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# Influence of Physiological Factors on Rooting Ability of Plum Rootstocks' Hardwood Cuttings

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**Summary**

The physiological basis of rooting is well studied, it is known that certain substances formed in the plant are related to rooting, but their exact chemical composition, way of action, and the relation among them have not been cleared yet. Our aim was to examine the phenological status of the mother plant, the environmental conditions, the biochemical features of the cutting material at the time of collection, and the state of dormancy of buds. We also tried to discover correlations between these factors and rooting potential of cuttings, and also among the factors. The conclusion is that the complex process of rooting can be influenced in direct or indirect ways by the factors under investigation. The possible connections between rooting and the factors examined are shown in a figure.

**Key words.** leaf fall – water content – sprouting – cold units – peroxidase – polyphenol oxidase – phenolics

## Introduction

In the 1980's, many studies concerning rooting focussed on almost each of the determining factors. Apart from the environmental and technological factors, several investigations covered the roles of the internal processes inside the mother plant, which can be even more determining, e.g. the phenological status and biochemical features of the mother plant influence rooting (Bassuk and Howard 1981; Loreti et al. 1985; Gur et al. 1988; Guskov et al. 1988). It is known that certain compounds formed in the plant are related to rooting, but their exact chemical composition, way of action, and the relation among them have not been clearly defined yet. The preparation of the cells for root primordia initiation are probably made by the active enzymes and their substrates, through forming auxin-phenol conjugates, but the presence and concentration of several other materials (sugars, peptides, growth regulators) is also important. Our aim was to examine the phenological status of the mother plant, the environmental conditions, the biochemical features of the plant material at the time of cutting collection, and the dormant state of buds. Therefore, investigations were carried out associated to the dormant state of the mother plant and the cutting material (sprouting of buds, leaf fall), the temperature at collection, the cold units, the free water content of the cuttings, the activity of peroxidase (POD) and polyphenoloxidase (PPO) enzymes in the cutting material collected and in their buds, as well as the total amount of phenolics. From the data gained we tried to find correlations between these factors and rooting ability of cuttings, and also among the factors.

## Materials and Methods

### *Plant material*

Hardwood cuttings and samples for laboratory investigations were collected from the mother plants of the following three rootstocks, that are recommended to Hungarian conditions:

**'Marianna GF 8-1'.** A *Prunus munsoniana* Wight & Hedrick x *Prunus cerasifera* Ehrh. hybrid with high vigour. Roots easily, it is propagated by soft- and hardwood cuttings.

**'Sainte Julien GF 655/2'.** A *Prunus insititia* Jusl. rootstock of moderate vigour range, propagated vegetatively by soft- and hardwood cuttings.

**'Fehér besztercei'.** A Hungarian selection, a *Prunus domestica* L. rootstock. Its growth vigour is medium. For its propagation softwood cuttings are advised.

The mother plants of these rootstocks were planted to 3 m x 0,4 m, trained to hedge, and pruned heavily.

### *Experimental methods*

For determining the **rooting ratio** on the different collecting dates, cuttings were collected between 1997 and

2002 from September to March, prepared to the length of 25 cm, from the basal part of shoots 7–8 mm in diameter. After collection the cuttings were dipped to fungicide solution, and put to cold storage for one day. The day after collection IBA treatment was applied, dipping the base of the cuttings to 50 % alcoholic solution of indole-butyric acid for 5 seconds, using 2000 ppm dose. After the treatment the cuttings were put into wet perlite and stored at 2°C, until planted to open field the next spring. Split-split-plot arrangement was used, ten cuttings in five replicates in each date and hormone dose. After planting, the cuttings received the usual treatments, like tilling, pest management, irrigation, etc. The rate of rooted cuttings was determined in October.

The ratio of leaf fall was examined, as each year on 31st August six trees were selected of each cultivar, and four shoots on each. The leaves on the marked shoots were counted every week untill total leaf fall; the rate was given in the percent relative to the 31 st August state. For the examination of sprouting one cutting per cultivar was put to room temperature in six replicates on each collection date. They were treated before with fungicide and the water was changed regularly. For measuring the free water content of the cuttings on collection, two cuttings per cultivar were cut into 1 cm pieces in five replicates on each date, and dried at 120°C for three days. The water content was counted from the difference between the fresh and dry weight of the cuttings. The daily maximum and minimum temperature data of the meteorological station nearest to the experiment (Budapest-Pestszentlörinc) were used for calculating the daily mean temperatures and cold units, the later was counted by the method of RICHARDSON et al. (1974), starting counting from 23 September.

The samples for the biochemical assays were prepared from the cuttings and from the buds of shoots used for the preparation of cuttings. The measurements were executed in four replicates, as four cuttings were analysed separately, and the buds of the 25–40 shoots collected on each date were processed divided into four parts. The shoots were transported in a cooler to the laboratory, where they were put to –20 °C until the immediate processing. The buds were removed from the shoots with a knife, and the 15 cm long basal part of the cuttings was grinded with a pencil sharpener. Afterwards the cuttings and buds were homogenized with liquid nitrogen. From this material 150 mg was measured to cooled mortars, and 800 µl cold 0,1 M Tris-HCl buffer and sand was added. The samples were than spinned at 13000 rpm for 18 min at 4 °C, and the supernatant was used for measuring the activity of peroxidase and polyphenoloxidase enzymes, and the protein content.

For measuring the total phenolics 300 mg sample was weighed to centrifuge tubes after the homogenization with nitrogen, and 900 µl methanol:water 80:20 mix was added and shaken. After 5 min of ultrasonic digestion the samples were spinned at 13000 rpm, the supernatant was used for analysis. The activity of peroxidase enzyme was measured by the method of SRIVASTAVA (1983), by spectrophotometry (λ=460 nm), in 0,1 M Na-acetate buffer (pH=5.0), in the presence of orthodianisidin (ε=11,3) colour reagent and $H_2O_2$ substrate. The activity of polyphenol oxidase enzyme was measured by the method of BASSUK et al. (1981) by spectrophotometry (λ=420 nm), in 0,1 M Na-phosphate buffer (pH=6,5), in the presence of 0,25 % catechol substrate. The activity of the enzymes is given in Unit mg protein. The determination of the protein content was accomplished by spectrophotometric measurement (λ=595 nm) according to BRADFORD (1976). The phenolics were assayed by the method described by SINGLETON and ROSSI (1965) (λ=760 nm), using Folin-Ciocalteu reagent. The results are given in µg/mg gallic acid.

The experiments were performed at the Experimental Station of the Corvinus University of Budapest, the laboratory analyses at the Applied Chemistry Department of the Food Science Faculty of the University. The water content measurements and the sprouting were accomplished at the Fruit Science Department. The results were analysed with statistical programs, using one- and two-factor analysis of variance, regression analysis and partial correlation analysis. Any effect of time was ignored.

## Results

### *The relation between rooting and the factors examined*

The cuttings collected in fall rooted better. This period could also be described by the extent of leaf fall. The quadratic figures fitted on the rooting results in relation to leaf fall reached their peak at 40–60 % leaf fall (Fig. 1, Table 1).

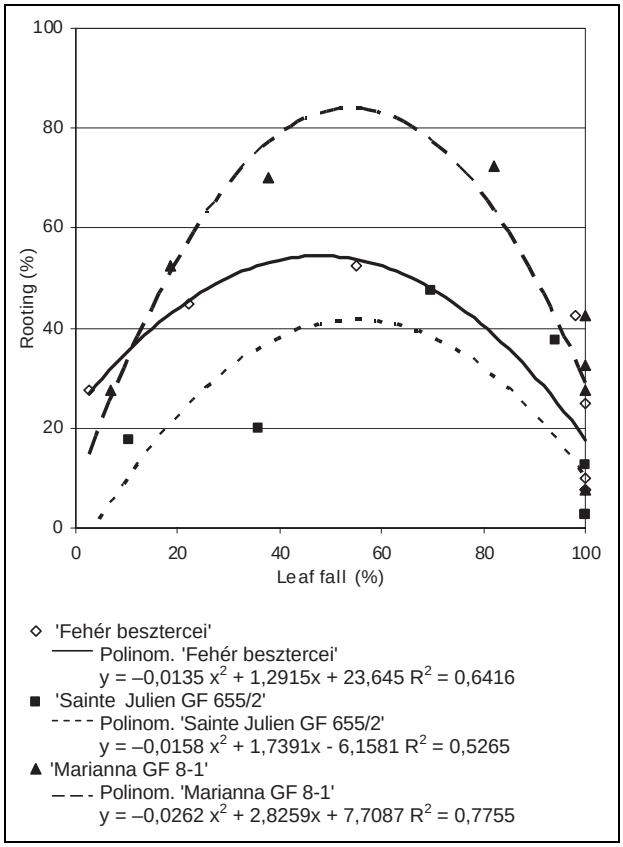



Fig. 1. Rooting of hardwood cuttings in relation to the extent of leaf fall.

Table 1. The correlation and regression coefficients of the connections between rooting and the different factors examined.

| | | 'Fehér besztercei' | 'Sainte Julien GF 655/2' | 'Marianna GF 8-1' | Number of years examined |
|---|---|---|---|---|---|
| Leaf fall – rooting | SR+ | 0.642* | 0.562 | 0.776** | 1 |
| Water content – rooting | LPC | 0.522** | 0.560** | 0.480* | 3 |
| CU – rooting | LC | −0.332 | −0.311 | −0.407** | 4 |
| Weeks until maximal sprouting – rooting | LPC | 0.514*** | 0.354* | 0.447** | 4 |
| POD activity – rooting | LPC | −0.572** (in bud) | −0.735*** (in cutting) | −0.825*** (in cutting) | 2 |
| Phenol in bud – rooting | SR | 0.408** | 0.425** | 0.656*** | 2 |

*Significant at P=10 % level; **Significant at P=5 % level; ***Significant at P=1 % level.
+LC: Linear correlation; LPC: Linear partial correlation; SR: Square regression

According to our results, the water content on collection influenced rooting (Table 1). Though the strength and features of the relation was varying in the years, based on three years' data, higher water content showed to be favourable. The higher (above 50 %) rooting percentages usually were reached when the water content of the cuttings was over 50 %, though lower establishment rates occurred as well. The 50 % water content on collection probably represents a threshold below which high rooting percentage cannot be expected.

Statistical analysis did not show any significant relation between the rooting of hardwood cuttings and the daily mean temperature at the day of collection except for for 'Marianna GF 8-1' . The strength of the relation was differing in years for the two other rootstocks.

The dormant state of the mother plants can be described with the capacity of sprouting of their buds. The number of weeks needed for reaching maximum sprouting was in linear connection with rooting rate, while the maximum rate of sprouting showed a varying relation (Table 1). The correlations analysed indicated that the extent of sprouting was less important concerning rooting than the time required for sprouting. So the deeper the dormant state of the buds, the higher rooting percentage can be expected. The correlation for 'Fehér besztercei' was strong, while 'Marianna GF 8-1' and 'Sainte Julien GF 655/2' data showed even less relation, so no further conclusion could be derived.

We found significant correlations between the rooting of all the three rootstocks and the enzyme activity measured in one of the organs (Table 1). The lower activity values were favourable. We have found similar results in the literature, with cuttings of cultivars with lower IAA-oxidase activity (which is mostly due to peroxidases) rooting better (Guskov et al. 1988). However, evidence for the contrary also can be found (Ryugo and Breen 1974).

Unlike earlier studies (Al Barazi and Schwabe 1984; Gur et al. 1998), we could not find clear correlations between rooting of the cuttings and the PPO activity measured in the buds and cuttings. Significant correlation occurred in some years, but it could not be proved in the two-year data.

The content of phenolics in the buds had square regression relation to rooting in all of the rootstocks (Fig. 2, Table 1). In the literature the lower phenol content of the mother plants is said to be favourable (Haissig 1986), but the total phenolics values measured by us were favourable in relatively wide intervals but near the measured maximum the rooting dropped.

*The relations between the different factors examined*

The connection between the daily temperatures, leaf fall, and sprouting is clear. The phenol content had relation with all the other factors but POD-activity. The higher temperatures and deeper dormant state occurred together with higher phenol content, the maximum of phenolics and PPO enzyme activity happened at 40–60 % leaf fall. The POD-activity had close correlation with sprouting (Table 2).

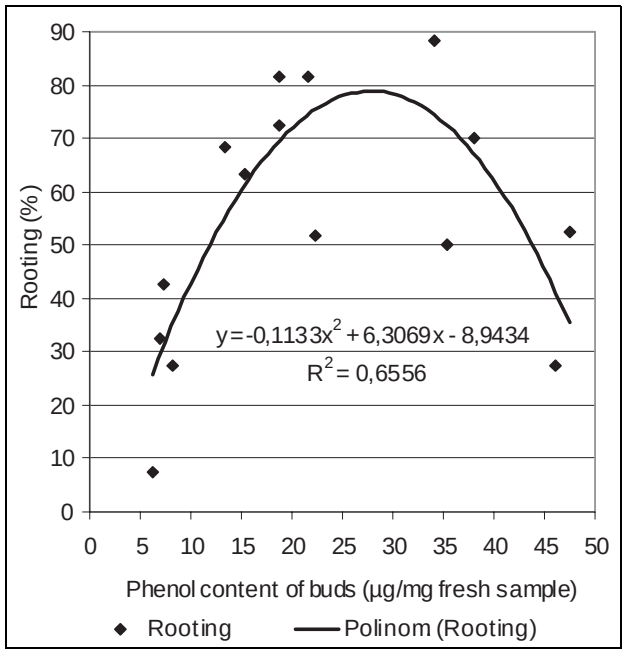



Fig. 2. Rooting of 'Marianna GF 8-1' hardwood cuttings in relation to the total phenol content of buds.

Table 2. The correlation and regression coefficients of the connection between the different parameters of 'Fehér besztercei' rootstock.

| | | Phenolics cutting | Phenolics bud | PPO cutting | PPO bud | POD cutting | POD bud | Water content |
|---|---|---|---|---|---|---|---|---|
| Phenolics – cutting | LPC+ | | | 0.166 | | −0.349 | | −0.749* |
| | SR | | | 0.101 | | 0.119 | | 0.761* |
| Phenolics – bud | LPC | | | | 0.545*** | | −0.346 | −0.809* |
| | SR | | | | 0.308** | | 0.078 | 0.827** |
| PPO – cutting | LPC | 0.166 | | | | 0.442 | | 0.476 |
| | SR | 0.022 | | | | 0.195 | | 0.284 |
| PPO – bud | LPC | | 0.545*** | | | | 0.242 | 0.016 |
| | SR | | 0.394*** | | | | 0.121 | 0.118 |
| POD – cutting | LPC | −0.349 | | 0.442 | | | | 0.427 |
| | SR | 0.122 | | 0.205 | | | | 0.608 |
| POD – bud | LPC | | −0.346 | | 0.242 | | | 0.131 |
| | SR | | 0.047 | | 0.152 | | | 0.297 |
| Water content | LPC | −0.749* | −0.809* | 0.476 | 0.016 | 0.427 | 0.131 | |
| | SR | 0.689* | 0.837** | 0.119 | 0.240 | 0.136 | 0.411 | |
| Leaf fall | LPC | −0.860*** | −0.619** | −0.632* | −0.559** | 0.000 | 0.218 | |
| | SR | 0.845*** | 0.632*** | 0.756** | 0.312* | 0.188 | 0.041 | |
| Max. sprouting | LPC | −0.080 | −0.510** | −0.077 | −0.339 | 0.392 | 0.148 | 0.212 |
| | SR | 0.050 | 0.293** | 0.025 | 0.115 | 0.163 | 0.010 | 0.327* |
| Weeks needed for max. sprouting | LPC | 0.187 | 0.582*** | 0.038 | 0.392* | −0.325 | 0.006 | 0.174 |
| | SR | 0.033 | 0.352** | 0.087 | 0.177 | 0.115 | 0.031 | 0.364** |
| CU | LC | 0.237 | −0.302 | 0.503* | −0.435** | 0.391 | −0.100 | −0.074 |
| | SR | 0.263 | 0.489*** | 0.256 | 0.246* | 0.210 | 0.246* | 0.019 |
| Daily mean temperature | LC | 0.271 | 0.499** | 0.257 | 0.339 | −0.014 | 0.015 | 0.218 |
| | SR | 0.107 | 0.357** | 0.131 | 0.115 | 0.104 | 0.063 | 0.072 |

+LC: Linear correlation; LPC: Linear partial correlation; SR: Square regression
*Significant at P=10 % level; **Significant at P=5 % level;***Significant at P=1 % level.

## Discussion

Rooting is a complex process that is determined by the relation of many factors. Rooting rate is highly influenced by the time of cutting collection, which includes the state of the mother plant and the environmental factors prevailing that day. We gained the higher rooting rates most frequently in the first half of the dormant season, or before (Szecskó et al. 2003). The environmental circumstances that we examined, such as the daily mean temperatures, and cold units derived from it (CU), – that means the temperature relations of the period between 23 September and the day of collection –, directly influenced rooting. Only in 'Marianna GF 8-1' direct correlation of rooting with cold units could be shown, for the other two rootstocks it varied.

We found a relatively loose connection between rooting and one of the factors describing the state of the mother plant, the leaf fall, which can be due to the indirect nature of relation. The phenolics that directly related to leaf fall also had close correlation to rooting. During leaf fall the content of phenolics continuously raised in the buds of the shoots (possibly in connection with the materials formed in the separating zone of the leaves during leaf fall – first of all phenolics and polyphenol oxidase enzyme). Since this is favourable for rooting to a certain extent, from the state of leaf fall the values of this important factor can be suggested.

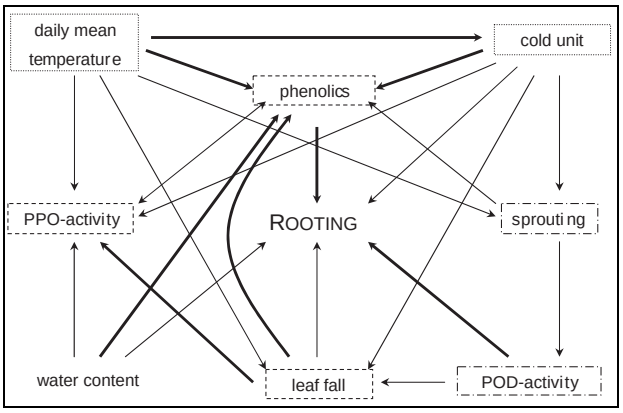



Fig. 3. The possible relation between the factors influencing rooting.

Many authors examined the role of phenolics in rooting (WILSON and VAN STADEN 1990; HARTMANN and KESTER 1997), often getting correlations between the amount of phenolics and the rate of rooting (RANA and CHADHA 1992), but there are examples for the contrary as well (HAISSIG 1986). We also found positive correlation between the phenol content of the hardwood cuttings at the time of collection and the rooting achieved later. Some phenolics preserve auxin from degradation by blocking the IAA-oxidase (peroxidase), on a way that peroxidases oxidize these phenolics instead, and auxin only later (JARVIS 1986). We did not find linear or square correlation between POD activity and total phenolics, but both was related to rooting. On those dates when POD activity was relatively high and phenolics relatively low, the rooting was worse than with high phenolics-low POD activity.

According to our opinion the phenol content of buds was one important factor in rooting of the three rootstocks. The rooting of all the three rootstocks had correlation with bud-phenolics, and the phenolics correlated to the other factors measured, so rooting – directly or indirectly – depends on all of the environmental, phenological and biochemical factors measured at the day of collection. Based on this, we can conclude that for example rooting depends indirectly on the temperature of the day of collection, that was known so far in a form that cutting collection is best to do on days without frost. The physiological base of it was not known, it is possible that the phenol content of buds is the link between the rooting of hardwood cuttings and the environmental circumstances.

Based on the statistical analysis, the polyphenol oxidase activity did not show relation with rooting, but had connection with the level of phenolics, leaf fall, and the temperature data, therefore indirectly may have effect on root formation as well. Water content is also related to the experienced rooting, and also with the two factors mentioned, but the correlation with phenol content and PPO-activity was also significant. Accordingly, it also takes part in the determination of rooting ability.

The other important feature of the mother plant is the level of dormancy, which cannot be measured. It only can be concluded from the extent and speed of the sprouting of the cuttings forced at room temperature. We experienced that the slower the sprouting of the buds, the better rooting can be expected, therefore cutting collection in deeper dormant state of the mother plant can be beneficial. Sprouting of the buds negatively influences the rooting of the cuttings, too early spouting results in the too early using up of energy and nutrient reserves. The link between sprouting and peroxidase activity and between POD activity and rooting suggests that sprouting has its effect on rooting through the total POD activity, and POD enzymes take part in that complex process that results in the sprouting of the plant. Peroxidases take part in indole-acetic-acid degradation, which we can confirm according to our results, as the rooting rate decreased as soon as the activity of this enzyme reached a certain level. Sprouting shows a slight correlation with phenol content, so it can be joined into the system described above.

The complex relation system influencing rooting ability we concluded is presented in Fig. 3.

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