	4,1	5,7	4,0	2,9
3	800	light	82,4	4,2	6,0	3,0	1,6	77,8	4,0	5,9	3,4	3,3	63,7	4,2	4,3	4,6	2,9
		dark	80,3	3,9	5,4	2,7	1,1	70,3	3,4	4,0	2,7	2,8	60,3	3,8	3,7	3,8	2,0

Table 4 Influence of light condition and cold stratification on germination characteristics of genus Tilia

№	Variants		*Tilia cordata*					*Tilia platyphyllos*					*Tilia tomentosa*				
			GP%	Avg root length	Avg. root number	Avg. leaf number	Avg. leaf area (cm^2)	GP%	Avg root length	Avg. root number	Avg. leaf number	Avg. leaf area (cm^2)	GP%	Avg root length	Avg. root number	Avg. leaf number	Avg. leaf area (cm^2)
1	6	light	50,4	2,5	1,2	1,4	0,9	38,7	1,7	2,9	1,5	0,9	58,8	2,3	2,7	2,7	1,5
		dark	55,6	3,7	2,9	2,9	1,7	40,5	2,7	3,4	2,5	1,6	63,4	3,8	3,9	3,1	2,7
2	9	light	86,3	3,1	2,1	2,3	1,3	50,8	2,4	3,4	2,1	1,3	78,9	3,8	3,9	3,5	2,7
		dark	88,4	4,2	3,5	3,7	2,8	60,1	3,1	4,5	3,2	2,7	81,3	4,1	4,3	4,4	3,5
3	12	light	84,8	2,7	2,7	1,7	1,7	72,0	1,9	3,7	1,7	1,5	83,7	3,9	3,9	4,6	2,9
		dark	86,9	3,4	3,1	2,9	2,8	79,3	2,5	3,9	2,4	2,5	88,3	4,3	4,8	5,1	3,7

Cold moist stratification was very successful in breaking dormancy of seeds as was reported by Roy and Huxley et all. Although Huxley et all. reported that ***Tilia*** seeds require four to six weeks of stratification. Karam and Al-Salem stated that at least 10 -12 weeks of stratification was needed to overcome dormancy in ***T. platyphyllos*** seeds. The present study showed that ***Tilia*** seeds required 9 or 12 weeks of stratification.
Germination percentage of the seeds treated with GA_3 was similar to that of seeds stratified for 9 or 12 weeks at 4°C. This implies that treatment of seeds with GA_3 may substitute for cold stratification as was reported for *Prunus persica, Corylus avellana.* GA_3 was shown to enhance seed germination in several species and to overcome physiological dormancy in seeds with dormant embryos. Germination percentage of ***Tilia*** decreased as concentration was increased above 800 mg L^{-1}. In this study, treatment of seeds above 800 mg $L"^{1}$ reduce germination of ***Tilia*** seeds.

Conclusions
The study revealed that the seeds were found dormant and dormancy can be attributed to physiological inhibitory mechanisms of germination. Stratification of seeds for 9 or 12 weeks,

or treatment with 600 and 800 mg L^{-1} GA_3 can successfully overcome dormancy in ***Tilia*** seeds and light ifnditions do not play a main role in the germination.

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The Evaluation of fruit and vegetable consumption among students of the Constantine the Philosopher University

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Abstract
Nowdays the topic of healthy lifestyle has become more and more actual. In this way, the monitoring of the fruit and vegetable consumption has been provided among the sample of college students (n=242) from the Constantine the Philosopher University and the differences between study programmes were evaluated by χ-method. The results of experiment proved that the majority of respondent preffered to consume fresh fruit and vegetable but only once a day. In respect of the fresh vegetable consumption there has been statistically significant differences between the field of study RTH –J, RTH – PEES, RTH –RTS and RTH – PEEH ($P<0.05$), in fresh fruit consumption between the following groups: RTH – RTS, J – RTH and J – RTS ($P<0.05$).

Key words: fruit, vegetable, college students, consumption, frequency

Introduction

Fruit and vegetable promotion is one of the key targets in healthy diet promotion in world-wide. The World Health Organisation (WHO) and Food and Agriculture of the United Nation reports recommend adults to consume at least five servings of fruits and vegetables per day excluding starchy vegetables. Despite an increasing focus on the health benefits of fruits and vegetables, their consumption is below the recommended intake among adults (Schneider et al., 2007).
The evaluation of regular fruit and vegetable intake (at least five times a day) has been evaluated mainly among pupils at primary school and students at secondary school (Babinska et al., 2007, 2008; Bašková, 2011). However, hardly any researches dealing with eating habits of college students because the major changes in eating behaviours are related to adolescence age (Gilbert et al., 2007). So our research focused attention on mapping of fruit and vegetable consumption (fresh or procesed form) in accordance with frequency of intake: more times a day, only once a day, 1-3 times a week, rarely or never.

Material and methods

In our research questionnaire method was applied including 242 respondents – students of Constantine the Philosopher University in Nitra in 2014. The university students aged 19-22 year had the following distribution according to field of study: Pre – school and elementary education in Hungarian language (PEEH n=32), Pre – school and elementary education in Slovak language (PEES n=55), Journalism (J=38), Regional Tourism in Hungarian language (RTH n=45) and Regional Tourism in Slovak language (RTS n=72). College students were

randomly selected including students have Health education in study programme (PEEH and PEES) and the rest groups have not incorporated the topic of health into the corpus of study programme. Students were asked for the filling the questionnaire in fruit (fresh, dried or canned) and vegetable consumption (fresh, tinned, legumes and potatoes) and chose the frequency: 3 and more times a day, daily, 1-3 times a week, rarely or never. The statistical evaluation was provided according to field of study and language of study (Hungarian – Slovak) by the method of χ - square statistic on the level of probability P = 0,05 resp. 0,01.

Results and discussion

First of all, we evaluated the frequency of consumption of fresh fruit in relation to study programme of students (fig. 1).

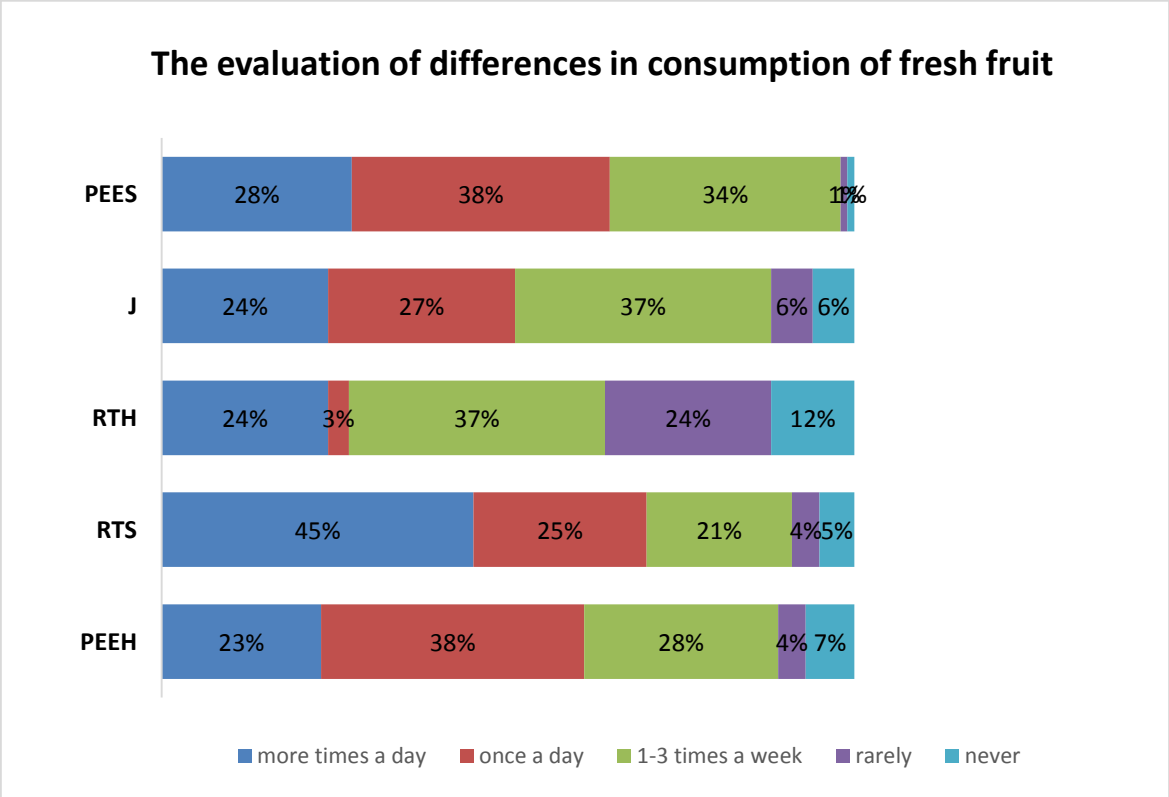

Figure 1 The evaluation of differences in consumption of fresh fruit in relation to study programme

Results of experiments pointed to the fact, that the majority of college students consumed fruit in fresh form. It can be considered as positive fact because according to WHO only 1/3 of adults took fresh fruit and vegetable more than three times a day (Beliveu – Gingras, 2008).
According to statistical evaluation of fresh fruit consumption taken into account all frequency of intake (more times a day, once a day, 1-3 times a day, rarely or never) there has been proved statistically significant differences between following field of study: RTH – RTS, J – RTH and J – RTS (fig 2).
On the basis of graph 3 it is clear that majority of probands consumed fruit in canned form only rarely (PEEH 29% - PEES 47%) or 1-3 times a week (RTS 21%- PEEH 48%) (fig 3). This fact can be explored by the fact that the most of the probands consumed lunch not at school canteen but at home or college (Juríková *et al*., 2015). In respect of canned fruit consumption the field of study has not been taken into account as statistically significant factor forming the eating habits of university students.

On the basis of fig 4 we can conclude that fresh vegetable has been eaten in fresh form (or salad form) more times a day (PEEH 23% - RTS 45%) or once a day (RTH 3% - 38% PEES, PEEH). There has been statistically significant differences between the field of study RTH – J, RTH – PEES, RTH – RTS and RTH – PEEH ($P<0.05$).
According to Unusan (2004) self-reported mean intake was found to be 3.67+/-1.81 servings of fruit and vegetables per day at seven selected universities in Turkey that represent higher value in comparison with our results of research.

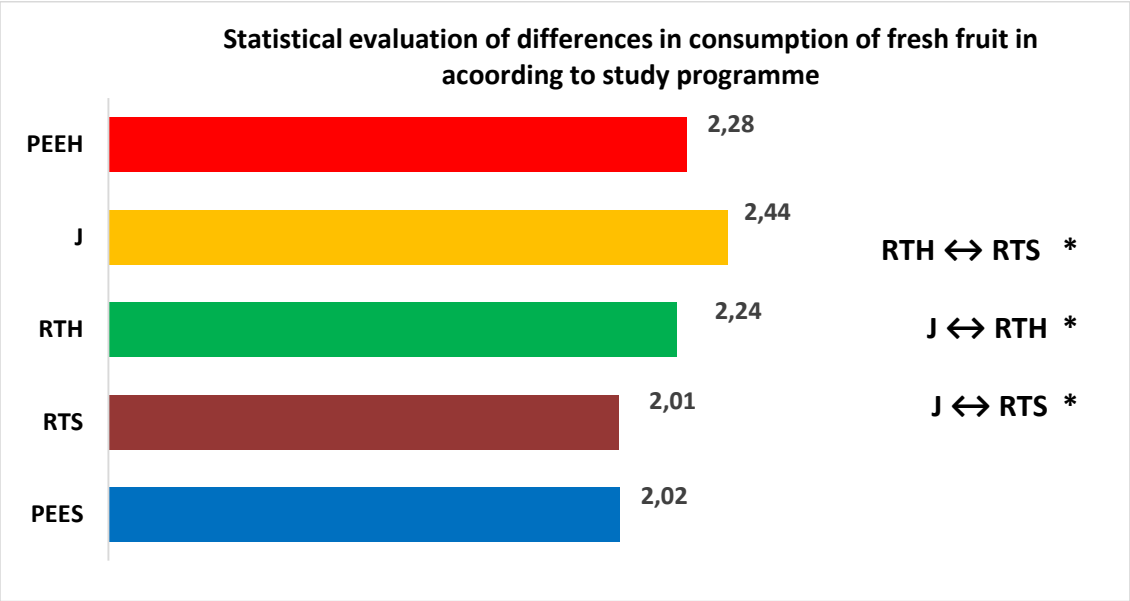

Figure 2 The statistical evaluation of differences in fresh fruit consumption in relation to study programme

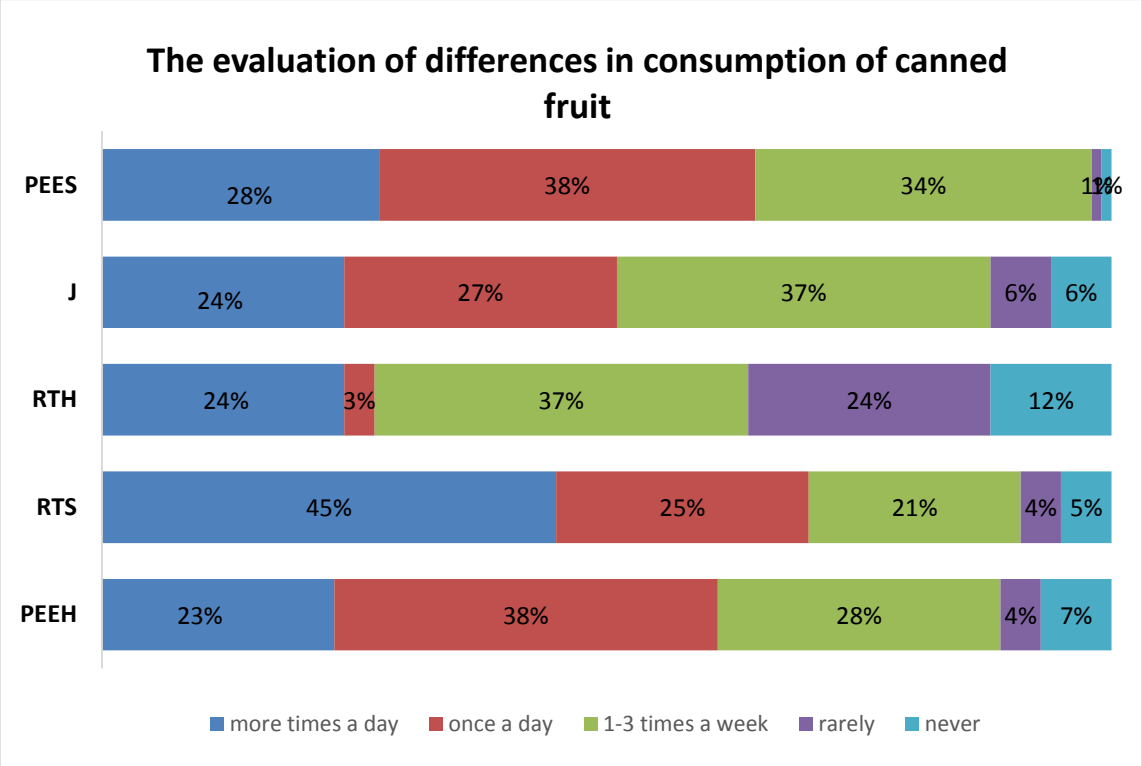

Figure 3 The evaluation of differences in consumption of canned fruit in relation to study programme

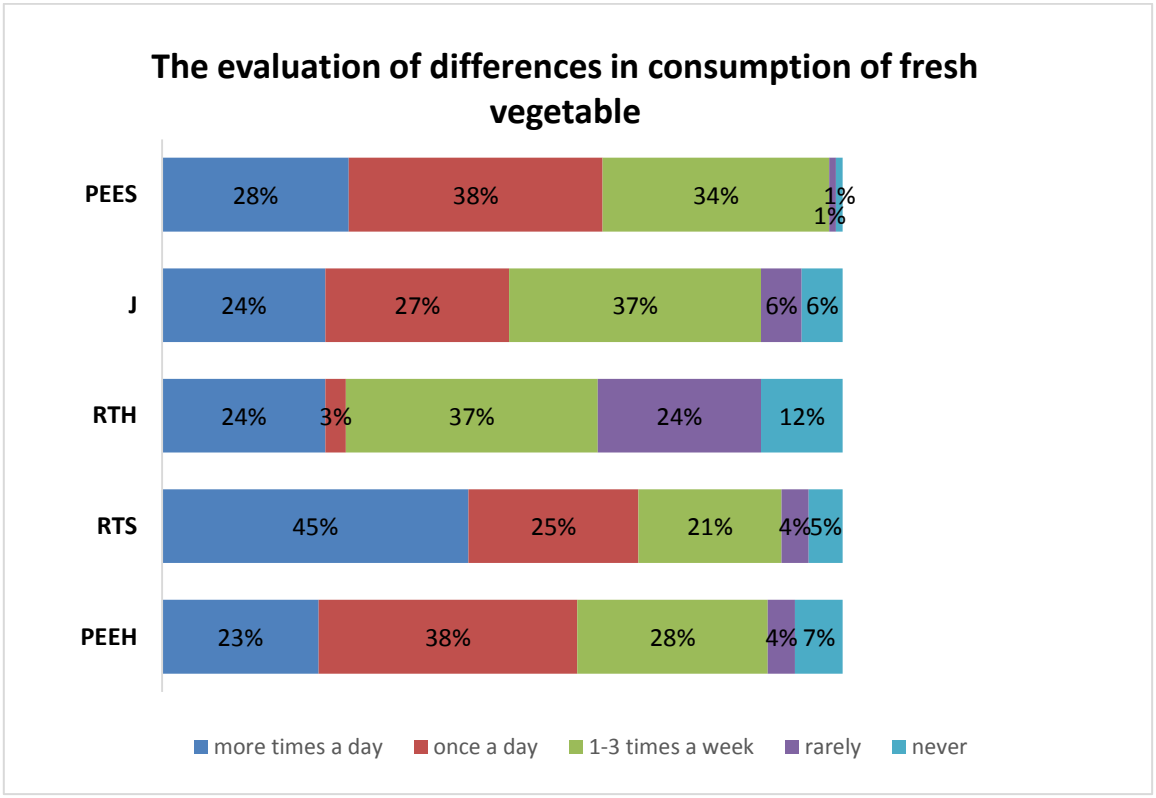

Figure 4 The evaluation of differences in consumption of fresh vegetable in relation to study programme

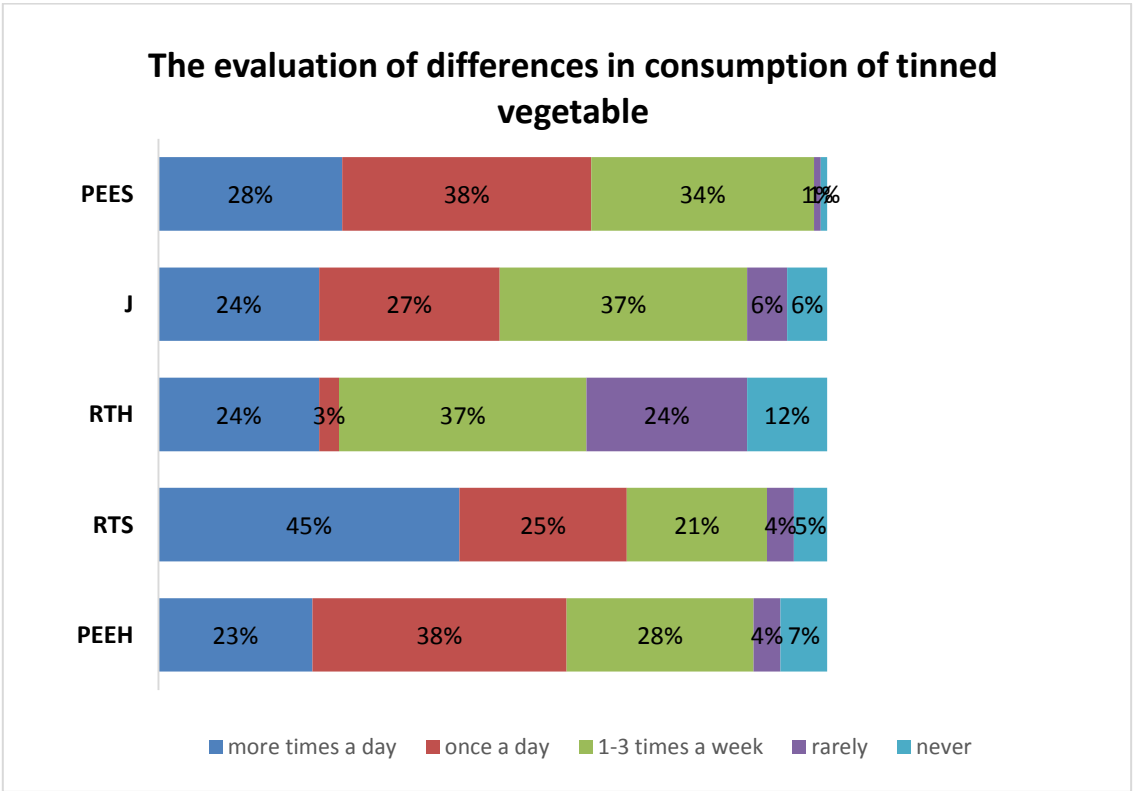

Figure 5 The evaluation of differences in consumption of tinned vegetable in relation to study programme

The consumption of tinned vegetable is lower than canned fruit that is in accordance with Habánová (2012). The prevalence of college students has preferred to consume tinned vegetable rarely (PEEH 29% - PEES 47%) or 1-3 times a week (PEES 34% - PEEH 58%) (fig 5). There has not been proved statistically significant differences between study programme.
The influence of nationality was proved but not in case of all evaluated parameters that is in accordance with results of te Welde et al. (2006) compared the Dutch population with minorities. Our results of reasearch are in agreement with the existing data suggests that despite the protective effects of fruits and vegetables, their intakes are still inadequate in many countries (Dhandevi- Jeevon, 2015). According to recent researches in USA, the mean intake of fruit and vegetable is only 1,1 times and 1,6 times a day (Centers for Disease Control and Prevention, 2013) that is similar to our results of research dealing with college students. The higher consumption of fruit and vegetable in nearby countries was noticed in Germany, according to German Health Interview and Examination Survey data report that women and men consume 3.1 and 2.4 servings of F&V per day respectively (Mensink et al., 2013).

Conclusion

The consumption of fruit and vegetable is higher in fresh form than processed (tinned and canned) that can be evaluated as a positive fact. The problem riced from the frequency of fruit and vegetable intake because the majority of respondents prefered to consume these comodities in fresh form only once a day. In respect of the fresh vegetable consumption there has been statistically significant differences between the field of study RTH –J, RTH – PEES, RTH – RTS and RTH – PEEH ($P<0.05$), in fresh fruit consumption between the following groups: RTH – RTS, J – RTH and J – RTS ($P<0.05$). We can conclude that increase in health education must have continual character from the early childhood up to adult age.

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Aphids' galls – damage or decorative value of host plants?

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Abstract
Gall formation is a unique group of interactions between the insect and the host plant. This paper presented the qualitative and quantitative structure of gall forming aphids (Hemiptera, Eriosomatinae) on elm and poplar trees and their influence on plants' decorative value. The research was carried out in 2012, on *Ulmus glabra*, *U. pumila*, *U. laevis* and *Populus nigra* var. Italica trees. The presence of two aphids species *Tetraneura ulmi* (L.) and *Colopha compressa* (Koch) causing true galls were found on elm trees. On *P. nigra* var. 'Italica' three galling aphid species were noted *Pemphigus spyrothecae* Pass., *P. bursarius* (L.) and *P. phenax* Börner&Blunck. Young galls of all observed aphid species were interesting neoplastic formations which doesn't exist in normal development of trees. But mature galls mostly wither in June and blemish plants. Only *Tetraneura ulmi* was considered to be important occasional elm pest.

Key words: *Tetraneura ulmi, Colopha compressa, Pemphigus spyrothecae, Pemphigus bursarius, Pemphigus phenax*

Introduction

Poplars (*Populus* sp.) and elms (*Ulmus* sp.) are grown in natural forests. And used as ornamental trees, with numerous cultivars used. Poplar trees with columnar branching are particularly popular, and are widely grown across Europe, especially as a part of town landscape. Many species and cultivars of elms were planted as ornamental street, garden, and park trees during the 19th and early 20th centuries. However, in recent decades, a lot of mature trees have died from fast spreading Dutch elm disease (Seneta and Dolatowski, 2006).
The feeding of insects is one of the biotic factors affecting the condition of plants. Gall formation is a unique group of interactions between the insect and the host plant. Approximately 10% of the 4,400 known aphid species worldwide are true gall formers (Blackman and Eastop, 1994; Wool 2004). They are sap sucking insects and have complex life cycles, with alternating sexual and parthenogenetic generations. Several types of true galls inducing by aphids belonging to the subfamily Eriosomatinae have been described on elm and poplar trees. In the Lublin region (Poland) these are species belonging to the genera Colopha, Eriosoma, Gootiella, Pemphigus, Tetraneura, Thecabius (Osiadacz and Hałaj, 2009). Galls are formed mostly on lamina, veins or petiole of host trees. Furthermore, gall structure is specific to species. Each aphid species can induce galls with different shape, size and galling site even they attack the same host plant and plant organ. Galls can be formed only on young, developing plants organs (Wool, 2004).
The most galling aphids inhabiting poplars and elms are heteroecious. In the spring the first instar of fundatrix hatch from eggs laid in bark crevices of trees. After hatching the larva migrates to developing leaves, finds a suitable site and starts galling process. Only that morph is capable of inducing a gall and each gall is induced by a single aphid. When gall mature, the fundatrix gives birth to offspring that develop into winged aphids. In early summer, after the

gall opens emigrants emerge and fly to the secondary host, where they start a new parthenogenetic colony. In autumn the sexuparae (mothers) develop on the roots of secondary host, reemigrate to the primary host and produce the sexual generation that will lay eggs (Wool, 2004). In the case of monoecious species e.g. *Pempighus spyrothecae* Pass. complete generation life cycle develop exclusively on a primary host (Suzuki et al., 2009; Urban, 2002).
The significance of galling aphids for primary hosts have not been satisfactorily revealed. The purpose of the present paper was to analyse the qualitative and quantitative structure of gall forming aphids (Hemiptera, Eriosomatinae) on elm and poplar trees and determine their influence on plants' damage.

Material and methods

The research was carried out in 2012, on *Ulmus glabra*, *U. pumila*, *U. laevis* and *Populus nigra* var. Italica trees, which are part of urban green areas of Lublin, Poland (51.24°N, 22.57°S). For each host species 15 trees were selected. In mid-May 30 random branches with a length of about 30 cm from each tree were collected for the qualitative and quantitative research (including shoots growing from a tree trunk on poplar). In the laboratory, the identification of galls and aphids was made at first (Blackman and Eastop, 1994). The number of leaves with galls of particular aphid species and their position on shoots were determined. From May to October, under field conditions, the development of galls and changes in leaves appearance were observed as well. Visible changes on leaves lamina were assessed in laboratory (Department of Entomology, University of Life Science in Lublin) using Laser area meter CI-202 (CID, Camas, USA).

Results and Discussion

The presence of two aphid species *Tetraneura ulmi* (L.) and *Colopha compressa* (Koch) causing true galls were found on elm trees. Galls of *T. ulmi* were stalked, approximately bean-shaped, smooth and shiny. Initially the galls were green, then they started to turn yellow and opened in mid-June (tab. 1). Near each gall stalk part of lamina was discoloured and corrugated. These galls were observed on two elm species - *U. pumila* and *U. glabra*. Galls of *T. ulmi* were noted on 31.8% leaves of *U. pumila* on average, while on *U. glabra* on 15.6% leaves. As in our previous studies (Kmieć and Kot 2007, 2010) damaged leaves contained 8 or 10 galls at most, according to elm species (tab. 2). Urban (2003) stated up to 21 galls per one leaf on *U. minor*. Present study revealed, that usually galls were situated on the distal part of the leaves. Although, if galls were numerous covered almost the entire surface of leaf blade. Fundatrix of *C. compressa* formed pocket galls on upper surfaces of leaves, near the midrib on *U. laevis* only, as in our previous study (Kot and Kmieć, 2013). Galls were yellowish tinged with red, laterally compressed, cockscomb-shaped. Damaged leaves contained from 1 to 3 galls, but leaves with one gall were the most numerous.
On *P. nigra* var. 'Italica' three galling aphids species were noted *Pemphigus spyrothecae* Pass., *Pemphigus bursarius* (L.) and *Pemphigus phenax* Börner&Blunck. *P. phenax* formed elongate, spindle-shaped, wrinkled galls which were reddish, often tinged with yellow and situated on upper side of leaf lamina along midrib. These galls were noted on 18.5% of poplar leaves. Slight discoloration of leaf lamina near the galls was observed. Galls of *P. bursarius* were purse-shaped always situated on leaves petioles. They were observed on 16.9% of *P. nigra* leaves. *P. spyrothecae* galls were smooth, formed by thickening, flattering and spiral twisting of petiole. In the period of fundatrix feeding galls were green, when the next generations appeared they started to reddish or yellowish. These galls were the most numerous on poplar shoots (tab. 2) and they were present on the petioles during the entire

growing period. These galls were opened in September (tab. 1). A similar pattern of development has been observed by Urban (2002).

Table 1. Life history traits of the Eriosomatinae examined in this study

Species of gall former	Host plant	Gall type	Gall localization	Time of first gall opening
Tetraneura ulmi L.	*Ulmus pumila* Mill.; *U. glabra* Huds.,	globose (bean shaped) on stalk/ one chamber	upper lamina	June
Colopha compressa (Koch)	*Ulmus laevis* Pall.	pocket, cockscomb-shaped/one chamber	upper side of leaf midrib	June
Pemphigus spyrothecae Pass.	*Populus nigra* L. 'Italica'	spirally twisted/ one chamber	petiole	September
Pemphigus bursarius (L.)	*Populus nigra* L. 'Italica'	purse	petiole	June
Pemphigus phenax Börner&Blunck	*Populus nigra* L. 'Italica'	spindly, wrinkled/ one chamber	upper side of leaf midrib	June

Table 2. The qualitative and quantitative structure of observed galls

Species of gall former	Number of galls per one leaf			% of galling leaves	Visible damage
	min	max	mean		
Tetraneura ulmi L.	1^a 1^b	8^a 10^b	3.26^a 3.76^b	31.8^a 15.6^b	Necrosis of leaf blade near gall stalk
Colopha compressa (Koch)	1	3	1.02	6.7	Discoloration of leaf lamina below the gall
Pemphigus spyrothecae Pass.	1	3	1.13	64.3	Not observed
Pemphigus bursarius (L.)	1	2	1.08	16.9	Not observed
Pemphigus phenax Börner&Blunck	1	2	1.03	18.5	Slight discoloration of leaf lamina near

					the gall

[a] data for *U. pumila*; [b] data for *U. glabra*

Galls dried after migrants of all aphid species left them in June, except *P. spyrothecae*. In the case of *T. ulmi* a part of leaf blade near the galls stalk also withered (Photo 1). Necrosis ranged from 15 to more than 70 percent of leaf blade. The most numerous were the leaves of damage about 30 percent of their area. The galls of *C. compressa* were situated mostly on the basal part of the leaf lamina. However, visible discoloration of leaf blades below the gall was observed, after gall opening lamina not withered. Discoloration ranged about 50% of leaf blade, on average. Samsone et al. (2012) observed decreased levels of photosynthetic parameters associated with direct damage to photosystem II in *U. laevis* leaves affected by *C. compressa*. In the case of petiole galls of *P. spyrothecae* and *P. bursarius* visible damage of leaves were not observed in our study. However, Urban (2002) reported that *P. spyrothecae* often appears in mass outbreaks and can be harmful.

Figure 1 Withering galls of *Tetraneura ulmi* L.

Conclusions

1. The occurrence of true galls formed by 2 aphid species on elms and 3 on poplar trees was stated.
2. The galls of *Tetraneura ulmi* (L.) on elms and *Pempighus spytothecae* Pass. on poplar were the most numerous.
3. Young galls of all observed aphid species were interesting neoplastic formations which doesn't exist in normal development of trees. Various shapes and colours of galls on the same host plant could be alluring elements enhanced decorative values of plants. But mature galls, after the aphids leaving, wither in June (except *P. spyrothecae*) and blemish plants.
4. From all observed galling aphids *Tetraneura ulmi* was considered to be important occasional elm pest. During mass outbreaks it is necessary to control this aphid species.

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Phenological diversity of *Lychnis coronaria* (L.) DESR. in different habitat

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Abstract
Lychnis coronaria (L.) DESR is a maximum 90 cm high perennialspecies, the plant has purplish pink flowers with 3 cm diameter (Jelitto et al., 2002; Phillips and Rix, 1994). Native in Mountains Bükk, Zemplén, Cserhát, Börzsöny, Pilis, Nyírség and Hills of Gödöllő, Hills of Zala, Balaton Uplands and Mecsek. Dispersedly: Alföld: Kis- Alföld, Duna-Tisza köze, Tiszántúl, Hungary (Simon, 1992; Soó, 1970).
During the experiment, 10-10 plants of *Lychnis coronaria* (L.) DESR was planted in the Buda Arboretum to places of different light intensity (sunny, semi shade, shade) in the beds. The parameters investigated are as follows: phenological periods of *Lychnis coronaria*, the height of plants, the beginning of flowering, the number of flowers per plant, the diameter of flowers.
We can state that sun is the optimal circumstances in Buda Arboretum for the species. Under such ecological conditions robust growth was observed, but black spots appeared on the leaves. In the shadow bed the plants grew poorly. The highest plants were observed in the sunny place (39-65 cm), but in the semi shadow circumstances the flowers were decorative too.

Keywords: *Lychnis coronaria* (L.) DESR., Buda Arboretum, light condition, development, morphologie

Introduction

Lychnis coronaria (L.) DESR. is a medium-high perennial in the family *Caryophyllaceae*. The species grows to 30-70 cm, but flowerstems up to 100 cm, 7-18 cm ground leaves. Stems and leaves are covered with bright silvery hair (Kárpáti and Terpó, 1968; Soó and Kárpáti, 1968; Száraz, 1993; Simon, 1992; Rubinstein, 2006).
Lychnis coronaria (L.) DESR. lives best on well-drained soil. In the nature it lives in dry oak or karst forest and meadows (Simon, 1992; Soó, 1970).The species has 7-15 cm long, hairy shaped leaves. The flower is brightly colored, pink, magenta and white rose with eye patch with 2-5 cm in diameter. Flowering period is Mai-June, or Mai-July and in September can reflorescence, in Hungary Juni-September (Simon, 1992). The fruit is capsule. Our experiment tended towards to get information how this species can grow among different light conditions. This experiment is a step in the *ex situ* gene reservation of this protected species (Soó and Kárpáti, 1968; Rubinstein, 2006; Tuba et al., 2007).
The plant has leaf rosette. In the apex develops the inflorescence. Flower formula is: K(5) C5 A5+5, 5, 1 G(5-2) (JELITTO et al., 2002; Kárpáti and Terpó, 1968; Soó and Kárpáti, 1968; Száraz, 1993; Nau, 1996; Simon, 1992).
Rose champion is the member of Sub-Mediterranean flora elements, is native in Europe. *Lychnis coronaria* is a calcareous plants, accepts the neutral or slightly acidic soil as well (Kárpáti and Terpó, 1968; Csapody et al., 1993; Simon, 1992; Bartha, 2012). It survives on very poor, dry sandy soils in full sun; but short living in rich soil and partial shade. Hardy to -20 °C (Bird, 1993; Száraz, 1993; Takács, 2014).

Rose champion is a short-life plant, best flowering is in the second year, especially when summer is hot. In these cases the species saws itself.

Material and Methods

The plants examined were propagated by seed sowing. We obtained the seeds from the Botanical Garden of Vácrátót. The seeds were sawn in the glasshouse of the Department of Floriculture and Dendrology, Szent István University. The one year old plants were planted in the Buda Arboretum in October, 2011 to three different environmental places: sunny, semi shade and shade, ten plants to each. The soil constituting foundation rock is limestone and dolomite, often settle down adobe type, clay restriction, calciferous, sedimentary rock. The soil is medium or drastic calciferous, poor in humus, pH 8 (Czigány et al., 2012).). During the vegetation period we investigated the morphological changes resulted by the various light conditions.

We measured the following parameters from April 2012 to July 2015 two weeks intervals:

1. height of stem (cm)
2. begin of flowering
3. number of flowers per plant (piece)
4. diameter of flowers (cm)

Results and Discussion

The results of the height are shown in Figure 1. The plant height differed according to the circumstances of flower beds. The plants grew equal high in the sunny and semi shadow beds, the intensive sunshine hadn't negative effect on the growth. But, in the deep shadow circumstances *Lychnis* plants grew shorter.

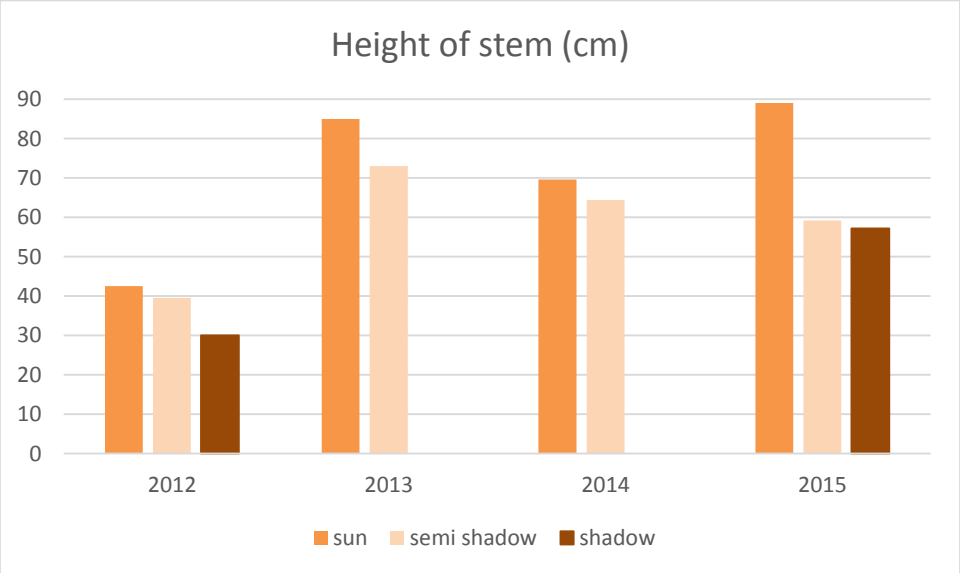

Figure 1 The mean height of stem in the Buda Arboretum

According to the authors *Lychnis coronaria* has 30-70 cm stem (Kárpáti and Terpó, 1968; Száraz, 1993; Rubinstein, 2006). This situation is typical in the natural habitat where competitors may hinder their development. Stems generally reach or overgrow 70 cm stem length as well in sunny conditions.

In the Buda Arboretum higher stems grew up to 82-89 cm, the shortest stems we measured 25-46 cm, but the common value was 50-70 cm.

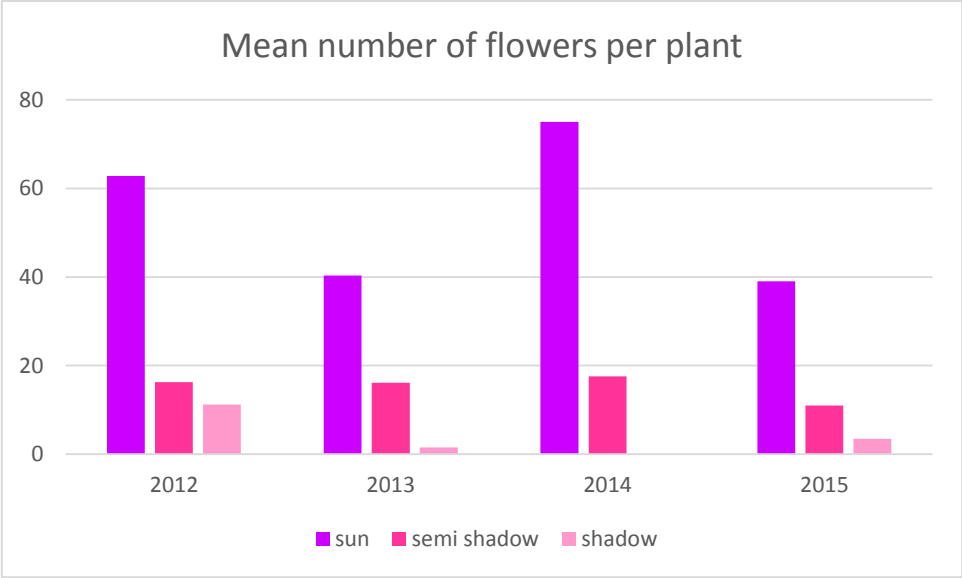

Figure 2 The mean number of flowers per plant

Great difference was observed in the case of flower number. The average number of flowers was twice big in sunny habitat comparing to the semi shadow (Figure 2). In shadow habitat plants had almost no flowers. In shadow place the plants had less stems than in sunny bed.

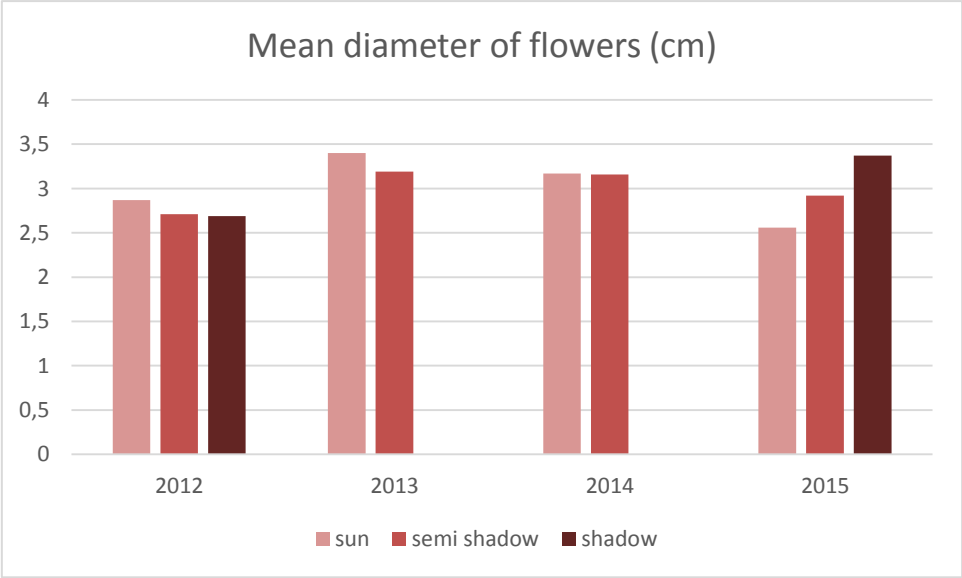

Figure 3 The mean diameter of flowers per plant

In the diameter of flowers we found differences between the shadow and the two other habitats. Biggest diameter was measured in sunny habitat (Figure 4), and in 2015 in shadow habitat (Figure 3). In sunny circumstances much smaller flowers developed than in the two other places. The lack of data in 2013 and 2014 leads back to the disappear of some plants. These were replanted in the autumn of 2014.

The diameter of most flowers was measured between 25 and 35 mm. But, in sunny and in semi shadow beds we observed bigger than 35 mm, and smaller than 25 mm flower size too. Around the end of flowering period the diameter of flowers decreased. The intensity of light had no effect on the flowers size, only on the number of per stem.

Figure 4 *Lychnis coronaria* flowers in sunny places in 2014

The flowering periods during the four years began in May, the longest were in the sunny places. The number of flowers increased steadily to end of blossoming.
Lychnis coronaria needs light for flowering. In the semi shadow and shadow beds 6 or 4 flowers per stem could be counted only, but here the flowering periods were shorter, began at the end of May, and in shadow bed in the middle of June.
Life of one flower was 14 days. The fruits (Figure 5) appeared two weeks after flowering.

Figure 5 Frutis of *Lychnis coronaria* (left) and the seeds (right) (Takács, 2013)

Conclusion

Based on the results we can conclude that sunny condition is the optimal habitat for the plant, the highest number of flowers per plant, the diameter of flowers per plant was observed in the Buda Arboretum, but in the nature is the optimal for plant semi shadow (Takács, 2014).

The flowering period lasts from the middle of May to the end of June (2012-2015).

The diameter of flowers were 2-5 cm according to Phillips and Rix (1994) and Rubinstein (2006), but in our experiment smaller flower diameter was registered too (2,5 cm). In semi shadow and shadow places the plants didn't flower as intensive as in full sun.

The competitors not, but little light abrogated the height of stem, in shadow and semi shadow places the plants were smaller than 40 cm. According to Száraz (1993) stems were 30-70 cm high, but this in not medium height, but rather small. In full sun stem grew up to 39-65 cm.

Rubinstein (2006) reported that *Lychnis coronaria* often self-saws itself producing seedlings aroun the stock plant. We observed the same situation in the Buda Arboretum in the sunny bed (Figure 6).

Figure 6 *Lychnis coronaria* can be self-sawing

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Leaf blister mites (*Eriophyes* sp.) as significant pests in orchards

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Abstract
Orchards constitute a place to live for different pests. Leaf blister mites belong to the family of eriophyoid mites and they can have significant economic meaning in fruit production. These are not free-living organisms and the majority of life they spend sheltered in blisters or under the bud scales, where they overwinter. In spring overwintered females leave the swelling buds and start to feed. In result of their feeding appear small blistered galls inside which females lay eggs. When the trees are numerously infested by leaf blister mites both efficiency of photosyntesis and vigor are significantly reduced, what directly influences on the yield of fruit. These pests can be effectively controlled only in such moments when they appear on the surface on plants, so it is particulary necessary to follow the deadlines in case of control leaf blister mites.

Key words: orchards, leaf blister mites, pests, galls, control

Introduction

In many countries within the Europe and in other continents fruit production is highly developed and constitutes an important branche of economy. Orchards in which are grown different species and cultivars of fruit trees cover a huge area counted in thousands of square kilometers. Fruit trees are a place of living for some kinds of pests. One of the groups of pests infesting fruit trees constitutes the superfamily of eriophyoid mites (Acari: Eriophyoidea). These are very tiny organisms and it is almost impossible to observe them by naked eye, without using any microscopic device. Eriophyoid mites are obligate phytophagous organisms and in majority exhibit a high level of host specificity and adaptability. They are able to inhabit practically all the parts of plants apart from roots. Many species of eriophyoid mites are considered economically important because of certain damage like spots, galls, rusetting, browning, stunting or rolling the leaf edges, which they are able to cause. Several species can also transmit phytopathogenic viruses (Vidović et al. 2014; Nasareen & Ramani 2014).
Leaf blister mites (*Eriophyes* sp.) are a genus inside the eriophyoid mites. These mites attack such plants like pear, apple or quince, but also can occur on other species of plants from the family Roseaceae, like mountain ash or hawthorn. In literature as significant pests are mentioned especially pear leaf blister mite (*Eriophyes pyri* Pagenstecher, 1857) and apple leaf blister mite (*Eriophyes mali* Nalepa, 1926). To the genus *Eriophyes* belongs also *Eriophyes sorbi* Canestrini, 1890 which infests mountain ash. Some scientists even suppose that pear leaf blister mite and *Eriophyes sorbi* may potentially constitute one species because of numerous similarities, but it is still not explained. The average body length at leaf blister mites is less than 0.3 mm. The same like other eriophyoid mites, they have got only two pairs of legs. The body is worm-shaped, what differs leaf blister mites from for example pear rust mite (*Epitrimerus pyri* Nalepa, 1898) coexisting with them on pear trees. Leaf blister mites are usually bright coloured. The most often are observed whitish specimens, but some of them can be tinged with pale brown. Specific symptoms of their presence are small blistered galls (Larsen 2011; Gratwick 1992).

Numerous populations of leaf blister mites infesting the trees is able to cause serious damage, but when the population of mites is not large, plants can well tolerate it and in such case it does not have especial impact both on the yield fruit (Murray & Alston 2011). The majority of life they spend hidden what effectively protect them from the impact of many plant protection products. Only for a short time, especially in spring, they are present on the surface of plants, what means that in case of controlling these pests it is particularly important to follow the deadlines (Daniel et al. 2006; Sekrecka & Hołdaj 2013).

Biology

Leaf blister mites do not belong to the free-living mites and majority of their life spend they in blisters or in buds. Only for a short time they leave these places. Their life cycle is the same like at other eriophyoid mites and consists of four stages: egg, larva, nymph and adult. Nymphal stage is similar to the adult specimen but is a bit smaller. During the year can usually occur two or three generations (Lindquist et al. 1996; Singh et al. 2016).

Leaf blister mites spend the winter in a semi-active state under the bud scales. Almost the whole overwintering populations constitute females, within such populations there are hardly some males. When the temperature permits, they carry on the feeding and breeding activities (Ali Hammad 2004). In spring when the buds swell mites become active, leave the buds and start to feed. In result of their feeding appear small blistered galls. After 1-2 weeks females begin to form small holes in blisters through which they enter and lay eggs inside these blisters. Usually only one female enters to one blister and each female is able to produce 7-21 eggs, so the reproductive potential is high (Singh et al. 2016; Daniel et al. 2006).

Usually the development of specimens of leaf blister mites within one generation lasts quite shortly, because only 20 - 30 days are required to go from egg to the adult stage. Their whole life cycle takes a place inside the blisters caused by feeding overwintered females. Mites belonging to the next generations can move out of the blisters and create new blisters and set up colonies on other nearby developing leaves. It may be observed especially in case when populations of mites are very numerous and the existing blisters become overcrowded. Apart from it eriophyoid mites are such small organisms that they can expand being transmitted by wind (Larsen 2011).

Harmfulness and methods of control

Leaf blister mites belong to the significant economic pests in orchards. The size of damage caused by them depends not only on atmospheric factors, but also on other factors like cultivar, place of setting the plantation and the presence of natural enemies. The symptoms of infestation of leaf blister mites are very clearly noticeable. Leaves developed in spring from the buds in which mites were overwintering may by often deformated (Sekrecka & Hołdaj 2013). Mites feeding on leaves invade the mesophyll and create small galls having a blistered structure (Fig. 1; Fig. 2). At the beginning they are greenish or slightly yellowish. After short time they become darker and change their colour into pinkish. Especially on pear trees is noticeable, that the blisters change the colour very fast. Finally after some time they turn necrotic and brown or blackish. When the occurrence of mites is very intensive and there is especially a lot of blisters, they can join together into bigger ones and cover a substantial part of leaves. It significantly reduce both the photosynthesis efficiency and plant vigor and even can lead to the partial leaf fall during the summer, what may be especially observed during August. The final consequence is a strong decline of yield (Daniel et al. 2006, Gratwick 1992).

Not only leaves can be attacked by leaf blister mites. They can cause forming the blisters either on the top parts of branches and on flower buds. Mites can also infest fruit at the time of blooming and feed on them. In consequence on the skin of fruit appear symptoms like

scarring and russet, which are quite similar to the symptoms of apple scab disease caused by fungus *Venturia inaequalis*. The commercial value of such fruit is lower (Larsen 2011).

Figure 1 Symptoms on apple (http://utahpests.usu.edu)

The significant influence on the degree of damage caused by leaf blister mites has got the cultivar of fruit tree. Some cultivars show bigger resistance and tolerance on such kind of pests. Badowska-Czubik et al. (2014) carried out the experiment in which six cultivars of pear were examined regarding to the impact of pear leaf blister mite. This experiment showed that the cultivars 'Erika', 'Amfora' and 'Faworytka' are distinctly more susceptible for the damage caused by leaf blister mites than the cultivars 'Concorde', 'Konferencja' and 'Radana'. In June 2011 it was noticed that over 70% of the leaves on the cultivars 'Erika', 'Amfora' and 'Faworytka' were damaged whereas on the rest of the cultivars such a huge percentage of infested leaves was not observed.

Figure 2 Symptoms on pear (author,s photo)

Leaf blister mites belong to the economically significant pests and in comfortable conditions they can constitute a real threat. Unfortunately dealing with a problem of them is quite a difficult task. The possibilities of their control are limited because of the fact, that like all the gall mites they appear on the surface of plants very rarely. Chemical protection from these pests is mostly inefficient, because the chemicals can usually control the pests only on the surface of plants (Wawrzynski 2005). In UE countries from the beginning of 2014 is required to apply integrated plant protection and according to it leaf blister mites the same like other pests must be controlled with a priority of using methods alternative to chemicals. Very

important is prevention. Newly setting orchards should be enough isolated from these places where the symptoms of occurrence of leaf blister mites were observed. Apart from that all the principals connected with fertilizing and other agrotechnical aspects should be followed. Referring to the results of research important matter is to choose the cultivars showing higher degree of resistance on leaf blister mites and what is more the growing material should be free of mites and any pests. As a biological control can be used biopreparates containing such predatory organisms like mite *Typhlodromus pyri* Scheuten, 1857 which belongs to the phytoseiid mites. The research showed that this biocontrol agent is efficient in controlling leaf blister mites in orchards. Moreover, *T. pyri* is able to survive and reproduce on the alternative food sources like fungi, pollen or plant juices, so the treatment with this mite is not necessary to be so often repeated (Praslička et al. 2011).

Chemical control is possible to be carried out only in a period before forming the blisters, otherwise it will be not efficient (Wawrzynski 2005). It was showed that satisfying effects can bring pesticides containing sulphur. Against leaf blister mites also mineral oil was tested and even if that significantly reduced the population of mites, there was still quite a lot of young fruit damaged. It means that the efficiency of using mineral oil is usually lower in comparison to the sulphur but both of them can be applied (Daniel et al. 2006).

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Effect of light quality on growth and development of *Cosmos atrosanguineus* (Hook.) Voss shoots *in vitro*

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Summary
Cosmos atrosanguineus (Hook.) Voss is a herbaceous perennial plant with dark red – brown flowers characterizing with chocolate fragrance. This plant does not produce seeds, so that it has to be propagated vegetatively, by division of tubers. As this method of propagation is very slow, a research on *in vitro* propagation has been conducted. A study was undertaken to determine the light quality (white, blue, red and yellow) influence and the medium without growth regulators or supplemented with BA in concentration of 1 $mg \cdot dm^{-3}$, on induction and growth of axillary shoots. The shoots were cultivated on the Murashige and Skoog basic media (MS) for six weeks. The biggest elongation of the main shoot was observed in presence of yellow and blue lights on the medium without growth regulators. The fresh weight of the main shoot was the highest in presence of BA in yellow light. The most axillary shoots, which were the longest and of the highest fresh weight, were obtained in presence of BA in yellow light, however, the results were not significantly different from those observed in white and blue lights. The red light significantly inhibited initiation and growth of axillary shoots.

Key words: *Cosmos atrosanguineus,* axillary shoots, light quality

Introduction
The effect of growth regulators to a great extent depends on light quality. It may influence their biosynthesis, transport and metabolism; it may also change sensitivity of tissue to endogenous and exogenous growth regulators (Kopcewicz et al., 1992; Gabarkiewicz et al., 1995). Application of light with definite colour might regulate the level of endogenous growth regulators in plants and in this way increase the propagation rate. The influence of light quality and its' joint effect with growth regulators in the process of shoots and roots differentiation in tissue culture of plants belonging to the Asteraceae family was examined by: Latkowska and Chmiel (1996), Kurilčik et al. (2008), Wang et al. (2011).
The effect of light quality on growth and development of shoot tips cultivated on growth regulator free MS medium or in presence of BA in concentration of 1 $mg \cdot dm^{-3}$ was examined in the presented paper.

Material and methods
Shoot tips of *Cosmos atrosanguineus* (Hook.) Voss of 10 -15 mm length with at least 2 fully developed leaves taken from aseptically grown shoot cultures were used in this experiment. The explants were placed on the basic medium containing mineral salts according to Murashige and Skoog (1962) and: thiamine – 0.4 $mg \cdot dm^{-3}$, pyridoxine – 0.5 $mg \cdot dm^{-3}$, nicotinic acid – 0.5 $mg \cdot dm^{-3}$, glycine – 2 $mg \cdot dm^{-3}$, myo-inositol – 100 $mg \cdot dm^{-3}$, sucrose – 30 $g \cdot dm^{-3}$, Agar-Agar ((Lab-Agar^{TM} Biocorp) – 6.5 $g \cdot dm^{-3}$, and supplemented with BA in concentration of 1 $mg \cdot dm^{-3}$. A medium without cytokinin was used as a control. The pH of the media was adjusted to 5.7 before autoclaving. The cultures were maintained at $22^{o}C \pm 2$ ^{o}C under 16-h photoperiod, and light intensity of 35 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Effect of different colours of light provided from Philips fluorescent lamps: white (TLD 33), blue (TLD 18), red (TLD

15), yellow (TLD 16) was tested in the experiment. There were four replications per treatment, each consisting of 5 explants cultivated in 250 ml Erlenmeyer flasks. The experiment was repeated twice.
The following features were evaluated after 6 weeks of cultivation: length and fresh weight of the main shoot, number of leaves on the main shoot, number of axillary shoots, length and fresh weight of axillary shoots (per explant). The results of the experiments were analyzed statistically using a standard statistical procedure with one factorial design, while the Tukey test was used to estimate the differences between the means at the 5% level of significance.

Results and discussion

The presented results indicate both significant influence of different types of light and combination of light quality and BA on morphogenesis of *Cosmos atrosanguineus*. Analyzing the main shoot length, it was observed that longer shoots were noted on the medium without growth regulators (Tab. 1). Yellow and blue lights had the strongest positive effect on shoots elongation (40.8 and 38.9 mm, respectively). Moreover, the yellow light had a positive effect on length and fresh weight of the main shoot in presence of BA in concentration of 1 mg·dm^{-3}. Lights white and red inhibited elongation of the main shoot, especially when the media was supplemented with 1 mg BA·dm^{-3} (24.0 and 23.8 mm, respectively).

Table 1 Effect of light colour on growth and development of *Cosmos atrosanguineus* main shoot after 6 weeks of *in vitro* culture

Light colour	BA (mg·dm^{-3})	Main shoot length (mm)	Number of leaves	Main shoot fresh weigth (mg)
White	0	27.1 bc*	8.7 ab	49.7 cd
	1	24.0 c	7.3 ab	72.2 ab
Blue	0	38.9 a	9.5 a	65.7 abc
	1	28.3 b	7.8 ab	63.1 a-d
Red	0	27.4 bc	6.9 bc	46.2 d
	1	23.8 c	4.9 c	57.6 bcd
Yellow	0	40.8 a	8.5 ab	51.4 cd
	1	36.7 a	7.3 ab	81.5 a

*Values in vertical columns followed by the same letter do not differ significantly at P = 0.05

Table 2 Effect of light colour on growth and development of axillary shoots formed on *Cosmos atrosanguineus* shoot tips after 6 weeks of *in vitro* culture

Light colour	BA (mg·dm^{-3})	Number of axillary shoots per explant	Length of axillary shoots (mm)	Fresh weight of axillary shoots per explant (mg)
White	0	0.8 c*	2.4 c	10.7 bc
	1	5.1 a	10.9 ab	69.0 a
Blue	0	1.1 c	3.5 c	14.8 bc
	1	5.6 a	12.9 a	58.2 a
Red	0	0.2 c	1.0 c	25.6 b
	1	3.1 b	7.4 b	57.6 a
Yellow	0	0.5 c	3.2 c	5.8 c
	1	5.8 a	14.9 a	73.2 a

*Values in vertical columns followed by the same letter do not differ significantly at P = 0.05

Number of axillary shoots depended on the presence of BA in media and on light colour. A similar number of shoots (5.1-5.8) was obtained in white, blue and yellow light. Red light had the weakest effect on shoot induction (3.1) (Tab. 2). Axillary shoots were the longest in presence of yellow light (14.9 mm), however, axillary shoots formed in presence of blue and white lights were insignificantly shorter. No significant differences in fresh weight of shoots cultivated under the tested colours of light and in presence of BA 1 mg·dm^{-3} were noticed.
The obtained results deviate from the available literature. Many authors report about the positive effect of red light on elongation of shoots. Latkowska and Chmiel (1996) inform that the longest shoots of *Dendranthema grandiflora* 'Escort' were formed on the medium without growth regulators in the red light. Similarly, Kim et al. (2004) observed the best elongative growth of *Dendranthema grandiflorum* 'Cheonsu' shoots in presence of red light. *Gerbera jamesonii* 'Ruikou' plantlets grown under red light were higher than those growing under blue or white lights (Wang et al., 2011). The red light also stimulated elongation of *Petunia hybrida* (Witomska and Ładyżyńska, 2001). The red light positively influenced elongation of shoots of *Ficus benjamina* 'Golden King' cultivated on the medium without growth regulators as well as on the medium supplemented with IAA in concentration of 0.5 mg·dm^{-3} (Gabryszewska and Rudnicki, 1997). Elongation of *Cosmos atrosanguineus* main shoot was the best in yellow and blue lights. The advantageous effect of yellow light was observed by Miler et al. (2005), who obtained the highest microshoots of *Chrysanthemum* x *grandiflorum* 'Richmond' in red and yellow lights on the media without growth regulators. Shoot length of *Zieria fraseria* was positively affected by blue light (Tapingkae and Taji, 2000).On the other hand, the blue light, advantageous in case of *Cosmos*, inhibited elongation of shoots of *Dendranthema grandiflora* 'Escort' (Latkowska and Chmiel, 1996), *Dendranthema grandiflorum* 'Cheonsu' (Kim et al., 2004), *Chrysanthemum morifolium* 'Ellen'(Kurilcik et al., 2008). It also inhibited elongation of *Ficus benjamina* 'Golden King' shoots cultivated on the media containing 2iP in concentration of 15 mg·dm^{-3} (Gabryszewska and Rudnicki, 1997).
The type of the light used had no effect on number of axillary shoots formed on shoot tips on the medium without growth regulators (Tab. 2). The number of axillary shoots obtained in white, blue or yellow lights on the medium supplemented with 1 mg BA·dm^{-3} was 5.1-5.8 and there were no significant differences between these treatments. The most shoots were formed in the yellow light and they were the longest (14.9 mm) and of the highest fresh weight (73.2 mg). In the studies on micropropagation of Asteraceae family members, cultures were in majority cultivated under cool white fluorescent lamps (Bascaran and Jayabalan, 2005; Lucchesini et al., 2009; Zayova et al., 2013). The number of axillary shoots of *Cosmos atrosanguineus* regenerating under white light was 5.1 and this amount was similar to a number of shoots formed under blue or yellow lights. Latkowska and Chmiel (1996) obtained the most axillary shoots of *Dendranthema grandiflora* 'Escort' under white and blue lights, in presence of BA in concentration of 0.6 mg·dm^{-3}. White light used jointly with 2iP in concentration of 10 mg·dm^{-3} had the strongest positive effect on regeneration of *Dieffenbachia* 'Compacta' shoots (Gabarkiewicz et al., 1997). In case of *Ficus benjamina*, the most shoots were formed under red light in presence of 5 mg 2iP·dm^{-3} (Gabryszewska and Rudnicki, 1997), and for *Gerbera jamesonii* 'Queen Rebecca' the most advantageous were red and green lights used jointly with kinetin in concentration of 5 mg·dm^{-3} (Gabryszewska and Rudnicki, 1995). Literature data is often discrepant, as the reaction of plants to the colour of light depends on many factors: genotype, physiological state, the level of growth regulators as well as the source and intensity of light.

Conclusion

1. Light quality has influence on the growth and development of *Cosmos atrosanguineus* shoot tips *in vitro.*
2. There were no significant differences in the number of axillary shoots induced under the white, blue and yellow light, in the presence of BA 1 $mg \cdot dm^{-3}$.
3. Yellow light has a most beneficial effect on the growth of axillary shoots.

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Effect of the type of explant and benzyladenine on growth and branching of *Cosmos atrosanguineus* (Hook.) Voss shoots *in vitro*

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Abstract
Cosmos atrosanguineus is a frost-sensitive tuberous perennial. It has velvety, dahlia-like, brown-red flowers with chocolate fragrance. The plant is sterile, as it does not produce seeds. It is usually used as a border plant in perennial gardens. A research was conducted in order to estimate the effect of benzyladenine (BA) in concentrations of: 0.2, 1, 2.5 and 5 $mg \cdot dm^{-3}$ on induction and growth of axillary shoots from two type of explants: shoot tips and nodes taken from *in vitro* grown plants. A medium without cytokinin was used as a control. The explants were cultivated on the Murashige and Skoog basic medium (MS) for six weeks. The obtained results show that shoot tips and nodal parts of shoots are suitable as a secondary explants for multiplication stage of *Cosmos atrosanguineus*. MS medium supplemented with BA in concentration of 1- 2.5 $mg \cdot dm^{-3}$ is the best for shoot proliferation from both types of explants. Elongation growth of axillary shoots induced from nodes is the best on the control medium, but shoots obtained from shoot tips are the longest in presence of 1 $mg \cdot dm^{-3}$ BA.

Key words: *Cosmos atrosanguineus,* benzyladenine, explant type, axillary shoots

Introduction

Cosmos atrosanguineus (Hook.) Voss (chocolate cosmos) is a frost-sensitive perennial which flowers have chocolate fragrance. The flowers' colours range from a brownish-red to a maroon and they are in a contrast with deeply green leaves. In areas with frosty winters, the plant should be dug up before the first frost and stored indoor. The plant is sterile, as it does not produce seeds. It has to be propagated through division of tuberous roots. In order to establish a method for production of healthy plants and to obtain a high propagation rate, an *in vitro* studies were undertaken. One of the main factors influencing plants' morphogenesis *in vitro* are growth regulators. The role of benzyladenine (BA) in bud induction has been recorded for many plants from Asteraceae family such as *Dendranthema* (Latkowska and Chmiel, 1996), *Cosmos atrosanguineus* (Hosoki et al., 2003), *Eclipta alba* (Husain and Anis, 2006), *Anthemis xylopoda* (Erdag and Emek, 2009), *Sphagneticola trilobata* (Sivanesan and Jeong, 2009), *Saussurea esthonica* (Gailite et al., 2010), *Inula racemosa* (Kaur et al., 2010), *Spilanthes acmella* (Singh and Chaturvedi, 2010), *Calendula officinalis* (Victorio et al., 2012), *Rhaponticoides mycalea* (Emek and Erdag, 2013). Kinetin and thidiazuron (TDZ) were rarely used. Kinetin was recommended for micropropagation of *Gerbera jamesonii* (Hempel et al., 1985), *Echinops spinosissimus* (Murch et al., 2003), and thidiazuron for *Stevia rebaudiana* (Lata et al., 2012). A use of BA (Lele et al., 1998; Keng et al., 2009; Taleie et al., 2012; Surmacz-Magdziak and Sugier, 2012; Zayova et al., 2013) or kinetin (Malarz et al., 1993; Wildi et al., 1998; Salazar et al., 2005; Mitra and Pal, 2007; John Peter Paul et al., 2012) jointly with auxins is also recommended.
Regeneration ability is greatly dependent on the explant type. For plants mutliplication the shoot tips or nodal explants are the most preferred parts, because it allows to eliminate callus or adventitious shoots phase and the associated problems of genetic variability. Hosoki et al. (2003) published the report on multiplication of *Cosmos atrosanguineus* through division of

nodal tissue and separation of axillary shoots, while shoot tip explants were taken for shoot proliferation of *Stevia rebaudiana* (Sivaram and Mucundan, 2003), *Eclipta alba* (Dhaka and Kothari, 2005), *Artemisia amygdalina* (Rasool et al., 2013). Single nodal explants containing axillary buds were used in study on *Eclipta alba* (Dhaka and Kothari, 2005; Husain and Anis, 2006) and *Vernonia amygdalina* (Khalafalla et al., 2007).
The aim of the undertaken research was to compare regeneration ability of shoot tips and nodal parts of shoots of *Cosmos atrosanguineus* cultivated on the Murashige and Skoog (MS) medium supplemented with BA in concentrations of: 0.2, 1, 2.5 or 5 $mg \cdot dm^{-3}$.

Material and methods

Shoot tips of 10-15 mm in length with at least 2 fully developed leaves and 1 cm long nodal parts of shoots of *Cosmos atrosanguineus* (Hook.) Voss were taken from aseptically grown shoots cultures to be used in the experiment. The explants were placed on the basic medium containing mineral salts according to Murashige and Skoog (1962) and: thiamine – 0.4 $mg \cdot dm^{-3}$, pyridoxine – 0.5 $mg \cdot dm^{-3}$, nicotinic acid – 0.5 $mg \cdot dm^{-3}$, glycine – 2 $mg \cdot dm^{-3}$, myo-inositol – 100 $mg \cdot dm^{-3}$, sucrose – 30 $g \cdot dm^{-3}$, Agar-Agar (Lab-Agar^{TM} Biocorp) – 6.5 $g \cdot dm^{-3}$, and supplemented with BA in concentrations of: 0.2, 1, 2.5 and 5 $mg \cdot dm^{-3}$. A medium without cytokinins was used as a control. The pH of the media was adjusted to 5.7 before autoclaving. The cultures were maintained at $22^{o}C \pm 2\ ^{o}C$ under 16-h photoperiod, and light intensity of 35 $\mu mol \cdot m^{-2} \cdot s^{-1}$. There were four replications per treatment, each consisting of 5 explants cultivated in Erlenmeyer flask of 250 ml capacity. The experiment was repeated twice.
The following features were evaluated in the experiment after 6 weeks of cultivation: length and fresh weight of the main shoot, number of leaves on main shoot, number of axillary shoots, length and fresh weight of axillary shoots (per explant). The results of the experiment were analyzed statistically using a standard statistical procedure with two factorial design, while the Tukey test was used to estimate the differences between the means at the 5% level of significance.

Results and discussion

The analysis of the conducted study results showed that the concentration of BA had a significant effect on the growth of the main shoot (Tab. 1). Shoot tips cultivated on the control medium or in presence of BA in the lowest concentration characterized with the highest elongation (36.5 - 40.9 mm). An inhibition of the main shoot growth was observed at higher concentrations of BA. Analysing a number of leaves per shoot, it was observed that the control medium and the media with low level of BA had the most beneficial effect on this feature. The fresh weight of the shoots was the highest on the control medium and in presence of BA in the highest concentration used.

Table 1 Effect of BA on growth and development of *Cosmos atrosanguineus* shoot tips after 6 weeks of *in vitro* culture

BA ($mg \cdot dm^{-3}$)	Main shoot length (mm)	Number of leaves	Main shoot fresh weight (mg)
0	40.9 a*	10.1 ab	84.6 a
0.2	36.5 a	9.3 ab	56.6 b
1.0	23.2 b	8.8 bc	51.0 b
2.5	28.5 b	7.6 c	41.2 b
5.0	29.7 b	8.5 bc	82.4 a
Mean	**31.8**	**8.9**	**63.2**

*Values in vertical columns followed by the same letter do not differ significantly at P = 0.05

The significant effect of the presence of BA in the culture medium on the regeneration of axillary shoots from shoot tips was noted (Tab. 2). It was observed that the number of axillary shoots increased strongly together with the increase of BA till the concentration of 1 mg·dm^{-3} (from 1.2 to 6.0). Further increase in BA concentration (2.5 - 5 mg·dm^{-3}) did not influence the axillary shoot induction significantly. Similar trends were observed in case of the length of shoots. There were significant differences in the fresh weight of axillary shoots, depending on the BA concentration. In presence of 5 mg BA·dm^{-3}, the fresh weight of shoots/explant was 8 times larger in comparison to the control.

Table 2 Effect of BA on growth and development of axillary shoots formed on *Cosmos atrosanguineus* shoot tips after 6 weeks of *in vitro* culture

BA (mg·dm^{-3})	Number of axillary shoots	Length of axillary shoots (mm)	Fresh weight of axillary shoots per explant (mg)
0	1.2 b*	3.4 c	21.4 c
0.2	1.8 b	8.9 b	19.3 c
1.0	6.0 a	17.5 a	88.9 b
2.5	5.1 a	12.1 ab	78.9 b
5.0	5.5 a	15.3 a	172.5 a
Mean	**3.9**	**11.4**	**76.2**

*Values in vertical columns followed by the same letter do not differ significantly at P = 0.05

The analysis of axillary shoots induction from nodal segments of stems demonstrated significant differences in shoot number, depending on concentration of BA (Tab. 3). The number of axillary shoots increased with the increasing BA concentration. On the control medium only 1.5 axillary shoots were obtained. The addition of BA in the highest concentration (5 mg·dm^{-3}) to the medium increased the number of axillary shoots by more than 6 times (9.2). It was found that the presence of BA in the medium had inhibitory effect on axillary shoots elongation but at the same time it was favourable for fresh weight of shoots.

Table 3 Effect of BA on growth and development of axillary shoots formed on *Cosmos atrosanguineus* nodes after 6 weeks of *in vitro* culture

BA (mg·dm^{-3})	Number of axillary shoots per explant	Length of axillary shoots (mm)	Fresh weight of axillary shoots per explant (mg)
0	1.5 c*	25.2 a	71.2 c
0.2	3.6 c	17.9 b	74.3 c
1.0	6.3 b	13.7 b	101.8 c
2.5	6.4 b	12.5 b	139.4 b
5.0	9.2 a	12.6 b	301.0 a
Mean	**5.4**	**16.4**	**137.5**

*Values in vertical columns followed by the same letter do not differ significantly at P = 0.05

Analysing the number and growth of axillary shoots formed on two types of *Cosmos atrosanguineus* explants, it was found that more shoots which characterized with the strong elongation growth, were obtained from nodes. Aseptic cultures of many plants from Asteraceae family were established with the use of: shoot tips (Salazar et al., 2005; Anantasaran and Kanchanapoom, 2008; Turker et al., 2009; Waseem et al., 2009; Surmacz-

Magdziak and Sugier, 2012; Kozak et al., 2013) or nodes (Hosoki et al., 2003; Murch et al., 2003; Chan et al., 2009; Sujatha and Kumari, 2008; Leal et al., 2009; Nencheva, 2010). On medium supplemented with BA in concentration of 1 - 5 $mg \cdot dm^{-3}$ the best shoot proliferation of *Cosmos atrosanguineus* from both types of explants was obtained. Although BA at concentration of 5 $mg \cdot dm^{-3}$ gave high response (5.5/shoot tip explant, 9.2/node explant), the use of this treatment resulted in light green leaves. Hence, it would be preferable to use lover level of BA. BA has been often used to induce shoots *in vitro* from explants of many species from the Asteraceae family. The proliferation of *Cosmos atrosanguineus* (Hosoki et al., 2003), *Eclipta alba* (Husain and Anis, 2006), *Inula racemosa* (Kaur et al., 2010), *Rhaponticoides mycalea* (Emeg and Ergad, 2013), *Saussurea esthonica* (Gailite et al., 2010) was stimulated by the application of BA alone at the concentrations of 0.2-1 $mg \cdot dm^{-3}$. In other experiments, shoot proliferation increased on media containing BA jointly with a small quantity of an auxin. The shoots of *Arnica montana* (Lele, 1998), *Gerbera jamesonii* (Cardoso and da Silva, 2012), *Gynura procumbens* (Keng et al., 2009), *Stevia rebaudiana* (Taleie et al., 2012; Zayova et al., 2013), branched better on the media containing BA in concentrations of 0.5 - 2 and 0.01-0.5 $mg \cdot dm^{-3}$ NAA. The elongation of *Cosmos atrosanguineus* shoots was inhibited by BA, especially when it was added to the medium in higher concentrations. Similar observations were found in studies on *Carlina* (Trejgell et al., 2009; Trejgel and Tretyn, 2011), *Eclipta alba* (Bascaran and Jayabalan, 2005), *Leontopodium alpinum* (Trejgell et al., 2010) and *Saussurea obvallata* (Joshi and Dhar, 2003).

Conclusions

1. Shoot tips and nodal parts of shoots are suitable as a secondary explants for multiplication stage of *Cosmos atrosanguineus*.
2. MS medium supplemented with BA in concentration of 1-2.5 $mg \cdot dm^{-3}$ is the best for shoot proliferation from shoot tips and nodes as a source of explants.
3. Elongation growth of axillary shoots induced from nodes is the best on the control medium, but shoots obtained from shoot tips are longest in presence of 1 $mg \cdot dm^{-3}$ BA.

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The effect of stimulators and plant density on the yield quality of broad bean (*Vicia faba* var. *major*)

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Abstract
The research was established in the Kraków region in years 2014-2015. The investigated factors were stimulators (Goëmar BM 86 i BioVigor) and plant spacing (45×15, 45×20 and 45×25 cm). Seeds of broad bean (cv. 'Figaro F_1') were sown in the end of March, directly to the ground. Spraying plants with stimulators were done three times, during the plant blooming. Seeds in the phase of harvest maturity were gathered in the beginning of July. Marketable yield of broad bean ranged from 8.12 to 12.47 t·ha^{-1} in 2014 and from 11.44 to 14.61 t·ha^{-1} in 2015. The positive effect on the marketable yield and content of dry matter, total sugars and ascorbic acid in seeds, to a greater extent, had density of plants than stimulators.

Key words: faba bean, plant spacing, seaweed preparations, nutrient value

Introduction

In many parts of the world *Vicia faba* belongs to the plants of great economic importance. More than 50% of global cultivation area of the plant is located in China (Lang et al., 1989). In the Mediterranean area (nearly 25% harvested area of *Vicia faba* on the global scale), considered to be the center of its origin, region with the largest production of broad beans is North Africa. The average area of cultivation ranges there from 23000 to 73000 ha (Aouar-Sadli et al., 2008). In Europe, the major producers of broad bean (considered as a vegetable) are Spain and Italy, and then the United Kingdom, France and Germany. In Poland this vegetable is grown on a relatively small scale. Larger plantations are located in the fertile soils in the Lublin region and the southern part of Poland. Immature green seeds are used for direct consumption and as raw material for refrigeration and preservation. In 2013 the cultivated area of broad bean was estimated at 2463 ha, which accounted for 6.2% of the total area of leguminous crops. The total production of its immature seeds amounted to 5.8 thousand tons (Łączyński et al., 2014a, b). From a practical point of view the most important disadvantage of leguminous plants is their instability in terms of the yield. The production capacity of this group of plants is very large, but they use only 20-30% of their biological potential. Therefore an important issue is searching for methods of breeding and agronomic methods allowing increase this potential (Prusiński and Borowska, 2002). Among latest is the use of preparations influencing the physiological processes of plants (Attiya et al., 1983; Przybysz et al., 2010; Kavipriya et al., 2011; Abbas, 2013; Amin et al., 2014; Halpern et al., 2015). Currently seaweed extracts are becoming increasingly popular, especially in organic farming, due to their possibility to increase growth, **c**hlorophyll levels, flowering, yield, and seed germination of different plants (Zodape et al. 2008; Zodape et al. 2010; Kavipriya et al., 2011; Zodape et. al. 2011; Pramanick et al. 2013; Abd El-Gawad and Osman 2014), including broad bean (El-Naggar at al. 2005; Salah El Din Abbas 2013). According to Abbas (2013) algae extract as a new bio-fertilizer containing N, P, K, Ca, Mg, and S as well as Zn, Fe, Mn, Cu, Mo, and Co, some growth regulators, polyamines, natural enzymes carbohydrates, proteins and vitamins can be applied to improve vegetative growth and yield. He also concluded that, the application of the biostimulators such as algal extract in small quantities

has been effected on several metabolic processes, enhances plant growth and development via the increasing of photosynthesis, endogenous hormones and ion uptake. They also enhance plant protection against pathogens and pests (Zodape et al. 2011; Baloch et al. 2013). Liquefied seaweed extracts are usually manufactured from *Ascophyllum nodosum*, brown seaweed that is commonly found in the North Atlantic (Halpern et al., 2015). Amin et al. (2014) suggest possibility of increasing production efficiency and nutritive value of faba bean seeds, by spraying plants with different concentrations of amino acid.
Increasing the yield and its quality can be also achieved by controlling the density of cultivation. It has long been known that the higher density of the plants within certain limits, increases yield, which does not always entail an increase in its quality. Growing in different spacing can affect climate conditions for plantations, causing an increase or decrease the seed yield and the level of certain nutrients (Graf and Rowland, 1987; Coelho and Pinto, 1989; Al-Rifaee et al., 2004; Lopez-Bellido et al,. 2005; Matthews et al., 2008; Khalil at. al., 2010; Bakry et al., 2011; Abou-Amer et al., 2014; Amany, 2014).
The aim of this research was estimating the influence of three plant densities and two plant stimulators on the quantity and quality of the bean yield in the spring cultivation.

Material and methods

The research in the Kraków region was done in years 2014-2015 on the soil describe as the typical brown. Two-factor experiment was established as a split-block with four replications. The investigated factors were: stimulators Goëmar BM 86® [BM] i BioVigor® [BV], in comparison with control which was water [C], and plant spacing: 45×15, 45×20 and 45×25 cm (14.8; 11.1 and 8.9 plants per 1 m^2, respectively). The results of the experiment were statistically analyzed by means of the analysis of variance. Significance of differences was determined by the Tukey test at the significance level of $\alpha = 0.05$.
Preparations used in the experiment are defined as blooming activators containing biologically active GA filtrate 142 obtained from the seaweed *Ascophyllum nodosum*. Important elements in Goëmar BM 86 are boron (2.03%), molybdenum (0.02%) and magnesium (4.8%), while in BioVigor - boron (0.3%) and zinc (1.7%).
Seeds of broad bean (cv. 'Figaro F_1') were sown in the end of March, directly to the ground. Spraying with stimulators and water were done three times, during the plant blooming (from end of May to half of June). Seeds at the phase of harvest maturity (immature, green) were harvested in the beginning of July (on 7 and 6 of July in 2014 and 2015, respectively). Representative samples of seeds were analyzed for the content of dry matter, L-ascorbic acid and total sugars.
Meteorological conditions during the growing season are presented in table 1. The average temperature of each month was similar to the multi-year average in both years, but in terms of precipitation more favorable conditions prevailed in 2015.

Table 1 Monthly mean air temperature and sum of precipitation [mm] in the vegetation period of broad bean in 2014 and 2015 against average data from the years 1981-2010

Years	Month							
	IV	V	VI	VII	IV	V	VI	VII
	Temperature [°C]				Precipitation [mm]			
2014	10.3	13.8	16.2	20.2	32.2	10.2	6.2	9.8
2015	8.7	13.0	17.5	20.6	42.2	103.9	35.8	42.4
1981–2010	8.7	14.0	16.8	18.8	46.2	81.4	86.8	87.9

Results and discussion

Marketable yield of broad bean ranged from 8.12 to 12.47 t·ha^{-1} in 2014 and from 11.44 to 14.61 t·ha^{-1} in 2015 (tab. 2) which is significant higher than an average yield in Poland reported on the level of 2.34 t·ha^{-1} by Łączyński et al. (2014a, b).

Table 2 Marketable yield [t·ha^{-1}] in 2014 and 2015 depending on stimulators and plant spacing

Stimulator	2014			2015			Mean	
	Spacing in rows [cm]			Spacing in rows [cm]				
	15	20	25	15	20	25	2014	2015
C	13.01 a	11.65 a	8.12 a	11.78 a	12.39 a	11.88 a	10.93 A	12.02 A
BM	12.33 a	12.47 a	8.21 a	13.35 a	11.44 a	11.41 a	11.00 A	12.07 A
BV	10.56 a	10.72 a	9.79 a	14.61 a	13.78 a	13.64 a	10.36 A	14.01 B
Mean	11.96 B	11.61 B	8.71 A	13.4 A	12.54 A	12.31 A	-	-

In 2014, irrespective of plant spacing, investigated preparations had no significant influence on its level in comparison to control; while in 2015 the highest yield (14.01 t·ha^{-1}) was obtained in case of BioVigor. Irrespective of the stimulators, the highest yield in 2014 was obtained from plots containing 14.8 and 11.1 plants per 1 m^2, while in the next year there were no significant differences between different spacing, however the tendency of yield decreasing was the same as in previous year. This tendency is in agreement with the results achieved by Khalil et al (2010) who reported that grain yield increased linearly with increase in plant density and reached the highest level of 2498 kg·ha^{-1} at density of 450,000 plants·ha^{-1}, and the lowest at density of 150,000 plants per 1 ha. According to Lopez-Bellido at al. (2005) most studies dealing with plant density in faba beans use minor or small-seeded varieties, but large-seeded cultivars are known to develop greater plant cover and require lower plant densities to achieve high grain yields. Further research on plant density should, therefore, be broadened to include cultivars with different seed sizes. Al-Rifaee at al. (2004) stated that the plant population of 12.5 plants·m^{-2} was found to be optimum in the second growing season, whereas 25 plants·m^{-2} was considered the proper population where chilling injury and drought were prevailing. Due to unpredictable environmental conditions a planting density of 25 plants per 1 m^2 should be used. Further increase of population will have no economical return on the produced yield In the investigation of Khamooshi et al. (2012) one of experimental factors was plant density (25×25 cm, 35×35 cm and 45×45 cm), and the highest seed yield was obtained in 25 × 25 cm plant density. According to Amany (2014) using of 25 plants·m^{-2} versus 33 plants increased seed yield. Bakry et al. (2011) investigated five faba bean cultivars which were seeded in rows spaced from each other by 20, 40 and 60 cm (42, 25 and 16 plants·m^{-2}); all cultivars produced the highest seed yield at the highest plant density. Plants treated with stimulators gave the yield on the same level as in the control in 2014 and on the higher level in the next year (not significantly in case of Goëmar BM 86 and significantly in case of BioVigor), what is partly consistent with results obtained by other authors. The data obtained in the works of Salah El Din et al. (2008) and Abbas (2013) show that the yield of faba bean was distinctly increased by seaweed extracts used in the form of foliar application. In the experiment conducted by El-Naggar et al. (2005) algal extracts increased the weight of seeds over the control by 1 to 5 fold. According to results obtained by Zodape et al. (2010) green gram (or mung bean) treated with seaweed extracts gave yield by 30.11% and according to Pramanick et al. (2013) - from 13.8 to 33.58% higher than control plants. Taking into account other vegetables crops one should say that in many cases seaweed extracts increased their yields. Abd El-Gawad and Osman (2014) reported that foliar application of 1000 and 2000 ppm seaweed extract increased the yield of eggplant in comparison with control by 27 to 23%, respectively. In the experiment of Zodape et al. (2008), compared to control, okra

sprayed with 2.5% extract of *Kappaphycus alvarezii* showed a significant increase in fruit yield by 20.47%, and in the another work conducted by this author number of tomato fruit per plant has increased by 75.11% for the plants receiving foliar application of 5.0% extract (Zodape et al. 2011), while in the experiment by Gajc-Wolska et al. (2010) positive influence of Goëmar BM 86 on the yield of tomato was not found. Although in this experiment the influence of used stimulants on the yield of broad bean was different in each year, however taking into account the results of other authors one can conclude that in the vast majority of cases, spraying plant with extracts derived from seaweed leads to an increase of the yield of vegetables.

Table 3 Dry matter content [%] in seeds depending on stimulators and plant spacing in 2014 and 2015

Stimulator	2014			2015			Mean	
	Spacing in rows [cm]			Spacing in rows [cm]				
	15	20	25	15	20	25	2014	2015
C	20.37 a-b	20.45 a-b	21.41 b	25.03 b-c	24.21 b	25.70 d	20.74 B	24.98 B
BM	20.11 a-b	20.51 a-b	20.98 b	25.44 d	21.91 a	24.0 b-c	20.53 B	24.08 A
BV	19.74 a	19.92 a	20.95 b	25.23 c	24.45 b-c	24.57 b-c	20.09 A	24.77 B
Mean	20.07 A	20.26 A	21.13 B	25.23B	23.64 A	25.11 B	-	-

Generally, dry matter content in seeds was greater in 2015 (from 21.91 to 25.70%) than in 2014 (tab. 3). In 2014 dry matter content in control seeds was on the same level as from plants treated with Goëmar BM 86 and in 2015 - from plants sprayed with BioVigor. In both years seeds obtained from plants growing at the lowest density (8.9 plants·m^{-2}) contained the highest dry matter (in 2015 at the same level as at spacing of 15 cm).
The content of sugars in seeds was comparably in both years (from 1.79 to 2.79% in 2014 and from 1.12 to 2.93% in 2015) - tab. 4. In both years Goëmar BM 86 had significant positive influence on its content (as well BioVigor in 2015). In the experiments conducted by El-Sheekh and El-Saled (2000) and El-Naggar et al. (2005) after the application of algae extract, an increase the content of soluble sugars in the leaves and pods of broad beans was observed. Extracts from *Kappaphycus alvarezii* caused increasing the content of carbohydrates by 3,4% in seeds of green plants compared with control (Zodape et al. 2010). Abbas (2013) showed that treatments with algal extract caused significant increase in total carbohydrate contents in faba bean shoots as compared with control.
Irrespective of stimulators in both years the lowest content of sugar was noticed in seeds obtained from plants growing at the lowest density (2.07% and 1.17%, respectively), however only in 2015 the difference was significant in comparison to two others densities.

Table 4 Total sugars content [%] in seeds depending on stimulators and plant spacing in 2014 and 2015

Stimulator	2014			2015			Mean	
	Spacing in rows [cm]			Spacing in rows [cm]				
	15	20	25	15	20	25	2014	2015
C	2.79 c	2.03 a	2.10 a-b	2.93 d	1.34 a-b	1.15 a-b	2.30 B	1.81 A
BM	1.84 a	2.71 b-c	2.13 a-b	2.61 d	2.38 a-b	1.24 a-b	2.23 B	2.08 B
BV	1.79 a	2.03 a	1.98 a	2.68 d	2.26 a	1.12 a	1.93 A	2.02 B
Mean	2.14 A	2.25 A	2.07 A	2.74 C	1.99 B	1.17 A	-	-

In both years content of ascorbic acid was higher in seeds obtained from plant sowing at lower densities, however only in 2015 the difference was significant (tab. 5). Bakry et al. (2011) stated that two of investigated cultivars of faba bean in their experiment gave high biological yield, when they seeded at the highest density. Irrespective of plant density more ascorbic acid gained seeds from plants treated with both stimulators (5.30 and 5.35 mg·100 g^{-1} f. m) and in 2015 from control plants (6.60 mg). Zodape et al. found a significant higher content of vitamin C in fruits of okra (2008) and tomato (2011) in plants sprayed with seaweed extracts.

Table 5 L-ascorbic acid content [mg·100 g^{-1} f. m] in seeds depending on stimulators and plant spacing in 2014 and 2015

Stimulator	2014			2015			Mean	
	Spacing in rows [cm]			Spacing in rows [cm]				
	15	20	25	15	20	25	2014	2015
C	5.05 a	5.05 a	5.22 a-c	5.00 a-b	7.29 d	7.50 d	5.11 A	6.60 B
BM	5.25 a-c	5.22 a-c	5.44 b-c	4.57 a-b	6.57 c	4.43 a-b	5.30 B	5.19 A
BV	5.30 a-c	5.69 c	5.05 b-c	4.14 a	4.71 a-b	6.50 c	5.35 B	5.12 A
Mean	5.20 A	5.32 A	5.24 A	4.57 A	6.19 B	6.14 B	-	-

Conclusions

1. The positive effect on the marketable yield and content of dry matter, total sugars and ascorbic acid in seeds of broad bean cv. 'Figaro F_1', to a greater extent, had density of plants than stimulators.
2. Considering the investigated stimulators only BioVigor in 2015 had a significant effect on increasing the marketable yield.
3. Irrespective of stimulators the highest marketable yield was achieved from plant growing at the highest density (14.8 plants per 1 m^2).
4. Considering used stimulators and both years of the experiment more positive influence on the content of dry matter, sugars and ascorbic acid had Goëmar BM 86.
5. Irrespective of investigated stimulators the highest content of dry matter and ascorbic acid in seeds was achieved from plots of the lowest density (8.9 plants per 1 m^2), while more sugars - in seeds from plants growing at the highest density.

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In vitro polyploidization of a hardy terrestrial orchid, *Bletilla striata* (Thunb.) Rchb. f.

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Abstract
Effect of antimitotic agents, colchicine (1.2 g L^{-1}) and trifluralin (10 mg L^{-1}) were evaluated with different exposure time (1, 3, 5 days) combined with different solvents (dimethyl sulfoxide and acetone) on *Bletilla striata in vitro* culture to induce polyploidization. After *in vitro* seed sowing, 6 weeks old protocorms were treated and 9 months after the treatments the plants were investigated. To assess the ploidy level of the specimens the stomatal guard cell length was measured with light microscope using phase contrast after creating replicas from the epidermal surface of the plants.
Colchicine treatment of 3 or 5 days resulted in high number of plants identified as polyploids, at the same time the mortality of plants after these treatments was high as well. The survival rate after trifluralin treatments was 100 % but polyploidy was induced at a lower proportion of plants compared to the colchicine treatment. No difference was observed between the effects of different type of solvents used during the treatments.

Keywords: polyploid induction, orchid, ornamental plant, trifluralin, colchicine

Introduction

More than two complete set of chromosomes in a cell nucleus, i.e. polyploidy plays a significant role in evolutional processes within the plant kingdom and it also has a high impact on horticultural production, since numerous of our cultivated plants are polyploid. In a horticultural aspect, polyploid plants possess more favourable features than diploid ones: in general they have more vigorous and more robust habit, thicker, stronger stem, larger leaves, flowers, fruits, longer flowering, and more intense colours. Since chromosome set multiplication does not occur naturally in all plant genera, plant breeders use artificial methods in practice for induction of polyploidy. The cell cycle can be disturbed by many chemicals but only those are suitable for polyploid induction which influence the cycle after synthesis phase but before cytokinesis. Most of the anti-mitotic compounds are inhibiting the metaphase. In practice the most wide-spread mitotic inhibitor is colchicine, an alkaloid type compound extracted from the seeds and corms of *Colchicum autumnale* (Eigsti and Dustin, 1955). Colchicine is used since the 1930's to induce polyploidy in plants. An advantage of this compound is its heat stability – thus sterile solution of colchicine can be prepared by autoclaving (Zhang et al., 2007). However, there are some disadvantages: side-effects like sterility, abnormal growth, loss of chromosomes, gene mutation can also occur in some cases (Luckett, 1989). In addition it is also known that colchicines has higher affinity to animal microtubuli than to those of plants, therefore higher concentration is required by plants to induce the required effect, and this also increases the rate of side-effects as well (Morejohn et al., 1984). To lower the occurrence of these undesirable effects during polyploidy induction, there is a need for searching for new compounds. Since approximately 25 % of all herbicide agents are mitotic disruptors, they could be potentially applied in polyploidy induction as well. Besides, herbicides have a greater affinity to plant microtubuli than colchicine, they can

be used in much lower concentration and their toxicity to humans is also significantly less, therefore these compounds can be seen as superior alternatives to colchicine (Bayer and Molé-Bajer, 1986; Hugdahl and Morejohn, 1993; Morejohn et al., 1987). Among antimicrotubular herbicides especially dinitroanilines, like trifluralin and oryzaline, became the general substitute for colchicine (Ramulu et al., 1991).

Bletilla striata (Thunb.) Rchb. f. is a 18–60 cm high terrestrial orchid from the subtropical and warm temperate region of Central and South China, Myanmar, Central and South Japan, Southwest Korea where it lives in evergreen forests and grasslands (Chen et al., 2009; Chung et al., 2013). Its inflorescence contains 3–10 purplish-red or pink, sometimes white flowers which are quite large compared to other terrestrial species. Flowering time is April–May. *Bletilla striata* is a self-pollinating species, it frequently develops clonal patches in which the rhizomes are connected to each other. Capsules are approx. 3.5 cm large and contain a lot of small seeds (Chung and Chung 2005; Sugiura, 1995). This species is very decorative, it has a high potential as an ornamental garden plant.

Several methods are available to determine the ploidy level of a plant. Measuring the stomatal guard cell length is a fast and cost-efficient way to estimate the ploidy level of a specimen in the L1 layer of meristem, since there is a high correlation between these parameters. However, this method itself is not suitable to determine the ploidy level of a plant exactly, rather it is proper way to screen the population for possible polyploid specimens. This method is already successfully applied in orchid species to detect induced polyploidy (Miguel et Leonhardt, 2006). Counting the chromosome number or cell nucleus content estimation with flow cytometry has been considered to most accurate methods to detect polyploids (Sattler et al., 2016). In this research our aim was to induce polyploidy in *Bletilla striata* to get tetraploid genotypes for breeding purposes.

Materials and methods

For the experiment seeds were harvested from a mother plant maintained in a personal collection. The seeds were stored refrigerated at 4 °C for one year long before the research started. The seeds remained viable, kept their ability to germinate. Seeds were surface-sterilized before sowing: first they were dipped in 70 % (V/V) ethanol for 1 minute then they were washed in 5 g L^{-1} sodium dichloroisocyanurate solution supplemented with few drops of Tween 80 for 15 minutes using a magnetic stirrer followed by rinsing is sterile distilled water 3 times in a laminar airflow bench. The sterilized seeds were sown in 100 ml Erlenmeyer flasks filled with 40 ml FAST orchid medium (Fast, 1980) solidified with 6 g L^{-1} plant agar.

The cultures were maintained in a laboratory growing room where 16/8 hours day/night cycle, 3000 lx light intensity and 22 ± 2 °C temperature was provided for them.

Mitosis inhibitor treatments were conducted at 6 weeks after sowing, at the end of the protocorm stage (3–5 mm elongated plantlets with a visible differentiating shoot). The treatment solutions contained 1.2 g L^{-1} (3000 μM) colchicine with 0.1 % (V/V) dimethyl sulfoxide (colchicine was dissolved first in DMSO) or 10 mg L^{-1} (30 μM) trifluralin. Since keeping trifluralin dissolved in water based solution is very difficult, two kind of solvent were tried: 0.1 % (V/V) DMSO or 1.2 % (V/V) acetone. For the sterilization of colchicine solution an autoclave (121 °C, 35 min) was used, trifluralin solutions were filter sterilized using a 0.22 μm pore sized membrane filter. In each treatment 20 ml of the respective solution were layered on the surface of the solid media, so all of the protocorms were immersed in the liquid. Treatment vessels (3 pieces in each group with hundreds of seedlings) were placed on rotary shaker for 1, 3 or 5 days, so 9 treatment groups were created. After decanting the solutions the seeds were washed with distilled water and passed on fresh FAST medium, 5-10 plantlets per a 100 ml vessels. 30, 60, 90 days after the treatments the mortality of the treated plants were assessed, and later each living plant was placed into fresh medium in a 50 ml

volume vessel individually. The media was changed after 120 days again. 9 months after the treatments the plants were large enough to evaluate their ploidy level using stomatal guard cell morphology analysis. For the creation of epidermal surface replica a colourless nitrocellulose-based varnish was applied to the middle section of the abaxial surface of the second leaf from the shoot tip. After the drying time (3–5 min) the solidified varnish layer could be removed from the leaves and placed on microscope slides. The slides were examined under light microscope with a 40× phase contrast objective (Zeiss AxioLab A1). Digital images were captured and analysed with AxioVision LE 4.8 software. The length of stomatal guard cells was measured in each sample at least in case of 10 stomata, and 10 samples were created at least in each treatment group. Number of stomatal density (per 1 mm^2 surface) was also calculated. Data were evaluated with IBM SPSS Statistics 22 software using analysis of variance combined with Dunnett and Games-Howell tests.

Results and discussion

Lethality caused by the mitotic inhibitor treatments

The plants treated with colchicine showed high mortality rate depending on the exposure time, whereas trifluralin treatments did not cause any mortality. Colchicine treatment for 1 day resulted only 4 % mortality but 3 or 5 days long treatments had much higher ratios: 38.9 % and 62.1 % (Table 1.). After trifluralin treatments the plants continued their growth nearly without intermission while colchicine caused a serious setback in their development: the longer they were exposed the longer was the recovery.

Table 1 Lethality caused by mitotic inhibitor treatments in *in vitro* culture of *Bletilla striata* (DMSO – dimethyl sulfoxide)

Treatments (sample sizes = 50 - 200)			Percentage of destroyed plants (%)		
agent (solvent)	concentration ($mg\ L^{-1}$)	exposition time (day)	30 days after	60 days after	90 days after
colchicine (DMSO)	1200	1	1.4	2.5	4.0
colchicine (DMSO)	1200	3	1.2	26.2	38.9
colchicine (DMSO)	1200	5	0	45.0	62.1
trifluralin (DMSO)	10	1	0	0	0
trifluralin (DMSO)	10	3	0	0	0
trifluralin (DMSO)	10	5	0	0	0
trifluralin (acetone)	10	1	0	0	0
trifluralin (acetone)	10	3	0	0	0
trifluralin (acetone)	10	5	0	0	0

Morphology of stomatal guard cells

Based on the length of stomatal guard cells we can state that colchicine treated plants have significantly larger guard cells compared to non-treated control plants (Figure 1. and 2.) while none of the trifluralin treatments caused any statistical difference in this parameter. Results are supported by the Dunnett-test (Figure 3.). Consequently we propose that colchicine treatments are proper ways of inducing high rate of polyploid plants while the examined trifluralin concentration and exposure time is not optimal compared to control plants although trifluralin also induced some increase in stomatal length. Difference between exposure time by the treatments could not be observed according to Games-Howell test (Figure 4.)

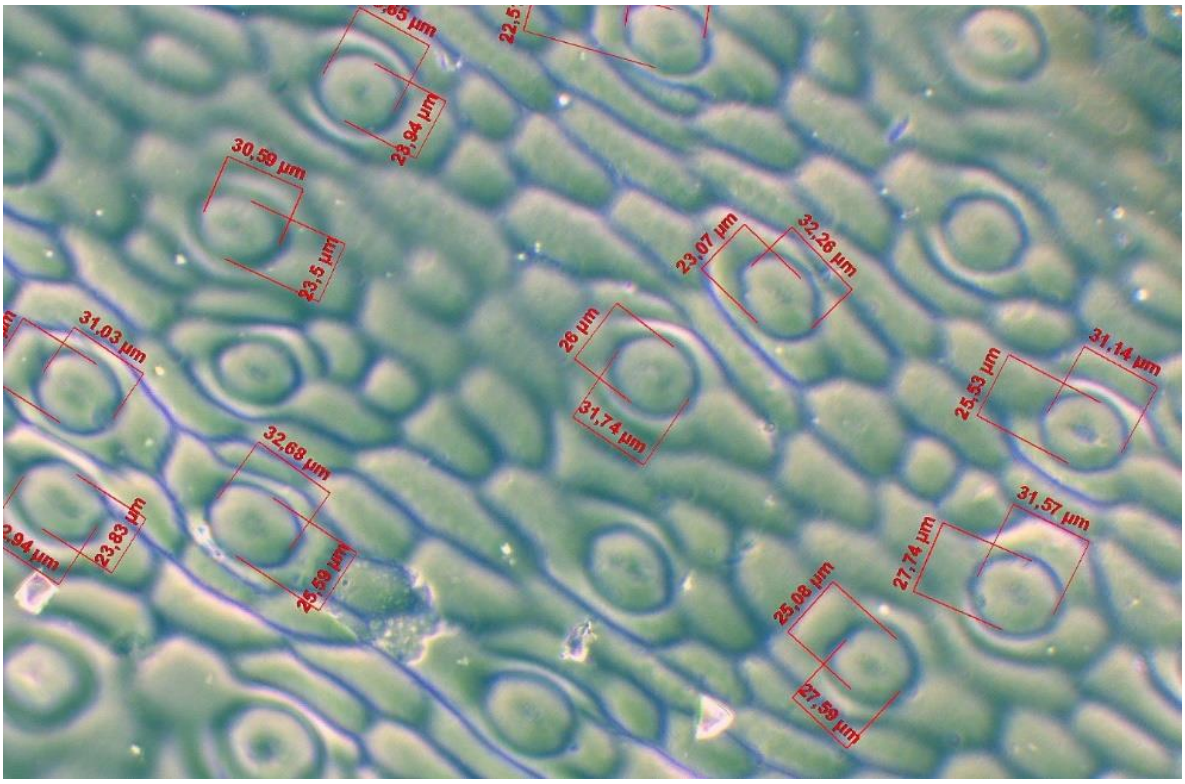

Figure 1 Epidermal replica of a non-treated plant with the dimensions of the stomatal guard cells

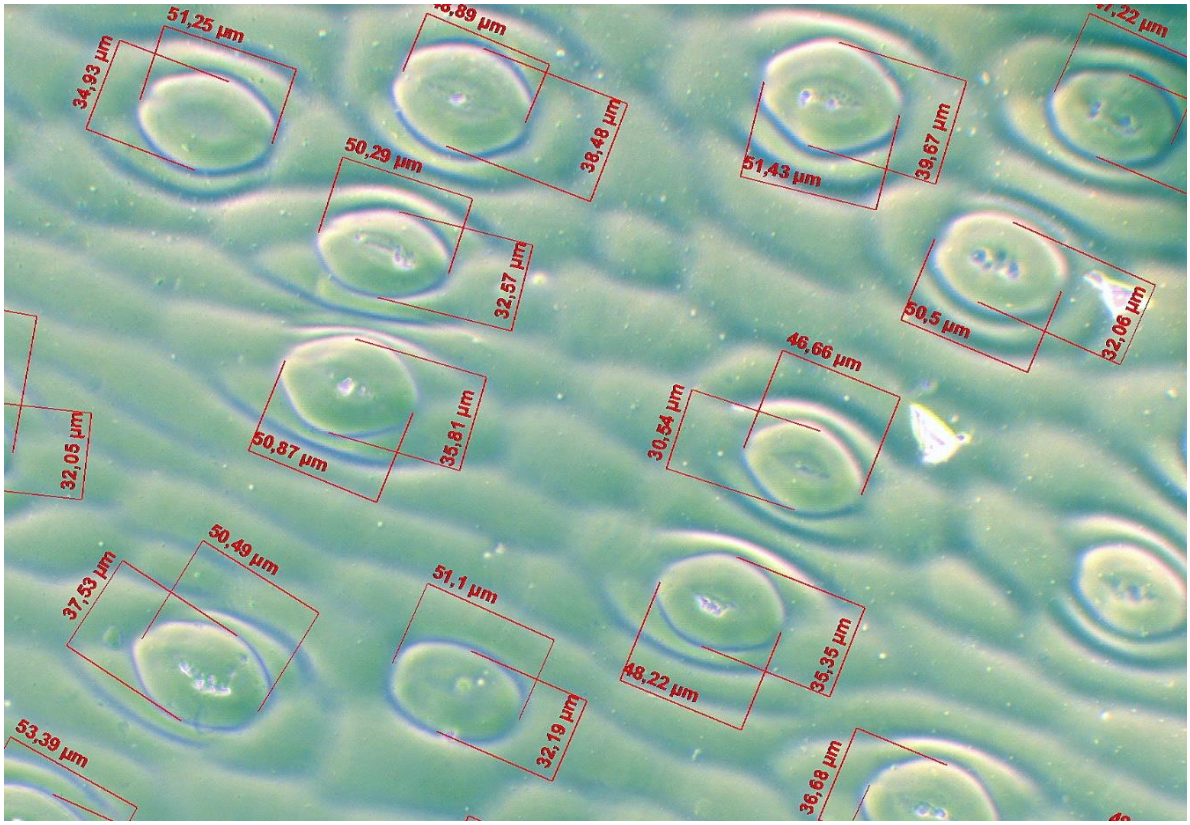

Figure 2 Epidermal replica of a colchicine treated (for 3 days) plant with the dimensions of the stomatal guard cells

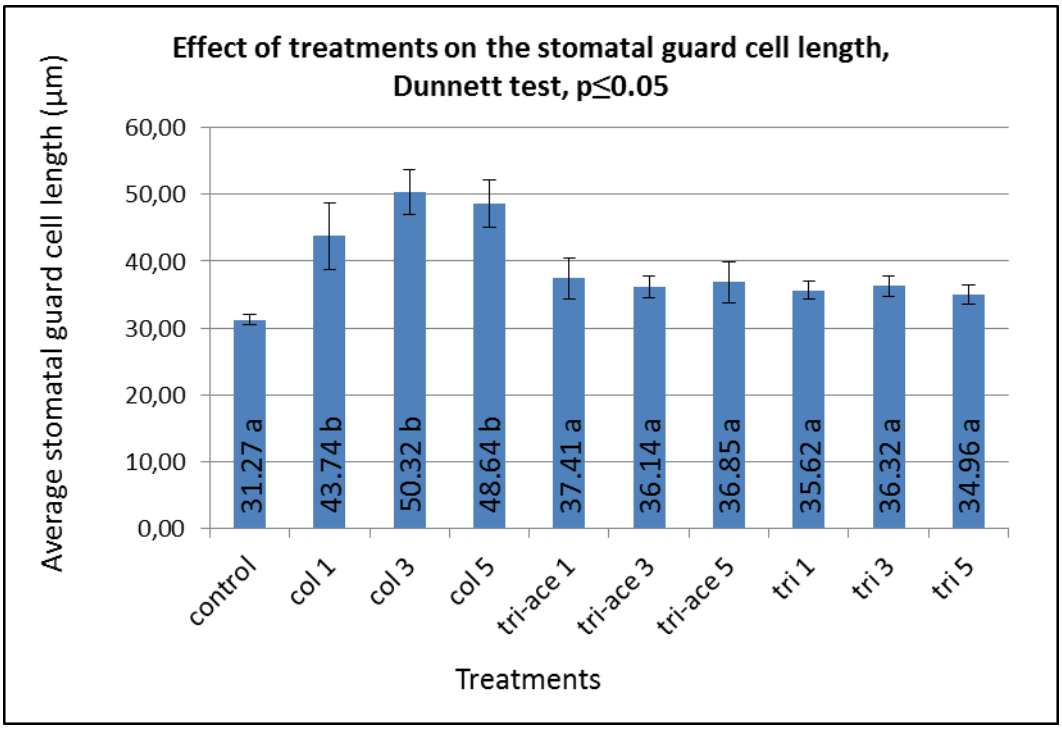

Figure 3 Effect of mitotic inhibitor treatments on the length of stomatal guard cells, comparison to control group (col = colchicine, tri = trifluralin, tri-ace=trifluralin solved in aceton, in the name of treatments the number mean the exposure time in days)

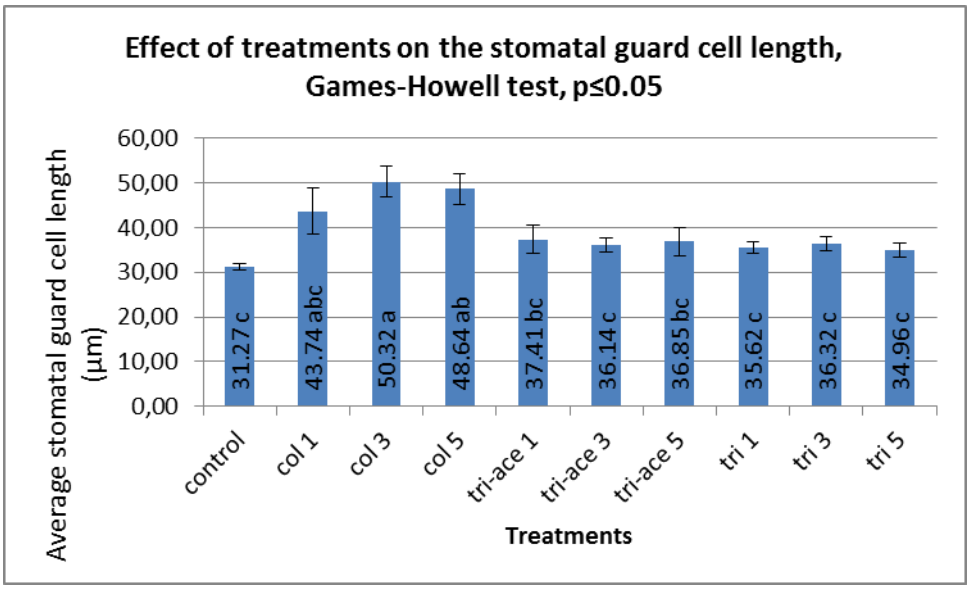

Figure 4 Effect of mitotic inhibitor treatments on the length of stomatal guard cells, comparison between all treatment group (col = colchicine, tri = trifluralin, tri-ace=trifluralin solved in aceton, in the name of treatments the number mean the exposure time in days)

Each plant specimen was assessed individually based upon their average stomatal guard cell length to evaluate ploidy level. To define the size limit for polyploid specimen we used the following relationship: the volume of a cell changes proportionately with the ploidy level. According to the statement of Russell (2004) tetraploid cells have twice the volume of a diploid one, hexaploid cells have thrice the volume, octaploid have 4 times etc. If we consider each of the spatial dimension of a cell equal we can say that one dimension of a tetraploid cell

is 1.26-times larger in average compared to diploid one. Since the average stomatal length of the non-treated plants was 31.3 μm, the size limit for tetraploid plants will be 39.4 μm in our case, and 45.1 μm, 49.8 μm for hexaploid and octaploid plants respectively (with a factor of 1.44 and 1.59). According to the Games-Howell test (Figure 4.) we can state that plants with higher ploidy level than tetraploid are not present in high ratio among the treated plants. Only the colchicine 3 and 5 days treatments have higher average values than the previously defined limits for hexa and octaploid plants, but since the numbers represent average values and they are very near the limit they should be identified only as a polyploid specimen without further classification.
The second table shows the results of the individual evaluation of specimens whether they are diploid or polyploid. The highest polyploid rate (94% and 90%) occurs after colchicine (3 and 5 days) treatments, and trifluralin treatments effected at best 22-27% poliploid rate after 3 or 5 days exposure time.

Table 2 The percentage of polyploid and diploid plants, identification based on the stomatal measurements

Treatments (sample sizes = 10 - 20 in each)			Ploidity level	
antimitotic agent (solvent)	concentration (mg L^{-1})	exposure time (day)	polyploid (%)	diploid (%)
colchicin (DMSO)	1200	1	63	37
colchicin (DMSO)	1200	3	94	6
colchicin (DMSO)	1200	5	90	10
trifluralin (DMSO)	10	1	18	82
trifluralin (DMSO)	10	3	22	78
trifluralin (DMSO)	10	5	27	73
trifluralin (acetone)	10	1	9	91
trifluralin (acetone)	10	3	22	78
trifluralin (acetone)	10	5	17	83

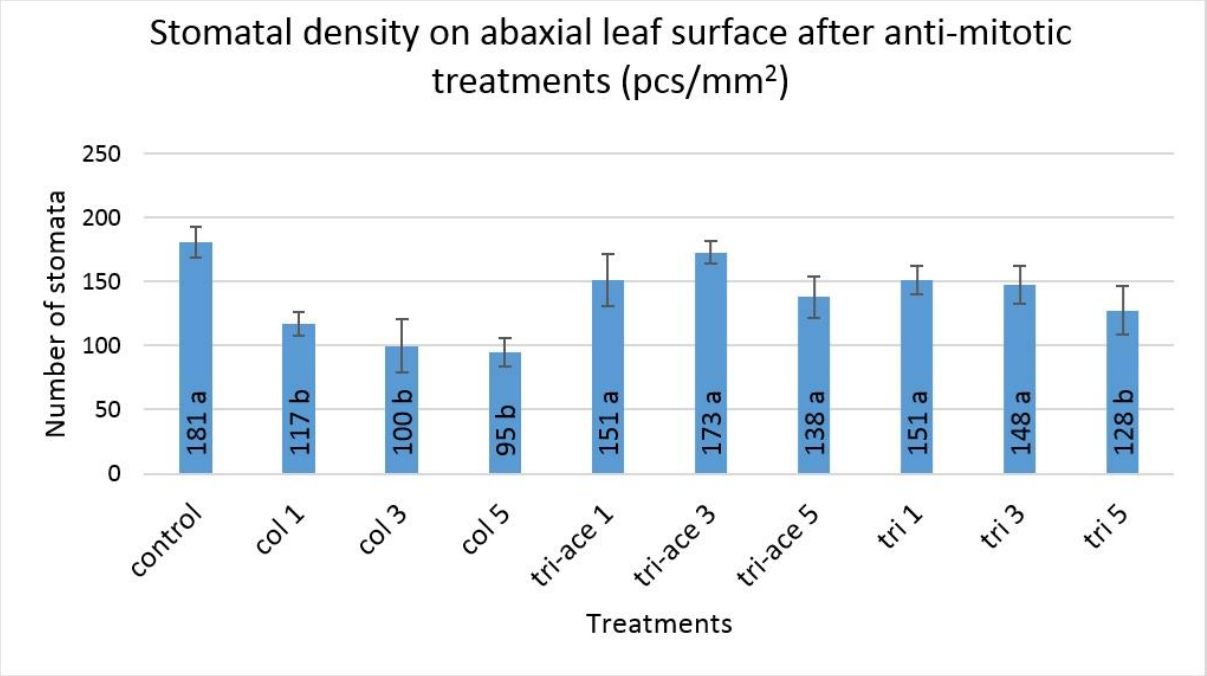

Figure 5 Effect of antimitotic treatments on the stomatal density of abaxial leaf surface of *Bletilla striata*, Dunnett-test $p \leq 0.05$ (col = colchicine, tri = trifluralin, tri-ace=trifluralin solved in aceton, in the name of treatments the number mean the exposure time in days)

Evaluation of stomatal density

The stomatal density of 1 mm^2 surface was also compared after the treatments. The colchicine treatments, in which the highest polyploidy rate was achieved, caused significantly lower stomatal density compared to the non-treated plants. This difference is mainly because of the the larger cell sizes of the polyploid plants, and supports or previous assumption about the efficacy of colchicine treatments.

Macromorphological differences

Although detailed morphological measurement was not made after the treatments, there were visible differences between the plants identified as polyploid and the non-treated or treated but identified as diploid ones. Plants which were identified as polyploid have wider leafblade in general than those of the control plants. The leaves of the control plants show an even narrowing from the leaf base to the leaf tip while the polyploid plants have the widest part in the middle section of the leafblade (Figure 6.). After colchicine treatments many of the plants developed a lot of small shoots around the original plant, trifluralin did not have such effect.

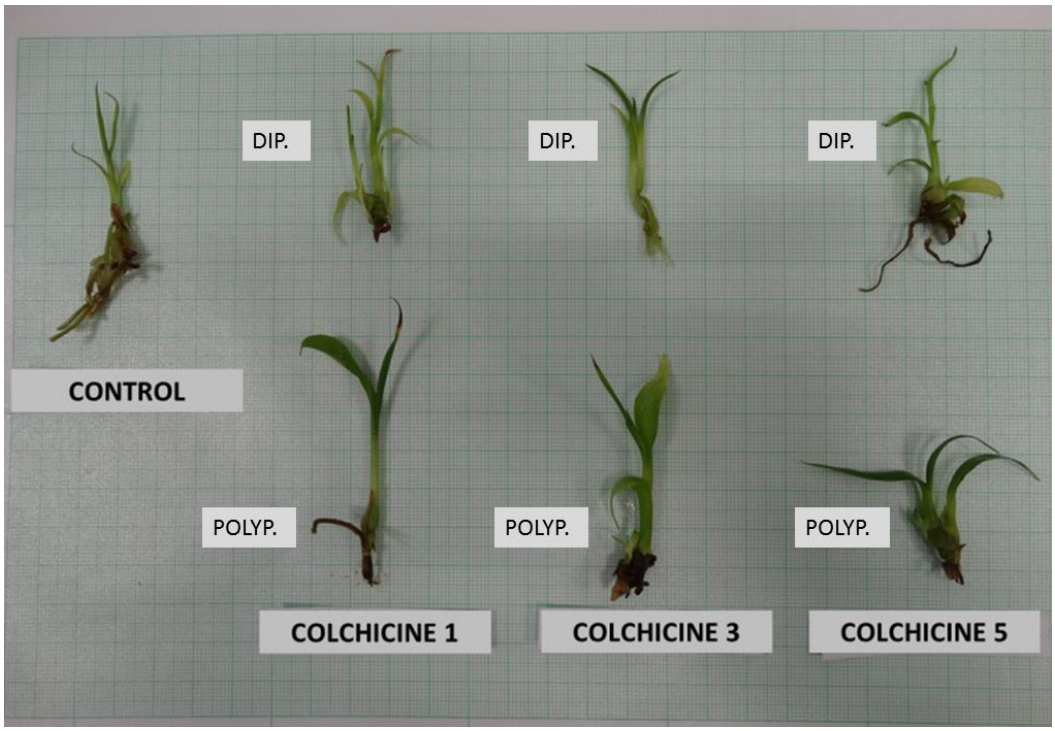

Figure 6 Effect of colchicine treatments on the morphology of the plants. Upper row shows treated plants but not identified as polyploid.

Conclusions

Among the evaluated treatments the 1.2 g L^{-1} (3000 μM) colchicine for 3 or 5 days caused the highest polyploid induction ratio in *Bletilla striata* seedlings, but high mortality rate was also bound to these treatments, especially with the longest exposure time. The development of the survival plants was set back by colchicine. Treatments with trifluralin caused no mortality but the ratio of polyploid plants was also lower than colchicine. Significant differences between the two used solvent (DMSO and acetone) can not be observed. From the treatments evaluated the 3000 μM colchicine for 3 days is recommended to induce polyploidy in in vitro culture of *Bletilla striata*. The evaluation of higher concentration of trifluralin is also suggested based on our results.

To state the exact ploidy level of the plants identified as polyploid requires further examinations. Based upon our assessment of epidermal replicas only the ploidy level of the epidermal layer (L1 layer of meristem) can be certified, however in many cases this method has a very good efficiency to screen the population of possible polyploid plants, it is relatively fast, cheap and reliable. It is applied frequently as a supplemental method to detect higher ploidy level in plants (Miguel and Leonhardt, 2011; Russel, 2004; Zlesak et al., 2005). Further examinations can include chromosome counting or measuring pollen sizes to determine the ploidy level of L3 or L2 layer of meristem, or flow cytometry. Until these examinations our selected genotypes should be regarded only as mixoploids.

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Biological control of parsley (*Petroselinum crispum* var. *tuberosum*)

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Abstract
In two-year field experiments (2014 and 2015) the effect of some biological preparations on health status of parsley leaves on three cultivars: 'Alba', 'Hablange-Berlińska' and 'Kinga' was studied. During investigations: Polyversum WP (*Pythium oligandrum* – 10^6 oospores in 1 g), Trifender WP (*Trichoderma asperellum* – $5x10^8$ spores in 1 g) and RhizoVital 42 (*Bacillus amyloliquefaciens* >$2.5x10^{10}$ CFU/ml) and standard fungicide Topsin M 500 SC (tiophanate methyl – 500 g in 1 l) were used. In 2015 to the experiments a preparation AQ10 (*Ampelomyces quisqualis* strain AQ10 >5.0 x 10^9 spores/g) at dose 50 g/ha was introduced. Unprotected plants presented control.
The standard fungicide Topsin M 500 SC efficiently protects parsley leaves against fungal pathogens. *A. quisqualis* (AQ 10) very effectively restrict the development of *E. heraclei* as well as *Alternaria* spp and *S. petroselini* on the leaves of parsley. *P. oligandrum* (Polyversum WP) effectively protect of parsley cultivars 'Alba' and 'Kinga' against fungal pathogens. *T. asperelleum* (Trifender WP) and *B. amyloliquefaciens* (RhizoVital 42) quite effectively protect most of the cultivars of parsley leaves against pathogens.

Keywords: *Pythium oligandrum, Trichoderma asperellum, Bacillus amyloliquefaciens*, tiophanate methyl, fungal pathogens of leaves

Introduction

Parsley leaves can be infected by a number of fungal pathogens, among which the most important are fungi of the genus *Alternaria*, infested the roots at the end of the growing season and during postharvest period. Infested leaves, under the influence of mycotoxins, are yellowing and dry quickly, which reduces the yield of leaves and roots. Alternaria spp., can also infect seed roots, plants and flowers leading to poor quality of harvested seed, and cause damping-off of seedlings. Also a dangerous pathogen of parsley is *Erysiphe heraclei* - perpetrator of powdery mildew, who damaging the leaves causes a loss of water, especially during hot summer days, which also significantly reduces the yield of parsley. *Septoria petroselini* occurs least frequently, but the occurrence of *Septoria* on the leaves causes their premature yellowing and drying (Davis and Raid, 2002; Koike et al., 2007). Little efficacy of currently registered chemicals to protect parsley makes it necessary to search for new, effective preparations, including biologics. Such preparations do not induce the dangerous phenomenon - compensation pathogens, prevent the formation of resistant populations of fungi, in particular of the genera *Alternaria* or *Fusarium*.
The aim of the study was to determine the effectiveness of the biological preparations for parsley protection against fungal pathogens of leaves.

Material and Methods

Field experiments were carried out in 2014 and 2015 on three cultivars of parsley: 'Alba', 'Hablange-Berlińska' and 'Kinga', at the Mydlniki - Experimental Station of the Agricultural University in Krakow. The following biological plant protection products were applied 4 times during the growing season: Polyversum WP (*Pythium oligandrum* – 10^6 oospores in 1 g) at dose 0,05%, Trifender WP (*Trichoderma asperellum* – $5x10^8$ spores in 1 g) at dose

0,05% and RhizoVital 42 (*Bacillus amyloliquefaciens* >2.5x10^{10} CFU/ml) at dose 0,05%. As the standard fungicide Topsin M 500 SC (tiophanate methyl – 500 g in 1 l) at dose 0,15% was used. In 2015 to the experiments a preparation AQ10 (*Ampelomyces quisqualis* strain AQ10 >5.0 x 10^9 spores/g) at dose 50 g/ha was introduced. Unprotected plants presented control. During the August and September the symptoms on the leaves, was determined with 4 points scale, with 0 - no symptoms, 1 - 5% infestation 2 - 6-25% infestation 3 - infestation of 25-50%, 4 - infestation greater than 51%. From the obtained data infestation indices were calculated (Mazur et al., 2004). The results were subjected to statistical analysis by Duncan's test in two-factor system: cultivar of parsley and the used preparation.

Results and Disscusion

In 2014 powdery mildew appeared on parley leaves and petioles quite early, at the end of July. All the tested preparations were significantly effective in protect all cultivars of parsley against *E. heraclei* compared to the control (Tab. 1).The most effectively limit the development of powdery mildew preparation Topsin M 500 SC cultivars 'Hablange-Berlinska' and 'Kinga', but for 'Alba' the best preparation was Trifender WP. The least effective was RhizoVital 42 for all cultivars of parsley.

Table 1 Health status of parsley leaves, infestation idices of *Erysiphe heraclei* in 2014 [%]

	'Alba' - infestation index [%]	'Hablange-Berlińska' - infestation index [%]	'Kinga' - infestation index [%]	Mean values - infestation index [%]
Polyvesum WP	28,75 f	22,48 de	16,95 bcd	22,54 c
Topsin M 500 SC	18,47 bcd	13,67 ab	9,86 a	13,18 a
Trifender WP	16,32 bc	20,05 cd	20,03 cd	18,77 b
RhizoVital 42	28,71 f	27,42 ef	29,17 f	28,43 d
Control	43,21 g	39,49 g	38,39 g	40,35 e
Mean values	26,59 b	24,13 ab	22,10 a	

Note: means folowed by the same letter do not differ with 5% of significance (Duncan's multiple range test)

Table 2 Health status of parsley leaves, infestation indices of *Alternaria* spp. in 2014

	'Alba' - infestation index [%]	'Hablange-Berlińska' - infestation index [%]	'Kinga' - infestation index [%]	Mean values - infestation index [%]
Polyversum WP	15.30 e	17.36 ef	9.37 cd	13.82 c
Topsin M 500 SC	6.28 abc	5.38 ab	4.08 a	5.21 a
Trifender WP	13.09 de	13.46 de	5.32 ab	10.27 b
RhizoVital 42	5.67 ab	7.72 bc	15.85 e	9.33 b
Control	22.42 fg	14.13 e	15.85 e	19.83 d
Mean values	11.87 a	11.20 a	10.65 a	

In the first year of research preparation Topsin M 500 SC most effectively protects all varieties of parsley leaves against infection by fungi of the genus *Alternaria* (Tab. 2). It was also very effective formulation RhizoVital 42 except 'Kinga' cultivar, because in this combination leaves were infected to the same degree as in the control. Protecting of parsey leaves cv. 'Kinga' was very effective with preparation Trifender WP. Polyversum WP was not effective on cultivar 'Hablange-Berlińska'

In this year was observed only single symptoms of *Septoria* blight on parsley leaves, regardless of the variety, which is not allowed to obtain reliable results.
In 2015 during the whole growing period was little rainfall and high air temperatures, long-term water deficit is not conducive to the development of pathogenic fungi. Only at the end of the growing season, autumn, observed numerous infections and the development of fungal diseases. This year, all preparations applied proved to be effective in protecting the parsley leaves from infections and development of *E. heraclei*, except Polyversum WP for cultivar 'Hablange-Berlin' (Tab. 3). The most effectively limit the development of this pathogen on parsley leaves was fungus *Ampelomyces quisqualis* included in the formulation AQ 10, hyperparasite of perpetrators of powdery mildew. The high efficiency of this fungus in the protection against powdery mildew on grapevines confirms previous publications (Falk et al., 1995). In Romero et al. (2007) study, both *A. quisqualis* and bacterium *Bacillus subtilis* showed high effectiveness, similar to standard chemical product azoxystrobin, in the protection of cucumber against powdery mildew. Also, Gilardi et al. (2008) confirm the very high performance of the two bioagents protect zucchini against powdery mildew, particularly in connection with chemical substances.

Table 3 Health status of parsley leaves - infestation indices of *Erysiphe heraclei* in 2015

	'Kinga'- infestation index [%]	'Hablange-Berlińska'- infestation index [%]	'Alba'- infestation index [%]	Mean values - infestation index [%]
Polyversum WP	7,46 cdef	10,97 fg	10,07 ef	9,44 c
Topsin M 500 SC	4,14 bcde	4,32 bcde	3,88 bcd	4,11 b
Trifender WP	4,72 cdef	6,87 cdef	8,52 def	6,62 bc
RhizoVital 42	5,51 cdef	5,23 cdef	11,01 fg	7.04 c
AQ-10	0,92 ab	0,37 a	2,45 abc	1,10 a
Control	19,71 h	18,63 gh	22,85 h	20,37 d
Mean values	6,07 a	6,53 a	8,84 b	

In 2015 fungicide Topsin M 500 SC most effectively protects parsley leaves, all cultivars, against *Alternaria* spp. (Tab. 4). Formulations RhizoVital 42 and AQ 10 were very effective, but only for cultivars 'Kinga' and 'Alba', in contrast 'Hablange-Berlińska' proved to be totally ineffective as compared to the control. Also effective was the preparation Polyversum WP. Similarly effective was this formulation in Patkowska (2006) study on protection of beans and peas against *Alternaria* spp.

Table 4 Health status of parsley leaves - infestation indices of *Alternaria* spp. in 2015

	'Kinga' - infestation index [%]	'Hablange-Berlińska' - infestation index [%]	'Alba' - infestation index [%]	Mean values - infestation index [%]
Polyversum WP	22,12 abc	24,64 bcd	18,11 ab	21,56 a
Topsin M 500 SC	18,27 ab	22,53 abc	19,87 abc	20,19 a
Trifender WP	19,53 abc	23,61 abcd	18,79 ab	20,60 a
RhizoVital 42	14,92 a	27,12 bcde	24,23 abcd	21,85 a
AQ-10	14,97 a	29,07 cde	22,88 abc	22,03 a
Control	33,42 de	36,66 e	36,93 e	35,66 b
Mean	20,23 a	27,16 b	23,22 a	

Table 5 Health status of parsley leaves infestation indices of *Septoria petroselini* in 2015

	'Kinga' - infestation index [%]	'Hablange-Berlińska' - infestation index [%]	'Alba' - infestation index [%]	'Mean values' - infestation index [%]
Polyversum WP	6.71 abc	5.23 ab	6.26 abc	6.05 ab
Topsin M 500 SC	6.17 abc	4.14 ab	9.78 bc	6.51 ab
Trifender WP	7.99 abc	9.00 bc	9.44 bc	8.80 bc
RhizoVital 42	4.97 ab	5.44 ab	6.38 abc	5.58 a
AQ-10	3.54 a	4.72 ab	5.44 ab	4.53 a
Control	9.69 bc	11.92 c	12.33 c	11.29 c
Mean values	6.37 a	6.50 ab	8.11 a	

In the second year were observed quite a number of symptoms *Septoria* blight on parsley leaves, wherein the cultivars 'Alba' and 'Kinga' only applicable AQ 10 significantly influence on restricting the development of the disease (Tab. 5). And for variety 'Hablange-Berlińska' only Trifender WP proved to be ineffective, the remaining preparations significantly, compared to controls, limited the development of *S. petroselini*. In research Perello et al. (2009) *Septoria tritici* was effectively limited by *Trichoderma harzianum* and *T. koningi*. Puzzling is the low efficiency of the standard fungicide Topsin M 500 SC, which confirms the need to constantly search for new substances to protect the vegetables against fungal pathogens.

Conclusion

The standard fungicide Topsin M 500 SC efficiently protects parsley leaves against fungal pathogens.

A. quisqualis (AQ 10) very effectively restrict the development of *E. heraclei* as well as *Alternaria* spp and *S. petroselini* on the leaves of parsley.

Pythium oligandrum (Polyversum WP) effectively protect of parsley cultivars 'Alba' and 'Kinga' against fungal pathogens.

T. asperelleum (Trifender WP) and *Bacillus amyloliquefaciens* (RhizoVital 42) quite effectively protect most of the cultivars of parsley leaves against pathogens.

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Effect of container type on growth of *Cornus alba* 'Sibirica' and *Prunus laurocerasus* 'Novita' nursery plants

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Abstract
The aim of this work was to study the growth of *Cornus alba* 'Sibirica' and *Prunus laurocerasus* 'Novita' nursery plants grown in conventional above ground (CAG) containers and in pot-in-pot (PIP) system. The investigations were carried out in the Jaroslaw Chabin Ornamental Nursery in Hungary in Páty. The system had been introduced in the USA in early 90-ies of last century. Morphological characteristics, fresh weight and dry weight of the plants were measured. Shoot length increment and seasonal growth of trunk diameter were calculated from differences between data of April and October. The crown diameter of *Cornus alba* 'Sibirica' in PIP was 789 mm and the seasonal growth was 504 mm contrast to 373 mm in CAG. In contrary trunk diameter was larger in CAG for *Prunus* (8.95 mm) compared with PIP (6.27 mm). *Cornus* root dry weight had shown by 33% more increase in PIP (171g) compared with CAG (114g), while *Prunus* shoot dry weight increment was by 30% more in CAG (64g) compared to PIP (45g). According to the leaf characteristics (single leaf size) larger leaves were measured in PIP for *Cornus. Cornus* plants in PIP presented 25% increase total fresh weight, while *Prunus* plants had shown higher value of shoot and root biomass in CAG. We concluded that our results confirmed the benefits of PIP system only for *Cornus alba* 'Sibirica'.

Keywords: pot-in-pot, fresh weight, dry weight, parameter of leaf

Introduction

From among the numerous cultivars of Tatarian Dogwood (*Cornus alba* L.) and Cherry laurel (*Prunus laurocerasus* L.) *Cornus alba* 'Sibirica' and *Prunus laurocerasus* 'Novita' are commonly produced by Hungarian nurseries. The dogwood cultivar has bright coral red stems; it is widespread offered in the trade. The Cherry laurel variety is suitable as hedge plant (Dirr, 1998). Both cultivars are produced as container-grown plants. The nursery production in conventional above-ground containers production has known many of disadvantages. The most limited factors in the commercial range of ornamental production are the overheating in summer and lack of root hardiness in winter (Mathers, 2003). Container grown nursery stock requires more manual labour and the substrate temperature is higher than that is in the soil when the container is exposed more to solar radiation and the heating effect of warm ambient air. To eliminate these problems, in the USA the pot-in-pot production system has been introduced in the 1990 (Parkerson, 1990). The system consists of a planted container, which is placed in a holder pot that has been permanently placed in the ground (Ruter, 1997b; Mathers, 2001a). Several studies showed that the pot-in-pot can moderate root temperature (Young and Bachman, 1996; Schluckebier and Martin, 1997; Ruter, 1997). Studies based on comparison of conventional production systems to pot-in-pot system had shown, the root-zone temperatures in pot-in-pot containers were lower than for conventional above ground containers (Martin et al., 1999) and the substrate temperature in the pot-in-pot system had much lower variation than the ambient temperature within a day (Zhu, 2005). The

aim of our work was to examine the effects of pot-in-pot production system to plant growth and quality for two widely-used broadleaf ornamental shrubs described above.

Materials and methods

As test plant material were used of *Cornus alba* 'Sibirica' and *Prunus laurocerasus* 'Novita' rooted cuttings. The plants were selected randomly (20 pieces per species) from the nursery's stock and each species were grown under same condition. The plants were transplanted to black plastic pots of 5 L volume; 23 cm upper diameter and 18 cm height (Interplast Plastic Products, Bytom, Poland). The pots were filled with a mixture of white peat (Pindstrup Substrate, Latvia) and fertilizer (Osmocote Pro).

The experiment was carried out in a plot of Jaroslaw Chabin Ornamental Tree Nursery (47° 31′ 1′′ N, 18° 48′ 27′′). The transplantation of one-year-old plants were performed on 1 April 2015 and the experiment was finished on the end of October 2015. Data on weather condition were taken from a meteorological station (imetos®) located 3 km from the experimental plot (Nr. 1, Petofi Sandor Street, Telki, Hungary).

The drip irrigation system was set up with one dripper per plant (2 L per hour). One spaghetti tube connected to each plant. Local irrigation was used with parameters pH: 7.1; EC: 0.9 mS/cm. All plants (container and pot-in-pot system) were irrigated with the same frequency and volume of water. Irrigation frequency was changed according to season, two time per day in spring and autumn, tree time per day in summer. The volume of daily water was set up to 0.2 L and 0.6 L per pot in each case (Chabin, 2015).

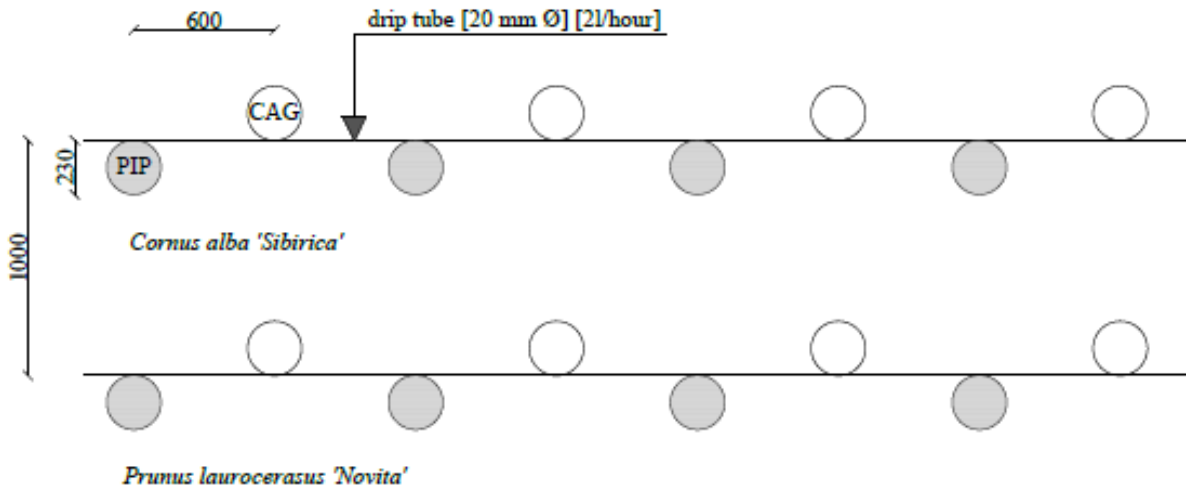

Figure 1 Experimental design. PIP: pot-in-pot; CAG: conventional above ground container; units are in mm

The experimental plot was assigned in the middle of the pot-in-pot system plot of the commercial nursery. The PIP system has been installed in 2006. The pot-in-pot system consisted of placing cultivation pot in pot below ground level. The buried pots were same type of used to transplanted plants.

Containers above the ground were placed in turn buried pots ensuring same condition (*Figure1*). The total 40 pots were placed to 2 rows. Each row had 20 pots 60 cm apart. Distance of rows was 100 cm.

Measurements of morphological characteristics

Plant height, crown diameter, trunk diameter, plant fresh weight and shoot evolution were measured at the beginning of cultivation and the end of the experiment for all species. Measurements were performed on 1st April and 30th October, 2015. Crown diameter was calculated from two direction of the widest part of the crown and trunk diameter was calculated from two measurements of root crown. Digital caliper (Garant) was used to determine trunk diameter. At the end of the experiment five plants were selected from each treatment of two species to determine mean area of leaves (600 piece of leaves were taken). The area of leaves and width and length of leaves were designed with portable area meter (AM350, ADC BioScientific Limited, UK).

Fresh weight and dry weight percentages

At the end of the experiment five samples were selected to determine the total fresh weight of plants, the fresh weight and dry weight of root, shoot and leaf separately. Drying of samples was carried out at the Laboratory of Department of Floriculture and Dendrology at Szent István University. Samples were drying in the drying oven (Binder) at 104°C for 24 hours.

Statistical analyses

Data (morphological characteristics) were analysed by One-way ANOVA. Normality was proved by K-S test ($p > 0.05$) while homogeneity of variances was checked by Fisher's F-test ($p > 0.05$). The parameters of leaves were analysed by Multivariate analysis of variance (MANOVA). The statistical analyses were performed using IBM® SPSS STATISTICS (Version 22) software (Tabachnick *et* Fields, 2013).

Results and Discussion

The comparison of production system had shown no significant effect on the plant height (Table 1). The seasonal growth of *Cornus alba* 'Sibirica' in CAG was 623 mm against 579 mm in PIP. Previous study had shown similar result in production of *Magnolia* x *soulangiana* and *Myrtus communis*: conventional production system resulted taller plants after 7 and 15 month (Ruter, 1993; Miralles *et al*., 2009). However, *Prunus laurocerasus* 'Novita' had shown little heigher increase in PIP system (Table 1). In the genus of *Prunus* was measured earlier 9 % growth of height in PIP (Ruter, 1998).

Table 1 Effect of production type on the plant height (CAG: Conventional Above Ground system, PIP: Pot-In-Pot system)

Plant scientific name	Plant height (mm)			
	Production System	April	October	Seasonal Growth
Cornus alba 'Sibirica'	CAG	998	1621	623
	PIP	977	1556	579
Prunus laurocerasus 'Novita'	CAG	538	725	187
	PIP	526	719	193

** = statistically significant at p <0.05 n =38*

At the end of the experiment, crown diameter was significantly different for two species. The average value of *Cornus alba* 'Sibirica' in PIP was 789 mm and the seasonal growth was 504 mm contrast to 373 mm in CAG. We detected in growth of crown diameter statistically significant difference (at $p<0.05$) in *Cornus alba* 'Sibirica' between the two treatment

(Figure2). In contrary the statistical analysis did not show any significant differences in crown growth of *Prunus laurocerasus* 'Novita'.

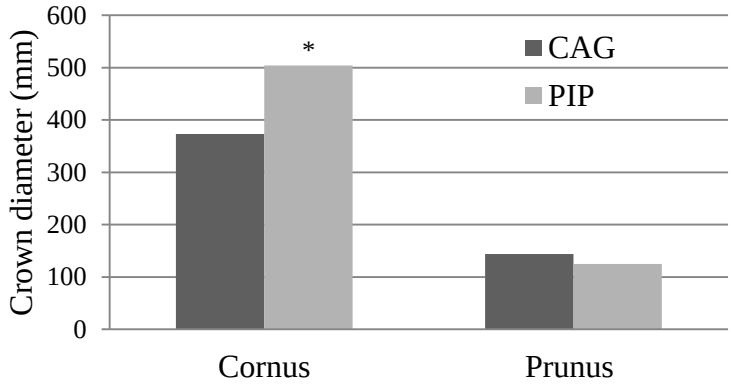

Figure 2 Mean growth of crown diameter in pot-in-pot (PIP) and conventional above ground system (CAG). Values are * statistically significant at p<0.05. Error bars are standard deviation (n=38)

Mean seasonal growth of trunk diameter and increment of shoot length calculated from differences between data of April and October did not show significant differences on *Cornus alba* 'Sibirica'. Albeit the trunk diameter was greater in CAG for *Prunus* (8.95 mm) compared with PIP (6.27 mm), the growth in shoot length did not show any significant differences on *Prunus laurocerasus* 'Novita' (Figure 3).

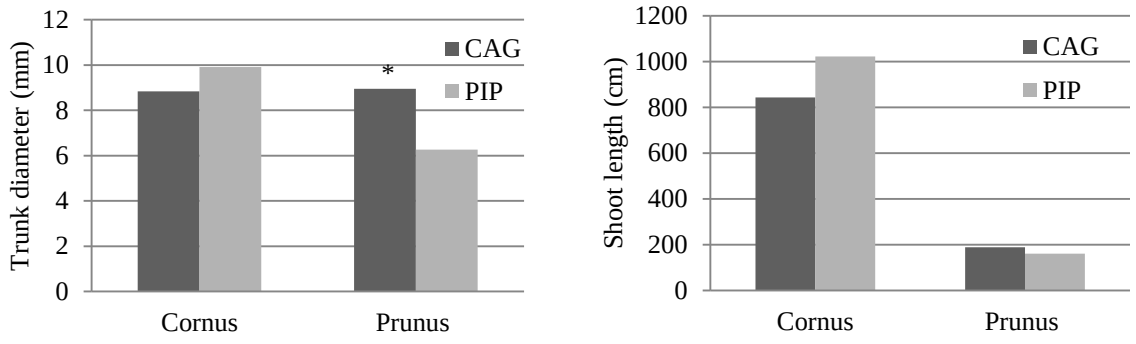

Figure 3 Mean growth of trunk diameter and shoot length in pot-in-pot and conventional above ground systems (control). Values are *statistically significant at p<0.05. Error bars are standard deviation (n=39 and n=38)

The foliage of plants is important source of assimilates on the other hand larger leaves and leaf area makes the plant more attractive. There are no significant differences found in leaf number and total leaf area of both plants grown in PIP and CAG. However, on *Cornus alba* 'Sibirica' we measured larger single leaves in PIP system compared to CAG, while the leaves of *Prunus laurocerasus* 'Novita' were significant larger in CAG system (Table 2).

Table 2 Effect of production system on leaf characteristics **(**CAG: Conventional Above Ground system, PIP: Pot-In-Pot system)

parameters	*Cornus alba* 'Sibirica'		*Prunus laurocerasus* 'Novita'	
	CAG	PIP	CAG	PIP
Leaf number of plant	299	275	185	168
Total area (m²)	0.69	0.72	0.49	0.40
Single leaf area (mm²)	2334	2615*	2682*	2372
Length (mm)	85	89*	95*	89
Width (mm)	46	48*	40	40

*** = statistically significant at p<0.05 n =600 (Leaf number of plant were calculated from average of five samples)**

Table 3 shows 33% increase in root dry weight in PIP (171g) for *Cornus* compared with CAG (114g) (statistically significant difference at p<0.05). MIRALLES et al., (2009) observed 14 % increase in root dry weight compared PIP with conventional container-grown for *Myrtus communis*. Authors of this study also refer to in earlier studies had been measured higher root dry weight in PIP. *Prunus* shoot dry weight had shown by 30% more increase in CAG (64g) compared with PIP (45g). In total dry weight of *Cornus* plants shows 28% higher value in PIP than CAG. Another difference was detectable in total dry weight of *Prunus* plants between the two production systems: 31% increase in CAG.

Table 3 Effect of production system for dry weight content (g) **(**CAG: Conventional Above Ground system, PIP: Pot-In-Pot system)

Production system	*Cornus alba* 'Sibirica'				*Prunus laurocerasus* 'Novita'			
	leaf	shoot	root *	total	leaf	shoot *	root	total
CAG	41	57	114	212	63	64	112	238
PIP	43	79	171	293	46	45	73	165

*** = statistically significant at p<0.05 n =20**

By the end of the season there is no statistically confirmed difference between the two production systems in the fresh weight of *Cornus* plants, albeit PIP plants presented 25% increase in total fresh weight. In contrary *Prunus* plants had shown higher value of shoot and root biomass in CAG, where the difference is statistically confirmed (*Table 4*).

Table 4 Effect of production system for fresh weight (g) (CAG: Conventional Above Ground system, PIP: Pot-In-Pot system)

Production system	*Cornus alba* 'Sibirica'				*Prunus laurocerasus* 'Novita'			
	leaf	shoot	root	total	leaf	shoot *	root *	total
CAG	109	123	547	779	181	165	414	760
PIP	121	170	735	1026	136	117	243	495

*** = statistically significant at p<0.05 n =20**

Conclusion

The nursery plants of tested taxa performed differently in the two production systems. Our study confirmed that PIP system can be particularly beneficial to *Cornus alba* 'Sibirica' during nursery production. We detect significant differences between pot-in-pot production systems and conventional above ground system, where PIP had shown higher values of seasonal growth of crown diameter, shoot length, dry weight content of root and size parameters of leaves. Our study could not confirm the advantages of PIP system for *Prunus laurocerasus* 'Novita' concerning the plant characteristics. We concluded that conventional container system can be suitable to *Prunus laurocerasus* 'Novita'. In these plants higher value of fresh weight and parameters of leaves in conventional above ground production were observed. Further research is needed to investigate the reasons and the temperature and water supply conditions in comparison of the PIP and CAG production systems.

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Root system of trees in urban areas - main hazards and solutions

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Abstract
The most damage and conflict situations of trees within urban areas are the result of damage to the roots and ignorance of their biological properties during planting and operational practice. The paper provides an overview of current knowledge on the root system of trees in urban conditions and about sources of harm and stress in the area of the root zone of trees. It focuses on some rules that help to protect the root system of trees and support their sustainability and operational safety in urban conditions.

Keywords: urban trees, roots, growth, development, stress

Introduction

Trees are long-lived organisms with large dimensions. Their impact in space is particularly evident through the above-ground organs - stems, leaves, flowers and fruits. The root system of trees is below the soil surface, so visually the less available. However, the volume and impact of root system in the environment are comparable and sometimes higher than for aboveground organs.
The present knowledge of the root system of trees is associated with the available instrumental equipment and influenced by the limited possibilities of study of the intact root systems of trees in the natural and urbanized areas. The device technics captures just selected parameters of root system and within sampling some faction of roots are lost. In addition, the study of the root system is also influenced by local conditions of the habitat.
Within the recent 20 years the information about tree roots were collected that fundamentally change the view on the root system of trees, their location, morphology, biological properties and importance in relation to durability, safety and operating safety of trees.
However, in practice incorrect concepts for the root system of trees still persist, especially in urban areas. Insufficient attention is paid to the conditions of growth and development of roots and to effective protection of the root system within standard operation and construction activities. The most damage and conflict situations of trees within urban areas are the result of damage to the roots and ignorance of their biological properties during planting and operational practice.
The paper provides an overview of current knowledge on the root system of trees in urban conditions and about sources of harm and stress in the area of the root zone of trees. It focuses on some rules that help to protect the root system of trees and support their sustainability and operational safety in urban conditions

Morphology and structure of tree roots

Tree roots are anatomically and structurally adapted to perform three basic functions - support and stabilization of the tree in the soil, uptake and distribution of water and nutrients, preservation and storage of organic substances. Therefore, the architecture of the root system is different from that of the above-ground parts of trees (Briddle 1998). Anchoring (structural) roots stabilize trees in the soil, absorbing roots provide uptake of water and nutrients.

From an anatomical point of view the root systems of trees consist of woody and non-woody roots. Woody roots represent the largest amount of biomass of the root system, but a lower rate of the overall length and surface of root system (Day et al. 2010). Their main role is mechanical stabilization of a tree and distribution of water and nutrients.
The surface of the root system consists primarily of a network of fine, non-woody roots (diameter up to 2 mm), without secondary growth. They are known as absorptive roots, whose role is uptake of water and nutrients. They have a short life span - up to several weeks (Pretziger et al., 2002). However, as mentioned Fahley & Hughes (1994) the secondary growth and functional changes may appear also in some non-woody roots, so they can be incorporated into the system of structural roots.
Structural roots are spread horizontally and eccentrically from the trunk. The root system of older trees rises above the soil surface and forms buttress roots. At a distance of 1-2 m from trunk, the structural roots have intensive secondary growth - thickening (Wilson 1964), while further away from the trunk thickening of the roots is not so significant.
It was assumed that massive buttress roots are related to specific soil conditions that do not allow development of tap roots. However tap root system have species with robust buttress roots as well as species with less marked root heave (Crook et. Al. 1997). The eccentric shape of the buttress roots more effectively distributes the mechanical load on the root system of the tree (Mattheck 1991) and increases the tensile and compressive resistance (Crook et al. 1997). Some woody plants create so called sinker roots that provide additional anchoring. These roots grow vertically down from the structural roots in the direction of gravity.

Growth and spread of tree roots in urban conditions

In general, tree roots need water, oxygen and space for growth. When complying with the above mentioned assumptions, tree roots can grow into great depth and their growth in the soil profile is species dependent. According to data submitted by Stone & Kalitz (1991) for different species of woody plants, the depth of the root system ranges from 1 to 53 meters.
In urban areas, the growth of roots in depth and width from the central axis of a tree is uneven and heavily influenced by conditions (Day et al. 2010). Urban soils have high vertical and spatial variability, modified and compacted soil structure, an impermeable crust on the soil surface, restricted aeration and water drainage, interrupted nutrient cycling, altered soil organism activity, presence of anthropogenic materials and other contaminants, and altered temperatures (Craul 1985; Bullock & Gregory 1991; Scheyer & Hipple 2005).
In urban areas tree roots tend to grow shallow under the soil surface (Crow 2005, Wang et al. 2006), where there are available more oxygen and water.
Urban soils often have less structure and greater bulk density than most undisturbed natural soils. The resulting reduction in pore space reduces plant available water (Letey 1985; Craul 1992). The loss of natural soil structure is one of the most important limitations to tree growth in urban areas (Stewart & Scullion 1989).
As soil begins to dry, the development of branch roots is inhibited more than the growth of primary roots (Wright et al. 1992). When roots are drought stressed, they mature rapidly toward the tip, decreasing absorption, and reducing future growth (Kaufmann 1968; Bilan 1974). As the effective absorbing surface is diminished, the roots do not regain their full capacity for water uptake until new root tips can be produced. When roots are rewatered immediately after cessation of elongation, roots may not resume elongation for at least one week. Resumption of root growth can take up to five weeks if water is withheld longer (Bilan 1974).
According to the optimal partitioning theory, plants should allocate relatively more carbon and nutrients to root growth than to aboveground growth when plant growth is limited by water shortage (Bloom et al. 1985). However, some research reports have shown a decrease in

root length density when water is withheld (Ruiz-Canales et al. 2006; Abrisqueta et al. 2008). This decrease may be explained by increased fine-root turnover —higher fine-root mortality concurrent with increased root growth (Meier and Leuschner 2008).
In wet soils, the growth of roots tends to be confined towards the soil surface. In dry soils, root growth can be shifted downward due to water depletion in surface soils (Torreano & Morris 1998). When urban soils limit rooting depth, the ability of tree root systems to respond to periods of drought and high soil moisture may be very limited.
Flooding of soil usually leads to greatly reduced root growth, and death of many of the fine absorbing roots. The small root systems of flooded trees reflect the combined effect of reduction in root initiation and reduced growth of existing roots, as well as decay of the original root system. Because root growth is usually decreased more than shoot growth by high soil moisture, drought tolerance of flooded trees is reduced after the flood waters recede. This change reflects the inability of the small root systems to supply enough water to meet the transpirational requirements of the crown (Kozlowski 1985).

Sources of stress and damage to roots in urban conditions
In the urban ecosystems the root system of trees is exposed to various stress factors with greater extent, than in the natural environment. In addition, the architecture of the root system of urban trees is altered by the nursery production and transplanting that can affect tree throughout its life (Watson et al. 2014)
The most common source of the direct damage of tree roots is building work or laying of underground utilities. These activities can be extremely damaging to trees and can make them very hazardous. They are associated with loss of the absorbing surface of root system and disruption of the anchoring roots.
Due to the damage and loss of roots, tree can't provide sufficient uptake of water and nutrients. Crown pruning is not sufficient solution of the problem. It reduces dispensing of water within transpiration, but also reduces size of the assimilation surface and sources of energy. It is therefore important to minimize the extent and volume of the damaged roots using trenchless methods for installation and replacing of distribution system.
When roots are severed, numerous roots are initiated at or just behind the cut (Wilson, 1967, Carlson 1974, Watson & Himelick 1982, Gilman et al. 2010). Although the root system is able to regenerate and compensate (to some extent) the loss of roots due to their damage, the tree stability and health can be seriously affected. Anchoring trees is particularly disturbed excavation along both sides of the trunk. Such damage reduces the force required to cause tree failure by two-thirds compared with a tree without injuries caused by trenching (O'Sullivan and Ritchie 1993).
The protection of a tree root zone should be respected at least in the range of 2.5-3 times of the diameter of a particular tree (Watson, 1998). The risk of damage is higher for trees with irregularly developed root system. Even relatively shallow excavation conducted within small range may cause the substantial reduction of their stability. In this regard, it is important to determine position and extent of the root system of a tree, for example using of non-destructive ground penetrating radar prior to construction (Nadezhdina & Cermak, 2003). The ground radar captures only the roots of size over 10 mm to depth of approximately 2 m. The rest of the root system is out of the record of this device.
Younger trees with a trunk diameter up to 0.3 m are more tolerant to interferences in their root zone without major loss in stability and crown decline (Watson et al. 2014). When planning the construction work associated with deep excavations the larger trees can be prepared for intervention in their root zone by installation of a root curtain. It is installed at least a year before excavation or trenching at a distance of 2.4 meters far from the tree trunk (Wenger 1984).

Figure 1 There is a high probability of the soil compaction and mechanical damage of roots due to storage of heavy material in the root zone of the tree

The big problem is the loss of space for root growth in connection with the building of structures - foundations of buildings, pavements etc. Then tree may not be able to compensate the loss of absorbing surface, neither the disruption of structural anchoring roots due to lack of space. In such situations, the potential for conservation of a tree on stand has to be considered. For conservation of trees it is important to encourage the regeneration of their root system by regular irrigation and improving the soil conditions. In this respect again younger trees have the advantage of a higher rate of thinner roots, because the ability of damaged roots to form new roots decreases with their increasing diameter (Balder et al. 1995, Balder 1999).
After excavation, the roots should not be exposed to the airflow and temperature extremes for a long time. They should be covered with a moist substrate or cloth. Uneven surface wounds must be aligned. Then earlier and effective regeneration of the root system is expected.
During construction the severe root damage occurs as a result of the movement of vehicles close to trees and storage of heavy materials in the area of their root zone. Mechanical pressure can cause bursting of the roots. Weight of vehicles and stored materials (Fig. 1) also cause soil compaction and change the water-air regime of the soil. Due to lack of air the roots suffer from hypoxia. The compaction results to poor water storage capacity of the soil and occurrence of the soil drought.
In the area of construction is a high probability of damage to trees due to destructive nature of the work. The serious damage to trees may occur within period of one to three years after completion of a construction. The most dangerous is the risk of sudden failure of a tree. That threatens property, health and life of humans. It is necessary to respect the biological properties of trees and implement timely measures to prevent, or reduce the impact of operations on the stability and longevity of trees.

Conclusion

Damage to root system may not be visible, but causes serious disorders in the organism of a tree, which threaten its stability and operational safety.
Within construction area, effective protection of trees requires detailed planning. The plan of tree protection should have outlined routes for movement of heavy machinery, suitable areas for storage of materials and locations, where there will be installed elements for active protection of trees. The plan contains instructions for regular maintenance of trees during construction and within at least three years after the completion.

Especially in urban areas should be avoided damage to buttress roots, excavations should not be conducted very close to tree trunk and trenchless technologies preferentially should be used for installation and replacing of distribution system. The safety units preventing soil compaction and protecting the root system of trees should be used on parking places, near the roads and on pedestrian zones.

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Studies on Herbicides Application in Vine Nurseries

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Abstract
This paper presents a review of the published data and conclusions from studies of different researchers, specialists in the field of Herbology and vine propagation material production. The use of herbicides in vine nurseries has been slightly developed and data in literature were quite few. This analysis of the results achieved in weeds control revealed the opportunities of herbicides application, both preventively and at the time of rooting of the cuttings. With the drastic reduction of the workforce in agriculture its application has been a necessary part for the solution of this problem.

Key words: herbicides, phytotoxicity, planting material, vine nursery, weeds

Review
The grafted cuttings in nurseries and young vines are particularly sensitive to the harmful effects of weeds. Their yet poorly developed and shallow root system makes them uncompetitive and very vulnerable, so maintaining the soil surface in good agricultural condition is an essential element of vine propagation material production technology. Weed control is carried out mainly by mechanical and manual practices however it's very labor-intensive and expensive. The use of herbicides in vine nurseries has been slightly developed and data in literature were quite few.
At the end of 20th c. triazine herbicides (simazine, atrazine, prometrin, terbuthylazine, metribuzin) have been well-researched and widely used in viticulture. Lyubenov (1972), Fetvadzhieva (1973), Nikov et al. (1978), Boychev (1985), Boubals (1991), Agulhon (1996), Janjic (1996) etc. have clarified in details their chemical composition, biochemical mechanism of action on the plant organism, their effects on photosynthesis and metabolism of weeds. A number of authors have published data on their application in grapevine nurseries and their impact on grafted cuttings and young vines. Most often the studies included also other active substances, scarcely used in vine propagation material production.
Lange et al. (1969) reported good results in annual weeds control in newly planted vineyards (up to 3 years) and nurseries after application of trifluralin. The herbicide was introduced after planting the young vines and cuttings before the bud development by incorporation into the soil and did not cause them any damages. In the same study DCPA (propanid) diphenamid, benzulid and trifluralin and simazine have been tested. The highest herbicidal effect was reported for simazine, and the lowest - after applying of DCPA (Lange et al., 1970).
In 1974-75, Melykumova tested simazine (2-4 kg/ha), atrazine (2-4 kg/ha) and monuron (2-3 kg/ha) in a vine nursery located on gray forest soils. The best weed control had simazine (4 kg/ha), atrazine (2 and 4 kg/ha) and monuron (3 kg/ha). The high doses caused growth depression at the onset of vegetation, chlorosis and withering of the shoot tops. (Melykumova, 1976; Melykumova, 1977).
Bravenec and Miŝa (1978) investigated the effect of Herbex, Bethanal, Tenoran, Venzar, Treflan and Afalon in a vine nursery. The best effect was recorded in Afalon, Herbex and

Treflan. Harmful effects on the vines were not observed. The authors recommended herbicides to be selected based on the soil conditions and the expected weed species.
As a result of soil herbicides testing in a vine nursery Chelebiev (1981) recommended after planting the cuttings and shaping the ridges to be treated with Herbazin 50 at a dose of 5 – 8 kg/ha or Caragard combi - 8 to 10 kg/ha. The trial was carried out on carbonate chernozem with Dunavska Gamza variety grafted to Kober 5BB and SO4. It was applied Herbazin 50 (doses – 5; 8; 10 and 12 kg/ha), Ceazin 50 (doses - 3; 4; 5; 6 and 8 kg/ha) and Caragard combi (doses – 5; 8; 10; 12 and 15 kg/ha). Visible signs of phytotoxicity were reported on the 40^{th}, 60^{th} and 90^{th} day after treatment with Ceazin 50 in all doses. On the 60^{th} day, similar signs were observed for the other two herbicides, especially in high doses. Ninety days after treatment the phytotoxic manifestations were limited only on some leaves at the base of the shoots. In the autumn noticeable difference in the length and color of the annual growth in the controls and the treated variants were not observed. As a result of the same experiment, Chelebiev (1981*) reported that in the tested doses all three herbicides had a very good action and after-effect against annual weeds as the effect increased with increasing the dose.
In the production of vine propagation material in greenhouses the density of the planted grafted cuttings was high and the mechanical weed control was difficult. Therefore, during the period 1977 - 1980, in Iaşi (Romania) the herbicides Pitezin, Livezin 50, Gezatop 50, Caragard combi A-50, Devrinol 50 W were applied. They were introduced before the weeds germination and planting of the grafted cuttings. The best results were achieved with Caragard combi A-50 at a dose of treatment 5 kg/ha, Gezatop 50 at a dose of 6 kg/ha and Livezin 50 at a dose of 5 kg/ha. It was found out that the tested herbicides did not cause phytotoxicity on the grafted vines (Calastru, 1982).
Introduced 25-30 days before planting in the nursery of the grafted cuttings of Moldova variety to V. riparia X V. rupestris (101-14 Mgt), the herbicides Caragard (terbumeton) at 4-10 kg a.i./ha and Devrinol (napropamide) at 3-6 kg a.i./ha did not affect negatively the germination, growth, maturation and the number of roots. At higher doses they controlled efficiently the annual weeds during the first part of the vegetation period. In the second part however their effect weakened and several mechanical treatments were recorded (Gromakovskiy et al., 1984).
Prabha Challa (1987) reported that oxyfluorfen at a dose of 1 – 2 kg/ha, diuron and atrazine at doses of 2.0 – 3.0 kg/ha effectively controlled weeds in a vine nursery, including *Parthenium hysterophorus L.*, *Cynodon dactylon L.*, *Cyperus iria L.* and *Eleusine indica L.* In the same study it was found that oxadiazol at a 2-3 kg/ha reduced the germination ratio of the cuttings, especially for Thompson seedless variety.
An area, highly infested with weeds, intended for rooting of cuttings in the Odessa region was treated with simazine, atrazine and Caragard (terbumeton + terbuthylazine) at doses of 3.75-7.5 kg/ha - simazine, 12.0-16.0 kg/ha - atrazine and 12.0 kg/ha - Caragard. Their application controlled the weeds to 76-94% in the first year and 59-88% in the second one. Basagran (bentazone) or glyphosate were applied for control of the especially persistent weeds *Setaria Virid*is L., *Xanthium strumarium* L., *Cirsium incanum* L., *Convolvulus arvensis* L., *Elymus repens* L., *Amaranthus sp.* and *Solanum nigrum* L. (Litvinov et al., 1987).
Chelebiev and Katerova (1988) investigated the influence of some herbicides on weeds and vines in the nursery. It was tested Herbazin 50 – 4; 5; 6; 8 and 10 kg/ha; Ceazin 50 – 4; 5; 6; 8 and 10 kg/ha; Caragard 3587 – 5; 6; 8; 9; 10 and 12 kg/ha; Devrinol 50 – 4; 6; 8; 10 and 12 kg/ha; Terbutrex combi – 6; 8 and 10 l/ha; Ronstar – 3; 4; 5; 6 and 8 l/ha; Racer – 3; 4; 6 and 8 l/ha and Goal 2E – 4; 6 and 8 l/ha. Herbazin 50 – 5 – 6 kg/ha, Caragard 3587 – 6 – 8 kg/ha and Terbutrex combi – 6 – 8 l/ha have shown the highest efficiency against annual weeds. In those doses they did not have a harmful effect on the vines. The herbicides Ronstar, Racer, Goal 2E and Ceazin 50 in the tested doses showed good herbicidal effect but caused

phytotoxic damages to vines and reduced the rate of produced first-class material. In the untreated control and all doses of Devrinol 50 mildew was developed due to strong weeding, which caused partial defoliation of the vines in late August and poor ripening of the annual growth.

According to Nikov et al. (1988) Herbazin 50 – 5 – 7 kg/ha, Caragard 3587 – 8–10 kg/ha, Afalon – 5–6 kg/ha, Ronstar – 5–6 l/ha and Terbutrex combi – 5–6 l/ha were efficient against weeds and harmless to vines in the nursery. The authors recommended the nurseries to be treated after planting of grafted cuttings and formation of the ridges, but before the weeds emergence. In their opinion, during spring drought spray irrigation after the treatment had a positive effect on the herbicides action. It was obligatory in the nurseries created by the classical technology (indoor rooting) the dewy roots to be cleared away. If they were not removed on time leaf chlorosis and blight appeared and vines were behind in their growth.

Moretti and Borg (1992) applied soil herbicides (diphenamid, propizanid + simazine and simazine) in a vine nursery with grafted cuttings to different rootstocks: SO4, 140 Ru, 1103 P, 5BB and 41B. The used herbicides have shown good effect in weeds control (with the exception of *Polygonum persicaria* L. and *Digitaria sanguinalis* L. species). The rootstocks 140 Ru and 41B were sensitive to the herbicides.

Further to simazine and atrazine in vine nurseries it could be applied Devrinol, Terbutrex combi and Fuzilad (Spasov et al., 1999).

For grafted cuttings rooted by the classical technology Todorov (2005) recommended immediately after planting and before emergence of the weeds the ridges to be treated with the herbicides Dirimal and Dirimal extra (oryzalin) at a dose of 3 kg/ha or Racer (fluorochloridone) in a dose of 4 l/ha.

Tonev (2000) determined the use of trifluralin and napropamide as admissible in young not fruit-bearing vineyards as well as for the production of vine propagation material. It was recommended the first one to be applied before planting necessarily with incorporation, while the second - after planting in well-cultivated and free of weeds soil. For control of the already grown annual and perennial gramineous weeds the author recommended the use of the foliar herbicides fluazifop-P-butyl, haloxyfop, clethodim, etc. A new method for introducing in the nurseries of fluazifop-P-butyl (Fuzilad) by photodegradable tape was reported by Petrov (2000).

According to Chelebiev and Encheva (2007) simazine based herbicides could be introduced without risk of causing damages to young vines and nurseries in lower doses – 2-4 kg/ha active ingredient.

With good effect and without the risk of phytotoxicity in the young vineyards and nurseries it might be applied Herbazin 50 at a dose of 5–8 kg/ha (Karanikolov and Draganov, 2003).

The authors mentioned here so far have developed the problem of weed control in the grapevine nurseries where the grafted cuttings were planted and grown by the classical technology (in ridges, under the soil surface).

Since 2007 at the Institute of Viticulture and Enology in Pleven (Bulgaria) it had been studied the opportunity of applying herbicides in open rooting of waxed grafted cuttings. The efficiency and selectivity of 6 soil herbicides in the vine nursery have been investigated for variety Muscat Kaylashki/SO4: Devrinol 4F, Stomp 33EC, Dual Gold 960 EC, Goal 2E, Dual Gold 960 EC + Goal 2E, Lumax 538 SC and Venzar 80 VP. The tested herbicides except Devrinol 4F, showed good herbicidal effect against the weeds found in the nursery. It was established different duration of the action, depending on the active substance. For Stomp 33 EC, Dual Gold 960 EC and Venzar 80 VP it weakened after the sixtieth day, while for Goal 2E, the combination of Dual Gold 960 EC + Goal 2E and Lumax 538 SC it lasted throughout the entire growing season. There was a negative effect of the herbicides on the grafted vine cuttings in the initial phase of shoot germination in the nursery. The tested products did not

affect adversely the yield of vine propagation material of Muscat Kaylashki/SO4 (Prodanova-Marinova et al., 2011; Tsvetanov et al., 2012; Prodanova-Marinova, 2012; Prodanova-Marinova et al., 2014; Dimitrova et al., 2014).

Studies have been carried out for determining the biological efficiency of the herbicides Wing P (*250 g/l pendimethalin + 212.5 g/l dimethenamid P*), Gardoprim Plus Gold 500 SC (*312.5 g/l s-metolachlor + 177.5 g/l terbuthylazine*) and Stratos ultra (*100 g/l cycloxydim*). Wing P and Gardoprim Plus Gold 500 SC efficiently controlled the annual weeds, except *Xanthium strumarium* L. That species was sensitive to Wing P while Gardoprim Plus Gold 500 SC at a dose of 6 l/ha inhibited its germination only to the thirtieth day after treatment. The root-sprouting species, especially *Convolvulus arvensis* L. were slightly affected by the tested products. Stronger herbicidal effects and greater persistence were reported at higher doses. The effect of Wing P weakened after the sixtieth day and Gardoprim Plus Gold 500 SC maintained the soil surface free of weeds until the ninetieth day after treatment. Stratos Ultra (2 l/ha) showed excellent herbicidal effect to *Sorghum halepense* L. and weaker, but satisfactory effect on *Cynodon dactylon* L. (Prodanova-Marinova, 2015). The impact of these herbicides on the vegetative manifestations of the grafted cuttings during their rooting was studied and it was found that their application did not result in negative changes in the growth and the number of roots of the obtained standard vines (Prodanova-Marinova, 2015.*)

Conclusions

The chemical means for maintaining the soil surface in the vineyards free of harmful vegetation have been studied and used long ago. The analysis of the results achieved in weeds control showed the opportunities of herbicides application, both preventively and at the time of rooting of the cuttings. The specific action of soil applied active substances, the time of their introducing and their relatively high persistence allowed for effective weed control already from the onset of the vegetation period. The selective foliar herbicides equally successfully control both annual and perennial gramineous weeds. Despite the global trends in organic farming development the chemical method had remained relatively inexpensive and popular. With the drastic reduction of the workforce in agriculture its application has been a necessary part for the solution of this problem.

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The effect of growing mixture additives on the development of *Viola x wittrockiana* 'Carrera'

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Abstract
During our work, newly developed growing mixture additives such as the Finnish BRT® EverGreen and Fainsoil Bioactivator were applied in *Viola x wittrockiana* 'Carrera' production. The morphological characteristics, chlorophyll content, peroxidase enzyme activity and bacteria number of medium were measured. The plants in mix of coconut coir, BRT® and FBA were higher and wider than those in the mix of peat, BRT® and FBA. The mix of coconut coir, BRT® and FBA resulted higher chlorophyll and carotenoid content and the medium contained more bacteria. The treatment groups in mix of peat, BRT® and irrigated with FBA had wider leaves and greater flowers than groups grown in mix of coconut coir, BRT® and FBA. The groups in 20% BRT® showed the most stressful state. Mix of coconut coir, BRT® and FBA contained more bacteria.

Keywords: BRT® EverGreen, Fainsoil Bioactivator, POD

Introduction

Nowadays, climate change and depletion of peat mines is a serious problem in the world. The water management has become increasingly important because of the temperature increasing and the precipitation decreasing. The water holding capacity of the growing media have major role in the ornamental plant production. There are several kinds of media for the ornamental plant cultivations. The Baltic peat is the most commonly used medium. Excavation without degradation of soils and peat moss is impossible. For the nature conservation and the decreasing of water consumption, the growing mixture additives such as the Finnish BRT® EverGreen and FainSoil Bioactivator have major role in the cultivation.
In the last decades, the growing mixture additives and soil amendments were tested in the horticultural production. Superabsorbents such as hydrogels or superabsorbent polymers are synthetic substances and water-insoluble polymers (Pora et al., 2015). It has got the ability to swelling in water and retaining of water within their structure (Zohuriaan-Mehr and Kabiri, 2008). The superabsorbents, Zeba® increased moisture conservation on cotton (Ngoben et al., 2007) and had positive effect on oat, onion and watermelon (Viztiu et al., 2014). Stockosorb®, water storing product, had beneficial effect on *Quercus virginiana* (Gilman, 2004), on turfgrass (Zheng et al., 2005) and had better water holding capacity on *Salvia officinalis* (Savi et al., 2014). The mineral-polyacrylate water absorbent Geohumus®, decreased stress response of *Dianthus chinensis* (Li et al., 2016), decreased irrigation application rate of *Hydrangea macrophylla* (Kapsimalis et al., 2011) and responded stronger drought stress on *Zea mays* (Duong and Asch, 2012). The superabsorbent polyacrylate Hydrogel enhanced the efficiency of water uptake and utilization of photosynthetic products of *Eucalyptus* species grown in soil (Orikiriza et al., 2009), increased salt stress tolerance of turfgrass (Hadam et al., 2011), improved nutrients uptake efficiency of *Solanum tuberosum* (Faried et al., 2014) and also increased root and shoot biomass of *Fagus sylvatica, Picea abies* and *Pinus sylvestris* (Orikiriza et al., 2013).

Biostimulators have important role in ornamental plants cultivation. Since the middle of the 20th century synthetic and natural regulators have been used in Floriculture. Their application inhibited or stimulated the growth of the plant, controlled flowering and enhanced stress tolerance (Basra, 2000; Ueber, 2007; Domanski and Hetz, 2007). The natural alga extract Kelpak® increases the size of roots in ornamental plant cultivations. It has positive effect on *Pelargonium zonale* 'Serena' (Köbli et al., 2012), on *Lilium* oriental hybrid 'Rialto' (Tilly-Mándy et al., 2012) and increase of Zn, Cu, Fe and Mn in *Dactylis glomerata* and *Festulolium braunii* (Godlewska and Ciepiela, 2016). Pentakeep®-V, which is formulated with 5-aminolevulinic acid, increased the content of chlorophyll in *Tillandsia usneoides* (Tilly-Mándy et al., 2010a), in *Begonia x tuberhybrida* (Kisvarga et al., 2015), in *Saintpaulia ionantha* leaves and induced two weeks earlier flowering (Tilly-Mándy et al., 2010b). Bistep® micronutrient containing nanofertilizer had positive effect on *Petunia x grandiflora* 'Musica Blue' (Kisvarga et al., 2014) and on *Lilium* orientale 'Rialto' (Tilly-Mándy et al., 2012) development. Radifarm®, a root stimulating agent, increased the fresh and dry weight of *Salvia splendens* (Zeljković et al., 2010a), nitrogen and potassium content of *Begonia semplerflorens* (Zeljković et al., 2010b) and *Tagetes patula* (Zeljković et al., 2013) as well as had positive effects on annuals germination (Parađiković et al., 2008).
The main aim of this study was to examine the effects of BRT® EverGreen and FainSoil Bioactivator on *Viola x wittrockiana* 'Carrera'.

Material and Methods

Viola x wittrockiana 'Carrera' seedlings were grown in the greenhouse of the Department of Floriculture and Dendrology, Szent István University, Budapest. The start of the research was 6th November 2015. The processing of samples was performed in the labour of the Department of Floriculture and Dendrology and Department of Soil Science and Water Management, Szent István University, Budapest.
Viola x wittrockiana is a popular biennial bedding plant for mid-fall to late-spring. Intensive breeding programs have selected for unique flower colours, large flower size and temperature tolerance (Bailey, 1995). *Viola x wittrockiana* 'Carrera' is a large-flower viola with a very compact and uniform habit (www.issuu.com).
BRT® EverGreen is a newly developed moisture control and soil amendment product. It is composed of methylene-urea resin. During the manufacturing, phosphorous acid is added and after the foaming, wetting agent is added. It is a slow releasing nitrogen and phosphorous fertilizer as well. The product is hydrophilic, able to store up to 90% of its volume and release it slowly to plant root systems and also provides roots with extra oxygen needed for growth. Using of this product, the number of irrigations and the amount of water used for irrigation will be reduced. The recommended concentration is between 10% and 30% (www.brt.fi).
FainSoil Bioactivator (FBA) is prepared with sterile fermenting process from EM-1 microbe mixture, sugar cane molasses and UV-cleansed water. The product also contains lactic acid bacteria, yeast and Actinomyces (aerobic and anaerobic), Fain special tenside and Greenfain product. This product is recommended for preventing or eliminating of odour problems, quickening composting process in composts and manures. The producer recommended the following application: meantime with the potting plants were irrigated with 0.1% of FBA solution. Two weeks later the plants were irrigated with 0.05% of FBA solution. 4 weeks after potting plants were irrigated again with 0.05% of FBA solution (www.faintend.fi).
The treatment groups can be seen in Table 1. Every treatment groups contained 18 plants. Plant height, plant diameter and size of leaves were examined. We also measured the size of flowers. With chlorophyll and carotenoid content and peroxidase enzyme activity the physiological conditions were examined. The bacteria number of medium was measured by

MPN method. In order to determine water capacity of growing mixtures, weight of the media was measured daily.

To determine chlorophyll and carotenoid content, 100 mg leaf sample were collected. With quartz sand the samples were homogenized and completed with 80 % Acetone solution to 10 ml. After 24 hour rest the light absorbance of the solution was measured on 663, 644 and 480 nm wave length (Arnon, 1949).

To determinate peroxidise enzyme activity, 200 mg leaf samples were collected and homogenized with 1500 µl potassium phosphate buffer. The samples were centrifuged at 4 °C, 13500 rpm/min for 20 minutes. The solutions were reacted with H_2O_2 substrate, Orthodianizidin reagent and Na-acetate buffer and were spectrophotometry measured on 460 nm wave length (Shannon et al., 1966, Blinda et al., 1996).

To count the number of bacteria, 1 g soil sample with 9 cm^3 physiological saline were mixed. 180 µl liquid nutrient broth (3.45 g peptone from meat, 3.45 g casein peptone, 5.1 g sodium chloride, pH=7.5) and 20 µl medium sample were added to the microtiter plate and then dilution series were made. The samples were incubated for 1 week. 40 µl iodonitrotetrazolium chloride was added and another dilution series were made. The positive tubes, key numbers, were determined. The set points of key numbers were determined from Hoskins's Table (Cochran, 1950).

set point x dilution = bacterial count (cell/cm^3)

Table 1 The treatment groups of *Viola x wittrockiana* 'Carrera'

Treatment groups		Medium	BRT	Added nutrients	potting	FBA 2WA. potting *	FBA 4WA. potting *
Group 1.	P (control)	100% p	-	1.5 g/l Osmocote	-	-	-
Group 2.	PBRT10	90% p	10%		-	-	-
Group 3.	PBRT20	80% p	20%		-	-	-
Group 4.	PBRT30	70% p	30%		-	-	-
Group 5.	PFBA	100% p	-	1.5 g/l PG mix	0.1 %	0.05%	0.05%
Group 6.	PBRT10FBA	90% p	10%		0.1 %	0.05%	0.05%
Group 7.	PBRT20FBA	80% p	20%		0.1 %	0.05%	0.05%
Group 8.	PBRT30FBA	70% p	30%		0.1 %	0.05%	0.05%
Group 9.	C	100% p+c	-	1 g/l Futor	-	-	-
Group 10.	CBRT10	90% p+c	10%		-	-	-
Group 11.	CBRT20	80% p+c	20%		-	-	-
Group 12.	CBRT30	70% p+c	30%		-	-	-
Group 13.	CFBA	100% c	-		0.1 %	0.05%	0.05%
Group 14.	CBRT10FBA	90% p+c	10%		0.1 %	0.05%	0.05%
Group 15.	CBRT20FBA	80% p+c	20%		0.1 %	0.05%	0.05%
Group 16.	CBRT30FBA	70% p+c	30%		0.1 %	0.05%	0.05%

*2WA. = 2 weeks after potting, 4WA. = 4 weeks after potting

** p=peat, c= coconut coir

Statistical analysis was performed by IBM® SPSS STATISTICS (Version 22) by 95% significance level. One-way ANOVA model was used to compare the varieties. The assumptions of homogeneity of variance were hold. Normality of residuals was proved by Kolmogorov-Smirnov test. Pairwise comparisons were run by Tukey's Post Hoc test (Tabachnick and Fields, 2013).

Results and Discussion

Morphological characteristics

The morphological characteristics with plant height, plant diameter, size of leaves and size of flowers measurements were examined *(Table 2.).*

We detected significant differences between the treatments for plant height [$F(15;266)=11.379$; $p<0.001$]. Only two treatments groups (CBRT20FBA and CBRT30FBA) were significantly higher than the control (P) and PBRT10 group. The other treatment groups did not show differences from those groups. The highest plants were measured in CBRT30FBA group (10 cm) which was 2 cm higher than the control group. Various biostimulators had similarly positive effects on plant height. Bistep® increased by 4 cm the plant height of lily (Tilly-Mándy et al., 2012) or Radifarm® significantly increased the height of marigold (Zeljković et al., 2013). Soil amendments, Hydrogel was doubled the height of potato seedlings (Faried et a.l, 2014). The height of plant is one of the most important quality properties. The results (2 cm higher) may be remarkable in the ornamental plant production, it could be more marketable seedlings.

Table 2 The results of morphological characteristics and flower size of *Viola x wittrockiana* 'Carrera'

Treatment groups	Height		Diameter		Leaf length		Leaf width		Flower length		Flower width	
P(CØ)	8,0	**a**	11,2	**a**	4,1	**a**	3,4	**abcd**	5,2	**a**	4,7	**a**
BRT10	7,9	**a**	11,0	**a**	4,1	**a**	3,4	**bcd**	5,3	**a**	4,6	**a**
BRT20	8,8	**ab**	11,9	**ab**	4,3	**a**	3,6	**cd**	5,7	**ab**	5,1	**ab**
BRT30	8,9	**ab**	12,1	**abc**	4,3	**a**	3,5	**cd**	5,5	**ab**	4,8	**a**
PFBA	9,0	**ab**	11,6	**ab**	4,6	**a**	3,5	**cd**	6,0	**ab**	5,3	**ab**
BRT10FBA	9,3	**ab**	12,2	**abc**	4,6	**a**	3,7	**d**	6,3	**b**	5,7	**b**
BRT20FBA	9,1	**ab**	13,3	**bcde**	4,3	**a**	3,5	**cd**	5,8	**ab**	5,1	**ab**
BRT30FBA	9,5	**ab**	12,7	**abcd**	4,4	**a**	3,5	**cd**	5,6	**ab**	4,9	**a**
C	8,4	**ab**	12,9	**abcd**	3,9	**a**	3,0	**abc**	5,3	**a**	4,8	**a**
CBRT10	8,7	**ab**	12,8	**abcd**	3,9	**a**	2,9	**ab**	5,5	**ab**	5,0	**ab**
CBRT20	9,0	**ab**	13,7	**cdef**	4,0	**a**	2,8	**a**	5,5	**ab**	4,9	**a**
CBRT30	9,2	**ab**	13,5	**bcde**	4,3	**a**	3,1	**abc**	5,7	**ab**	5,2	**ab**

CFBA	9,9	**ab**	15,7	**f**	4,5	**a**	3,4	**abcd**	5,8	**ab**	5,1	**ab**
CBRT10FBA	8,7	**ab**	14,0	**cdef**	4,3	**a**	3,2	**abcd**	5,3	**a**	4,8	**a**
CBRT20FBA	9,9	**b**	15,2	**ef**	4,3	**a**	3,3	**abcd**	5,6	**ab**	5,1	**ab**
CBRT30FBA	10,0	**b**	14,6	**def**	4,6	**a**	3,3	**abcd**	5,5	**ab**	5,0	**ab**

Significant differences were detected between the treatments groups of plant diameter [F(15;266)=11.374; p<0.001]. Seven treatment groups (PBRT20FBA, CBRT20, CBRT30, CFBA, CBRT10FBA, CBRT20FBA, CBRT30FBA) differed from the control group. The widest plants grew in CFBA group which was 4.5 cm wider than the control group. The result of CFBA group may be remarkable in the plant production.

We were not able to detect significant differences between the treatments groups in leaf length [F(15;266)=1.775; p=0.038]. The longest leaves formed in three groups (PFBA, PBRT10FBA and CBRT30FBA). However, the results of leaf width showed significant differences between treatment groups [F(15;266)=4.849; p<0.001]. Seven treatment groups (PBRT20, PBRT30, PFBA, PBRT10FBA, PBRT20FBA, PBRT30FBA) differed from CBRT20 group. The widest leaves in the group PBRT10FBA developed 1 cm wider than the narrowest group CBRT20. The result of PBRTR10FBA group may be remarkable in the plant production. The wider leaves have got greater leaf area with greater photosynthetic surface which increases plant productivity.

We detected differences between the treatments groups for both flower length [F(15;266)=2.716; p<0.001] and width [F(15;266)= 2.690; p=0.001]. PBRT10FBA group had significantly longer (approx. 1 cm longer) flowers than the control group. The other treatment groups did not differ from each other. The results of flower width were similar to the results of flower length. Also in the PBRT10FBA group were found the widest flowers. In other experiments, Pentakeep®-V was increased the flower size of *Hosta* (Denisow et al., 2016). The most important characteristic for the customers is the decorativeness. Improving the trait characteristic of plants is a main point of the ornamental plant production.

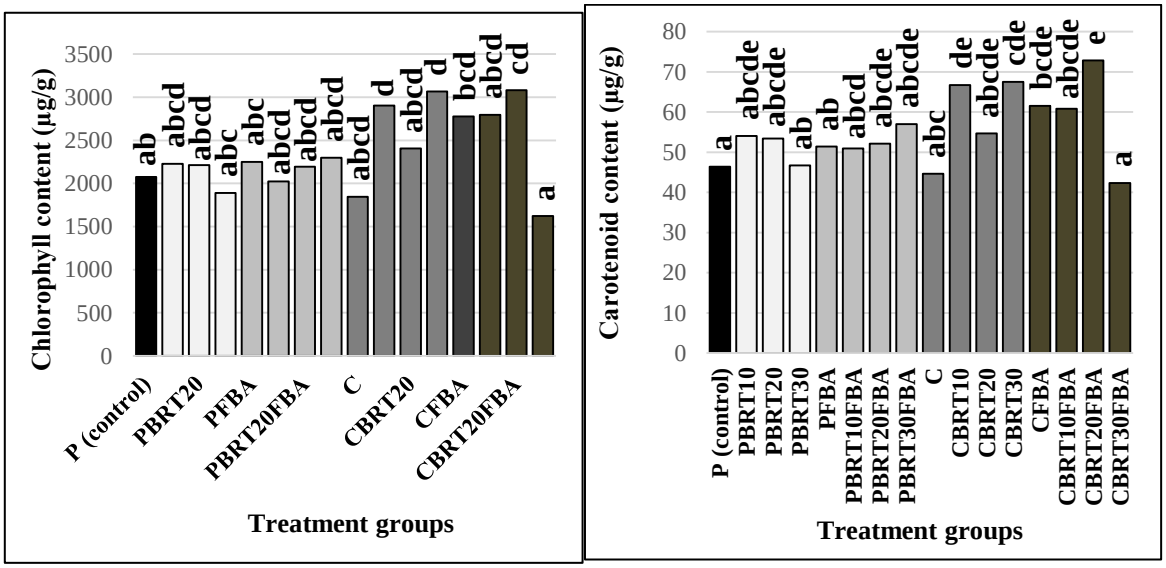

Figure 1 Chlorophyll content and carotenoid content of *Viola x wittrockiana*

Physiological conditions

Physiological conditions were examined by chlorophyll content, carotenoid content and peroxidise enzyme activity. The chlorophyll content was detected by two laboratory measurements, on average samples. Significant differences were detected between treatment groups [F(15;32)=11.761; p<0.001] *(Figure 1.)*. Four treatment groups (CBRT10, CBRT30, CFBA, CBRT20FBA) differed from control group. The results of chlorophyll content were similar to the results of carotenoid content [F(15;32)=6.365; p<0.001] *(Figure 1.)*. The same four groups differed from the control group. In other experiments with biostimulators, such as Pentakeep®-V also increased the chlorophyll content of leaves of *Tillandsia usneoides* (Tilly-Mándy et al., 2010) or of *Saintpaulia ionantha* (Tilly-Mandy et al., 2010). PZB, B9 and CCC were applied in pansy production. Using these products the chlorophyll content was significantly higher than in the control group. Nearly 150% more pigment was measured comparing to the control. The carotenoid content was 1.5-fold higher than the control (Gliožeris et al., 2007). 110% more chlorophyll content and 30% more carotenoid contents were measured on *Pelrgonium peltatum* 'Ville de Paris Red' using 0.5% Kelpak® (Urbanek Krajnc et al., 2012). Hydrogel superabsorbent increased 1.5-fold the chlorophyll content of potato (Faried, 2014). We got the same result for chlorophyll and carotenoid content. CBRT30 and CBRT20FBA had 1.5-fold more content than the control. Higher concentration of chlorophyll increases photosynthetic activity which makes better "fitness" of plants.

The peroxidase enzyme activity was detected by three laboratory measurements, on average samples. Significant differences were detected between treatment groups [F(15;30)=5.016; p<0.001] *(Figure 2.)*. Four treatment groups (PBRT30, PBRT30FBA, C, CBRT20FBA) were significantly more stressful than the control. The most stressful treatment group was PBRT30FBA, it had three-fold higher activity than the control. The less stressful treatment group was PBRT30, it had 2.5-fold lower POD activity than the control. Generally, it was observed that the value of 10% BRT concentration was followed by a higher value of 20% BRT concentration and then a lower value of 30% BRT concentration. Mixture of 75% vermicompost as soil amendment and peat, resulted 1.5-fold increase in peroxidase enzyme activity of *Dracaena marginata* (Tatlari et al., 2013). Trends are observed but further studies are needed.

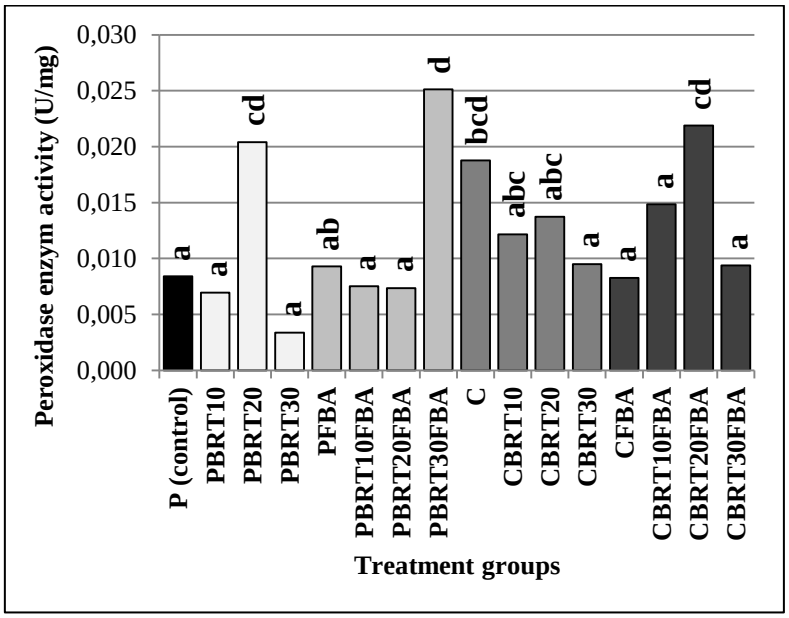

Figure 2 The results of peroxidase enzyme activity of *Viola x wittrockiana* 'Carrera'

Table 3 The results of bacteria number of media treated with FBA

	18th November	7th December

Treatment groups	**key number**	**dilution**	**set point**	**bacterial count (cells/cm^3)**	**key number**	**dilution**	**set point**	**bacterial count (cells/cm^3)**
P (control)	311	E (10^6)	7.5	7 500 000	310	E (10^6)	4.3	4 300 000
PFBA	300	D (10^5)	2.3	230 000	322	D (10^5)	21	2 100 000
PBRT10FBA	300	D (10^5)	2.3	230 000	321	D (10^5)	15	1 500000
PBRT20FBA	310	C (10^4)	4.3	**43 000**	321	D (10^5)	15	1 500 000
PBRT30FBA	320	D (10^5)	9.3	930 000	311	D (10^5)	7.5	750 000
CFBA	321	E (10^6)	15	15 000 000	310	E (10^6)	4.3	4 300 000
CBRT10FBA	321	E (10^6)	15	15 000 000	321	C (10^4)	15	**15 000**
CBRT20FBA	320	F (10^7)	9.3	**93 000 000**	310	F (10^7)	4.3	43 000 000
CBRT30FBA	310	E (10^6)	4.3	4 300 000	321	E (10^6)	15	**15 000 000**

Bacterial count

The number of bacteria was counted (MPN method with Hoskins's Table) in the medium treated with FBA *(Table 3.)*. During the statistical analysis, we were not able to detect significant differences between the treatment groups because of the standard errors. At the first sample time, the highest number of bacteria were in CBRT20FBA group and the least number of bacteria were in PBRT20FBA group. Groups with coconut coir contained more bacteria than groups with peat. At the second sample time, the bacteria number was mostly decreased in coconut coir containing groups. The trend of groups containing peat had opposite tendency comparing to group with coconut coir. The bacterial number of control group was decreased.

Water content

Differences were found between the treatment groups *(Figure 3.)*. It was observed that the groups containing peat dried up more slowly than the groups with coconut coir. Best result was obtained with group C. The treatment groups with peat and high BRT concentration contained water for longer time during the drying out period than the peat with lower BRT concentration. Opposite situation was observed in the treatment groups with coconut coir. At the end of the experiment, groups with peat and BRT contained more water than the groups with coconut coir and BRT. In an experiment on *Pentas lanceolata* and *Ixora coccinea*, the peat-based medium had the lowest water-holding capacity. The coconut coir-based medium showed the least change (Meerow, 1994). Experiment with *Petunia x hybrida*, *Tagetes patula* and *Pelargonium x hortorum*, the water-holding capacity of medium increased as the proportion of peat and coconut coir increased. Coconut coir-based medium had greater water-holding capacity than the peat-based medium (Evans and Stamps, 1996). On *Dieffenbachia maculata* the coconut coir-based medium had greater water-holding capacity than the peat (Evans and Stamps, 1997). Our results were been opposite results. Maybe, the BRT® is being affected the peat and the coconut coir.

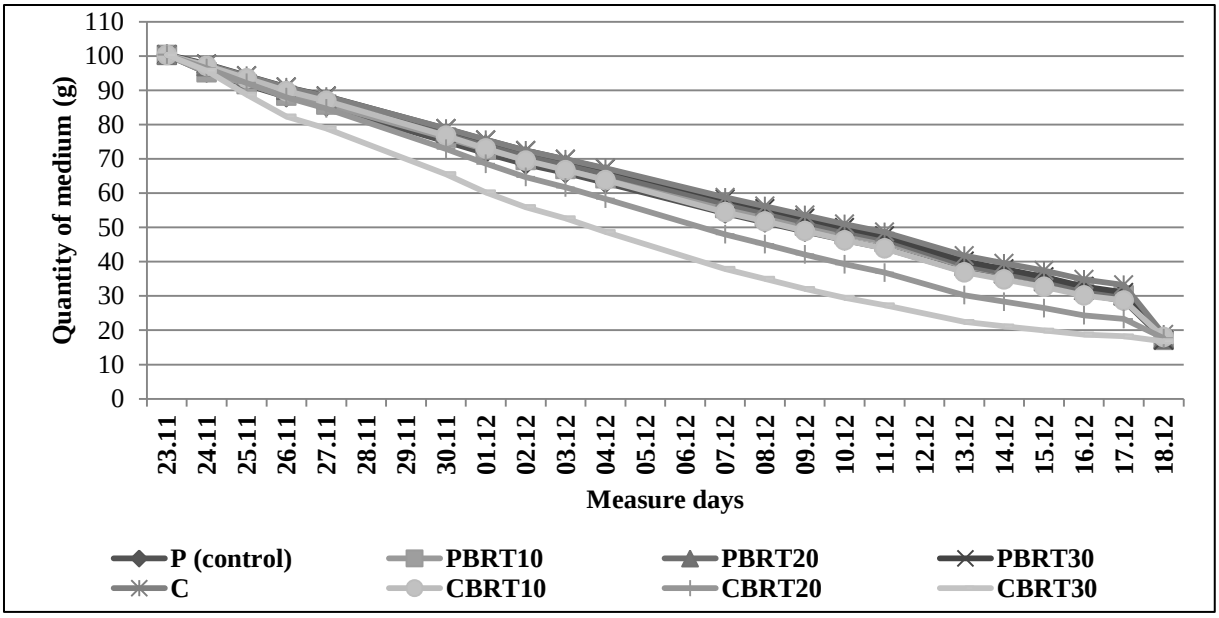

Figure 3 The drying out of the medium

Conclusion

Plant groups treated with BRT® EverGreen and Fainsoil Bioactivator. The morphological characteristics showed significant differences. The plants in treatment groups with mix of coconut coir, BRT® and FBA were higher and wider than in the mix of peat, BRT® and FBA. CBRT20FBA and CBRT30FBA group (10 cm) were 2 cm higher than the control group. The treatment groups with mix of peat, BRT® and FBA had wider leaves and larger flowers than groups with mix of coconut coir, BRT® and FBA. These are remarkable in practical aspect as well. These results may be important for growers to improving the most important morphological characteristics during the ornamental plant production.

The physiological conditions showed differences. The chlorophyll and carotenoid content of the leaf is an important marker of the physiological state of the plant. The mix of coconut coir, BRT® and FBA resulted higher chlorophyll and carotenoid content than the mix of peat, BRT® and FBA. The peroxidise enzyme activity shows the stress state. The groups in 20% BRT® showed the most stressful state.

The mixture of coconut coir with BRT® and FBA contained more bacteria than the peat. Mixture of peat with BRT® had higher water-holding-capacity than the group of coconut coir.

Summarizing, further experiments suggested. Using of these agents is recommended on *Viola x wittrockiana* ‘Carrera’.

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Growth characteristics of urban trees in Budapest

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Abstract
Modelling the capacity of urban trees in environmental benefits (e.g. CO_2 sequestration, vapor release, shading, deposition of air pollutants) requires models on their growth characteristics and leaf canopy development. In our project we selected for the measurements from among *Acer*, *Fraxinus* and *Tilia* species and varieties around 40 trees with different age and measured trunk and canopy characteristics for setting up models for their growth characteristics over the lifespan. Regression analysis was used to evaluate the relationship between trunk circumference and crown volume, crown projection area, tree height and leaf area index. Statistically significant relationship was found between trunk circumference and crown volume, crown projection area and tree height. Further on LAI development showed correlation to trunk circumference, however LAI might be influenced by other factors (e.g. environmental and year effect) too. On trees of four taxa (*Acer platanoides* L., *A. platanoides* 'Globosum', *Tilia cordata* Mill. and *T. tomentosa* Mill.) seasonal course of leaf area index (LAI) was also measured in 2013 and 2014. It can be well observed on all of these four taxa that there are significant differences in leaf falling period compared the two years, however sprouting was detected in same time.

Keywords: canopy modelling, canopy volume, leaf area index, seasonal course, trunk circumference

Introduction

Urban trees play important role in green spaces due to their environmental benefits such as carbon dioxide fixation, oxygen and vapor release, modification of microclimate, screening of noise and deposition of air pollutants. It is commonly accepted that urban trees diminish the air pollution, but the interactions between atmospheric contamination and trees may involve both positive and negative effects. The above environmental benefits of urban trees are provided mainly by the leaf canopy, which shows yearly course and typical growth performance by tree ages (Hrotkó et al., 2014).

In the frame of a larger project we plan modelling of carbon dioxide fixation, vapor release, and deposition of air pollutants on the most commonly planted urban trees as *Acer, Fraxinus* and *Tilia* species and varieties. Modelling the capacity of urban trees in environmental benefits requires first models on their growth characteristics and leaf canopy development. Although measurements by sophisticated equipment may provide actual data for a given site (Fernández-Sarría et al., 2013) but our planned modelling would need exact data on trees' canopy, dynamic performance of canopy characteristics over the season and over the lifespan. These data on deciduous tree species obviously show specific dynamics over the lifespan of the tree species as well as a yearly course of leaf mass and surface development during the season (Hrotkó et al., 2014; Tóth et al., 2014, 2015). Further on the overview on the planted species in Budapest (Szaller et al., 2014) showed that *Acer, Fraxinus* and *Tilia* species and varieties are widespread planted in Budapest urban area but the lifespan of recently planted trees is rather short, in average is around 30 years. This is traced back to urban environment (air pollution, salting under wintertime, injuries by human, etc.).

In the literature there are found very few data on growth dynamics, canopy covered surface, canopy volume, leaf mass and LAI (leaf area index) of investigated tree species. Niklas (1995) provides correlation between stem diameter and tree height in the case of *Robinia pseudoacacia* and other 12 species, Duchaufour (1903) (cit. Hemery et al., 2005) shows results about the relationship between crown diameter and stem diameter (*K/d* relationship) in *Fagus*, while Hâruţa (2011) and Paganová et al. (2015) between stem diameter and crown volume (*Quercus pertea* and *Sorbus domestica*), but these papers do not give information about taxa investigated in present paper. Schmidt (2003) gives estimated data on the expected canopy height and diameter. Detecting trends in tree growth is also difficult job, because growing speed is changing during the life of the tree: initial fast growing slows down later (Bowman et al., 2013). Data on the leaf mass and leaf area development over the season are not found in the literature. Results of above authors suggest possible correlation of the most important growth and leaf canopy characteristics to the stem circumference, which is an easy measurable parameter of trees.

In our project we selected for the measurements from among *Acer, Fraxinus* and *Tilia* species and varieties around 5 trees from 8 age groups and measured trunk and canopy characteristics for setting up models for their growth over the lifespan. The age of the trees was estimated by the method of Radó (1997). Further, we measured the leaf mass and LAI development over the season.

Our paper presents the data and growth models of the investigated tree species under Budapest site conditions within the lifespan of measured trees.

Materials and methods

Investigated taxa

Acer platanoides L.

Native to Europe with continental centred distribution. It can characterized by fast juvenile growing. Large leaf surface with fine venation evaporates a lot, therefore it requires much moisture. It prefers mildly acidic soil and moderate humidity. Shade tolerant during its youth, but later its light demand increases. Since it tolerates flue gases and salinization quite badly, it can use only in low traffic streets in urban forestry (Krüssmann, 1986; Dirr, 1996; Schmidt and Tóth, 2006).

Acer platanoides L. 'Globosum'

It is 3-5 m high tree, the canopy is flat globose with diameter 5-6 m. Nurseries produce it mostly as top-grafted tree. Due to its smaller canopy size it may be planted under overhead powerlines as well, therefore it is perfect as street tree. Leaf characteristics and other attributes are the same as in the case of *A. platanoides*. (Krüssmann, 1986; Schmidt, 2003; Schmidt and Tóth, 2006; Tóth, 2012).

Tilia cordata Mill.

Native to Europe, forming climax forests on moist soil. Its growing is slow. Its drought tolerance is relatively good, but tolerates urban stresses badly. In air polluted urban environment mass appearance of spider mite has high risk. Prefers deep, calcareous, humid and rich soils. Its heat demand is moderate, and not sensitive to low winter temperatures (Schmidt and Tóth, 2006; Johnson and More, 2007; Radoglou et al., 2008; Tóth, 2012; Tóth et al., 2015).

Tilia tomentosa Mill.

Native to Asia Minor and South-East Europe forming mixed deciduous and evergreen forests. Prefers humid, deep, calcareous, humous, rich, loess, loam, or sandy soils, but it adapts also to unfavourable conditions well. It is moderately drought tolerant, thermophilous, shade tolerant during its youth, later light demand, fast-growing species. Although spider mite does not damage its foliage, it is sensitive to salinity (similar to

other linden species). Out of linden species it tolerates urban conditions the better. (Dirr, 1996; Schmidt and Tóth, 2006; Radoglou et al., 2008; Tóth, 2012, Gülz et al. 2014).

Fraxinus excelsior L. 'Westhof's Glorie'

Fraxinus excelsior is native to Europe, Asia Minor and Caucasus forming mixed deciduous forest mainly with beech hornbeam and oak. 'Westhof's Glorie' is fast-growing variety, according to some authors 20-25, while to others 30-40 m high tree. It is moderately warm, water and nutrient demanding. It requires many light and calcareous soils. Thanks to its quick growing and good tolerance against urban conditions it is wide used in urban forestry (Schmidt and Tóth, 2006; Johnson and More, 2007; Dobrowolska et al., 2011; Tóth, 2012; Douglas et al., 2013).

Fraxinus ornus L. 'Mecsek'

Fraxinus ornus originates from southern Europe and Asia Minor mainly in dry thickets, open woodland forests on karst. 'Mecsek' is 6-8 m high tree with globular, later oblate crown. It grows well in calcareous, well drainaged, moderately dry soil and sunny place. Its main value is the drought tolerance, and tolerance against urban conditions, so it may be used as street tree. Disadvantage that its leaves languish in drought spectacularly; however after rain or irrigation they recover. This species is supposed to be resistant against bacterial disease of ash caused by *Pseudomonas savstanoi* pv. *fraxini*. (Schmidt and Tóth, 2006; Gortan et al., 2009; Fini et al., 2012; Tóth, 2012).

Examined specimens are located in Budapest and in the outskirts of the city (within 20 km from city centre), they were appointed randomly. Every tree stood in heavy traffic areas; they habitats can be divided into 3 different types:

- heavy traffic on both side of tree (tree stands e.g. on green median divider or between main road and service road),
- heavy traffic on one side of tree, building at the other side (tree stands within 5 m from road),
- heavy traffic on one side of tree, open space (e.g. park) at the other side (tree stands within 5 m from road),

Seasonal leaf area index (LAI) development was measured on trees standing in Experimental Farm of Szent István University in Soroksár, Budapest.

Trunk and canopy characteristics measurements

The following tree parameters were investigated: trunk circumference, trunk height, tree height, crown diameter, crown projection area, crown volume and leaf area index (LAI). Data were collected in the middle of summer when tree growing stopped. Trunk circumference was measured by measuring tape at height 1 m above the ground. Crown diameter was measured perpendicularly in two directions (d_1, d_2) by measuring tape, as well. For measuring tree height (total height of tree from the base to the uppermost tip of tree) and trunk height (height of unbranched trunk measured from the base) Leica DISTO D510 laser distance meter was used. Crown projection area (A_c) was calculated with the following equation:

$$A_c = (d_1 / 2) \times (d_2 / 2) \times \pi$$

Crown length (l_c) was determined as the difference between tree height and trunk height. For calculation of crown volume three different equations were used according to Coder (2012) and Fernández-Sarría et al. (2013):

- Spheroid: $V_c = d_1 \times d_2 \times l_c \times 0.5236$
  applied for trees with spherical crown (*F. ornus* 'Mecsek')
- Elongated spheroid: $V_c = d_1 \times d_2 \times l_c \times 0.5891$
  applied for trees with ovate crown (*A. platanoides*, *F. excelsior* 'Westhof's Glorie', *T. cordata*, *T. tomentosa*)
- Hemispherical crown: $V_c = (\pi \times d_1 \times d_2 \times l_c) / 12$

applied for trees with hemispherical crown (*A. platanoides* 'Globosum')

Leaf area index (LAI) measurement

For measuring leaf area index (LAI) AccuPAR LP-80 PAR/LAI ceptometer (Decagon Devices, Inc.) was used. For correlation models LAI was measured on appointed specimens parallel with tree survey in the middle of summer. For following seasonal LAI development measurements were done monthly, in the sprouting and leaf falling period in every two weeks. This examination was done in Experimental Farm of Szent István University in Soroksár, Budapest.

Measurement process was as follows: one above-canopy PAR measurement was done on every tree. Number of measurements below the canopy was depended on crown diameter: over 2 m eight, under 2 m four measurements were done. Measurements were distributed radially around the trunk. Value of below-canopy PAR was given as the mean of these measurements. LAI was measured on trees with trunk circumference over 15 cm.

Statistical analysis

Regression analysis was applied to evaluate the relationship between trunk circumference and crown volume, crown projection area, tree height and leaf area index. For statistical analysis different linear regression models were applied. Those data, which were outside of prediction intervals of the model at 95% confidence level were eliminated. Software IBM SPSS Statistics 22 was used for mathematical and statistical assessment.

Results

Correlation of trunk circumference to crown volume, crown projection area, tree height and leaf area index of investigated species over their lifespan

Trunk circumference correlated highly with development of crown volume in the case of every taxa (Table 1). R^2 values were between 0.91 (*A. platanoides* 'Globosum') and 0.974 (*Tilia cordata*). Every cases power model was applied.Correlation was also very strong with crown projection area. Model was the strongest in *A. platanoides* (R^2 = 0.988), while in *A. platanoides* 'Globosum' the weakest (R^2 = 0.888). Power model was used also in every taxa unless *Fraxinus excelsior* where linear relationship was established.

Table 1 Correlation between trunk circumference, crown volume and crown projection area of the investigated taxa (***: SL < 0.0001)

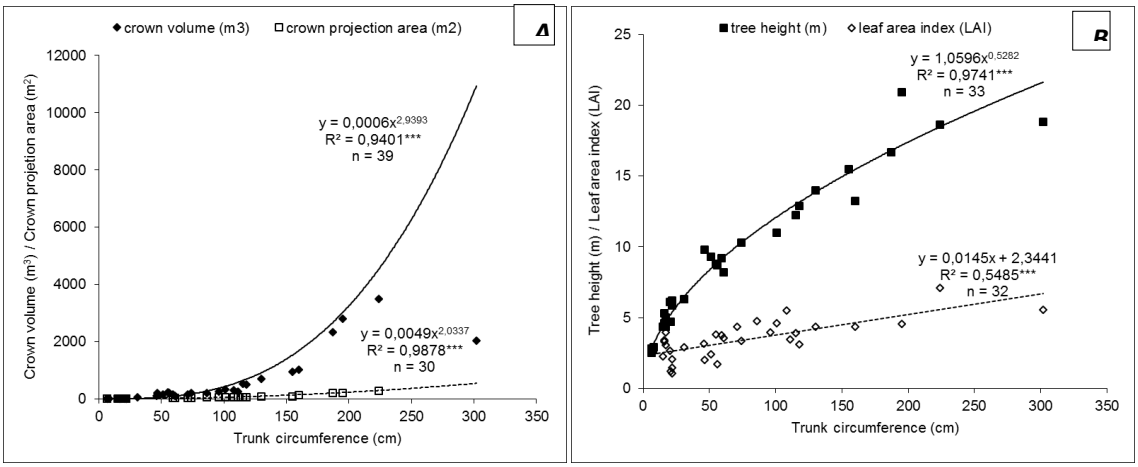
	crown volume (y; m^3) in function of trunk circumference (x; cm)			crown projection area (y; m^2) in function of trunk circumference (x; cm)		
	Model	R^2	n	Model	R^2	n
Acer platanoides	$y = 0.0006x^{2.939}$	0.94***	39	$y = 0.005x^{2.034}$	0.988***	30
A. platanoides 'Globosum'	$y = 0.0002x^{2.823}$	0.91***	36	$y = 0.005x^{2.04}$	0.888***	36
Fraxinus excelsior	$y = 0.011x^{2.216}$	0.969***	24	$y = 0.992x - 11.607$	0.978***	22
F. ornus 'Mecsek'	$y = 0.0003x^{2.873}$	0.955***	23	$y = 0.003x^{2.092}$	0.959***	23
Tilia cordata	$y = 0.0006x^{2.866}$	0.974***	35	$y = 0.002x^{2.246}$	0.97***	35
Tilia tomentosa	$y = 0.0021x^{2.537}$	0.967***	31	$y = 0.013x^{1.788}$	0.953***	31

Table 2 Correlation of tree height and leaf area index (LAI) of the investigated taxa in function of trunk circumference. (***: SL < 0.0001; **: SL< 0.005; *: SL < 0.01)

	tree height (y; m) in function of trunk circumference (x; cm)			leaf area index (LAI) in function of trunk circumference (x; cm)		
	Model	R^2	n	Model	R^2	n
Acer platanoides	$y = 1.06x^{0.528}$	0.974***	33	y = 0.015x + 2.344	0.549***	32
A. platanoides 'Globosum'	$y = 1.309x^{0.323}$	0.856***	36	$y = 0.646x^{0.561}$	0.463**	34
Fraxinus excelsior	$y = 1.17x^{0.48}$	0.975***	23	$y = 1.678x^{0.15}$	0.529**	12
F. ornus 'Mecsek'	y = 0.047x + 2.644	0.951***	25	$y = 0.452x^{0.494}$	0.407***	26
Tilia cordata	$y = 0.852x^{0.53}$	0.937***	35	y = 1.822ln(x) – 1.975	0.392**	16
Tilia tomentosa	$y = 3.935\exp^{0.007x}$	0.885***	31	$y = 1.1725x^{0.269}$	0.297**	29

Strong relations (R^2 between 0.856 and 0.975) was confirmed between trunk circumference and tree height, too. Mostly, power model was the better to describe relationship, however in case of *F. ornus* 'Mecsek' linear, and in *Tilia tomentosa* exponential model was applied (Table 2).

Leaf area index (LAI) showed lower relationship with trunk circumference (R^2 between 0.297 and 0.549). Power model was used in 4 taxa, while in *A. platanoides* linear and in *Tilia cordata* logarithmic relation was detected.Figure 1 display the functions in graphical form of most representative two taxa *Acer platanoides* and *Tilia tomentosa*.



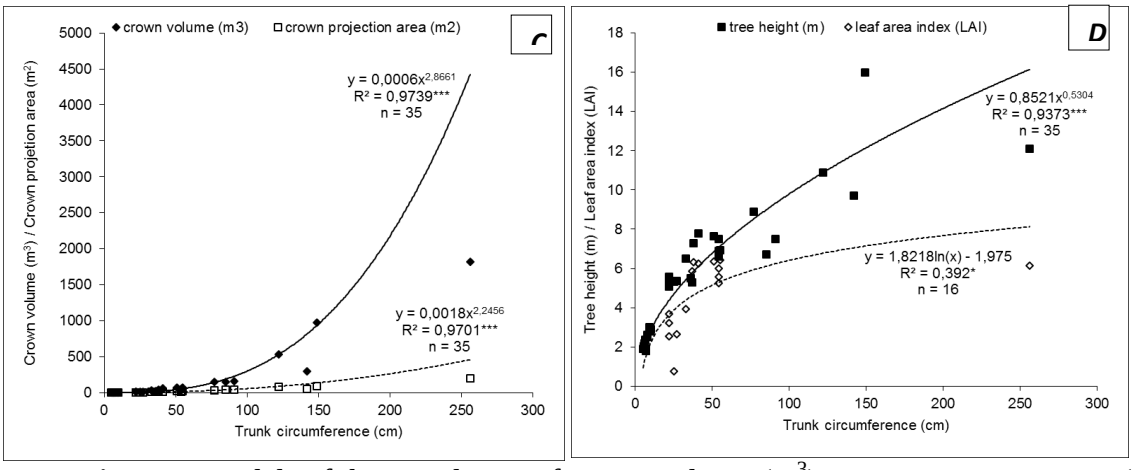


Figure 1 Models of the correlation of crown volume (m^3), crown projection area (m^2), tree height (m) and leaf area index (LAI) in function of trunk circumference in the case of *Acer platanoides* L. (A-B) and *Tilia cordata* Mill. (C-D). (***: SL < 0.0001; *: SL < 0.01)

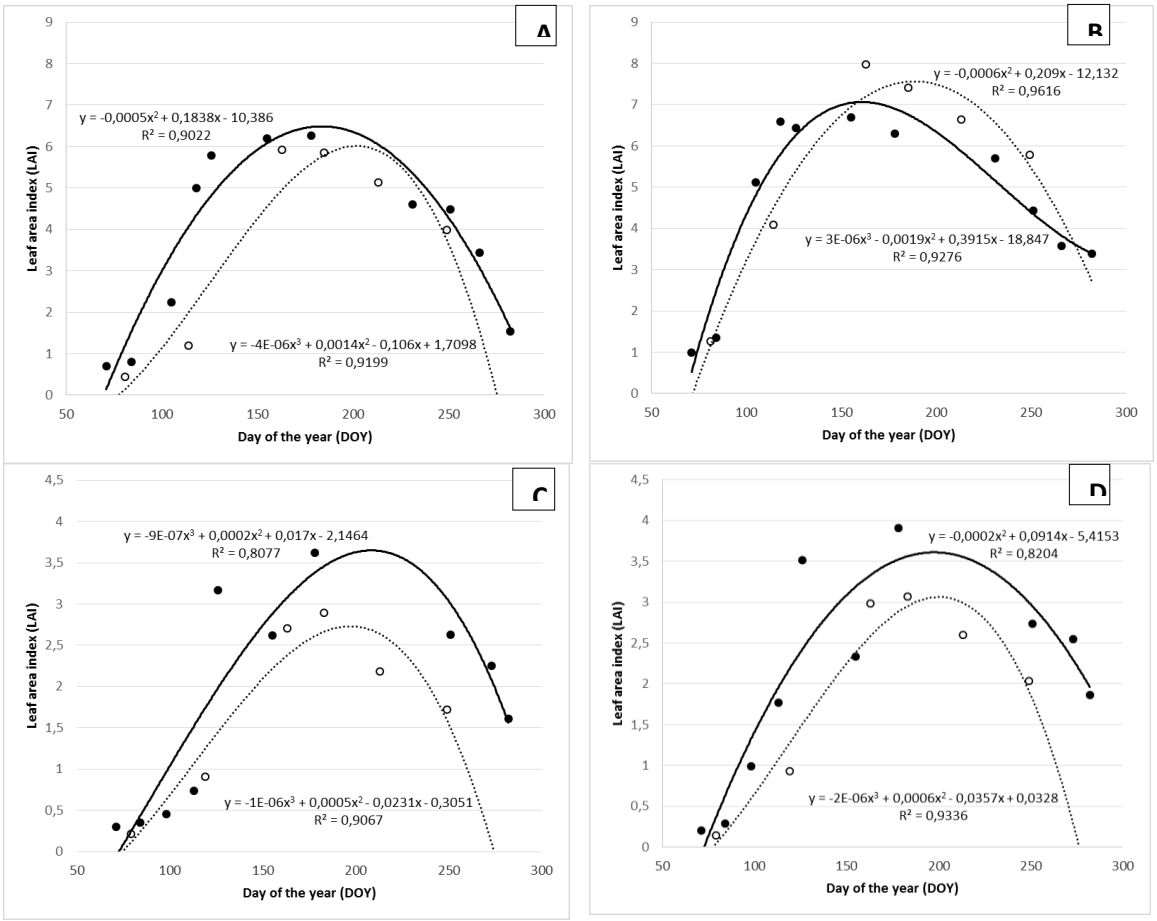


Figure 2 Seasonal development of leaf area index (LAI) on *Acer platanoides* L. (A), *A. platanoides* 'Globosum' (B), *Tilia cordata* Mill. (C) and *T. tomentosa* Mill. (D) in 2013 (dotted line and empty circle) and in 2014 (solid line and filled circle).

Leaf area index (LAI) development of investigated species over the season

On trees of four taxa (*Acer platanoides*, *A. platanoides* 'Globosum', *Tilia cordata* and *T. tomentosa*) seasonal course of leaf area index (LAI) was measured in 2013 and 2014. Results are shown on Figure 2.

The LAI on two examined *Tilia* species showed very similar performance in both years. They had lower LAI in 2013 (peak around 3), while in 2014 both taxa had LAI maximum between 3.5 and 4. It can be well observed there are significant differences in leaf falling period compared the two year, however sprouting was detected in same time.
Acer platanoides and *A. platanoides* 'Globosum' had much higher LAI maximum. It was next to 6 in case of *A. platanoides* in both years and between 7 and 8 in *A. platanoides* 'Globosum'. Later leaf falling can be detected on these taxa as well in 2014.

Discussion

It is very important to know growth characteristics of trees in urban conditions; however the most published papers give data about just one or two growth parameter(s) and not in urban conditions. Our measurements and data analysis provide information about growth characteristics of six important taxa in urban forestry and correlation models for canopy size, and LAI_{max}. Further on our data provide valuable information on the course of LAI development over the season under urban conditions.
Hâruţa (2011) found linear relationship between stem diameter and crown volume in *Quercus petraea* (Matt.) Liebl. forest stands. It is supported by the data of Duchaufour (1903) (cit. Hemery et al., 2005) in *Fagus sylvatica* L. forest, where linear relationship was found as well between stem diameter and crown diameter. In contrary Paganová et al. (2015) described exponential relationship between these two parameters on *Sorbus domestica* L. urban trees. Looking at our data the relationship between trunk circumference and crown volume by exponential function was described in the case of both taxa which confirms Paganová et al. (2015). This different performance of growth characteristics might be caused by the different site conditions between forest stands and under urban conditions.
However, in the case of LAI development relationship is not too close, because LAI is determined not only by the growth of the tree but also by other factors (e.g. environmental and year effect) (Vose and Allen, 1988). It is supported also by the difference of annual development of LAI in the examined two years (Figure 2): it was especially significant in the case of the two *Tilia* species, however variance in the beginning of leaf falling period could be observed in both taxa.
Our investigations show due to the strength of models that the measurements of trunk circumference explain well the development of the examined factors. Further on using our models provide useful data about canopy size development for such sophisticated modelling for the local scale simulations of the atmospheric dispersion of air pollutants among buildings and trees (Lagzi et al., 2004, 2006; Mészáros et al., 2006, 2009a, 2009b, 2011; Molnár et al., 2010). Regarding geometry of different arrangement of buildings and trees, using our in situ collected data and functions, applicable in the model simulation simplifies the measurements need for the complex model of interactions in the "plant – air pollution – urban site" multiple system.
Our results would assist for professionals (landscape architects, horticulture engineers) in urban planning with more detailed data on tree size development over the lifespan and LAI development over the season.

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Production of spice pepper (*Capsicum annum* L. var. *longum*) in Slovakia

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Abstract
Red spice pepper is presently the most grown spice plants in Slovakia. This pepper was occupying most areas in the 80s. The record area was reported in 1991 (2 546 hectares). Then there was a significant decrease in a downward trend of culinary peppers growing in Slovakia lasts until today. Since 1989, the highest production achieved in 1991 (3,080 tons). In 1997 the total harvest was 666.3 tons and from 1999 until now, the production is declining. In 2015 has produced 98 t of red spice pepper an area of 68 ha and fecundity was 1.45 t ha $^{-1}$. Most of red pepper is grown in the Nitra and Trnava regions (Rozborilová, 2014)..

Key words: red spice pepper, cultivated areas, production, yield

Introduction
Fruits of red spice pepper contain about 0.04 to 1.5 percent of alkaloids, in particular capsaicin and its derivatives, which cause a hot, burning taste (Ivanišová, 2014). In peppers are present pro-vitamin A, alpha and beta-carotene and cryptoxanthin. Present is also vitamin B1 and B2 and 150 up to 400 mg.100g^{-1}of important vitamin C (Hegedűsová et al., 2016). In fruits of the pepper is also a quantity coloring matters, mainly capsanthin (2.5 - 11.5 g.kg^{-1}). The consumption of sweet spice pepper is higher than the consumption of hot spice proper. The production of sweet paprika in Slovakia is mainly in hands of private farmers (Valšíková et al., 2015).

Materials and methods
The essence was to process all available data on the cultivation of spice pepper in Slovakia. We used the evaluation reports of the Statistical Office of the Slovak Republic and other professional and scientific publications.

Result and discussion
The spice pepper contains more hundreds compounds in different concentrations. These compounds give to peppers its specific features as flavor, aroma, **color** (Luning, Yuksel and Vries, 1995)**.** We can find in spice proper mainly carbohydrates, lipids, proteins, cellulosic substances, minerals, vitamins and organic acids which enrich paprika with a great nutritional valuem (Holub et al., 1999; Bosland and Votava, 2013).
The production of spice pepper was extended in more areas in the past. Cultivation has been extended on larger areas in regions Nitra, Trnava, Banská Bystrica and Košice. Today, the cultivation of spice pepper is only limited to the Nitra region, Trnava region and Košice region.
In Table 1, graph 1 and 2 is shown course of growing of spice pepper since 1989, includes information about harvested area, total harvest and yield in individual years. The biggest areas (above 2000 ha) were in the 80s. The first decline in production was recorded in the 90s and the trend has been continuing up to now. Nowadays, only about 68 ha of spice peppers are recorded on arable land. The production has been decreasing in accordance with the decrease in areas too. In comparison with 1991 when 3080 t of fresh spice pepper were harvested, the

production from the year 2015 represented only 98 t. The productivity in $t.ha^{-1}$ has been fluctuating from 0.56 to 1.89 $t.ha^{-1}$.

Table 1 Evalution of red spice pepper production in Slovakia

Year	Harvested area (ha)	Yield (t)	Production ($t.ha^{-1}$)
1989	2 204	2 116	0.96
1990	1 835	1 029	0.56
1991	2 546	3 080	1.21
1992	1 480	1 388	0.94
1993	1 031	1 218	1.18
1994	1 070	1 340	1.25
1995	1 343	1 727	1.29
1996	959	1 090	1.14
1997	552	666	1.21
1998	718	1 024	1.43
1999	560	827	1.48
2000	536	540	1.01
2001	333	482	1.45
2002	272	378	1.39
2003	254	228	0.90
2004	460	450	0.98
2005	430	538	1.25
2006	195	225	1.15
2007	152	181	1.19
2008	105	87	0.83
2009	60	73	1.20
2010	65	97	1.50
2011	49	48	0.98
2012	27	51	1.89
2013	36	52	1.43
2014	42	72	1.69
2015	68	98	1,45

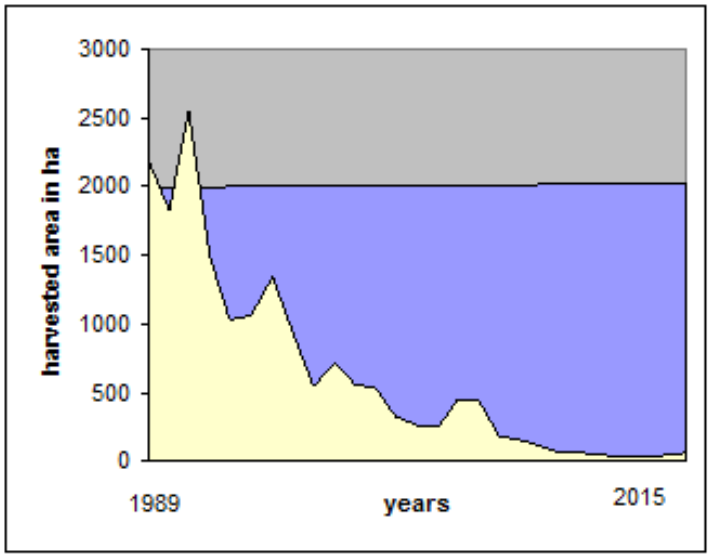

Figure 1 Harvested area of red spice pepper in last 27years (ha)

The domestic consumption of spice pepper per capita is increasing and nowadays it is around 100 – 150 grams. Even if the consumption is increasing, production has been decreased. Decreased total yield is replaced by imports of cheap foreign spice peppers, often of lower quality.

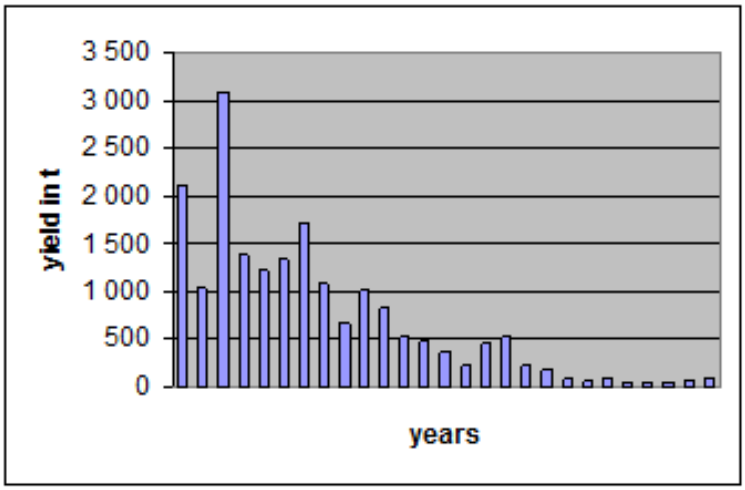

Figure 2 Total yield of red spice pepper in last 27 years (t)

Conclusion

Paprika becomes popular again and paprika is required on both national and international markets. Slovak paprika is renomous for its unique properties, compared to paprika coming from abroad.

Especially Slovak spice pepper – Žitava is highly appreciated in the world. Spice pepper Žitava is protected designations of origin and protected geographical indications by the European Commission. Spice pepper Žitava can become a new opportunity for Slovak agriculture in international competition.

The product known as pepper Žitava was entered in the register of protected designations of origin and protected geographical indications by the European Comission on the 11th of February 2014 and published in the Official Journal of the European Union.

„Pepper Žitava“ is sweet ground spice paprika made by grinding of dried spice peppers, that are harvested in the area Podunajská nížina (Danubian Lowland) from state- recognized varieties developed and harvested in ripe flawless condition and processed in a specific post-harvest treatment.

The final phase of grinding on a so called „coloring stone“ gives the product its intense color. The pressure used in this process increases heat and lipids that are released from the seeds cause the orange-red coloring.

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Cultivation of Japanese horseradish (*Wasabia japonica* (Miq.) Matsumara) under polycarbonate cover in Poland

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Abstract
Studies were carried out to verify a possibility to cultivate Japanese horseradish (*Wasabia japonica* (Miq.) Matsumara) under covers in Poland. The transplants needed for the experiment were purchased in the Great Britain. They had been propagated in artificial conditions. They were then grown in rows and in pots, in a polycarbonate greenhouse on peat and sand medium, with drainage and closed water circulation. During the vegetation period, some biological characteristics of the plant, as well as changes in chemical composition of both leaves and the medium were measured. The wasabi plants propagated in the Great Britain were successfully used in cultivation under a polycarbonate greenhouse in Poland. At the beginning, the best results were received for the plants grown in the pots. However, after 5 months of vegetation, the plants were already at the same stage of development in both methods. Moreover, they did not tolerate direct exposure to sunlight. Those grown in rows had bigger and greener leaves than the ones grown in pots. The methods of cultivation did not affect the phosphorus (P) and the potassium (K) levels in leaves. However, the plants grown in rows had more calcium (Ca) and magnesium (Mg) and less organic nitrogen (N) than the ones grown in pots. During the vegetation, the plants took P, K, and chlorine (Cl) from the soil and, at the same time returned Ca and Mg to it. The plants growing alongside the water stream developed better than the ones which were growing near water. The plants cultivated in Poland produced flower stalks and bloomed. The experiment needs to be repeated with bigger number of specimens and should be extended to seed setting and plant yielding.

Keywords: *Eutrema wasabi* Maxim, *in vitro* wasabi plants, elements changes in wasabi plants, carbonate greenhouse in wasabi production

Introduction

Wasabi, also known as Japanese horseradish (*Wasabia japonica* Matsumara or *Eutrema wasabi* Maxim), is a vegetable originated from Japan (Ma et al., 2009). The first mention of this plant can be found in a Japanese medical encyclopedia called Honzo-wamyo, published as early as 918 A.D. The locals referred to it as "wild ginger". However, its medicinal properties were first described by Sukahito Fukae (Hedge, 1974). The first documented location dedicated to the plant is believed to have been the town of Utogi, situated along the upper bank of river Age in Shizuoka prefecture in Japan (Anonymous, 2015). Wasabi belongs to the *Brassicaceae* botanical family (Chadwick et al., 1993). Morphologically, it most resembles our common variety of horse radish (*Armoracia rusticana* G. M. Sch.), quite widespread in Europe and North America. In Japan, it is anherbaceous perennial, with heart-shaped pointy leaves of radiant green color. Its stems reach up to 30-50 cm, measuring from the root. In its main segment, it is between 2 and 4 cm thick and can be as long as 5-20 cm (Anonymous, 2015). Its flowers are white, with four petals, raised sepal and straight stigma (Chadwick et al., 1993). The blooming begins in fall and may and can last until early spring - depending on the growing conditions provided. Each stem can set up to a few flower spikes

(Palmer, 1990).Usually, the developed fruit - silique - can produce up to 8 seeds. Each of them is about 2-3 mm long, 1 mm wide and covered by very thin coat (Chadwick et al., 1993). Fresh seed is naturally dormant and remains in that state until it is vernalized by a period of low temperatures. In recent years, the species has been commonly cultivated through vegetative propagation, tissue culture and fragmentation; seeds are a rarer choice (Miles, Chadwick, 2008). The use of wasabi for health curing has been known for years but not yet completely proved in research (Anonymous, 2015).
At present, wasabi is grown in many other countries across the world, such as: New Zealand, Taiwan, South Korea, Israel, Brazil, Thailand, Columbia, Canada, USA, the Great Britain and China (Forde, 1982; Van Mellaerts, 2015). The vegetable has never been cultivated commercially in the Central Europe, including Poland.
The main purpose of the experiment was to find out if the Japanese horseradish can be grown commercially in Poland.

Material and Methods

All seedlings of Japanese horseradish used in the experiment were obtained in May 2015 from Wasabi Co., a British branch of the Japanese company Miyoshi. The used plants were propagated through tissue culture and then sent to Poland. Next, they were planted in 9 cm pots. For the first month they were kept under a foil tunnel in order to adapt to Polish conditions. In such protective conditions, the plants eventually acclimated and grew considerably, thanks to which they responded well to the final replanting. In order to conduct the experiment, it was necessary to provide a separate construction that would make it possible to maintain specific parameters of humidity and temperature. This was because in Japan the wasabi is originally cultivated in river valleys or foil tunnels where the temperature does not exceed 20°C. Due to differing climatic conditions in Poland, it was decided to build a special polycarbonate construction which enables partial control over the plants' growing conditions. The entire surface within was covered the thick foil in order to prevent water loss.
In order to prepare the ground for wasabi cultivation, stones of 7-10 cm in diameter were laid out on the foil to serve as drainage. To obtain the optimum pH of 6.0-7.0, the soil consisted of 70% sphagnum peat, 25% slow-release pine bark, and a small addition of fine gravel and about 800 kg of fine stone per 1 ton of the soil. Water circulation in the experiment was closed and automated with the use of a pump device. The water from the residual tank was pumped into the drainage pipe which directed the water to the starting point and reintroduced it to the watering system, making the water flow down the slope back again. The closed circulation system was implemented as a means of minimizing the cost of water, drawn from a nearby drilled well. The water was replaced on a weekly basis, thanks to the piping system installed. The summer of 2015 turned out to be extremely hot for Poland. To protect the plants from high room temperature, an impulse sprinkler was placed in the tunnel. Thanks to this solution, the possible lowest temperature was ensured, which combined with the polycarbonate structure minimized the heating up.
The experiment included two groups of plants: the first one, in which the plants were grown directly in the medium and the latter, in which they were grown in large pots. The carried out plant observations included: plants' condition after shipment from England, their optimal size, their hardening off, the plants' condition after replanting, growth of them after replanting, their resistance to frost and their blooming time.
During the vegetation of the plants, also chemical routine analyses of foliage and soil were done by the state chemical laboratory in Poznań.

Results and Discussion

Transplants' shipment: The plants were dispatched on 29th April 2015 and were shipped to their destination, Poland, the very next day. Thanks to swift transport and proper protective packaging of the plant material, the Japanese horseradish seedlings were in a pretty good condition.
Optimum seedling size: The seedlings were very similar in terms of size. All plants had a very well-developed root system and foliage.
Acclimating the seedlings: in order to adapt the seedlings to Polish climatic conditions, they were planted in pots (9cm in diameter each) and placed in a foil tunnel, where the temperature was kept at similar level throughout the day. This enabled observation and gradual transition to less and less controlled temperature.

Photo 1 Wasabi transplants in rows (left and middle) and pots (right) after planting on a permanent place. Between the rows, there was permanently circulating water. The picture was taken on 28thJune, 2015, in the village Marzenin near Września (Western Poland)

Seedlings replanting: the seedlings, after being initially planted in pots, were consequently replanted to their permanent growing site on 28th June, 2015 (photo 1).The plants obtained in laboratory conditions were quite brittle, with very well developed roots. They were in very good biological condition (were fully fit for huge farming operations) and required hardening off before replanting, they showed regenerative properties. The plants were planted in double rows and pots measuring 26 cm in diameter. Plants' growth and formation of new leaves:
Somewhere between 28th June and 28 July it could be concluded that the plants adapted well (no losses recorded). Within two weeks, since they had been planted, the seedlings started growing, responded well to mineral fertilization and watering, as well as to fogging. At that time, the plants grew rapidly, which was measured on a basis of new leaves forming. The plants cultivated in rows adjacently to the water flow grew better than those who were merely situated near to the water (the potted ones).
Diseases and pests: In the course of the experiments, symptoms of following diseases were observed: chlorosis, mildew and black spot (*Alternaria brassicicola*). However, typical diseases described in Japanese literature, such as the rotting of the root caused by *Erwinia carotovora* or fungal infection caused by *Phoma wasabiae* (Ma et al., 2009), were not recorded.
The plants also suffered attacks from a few species of larvae, such as the cabbage butterfly (*Pieris brassicae*), as well as the larvae of *Otiorrhyn chusniger* and *Derocera sagreste*.
Resistance to frost: the frosty character of Poland's late autumn had no negative effect on

further growth and development of the plants. The material which was used to build the greenhouse effectively prevented the cold from getting inside. Blooming (photo 2) began five months after the seeds were permanently replanted, the first ever inflorescence was recorded on 2nd October, 2015. On 20th May,2016, the plants' blooming started again. Poorer light conditions, along with days becoming shorter caused a rise in number of leaves that were showing symptoms of aging. As the blooming started, so did the examination of roots' development.

Photo 2 Wasabi plants in the experiment with first flower spike. The picture was taken 2nd October, 2015, in Marzenin

Photo 3 Wasabi plants in fool winter blooming. The picture was taken on March 26th, 2016, in Marzenin

Number of leaves in plants from rows and from pots

The measurements taken were assessed statistically in specified iterations. A variance was calculated for all results obtained, while significant differences of the mean values were determined according to Duncan's test at $\alpha = 0,05$

One month after replanting, the number of leaves in pots was bigger than in rows. However, the measurements taken 5 months after replanting showed that not only did the plants in rows caught up with their potted counterparts in terms of average number of leaves, but they were also found to increase foliar growth. The conducted analyses have shown that the cultivation method had no effect on phosphorus (P) and potassium K) levels in wasabi leaves (table 2).

However the plants which were grown in rows showed a higher concentration of calcium (Ca) and magnesium (Mg) than the potted plants, as well as lower levels of organic nitrogen. The chemical analyses of the soil before cultivation (straight from the original packaging), compared with results obtained after six months of cultivation showed that the chemical composition of the soil changed (table 3). Its bulk density rose considerably, and so did the acidity, and levels of N, K and Cl (table 3).

Table 1 The effect of cultivation method on the number of leaves in a plant, measured 1 and 5 months after replanting

Method of cultivation	Observation time							
	1 month after replanting				5 months after replanting			
Rows	1	2	3		1	2	3	
	36	43.8	44.4	41.3333 a	164.2	125.5	161.2	150.3333 a*
Pots	1	2	3		1	2	3	
	48.6	49	50.4	49.3333 b	132.8	121.4	128.2	127.4667 a

*means followed by the same letters are not significantly different according to the Duncun's test for $\alpha=0.05$

Table 2 The influence of the cultivation method on the chemical composition of the plants' leaves

Method of cultivation	Chemical composition (%) – dry matter content				
	Organic Nitrogen	P	K	CaO	MgO
Rows	2.16	0.44	3.11	9.66	1.72
Pots	2.32	0.50	3.11	6.64	1.23

Table 3 The influence of wasabi cultivation on soil parameters

Type of soil	Bulk density (g/dm^3)	pH in H_2O	Chemical composition (g/soil)						Salinity $gNaCl/dm^3$
			N	O	K	Ca	Mg	Cl	
before cultivation	580	6.1	5	22	125	1356	90	21	0.30
during cultivation	740	8.0	11	18	35	3448	416	<11.5	0.36

Before even setting out to conduct the experiment, talking about it for many months, after contacting Japanese company, we found it enormously changeling, eventually, to get access to *in vitro* propagated wasabi plants. According to our field research, wasabi had never been grown by anyone or by any method, whatsoever, in Poland. In order to start the experiment, plants that had been artificially propagated were used, shipped from the British subsidiary of Miyoshi company (based in Japan). The artificially propagated transplants from the UK were then successfully implemented for cultivation in a polycarbonate greenhouse in Poland. The transplants supplied from the UK were of high quality and not even a single transplant went to waste.

The experiment has shown that the climatic conditions of Western Poland (near the town of Września) were suitable to grow Japanese horseradish under polycarbonate covers with partly controlled growing conditions. After 6 months of cultivation, the plants obtained were healthy and developed normally.

The wasabi plants cultivated in Poland did not tolerate direct exposure to sunlight. This observation is in agreement with the information provided by Douglas and Follet (1992), according to whom slightly shaded mountain valleys constitute the optimal growing environment to produce commercially wasabi. In our experiment, the plants cultivated in rows alongside the water flow grew better than those growing at a larger distance from the water (in pots). This observation followed data provided by many other researchers, e.g. such as Ma et al. (2009) or Chadwick et al. (1993).

In the experiment, two methods of cultivation were used: in rows and in pots. Initially, the potted plants grew more vigorously, however, the second measurement of growth showed that the number of leaves in row-based cultivation not only reached the level of potted plants, but eventually exceeded it. These observations should be extrapolated with caution due too relative small number of specimens used in the experiment. With such low number, statistical evaluation of the results was not possible. There is also no relevant data in the available English literature about the chemical composition of the leaves or the soil (namely, the changes in the soil caused by cultivation). Therefore, undoubtedly, the experiment should be repeated on a much larger amount of plants. A separate experiment should also be carried out regarding the seed formation and yielding.

Another area for research is also to determine the existence of varietal variability for growing conditions in Poland, and whether the plants which have bloomed or are to bloom in Spring will eventually produce seeds and whether the seeds will sprout. The information that I personally gathered during my visit to Japan in August 2015, seeds would be the easiest and most cost-effective method of wasabi propagation. In such case, however, Japanese experts (Anonymous, 2015) emphasize the need to very carefully select cultivars and cultivation sites. Fresh seeds are naturally dormant and remain in that state until they are vernalized by a period of low temperatures. Dormancy can also be broken artificially by soaking the seed in gibberellic acid (Palmer, 1990). Wasabi is a widely recognized condiment used world-wide, and the market for it is steady and the plant has never sold badly. The wholesale price of 1 kg of paste can exceed USD 100.

Conclusions:

1. Wasabi transplants, artificially propagated in the UK, were successfully planted and grown in polycarbonate greenhouse in Poland. Their quality was very good. All the provided transplants were used, without even a single loss.
2. It was possible to grow wasabi under polycarbonate cover in Poland. Both potted and rowed plants production methods can be used for it.

3. The plants cultivated in Poland did not tolerate direct sun light. The ones in rows had larger and greener leaves than those in pots. The cultivation method had no effect on the P and K levels in the leaves. However, the plants in rows were richer in Ca and Mg, with simultaneously lower levels of organic N. In the course of their vegetative process, the plants derived P, K, and Cl from the soil, while eliminating Ca and Mg.
4. The plants which grew alongside the water stream grew better than those situated just not too far from the water.
5. The plants cultivated in Poland produced flower stalks and they all bloomed.
6. The experiment needs to be repeated on a larger number of specimens and it should be extended to examine seed formation and yielding.

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Production of vegetables in Slovakia

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Abstract
Vegetable production in Slovakia was followed from the year 1989 to 2014. During this period, the highest total production (arable land + gardens) was in 1999. That time, the vegetables were cultivated on the area of 46903 hectares and 685379 tons were harvested. Average yield was 14.6 t / ha. Least of vegetables (284,429 tons) were produced in 2010. The lowest area (28320 ha) was occupying with vegetables in 2013. Largest share on production have sweet corn, garden peas, onions, and pumkins. Most of vegetables are grown in the Nitra and Trnava regions.

Keywords: vegetables, area, production

Introduction

Increased consumption of fruits and vegetables is closely linked to the reduction in the risk of various diseases (Jedlička, 2012). Significant preventive effects have antioxidants in vegetables (Mlček et al., 2015). Antioxidants are reacting with free radicals and inactivate them, thus preventing short and long term oxidative stress, which has an adverse impact on the development of diabetes, cataracts, cancers and cardiovascular diseases (Keresteš et al., 2011).

Results and discussion

Total harvested area of arable land and home gardens is estimated above 28000 hectares and together on this area produces more than 300,000 tons of vegetables (tab.1). Vegetables are currently cultivated on arable land of area around 7100 ha and production is approximately 108,000 tons (tab. 2) (Valšíková, 2015).
In 2013 the largest area of arable land was occupying by sweet corn (1424 ha) and garden pea (886 hectares). Onions, pumpkins, cabbages and tomatoes were grown on plots between 682 to 435 hectares. Another seven kinds were grown on 291 to 118 hectares and the remaining species on smaller areas than 100 ha. In 2015, the first place belonged to pumpkin. Behind it followed sweet corn, garden pea and onion (Table 3).
On arable produced most Nitra region, followed Trnava, Bratislava and Košice region. Production on arable land in 2015 is expressed in Table 4 and Figure 1.

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Table 1 Total area, production and average yield of vegetables on arable land and including gardens, since 1989

Years	Areas in ha	Production in t	Yield in t.ha^{-1}
1989	30 606	571 022	18.65
1990	29 964	496 576	16.57
1991	32 449	552 123	17.02
1992	31 291	459 073	14.67
1993	32 860	535 467	16.20
1994	34 195	483 524	14.10
1995	37 009	498 421	13.50
1996	38 389	559 588	14.58
1997	39 921	594 741	14.98
1998	42 157	593 025	14.07
1999	46 903	685 379	14.60
2000	43 834	468838	10.70
2001	32 896	406 064	12.30
2002	33 572	363 482	10.83
2003	34 538	368 847	11.00
2004	32 017	380 626	11.89
2005	30 241	353 567	11.69
2006	29 795	351 526	11.80
2007	28 870	307 756	10.66
2008	28 426	332 954	11.71
2009	28 547	312 084	10.96
2010	30 559	284 429	10.38
2011	30 334	314 855	11.49
2012	29 164	310 148	10.63
2013	28 320	325 378	11.49
2014	28 065	326 074	11.62

Data source: Rozborilová a klol. (2014, 2015, 2016),Statistical Office of Slovak Republic

Table 2 Production of vegetables on arable land (t)

Vegetables	2007	2008	2009	2010	2011	2012	2013	2014
Cabbage	20 160	18 904	14 729	11 252	15 571	12 079	14 957	16 304
Cauliflower	1 925	1 367	1 194	981	1 333	2 796	1 169	424
Broccoli	184	119	99	30	185	2 572	1 089	552
Savoy cabbage	713	437	543	508	476	190	280	109
Kohlrabi	919	457	495	378	688	583	484	449
Celery	576	581	398	340	303	375	550	552
Carrot	7 801	9 435	7 934	7 159	7 024	6 685	6 523	6 502
Parsley	1 755	3 257	2 124	2 601	3 657	5 602	4 496	4488
Beet root	514	402	426	610	872	468	421	707
Radish	86	82	131	46	140	125	53	58
Cucumbers - pickling	2 300	1 540	1 531	1 130	1 235	1 144	534	656
Cucumbers - salad	2 728	3 207	3 766	3 580	4 531	3 034	2 987	20608
Honey melons	568	329	455	190	321	375	384	440
Water melon	4 033	2 497	2 888	1 519	1 847	2 491	2 519	3038
Sweet pepper	7 815	5 108	6 727	4 073	6 266	5 746	5 413	5 083
Tomatoes	29 166	30 598	25 985	10 469	19 088	14 246	9 731	21 459
Onion	8 938	14 981	12 831	14 659	21 594	12 983	14 930	24 247
Garlic	435	91	84	104	269	118	228	41
Lettuce	163	459	435	306	152	221	241	223
Spinach	640	1 994	1 627	1 079	2 041	2 473	1 377	2 062
Peas	398	381	2 892	2 428	4 749	2 062	2 272	2 769
Asparagus	1 112	1 242	1 296	744	1 003	801	975	746
Sweet maize	4 966	18 743	6 887	9 432	11 648	9 913	8 527	12 610
Zucchini	770	706	555	181	582	534	85	498
Pumkin	421	771	976	3 676	2 776	3 013	559	1 480
Other vegetables	253	91	324	237	241	152	870	238
Total Vegetables	99 393	117 805	97 250	77 721	108 627	90 806	81 671	108 332

Meravá a kol., (2008, 2014, 2015),

Table 3 Harvested area of vegetables on arable land in 2013 and 2015 (ha)

Vegetables	Harvested area in 2013	Harvested area in 2015
Sweet corn	1424	1008
Garden pea	886	897
Onion	682	704
Pumkin	614	2248
Cabbages together	492	384
Tomatoes	435	574
Sweet pepper	291	322
Parsley	276	185
Asparagus	266	184
Carrot	243	312
Spinach	139	147
Water melon	132	119
Cucumbers	118	117
Cauliflower	74	52
Garlic	72	67
Honey melon	69	38
Kohlrabi	53	29
Celery	44	30
Broccoli	40	76
Beet root	22	61
Head salad	19	36
Savoy cabbage	14	8
Zucchini	11	25
Radish	8	15
Other vegetables	814	243

Table 4 Vegetable production according to regions on arable land in 2015

Regions	Production in tones
Bratislava	8659
Trnava	35387
Trenčín	1939
Nitra	37630
Žilina	544
Banská Bystrica	3570
Prešov	1656
Košice	3584

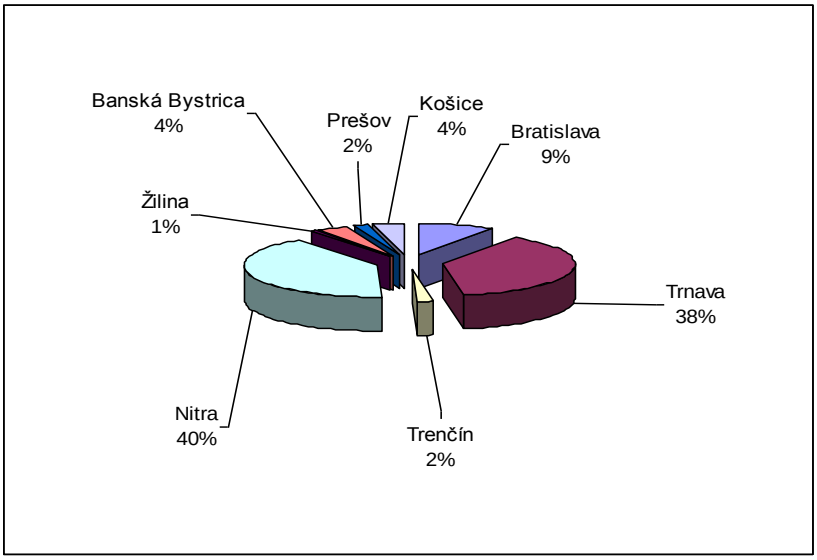

Figure 1 Production of vegetables by region in 2015 expressed in %

The effects of the use of modern SSL LED technologies in supplemental lighting of horticultural crops

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Abstract

Rising costs of energy and the concern for a natural environment contribute to the intensive search for alternative light sources that could replace high pressure sodium lamps (HPS), commonly used in horticulture cultivations. For that purpose, light emitting diodes (LEDs) are very promising light source. The aim of this study was to briefly review of the applications of LED lighting in horticulture. The results of some research concerning the effects of LED light with various spectral characteristics on plant growth and development are presented. The most effective in photosynthesis and photomorhogenesis is blue (400-500nm) and red (600-750nm) light due to the specific plant photoreceptors absorbing these range of wavelengths. Therefore blue + red light is treated as a basal in the lighting spectra for plant growth. This work is also the short summary of our own studies conducted in Agricultural University in Cracow with using of the SSL LED (solid state lighting light emitting diodes) technology to stimulate some horticulture crops. For now, the LED light systems are widely used in in vitro cultures or growth chamber cultivation in growth chamber, but the question of such applications for greenhouse plant production on larger scale is still open.

Keywords: light spectra, plant growth, greenhouse plants, SSL LED, HPS

Introduction

Life on Earth is inseparably related to sunlight. For plants, solar radiation acts both as a trophic (necessary for photosynthesis) and morphogenetic factor which affects the plant development (germination, leaf expansion, stomatal opening, flowering etc.). Taking into account the requirements of plants, both the number of absorbed photons and the spectral characteristics of light (referred as quantity and quality of light, respectively) are important. Besides, light influences the synthesis of plant compounds valuable for human healthy diet thereby modifying the nutritional value of plant yield.

In autumn and winter, to obtain a commercial yield at northern latitudes, supplementation of the natural light by artificial lighting is necessary in greenhouse crop production. Worldwide, high pressure sodium lamps (HPS) are still the first choice source of artificial light in horticulture. Because the energy consumption of these lamps is high, supplemental lighting considerably increases expenses in greenhouse industry. Despite the fact that HPS have a high luminous efficiency, a huge amount of consumed electrical energy is converted into heat (Kurpaska 2007). The high radiant heat emission results from high operating temperature (above 200°C). A lot of HPS light is wasted, what increases not only costs of energy but also can contribute to the "light pollution" (Morrow 2008). Moreover, spectral characteristics of sodium lamp (Grzesiak et al. 2012) do not coincide with real needs of plants in the case of light. The emission spectrum of HPS is dominated by yellow light (550-600nm), which is not effective in stimulation of physiological processes. Plants' needs for light are determined by the curves of light absorption of specific photoreceptors. The maxima of absorption for photosynthetic pigments are concentrated on blue and red light, that is for chlorophyll a - about 430 and 640nm, chlorophyll b - 450 and 660nm, or carotenoids - 440 to 480nm (Taiz and Zeiger 2015). Many photomorphogenic responses depend on the presence of

phytochromes with maxima absorption of red (form PR - 660nm) and far-red (form PFR - 730nm) light. Cryptochromes, phototropins and Zeitlupe family of proteins (ZTL/ADO) belong to the important classes of photoreceptors which absorb light in the blue and UV-A wavelength range (Devlin et al. 2007). Photoreceptors are involved in the initiation of a huge number of morphogenetic processes. For example, red to far red ratio determines the PR/PFR equilibrium affecting germination, de-etiolation, shoot elongation etc. Both phytochromes and cryptochromes regulate flowering time due to duration of light exposure. Phototropins are responsible for phototropic curvature, regulate stomatal opening, leaf expantion etc. ZTL family is associated with circadian clock and flowering regulation (Devlin et al. 2007). Therefore, plants demand for light is concentrated mainly around the blue and red light. However, plants respond also to other wavelengths i.e. green light (500-550nm) which appears necessary in regulatory and signaling functions in plants (Folta and Marunich 2007). Recently, the specific photoreceptor UVR8 absorbing UV-B in Arabidopsis was described (Heijde and Ulm 2012).

Taking into account plant needs to specific wavelengths, light emitting diodes (LEDs) which can be manufactured to emit narrow-spectrum wavelengths according to desired applications, seem to be a very promising source of light. Compared to conventional light sources, LED systems can fulfill a lot of desirable functions, such as energy-saving, efficient space utilization or environment protection. The main benefits of LEDs are low energy input, long operating life, high resistance to voltage changes or possibility of wavelength regulation through computer system (Morrow 2008, Olvera-Gonzales et al. 2013). Because of their low operating temperature, LED lamps can be used as inter-lighting to improve light distribution within canopy (Trouwborst et al. 2011). The constantly analyzed question is: what proportion of red to blue LED light will give the best results and this issue seems to be related to species, cultivar or development stage (Hogewoning et al. 2010, Fan et al. 2013, Randall and Lopez 2014).

Several studies showed that LED light might be useful for improving quality of vegetables, for example by increasing the antioxidant activity (Wu et al. 2007, Urbonaviciute et al. 2009, Li and Kubota 2009). Supplemental red LED in combination with HPS light may enhance antioxidant capacity and phenolic compounds content, what was observed in several cultivars of lettuce (Lactuca sativa L.) (Žukauskas et al. 2011, Samuolienė et al. 2012a). The use of red LED lighting in greenhouse may also result in reduction of nitrate concentration (Samuolienė et al. 2009, Samuolienė et al. 2011, Bliznikas et al. 2012), as well as higher carbohydrates synthesis, or increase in ascorbic acid content (Bliznikas et al. 2012) in several green vegetable species. Lu et al. (2012) reported enhanced tomato yield after red LED light addition to fluorescent supplemental lighting in a developmental stage of transplants. Some research suggest the positive influence of blue LED light on concentrations of glucosinolates and essential mineral elements (Kopsell et al. 2015) or β-carotene levels (Lefsrud et al. 2008) in selected Brassica sp. crops. Trials of applying green LED light in greenhouse lettuce cultivation revealed a possibility to increase antioxidative properties (Samuolienė et al. 2012b) and saccharide content with simultaneous reduction of nitrate (Samuolienė at al. 2012c). Currently, at Michigan State University was held 8th International Symposium on Light in Horticulture (May 22-26, 2016). The main issue of this conference was concentrated on improving the horticultural crops with artificial light using LEDs. Many research concerned LED light also in large-scale cultivations.

The use of LEDs for horticulture plants - historical overview

Studies on the light-emitting diodes in plant culture systems have been conducted for over 25 years. As a consequence, LEDs have been already applied in horticulture (Wright 2011).

Because of high costs of LED production, most research and applications are used on small scale like growth chamber cultivations or in vitro cultures.

One of the earliest trials of LEDs tests for plant growth and development were conducted in United States with lettuce, spinach, potato and wheat (Bula et al. 1991). In cited experiment red LEDs (660nm) solely or in combination with blue fluorescent lamps were used. In subsequent studies, advantageous effects of LEDs for plant growth and development in space-based culture systems were shown. In this area, Brown et al. (1995) noticed that red LEDs in combination with blue light might be suitable for pepper plants. Goins et al. (1997) showed usefulness of red LEDs in the whole life cycle of wheat. Following the development of LED technology, blue (450 nm), green (550nm) or far-red (730 nm) diodes were applied (Lian et al. 2002, Kim et al. 2004a). Studies in growth chambers without natural sunlight are continuously conducted to produce high quality crops year-round. Very promising source of light in such closed-type plant production systems are LEDs, due to their small size and relatively low heat radiation. Lin et al. (2013) revealed the usefulness of mixture of red, blue and white LED light in chamber-grown lettuce, obtaining higher soluble sugars and lower nitrate contents in yield. Also the use of red and blue LED supplemented with green light resulted in high biomass accumulation of lettuce (Kim et al. 2004b). Recently Lee et al. (2016) showed the significant impact of far-red LED light supplemental to red and blue in increasing of biomass and bioactive compounds in lettuce.

LEDs have found use in in vitro cultures for many years. In Japan, LED light was implemented for one of the first tissue culture systems (Miyashita et al. 1995). Lian et al. (2002) observed that light generated by red plus blue LEDs (1:1 photon flux density) was suitable for growth of Lilium bulblets. Kim et al. (2004a) showed that net photosynthetic rate of chrysanthemum plantlets after 5 weeks of culture under red + blue (RB) have been the highest in comparison to other combinations (with separately blue, red, far red LED or fluorescent light). These and other works (Shin et al. 2008) showed that leaves in in vitro cultures under red plus blue LEDs contained more chlorophylls, carotenoids and carbohydrates in comparison to other treatments. The 3: 1 ratio of blue to red LED light was the most suitable for rapeseed plantlet growth in vitro, included the survival rate of transplanted plantlets (Li et al. 2013). Many research showed that LED irradiation at proper wavelength may successfully replace fluorescent lamps improving the quantity of plantlets (Bornwaßer and Tantau 2012, Dutta Gupta and Jatothu 2013, a review).

Interesting question is, if SLL LED technology could be used in a large-scale greenhouse crop production. This issue is now on the topic in horticulture studies concerning commercial production. First prototypes of LED supplemental greenhouse lighting appeared in 2006 (Morrow 2008), following the appearance of high power LED units - above 1 Watt. According to some authors the light output increases while device costs decrease, so LEDs used for large-scale in horticulture might be economically feasible (Morrow 2008, Massa et al. 2008, Sabzalian et al. 2014). Application of LEDs in greenhouse as a light source is still relatively new. In Europe, one of the first studies with LEDs dedicated to greenhouse environment was conducted in Finland (Pinho et al. 2007, 2008). Compared to HPS lamps, the lettuce biomass was improved under LEDs emitting red-orange (630nm) and blue (450nm) light. In Netherlands, greenhouse cucumber was lighted with red and blue LEDs intra canopy as interlighting radiation (Trouwborst et al. 2010). The authors showed that it is possible to decrease costs of power used for supplemental irradiance without decrease of total plant biomass or fruit production. In other studies supplementation of HPS light with 450, 470 and 505 nm LEDs increased the biomass and photosynthetic pigments content of vegetable transplants in greenhouse cultivation (Samuolienė et al. 2012d). In bedding plant seedling production the quality of plants under LED lamps emitted red + blue light in various ratios were similar or higher compared to HPS ones (Randall and Lopez 2014). Studies on energy

reduction and increase of plant production in consequence of using LEDs in greenhouses are now the focus of many research centers in the World (Wright 2011, 8th International Symposium on Light in Horticulture 2016).

Modern SSL LED technology – studies in University of Agriculture in Poland

There are some attempts to use LED lamps for greenhouse crops in Poland, too.

According to Grzesiak et al. (2009) modern SSL LED technologies can provide high light intensities with low radiant heat and optimal spectral characteristics. In several scientific centers in Poland, some research on the effectiveness of originally designed LED lamps for winter supplemental lighting of bedding plants (Treder et al. 2012), tomato (Klamkowski et al. 2012) or selected plant seedlings (Puternicki et al. 2012) were conducted.

The interesting prototype of LED lamp dedicated to greenhouse crops was described by Grzesiak et al. (2011). Using this kind of lamps, several experiments were carried out in University of Agriculture in Cracow. In autumn growing, a clear stimulation of photosynthesis in lamb`s lettuce (Valerianella locusta) leaves under red + blue (1:0.8) and white LED lamps in the evening hours was showed (Wojciechowska et al. 2013). But finally, mean weight of V. locusta rosette was greater as a result of red and blue lighting compared to white light. Beneficial effect of tested LED lamps on growth and flowering of Begonia semperflorens was also observed (Kołton et al. 2013). After these preliminary experiments, innovatory SSL LED system was developed in adjustment to the needs of new experiments in which the spectral composition of light or emission time are changing (Grzesiak at al. 2012, Żupnik et al. 2012). Such intelligent programmable system which consisted of 24 LED lamps was used in the scientific project realized in 2012-2014 in greenhouse of Faculty of Biotechnology and Horticulture, University of Agriculture in Cracow (the study was supported by the National Science Centre in Poland, Project no. 2011/01/B/NZ9/00058). The main aim of this project was to evaluate the physiological processes connected with carbon and nitrogen metabolism (photosynthesis, metabolism of nitrates) and the estimation of quality and pro-healthy compounds of lamb‘s lettuce (Valerianella locusta) as an effect of supplemental lighting with LED lamps of various spectral characteristics (different ratios of red to blue light and white light). Control plants were lighted with sodium lamps (HPS). Studies allowed to precise expenses of energy and costs of production. The final effect was connected with the choosing of appropriate kind of lamps which assured the best yield of high biological quality simultaneously with decreasing the costs of cultivation. The most effective in stimulating both the size and quality of the yield (in terms of health-promoting substances such as ascorbic acid or soluble sugars) were LED lamps which emitted red and blue light in proportion 7: 3 in autumn cultivation, and 9: 1 in winter. The latter ratio affected the significant reduction of nitrates in lamb’s lettuce yield in each cultivation cycle. Costs of the supplemental lighting were the lowest in the case of LED lamps emitting red and blue in a ratio of 9: 1. Compared to the control cultivation (HPS), power consumption in this light combination was about 50% lower and the yield was significantly higher (more than 10% in autumn and 20% in winter). Some results have been already published (Kołton et al. 2014, Wojciechowska et al. 2015), some of them are currently in review (Wojciechowska et al. 2016a, Wojciechowska et al. 2016b) or after a positive review (Długosz-Grochowska et al. 2016) and other results are prepared for publication.

Conclusions

It is shown that the high emitting diodes (LEDs) can be a good source of artificial light in various applications in horticulture. Available data indicate that LED light of proper spectral characteristics might be useful for: (1) improving the growth, development and chemical composition of plants (including vegetables); (2) using in close-type plant production

systems; (3) improving the quantity and quality of plantlets in in vitro cultures; (4) using for supplemental irradiation of plants in crop production under glass (the hot issue in recent studies in the World). The last usefulness was confirmed in the studies conducted in Agriculture University of Poland.

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Seasonal variation of the nitrates and ascorbic acid content of selected basil (*Ocimum basilicum* L.) varieties

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Abstract
Basil (*Ocimum basilicum* L.) a most commonly used culinary herbs in the world in fresh, dried or otherwise prepared. Aerial parts of plants or leaves are suitable to consumption. Basil plant contains substances that are beneficial to health, as well as substances acting negatively on the human organism. Some research has been reported on the greenhouse production of this plant. Seven basil varieties were evaluated for content of ascorbic acid and nitrates during the years 2014 – 2015. Plants were cultivated as pot culture in greenhouse of Mendel University in Brno at Faculty of Horticulture. High Performance Liquid Chromatography (HPLC) was determined the ascorbic acid content. The ascorbic acid contents of basil ranged from 56.2 to 234.5 $mg \cdot kg^{-1}$ f. w. Ion Selective Electrode was determined the nitrates content. Nitrates content during the reporting period ranged from 178.7 to 17918.2 $mg \cdot kg^{-1}$ f. w. Ascorbate-nitrate index was calculated as a ratio of ascorbic acid to nitrate levels in f. w. Among the cultivated samples were found significant differences in the content of ascorbic acid and nitrates in different varieties, years and cultivation seasons. Most of the tested samples basil had ascorbate-nitrate index value of less than 0.5.

Keywords: basil (*Ocimum basilicum* L.), greenhouse production ascorbic acid, nitrates, *ascorbate-nitrate index*

Introduction

Basil (*Ocimum basilicum* L.) herb is one of the most frequently used culinary and raw materials, containing a significant amount of biological compounds with strong curative properties. Basil contains essential oil with different chemical composition, chlorophyll, vitamin C, carotenoids, a wide range of phenol compounds displaying various antioxidant activities, depending on the basil species and varieties (Nurzynska-Wierdak et al., 2011; Politeo et al., 2007). The herb is used not only for cooking but also in commercial fragrances, flavourings, and for increasing the shelf life of food products (Suppakul et al., 2003). Basil is widely used in traditional medicine as a digestive tonic and for curing ailments such as warts, inflammations, colds, and headaches (Chanwitheesuk et al., 2005). Basil extract has known sedative and anticonvulsant properties (Freire et al., 2006) as well as antimicrobial and antifungal activity (Holm, 1999; Suppakul et al., 2003).
Ascorbic acid belongs among one of the most important and frequent monitoring of the constituents in the culinary herbs. Ascorbic acid is a very effective antioxidant (Harris, 1996). According to World Health Organization should be area daily amount of vitamin C is 45 - 50 mg (WHO and FAO, 2004). According to USDA (2016) 100 g of fresh basil leaves contain 18 mg of ascorbic acid. Favell (1998) believes that ascorbic acid can serve as a sensitive marker for determining nutritional quality. Frank et al. (2001) states that for consumers is more significant colour and retail price than nutritional quality. Ascorbic acid is by far the least stable nutrient during processing. It is highly sensitive to oxidation and leaching into water-soluble media during processing, storage and cooking (Franke et al., 2004). Basil herbs are characterized by significant variability in terms a broad range of the constituents.

Influences, which determine the content of ascorbic acid, there are several. Here we can include external factors (climatic and growing conditions, fertilization, agronomic methods) as well as internal factors (genotype, development stage) (Dzida, 2011; Fraszczak et al., 2015; Nurzynska-Wierdak et al., 2011; Saadatian et al., 2014).
In addition to health promoting compounds in basil are also substances that may be toxic an excessive amount. For such substances, we advise nitrates. Nitrates are not common for adults dangerous because relatively quickly excreted in the urine. Potential danger stems from nitrites or nitrates when their conversion to nitrite. In case the nitrite absorbed into the blood, they can cause methemoglobinemia. European Commission (1997) established the maximum permissible levels from 3500 to 4500 mg $N\text{-}NO_3^-$ kg^{-1} f. w. for the winter season and 2500 mg of $N\text{-}NO_3^-$ kg^{-1} for the summer crops. Accumulation of nitrate levels is higher in the absence of light radiation (Buwalda and Warmenhoven, 1999). Therefore, most greenhouse crops have higher nitrate content compared field culture (Gruda, 2005). The factors responsible for nitrate accumulation in plants are mainly nutritional, environmental and physiological. Nitrogen fertilization (Dzida, 2011; Nurzynska-Wierdak et al., 2011) and light intensity (Urbonavičiute et al., 2008) have been identified as the major factors that influence the nitrate content. Purple leaves have a higher tendency to accumulate nitrates (Wojciechowska et al., 2000). It is caused by the predominance of anthocyanin colorants in the plastids.
The ratio of ascorbic acid and nitrate content, so called ascorbate nitrate index is a parameter that can be used to evaluate the nutritional quality and possibly harmlessness horticultural products. Horticultural products with a higher content of ascorbic acid compared to nitrate are safer and more favorable for human nutrition (Pokluda 2005). Lachman et al. (1997) categorized vegetables according to this index into three groups: risk species with values below 0.5, neutral species with values ranging from 0.5 to 1.0, and positively beneficial species with values over 1.0.
The aim of the present investigation was to determine the level of total nitrates and ascorbic acid according to the growing season and varieties. For evaluating the health quality of basil was calculated ascorbate-nitrate index.

Materials and Methods

Plant materials and cultivation conditions

The control variety 'Ohře' produced by Seva Moravia (Czech Republic) and six varieties ('Lettuce Leaf', 'Purple Opaal', 'Dark Green', 'Mammolo Genovese', 'Mánes', 'Red Rubin') produced by Semo Smržice Ltd. (Czech Republic) were used in the experiment.
The plants were grown in greenhouse of Mendel University in Brno, Faculty of Horticulture in Lednice as a pot plants during the years 2014 – 2015 (Tab. 1). Greenhouse experiments were established in 4 replications for each variety with 30 plants for each variant.

Tab. 1 Cultivations detail for basil plants

Time of sowing and harvesting	**2014**	**2015**
Pot culture 1	11.2. - 9.4.	23.3. -1.6.
Pot culture 2	23.4. - 23.6.	27.4. - 14.7.
Pot culture 3	26.8. - 15.10.	1.9. - 26.10.

Plants were cultured in medium (Horticultural substrate B with active humus, Rašelina Soběslav Ltd., Czech Republic), as needed irrigated, protected from pests and diseases and once fertilized (Kristalon Gold, Agro CS, Czech Republic; dosage: 10 g of fertilizer per 10 l of water). Basil was grown in 0.5 dm^3 plastic pots, at 20 plants per pot. Plants were harvested (for determination ascorbic acid around 7.00 a. m., for determination nitrates around 7.00 a.m. (years 2014) and around 11.00 a.m. (years 2015)) before their flowering (they were planted as

culinary herbs). Samples were immediately transported to the laboratory for their chemical analysis.

Extraction and determination of ascorbic acid

The concentration of ascorbic acid was determined by HPLC according to Arya et al. (2000) with slight modification. Aerial parts of basil (10 g) were homogenized in a blender with 30 ml of 0.1 M oxalic acid. The homogenate was topped up with oxalic acid to the volume of 100 ml, filtered, centrifuged (3800 rt/min for 10 minutes at room temperature) and the supernatant was used for measurement. The analyses were performed by RP-HPLC (ECOM, Praha) at 254 nm using UV-VIS detector. All samples were measured triplicate. The amount of ascorbic acid was expressed as $mg \cdot kg^{-1}$ f. w.

Extraction and determination of nitrates

The content of nitrates was determined by ion-selective electrode according to Javorský et al. (1987). 20 g of the sample were mixed with 20 ml of aluminium sulphate. Homogenized sample (20 g) was accurately weighed and boiled for 5 min with 20 mL of aluminium sulfate and 1 ml of 30 % hydrogen peroxide. After cooling, the solution was made up to 100 ml aluminium sulphate into Erlenmeyer flask. Measurements were done using the device Ionalyzer MPH 171 (Monokrystaly, Turnov, Czech Republic). The potential was recorded and compared with the calibration graph. Nitrate content was expressed as mg $NO_3 \cdot kg^{-1}$ f. w.

Statistical analysis

All analyses were conducted in four replications, each in two parallel samples. For statistical evaluation of the results the program Statistica Cz v. 12 (StatSoft) was used. Data are expressed as means ± SD (standard deviation). Differences were analyzed for sample data using a non-parametric Kruskal-Wallis test.

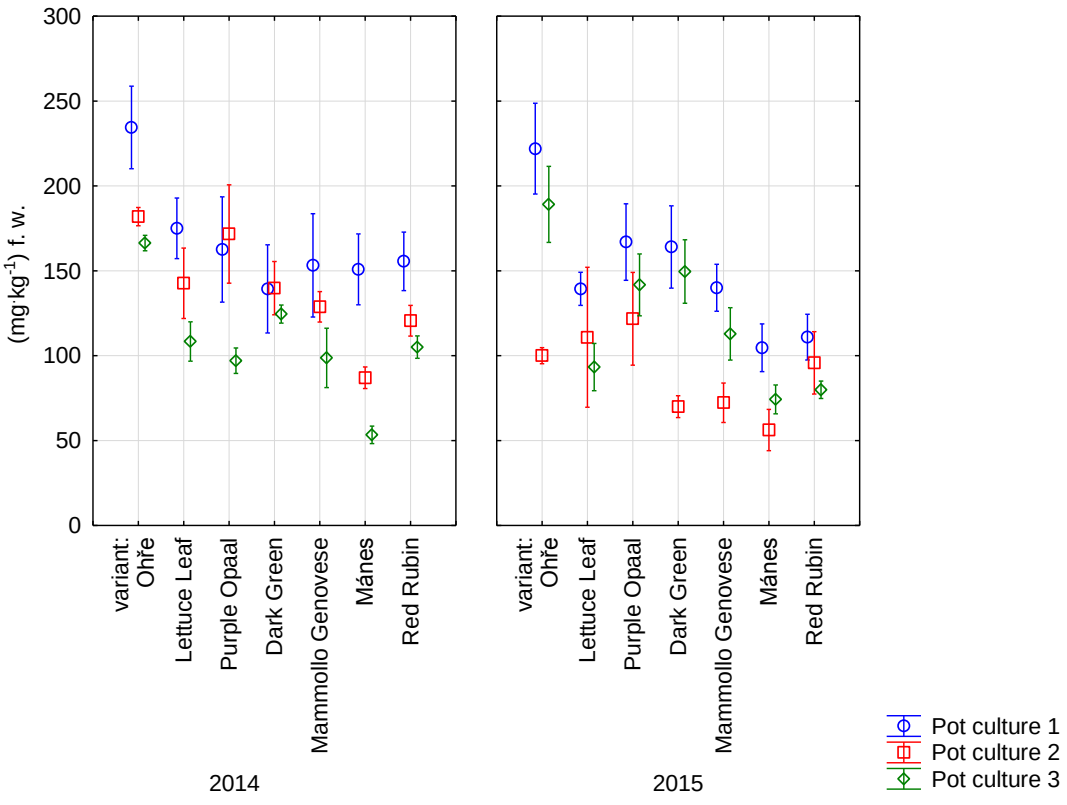

Fig. 1 Total ascorbic acid content of selected basil varieties

Results and Discussion

The results of the analysis of ascorbic acid content in the selected basil varieties are shown in Tab. 2 and Fig. 1. The ascorbic acid contents of basil ranged from 56.2 to 234.5 mg·kg^{-1} f. w. The lowest content of ascorbic acid throughout the period 2014 - 2015 were found in the variety 'Mánes', and the highest was measured in variety 'Ohře'. The effect of years, cultivated season and variety on ascorbic acid content was found statistically significant.

According to Holm (1999) fresh basil contains a lot of ascorbic acid. Authors Dumbrava et al. (2012) observed of ascorbic acid content in basil and rosemary leaves. They found that the basil leaves are richer in vitamin C (271 mg·kg^{-1}) than rosemary leaves (185 mg·kg^{-1}). Martyniak-Przebyszewska and Wojciechowski (2004) recorded lower concentrations of ascorbic acid in basil (119 mg·kg^{-1}). Dzida (2010) reported that Polish variety of basil 'Kasia' accumulated more ascorbic acid (265 mg·kg^{-1}) than variety 'Wala' (204 mg·kg^{-1}). Authors Vábková and Neugebauerová (2009) investigated the content of ascorbic acid in different varieties of basil. The amount of ascorbic acid was highest in basil varieties 'Ohře' (271.7 mg·kg^{-1}). The lowest ascorbic acid content was found by basil varieties 'Lettuce Leaf' (150.1 mg·kg^{-1}). Our results are similar to the results that indicate the above-mentioned authors. The results in 2015 were lower than in 2014.

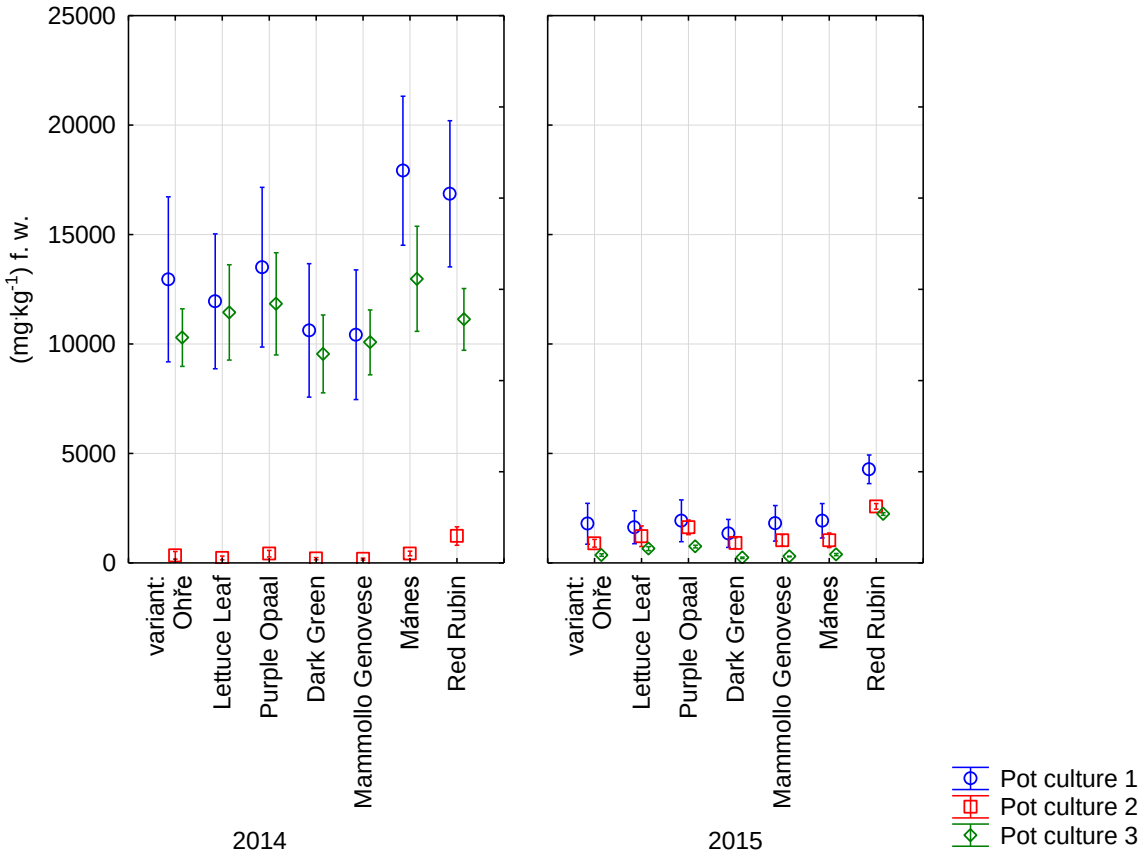

Fig. 2 Content of nitrates of selected basil varieties

The results of the analysis of nitrates content in selected basil varieties are shown in Tab. 3 and Fig. 2. The content of nitrates in basil varies depending on the year, pot cultivation and varieties. Content of nitrates was in the range from 178.7 to 17918.2 mg·kg^{-1} f. w. The standard deviations indicate a great variation of nitrates in researches years and differences pot cultures. Varieties with purple leaves ('Purple Opaal' and 'Red Rubin') had higher nitrates

content. A significant impact of a cultivar on the total nitrogen content was recorded in the study by Dzida (2010). Experiment conducted by Nurzynska-Wierdak et al. (2011) revealed the relationship between the cultivar grown vs. nitrates content in the plant.

The nitrates content of culinary herbs is an important consideration in the greenhouse production. The potential health hazards of nitrates were well studied. The results (Majkowska-Gadomska, et al., 2015) of chemical analyses indicate that lemon basil plants had the highest nitrates content (1904 $mg \cdot kg^{-1}$ f. w.). According to Telesiński et al. (2013), the nitrates content of basil herbs can be as high as 9950 $mg \cdot kg^{-1}$ f. w. In the studies performed by Vábková and Neugebauerová (2011), the range of the content of that component for dill was from 252 to 617 $mg \cdot kg^{-1}$ f. w. In kale plants obtained in the first harvest, the content of nitrates was significantly higher compared with the remaining dates. It varied from 1643 to 1730 $mg \cdot kg^{-1}$ f. w. on average, depending on the year of the investigation and the cultivar (Korus and Lisiewska, 2009). Jaworska (2005) noted a much higher content of 2804 $mg \cdot kg^{-1}$ f. w. in *Tetragonia expansa*.

The mean ascorbate-nitrate index (Tab. 4) was found in range of 0.08 to 0.721. Neutral values were found in the pot culture 2 in 2014 (variety 'Ohře' 'Lettuce Leaf', 'Dark Green', 'Mammolo Genovese') and pot culture 3 in 2015 (variety 'Ohře', 'Dark Green'). Low levels of this parameter were determined in other variety.

Tab. 2 Ascorbic acid content in basil ($mg \cdot kg^{-1}$) f. w.

	2014			2015		
	Pot culture 1	**Pot culture 2**	**Pot culture 3**	**Pot culture 1**	**Pot culture 2**	**Pot culture 3**
'Ohře'	234.5±48.7^{b}	181.9±10.7^{ab}	166.4±9.1^{a}	222.0±53.5^{b}	100.1±9.5^{a}	189.1±44.8^{ab}
'Lettuce Leaf'	175.1±35.7^{a}	142.7±41.4^{a}	108.4±23.1^{a}	139.4±19.5^{a}	110.9±82.4^{a}	93.3±27.9^{a}
'Purple Opaal'	162.6±62.1^{ab}	171.7±58.0^{b}	97.1±14.9^{a}	166.9±45.1^{a}	121.8±54.7^{a}	141.7±36.5^{a}
'Dark Green'	139.3±51.9^{a}	139.8±31.4^{a}	124.5±10.7^{a}	164.0±48.5^{b}	70.0±12.9^{a}	149.6±37.4^{ab}
'Mammolo Genovese'	153.2±60.9^{a}	128.8±18.0^{a}	98.7±35.0^{a}	140.0±27.7^{b}	72.3±23.3^{a}	112.9±30.8^{ab}
'Mánes'	150.9±41.8^{b}	87.0±12.7^{ab}	53.4±10.3^{a}	104.7±28.1^{a}	56.2±24.3^{a}	74.3±17.0^{a}
'Red Rubin'	155.6±34.5^{b}	120.6±18.1^{ab}	105.1±13.3^{a}	111.0±26.9^{a}	95.8±36.7^{a}	79.9±10.3^{a}

*2014: Pot culture 1 (11.2. – 9.4.); Pot culture 2 (23.4. – 23. 6.); Pot culture 3 (26.8. – 15.10.); 2015: Pot culture 1 (23.3. – 1.6.); Pot culture 2 (27.4. – 14.7.); Pot culture 3 (1.9. – 26.10.)

Tab. 3 Nitrates content in basil ($mg \cdot kg^{-1}$) f. w.

	2014			2015		
	Pot culture 1	**Pot culture 2**	**Pot culture 3**	**Pot culture 1**	**Pot culture 2**	**Pot culture 3**
'Ohře'	12957.3±7545.3^{a}	347.3±345.0^{a}	10294.6±2634.2^{a}	1790.9±1866.1^{a}	899.3±352.2^{a}	361.8±115.0^{a}
'Lettuce Leaf'	11951.1±6161.5^{a}	228.4±172.8^{a}	11447.4±4358.6^{a}	1631.8±1508.1^{a}	1223.1±943.1^{a}	654.9±185.8^{a}
'Purple Opaal'	13508.9±7294.1^{a}	425.1±301.8^{a}	11837.3±4673.6^{a}	1925.8±1916.8^{a}	1627.8±675.0^{a}	748.2±125.6^{a}
'Dark Green'	10623.6±6095.2^{a}	195.5±98.3^{a}	9550.0±3557.8^{a}	1348.1±1273.5^{a}	903.3±490.6^{a}	237.7±65.6^{a}
'Mammolo Genovese'	10425.4±5926.8^{ab}	178.7±88.4^{a}	10076.9±2964.5^{b}	1811.4±1623.3^{a}	1033.7±525.9^{a}	294.9±33.9^{a}
'Mánes'	17918.2±6804.0^{b}	434.2±209.1^{a}	12977.5±4800.2^{ab}	1924.9±1570.5^{a}	1036.9±660.7^{a}	381.6±101.6^{a}
'Red Rubin'	16861.8±6677.4^{b}	1230.1±841.6^{a}	11125.9±2823.9^{ab}	4280.6±1306.5^{b}	2583.8±254.9^{ab}	2235.7±121.8^{a}

*2014: Pot culture 1 (11.2. – 9.4.); Pot culture 2 (23.4. – 23. 6.); Pot culture 3 (26.8. – 15.10.); 2015: Pot culture 1 (23.3. – 1.6.); Pot culture 2 (27.4. – 14.7.); Pot culture 3 (1.9. – 26.10.)

Tab. 4 Ascorbate-nitrate index in selected basil varieties

	2014			2015		
	Pot culture 1	**Pot culture 2**	**Pot culture 3**	**Pot culture 1**	**Pot culture 2**	**Pot culture 3**
'Ohře'	0.018	0.524	0.016	0.124	0.111	0.523
'Lettuce Leaf'	0.015	0.625	0.009	0.085	0.091	0.142
'Purple Opaal'	0.012	0.404	0.008	0.087	0.075	0.189
'Dark Green'	0.013	0.715	0.013	0.122	0.078	0.629
'Mammolo Genovese'	0.015	0.721	0.010	0.077	0.070	0.383
'Mánes'	0.008	0.200	0.004	0.054	0.054	0.195
'Red Rubin'	0.009	0.098	0.009	0.026	0.037	0.036

values are below than 0.5; values between 0,5 - 1,0; values are higher than 1.0

Conclusion

Ascorbic acid content which was evaluated in basil varieties grown under greenhouse conditions is identical to the content that is in the field area. The amount of nitrates contained

in basil is low to the amount that is consumed for seasoning dishes. Serious health complications are unlikely of commonly consumed amount. Similarly, low levels of ascorbate-nitrate index does not adversely effect on human health.

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Medicinal and useful plants in landscape creation

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Abstract
The Botanical garden as a scientific – pedagogical workplace, within the frame of own activities deals with formation of collection and collections for research and educational purposes. In 2016, we focused on formation of plantings meeting not only aesthetic but also educational function. For this purpose, new exposure areas were created, the assortment of plants adapted and even collaboration outside the campus was developed. The main idea was to build up the landscape interesting modifications using medicinal and useful plants. In total, an area of approximately 690 m2 was planted. Together, 4015 pieces of plants was used.

Keywords: medicinal plants, useful plants, collection, landscaping creating, education

Introduction

One of the main objects of landscape formation is to take care of the environment. Just the green is an essential component of the environment and becomes an equivalent partner of other city and village amenities (Hurych, 1995).
Usage of perennial medicinal plants in the formation of landscaping is not as rare as it may seem. However, medicinal and aromatic plants are often used in this area without a deeper understanding of their values. The use of one-year or two-year useful plant species and vegetable species is no longer uncommon. Useful plants are often perceived as a crop for vegetable gardens only without considering their aesthetic importance.

Material and Methods

In Y2016 the implementation activities of the Botanical garden SUA in Nitra focused primarily on the utilization of commercial plants in the landscaping. The aim was to realize the outplanting of education while maintaining their aesthetic features. To this objective the assortment composition has been adjusted.
The plants were selected according to different parameters. Not only ornamental nature and specific growth characteristics were priorities but also the usefulness in other areas. Outlantings were made in three main areas: planting of annuals decorative flower beds, building collection of useful plants of the family Solanaceae and Cucurbitaceae and permanent planting of Orangery in Topoľčianky park.
Annual flower beds were made not only in the collections of the Botanical Garden on the campus of exposure, but also on the grounds of the Slovak agricultural University, as representative plantings in front of the main building. Materials for planting of flower beds have been acquired through the Index seminum, purchase of seeds or as a gift.
Renovation of the Orangery in Topoľčianky originated from the impulse of the Topolčiansky Park and the Botanical garden arranged the assortment composition and plantings. Plants were selected from the collections of the POI with respect to their specific positioning requirements. Major part of the assortment form medicinal and useful plants with origin in the Mediterranean area or subtropical areas.

In addition to flower beds and perennial plantings the short-term collections of useful plants were built, primarily we focused on the family *Solanaceae* complemented by some species of the family *Cucurbitaceae*.

Results and Discussion

Annual flower beds in the SUA and in the park of the Botanic garden.

In the year 2016, 8 beds have been planted on the total area of 290 m^2. Exposed areas in front of the SUA consist of 4 flowerbeds of rectangular shape with dimensions 2x10 m and the one of ring shape with a diameter of 10m. Proper implementation proceeded in three stages: the first stage consisted of crops of the species with shorter life cycle and early start in the vegetation. This phase should provide an early spring effect and was realized in the week 8 (end of February). In the week 18 (early May) the second phase consisting of seedlings planting has running. The original vegetation was reduced and supplemented by mostly vegetable plants. Annuals without medical or useful characteristics were used as complementary plants.

In the last phase, in early July, finally herbs of interest were planted. Together, 1896 pieces of plants were used for the planting of seedlings on the total area of 80 m^2. Composition of these areas is shown in the table 1.

Table 1 List of species used for planting of 4 representative flower beds in front of the SUA

Species	Number of pieces	The planting stage
Acmella oleracea (L.) R.K.Jansen *(syn. Spilanthes oleracea)*	96	3.
Anethum graveolens L.	Sowing seeds	1.
Apium graveolens L. var. *dulce*	120	2.
Beta vulgaris L. var. *cicla*	144	2.
Coix lacryma-jobi L.	48	2.
Foeniculum vulgare Mill.	Sowing seeds	1.
Lactuca sativa L. *´Lollo Bionda´*	360	2.
Lactuca sativa L. *´Lollo Rossa´*	360	2.
Linum usitatissimum L.	Sowing seeds	1.
Ocimum basilicum L. ´Green Dark´	96	3.
Ocimum basilicum L. ´Purple Ruffles´	96	3.
Ocimum basilicum L. ´Purple´	96	3.
Ocimum basilicum L. ´Siam Queen´	96	3.
Papaver somniferum L.	Sowing seeds	1.
Petroselinum crispum (Mill.) Fuss. convar. *vulgare ´Kinga´*	144	2.
Tagetes erecta L.	96	3.
Verbena bonariensis L.	144	2.

In the second stage also planting of circular flower bed ran, this was remained without the early spring effect. The total area of this bed is approximately 78 m^2 and 324 plants together were planted on it. This makes about 4 plants for one square meter. The species are shows in the table 2.

In the exposure part of the Botanic garden 3 annual flower beds were planted as a combination of educational and aesthetical planting. The dimensions of these beds are different and depend on the location of the area. The first flower bed with dimensions 2 m x 22m is a mixture of vegetable and spice plants with an impressive colourity.

Table 2 List of species used for planting of the circular flower bed in front of the SUA

Species	**Number of pieces**
Cleome houtteana Schltdl. (syn.: *C. hassleriana*)	20
Foeniculum vulgare Mill.	20
Leonotis leonurus (L.) R.Br.	96
Rudbeckia hirta L.	20
Sorghum bicolor (L.) Moench. (syn.: *S. nigrum*)	72
Tithonia rotundifolia (Mill.) S.F.Blake	48
Verbena bonariensis L.	48

The second flowerbed, which is the largest in size, wide 2 m and long 34 m, enabled using of high and rugged types. In the last flower bed, 1,2 m x 17 m of size, the combination of herbs and annuals was used. Each of the beds is depicted in different style, eventually color contrast and is complemented by interesting structures for climbing plants. Useful plants are complemented by common annuals. The total area of flower beds is approximately 132 m^2 and together 1495 plants were planted on it. Specific composition of these flower beds is shown in the table 3.

Table 3 List of species used for planting of flower beds in the Botanic gardens

Species	Number of pieces	Flowerbed
Ageratum houstonianum Mill. (blue)	96	2.
Ageratum houstonianum Mill. (white)	72	2.
Beta vulgaris L. var. *cicla* (mix)	40	3.
Beta vulgaris L. var. *cicla ´Green´*	60	1.
Beta vulgaris L. var. *cicla ´Ruby Red´*	60	2.
Cobaea scandens Cav. *´Alba´*	2	2.
Cobaea scandens Cav. *´Blue Bell´*	2	
Coix lacryma-jobi L.	72	1.
Cosmos sulphureus Cav.	48	1.
Cyclanthera pedata (L.) Schrad.	2	2.
Dahlia hyb.	96	3.
Ipomoea lobata (Cerv.) Thell. *´Citronella´*	3	3.
Ipomoea quamoclit L. *´White´*	4	2.
Ipomoea rubriflora O´Donell (syn.: *I. coccinea*)	2	3.
Lablab purpureus (L.) Sweet	6	2.
Leonotis leonurus (L.) R.Br.	144	1.
Ocimum basilicum L. *´Cinnamon´*	96	2.
Phaseolus vulgaris L.	6	2.
Ricinus communis L.	24	1.
Rudbeckia hirta L.	96	3.
Salvia nemorosa L.	120	2.
Sorghum bicolor (L.) Moench. (syn.: *S. nigrum*)	144	1.
Tagetes erecta L.	72	1.
Tithonia rotundifolia (Mill.) S.F.Blake	120	1.
Verbena bonariensis L.	36	3.
Verbena bonariensis L. *´Lollipop´*	72	2.

Orangery in Topoľčianky

Thanks to the collaboration with Topoľčianky park, in early 2016, the Botanic Garden SUA NITRA started to participate in the implementation of the interesting project of the Orangery restoration. This is a tempered greenhouse with an area of almost 200 m^2 and ground clearance of approx. 6m. The greenhouse is divided in several flower beds with possibility of plantings in open soil and with raised concrete beds of the depth up to 30cm. Even hanging wooden trellises that serve not only as decorative elements but also as a stay for epiphytic types of plants and climbing plants were built. The area of planted field with few mature plants in the central part has 108m2. Together 44 species of mostly subtropical plants of different origins and values were planted. The assortment was complemented by the epiphytic plants (Orchidea, Bromelia, Tillandsia), placed on raised beds and trellises, in total by 120 pieces. The list of plants is shown in the table 4.

Table 4 List of species used for planting in Orangery in the Topolčianky park. .

Species	Position
Brachychiton rupestris (T.Mitch. ex Lindl.) K.Schum.	border flowerbed 2.
Malpighia coccigera L.	border flowerbed 2
Brachychiton populneus (Schott. et Endl.) R.Br.	border flowerbed 2
Schinus molle L.	border flowerbed 2
Catha edulis (Vahl) Endl.	border flowerbed 2
Phyllanthus arbuscula (Sw.) J.F.Gmel.	border flowerbed 2
Hakea suaveolens R.Br.	border flowerbed 2
Psidium cattleianum Afzel. ex Sabine	border flowerbed 2
Jacaranda acutifolia Bonpl.	border flowerbed 2
Pittosporum sp.	border flowerbed 2
Casuarina glauca Sieber ex Spreng.	border flowerbed 2
Fatsia japonica (Thunb.) Decne. et Planch.	border flowerbed 2
Cyrtomium falcatum (L.f.) C.Presl	border flowerbed 2
Justicia carnea Lindl. (syn.: *Jacobinia carnea*)	border flowerbed 2
Pachira aquatica Aubl.	border flowerbed 1.
Araucaria sp.	border flowerbed 1.
Psidium guajava L.	border flowerbed 1.
Annona glabra L.	border flowerbed 1.
Syringa sp.	border flowerbed 1.
Annona muricata L.	border flowerbed 1.
Punica granatum L. ´*Nana*´	border flowerbed 1.
Laurus nobilis L.	border flowerbed 1.
Howea forsteriana (F.Muell.) Becc.	central flowerbed 1
Serissa japonica (Thunb.) Thunb.	central flowerbed 1
Phormium tenax J.R.Forst et G.Forst.	central flowerbed 1
Plectranthus ciliatus E.Mey.	central flowerbed 1
Spathiphyllum wallisii Regel.	central flowerbed 1
Bauhinia purpurea L.	central flowerbed 1
Setaria palmifolia (J.Koenig) Stapf	central flowerbed 1
Plectranthus (modrý)	central flowerbed 1
Plectranthus (biely)	central flowerbed 1
Cymbopogon citratus (DC.) Stapf.	central flowerbed 1
Myrtus communis L.	central flowerbed 1
Hedera	central flowerbed 1
Strelitzia nicolai Regel et K.Koch	Central flowerbed 2..

Yucca aloifolia L.	Central flowerbed 2
Tradescantia sp.	Central flowerbed 2
Tamarindus indica L.	Central flowerbed 2
Chlorophytum comosum (Thunb.) Jacques	Central flowerbed 2
Chlorophytum orchidastrum Lindl.	Central flowerbed 2
Gynura aurantiaca (Blume) Sch. Bip. ex DC.	Central flowerbed 2
Clerodendrum thomsoniae Balf.f.	Central flowerbed 2
Bougainvillea spectabilis Willd.	Central flowerbed 2
Jasminum officinale L.	Central flowerbed 2

The short-term collection of useful plants collection of Solanaceae family.
The main aim of this collection is to show the diversity of the family Solanaceae. The base of the collection consists of different varieties of tomatoes and peppers, grown in containers. Overall, 135 containers with 46 varieties of peppers and 18 varieties of tomatoes there are set. These are complemented by several less known species of the family and by the climbing plants of Cucurbitaceae family, planted in open soil. Collection includes 8 different genera, 20 species and 62 different varieties of tomatoes and peppers. Together there are situated 180 plants. The entire collection is concentrated in the greenhouse of botanical gardens, on an area of approximately 200 m^2. A list of the different species is described in the table 5.

Table 5 List of species used for planting *Solanaceae* a *Cucurbitaceae*

Species	**cultivar**	**Species**	**cultivar**
Capsicum annuum L.	*Arlequin* F1	*Capsicum baccatum* L.	*Bishops Crown Yellow*
	Bianca F1		*Criolla Sella*
	Emma		var. *pendulum ´Escabeche´*
	Flamingo F1		*Zvonček*
	guľatá - zmes	*Cyclanthera pedata* (L.) Schrad	
	Hamík	*Lagenaria siceraria* (Molina) Standl.	*Birdhouse Bottle*
	Hamík-mix		*Cobra*
	Hodonínska Sladká Vzpřímená		*Dipper*
	Chilli Peter Pepper Red		*Froggy*
	Kilian		*Marenka Limegreen*
	Kiowa F1		*Plate de Corse*
	Kolora	*Lycopersicon esculentum* Mill.	*Banana Legs*
	Koral		*Citrina*
	Kristian		*Cream Sausage*
	Lozorno		*Fuzzy Wuzzy*
	Mini Bell Chocolate		*G – 29*
	Mini Bell Red		*Charlie Chaplin*
	Mini Bell Yellow		*Maiglöckchen*
	Pepper Red		*Noir de Crimée*
	Pepperoncini Greek		*Orange Banana*
	Plamínek Červený		*Red Zebra*
	Plamínek Žlutý		*Ríbezľová*
	Shakira F1		*Ruen F1*

	špicatá - zmes		*Rugby F1*
	Tabasco		*San Marzano*
	Turkisch Sweet		*Semo Locarno*
	var. *glabriusculum*		*Shimmeig Striped Hollow*
	Victoria		*Thai Pink Egg*
Capsicum chinense Jacq.	*7 pot Primo Choco*		*Tlacolula*
	7 pot/7 pod	*Passiflora mollissima* (Kunth) L.H.Bailey	
	Fatalii Red	*Physalis angulata* L.	
	Five Colour	*Physalis philadelphica* Lam cv. *Grose Gelbe*	
	Habanero Red	*Solanum aethiopicum* L.	
	HP22B Carolina Reaper	*Solanum atropurpureum* Schrank	
	Naga Morich	*Solanum caripense* Dunal	
	Scotch Bonet	*Solanum laciniatum* Aiton	
	Trinidad Moruga Scorpion	*Solanum melongena* L. ´*Bartok* F1´	
	Trinidad Perfume	*Solanum muricatum* Aiton	
	Trinidad Scorpion Orange	*Solanum pyracanthum* Jacq.	
Capsicum nigrum Willd.	*Black Prince*	*Solanum sisimbriifolium* Lam.	
		Solanum uporo Dunal	
		Withania somnifera (L.) Dunal	

Conclusion

The aim of outplantings realized in 2016 was to draw an attention to the aesthetic function of many commonly used vegetables and useful plants. By their application in landscaping we have combined the educational potential of plantings with aesthetic representation in modern composition. The selected range combined the modern varieties and flavors of some commonly used herbs and vegetables with traditional botanical species and annuals. The appropriate combination of colors and structures created interesting plantings attractive not just at first glance but also after closer analysis of the species composition.

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