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PROPAGATION OF SELECTED PEAR AND QUINCE ROOTSTOCKS BY HARDWOOD CUTTINGS

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Abstract

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As long as new rootstocks continue to be developed, there will always the need for new genotypes to be controlled and evaluated. This study involves verification of selected rootstocks with reference to growth, propagation ability and other characteristics. Different rootstocks, different growth stimulators and different kinds of cuttings techniques were adopted for these experiments. Stimulators used for evaluations included: 1 % Racine (2.5 % naphthalene acetic acid - NAA), 0.5 % Rhizopon AA (2.5 % indole-3-butyric acid - IBA) and 0.25 % IBA in 50 % ethanol solution. There were three sets of experiments in all. In Experiment 1, the bases of cuttings were treated with the stimulators and part of them was also treated with heat before planting. Results from Experiment 1 indicated that cuttings treated with Rhizopon AA produced the best effect after heat treatment (with an overall average of 36.9 % of rooted cuttings). Pyrodwarf (78.7 %), Cydomalus (73.9 %) and Pyroplus (60.6 %) were the best performing rootstocks among the evaluated ones. In Experiment 2, we evaluated the influence of the date of harvesting the cuttings on rooting (comparisons were made between cuttings prepared in December [early] and March [late]). For BA29 rootstock cuttings, the best performance was attained by cuttings taken in March. The difference in rooting between March and December was 43.9 %. On the other hand, early harvested rootstocks (cuttings taken in December) for Cydomalus showed the best performance, with an 18.7 % difference between cuttings made in December and in March. Results from other evaluated rootstocks were not significant enough. In Experiment 3, the effect of chemical (phytohormone) stimulation and heat stimulations was compared. The best result was obtained by combinations of treatments without heat stimulations, when the cuttings were stored at 5°C and later treated with 0.25 % IBA in 50 % ethanol solution. The least rooting results, on the other hand, were observed in heat treated cuttings, in cuttings stimulated with IBA and subsequently with heat and in cuttings treated with a 3 cm radial cut at the base. Based on our findings we cannot prove clearly that heat stimulation and the phytohormones used have beneficial effects on rooting of especially new rootstock genotypes with an unknown propagation coefficient. This means that propagation using cuttings still remains an interesting topic to be further researched.

Keywords: plant hormones, vegetative propagation, nursery, *Pyrus*, *Cydonia*

INTRODUCTION

For most fruit trees of temperate origin the use of rootstocks is common. Rootstocks remain essential because of the characteristics and demands of most of the modern varieties. One way in which vegetative propagated rootstocks can be produced is by using hardwood cuttings (Brewer, 2011). Although propagation of rootstocks by hardwood cuttings is more common, Loreti and Morini (1977) revealed that propagation of Pyrus betulaefolia was more successful by softwood cuttings treated with 3000 ppm Butyric acid (IBA). The softwood cuttings rooted several levels higher than the hardwood ones. Also, some pear varieties, such as 'Bartlett', do not need to be grafted on rootstocks, they can be self-rooted. Thus, it is possible to propagate this variety by cuttings, but rooting might be difficult (Janick and Moore, 1996). For vegetative propagated rootstocks, it is important to have a good rooting ability and to exert no negative influence on fertility of the grafted scion variety. Common life span of most fruit trees in orchards is usually around 25 years, but popularity of a lot of fruit varieties offered on the market is changing at a very fast rate. For this reason there is a high demand for rootstocks. This only applies to pears, which is partly caused by the fact that market in Europe was dominated by a few varieties for a long time, with 'Conference'´ being the leading one (Brewer and Palmer, 2011).

Pear and quince rootstock cuttings are mostly used for pear varieties. While quince is a popular rootstock for pears, it has some negative characteristics. In some countries, exclusively quince rootstocks are used and these might face problems such as chlorosis and incompatibility, if planted on soils with high calcium content. Yet, rootstocks bred from *Pyrus communis* do not carry these problems but they are usually too vigorous, which causes other problems (Quartieri, *at al.*, 2011). *Pyrus ussuriensis* rootstocks are also suitable and are used in areas where temperatures are low during winter, as the variety has good frost resistance. Rootstocks bred from *Pyrus calleryana* are commonly used for pears in Australia, South America and China because of their good resistance to aphids, fire blight and other diseases. Similar resistance can be found in *Pyrus ussuriensis* rootstocks, but they are sensitive to waterlogging (Hancock, 2008).

Some pear rootstocks, such as 'Pyrodwarf', 'Rhenus' and 'Pyriam', and possibly quince rootstocks S1, (Simard and Michelesi, 2004) or quince A, quince C and the CQ set (127, 129, 130, 131, 132, 133 a 134), are very amenable to propagation by hardwood cuttings. Some of them, on the contrary, perform very poorly, e.g. BP 1 and BP 3 (clonal selections from *P. communis*), OHF 51 (Broklyl®), OHF 69 (Daynir®), OHF 97 and OHF 217 (Noad, 2003). Rootstocks OHF 333 (Brokmal®) and BA 29 (BAC 29) are often quoted as giving inconsistent results.

Cuttings are mostly taken in January or February and are 15-20 cm long. They are often stored in moist perlite at a temperature of 5°C and later planted into soil after callusing (Necas and Kosina, 2008).

One of the things that affect rooting of cuttings is concentration of Auxins (AU). While higher auxin concentration is necessary for good rooting, too high concentrations can cause the opposite and slow down the rooting. Concentration of AU increases with growth of the buds, where AU is synthesized. The main adversary substance of AU is Abscisic acid (ABA), which inhibits growth of the roots and reduces the effect of AU.

Other substances affecting rooting of cuttings are the so-called root cofactors. These are substances which can stimulate rooting and protects auxins from degradation. Rooting is also affected by hormones, such as Gibberellin (GA) and cytokinins (CK). These influence elongation and growth. GA stimulates formation of AU and can be applied exogenously. Such application, however, is not as effective as when applied endogenously. CK only stimulates rooting at low concentrations; when its concentrations are high, then, on the contrary, rooting is inhibited (Kutina, 1988).

MATERIALS AND METHODS

This study involved three sets of experiments, each of them focusing on a different technique of preparing the cuttings before planting. The three experiments comprise of several treatment variants, i.e. several combinations of the chosen phytohormones, implemented heat treatment and the way the cuttings were prepared.

Experiment 1

was conducted from 2011 to 2014 (in the Experimental Field of the Faculty of Horticulture in Lednice). The following rootstocks were used: 'OHF 333', 'Pyrodwarf', 'Pyroplus', seedlings of *Pyrus communis*, the inter-generic hybrid 'Cydomalus', quince rootstock 'BA 29' and two Asian cultivars 'Mansan' and 'Shinseiki'. Mother plants were grown, maintained and protected by standard techniques. One year old twigs were harvested in January or in February, 15-20 cm long cuttings were made and then disinfected with 1 % Previcur (propamocarb+fosetyl-Al). There were 8 treatments and 6 replications in each treatment. For all 8 treatments, there was a total of 4320 cuttings. Each of the treatments involved 540 cuttings and thus, 90 cuttings in each of the replications. A completely randomized design was used for this experiment.

Two main methods of hardwood cutting treatment were used in this experiment. The first method involved heat stimulation of the bases of cuttings - they were stored in a damp perlite at a temperature of 21°C for 25–35 days before planting. In the second method, cuttings were prepared without heat stimulation - they were stored in a damp perlite at a temperature of 5°C until the planting. In each of the two preparatory methods two different rooting hormone formulations were applied: 1 % Racine (2.5 % naphthalene acetic acid – NAA) and 0.5 % Rhizopon AA (2.5 % indole-3-butyric acid - IBA), plus untreated control. The number of well callused cuttings was determined at the end of the treatment process and cuttings were then stored in a damp perlite (5°C) until plating outside in the nursery. At the end of the growing seasons, rootstocks were collected from the nurseries and rooted cuttings were counted.

Data obtained from the experiment were analysed by statistical analysis methods based on multi-factorial analysis of variance (ANOVA) utilizing Tukey's HSD test (by menas of Statistica 10 software).

Statistical comparisons were elaborated for each treatment by rootstocks and for each rootstock by the individual treatments. Statistical testing was done for all the interactions between a rootstock and a variant of treatment of the cuttings. For the sake of clear representation also percentage values were calculated and transformed into tables.

Experiment 2

was carried out within a period two years (2013 and 2014) (in the Experimental Field of the Research and Breeding Institute of Pomology in Holovousy). The goal was to compare two different time of the year for collection of cuttings (early and late). The early harvest and preparation of cuttings was done on December 20, 2012 and December 18, 2013. The second collection date for cuttings (late harvesting) was March 15, 2013 and February 26, 2014. Cuttings 35-40 cm long were taken from seven rootstock varieties, namely: 'Pyrodwarf', 'Pyriam', 'Fox 11', 'Farold 40', 'Farold 87', 'Cydomalus' and 'BA 29'. There was a total of 1260 cuttings, two treatments (early and late) and 90 cuttings per rootstock variety. A completely randomized design was used for the experiment.

The bases of all cuttings were stimulated with 0.25 % IBA in 50 % ethanol solution for 5 seconds and then allowed to dry. When the stimulants had dried, the basal parts of cuttings were dipped in 1 % Previcur (Propamocarb 530 g/l, fosetyl-Al 310 g/l). Immediately after the chemical stimulation the cuttings were stored in a heating box, where basal parts were kept at the temperature of 21°C for four weeks. The aim of the heat treatment was to stimulate formation of callus. When heat treatment finished, the cuttings were lifted from the heating box and stored in a cooling box with dry perlite at a temperature of 5°C until the time of planting. The cuttings were planted onto the field in April. Assessment and counting of rooted cuttings was executed in autumn of the year of the planting. Data obtained from the two year experiment were statistically analysed in the same way as in Experiment 1. For the sake of clear representation percentage values were calculated and transformed into a table.

Experiment 3

was conducted in the same years and at the same location as Experiment 2. The goal of this experiment was to compare six different rooting preparation techniques for the following rootstocks cuttings: 'Farold 40', 'Farold 87', 'Pyrodwarf', 'MA' and 'MC'. This experiment involved 6 treatments and 5 replications in each treatment. There was a total of 2700 cuttings: 540 cuttings in each of the 5 treatments and 90 cuttings in each of the replications. A completely randomized design was used for the experiment.

The first two treatments did not involve heat stimulation. *Treatment 1* was chemical stimulation with 0.25 % IBA in 50 % ethanol solution but without heat treatment. *Treatment 2* did not involve the chemical stimulation with IBA nor the heat treatment. Cuttings in Treatment 1 and 2 were then stored in a box with damp perlite at 5°C for 4 weeks. The bases of cuttings in the other four treatments (*Treatments 3-6*) were heat stimulated for four weeks at 21°C. In this group, *Treatment 3* was without chemical stimulation before heating (21°C). *Treatment 4* involved chemical stimulation with IBA before heating (21°C+S). *Treatment 5* involved chemical stimulation with IBA and a 3 cm long radial cut made on the base of the rootstock cuttings before heating (21°C+S+R). *Treatment 6* involved chemical stimulation with IBA after the heating (21°C+SA).

Harvesting of the twigs and preparation of the cuttings was done in February and in March in both years. All other measures and statistical analyses were identical as the ones implemented in Experiment 1.

RESULTS

Exp. 1.

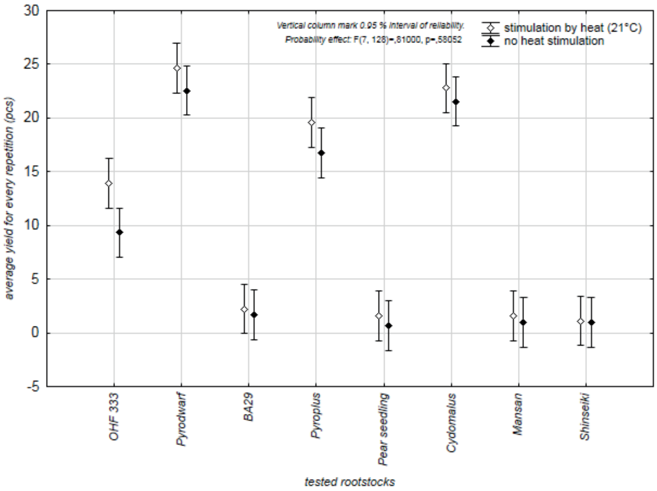
The results presenting the ability to develop callus are summarized and shown in Table 1. There were significant differences among all the studied rootstocks, but no significance difference was observed in the effect of the treatments on the ability to form callus (data not shown). The best callusing results were attained by the following rootstocks: 'Pyrodwarf', 'Cydomalus', 'OHF 333' and 'Pyroplus'. The worst callus formation result was recorded in pear seedling. Nevertheless, the remaining rootstocks ('BA29' and the variety 'Mansan') did not develop enough calluses either.

Significant differences in the rooting of the cuttings are shown in Graph 1. Graph 2 presents assessed differences between the Treatments. It is obvious the data that, except for rootstock 'OHF 333', there were no significant differences registered. The best rooting results in general, for all the treatments, were attained by 'Pyrodwarf' and 'Cydomalus' rootstocks. 'Pyroplus' rootstock also produced sufficient roots. The '*Pyrus* seedling' and 'BA 29' plus the two Asian varieties showed poor rooting.

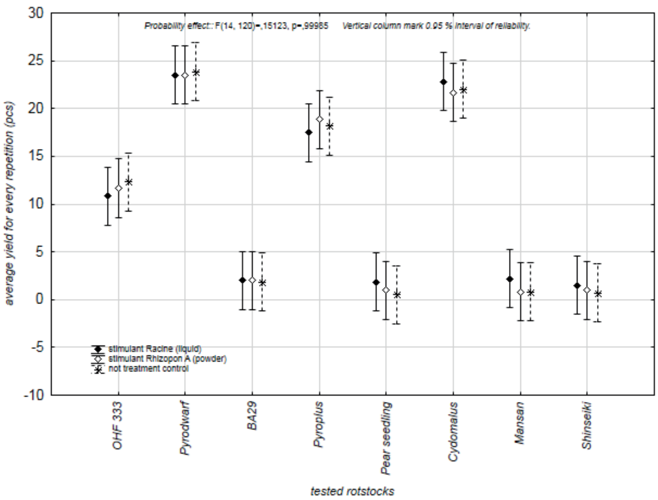
I: *Average stimulation effect (%) on rooting of the rootstocks (2011-2014). Results are comparable within each column and each row*

Rootstock	Heat stimulation			No heat stimulation		
	Racine	Rhizopon A	Control	Racine	Rhizopon A	Control
OHF 333	40.00 bc*	43.33 bc	55.57 ab	32.23 abcd	34.43 abcde	26.67 abc
Pyrodwarf	83.33 a	83.33 a	80.00 a	73.33 de	73.33 de	78.98 e
BA29	7.77 d	8.90 d	5.57 c	5.57 a	4.43 a	6.67 ab
Pyroplus	52.23 ab	73.33 abc	70.00 ab	64.33 cde	52.23 cde	51.10 bcde
P. seedling	8.90 d	3.33 d	3.33 a	3.33 a	3.33 a	0.00 a
Cydomalus	80.00 a	75.57 ab	72.23 ab	72.23 de	68.90 cde	74.43 de
Mansan	10.00 cd	3.33 d	2.23 c	4.43 a	2.23 a	3.33 a
Shinseiki	6.67 d	4.43 d	0.00 c	3.33 a	2.23 a	4.43 a
average	36.11	36.95	36.12	32.36	30.14	30.69

*letters characterize statistically significant homogenous subgroups



1: *Statistical analysis of root yield. Comparison between heat stimulation and cuttings without heat stimulation used for pear and quince rootstock cuttings.*



2: *Statistical analysis of root yields. Comparison of different stimulators used for the pear and quince rootstock cuttings.*

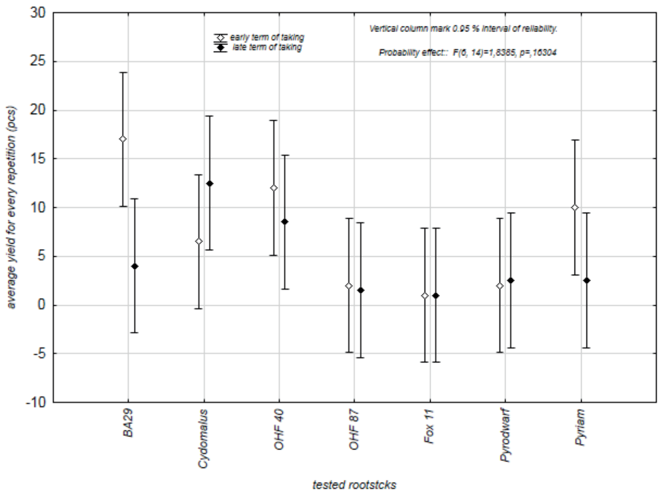
Exp. 2.

The goal of this experiment was to compare the early collection and preparation date of rootstock cuttings and a late one. The results are shown in Table 2. Significant differences were only observed in 'BA 29', 'Cydomalus' and 'Pyriam' rootstocks. In these rootstocks better rooting was attained among the ones taken in late winter (see Graph 3). Though not significantly, yet slightly better rooting was also attained by 'Farold 40', 'Fox 11' and 'Farold 87' rootstock cuttings harvested at the late collection date, in winter. 'Cydomalus' and 'Pyrodwarf' harvested in the early collection also produced better results, but not significantly more favorable. When comparisons between rootstock cuttings were made (statistical assessment not shown), the differences recorded were as in Experiment 1, nevertheless, none of the tested rootstocks attained sufficient rooting level. The data obtained from the early and the late preparation of the cuttings showed no significant statistical differences (Table 2). Statistically, a significant difference was only present in 'Farold 40' and 'Cydomalus' rootstock cuttings collected at the early date, revealing a positive statistical significance compared with the cuttings collected late in winter. In rootstocks 'Pyriam' and 'BA' 29, the case was exactly the opposite.

II: *Average number of rooted cuttings (in percentages) for the two different collection and preparation dates (2013 and 2014 winter season). Results are comparable within each column and each row.*

term	BA 29	Cydomalus	Farold 40	Fox 11	Farold 87	Pyrodwarf	Pyriam	average
Early	12.78 ab	41.11 b	28.34 b	2.78 a	5.56 a	8.34 a	8.89 a	15.40
Late	56.67 b*	22.44 ab	34.17 b	3.34 a	6.67 a	6.67 a	33.33 b	23.32

*letters characterize statistically significant homogenous subgroups



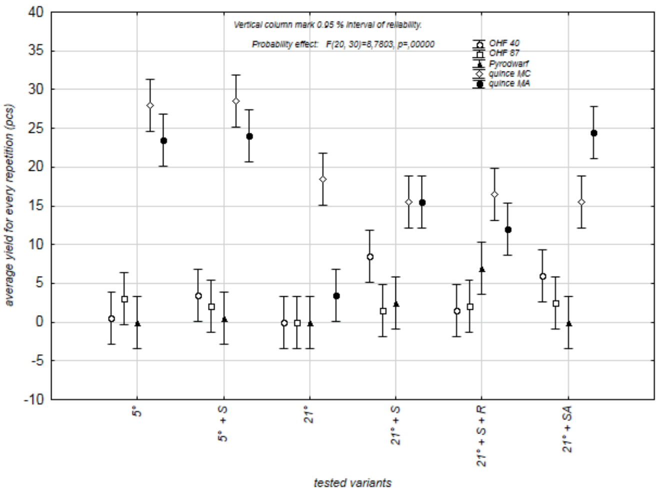
3: *Statistical analysis of root yield of the rooted cuttings; comparison between the different dates of collection of the pear and quince rootstock cuttings.*

Exp. 3.

Summarized results obtained from individual treatments are shown in Table 3. Significant differences were recorded in all the rootstocks except 'Farold 87' rootstock cuttings, which had the least rooting, see Graph 4. Heat treatment resulted in better rooting in Pyrus rootstocks 'Farold 40' and 'Pyrodwarf'. On the other hand, quince rootstocks 'MA' and 'MC' produced better results without any heat treatment. Both these rootstocks ('MA' and 'MC') achieved a rooting level above 75 %. After the overall average for a single treatment was calculated and rooting ability was compared, it was obvious that 'MA' and 'MC' rootstocks were very/highly suitable for propagation by hardwood cuttings. Pyrus rootstocks had very low rooting results with none of the tested treatment variants causing a marked improvement in rooting. 'Pyrodwarf', on the contrary, usually has high rooting results as compared to the results obtained in this experiment. Pear rootstocks treated at the temperature of 21°C produced the worst rooting results. Similarly, pear rootstocks treated at the temperature of 5°C did not produce encouraging results. Both quince cultivars generally responded well to all treatment methods, better than the pear rootstocks.

III: *Average number of rooted cuttings in percentages, by rootstock and by treatment for years 2013-2014: 21 °C = heated / 5 °C = unheated basal part of the cuttings; S = stimulation by IBA before heating or cold storage, SA = stimulation by IBA after heating, R = 3 cm long radial cut of the base. Results are comparable within each column and each row.*

treatment	Farold 40	Farold 87	Pyrodwarf	MC	MA	average
5°C	1.11 a	10.00 ab	0.00 a	93.33 gh	77.78 fgh	36.44
5°C + S	12.22 ab	6.67 a	2.22 a	95.00 h	80.00 fgh	40.22
21°C	0.00 a	0.00 a	0.00 a	62.22 ab	11.11 efg	14.67
21°C + S	28.34 abcd	5.56 a	8.34 ab	51.11 cdef	51.11 cdef	28.89
21°C + S + R	4.45 a	7.22 a	23.89 abcd	55.56 def	40.00 bcde	26.22
21°C + SA	20.56 abc	8.89 ab	0.00 a	51.11 cdef	82.22 fgh	32.55



4: *Statistical analysis of the yield of rooted cuttings – comparison between the different treatment variants in Experiment 3 for the rear and quince rootstock cuttings.*

DISCUSSION

The best rooting results without any use of phytohormones were attained by the quince rootstocks Pyrodwarf and Cydomalus. Pyrodwarf rootstock showed a success rate of 79 %. Jacob (1998) states that this rootstock has a good rooting ability, or to put it in a better way, a perfect coefficient of propagation ability. Cydomalus rootstock also obtained a rooting ability of a level of 74 %. However, in this study, Cydomalus rootstock demonstrated a lower root yield in the nursery as compared to Pyrodwarf rootstock. Both these rootstocks can also be propagated by *in-vitro* technique (De Paoli *et al.*, 2002).

Root yields obtained by Cydomalus MC (68 %) and Cydomalus MA (57 %) are within average. According to Rufato *et al.*, (2004) the use of IBA phytohormone in a concentration of 3000 mg.L^{-1} to stimulate rooting of quince cuttings can result in more than 50 % root yield. Barbosa *et al.*, (2008) also achieved an optimal rooting percentage in pears by using IBA at a concentration of 6000 mg.L^{-1}. Propagation of quince by rootstock cuttings according to Morini (1984) achieved 98 % of rooted cuttings. Moreover, he compared different rooting methods of the so-called "on-line grafting" of softwood cuttings. There were no significant differences for cuttings of Asian species of pears or for the varieties derived from *P. pyrifolia* (varieties Mansan and Shinseiki). However, Asian species like *P. calleryana* clone D-6 can attain rooting of 50 % (Chalfun *et al.*, 2002). In contrast, Loreti and Morini (1977) worked with clones of *P. betulaefolia* rootstock and they obtained about 15-56 % of rooting for an individual clone.

The effect of the cuttings collection date on root yield was significant for rootstock cuttings treated with BA29. The difference between early harvested cuttings prepared in December and later harvested cuttings prepared in March reached 43.9 %. Rootstock cuttings harvested in March (late) demonstrated better results than the early harvested cuttings of December. Similarly, the difference rooting between the early and the late prepared cuttings concerning Pyriam rootstock was 24.4 %. For Cydomalus rootstocks, the effect was more positive in cuttings prepared in December, with a difference of 18.7 %. Although Szecskó's and Hrotkó's (2004) results are not statistically significant, their experiments proved significant effect of the cutting time on root yield. They verified rooting at 5 different dates in a three year experiment.

In the experiment involving heat treatment, the effect on rootstock Farold 40 and Pyrodwarf was significant but it was not significant for Farold 87 rootstock. The heat treatment had a negative effect on quince rootstocks – the difference between heat treated cuttings and cuttings without heat treatment was 30 %.

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