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The effect of timing and IBA treatments on the rooting of plum rootstock hardwood cuttings

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Summary: In propagation of plum by hardwood cuttings, the success of rooting is affected by several factors. Many authors deal with the timing of cutting collections, others investigate the optimal extent of hormonal stimulation. However, there is no data, as yet, about the coherence between these two factors. The aim of this experiment was to find the most advantageous conditions, with regards to both timing and IBA doses, for rentable propagation of the examined varieties. Five varieties were studied: 'INRA Marianna GF 8-1', 'Myrobalan B', 'MY-KL-A', 'INRA Saint Julien GF 655/2' and 'Fehér besztercei'. Cuttings were collected on seven occasions, during late autumn and winter, and were treated with IBA solutions of different concentrations. The optimal dose of IBA was found to be dependent both on the variety and the actual date of cutting collection. Results are reported, along with suggestions for optimal doses and collection periods.

Key words: hardwood cutting, IBA, plum, rooting, adventitious root formation, propagation.

Introduction

There is a certain interest in improving the homogeneity of plum orchards. To have a true-to-type rootstock, one possible solution is using vegetatively propagated plant materials, which have recently become more and more the centre of attention. However, in Hungary, for making propagation by clonal rootstocks more widespread, it is necessary to develop cheap and efficient propagation methods.

There are several factors affecting the success of rooting. Among them, the time frame of collection of cuttings is one of the most important, because the phenological state of stock plants has a strong influence on the rooting process (*Guerriero Loreti*, 1975). It is widely accepted that the beginning, and also the end of the winter dormancy give the highest rooting percentage (*Bassuk Howard*, 1981, *Erbil* 1997). Thus, for propagation of plum rootstocks by hardwood cuttings, the period between middle of December and end of January should be the most disadvantageous one. Domestic studies corroborate this expectation. *Szecskó* et al. (2002) reported that the end of autumn and the beginning weeks of winter give the highest rooting percentage. It is followed by a less successful dormancy period, then another, although only local, maximum.

Another very important issue is hormonal treatment. In the autovegetative propagation of plum rootstocks a certain type of synthetic auxin, the IBA (indole-3-butyric acid) is widely used to stimulate rooting. Most of the experiments carried out in this field seek to determine the optimal IBA concentration for reaching the highest rooting percentage. Unfortunately, literature is full of contradictory reports. Usually, authors share the opinion that IBA treatment is necessary. It is also accepted that treatment with above-optimal concentrations is contra-productive, as it decreases the number of the survival plants (*Mindello Neto* et al., 2006; *Kersten* et. al., 1993).

On the other hand, many experts believe that the timing of cutting collection is much more important than the exogenously added auxin. With proper timing, the number of surviving plants will definitely be high, which might make IBA treatment seem insignificant, or even unnecessary.

Even where hormonal stimulation is regarded beneficial or necessary, there is no accord about the optimal concentration. It is generally reported that a treatment with 2000 $mg \cdot kg^{-1}$ IBA gives the highest rooting percentage, so it is extensively used in experiments (*Nahlawi Howard,* 1972; *Nahlawi Howard,* 1973; *Nicotra Damino,* 1975; *Lemus,* 1987; *Rana Cadha,* 1992). However, *Swedan* et al. (1993) found that 1000 $mg \cdot kg^{-1}$ is the best for 'Marianna GF 8-1' rootstock. *Kracikova* (1996) reported that 2500 $mg \cdot kg^{-1}$ is more efficient, while other researchers found 3000 $mg \cdot kg^{-1}$ to be optimal (*Sharma Aier,* 1989, *Kapetanovic* et al., 1972, *Fontanazza Ruigini,* 1980, *Rathore,* 1983). However, *Szecskó* et al. (2003) reported unsuccessful IBA stimulation of rooting of 'Marianna GF 8-1' stock. Also, *Tofanelli* et al. (2001) reported a total ineffectiveness of stimulation agents.

A logical explanation of the contradictory results might be that the optimal IBA concentration and the success of treatment depend on a lot of conditions, such as climate, varieties, weather, soil, etc. Furthermore, it seems to be certain that the degree of dormancy of the propagation material plays a main role in the effectiveness of root stimulation. The concentration, activity, synthesis and metabolism of some biochemical components (such as sugars, polyphenols, other hormones, anti-auxins, etc.) are regulated by this stage, and these factors are very important in the process of rooting (*Szecskó* et al., 2004). Following this line of thought, we investigated the effect of degree of dormancy on the optimal IBA concentration.

Materials and methods

The experiment was carried out in the dormant season of 2006-2007. The following plum rootstocks were used as plant material: 'INRA Marianna GF 8-1', 'Myrobalan B', 'MY-KL-A', 'INRA Saint Julien GF 655/2' and a Hungarian selection, the 'Fehér besztercei' (*Hrotkó,* 1999). All mother plants were grown in the Research and Experimental Farm of the Faculty of Horticultural Sciences in Soroksár, Hungary.

The cuttings were collected between October and February. It is known that the middle of winter is the worst time for taking hardwood propagation material. Rooting success of the cuttings collected in the middle of winter (i.e. from the end of December to the end of January) is very low, and basically independent of the exact date of taking, presumably due to the low rate of metabolism and, consequently, the low rate of hormonal synthesis in this time of year. So, following the advice of *Szecskó* et al. (2003), our efforts were focused on October, November and February. The actual dates of preparation were 26th Oct., 08th Nov., 29th Nov., 18th Dec., 22nd Jan., 13th Feb., and 28th Feb.

The cuttings were 15 cm long, with diameters between 7 and 12 mm. On the basal part of each cutting, a straight cut was made about 0.5 cm below the node. Also, on the apical end, a slanting cut was made about 1 cm above the last bud. After preparation, the cuttings of each variety were equally divided into three groups, which were then treated with stimulation agent of different concentrations. The stimulation agent (IBA, indole-3-butyric acid) was prepared in three concentrations (one for each group): 0 $mg{\cdot}kg^{-1}$ (solvent only), 2000 $mg{\cdot}kg^{-1}$ and 4000 $mg{\cdot}kg^{-1}$. A 1:1 water-ethanol mixture was used as solvent. The method of treatment was to dip the lowest 1 cm of the cutting for 3 seconds into the appropriate IBA solution.

The groups that were treated with solvent only will be referred to as "control" or "control groups" henceforth. Their purpose is to ensure that possible differences between the treatments do not originate from the damage and necrosis caused by ethanol.

After treatment, the cuttings were put into cool storage, in perlite, at 4 °C. All of them were planted out in the middle of March, 2007 in Soroksár. Each repetition was planted in one long row using 5–8 cm distance. The field was irrigated during the rooting period, and in the summer, too. The winter climate of 2006–2007 was much more moderate than usual, it was short and the minimum temperature was much higher *(Figure 1).*

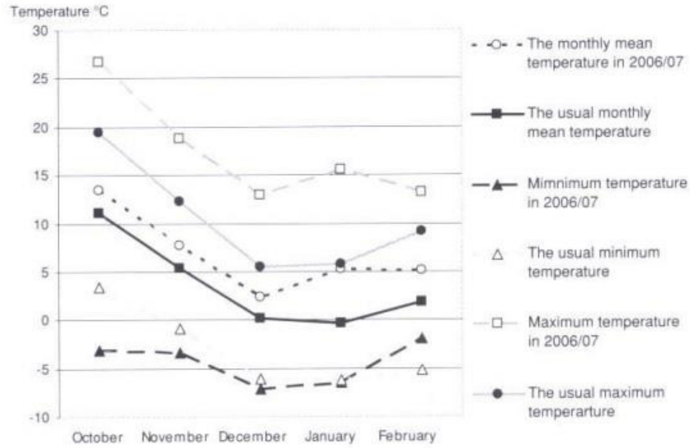

Figure 1. Monthly temperature changes during the propagation period

For each combination of treatment, variety and collection date, 100 cuttings were prepared. The statistical analysis was made by SPSS 14.0. The RSD value of the determination was 5% (n=5). In case of the statistical data analysis the rooting percentage on the end of February (28) was not submitted (Table 2. and general analysis), because there were no survival of plants, and there was no difference between the effect of treatments and varieties on rooting.

Results and discussion

The aim of this experiment was to find the optimal period for preparing hardwood cuttings with the optimal IBA doses. *Figures 2–4* show the rooting percentage of the control, 2000 $mg{\cdot}kg^{-1}$ and 4000 $mg{\cdot}kg^{-1}$ IBA treated cuttings, for the seven preparation dates described earlier. According to our experiments, the end of autumn and the beginning of winter

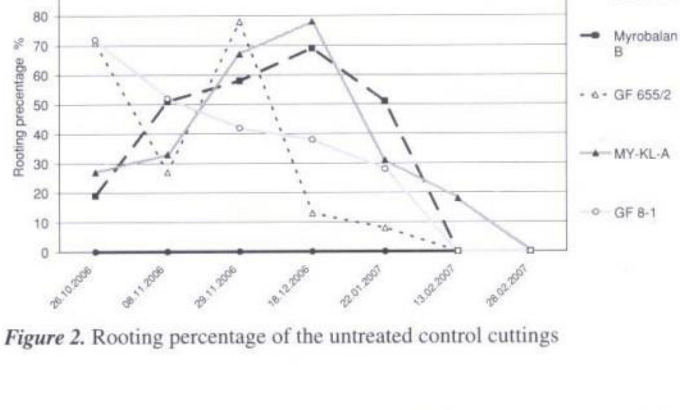

Figure 2. Rooting percentage of the untreated control cuttings

Table 1. The optimal IBA treatment levels for the plum rootstock varieties and statistical analyses of the treatments

Varieties / IBA treatment		'Fehér Besztercei'		'Myrobalan B'		'GF 655/2'		'MY-KL-A'		'GF 8-1'	
		Statistical group		Statistical group		Statistical group		Statistical group		Statistical group	
Optimal treatment	dose (mg/kg)	a	4000	a	2000	a	control	a	control	a	2000
	mean survival (%)		18,50		41,50		32,89		42,28		48,50
Suboptimal treatment	dose (mg/kg)	ab	2000	ab	4000	ab	2000	abc	2000	a	4000
	mean survival (%)		7,30		37,33		23,33		30,33		45,17
Worst treatment	dose (mg/kg)	c	control	bc	control	c	4000	bc	4000	ab	control
	mean survival (%)		0,00		21,83		14,00		15,00		38,83

proved to be most advantageous for preparing the cuttings. Under domestic conditions, it was this period that resulted in the most successful and reliable rooting.

As opposed to literature data (*Szecskó* et al., 2002), at the end of the winter no second maximum of rooting was found, moreover, no rooting at all. Our suggestion is that the short and moderate winter must have been the reason for the short effective period for taking hardwood cuttings. Under climatic conditions warmer than the Hungarian, *Abd Alhamed* et al. (1993) reported unsuccessful plum rooting experiments during January and February. This statement confirms our theory.

For any given variety, it was considered optimal treatment that gave the highest rooting percentage and had the longest effective period for preparing the cuttings. First row of *Table 1* shows the optimal IBA treatment level for each variety during the winter of 2006–07, and the overall average of rooting percentage, too. It is obvious that there are differences. It was found that the generally recommended 2000 $mg \cdot kg^{-1}$ IBA treatment did not always (i.e. hardly ever) promote the highest rooting percentage.

IBA treatment was found to be absolutely necessary for the difficultly rooting *Prunus domestica* L. 'Fehér Besztercei', and the highest rooting percentage was reached with 4000 $mg \cdot kg^{-1}$ IBA. 2000 $mg \cdot kg^{-1}$ IBA treatment was the best for 'GF 8/1', but there was no significant difference between this concentration and 4000 $mg \cdot kg^{-1}$. For 'GF 655/2' and 'MY-KL-A' the control treatment was the most effective. In the case of 'Myrobalan B', the absolute maximum of rooting percentage was 72%, which was measured at 2000 $mg \cdot kg^{-1}$ IBA treatment. However, it was only slightly better than the best value of control treatment (69%), and the effective period of the latter was considerably longer. As a result, no IBA was judged to be better for 'Myrobalan B', too.

Focusing on the rooting percentages *(Table 1, Figure 2–4)* it is apparent that the difficult to root *Prunus domestica* L. 'Fehér Besztercei' had the lowest rooting percentage, with a value less than 50%. *Prunus insititia* Jusl. 'GF 655/2' had a much higher maximum, at 78% *(Figure 2)*, but the optimal period for cutting collection was only two weeks, which makes it difficult to propagate by hardwood cuttings. The 'GF 8/1' and 'Myrobalan B' both had a long propagation period, and also high rooting percentages. Though for 'Myrobalan B' the rooting percentage was somewhat lower (69-72%), the long lasting rooting affinity still makes this variety an excellent choice. The 'MY-KL-A' had the longest propagation period, with a mediocre aptitude for survival.

In terms of rooting success, *Prunus insititia* Jusl. 'GF 655/2' was the most susceptible to the inhibiting effect of excessive IBA treatment, especially in the 2nd half of the effective period. It is our suggestion that IBA overdose should by all means be avoided when propagating this variety.

When exposed to too high rooting hormone concentrations, the callus and the bottom of the cutting turn black. These are symptoms of necrosis. We noticed that necrosis did not always induce the decay of the whole cuttings, and some of them have survived, despite the necrosis. Nevertheless, these symptoms make for a reliable indicator of IBA overdose, and provide valuable help in determining the appropriate hormone doses.

In terms of necrosis, the variety most sensitive to excessive IBA treatment was 'MY-KL-A', in case of which even 2000 $mg \cdot kg^{-1}$ IBA killed about 10% of the cuttings,

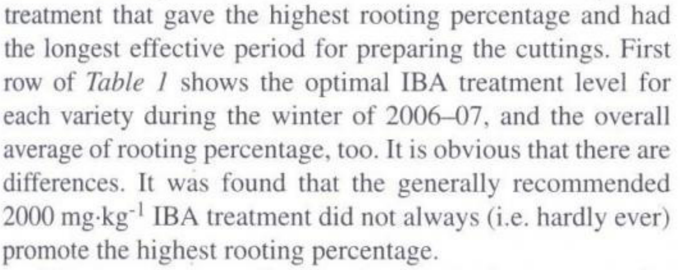

Figure 3. Rooting percentage of the 2000 mg·kg-1 IBA treated cuttings

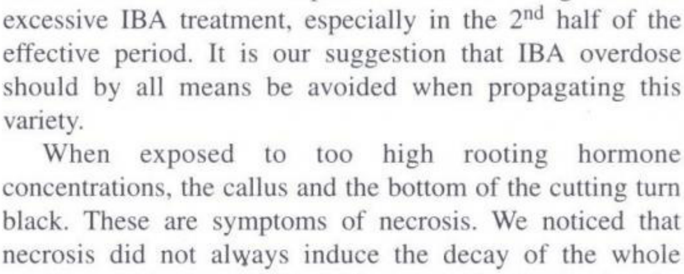

Figure 4. Rooting percentage of the 4000 mg·kg-1 IBA treated cuttings

Table 2. The effect of timing on the optimal IBA doses and the mean of rooting percentage in case of the exogenous auxin treatments

Varieties / IBA treatment		'Fehér Besztercei'		'Myrobalan B'		'GF 655/2'		'MY-KL-A'		'GF 8-1'	
		Statistical group		Statistical group		Statistical group		Statistical group		Statistical group	
Optimal treatment	dose (mg/kg)	a	4000	a	2000	a	control	a	control	a	2000
	mean survival (%)		18,50		41,50		32,89		42,28		48,50
Suboptimal treatment	dose (mg/kg)	ab	2000	ab	4000	ab	2000	abc	2000	a	4000
	mean survival (%)		7,30		37,33		23,33		30,33		45,17
Worst treatment	dose (mg/kg)	c	control	bc	control	c	4000	bc	4000	ab	control
	mean survival (%)		0,00		21,83		14,00		15,00		38,83

whereas 4000 mg·kg^{-1} IBA caused necrosis in nearly 40% of the cases. 'Myrobalan B' was less sensitive, as 2000 mg·kg^{-1} IBA caused only 1% necrosis, while at 4000 mg·kg^{-1} less than 10% of the plants died. In the case of 'GF 655/2' only 1% necrosis occurred at the highest IBA level, which is somewhat surprising in view of the fact that this is the variety that reacted most negatively to excessive rooting stimulation. With 'Fehér Besztercei' and 'GF 8/1' no necrosis was observed, and the IBA treatment increased the number of surviving plants.

Timing also had an effect on the extent of the damage. Cuttings suffered the least necrosis at the beginning of the propagation period.

Analyzing the combined effect of timing and IBA treatment *(Figure 2–4 and Table 2)* we concluded that, at the beginning of the propagation season, an IBA dose that is higher than the one generally considered ideal, is likely to result in higher rooting percentage.

Table 2 shows, the overall optimal doses for all cuttings, all five varieties included, according to collection date of cuttings. At the end of October 4000 mg·kg^{-1} IBA was best, but a scant fortnight later, 2000 mg·kg^{-1} proved to be the most effective. It was even true for the highly necrosis sensitive 'MY-KL-A', where on 26th October 2006 the 4000 mg·kg^{-1}, 2000 mg·kg^{-1} and control treatments resulted in 41%, 29% and 27% rooted cuttings, respectively. On the second date (8th November, 2006) the same survival rates were 29%, 62% and 33%. In case of the other five dates the control was the most successful.

We found that, in the highest degree of dormancy, too high levels of IBA treatment are likely to be the lethal for most plum cuttings. With the exception of the IBA dependent 'Fehér Besztercei', it was the control cuttings that retained their rooting ability for the longest time. With most varieties, even the control groups taken on 22nd January 2007 showed some extent of survival.

With regards to the 2000 mg·kg^{-1} IBA treated groups, the last date when surviving cuttings were taken was 18th Dec. 2006, except for 'Fehér Besztercei' and 'GF 8/1'. In case of 4000 mg·kg^{-1} treatment 'GF 655/2' was the most sensitive, with 8th Nov. 2006 as the last productive date, and only 13% rooting success *(Figure 3)*.

Conclusions

In the winter of 2006–2007, the studied plum rootstocks had successful rooting period only at the end of autumn and beginning of winter. In contrast to the literature data, no rooting was observed at the end of the dormancy period. The widely used 2000 mg·kg^{-1} IBA treatment was not optimal for most varieties.

For the difficult to root *Prunus domestica* L. 'Fehér Besztercei' the IBA stimulation is necessary in order to develop roots. 4000 mg·kg^{-1} was the most effective dose. In any case, this variety shoved a low rooting ability during the year of experiment. The *Prunus insititia* Jusl. 'GF 655/2' presented high rooting percentages, but the optimal period was very short, and IBA treatment decreased the survival ratio. *Prunus cerasifera* cv. myrobalana and its hybrids had similarly long optimal rooting periods and high number of survival plants too, which makes them easy and cheap to propagate. For 'GF 8/1' the IBA treatment is not necessary, but if used, the recommended concentration is 2000 mg·kg^{-1}. In case of 'Myrobalan B' and 'MY-KL-A' the untreated control was the most successful. Necrosis was also observed, and 'MY-KL-A' was found to be very susceptible to it.

The main conclusion of the whole experiment is that the optimal timing of cutting collection and the optimal dose of IBA treatment are interdependent *(Table 2)*. Neither of them can be determined without the other. It can be the reason for so many discrepancies concerning the effectiveness and, much more, the optimal level of IBA stimulations in the literature.

Under our climatic conditions, only the beginning of October, November and December seems to be effective and economic for clonal plum rootstocks propagation. Through the first weeks of this period higher IBA doses can be promising but later the optimal dose is to be used. For cuttings taken after December, IBA treatment should be avoided altogether.

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