

# THE EFFECT OF SOME CULTIVATION CONDITIONS ON PROPERTIES OF LEAVES OF THE CONTAINER-PRODUCED SPECIES *ALNUS GLUTINOSA* (L.) GAERTN

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## Abstract

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Plants differ in their reactions to unfavourable environmental conditions and the effect of the acting factors directly and indirectly results in changes in a number of plants organs. The leaves are vegetative organs and as such they are completely exposed to external conditions. Most markedly these conditions affect the assimilative organs. The objective of the present study was to evaluate the effect of four substrates and two sites differing in light intensity on the leaf morphology of the model plant species *Alnus glutinosa* (L.) Gaertn. The used substrates were the commercial peat-bark substrate (RKS II), sand and bark substrate. With these substrates the soil conditioner TerraCottem was applied at a rate of 5 g per one litre of the substrate. The experimental plants were grown in 2-litre containers. The focus of evaluations of the individual treatments was on some leaf properties. TerraCottem showed that it had a positive effect also on the leaf area ratio and on leaf efficiency. The number of leaves varied. The effect of the substrate and of the light intensity was identical; the latter parameter was strongly correlated with leaf weight. The shape of the leaves (leaf length/width ratio) and the SLA index responded to the different light intensities. Other parameters were calculated from the results of the measured values which specified in greater detail the effect of the year, media, hydro absorbent and light. Treatments A3 (1 697.60 mm<sup>2</sup>) and a control (1 708.10 mm<sup>2</sup>) had the smallest leaf area. These two treatments significantly statistically differed from those in the shade location. In the 2005 year the highest values were measured at the treatment B1 (41.22 m<sup>2</sup>.kg<sup>-1</sup>). Next year the values of the SLA were similar.

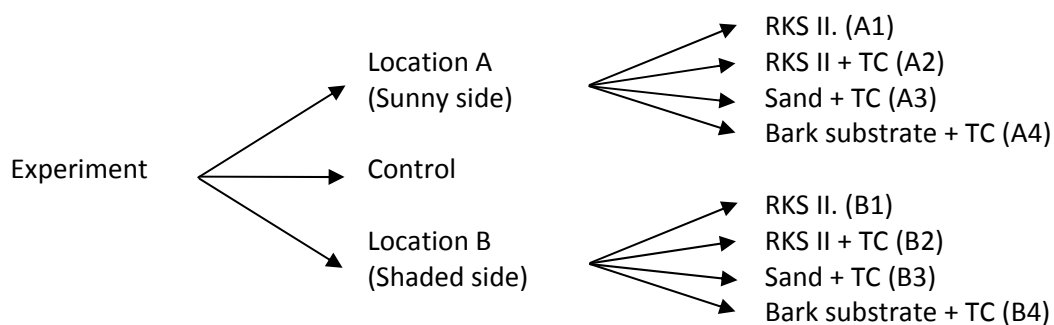
*Alnus glutinosa*, leaves, light intensity, hydro absorbent, RKS II, sand, bark substrate

Almost all unfavourable environmental conditions are directly and indirectly reflected in the development of the majority of plant organs. The most marked effect of these conditions is seen on the assimilative organs. Literary sources in general report that the leaf area changes in dependence on the light intensity (Šantrůček, 1998; Pallardy, 2008). Changes in the leaf size and leaf morphology may indicate tolerance of the species to shading (Niinements, Kull, 1994); especially the leaves growing in shade are larger and thinner (Veen,

Meijer, 1962; Pallardy, 2008) but also their shape is changed (Pallardy, 2008). Jurik *et al.* (1982) discovered that strawberry leaves growing in light were stronger than the shaded ones which contained more mesophyll cells. Xing Hua *et al.* (2010) monitored changes in the shape of leaves of the species *Camellia nitidissima* under different light conditions and they proved that shaded plants have a tendency to make shorter and narrower leaves. As a general rule, woody species growing under higher light intensity produce longer leaves and leaves with a wider leaf

blade. The length and width of leaf blade, as well as the leaf area, can be measured using simple calculation methods or using instrumentation in the form of scanners, while leaf thickness is measured by using formulas and the specific leaf area (SLA). The SLA is calculated as a rate of the total leaf area and leaf weight and is dependent on the leaf anatomy. SLA not only demonstrates the effect of light intensity but can also prove the effect of water and other conditions of the environment (Evans, Poorter, 2001; Xu *et al.*, 2009). In addition Xu *et al.* (2009) reported that after watering the length and width of leaves did not change. The production of biomass of container-cultivated plants is dependent on the relation between the water content in plants and the amount of irrigation water. Each cultivation medium has its own individual water regime which is dependent on the size of particles, amount of organic matter, ability to bind water and the permeability of the substrate. That is the reason why different cultivation media pose different demands for the amount of watering (Bunt, 1988; Raviv *et al.*, 2002). The most important cultivation substrates for container-grown plants are peat, bark, perlite, sand and vermiculite (Bunt, 1988). Sand is important in most of the cultivation mixtures. It has a low water-retaining ability (i. e. high water permeability) and that is why sandy substrates require frequent watering at low rates (Bunt, 1988). In order to improve the efficiency of water in the soil or the container cultivation mixture we use organic materials and synthetic polymers (Abedi-Koupai *et al.*, 2008). TerraCottem is a multi-component soil conditioner consisting of more than 20 substances which belong to the following groups: water-retaining and releasing polymers, mineral fertilisers, organic substances, natural root growth activators and volcanic rock. It has been proved that an application of TerraCottem improved the properties of sandy soils and of cultivation substrates in vegetable and ornamental plant production (Singh, Cotthem, 2002; Vieira *et al.*, 2005; Reis, Coelho, 2007). The objective of the present study was to evaluate the effect of four substrates and two sites of different light intensities on the leaf morphology of the model plant species *Alnus glutinosa* (L.) Gaertn.

Scheme of experiment



## MATERIALS AND METHODS

The experiment was established at a multi-purpose scientific experimental workplace on plots of the Faculty of Horticulture in Lednice in 2005–2006. The same method was used in these two years of the experiment. The experiment was assessed in three repetitions and 9 treatments. Each treatment was represented by 75 plants.

One year old seedlings, which were genetically identical, were planted out into special 2-litre nursery containers of the BC type. For the individual treatments of the experiment a substrate was pre-mixed with an addition of the hydro-absorbent TerraCottem at a rate of 5 grams per liter of the substrate. Based on the respective treatments the plants were planted out into different substrates and placed outdoors where they were exposed to natural climate effects and two different light intensities. The shading was made by the cover of the raschel net with 50% transparentness. The composition of the substrates for the individual treatments was as follows:

**Sunny:** A1: substrate RKS II (peat-bark substrate); A2: substrate RKS II + TC (hydro-absorbent TerraCottem); A3: sand + TC; A4: bark substrate + TC. **Shaded:** B1: substrate RKS II (peat-bark substrate); B2: substrate RKS II + TC (hydro-absorbent TerraCottem); B3: sand + TC; B4: bark substrate + TC.

**Control** – plants were grown on open land and planted in the conventional nursery manner on an open site.

**Studied parameters:** Morphological characters of the plants were measured on all leaves of the 9 representative plant sample at the end of vegetation. We monitored the leaf properties, number of leaves, leaf area, total leaf area and the length and width of the leaf blade. We calculated the average leaf size, size category and the length/width ratio of the leaf. We also explored the specific leaf area (SLA).

### Leaf properties

The morphological parameters of the leaves were measured before the end of vegetation (24 October 2005; 23 October 2006), i.e. 3 plants from each treatment and repetition.

**Number of leaves [specimens]:** For analysis of the leaf apparatus during the evaluation of the growth properties and differences the number of leaves was calculated in selected plants.

**Leaf area [mm<sup>2</sup>], length and width of the leaf [mm]:** The leaf blades of all the leaves on the plant were measured using the AM 200 apparatus. **The length/width ratio of the leaf** was also measured on the basis of these parameters. **The specific leaf area [m<sup>2</sup>.kg<sup>-1</sup>]:** SLA = was calculated as the total leaf area/weight ratio. The leaves were divided into **5 categories based on their size [mm<sup>2</sup>]:** 0–500 mm<sup>2</sup>; 501–1 000 mm<sup>2</sup>; 1 001–2 500 mm<sup>2</sup>; 2 501–5 000 mm<sup>2</sup>; < 5 000 mm<sup>2</sup> and their percentages in the individual classes were calculated. According to their length the leaves were divided into categories (0.1–30mm; 30.1–50.0mm; 50.1–75.0mm; 75.1–100.0mm; < 100 mm).

In the statistical processing was used the multifactorial analysis of variance (ANOVA) for comparisons of the treatments. The statistical analysis of the treatments in pairs used in the case of equal variances was the Tukey-Kramer method and in the case of unequal variances was the Games-Howell method. In all statistical evaluations were also used correlations.

## RESULTS AND DISCUSSION

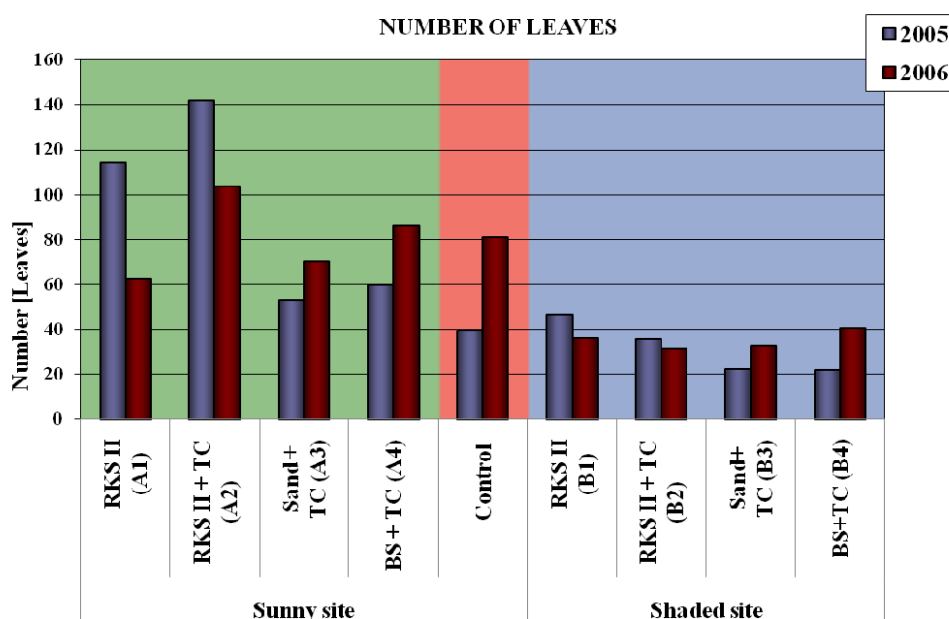
### Number of leaves

In 2005 treatments A2 and A1 resulted in the highest number of leaves (142.11 and 114.22 leaves per plant, respectively) and the fewest in treatments B3 and B4. On the sunny site the number of leaves in treatments A3 and A4 was 52.89 and 59.89, respectively. When comparing the sunny

and shaded localities of the experiment the effect of direct light was statistically highly significant. Conventional production of nursery stock using the method of bare-rooted plants did not result in more leaves (39.33 leaves per plant). In 2006, as in the previous year, treatment A2 resulted in the highest average number of leaves (103.67 leaves per plant). Owing to the great variability of the plants no statistically significant differences were discovered between the treatments on the sunny site. Plants of treatment B2 produced the least number of leaves (31.33 leaves per plant) but no statistically significant difference was seen on this site. In 2006 the plants of treatment B4 produced the highest number of leaves per plant, i.e. 40.7 leaves. Comparisons of substrate media under two different light sites showed highly significant differences in the number of leaves among treatments A2, A3 and A4. Plants grown in soil on open land produced more leaves (81.00 leaves per plant) than plants growing on a shaded site where the average values reached values similar to plants growing on a sunny site (Graph 1).

### Average size of leaf

In 2005 it was discovered that the average leaf area of plants growing on a shaded site was statistically larger than when growing on a sunny locality. This was confirmed by Pallardy (2009) who described this reaction in forest woody species. Treatment A3 and the control plants produced the smallest leaf area diameter (1 697.60 mm<sup>2</sup> and 1 708.10 mm<sup>2</sup>, respectively). These two parameters statistically significantly differed from treatments on a shaded site; treatment A1 was highly significantly different, treatment A4 only significantly different. In 2006 plants grown on a shaded site did not develop larger leaves. On both sites (sunny and shaded) a similar



1: Total number of leaves in the respective treatments

trend was discovered in the leaf area diameter; with treatment RKS II + TC the leaf area diameter was larger (on a sunny site: 3 433.80 mm<sup>2</sup>; on a shaded site: 2 120.30 mm<sup>2</sup>); the effect of the bark substrate on the leaf area diameter was very similar. The experiments of Xing Hua *et al.* (2010) showed that the leaf area diameter was larger in treatments on a sunny site compared to the contrary opinion of a number of other authors.

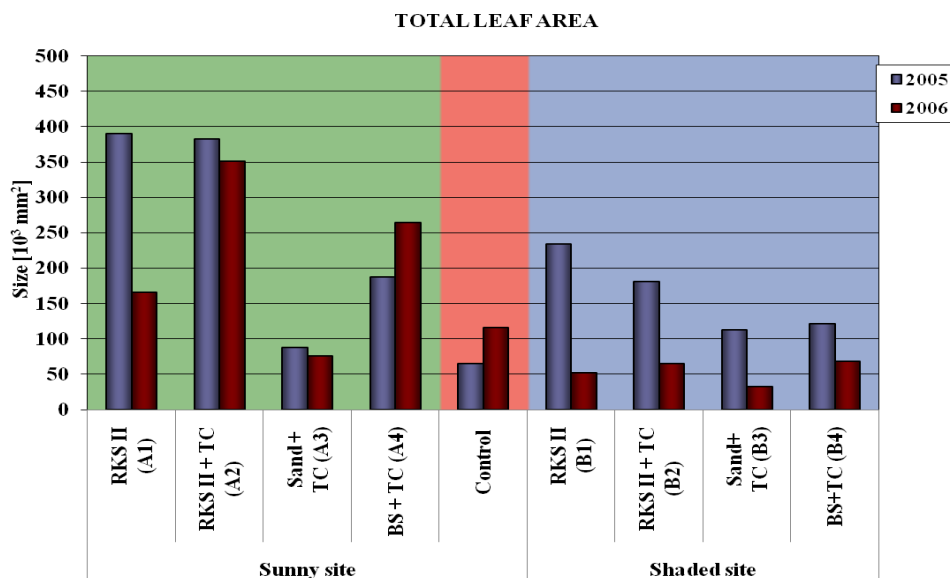
### Distribution of leaves into size categories

In 2005 most of the large leaves (over 5 000 mm<sup>2</sup>) developed under treatments A2, A1 and B1 (i.e. 17.71–19.30 leaves per plant). Nevertheless the proportion of large leaves was the highest on the shaded site (36.5–39.7%) where the total number of leaves was relatively low. Most of the leaves (of less than 500 mm<sup>2</sup>) developed in treatment A2 (13.72 leaves per plant, i.e. 9.7 %). Control plants growing on open land developed relatively small leaves and

in small numbers. On the shaded site in 2006 the leaf area was smaller than in 2005; the category of the largest leaves was a mere 0.43–7.17% of the leaves. Plants grown on open land developed small leaves with a leaf area smaller than 5 000 mm<sup>2</sup>. On both sites the used substrate medium (sand in a mixture with the hydro-absorbent) induced the production of very small leaves. The number of leaves smaller than 500 mm<sup>2</sup> was 27.98 leaves per plant (39.80%) and 13.43 leaves per plant (40.70%) on the sunny and shaded site, respectively.

### Length and width of leaves

In 2005 higher values were measured on plants growing in the shade. Comparisons of the effect of various substrates on the sunny and shaded sites revealed that the length of the leaves was statistically highly significant in treatments B2 and B3 and significant in treatments B1 and B4. The width of the leaves on these two sites differed; it was highly



2: Total leaf area of the individual treatments

I: Effect of treatments on some morphological parameters of leaves in 2005

Treatments		Leaf area (mm <sup>2</sup> )		Leaf length (mm)		Leaf width (mm)		Length/width ratio (mm.mm <sup>-1</sup> )		SLA (m <sup>2</sup> .kg <sup>-1</sup> )	
Sunny site	RKS II (A1)	3513.70	abc	85.06	b	58.15	b	1.49	b	25.57	b
	RKS II + TC (A2)	2735.00	bcd	76.31	bc	52.03	bc	1.51	ab	24.09	b
	Sand +TC (A3)	1697.60	d	61.01	cd	38.06	cd	1.63	a	27.87	b
	BS + TC (A4)	3253.90	b	81.17	b	52.94	b	1.57	ab	29.99	ab
Open land (C)		1708.10	d	58.46	d	39.97	d	1.48	ab	17.65	c
Shaded site	RKS II (B1)	5279.10	abc	98.95	a	63.98	ab	1.57	ab	41.22	a
	RKS II + TC (B2)	5145.90	a	105.04	a	67.34	ab	1.59	ab	36.18	a
	Sand +TC (B3)	5602.40	ab	108.83	a	68.60	ab	1.63	ab	35.58	a
	BS + TC (B4)	5778.70	a	109.70	a	68.13	a	1.63	ab	36.82	a

Note: identical letters placed beside the averages designate (column by column) the treatments that are statistically different at a p < 0.05 level

II: *Effect of substrate and light intensity on some morphological parameters of leaves in 2005*

	Leaf area (mm <sup>2</sup> )		Leaf length (mm)		Leaf width (mm)		Length/width ratio (mm.mm <sup>-1</sup> )		SLA (m <sup>2</sup> .kg <sup>-1</sup> )	
<b>Effect of medium</b>										
RKS II (A1)	4396.40	a	92.01	a	61.07	a	1.53	a	33.40	a
RKS II + TC (A2)	3940.45	a	90.68	a	59.69	ab	1.55	ab	30.14	a
Sand +TC (A3)	3650.00	a	84.92	a	53.33	b	1.63	b	31.73	a
BS + TC (A4)	4516.30	a	95.44	a	60.54	ab	1.56	ab	33.41	a
<b>Effect of location</b>										
Sunny site	2800.05	b	75.89	b	50.30	b	1.55	b	26.88	b
Shaded site	5451.53	a	105.63	a	67.01	a	1.60	a	37.45	a
<b>Results of two-way ANOVA (p value)</b>										
Place	0.0000	**	0.0000	**	0.0000	**	0.0216	*	0.0320	*
Medium	0.1059		0.0683		0.0183	*	0.0059	**	0.4484	
Place x medium	0.0107	*	0.0008	**	0.0003	**	0.5330		0.4668	

Note: identical letters placed beside the averages designate (column by column) the treatments that are statistically different at a  $p < 0.05$  level

III: *Effect of treatments on some morphological parameters of leaves in 2006*

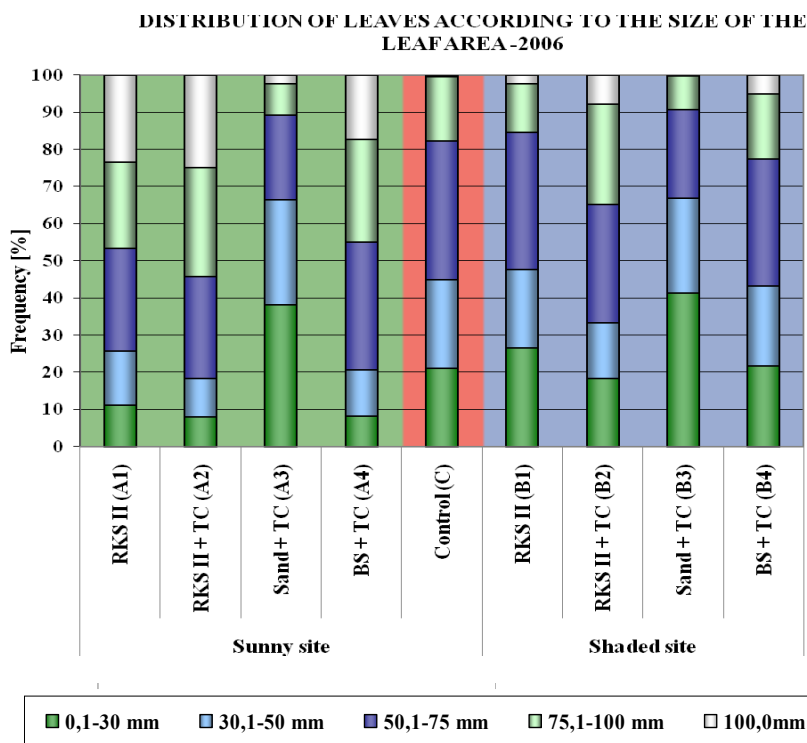
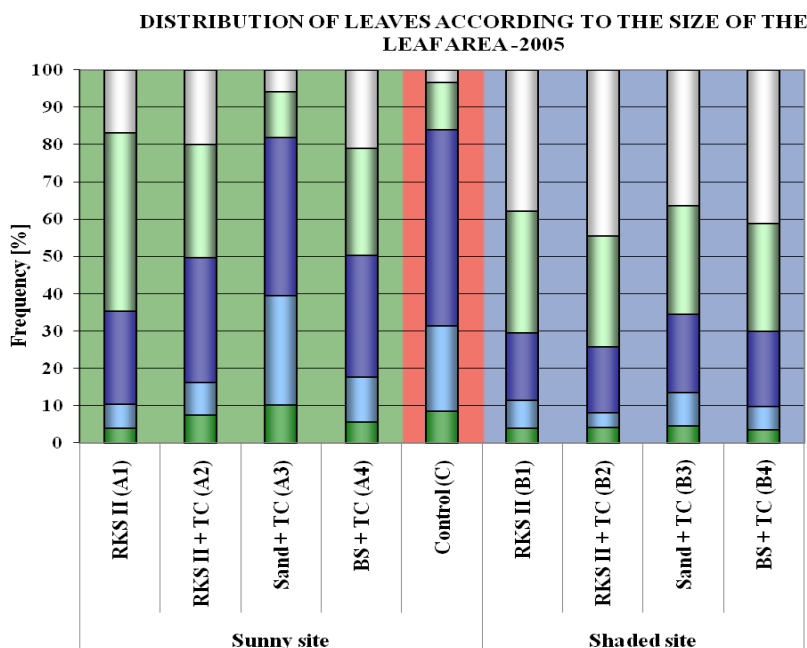
Treatments		Leaf area (mm <sup>2</sup> )		Leaf length (mm)		Leaf width (mm)		Length/width ratio (mm.mm <sup>-1</sup> )		SLA (m <sup>2</sup> .kg <sup>-1</sup> )	
Sunny site	RKS II (A1)	2870.00	ab	79.38	a	50.69	ab	1.60	ab	22.07	abc
	RKS II + TC (A2)	3433.80	a	82.21	a	53.81	a	1.56	b	24.73	a
	Sand +TC (A3)	1127.30	d	44.36	c	29.44	c	1.58	ab	21.40	b
	BS + TC (A4)	3193.40	a	80.31	a	51.53	ab	1.60	ab	24.03	ab
	Open land (C)	1449.00	cd	54.14	b	35.06	c	1.59	ab	16.02	c
Shaded site	RKS II (B1)	1448.60	cd	53.72	bc	33.99	c	1.66	ab	26.55	a
	RKS II + TC (B2)	2120.30	bc	69.48	ab	41.23	bc	1.74	a	25.50	ab
	Sand +TC (B3)	1022.30	d	46.00	bc	27.62	c	1.72	ab	29.22	ab
	BS + TC (B4)	1920.80	bcd	65.41	ab	37.77	bc	1.83	ab	28.39	ab

Note: identical letters placed beside the averages designate (column by column) the treatments that are statistically different at a  $p < 0.05$  level

IV: *Effect of substrate and light intensity on some morphological parameters of leaves in 2006*

	Leaf area (mm <sup>2</sup> )		Leaf length (mm)		Leaf width (mm)		Length/width ratio (mm.mm <sup>-1</sup> )		SLA (m <sup>2</sup> .kg <sup>-1</sup> )	
<b>Effect of medium</b>										
RKS II	2159.30	a	66.55	a	42.34	a	1.63	a	24.31	a
RKS II + TC (A2)	2777.05	a	75.85	a	47.52	a	1.65	a	25.12	a
Sand +TC (A3)	1074.80	b	45.18	b	28.53	b	1.65	a	25.31	a
BS + TC (A4)	2557.10	a	72.86	a	44.65	a	1.72	a	26.21	a
<b>Effect of location</b>										
Sunny site	2656.13	a	71.57	a	46.37	a	1.58	b	23.06	b
Shaded site	1628.00	b	58.65	b	35.15	b	1.74	a	27.42	a
<b>Results of two-way ANOVA (p value)</b>										
Place	0.0000	**	0.0000	**	0.0000	**	0.0000	**	0.0006	**
Medium	0.0000	**	0.0000	**	0.0000	**	0.2517		0.9212	
Place x medium	0.0019	**	0.0017	**	0.0094	**	0.2390		0.3684	

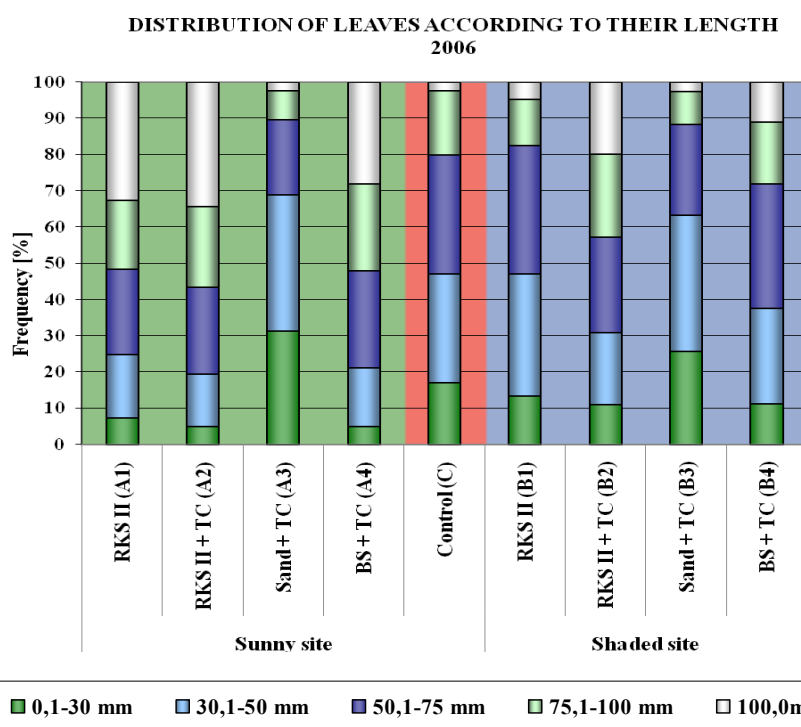
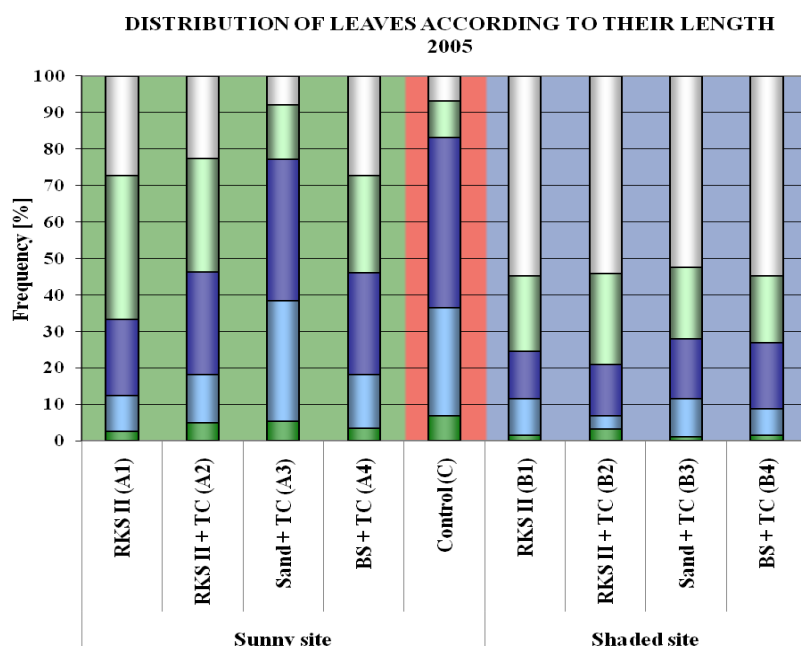
Note: identical letters placed beside the averages designate (column by column) the treatments that are statistically different at a  $p < 0.05$  level



3: Distribution of leaves according to the size of the leaf area

significant when grown on the sand substrate and significant on the bark substrate. The length and width values of the leaves were the lowest on control plants and in treatment A3. The effect of the hydro-absorbent was not significant; on site A the effect was seen as slightly declining and on site B slightly increasing but insignificant. **In 2006** the width/length ratio was lower on the shaded site. The

highest average values were achieved in treatments A2, A1 and A4 (length 79.38–82.21 mm; width 50.69–53.81 mm). These three treatments were statistically identical in both parameters. The lowest values were detected in the sand-grown plants (in the sun and shade: length 44.36–46.00 mm; width 27.62–29.44 mm). Likewise treatment B1 and the control developed relatively small leaves. The addition



4: Distribution of leaves according to their length

of the hydro-absorbent into the RKS II substrate had a positive effect on the average value but the difference was not significant.

#### Length/width ratio of leaves

Comparisons of the length/width ratio of the leaves showed that the differences among the treatments were not great; average values ranged between 1.58 and 1.84. In 2006 statistical differences

were found only between treatments A2 and B2 (B2 – 1.74; A2 – 1.56). Adding the hydro-absorbent to the RKS II substrate had a slight positive effect on the length/width ratio of the leaves, with the exception of 2006 on the sunny site. Using statistical analyses we detected no differences among the substrates on sites of different light intensities; slightly higher values were monitored on the shaded site in 2005 and 2006. In 2005 and 2006 the treatments A1 and



A2 were on average highly significant with a shorter length and leaf ratio (leaf ratio was 1.54–1.55) compared to treatments B2, B3 and B4 (leaf ratio 1.67–1.73). The effect of light was highly significantly higher on the shaded locality. TerraCottem did not markedly affect the length/width ratio of the leaves of plants grown on the RKS II substrate. The results of Pallardy (2008) confirmed that the light intensity changed the shape of the leaves. A higher length/width ratio indicates an extended length in relation to the width; Veen, Meijer, (1962) reached the same conclusion.

### Specific leaf area

In 2005 the highest values were monitored in treatment B1 (41.22 m<sup>2</sup>.kg<sup>-1</sup>). The lowest average values were achieved in plants growing on open

land (17.65 m<sup>2</sup>.kg<sup>-1</sup>). On a shaded site the SLA values in treatments B2, B3 and B4 were very similar (35.58–36.82 m<sup>2</sup>.kg<sup>-1</sup>). We compared the effect of shading and discovered that the specific leaf area with each substrate was higher on the shaded site than on the sunny site. In 2006 the same effects of shading were observed as in 2005. The values were the highest in treatment B3 (29.22 m<sup>2</sup>.kg<sup>-1</sup>). In this year the SLA of plants grown in soil reached the lowest values (16.02 m<sup>2</sup>.kg<sup>-1</sup>). In all the treatments the SLA values were higher than those measured by Vares (2000) in mature plants. In experiments with leaves of *Alnus* seedlings the SLA values were higher than reported in literary sources. The lowest values were monitored in the control plants. On the shaded site the leaves were thinner as was confirmed by a number of authors (Veen, Meijer, 1962; Jurik *et al.*, 1982; Pallardy, 2008).

## SUMMARY

The correlation between environmental conditions and possibilities of affecting the commercial properties of plants has been permanently studied in all its aspects. The focus of the present experiment was on evaluations of the properties and actions of four substrate media and two sites of different light intensities on the properties of leaves of the model plant *Alnus glutinosa* (L.) Gaertn. The experiment was established in 2005–2006 on a multi-purpose plot of the Faculty of Horticulture in Lednice, MENDELU. The experimental plants were cultivated in 2-litre containers. For comparison purposes plants were grown conventionally in soil on open land where they were exposed to natural influences of the open-air environment. In the experiment we explored the development of the plants and properties of the leaf apparatus with a focus on the following: number of leaves; average size of leaves; size category; evaluations of the length and width of the leaves incl. the length/width ratio; and the specific leaf area. Statistical results of the evaluated parameters resulted in highly significant differences among the treatments and sites. The best results were achieved with experimental treatment II (RKS II – peat-bark substrate with an addition of hydro-absorbent). In all the experimental years the most suitable substrate for most of the parameters was the peat-bark substrate RKS II + TerraCottem. Sand with the hydro-absorbent was unsuitable independent on the effect of the year. TerraCottem had a favourable effect on the leaf weight ratio and also on the efficiency of leaves. The number of leaves in the individual treatments was variable as was the effect of the used substrate. The effect of light was strongly correlated with the leaf weight. The results of 2-year evaluations of some parameters of leaves of plants cultivated in different substrates and sites confirmed the positive effect of the hydro-absorbent on a sunny site in the treatment with the peat-bark substrate. The positive effect of the hydro-absorbent in the sand medium did not improve the leaf parameters; this treatment can partly be compared with the control treatment on open land. The present study summarises important indicators of properties of the leaf apparatus and its changes under the action of different conditions of the substrate and light intensities. Treatments A3 (1 697.60 mm<sup>2</sup>) and a control (1 708.10 mm<sup>2</sup>) had the smallest leaf area. These two treatments significantly statistically differed from those in the shade location. In the 2005 year the highest values were measured at the treatment B1 (41.22 m<sup>2</sup>.kg<sup>-1</sup>). Next year the values of the SLA were similar.

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