

## **EFFECTS OF BIOSTIMULATOR AND LEAF FERTILIZERS ON *Prunus mahaleb* L. STOCKPLANTS AND THEIR CUTTINGS**

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**Abstract.** The effect of Kelpak<sup>®</sup> biostimulator, Wuxal<sup>®</sup> Ascofol and Pentakeep<sup>®</sup>-V leaf fertilizers were tested on *Prunus mahaleb* L. 'Bogdány' stockplants. Shoot production and characteristics, rooting and quality of their cuttings were evaluated. Biostimulator and fertilizers were sprayed four times on leaves before cuttings were taken. The number and fresh masses of the shoots, cutting weights, leaf chlorophyll indexes (SPAD) and indole-3-acetic-acid (IAA) levels in shoot tip were measured on stockplants. Rooting rates, IAA-levels in the rooting zone of cuttings, fresh and dry masses of rooted cuttings, both weights of the root and shoot were measured. Based on these results Kelpak<sup>®</sup> pretreatment could be considered as the most effective in improving the productivity of *Prunus mahaleb* L. stockplants, leaf chlorophyll indexes and IAA-level in shoot tip of stockplant and in cutting base. The stockplant pretreatment by Kelpak<sup>®</sup> increased both the single shoot mass and consequently the fresh mass of prepared cuttings. While these pretreatments alone did not affect the rooting rate, the pretreatment of shoots by Kelpak<sup>®</sup> and Pentakeep<sup>®</sup>-V increased the dry mass production of cuttings during rooting. Kelpak<sup>®</sup> pretreatment affected the root dry mass positively, while Pentakeep<sup>®</sup>-V increased the shoot dry mass of rooted cuttings.

**Key words:** fresh and dry mass production, IAA-level, Kelpak<sup>®</sup>, Pentakeep<sup>®</sup>-V, pretreatment, rooting rate, stockplant productivity, Wuxal<sup>®</sup> Ascofol

### **INTRODUCTION**

Stockplants' shoot production and cuttings' quality is essential for propagation efficiency. Cutting size, its morphology and physiological condition (nutrient status, native hormonal level) are determining factors in adventitious root formation and subsequent growth after rooting [Hartmann et al. 1997, Leakey 2004]. Improvement in above char-

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acteristics of shoots used for propagation may considerably increase the propagation efficiency.

It is commonly accepted that environmental factors such as light, water, nutrients, management and pretreatment of stockplants impact the rooting ability of cuttings through their effects on the morphology or physiological condition of the shoots [Hartmann et al. 1997, Leakey 2004].

Several studies showed that photosynthesis takes place in severed leafy stem cuttings during propagation which is itself influenced by propagator microclimate and cutting leaf area. Cuttings' root formation requires a positive carbon balance (faster assimilate production, than loss of assimilates during respiration and formation of new organs). Rooting ability presumably depends on the balance between photosynthesis and transpiration. However, the number of roots is produced, closely related to photosynthetic rate [Mesén et al. 1997, Leakey 2004]. The results of Leakey and Storeton-West [1992] suggest that rooting is promoted by the production of specific sugars during the period when the cuttings are in the propagator.

Photosynthesis and also the initial pre-severance store of carbohydrate [Mesén et al. 2001] influence the rooting of leafy cuttings. Despite the importance of current assimilates for rooting in leafy softwood cuttings, Magingo and Dick [2001] and Mesén et al. [2001] showed evidences that the level of dependency does vary between species. Perhaps, the variance reflects the differences in stem anatomy. This underlines the importance of stored assimilate from the pre-severance stage of leafy stem cuttings.

Leakey and Storeton-West [1992] found complex interactions between nutrients and the quantity and quality of light, which affected photosynthesis and the carbohydrate status of cuttings. The characteristics of pre-severance physiology and the morphology of stockplants enhanced subsequently the cuttings post-severance physiological status and promoted high rooting ability [Hoad and Leakey 1996]. The pre-severance auxin levels in stem parts used for cuttings affected the rooting too: the earlier injection of auxins was found to enhance rooting ability [Leakey 1992].

Leaf fertilizer treatments increase efficiently the shoot growth of stockplants. However, they may have both positive and negative effects on subsequent rooting of cuttings [Leakey 2004]. Based on their positive effects on intact plants [Beckett and van Staden 1989, Hotta et al. 1997, 1998, Watanabe et al. 2000, Kositorna and Smolinski 2008, Gajc-Wolska et al. 2009, Memon et al. 2009, Kwiatkowski et al. 2013] some of the recently introduced biostimulators and leaf fertilizers seem to be promising in improving the biomass production, which may increase the shoot production and shoot quality of stockplants [Szabó et al. 2011]. Further on as a subsequent effect, they may influence the rooting.

Kelpak<sup>®</sup> biostimulator is derived from brown kelp (seaweed, *Ecklonia maxima*). It contains auxin-like and cytokinin-like natural compounds. Indole-3-acetic acid (IAA) is dominant in Kelpak<sup>®</sup>, which improves the cell growth and the cell elongation, root growth and plants' activities [Crouch and van Staden 1991, Crouch et al. 1992, Jones and van Staden 1997, Jenkins and Mahmood 2003]. Wuxal<sup>®</sup> Ascofol is a leaf fertilizer with seaweed (*Ascophyllum nodosum*) extract, containing N, K and micronutrients. Wuxal<sup>®</sup> Ascofol increases the yield and quality of fruits and grape [Colapietra and Alexander 2006]. Besides nutrients, Pentakeep<sup>®</sup>-V contains 5-aminolevulinic-acid (ALA).

This compound stimulates chlorophyll-synthesis in low concentration (10 mM) and improves photosynthesis which results in higher yields and improved crop quality [Hotta et al. 1997, Bingshan et al. 1998, Babik et al. 2008, Dwornikiewicz 2008, Smoleń et al. 2010a, b]. This product moderated the salt stress on spinach [Nishihara et al. 2002] and on cotton [Watanabe et al. 2000].

There is no or very little information on the effects of the above-mentioned biostimulator and fertilizers on shoot production of stockplants' or cuttings' quality, rooting capacity and subsequent growth of cuttings. That is why we targeted in our investigations the improvement in propagation efficiency by using biostimulator and fertilizers on test plant *Prunus mahaleb* 'Bogdány' clonal rootstocks.

## MATERIAL AND METHODS

The investigations were carried out at Experimental and Research Farm of Corvinus University of Budapest, Faculty of Horticultural Science. The farm is located in Central Hungary on light sandy soil with pH of 7.8, the yearly average temperature is 11.3°C, the hours of sunlight are 2079, annual precipitation is 550 mm.

### Applied biostimulators and fertilizers.

– Kelpak<sup>®</sup> contains 11 mg·l<sup>-1</sup> natural auxins, 0.03 mg·l<sup>-1</sup> natural cytokinin substances (Kelpak Guide Manual).

– Wuxal<sup>®</sup> Ascofol consists of 2.5% N, 1.25% K<sub>2</sub>O, micronutrients (0.8% S, 3% B, 0.14% Ca, 0.0003% Cu, 0.003% I, 0.005% Fe, 0.8% Mn, 0.05% Zn) and plant hormones such as cytokinin, gibberellic acid and auxin [Kwizda Agro 2009].

– Pentakeep<sup>®</sup>-V contains 9.5% N, 5.7% Mg, 0.3% Mn, 0.45% B, DTPA-Fe, ZnSO<sub>4</sub>, CuSO<sub>4</sub>, dinatrium-molibdenat, and 0.3% ALA (5-aminolevulic-acid) [Hotta et al. 1997].

**Stockplant treatments.** Test plant was *Prunus mahaleb* L. 'Bogdány', a clonal cherry rootstock, propagated by softwood cuttings [Hrotkó 1982, Hrotkó and Magyar 2004]. Stockplants were 1.2 m high, planted in the spring of 2009 in 2.5 m rows and 0.6 m plant distance trained to hedge form. The following treatments were applied to 'Bogdány' stockplants: Kelpak<sup>®</sup> 0.2%, Wuxal<sup>®</sup> Ascofol 0.2% and Pentakeep<sup>®</sup>-V 0.05% by foliar spray, four times at 7 days interval from the end of April to the middle of May. Each treatment was applied on three stockplants, the control trees remained untreated. One tree was one replication. One week after the last treatment the number of shoots appropriate for cutting (more than 3 mm basal thickness) [Hrotkó 1982] on each tree was counted. At this time, the leaf chlorophyll content of the stockplants also was measured on 20 leaves as replicates per tree, using Konica Minolta SPAD-502 Chlorophyll meter. At the end of May, one week after the last foliar spray, the shoots were taken from each stockplant to measure total shoot mass, and those shoots, what thicker than 3 mm basal caliper, were prepared for cuttings.

**Measuring photosynthetic activity on stockplants.** Stomatal conductance, transpiration and photosynthetic activity were measured on treated and control stockplants using portable infrared gas analyzer (LCi, BioScientific Ltd. UK) over the course of years (2010–2012). We measured the leaf gas exchange on healthy and well developed leaves from the middle of shoot sampling 20 leaves from each treatment, possibly with

similar exposure to photosynthetic active radiation (PAR), on each side of trees. The measurements were carried out after the fourth biostimulator treatment, one week before taking cuttings, in the morning hours (10:00–12:00).

**Evaluation of rooting and cuttings' quality.** Shoots from stockplants pretreated by biostimulator and fertilizers, as well as untreated (control) ones were taken by the end of May, prepared for cuttings from the basal part of the shoot. The cuttings were 20 cm long, each with three leaves cut in half. Their fresh mass (FM) and dry mass (DM, after drying them at 80°C to constant weight) was measured. All the cuttings of pretreated and control stockplants were treated later uniformly, applying 0.2% IBA in 50% ethanol solution for 5 s, then douse for 30 s. Cuttings were rooted under intermittent mist in crate filled with perlite. The rooting trial was designed in randomized blocks repeated ten times, each plot contained 8 cuttings. Eight weeks after taking cuttings, the rooting rate (%) was calculated relating the rooted cuttings to the total number (8) in each plot. After rooting, samples were taken of rooted cuttings for measuring of fresh and dry mass. Roots and shoots were measured separately. New shoots formed during rooting were counted and averaged per treatment. The difference between starting and final mass (mass increment) of cuttings was calculated. In 2010 due to technical difficulties in the mist propagation facility, cuttings died in large number and the rooting failed, so data from this year on rooting and rooted cuttings was not evaluated.

**Measurements on indole-3-acetic acid (IAA) level in shoots and cuttings.** Sample preparation: Firstly samples were collected before taking cuttings for measuring of IAA-level from stockplants' shoot tips. Other hands basal part of one-week-old cuttings was sampled from each treatment. The IAA level of shoot tips was investigated in three years (2010, 2011 and 2012), while we took samples from the seven-days-old rooting base of cuttings in 2011 and 2012. The samples were measured and cut fine, then 80 mg·l<sup>-1</sup> BHT (butylated hydroxytoluene), containing 80% methanol, was added for each sample, at -20°C in the dark to avoid IAA degradation. 500 µl were calibrated from this solution to an Eppendorf-tube, and 300 µl of 4 M NaOH were added to precipitate the chlorophyll. 100 µl of acetic acid were added to the solution to move the pH-level below 7, and the mixture was centrifuged for 5 min at 15 000 rpm. The supernatant was filtered through a 0.45 µm PVDF Millex-HN Syringe Driven Filter Unit and injected into the HPLC-instrument.

**Chemicals:** All chemicals (the standards, the methanol, the HPLC grade acetic acid and BHT) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, Missouri 63178).

Analytical grade indole-3-acetic acid (IAA) was used as standard. IAA was prepared in a methanol stock-solution (0.2 mg·kg<sup>-1</sup>) and a 50× diluted solution was used as a standard for the HPLC measurements.

**HPLC analysis:** WATERS High Performance Liquid Chromatograph (WATERS Co.) equipped with a 2487 Dual Absorbance UV/Visible Detector, a 717plus Autosampler, Inline Degasser AF and a 1525 Binary HPLC Pump, controlled with EMPOWER™ 2 software, was used. A SYMMETRY C18 5 µm 4.6 × 150 mm column was installed. The mobile phase was a combination methanol: water 60 : 40 v/v % containing 0.5% glacial acetic acid. The isocratic flow rate was 1 ml min<sup>-1</sup>. The pressure on the column was 2300 ±15 psi at ambient room temperature. Each injected volume was 20 µl, and

the running time was fixed at 10 min. The IAA was monitored at a wavelength of 280 nm. In the standard solution IAA had the 4.5 min retention time. The concentrations were calculated in  $\mu\text{g}\cdot\text{g}^{-1}$  fresh plant weight.

**Statistical analysis.** All data were statistically analyzed by ANOVA using the statistical package IBM SPSS Statistics 20. Means were separated by Duncan-test at level  $p = 0.05$ . Different letters in the same column of the tables indicate significant differences at  $p = 0.05$ .

## RESULTS

**Stockplant productivity and leaf chlorophyll index.** Data of total shoot weight, shoot number appropriate for cuttings (3 mm  $\leq$  basal thickness) are displayed in Table 1. The number of appropriate shoots for cutting preparation (3 mm  $\leq$  basal thickness) on each stockplant increased from 2009 onward year by year reaching 240 shoots per plant. We found significant differences between the treatments. The average of shoot number on stockplants treated by Kelpak<sup>®</sup> in 2010, 2011, 2012 and in average of four years was significantly larger than on the control plants (tab. 1). The average of shoot number on stockplants treated by Wuxal<sup>®</sup> Ascofol was also considerable larger than on the control in 2010 and 2012, furthermore in average of four years (tab. 1).

Table 1. The stockplant productivity and shoot traits of *Prunus mahaleb* 'Bogdány' treated by different biostimulator/fertilizers (2009–2012)

	Treatments	2009	2010	2011	2012	Average
Shoot number on stockplant (pcs/plant)	untreated	26 a	38 a	84 a	185 a	83 a
	Kelpak <sup>®</sup>	32 ab	45 b	113 b	238 b	107 b
	Wuxal <sup>®</sup> Ascofol	21 a	43 b	103 ab	240 b	102 b
	Pentakeep <sup>®</sup> -V	42 b	42 b	92 ab	199 ab	94 ab
Mean single shoot mass (g)	untreated	9.6 a	10.3 a	7.4 a	7.3 a	8.7 a
	Kelpak <sup>®</sup>	10.4 a	11.1 b	7.8 a	8.3 b	9.4 b
	Wuxal <sup>®</sup> Ascofol	9.4 a	10.1 a	7.3 a	7.4 a	8.6 a
	Pentakeep <sup>®</sup> -V	10.1 a	10.6 a	7.7 a	7.9 a	9.1 ab
Total shoot mass on stockplant (g)	untreated	250 ab	390 a	600 a	1348 a	647 a
	Kelpak <sup>®</sup>	333 ab	498 c	865 b	1980 b	919 b
	Wuxal <sup>®</sup> Ascofol	197 a	434 b	755 ab	1745 ab	783 ab
	Pentakeep <sup>®</sup> -V	424 c	443 b	708 ab	1552 ab	782 ab
Leaf chlorophyll index (measured by SPAD)	untreated	33.3 a	40.9 a	43.3 b	46.0 a	40.9 a
	Kelpak <sup>®</sup>	35.0 b	40.8 a	43.3 b	46.0 a	41.3 b
	Wuxal <sup>®</sup> Ascofol	34.6 ab	40.0 a	42.9 ab	46.1 a	40.9 a
	Pentakeep <sup>®</sup> -V	33.3 a	40.5 a	41.4 a	46.8 a	40.5 a
Mean fresh mass of single cutting before rooting (g)	untreated	2.17 a	3.38 a	2.89 a	2.18 a	2.66 a
	Kelpak <sup>®</sup>	2.24 a	3.62 b	3.32 b	2.71 b	2.98 b
	Wuxal <sup>®</sup> Ascofol	2.04 a	3.56 ab	3.12 b	2.38 ab	2.78 ab
	Pentakeep <sup>®</sup> -V	2.06 a	3.43 a	3.12 b	2.23 a	2.71 a
Mean dry mass of single cutting before rooting (g)	untreated	0.65 a	0.79 a	0.94 a	0.74 a	0.78 a
	Kelpak <sup>®</sup>	0.62 a	0.89 b	0.88 a	0.84 a	0.81 b
	Wuxal <sup>®</sup> Ascofol	0.65 a	0.86 ab	0.81 a	0.82 a	0.79 a
	Pentakeep <sup>®</sup> -V	0.56 a	0.90 b	0.91 a	0.76 a	0.78 a

Note: means are separated by Duncan-test, different letters in the same column indicate significant differences at  $p = 0.05$ . If there are two letters in one cell, there are not any significant differences

The mean single shoot mass varied between 7.3 and 11.1 g, our results showed significant differences in 2010 and 2012, furthermore in average of four years between Kelpak<sup>®</sup> and other treatments. The single shoot mass was largest in those years on stockplants treated by Kelpak<sup>®</sup> significantly larger than on the control and Wuxal<sup>®</sup> Ascofol treated plants. Considering the average of four years, stockplants treated by Kelpak<sup>®</sup> produced significantly larger single shoot weights than control and Wuxal<sup>®</sup> Ascofol treated plants. Significant differences were found between the total shoot mass on stockplants. The stockplants treated by Kelpak<sup>®</sup> had got significant larger shoot mass than on control, while the results of Wuxal<sup>®</sup> Ascofol and Pentakeep<sup>®</sup>-V treatments were in between them (tab. 1).

In 2009 and 2011 we found significant differences in leaf chlorophyll indexes in response to different treatments. However the leaf chlorophyll index of stockplants compared to other three treatments in four-years-average was significantly larger only, when treated with Kelpak<sup>®</sup>. The mean fresh weight (FM) and mean dry weight (DM) of prepared cuttings before rooting over the four years showed the largest mass on Kelpak<sup>®</sup> treated plants, significantly larger than in case of control and Pentakeep<sup>®</sup>-V treated ones, while the FM of Wuxal<sup>®</sup> Ascofol treated plants fell between these two groups (tab. 1).

Table 2. Stomatal conductance, transpiration and photosynthetic activity on stockplants

Treatments on stockplant		2010	2011	2012	Average
Stomatal conductance (gs), mol·m <sup>-2</sup> ·s <sup>-1</sup>	untreated	0.521 a	0.401 ab	0.196 a	0.373 a
	Kelpak <sup>®</sup>	0.718 b	0.419 b	0.209 a	0.448 b
	Wuxal <sup>®</sup> Ascofol	0.801 b	0.412 b	0.206 a	0.473 b
	Pentakeep <sup>®</sup> -V	0.590 a	0.301 a	0.198 a	0.363 a
Transpiration (E), mmol·m <sup>-2</sup> ·s <sup>-1</sup>	untreated	8.837 b	7.290 a	4.097 a	6.741 ab
	Kelpak <sup>®</sup>	7.822 a	7.293 a	4.131 a	6.415 a
	Wuxal <sup>®</sup> Ascofol	9.517 c	7.040 a	4.137 a	6.898 b
	Pentakeep <sup>®</sup> -V	9.912 c	6.338 a	4.072 a	6.642 ab
Photosynthetic activity (A), μmol·m <sup>-2</sup> ·s <sup>-1</sup>	untreated	23.102 a	19.390 ab	14.343 a	18.945 a
	Kelpak <sup>®</sup>	23.478 a	20.805 b	15.089 a	19.791 b
	Wuxal <sup>®</sup> Ascofol	22.498 a	20.839 b	15.133 a	19.490 ab
	Pentakeep <sup>®</sup> -V	24.113 a	17.202 a	14.235 a	18.516 a

Note: means are separated by Duncan-test, different letters in the same column indicate significant differences at  $p = 0.05$ . If there are two letters in one cell, there are not any significant differences

**Stomatal conductance, transpiration and photosynthetic activity on stockplants.** The three years average showed that stomatal conductance was significantly higher on Kelpak<sup>®</sup> and Wuxal<sup>®</sup> Ascofol treated leaves, compared to control plants (tab. 2). The transpiration ( $E$ ) of the stockplants showed significant differences in 2009 only: leaves of Kelpak<sup>®</sup> treated stockplants transpired less water than control and other

treatments. The average over three years showed no significant difference related to control (tab. 2). In the three-years average, the leaves of Kelpak<sup>®</sup> treated stockplants assimilated significantly more CO<sub>2</sub> than either those of the control plants or those of the Pentakeep<sup>®</sup>-V treated plants, while Wuxal<sup>®</sup> Ascofol treated plants assimilated CO<sub>2</sub> at rates between these two groups.

**IAA level of pretreated shoots and cuttings, rooting rates and masses of fresh cuttings after rooting.** The indole-3-acetic-acid (IAA) level in shoot tip samples taken from differently pretreated shoots ranged between 179.2 and 253.5 µg·g<sup>-1</sup>. The levels varied moderately across the three years under investigation (2010–2012). The IAA-levels measured in shoot tips showed significant differences in 2010 and 2011. The IAA-level of Pentakeep<sup>®</sup>-V and Kelpak<sup>®</sup> treated plants was higher than that of the control in these two years. The shoot tips of Wuxal<sup>®</sup> Ascofol treated plants only once (2011) had a significantly higher IAA-level. In the three-years-average, any treatments did not affect the IAA-levels measured in shoot tip (tab. 3).

Table 3. IAA-level in shoot tip, basal part of one-week-old cuttings of rooted cuttings prepared from pretreated stockplants' shoots

Treatments on stockplants		2010	2011	2012	Average
IAA-level in shoot tip, µg·g <sup>-1</sup>	untreated	234.4 ab	179.2 a	221.5 a	211.7 a
	Kelpak <sup>®</sup>	245.0 bc	216.8 b	200.7 a	220.8 a
	Wuxal <sup>®</sup> Ascofol	221.5 a	218.1 b	224.2 a	221.3 a
	Pentakeep <sup>®</sup> -V	253.5 c	207.5 b	222.1 a	227.7 a
IAA-level in cuttings' base, µg·g <sup>-1</sup>	untreated	–	10.0 a	31.6 a	20.8 a
	Kelpak <sup>®</sup>	–	14.3 b	48.4 b	31.4 c
	Wuxal <sup>®</sup> Ascofol	–	9.2 a	43.4 b	26.3 b
	Pentakeep <sup>®</sup> -V	–	10.5 a	24.3 a	17.4 a

Note: means are separated by Duncan-test, different letters in the same column indicate significant differences at  $p = 0.05$ . If there are two letters in one cell, there are not any significant differences

Table 4. Rooting rate and fresh mass (FM) of rooted cuttings prepared from pretreated stockplants' shoots

Treatments on stockplants	Rooting rate (%)	Total FM (g) of rooted cuttings
Untreated	83.4 a	3.54 a
Kelpak <sup>®</sup>	82.8 a	4.10 b
Wuxal <sup>®</sup> Ascofol	78.5 a	3.47 a
Pentakeep <sup>®</sup> -V	76.9 a	3.87 ab

Note: means are separated by Duncan-test, different letters in the same column indicate significant differences at  $p = 0.05$ . If there are two letters in one cell, there are not any significant differences

The IAA-level measured in the basal part of the rooting cuttings on the 7<sup>th</sup> day was the highest in cuttings prepared from shoots on Kelpak<sup>®</sup> pretreated stockplants in both 2011 and 2012 (tab. 4). In the two-years-average, Kelpak<sup>®</sup> and Wuxal<sup>®</sup> Ascofol treatments resulted significant differences. No significant differences were found in the rooting rate of cuttings prepared from pretreated shoots (tab. 4).

The total FM of rooted cuttings was significantly higher in 2009 and in three-years-average when prepared of the shoots from Kelpak<sup>®</sup> pretreated stockplants. In other years similar tendencies were observed (tab. 4).

Table 5. The DM production and partitioning of rooted cuttings of *Prunus mahaleb* 'Bogdány' pretreated by biostimulator on stockplants (average of years 2009, 2011, 2012)

Treatments on stockplants	Increment in total DM (g)	DM total (g)	Shoot DM (g)	Root DM (g)
Untreated	0.338 a	1.154 a	1.062 a	0.091 a
Kelpak <sup>®</sup>	0.526 bc	1.310 b	1.180 ab	0.132 b
Wuxal <sup>®</sup> Ascofol	0.378 ab	1.137 a	1.040 a	0.096 a
Pentakeep <sup>®</sup> -V	0.613 c	1.355 b	1.267 b	0.087 a

Note: means are separated by Duncan-test, different letters in the same column indicate significant differences at  $p = 0.05$ . If there are two letters in one cell, there are not any significant differences

**The dry mass (DM) of cuttings measured after rooting.** The total DM of cuttings after rooting showed significant difference. The DM of cuttings prepared from shoots of Pentakeep<sup>®</sup>-V and Kelpak<sup>®</sup> pretreated stockplant were significant higher than that of control and Wuxal<sup>®</sup> Ascofol treated plants. Significant larger shoot DM showed only on Pentakeep<sup>®</sup>-V pretreated stockplants, while the root DM was larger on Kelpak<sup>®</sup> pretreated stockplants (tab. 5). The dry mass increment of cuttings prepared from differently pretreated shoots showed significant differences (tab. 5). Larger DM increment was measured in cuttings prepared from Pentakeep<sup>®</sup>-V pretreated shoots, followed by Kelpak<sup>®</sup> treated ones showing significant difference to the control. Cuttings from Wuxal<sup>®</sup> Ascofol treated stockplants did not differ from control.

## DISCUSSION

**Stockplant productivity.** Based on our results (tab. 1) we can state that the applied leaf spray treatments affected both the productivity and shoot characteristics of *Prunus mahaleb* 'Bogdány' (L.) stockplants. The number of shoots thicker than 3 mm in four-years-average increased on stockplants when treated by Kelpak<sup>®</sup> and Wuxal<sup>®</sup> Ascofol. The single shoot mass also was larger on stockplants treated by Kelpak<sup>®</sup> while the Pentakeep<sup>®</sup>-V treatment resulted in light increasing tendency. So the stockplant treatments increased the total biomass production on stockplants, especially the shoot mass appropriate for preparation of cuttings (3 mm  $\leq$ ). This is in line with those authors reporting



higher biomass, yield and crop on Kelpak<sup>®</sup> treated plants [van Staden et al. 1995, Arthur et al. 2003, Colapietra and Alexander 2006, Rathore et al. 2009]. However, the effect of Wuxal<sup>®</sup> Ascofol confirms Abetz and Young [1983] and Kwizda Agro [2009] but in this case the fertilizer effect cannot be separated from the effect of seaweed extract. The most efficient treatment was Kelpak<sup>®</sup>, which improved both the number and total shoot mass production of stockplants without any leaf fertilizer. Since Kelpak<sup>®</sup> contains hormonal substances and polyamines [Crouch and van Staden 1991, Crouch et al. 1992, Papenfus et al. 2012], its effect could be based on improved vigor caused by the active substances.

The higher chlorophyll index of Kelpak<sup>®</sup> treated stockplants in correspondence with higher stomatal conductance and photosynthetic capacity of leaves could cause increased growth intensity of shoots and enhanced mineral uptake which was shown by Rathore et al. [2009]. In contrary to results of Hotta et al. [1997], Babik et al. [2008] and Dwornikiewicz [2008] who indicated that the ALA, the active agent of Pentakeep<sup>®</sup>-V increases the leaf chlorophyll content and so improves the photosynthetic activity, we could not confirm this effect on *Prunus mahaleb* L. stockplants at the applied concentration.

In 2011, IAA level was higher in all the biostimulator treated stockplants shoots, in the shoot tip than that of untreated ones. Because Kelpak<sup>®</sup> contains considerable amount of IAA [Crouch and van Staden 1991, Crouch et al. 1992], the increased auxin level could be caused by the increased growth of stockplants due to the treatment. On the other hand, the IAA sprayed on the shoots and leaves were not excluded in the measurement, which may contribute to the higher measured levels. The higher IAA-level measured in the shoot tips, could be linked with the increased growth and the fertilizer effect.

**Effect of treatments on pre- and post-severance traits of cuttings prepared from treated shoots and rooting of cuttings.** The mean rooting rate of cuttings over three years ranged between 76.9 and 83.4%, similarly to the results of Hrotkó [1982] with IBA treated cuttings. So we can conclude that the stockplant pretreatments and the pre- and post-severance cutting traits do not influence the rooting rate (tab. 4). In contrary, our results suggest considerable effect of pretreatments on root and shoot mass increment of cuttings during rooting.

Albeit the cuttings were prepared uniformly, the increasing effect of Kelpak<sup>®</sup> on cuttings FM and DM (tab. 1) was still detectable. The FM of single cuttings taken from Kelpak<sup>®</sup> treated plants was the highest in 2010, 2011 and 2012, furthermore in average of years significantly larger than that of control. Results and statements of Leakey and Mohammed [1985], Leakey [2004], Magingo and Dick [2001] and Mesén et al. [2001] underline the importance of cutting's volume, reserved assimilate from the pre-severance stage of leafy stem cuttings and the storage capacity for current assimilates. Our data show a significant larger FM of cuttings pretreated by Kelpak<sup>®</sup>, but the FM of cuttings prepared from Wuxal<sup>®</sup> Ascofol and Pentakeep<sup>®</sup>-V treated shoots did not differ from control cuttings. The larger cutting' mass of Kelpak<sup>®</sup> pretreated shoots supported by higher IAA-level seem to be one of the reasons resulting in higher root mass formation capacity. This is in line with Leakey and Mohammed [1985], Magingo and Dick [2001] and Mesén et al. [2001].

Štefančič et al. [2007] measured similar level of IAA in *Prunus* hybrid GiSelA 5 cuttings and Sándor et al. [2010] in *Prunus marianna* cuttings. In the basal part of rooting cuttings after seven days, when the shoots were pretreated by Kelpak<sup>®</sup>, we found

higher IAA-level than that of control. Considering the higher IAA level, the total FM and DM of rooted cuttings and the separated root DM resulted by Kelpak<sup>®</sup>, our data are in correspondence to Leakey [1992], who found that the pre-severance auxin levels in stem part used for cuttings enhance rooting ability. As the cuttings' basis, all were uniformly treated by IBA the cause of the increased root mass production could be partly the increased IAA-level in the basal part of cuttings or rather the possible interaction between hormonal substances of Kelpak<sup>®</sup> and the IBA. Sándor et al. [2008] indicated certain interaction between the IBA treatment and the IAA-level of rooting cutting's base. Because of the basal IAA-level of the Pentakeep<sup>®</sup>-V treated sections did not increase significantly, this indicates that the improving effect on rooting of shoot IAA-level manifested in interaction with other factors of Kelpak<sup>®</sup> [Crouch and van Staden 1991, Crouch et al. 1992, Jones and van Staden 1997, Papenfus et al. 2012].

**Effect of treatments on DM production and partitioning of cuttings prepared from pretreated shoots.** In agreement with Mesén et al. [1997] we consider the significant differences of DM increment of cuttings (tab. 5) as evidence that the leaves of cuttings produced DM (assimilates) during rooting, and this DM production is influenced by pretreatments of stockplants. The Kelpak<sup>®</sup> and Pentakeep<sup>®</sup>-V treatments on shoot in the pre-severance stage resulted later the highest DM increment of cuttings, higher than that of the control. This indicates that the pretreatments still influence the photosynthetic activity of leaves in the subsequent rooting phase. Further on specific substances are produced in the assimilating leaves during the period in the propagator may promote DM production as it is suggested by Leakey and Storeton-West [1992].

The partitioning of DM produced during the rooting period, when the cuttings were in the propagator showed significant differences. Kelpak<sup>®</sup> resulted the highest root DM of cuttings, which could be caused by the higher IAA-level measured in the basal stem section of rooting cuttings. This is in line with the positive effects of Kelpak<sup>®</sup> on root mass production of intact plants [Crouch and van Staden 1991, Crouch et al. 1992, Jones and van Staden 1997], however, such as a subsequent effect of treatment on root mass formation capacity has not been reported yet. On the other hand, the Pentakeep<sup>®</sup>-V pretreatment on stockplant resulted higher shoot DM of rooted cuttings (tab. 5). Since the FM and DM of the Pentakeep<sup>®</sup>-V pretreated cuttings before rooting did not differ from control, the tendency to produce largest shoot mass during rooting indicates a subsequent effect of Pentakeep<sup>®</sup>-V pretreatment. This is in agreement with results of Hotta et al. [1997], Babik et al. [2008] and Dwornikiewicz [2008] obtained with other crops where the active agent (ALA, 5-aminolevulinic-acid) and leaf fertilizers of this biostimulator increases the biomass production.

## CONCLUSION

1. Kelpak pretreatment is the most effective in improving the productivity and shoot quality of *Prunus mahaleb* L. stockplants.

2. Kelpak and Pentakeep-V pretreatments did not affect the rooting rate but increased the dry mass production of cuttings. The Kelpak<sup>®</sup> pretreatment increased the root mass production, while Pentakeep<sup>®</sup>-V increased the shoot weight on cuttings.

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## WPLYW BIOSTYMULATORA ORAZ NAWOZÓW DOLISTNYCH NA ROŚLINY MATECZNE I SADZONKI *Prunus mahaleb* L.

**Streszczenie.** Przetestowano wpływ biostymulatora Kelpak<sup>®</sup> oraz nawozów dolistnych Wuxal<sup>®</sup> Ascofol and Pentakeep<sup>®</sup>-V na rośliny mateczne *Prunus mahaleb* L. ‘Bogdány’. Oceniono tworzenie i cechy pędów, zakorzenienie oraz jakość sadzonek. Przed pobraniem sadzonek czterokrotnie opryskano liście biostymulatorem i nawozami. Na roślinach matecznych określono liczbę oraz świeżą masę pędów, masę sadzonek, wskaźniki chlorofilu w liściach (SPAD) oraz poziom kwasu indoliloctowego (IAA) na wierzchołku pędu. Zmierzone wskaźnik zakorzenienia, poziom IAA w strefie korzeniowej sadzonek, świeżą i suchą masę zakorzenionych sadzonek, a także obie masy korzeni oraz pędów. Na podstawie wyników badań wnioskuje się, że wstępny zabieg Kelpakiem<sup>®</sup> można uważać za najskuteczniejszy w polepszaniu wydajności roślin matecznych *Prunus mahaleb* L., wskaźników chlorofilowych oraz poziomu IAA w wierzchołkach pędów roślin matecznych oraz w podstawie sadzonek. Wstępny zabieg Kelpakiem<sup>®</sup> zwiększał masę pojedynczych pędów, a w konsekwencji – świeżą masę przygotowanych sadzonek. O ile wstępne zabiegi same w sobie nie wpływały na wskaźnik zakorzenienia, to wstępny zabieg Kelpakiem<sup>®</sup> i Pentakeepiem<sup>®</sup>-V na pędach zwiększył tworzenie suchej masy sadzonek podczas zakorzeniania. Wstępny zabieg Kelpakiem<sup>®</sup> miał pozytywny wpływ na suchą masę korzenia natomiast Pentakeep<sup>®</sup>-V zwiększał suchą masę pędów zakorzenionych sadzonek.

**Słowa kluczowe:** produkcja świeżej i suchej masy, poziom IAA, Kelpak<sup>®</sup>, Pentakeep<sup>®</sup>-V, wstępny zabieg, wskaźnik zakorzenienia, wydajność roślin matecznych, Wuxal<sup>®</sup> Ascofol

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