Thesis of PhD Dissertation

CHANGES OF AUXIN CONTENT IN THE ROOTING ZONE OF HARDWOOD PLUM CUTTINGS

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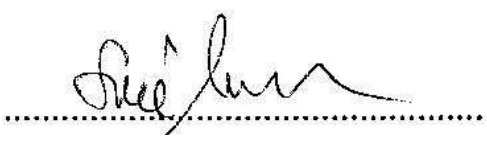

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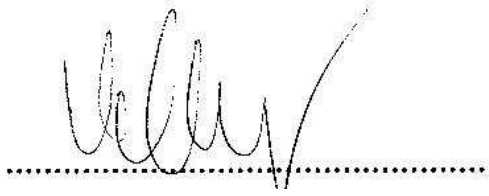

Supervisor

Introduction and Aims

Plum (*Prunus domestica* L.), which not only has many uses as a plant but is easily preserved, has been grown in Hungary ever since the Middle Ages. Its origin lies in the countryside surrounding the Black Sea and the Caspian Sea, prehistoric peoples appreciated and cultivated it. In terms of quantity, plum is second only to apple in Hungarian fruit production (Surányi, 2009). Average annual crop for the past several years stands at over 70,000 tonnes (FruitVeB, 2009). The Hungarian climate is well-suited to plum cultivation, thus it has spread widely. Today it has gone wild, and many different spontaneous and subspontaneous populations can be found here.

In plum cultivation, one of the main pillars of technological development is the use of appropriate rootstocks; thus with plum, similar to other plants which are common throughout wide climatic areas, propagation is carried out with graftings. The price of a grafted shoot is influenced by the price of the stock, and the price of the stock depends on how it was produced (technology) and how many shoots it will bear. One basic condition of good management is to use saplings derived from technology that does not waste money.

In Western Europe, more than 70 % of stone-fruit and plum rootstocks are propagated vegetative, whereas in Hungary and the neighboring countries, seedlings dominate the stock selection. Vegetative propagating clone rootstocks have numerous valuable characteristics, not the least of which is their uniform growth, a key factor in modern homogeneous plantations.

For apple, the using of layering propagated rootstocks, has become commonplace in Hungarian apple orchards. This method was the base of modern, intensive apple plantations. For apple growers, the pre-condition for this change was cheap, mechanized slip propagation, a method which usually fails with stone-fruits. It is possible to vegetative propagate the stone fruits within species using cuttings (hardwood or softwood), but this means plant tissue is much more sensitive. Rooting in propagation by softwood cuttings is effective but expensive, and great technological discipline is called for. The high cost prevents this method from becoming widespread.

Propagation by hardwood cuttings is potentially the cheapest and simplest asexual method for plum stocks. It requires no special equipment, the plant material is not delicate, and can easily be stored and transported. On top of that, it yields good quality seedlings.

This method, which has already become standard in Western Europe, should be adapted for the Hungarian environment. Szecskó et al. (2002, 2003) have studied propagation of plum by

hardwood cuttings as a technology for Hungary, but in spite of their results, clone rootstocks have not become popular in the production area. One reason for this is the climate, but other reasons can be found in the local orchard systems and their ways of organizing work. In the Western European nurseries practice, there are propagators, specialized for rootstock propagation. This is not the common yet in Hungary, where rootstock production is just one of an nurseries many tasks. Just when they should be doing propagation by hardwood cuttings, the orchards are at peak labor intensity, harvesting and selling the crop, not to mention their lack of expertise. Winter means work must be stopped, and workers find it very difficult to find employment. If the winter period were used for propagation by hardwood cuttings, this technology would promote its widespread use.

According to the literature, propagation by hardwood cuttings is warranted in 50 % of the species which put out roots. In orchard practice, plants must be stimulated in order to achieve the correct level of rooting (several possibilities for stimulation are known). Bottom heated treatment, which in practice means heating of the rooting zone, has hardly been tried in Hungary, due to its being expensive.

Although numerous experiments have been done with plum rootstocks to determine factors of propagation by hardwood cuttings such as external conditions, environmental factors, and technologies, studies of the internal biochemical processes are quite rare. Experiments dealing specifically with plum rootstocks are also scarce. Researchers working with adventitious root studies agree that the determining factors of successful rooting are the plant material's phenological and biochemical traits. The majority believe that materials that stimulate adventitious root growth originate in the buds, and the research literature lumps all of these producers of various compounds together as a co-factor in stimulating root formation (Darvis 1986). Their exact mechanism of influence is not known yet.

The auxin group plays a decisive role in the development process of adventitious roots, but only in the past few decades have researchers been able to make exact measurements of this and establish the necessary level of precision in analytic equipment. Auxin complexes are believed to affect the root induction period, but their presence is indicated in the subsequent, non-auxin-dependent periods as well.

The present study aimed to clarify the role of native auxins and exogenous auxins acting as stimulators in plum shoots. Further, we wished to accomplish this from the point of view of

adventitious root development. Our goal was to do more than simply describe the outcomes of technology and rooting.

The research focused on two main goals. First was the development of hardwood cutting propagation technology, and parallel with that were the examinations of variations in auxin content in the cuttings rooting zone. We aimed to blend traditional orchard propagation methods with data from the latest laboratory equipment and scientific measurements, in order to produce results that could be used in the practice of propagation. Further, the goal of the research was to observe how removal of various buds in various places or heated base treatment affect the indole-3-acetic acid level in the root formation area. Additionally, effects of IBA treatments were studied. The authors of this study wanted to know the rate of absorption and breakdown, as well as the affect of stimulators on the level of native auxin.

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Materials and Methods

Locations of the experiments

Open field experiments of propagation by hardwood cuttings were carried out in Soroksár, Hungary at the Corvinus University of Budapest Faculty of Horticultural Sciences Research and Experimental Farm. Cuttings were collected from the plantation of the Department of Fruit Science, mother plant stocks. Bottom heated treatments were done in electrically heated beds of the Department Ornamental Plants and Dendrology. Laboratory work proceeded in the HPLC Laboratory of the Corvinus University Fruit Science Department.

The study of propagation included myrobalan (*Prunus cerasifera* EHRH. *var. cerasifera* SCHNEID. *cv. myrobalana)* stocks 'Myrobalan B', 'INRA Marianna GF 8-1', 'Ishtara', 'MY-BO-1', 'MY-KL-A', and bullace rootstocks *(Prunus insititia JUSL.)* the 'INRA Saint Julien GF 655/2' subspecies, while for domestic plums, *(Prunus domestica L.)* 'Fehér besztercei', and 'Kisnánai lószemű' varieties were used. Cuttings were collected at the times given in **table 1**.

Technology of propagation

Cuttings of the various species were collected from the end of October to the beginning of March as shown in **table 1**. Shoots measuring 20 cm were cut from 6-9 mm twigs. The basal ends were cut straight so that the lowest bud was no less than 0.5 cm distant from the cut, and the upper end was cut on an angle. The basal ends of the cuttings were cut at 2 cm to wound them as a stimulus for rooting. After a few hours in storage the cuttings were immersed in a 50 % alcohol solution containing 0 ppm, 2000 ppm, and 4000 ppm IBA for 3 seconds. After drying, the plant material was stood upright in a planter box containing moist perlite and kept at 4 °C in a cool room until planting time at the end of March.

Table 1. Varieties used in the experiments and times of cutting collection

year/time								
2006/07	10.28.	11.08.	11.29.	12.08.	1.22.	02.13.	02.28.	
varieties	'Fehér besztercei', 'St. Julien GF655/2', 'Marianna GF 8-1', 'Myrobalan B', 'MY-KL-A'							
2007/08	10.31.	11.12.	11.26.	12.13.	01.22.	02.04.	02.21.	
varieties	'Kisnánai lószemű'				'Fehér besztercei', 'Kisnánai lószemű', 'St. Julien GF655/2', 'Marianna GF 8-1'			
2008/09	10.28.	11.12.	11.26.	12.15.	01.19.	02.10.	02.24.	03.11
varieties	'Fehér besztercei', 'Kisnánai lószemű', 'St. Julien GF655/2', 'Marianna GF 8-1', 'Myrobalan B', 'MY-BO-1', 'Ishtara'							

The technology of bottom heat treatments

Treatments were carried out using an electrically heated bed. The hardwood cuttings, which had been treated with various concentrations of IBA solution, were set up in moist Perlite in a plastic planter box, the base of which was heated with electricity. In January 2008, 18 °C base heat was applied for four weeks, and in the following year the temperature had to be reduced. From January 19, 2009, 15 °C heat was applied for three weeks. During this growth period the following varieties were examined using the usual stimulant concentrations: ’Fehér besztercei’, ’Kisnánai lószemű’, ’St. Julien GF 655/2’, ’Ishtara’, ’Myrobalan B’, ’MY-BO-1’ and ’Marianna GF 8-1’

We also examined the required time for bottom heating in the case of the ’Marianna GF 8-1’ rootstock. Three treatment periods -- one week (January 26) two-weeks (February 2) and three weeks (February 9) -- were involved, along with immersion in 0 ppm, 2000 ppm , and 4000 ppm IBA solution. Measurements were taken at the cuttings tips and basal ends for amount of IAA and the IBA decomposition during both storage and rooting time.

At the start of the bottom heat treatment, the cuttings were sprinkled with a solution containing Captan fungicide (Orthocid).

Removal of bud eyes from cuttings

Cuttings of the 'Marianna GF 8-1' variety collected on November 26, 2008 were subjected to a series of bud eye removal tests. Seven types of treatment were administered, and in the eighth operation, the cutting tip IBA stimulus (F) was also examined. Cuttings were divided into 3 parts and the base, middle and tip were examined for presence of bud eyes. Four types of bud removal were performed (V1, V2, V3, V4), and in each case buds were left on the cutting only in the locations shown in **table 2.** We then examined the combined effect of bud removal and IBA dip (B0, B1) in relation to artificial basal replacement of native auxins.

Bud removal was done with the sharp blade of a grafting knife. Buds were removed entirely so that no stubs remained and no sleeping buds could form.

Table 2. Bud removal experiment: removal sites, remaining buds, and IBA dip sites on the cuttings

	control	V1	V2	V3	V4	B0	B1	F	2000 ppm	4000 ppm
cutting part	no IBA					2000ppm IBA				IBA
tip	+	+	–	+	–	–	+	+	+	+
middle	+	–	–	–	–	–	–	+	+	+
base	+	–	+	+	–	–	–	+	+	+

IBA treatment site: bud eye removal: – condition of remaining bud eye: +

The following varieties' cutting tips were examined for effects of 2000 ppm IBA dip in November 2008: ’Fehér besztercei’, ’St. Julien GF 655/2’ and the ’Marianna GF 8-1’.

Sample collection times

Collection of laboratory samples always occurred at the same time cuttings were collected, according to the times shown in **table 1**. After the last cutting collection, we collected samples every two weeks: in the second and third ten day periods of March, the second and third ten day periods of April, and the first ten days of May.

Preparation of sample for determination of auxin

Samples (cuttings) were taken from the open field in Soroksár and from a cooler and transported in cooler box to the laboratory, where they were handled on the same day. Prior to handling they were stored at -20 °C to prevent decomposition of auxins.

Each sample was made from three cuttings containing nodes and measuring 1 to 1.5 cm. The shoots were destructed by hammer. After measuring the mass of the destructed plant material, we placed them in 10 ml of -20 °C extracting solution. The extracting solution was 80 % methanol containing 100 mg BHT per litre (Kim et al. 1992). An antioxidant, this protected the hormones from decomposing. Measurements were repeated three times and the sample extraction lasted one week at -20 °C.

300 µl 4 mol NaOH solution was added to 500 µl extract, to remove chlorophyll and proteins which could disturb detection. The compound was then neutralized with 100 µl acetic acid in order to prevent erosion of the column. The extract was centrifuged at 15000 rpm, after the supernatant has been filtered on Milex SLHN 13mm 0.45 µm filter.

HPLC measurement of auxins - conditions of analysis

Measurements were taken with a WATERS 2487 dual detector (UV-Vis), 1525 two channel pump and 717 plus automatic injector HPLC unit. The hardware was run by the EMPOWER^{TM} 2 program.

Indole-3-butyric acid [133-32-4] was identified in winter, 2006/07 in isocratic conditions according to the Végvári, László (2004) and Sándor et al. (2008) method. A 60 % methanol compound containing 0.5 ml acetic acid per litre was used in the mobil phase. Separation occurred with a Symmetry C18 (5 µm 4,6 x 150 mm) column at 20 °C, at 1 ml/minute flow rate. Pressure on the column was 2300±15 psi, the injected volumes were 20 µl. Detection was done in a 220 nm. The IBA retention time was 4.45 minutes. Each sample ran out in 6 minutes.

In 2008/09, because of determination of the quantity of indole-3-acetic acid [87-51-4], a different measurement method was put into use when we took the Trobec (2005) gradient elution method as a basis. The separation was done with the same Symmetry C18 (5 μm 4,6 x 150 mm) column. The summary of mobil phases is as follows: A: H_2O : MeOH : H_3PO_4 = 940 : 50 : 1, B: MeOH (pure methanol). With the A solution, the gradient reduced linearly from 100 % to 10 % in 30 minutes, and regained its original level in the next minute, back to 100 %, and finally, we waited 1 minute until the balance was regained, thus measurement of one sample took 32 minutes total. Samples were measured in 220 and 280 nm, and results were evaluated in 220 nm. The flow rate was 1 ml/minute and measurement was done at 20 °C. The IAA retention time was 17.8 minutes and the IBA time was 22.45 minutes.

Summary of laboratory measurements

In the course of comparing the varieties during winter 2006/07, the ’Fehér besztercei’, ’St. Julien GF 655/2’, ’Myrobalan B’, ’MY-KL-A’, and ’Marianna GF 8-1’ varieties were measured for IBA content in the rooting zone. Cuttings were collected at 7 different times (**table 1**) and the collection times were followed all the way to complete decomposition of IBA at the end of April. During the 2008/2009 growing period, fluctuations in indole-3-acetic acid level were noted during the cuttings collection period.

Varieties collected on November 26, 2008 and January 19, 2009 (’Fehér besztercei’, ’St. Julien GF 655/2’, and ’Marianna GF 8-1’) were examined for indole-3-acetic acid content during the rooting period, until complete degradation of the stimulant. Also, at the same time, the IAA content was measured through the first ten days of May.

The analysis of bottom heat treatment occurred with the following varieties, collected January 19, 2009: ’Fehér besztercei’, ’St. Julien GF 655/2’, and ’Marianna GF 8-1’; in which the base and tip areas were checked for IAA content during the rooting period, and then examined again from the time they were set out until the end of April. In addition to this, the IBA decomposition of cuttings was studied.

’Marianna GF 8-1’ rootstock was subjected to bottom heat treatments of various lengths, one- two- and three-week periods, and the cuttings were checked for IAA and IBA content as described in the previous paragraph.

In terms of bud removal, on November 26, 2008 the ’Marianna GF 8-1’ variety were collected and subject to bud removal in various ways; they were also treated with IBA (**table 2**). The base, middle, and tip areas of the cuttings were examined for indole-3-acetic acid

levels until the first part of May. The cuttings which had been dipped in IBA solution were also measured for indole-3-butyric acid concentration.

There were also questions about possible activity of the stimulant with the cuttings whose tips were treated at 2000 ppm with IBA. For this reason, all three twig areas were measured for exogenous auxin concentrations.

Examinations of the variations in cutting phenology and morphology

In 2009, on March 20, when hardwood cuttings were set out, they were compared in terms of phenological condition, with a focus on the possible influences of the various treatments.

The budding stage was compared, which enabled us to conclude when buds would open. One group of buds was still covered by scale, while others were not, and the green bud tip was showing. The percentage of cuttings with buds was calculated. Callus formation was also measured, as well as existence of primordia and adventitious roots.

Not counting the shoots with no active callus formatting basal part, the cuttings fell into three groups in terms of callus formation. A count was taken of cuttings having weak callus, the callus itself was examined, and we also studied cuttings which had swelled and split. Necrotic symptoms of the cuttings were noted, which offers information about their sensitivity to IBA. In winter 2006/2007 the open field cuttings were examined and notes made on the number and percentage of necrotic ones. In January 2009 the cuttings exposed to base heating were likewise examined for necrosis and severity of symptoms.

During the autumn production time, the number of roots on the rootstocks was counted.

Statistical analysis

The SPSS 14.0 program was used to analyze the results of the experiments. In addition, Microsoft Excel 2003 was used to interpolate functions. One and two-factor variation analysis occurred using the Duncan test. Work was always done with a 5 % significance level. In the case of function interpolation, in addition to the mathematic formula which describes the process, the R quarter value was input, which signals the exact interpolation of the curve, that is the "rightness" of the model.

Results and Discussion

Ability of hardwood plum cuttings in this study to produce roots

The rooting abilities of three plum varieties hardwood cuttings were examined and evaluated, from the end of October to spring budding. The *Prunus domestica* varieties, 'Kisnánai lószemű', is capable of being propagated with hardwood cuttings, the 'Fehér besztercei' subspecies produced disappointingly low results (20-40 %), and the 'St. Julien GF 655/2' (*Prunus insititia* variety) and myrobalan-type rootstocks proved much more reliable.

The length of the growing period is decreased by the short, mild winters. Good autumn rooting was observed in all three years studied, and this finding agrees with the literature (Erbil 1997, Guerierro and Loreti 1975, Szecskó 2004, Abd Alhamed et al. 1993); however, growth did not start in spring, which contrasts with others' results (Szecskó 2002, 2003). In the presence of unusually mild winters, drastic reduction of the growing period was observed.

Our results show that the optimal auxin treatmen concentration is not independent of the time when cuttings are collected. After December, the untreated control cuttings have the best rooting, and starting in January the sensitivity to IBA increases. For 'Fehér besztercei' the largest concentration of IBA dip, 4000 ppm, was the best; for this subspecies, stimulant was essential.

The *Prunus insititia* variety, 'St. Julien GF 655/2', reacts with the greatest sensitivity to IBA over-dosage. Among the myrobalans, 'MY-KL-A' is the most vulnerable, followed by 'Myrobalan B'.

Table 3. IBA treatments evaluated according to cutting collection time and rooting results (%), winter 2006/07

IBA treatment/cutting collection	Oct. III.	Nov. I.	Nov.III.	Dec.II.	Jan.III.	Feb.II.	Feb.III.
treatment producing best results	4000 ppm	2000 ppm	0 ppm	0 ppm	0 ppm	0 ppm	0 ppm
and corresponding average	54,8 c	53,8 c	49,1 c	39,8 b	23,7 b	3,4 a	0,0 a
second best treatment and	2000 ppm	4000 ppm	2000 ppm	2000 ppm	2000 ppm	2000 ppm	2000 ppm
corresponding average	47,8 c	41,8 c	45,8 c	25,2 b	1,6 a	0,0 a	0,0 a
worst treatment	0 ppm	0 ppm	4000 ppm	4000 ppm	4000 ppm		4000 ppm
corresponding average	38,0 c	32,8 c	29,4 b	9,2 ab	0,2 a	0,0 a	0,0 a
average	46,9 C	42,8 C	41,4 C	24,7 B	8,5 AB	1,1 A	0,0 A

The following varieties returned the above results: 'Fehér besztercei', 'St. Julien GF 655/2', 'Marianna GF 8-1', 'Myrobalan B', 'MY-KL-A'

Note: I., II., and III. denote first, second, and third ten days of the month, respectively.

For most of the varieties we observed at the end of October, the start of the propagation period, 4000 ppm IBA dip yielded the best results; however, in early November, 2000 ppm dose proved significantly better (**table 3**). Continuing, most control cuttings rooted; in fact, from December the overly high concentration was lethal, and cuttings treated with 4000 ppm IBA solution died. 'St. Julien GF 655/2' was the variety that was most vulnerable to high IBA stimulant concentrations, in spite of showing misleading necrotic symptoms.

Changes in auxin content in the rooting zone of plum rootstocks

Native auxin content was measured at the time of collection. IAA concentration was calculated during the growing period using a bell curve model. The *Prunus domestica* rootstocks' IAA level was twice that of the other variety, with the values standing around 25-35 µg/g, which represents quite a modest change (30-40 %). The influence of the particular time is not significant for native auxin concentration. For myrobalan rootstocks, the most noticeable is this change, the minimum value is nearly 5 µg/g, whereas the maximum reaches 20 µg/g, which is practically a quadruple increase. 'St. Julien GF 655/2' rootstock: the maximum value is hardly twice the minimum, which indicated less intensive change in auxin level.

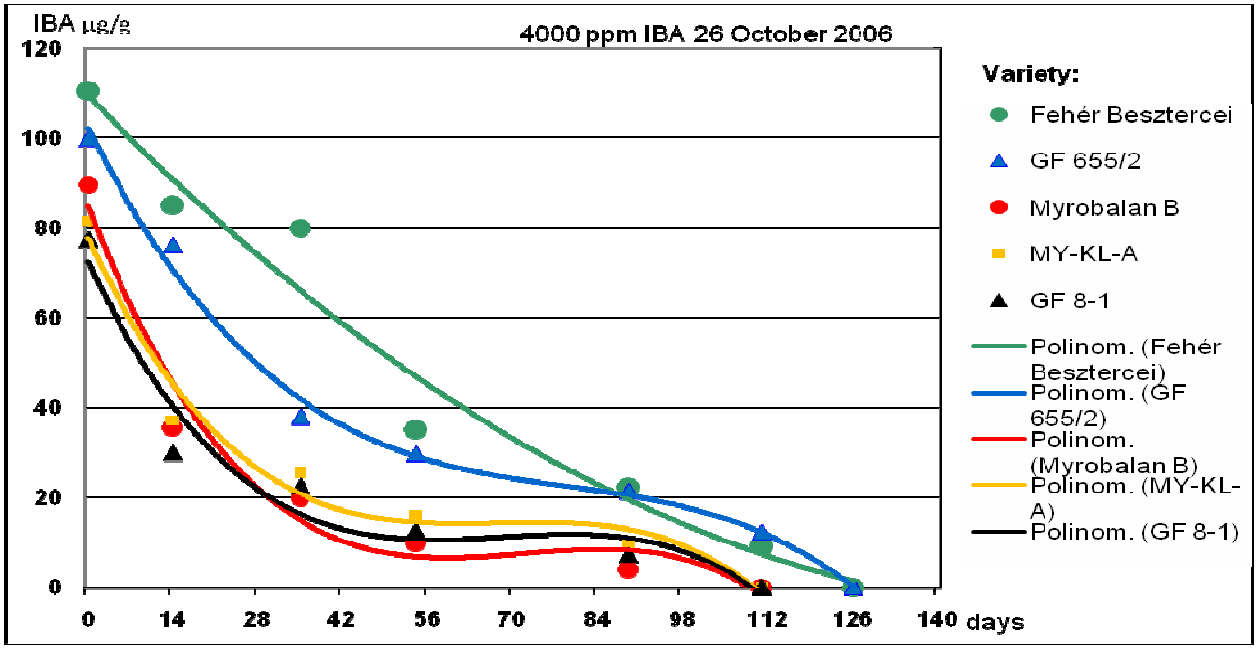
Examination of IBA decomposition among the cuttings resulted in considerable variations (**figure 1**) Myrobalan types broke down the stimulant at the fastest rate. Cuttings collected on October 26, 2006 and dipped in 4000 ppm IBA solution showed no detectable stimulant 111 days later, while 'Fehér besztercei' and 'St. Julien GF 655/2' metabolised stimulant in 126 days. Similarly, for IBA half-life, the myrobalan types varieties were quick (14 days), as opposed to 'St. Julien GF 655/2' (30 days) and 'Fehér besztercei' (45 days) regarding the gradual disintegration of stimulant.

Calculating the effect of IBA treatments on native auxin levels, we found that the stimulant caused an increase of IAA in the cutting base. The level of IAA in 'Marianna GF 8-1' was 9 µg/g at collection time (11.26.2009), which by 12.15.2009 was 10,4 µg/g in the untreated control, and the 2000 ppm-IBA dip raised it to 15,0 µg/g, while the 4000 ppm stimulant resulted in 23,4 µg/g native auxin level. Treatments of 'My-BO-1' and 'St. Julien GF 655/2' were found to have similar tendencies to increase IAA.

On the other hand, the untreated control of 'Fehér besztercei' had an initial native auxin level of 20,1 µg/g, which decreased by mid-December (13,1 µg/g), and 2000 ppm IBA dip (20,8 µg/g) was just enough to prevent reduction of IAA in the basal area. For this variety to raise its native auxin level, a 4000 ppm IBA immersion was necessary, and the greatest effect of this was

found in the rooting rate. The untreated control cuttings were unable to put out roots, the reason for which is presumably the rapid reduction in native auxin.

IBA treatments resulted in increased numbers of buds in the myrobalan varieties for the 2009 open field plantation period.



1. fig Plum rootstock varieties rooting cuttings and their IBA decomposition

Removal of bud from cuttings, and influences on rooting and auxin content

’Marianna GF 8-1’ rootstock cuttings were de-eyed in certain areas according to the **table 2** in "Materials and Methods." The de-eyeing process was combined with basal exogenous auxin treatments, whereby rooting was artificially stimulated with 2000 ppm IBA to replace the rooting which was aborted by de-eyeing. Due to de-eyeing, a significant number of cuttings were unable to survive because they could not bud, but they did in fact develop roots. Totally disbudded cuttings rooted most vigorously (96 %), whereas 40 % of cuttings which were not de-eyed developed roots. This difference is due to removal of buds which produce a substance that prevents rooting. The literature confirms this for autumn-collected cuttings (Howard 1980). Cuttings treated with 2000 ppm IBA rooted at 50 %, completely de-eyed and stimulant-treated cuttings at 78 %, and the highest rooting rate was achieved by cuttings treated at the apex.

Combined scooping (de-eyeing) and IBA treatments displayed much stronger (doubled) stimulant influence during the rooting period, and the influence was evident in the cutting base initial IAA content. Effects of bud removal were much weaker, hardly noticeable. Only with the completely disbudded cuttings was it apparent that the IAA concentration very slowly decreased from 8,3 µg/g-to 2-3 µg/g, one-third to one-fourth, over 2 months, in spite of the fact that these cuttings rooted at the best rate. That is to say with the ’Marianna GF 8-1’ variety the cause of no rooting is not low native auxin, but rather endogenous factors. According to the results of our experiments, the plant contains the minimum level of auxin needed for rooting throughout the propagation period.

At apex IBA immersion, the basal callus formation increased, but the buds were unable to open. They were inhibited, but rooting showed an excessively high value (94 %) In the case of cuttings, instead of budding, rooting predominated; budding was prevented by the plant's production of other substances.

Effects of bottom heat treatments

In 2008/09 plum rootstock cuttings were treated with base heating at 15 °C, from January 19 to February 9. Most varieties rooted poorly when kept in a cooler, and the heated base treatment could not amend this either. ’Ishtara’ (9 %) and ’GF 655/2’ (11 %) varietcies improved rooting slightly, but the myrobalan rootstocks became worse. The winter 2007/08 bottom heat treatment of 18 °C proved to be too warm; our results show that IBA treatments yielded complete absence of rooting.

In the presence of heated base treatment, the varieties examined experienced accelerated IBA decomposition. The amount of absorbed stimulant in the cuttings decreased to one-third in one week. The ‘Marianna GF 8-1’ variety cuttings, after a 2000 ppm stimulant dip, had a level at the end of February that was too low to measure, on a par with the amount metabolised by subjects kept in a cooler until April.

In order to express the ratio of bud hormone content and excessive amount of auxin, changes in IAA content at the cutting apex and base for the entire time of the rooting process were recorded. A new indicator, the A/F value, was used, which shows the fraction of hormone concentrations in the apical bud.

Grouping of species by sensitivity to IBA and need for stimulation

In determining the optimal IBA dose, an attempt was made to consider as many factors as possible (**table 4**). "Need for treatment" is understood to mean whether the variety was capable of rooting without immersion in stimulant. According to our results, ’Fehér besztercei’ was incapable of this. This result does not agree with the studies of Szecskó (2004), where a maximum of 10 % growth (rooting) was achieved. In our apical stimulation experiment, the cuttings base rooted without direct stimulation.

Table 4. Plum rootstock cuttings grouped by need for IBA treatment and sensitivity

Group	**1**	**2**	**3**	**4**
Varieties	**Fehér besztercei**	**Marianna GF 8-1, MY-BO-1, Ishtara**	**1. MY-KL-A 2. Myrobalan B**	**St. Julien GF 655/2'**
Need for treatment	**essential**	**–**	**–**	**–**
IBA decomposition*	**slow**	**fast**	**fast**	**fast**
IBA half-life*	**slow (50 days)**	**fast (14 days)**	**fast (14 days)**	**medium (30 days)**
sensitivity to necrosis*	**–**	**–**	**++,+++**	**–,(+)**
IBA effect on the IAA level (%)	**30%**	**65–100%**	65–100%**	**65%**
likelihood of death	**–**	**–,+**	**+,++**	**++,+++**

*** these capabilities determine that in the case of IBA over-dosage, how quickly and how much the species is able to rid itself of superfluous stimulant**

** hypothesized value

1. 2. order of necrosis sensitivity

The characteristics described in this paragraph show the varietiy' sensitivity to excessively high concentrations of stimulant. The species have potential internal defense mechanisms against "over treatment." For the myrobalans, some of whom show necrosis as a hypersensitive reaction, this is a fast and effective method, because they rid themselves of superfluous stimulant through death of tissue. We also found damaged, but surviving cuttings, often with considerable growth. Blackened tissue and decrease of growth rate are considered signs of overdose. Fast disintegration of IBA in the rooting zone is likewise an effective mechanism, as is the short half-life time (14 days), and these are all characteristics of the myrobalan rootstocks. The fact that the IBA half-life time is rather long (30 days), and the stimulant breaks down slowly in ’St. Julien GF 655/2’ cuttings makes it extremely sensitive. The ’Fehér besztercei’ actually needed stimulant for rooting, and in this subspecies no sensitivity was found, up to the 4000 ppm IBA dose.

IAA producing ability of the tissues increases with IBA treatments. In many different woody taxons it has been proved that IBA can convert to IAA (Baraldi et al 1993, van der Krieken 1992, Epstein and Lavee 1984, Epstein and Ludwig-Müller 1993), that is, the native auxins can behave as protective substrate (Breen and Muraoka 1973, 1974, Hartman 1997). The various varieties show considerable differences among the quantities of native auxins that appear in the rooting zone and create an influence on the stimulant dose. The ’Fehér besztercei’ gave the weakest reaction, but in the ’Marianna GF 8-1’rooting zone the same dose induced synthesis of more than three times the IAA concentration.

It is not shown in the table, but the IBA treatments provoked early sprouting in the myrobalan varieties. Influences by 4000 ppm IBA treatments on the four varieties studied were that the ’Marianna GF 8-1’ and ’Myrobalan B’ rootstock cuttings had faster phenological processes, but this appeared to a lesser extent in ’Ishtara’ and ’MY-BO-1’. Early budding frequently leads to drying (Szecskó 2004), since the plant does not yet have a root system capable of vaporizing the surface.

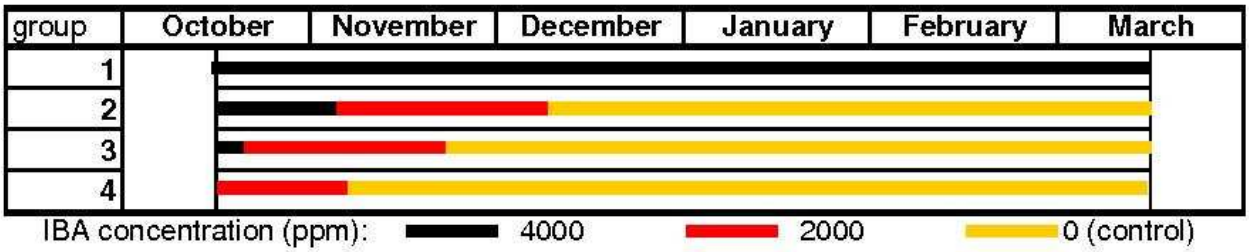
From the stimulant treatment standpoint, probably the variety' most salient capability is vulnerability to dying out, which is influenced by the factors that have already been mentioned. The varieties were categorized into four groups based on these main characteristics. Some members of the groups needed similar amounts of stimulant, and for them the IBA treatment technology was adapted (**table 5**).

Technological recommendations:

The varieties were grouped by sensitivity to IBA and capacity to bond to stimulant. For the four groups formed in this way (**table 4**), we created a technology of optimal dose IBA stimulant for the propagation period, which is shown in **table 5**. In the case of ’Fehér besztercei’ only the maximum 4000 ppm IBA dose was tested, but it would be worth it to try an even higher concentration, since others recommend the 5000 ppm dose for the treatment of *Prunus domestica* (Hartmann et al. 1997, Nahlawi and Howard 1973, Macdonald 1993). Considering our results, the autumn propagation period gives the best and most reliable results from the point of view of the rooting of hardwood plum cuttings.

In terms of applying base heating, for domestic conditions, the short, one to two-week treatment at under 15 °C is advisable, and IBA stimulant should be left off completely. The cuttings should then be stored in a cooler until they are planted outdoors.

Table 5. The IBA stimulant technology recommended for the **table 4** groups (dose/time)



New scientific results

1. The cuttings of some plum rootstock varieties were grouped according to need for and sensitivity to IBA treatment. The technology of IBA treatment for some variety was developed in conjunction with concentrations of stimulant, administered at the times we considered to be ideal.
2. In the case of myrobalan shoots, dipping of cutting bases in IBA may cause plants to start growing earlier than normal, which has a negative effect on their development. They dry out easily, and concentrate their internal strength on putting out new growth instead of developing roots.
3. We exhibited and compared IAA levels among plum rootstocks over the propagation period. According to our studies, beginning in autumn, the level of native auxin decreases, reaching a low point in deep dormancy (midwinter), after which it begins to increase.
4. Four plum rootstocks cuttings were studied: we modelled the effects of solutions containing various concentrations of IBA on the native auxin level. It was determined to what extent IBA treatment raises the native IAA concentration in the rooting zone. Based on our results ’Fehér besztercei’ showed the least effect, whereas IBA treatment of ’Marianna GF 8-1’ hardwood cuttings significantly raised the IAA content.
5. Plum cuttings which had been treated in warm beds and placed in cold storage were examined for their capacity to absorb and metabolise IBA. We concluded that these varieties metabolised the stimulant in irregular order and half the normal time. This in turn demonstrates their sensitivity to stimulant.
6. During studies of ’Fehér besztercei’, ’St. Julien GF 655/2’, and ’Marianna GF 8-1’ hardwood cuttings, data was collected on the influence of bottom heating treatment on the cutting base and tip in terms of IAA levels.
    - We found that warm bed treatment raised the predominance of IAA in the rooting zone of plum rootstocks.
    - Regarding ’Marianna GF 8-1’ cuttings, influence on auxin level during the treatment period revealed that high auxin concentration already manifests in the first week, after which warm bed treatment is no longer necessary.
    - We characterized cutting base and tip fractional amounts of IAA using the A/F value, and noted that excessive auxin developed in the rooting zone--relative to the tip--due both to warm bed treatments and administration of IBA.

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