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PRODUCTION OF WALNUT GRAFTS UNDER CONTROLLED CONDITIONS AND PLANTS DEVELOPMENT IN THE NURSERY

Svetlana M. Paunović^{1,*}, Mira Milinković², Žaklina Karaklajić-Stajić¹, Jelena Tomić¹, Boris Rilak¹

¹Fruit Research Institute, Čačak, Serbia

²Institute of Soil Science, Belgrade, Serbia

*Corresponding author: svetlana23869@gmail.com

ABSTRACT

Walnut grafting success under controlled conditions and the effect of foliar fertilization on the vegetative growth of walnut nursery plants were studied in this experiment. The study involved four cultivars and selections of walnuts, including 'Šeinovo', 'Šampion', 'Elit', and 'G-139'. The experiment included two parts. The first was conducted under controlled air temperature and humidity conditions with three different Treatments (Treatment 1 - grafting without paraffin treatment of the scion and graft union; Treatment 2 - grafting involving paraffin treatment of the scion and Treatment 3 - graft union covered with sawdust, and grafting involving paraffin treatment of the scion and graft union covered with sawdust and foil). The best results on the parameters tested were obtained in Treatment 3 involving dipping the graft into paraffin and graft covering with both sawdust up to the scion and polyethylene foil. The second part involved research in the nursery and included four treatments (Treatment 1 - without foliar fertilisation and Treatments 2, 3, 4 - with foliar fertilisation (Nitrophoska foliar, Nitrophoska solub and Humisol, respectively). Walnut plants treated with Humisol had a significantly higher survival rate in the first year, greater height and diameter in both years, as well as a higher percentage of first-class plants at the end of the second year compared to other treatments.

Keywords: *Walnut, cultivar and selection, graft, foliar fertilisation, nursery.*

INTRODUCTION

Walnut production in Serbia is insufficient to meet the country's needs, as it supplies less than 50% of national requirements. The reason is the decades-long of propagation walnuts by seed from natural populations dominated by walnuts that produce fruit of poor quality. To achieve improvement of walnut fruit quality, systematic work has been undertaken on walnut grafting. The technology employed in grafted walnut production is rather complex. Particular attention should be given to grafting and stratification methods, as well as to the proper care of grafted walnut plants in the nursery, in order to obtain quality planting material. The best and most effective results under continental climate conditions are produced by bench grafting using whip and tongue grafting techniques. Grafting success is dependent upon a multitude of factors, including choice of cultivars and rootstocks, time of scionwood collection from parent trees, time and method of grafting, air temperature, and humidity inside the heated room (Achim and Botu, 2001; Tshering et al., 2006; Mitrović et al., 2008; Paunović et al., 2011; Paunović et al., 2012a).

On the other hand, nursery plant cultivation is contingent upon environmental conditions, care, and handling operations. The survival rate planted of grafts in nurseries after the stratification period can be as high as 80%, with some of them being damaged during planting and further care, and the number of class 1 nursery plants produced realistically accounting for 50-60% of the number of grafted rootstocks (Paunović, 2010; Paunović et al., 2012b). According to Lučić et al. (1997), better graft growth and development in the nursery necessitate balanced fertilization, i.e. use of foliar fertilization, considering that in this way nutrients are more readily absorbed by the plant and more rapidly introduced into the metabolism than through soil nutrition. Apart from providing an optimum supply of water and minerals, diverse foliar fertilization has been proven to improve the survival rate and growth of grafted plants in numerous experiments (Ferrini and Nicese, 2002; Bostan and Islam, 2003; Salifu et al., 2006). However, the use of foliar fertilisation in walnut plant production has been negligible, regardless of its positive effect in terms of enhanced development of the root system, improved lignification and apical bud development, increased graft survival rate, increased percentage of class 1 plants and intensive vegetative growth of walnut plants (Solar, 2003; Paunović et al., 2012b; 2013a).

For that reason, the objective of this study was to evaluate the effect of different treatments on graft-take success under controlled conditions and observe the effect of foliar fertilisation on the survival rate and vegetative growth of nursery plants at the end of the first and second growing seasons.

MATERIALS AND METHODS

The experiment was conducted at the Fruit Research Institute, Čačak, Western Serbia. The first part of experiment was conducted under controlled air temperature and humidity conditions. Scions of cultivars 'Šeinovo', 'Šampion', 'Elit', and 'G-139' were grafted onto one-year-old seedling rootstocks of common walnut (*Juglans regia* L.) 8–14 mm in thickness. Grafting was performed during April by whip-and-tongue grafting in the root crown zone on a sample of 30 grafts per cultivar per treatment, following a randomised block design (4 cultivars x 3 treatments x 3 replications), totaling 1080 grafted rootstocks. The grafts were stored indoors for 28 days under controlled conditions at a temperature of 27–29°C and relative air humidity of 60–70%. On day 20 after grafting, shoots were removed from the grafted rootstocks. Treatments under controlled conditions included the following: Treatment 1 - covering the graft with conifer sawdust up to the top of the scion without dipping the scion and graft union in paraffin; Treatment 2 - covering the graft with conifer sawdust up to the top of the scion and dipping the scion and graft union in paraffin (paraffin temperature 60–70°C) and Treatment 3 - covering the graft with both conifer sawdust, up to the top of the scion, and polyethylene foil, and dipping the scion and graft union in paraffin.

Air temperature and relative air humidity inside the heated room were measured by a Wilh Lambrecht KG Gottingen 252 thermo-hygrograph. Sawdust temperature was determined by an NTOS M- 1718-80 soil depth thermometer having a measurement range of -20°C to +50°C. The following parameters were determined during the experiment: percentage of grafting success on day 20th after grafting and percentage of grafting success on day 28th after grafting.

Grafts were planted in the nursery in May at an intra-row spacing of 25 cm and inter-row spacing of 100 cm, following a randomised block design (4 cultivars × 2 treatments × 4 replications), 30 grafts per cultivar per treatment, giving a total of 1440

planted grafts. The grafted plants were grown in the nursery over two years, using standard cultivation practices. The experiment involved the use of the following treatments: Treatment 1 - without foliar fertilization (control); Treatment 2 - Nitrophoska foliar (N-20%; P-20%, K-20%, Mg-0.5%; S-0.8% and micro-nutrients (B-0.013%; Cu-0.04%; Fe-0.1%; Mn-0.1%; Mo-0.003%; Zn-0.038%); Treatment 3 - foliar fertilization with Nitrophoska solub (N-15%; P-5%, K-30%, Mg-1.3%; S-3.7% and micro-nutrients (B-0.011%; Cu-0.019%; Fe-0.05%; Mn-0.05%; Mo-0.001%; Zn-0.019%) and Treatment 4 - foliar fertilization with Humisol (humic acid - 15%; N-0.5%, K-2%, and other macro- and micro-nutrients (2%) (Ca, S, Mg, Zn, Fe, Cu, B, Mn). The foliar fertilizer was applied in three replications over the growing season, during May, July, and August. During both seasons, at a 20 day-interval upon shoot emergence, plant height and diameter were measured from the union upwards with a ruler and meter.

The following parameters were tested: percent survival of grafted plants at the end of the first growing season in the nursery; plant height at the end of the first and second seasons (as measured from the graft union up to the top of the grafted plant); trunk diameter at 3 cm above the graft union of the rootstock and the scion; and number of first-class nursery plants (plants with a height of more than 120 cm above the grafting point) at the end of the second season.

The data obtained were statistically analysed using Fisher's model of two-factorial analysis of variance – ANOVA with the probability level of 0.05.

RESULTS AND DISCUSSION

The grade of callus formation and percentage of grafting success were assessed on day 20 after grafting (Table 1). Cultivar-specific characteristics became evident in the experiment. Cultivar 'Šeinovo' had a significantly higher percentage of complete callus formation (63.1%) as compared to other selections, followed by 'Šampion' (52.5%), while no differences were observed between 'G-139' (38.7%) and 'Elit' selections (35.9%). After that grafts were subjected to further callusing for another 8 days. The percentage of complete callus formation after 28 days was higher, as compared to the grade of callus formation and grafting success on day 20 after grafting. At the end of the stratification period, the percentage of grafting success, was highest in cultivar 'Šeinovo' (83.5%), followed by 'Šampion' (74.6%) and 'G-139' (68.4%), while the lowest in 'Elit' (59.7%). The obtained results lead to the conclusion that medium early-season cultivars ('Šeinovo' and 'Šampion) show higher graft-take percentages than the medium late cultivar 'G-139' and the late cultivar 'Elit', which agrees with the results of Paunović et al. (2010) and Paunović et al. (2011), who achieved a percent graft-take of 65.3%–87.7% under controlled conditions depending on the cultivar, while Achim and Botu (2001) and Ozkan and Gumus (2001) reported that grafting success ranged from 63.0% to 70.0%.

In terms of treatments, grafting success was significantly lower both after 20 days and at the end of the stratification process in Treatment 1 (28.7 and 56.8%, respectively) than in other treatments. Treatment 1 did not involve the use of paraffin, whose role is to prevent desiccation of the graft union of graft components and excessive loss of water from the scion. Paraffin helps in making close contact between the cambial zones of the rootstock and the scion, thus fixing them and preventing further movement, particularly upon initiation of multiplication of parenchymal cells at the cross-sections of graft components. This enables more efficient adhesion between the rootstock and the scion, faster callus bridge formation at the graft union, and faster fusion between the graft

components. In the absence of paraffin in the treatment involving non-paraffined grafts, the rootstock and the scion developed an insufficiently tight union generally resulting in the separation of the graft components and higher fluctuations in sawdust temperature.

Table 1. Assessment of the grafting success on days 20 and 28 after grafting

Cultivar/ Treatment	Assessment of the degree of callus formation and grafting success on day 20 after grafting			Grafting success in walnut on day 28 after grafting	
	Complete callus formation (%)	Grafts subjected to further callusing (%)	Unsuccessful grafts (%)	Successful grafts (%)	Unsuccessful grafts (%)
‘Šeinovo’	63.1±0.99 a	36.4±1.10 c	0.50±0.11 d	83.5±0.63 a	16.50±0.13 c
‘Šampion’	52.5±1.05 b	45.3±1.01 b	2.01±0.21 c	74.6±0.91 b	25.4±0.21 b
‘G-139’	38.7±1.12 c	58.5±0.98 a	2.82±0.24 b	68.4±0.82 c	31.6±0.32 a
‘Elit’	35.9±1.18 c	59.8±1.10 a	4.29±0.29 a	59.7±1.03 d	40.3±0.39 a
Without paraffin	28.7±0.57 c	67.1±0.53 a	4.18±0.22 a	56.8±0.70 c	43.1±0.20 a
With paraffin	49.4±0.58 b	48.4±0.47 b	2.21±0.15 b	72.5±0.48 b	27.4±0.41 b
With both paraffin and foil	64.5±0.65 a	34.6±0.34 c	0.89±0.06 b	85.9±0.44 a	14.4±0.34 c
ANOVA					
Cultivar (A)	**	**	**	**	**
Treatment (B)	**	**	**	**	**
A × B	**	**	**	**	**

Means followed by different letters within the cultivar columns are significantly different at $P \leq 0.05$ according to LSD test and ANOVA (F-test) results

These results are comparable with the findings by Paunović et al. (2013b), who studied the effect of paraffin treatment on graft-take percentage in walnuts and obtained better results in paraffin-treated grafted plants as compared to untreated grafts. Also, Solar et al. (2001) employed grafting with and without the use of paraffin and recommended the use of paraffin in walnut grafting as a way to retain graft moisture for a longer period and, accordingly, enhance callus formation. In the present study, good results were achieved in the treatment by covering the graft with conifer sawdust up to the top of the scion and dipping the scion and graft union in paraffin (49.4% and 72.5%, respectively), while the best results were produced by the treatment involving dipping of the grafts into paraffin and their covering with both sawdust up to the scion and polyethylene foil (64.5% and 85.9%, respectively). Polyethylene foil in Treatment 3 prevented heat emission and provided longer maintenance of sawdust moisture, reduced temperature variations, and prevented desiccation of the substrate, thereby leading to increased sawdust temperature and positively affecting callus formation and overall grafting success. Paunović et al. (2011; 2012b) recommend covering the grafts with foil in whip and tongue walnut grafting due to the foil’s ability to provide more uniform and higher sawdust temperature, affecting better grafting success.

Walnut plants remain in the nursery for at least two years to satisfy the requirements concerning height, hardening, top bud development, resistance to low winter temperatures, and root system development, because after the stratification period are not sufficiently developed therefore they can not be transferred for conventional planting. Foliar fertilisation in the present experiment resulted in a higher survival rate, a higher

percentage of class 1 nursery trees, and higher vegetative growth and diameter of nursery plants at the end of the first growing seasons (Table 2). Differences in the number of surviving plants, the percentage of first-class plants, and vegetative growth among the foliar nutrition and the control treatments varied among the cultivars and selections. The results showed that medium-early season cultivar ‘Šeinovo gave a significantly higher number of surviving plants (85.1%) in the nursery, followed by cultivar ‘Šampion’ (77.3%), whereas the lowest values were recorded in medium-late season cultivar ‘G-139’ (70.8%) and late-season cultivar ‘Elit’ (69.5%) (Table 2).

Table 2. Survival and vegetative growth of walnut plants during the first season in the nursery

Cultivar/ Treatment	Survived plants at the end of the 1 st growing season (%)	Vegetative growth of nursery plants at the end of the 1 st growing season (cm)	Plant diameter at the end of the 1 st growing season (mm)
Šeinovo	85.1±0.88 a	19.1±0.80 a	8.02±0.70 a
Šampion	77.3±0.72 b	17.4±0.65 b	6.65±0.57 b
G-139	69.5±0.58 c	16.3±0.51 c	5.84±0.43 c
Elit	70.8±0.64 c	16.7±1.56 c	6.07±0.48 c
Control	65.9±0.44 d	15.3±0.25 d	5.89±0.36 d
Nitrophoska Foliar	73.4±0.47 c	16.9±0.36 c	6.24±0.40 c
Nitrophoska Solub	79.6±0.53 b	17.7±0.39 b	6.63±0.47 b
Humisol	83.8±0.62 a	19.4±0.41 a	7.85±0.55 a
ANOVA			
Cultivar (A)	**	**	**
Treatment (B)	**	**	**
A × B	**	**	**

Means followed by different letters within the cultivar columns are significantly different at $P \leq 0.05$ according to LSD test and ANOVA (F-test) results

The same results were obtained for vegetative growth and plant diameter. At the end of the first growing seasons, ‘Šeinovo’ had higher vegetative growth and plant diameter (19.1 cm and 8.02 mm, respectively) than ‘G-139’ (16.7 cm and 6.07 mm, respectively) and ‘Elit’ (16.3 cm and 5.84 mm, respectively). Cultivar ‘Šampion’ had 17.4 cm of vegetative growth and 6.65 mm of plant diameter. In the second season, ‘Šeinovo’ achieved the highest percentage of first-class plants (19.1%), followed by the ‘Šampion’ cultivar (17.8%), and the lowest by cultivars ‘G-139’ (16.7%) and ‘Elit’ (16.3%) (Table 3). Also, in terms of vegetative growth, ‘Šeinovo’ showed the highest vegetative growth (197.5 cm), in contrast to the lowest vegetative growth in cultivars ‘G-139’ (172.0 cm) and ‘Elit’ (173.4 cm), while vegetative growth in ‘Šampion’ was 185.2 cm. A similar effect was observed in plant diameter.

The present results are in agreement with the findings of Paunović et al. (2012b) and Paunović et al. (2013a), who obtained a survival percentage of 60.0 to 91.8% and plant height of 14.7 to 20.8 cm at the end of the first growing season, plant height of 170.6 to 225.9 cm at the end of the second growing season, and percentage of first-class plants of 52.2 to 80.4%, depending on the cultivar. Also, Stanisavljević and Mitrović (1997)

recorded a survival rate of 86.5% and growth of 17.8 cm and 170 cm in the first and second growing seasons, respectively, under Čačak (Western Serbia) conditions. In a study by Solar et al. (2001) in Slovenia and Erdogan (2006) in Turkey, the survival rate of nursery plants at the end of the second growing season was 64% and 82%, while Achim and Botu (2001) in Romania found slow growth of 30–40 cm in nursery plants at the end of the first year of cultivation and 168–172 cm at the end of the second year. In general, Korać et al. (1997) reported the typical height of nursery plants of 10–25 cm at the end of the first growing season and about 150 cm at the end of the second growing season.

Table 3. Survival and vegetative growth of walnut plants during the first and the second seasons of growth in the nursery

Cultivar/ Treatment	Class 1 plants at the end of the 2nd growing season (%)	Vegetative growth of nursery plants at the end of the 2nd growing season (cm)	Plant diameter at the end of the 2nd growing season (mm)
Šeinovo	19.1±0.80 a	197.5±7.64 a	22.9±0.46 a
Šampion	17.8±0.65 b	185.2±6.73 b	21.4±0.35 b
G-139	16.7±0.51 c	172.0±5.92 c	20.7±0.27 c
Elit	16.3±1.56 c	173.4±5.58 c	20.6±0.24 c
Control	15.3±0.25 d	167.6±2.35 d	18.5±0.10 d
Nitrophoska foliar	16.9±0.36 c	176.8±3.41 c	20.9±0.13 b
Nitrophoska solub	18.1±0.39 b	189.4±4.50 b	22.4±0.17 b
Humisol	19.4±0.41 a	194.5±5.09 a	23.7±0.21 a
ANOVA Cultivar (A)	**	**	**
Treatment (B)	**	**	**
A × B	**	**	**

Means followed by different letters within the cultivar columns are significantly different at $P \leq 0.05$ according to LSD test and ANOVA (F-test) results

In terms of foliar fertilisations, the percent survival of nursery plants was the lowest in control plants (65.9%), while the highest survival of plants (83.8%) was found in foliar Humisol application by 18.3%. The application of Nitrophoska solub also showed good results, while the use of foliar fertilizer Nitrophoska foliar indicated slightly worse results compared to the Humisol and Nitrophoska solub. In terms of Class 1 plants at the end of the 2nd growing season, the treatment without foliar fertilisation yielded the lowest number of Class 1 nursery plants as compared to the treatment involving foliar fertilisations. Nitrophoska foliar application led to 6.8% higher number of Class 1 plants than in the control treatment, whereas the Nitrophoska solub resulted in a 13.6% increase in Class 1 plants. The number of Class 1 nursery plants at the end of the 2nd growing season was the highest in the treatment involving Humisol by 18.2%. In all cultivars, in both growing cycles, the lowest height and diameter growth was observed in control plants. Walnut plants treated with Nitrophoska foliar application exhibited significantly higher growth compared to control plants by an average of 9.47% and 5.61% in terms of height and diameter, respectively, at the end of the first growing season. Also, foliar Nitrophoska solub spraying gave a significant increase of 13.5% and 11.55%, respectively, in plant height, with a difference in plant diameter by 11.2% and 17.4%, respectively, than

to control plants. Plants supplied with Humisol attained the highest height (21.1% and 13.8%, respectively) and diameter growth (24.9% and 21.9%, respectively), compared to control groups.

The spray application of the complex organic fertiliser containing humic substances in studies by Paunović (2010) and Paunović et al. (2013a) led to an average plant growth of 16.9 cm and 207.8 cm at the end of the first and second growing seasons, respectively, as compared to the treatment without foliar application, which is in agreement with the results of the present experiment. Also, Solar (2003) emphasizes that foliar nutrition in the nursery promotes earlier termination of the growth in autumn, good lignification, development of the root system, a higher number of first-class nursery plants, and better vegetative growth. Namely, Solar and Stampar (2004) recommend additions of P, K, Ca, and Mg which have a positive effect on general plant growth, and those of micronutrients B, Mn, and Mo which have a direct impact on plant lignification. Fathi et al. (2010) reported that apricot trees treated during the growing season with a solution of organic fertilizer (2.9% humic acid) improved the growth and fruit yield of the treated trees. Therefore, Salifu et al. (2006) investigated the growth of grafted black walnuts as affected by N, P, K, and micronutrient applications and underline that foliar fertilization can be applied in nurseries for the purpose of enhancing the nutritive quality of grafted plants and storage of nutrients in the root, as advisable for walnut planting establishment, while Zhang and Brown (1999) investigated the properties and mechanisms of foliar application in walnuts and revealed that foliar fertilisation had positively affected isotope stability in the leaf of walnut.

CONCLUSION

The results obtained suggest the necessity to treat grafts with paraffin and cover them with polyethylene foil during walnut grafting under controlled conditions. The use of paraffin played an important role in this treatment, considering that paraffin enables better adhesion of the rootstock and the scion, prevents water loss from both the scion and the graft union, and enhances callus bridge formation, which positively affects the grafting success. On the other hand, polyethylene foil prevents heat emission and provides longer maintenance of sawdust moisture, thereby leading to increased sawdust temperature and positively affecting callus formation and overall grafting success.

The results indicated that cultivar characteristics varied remarkably depending on the genetic background, considering that medium early-season cultivars show better results in tested parameters under controlled conditions and in the nursery than medium late and late cultivars.

Plants treated with foliar fertilizers had better hardening, and foliage, top buds and the root system were well developed., while their resistance to adverse weather conditions was higher than in the control plants. Humisol application as foliar fertilizers was especially effective at stimulating plant survival and vegetative growth, considering humic acids show a positive effect on plant nutrition and growth. In general, the production of good quality healthy walnut plants under nursery conditions should necessarily involve the use of foliar treatment as an essential part of plant care, particularly during unfavourable weather, towards reaching an increased survival rate and improved vegetative growth of nursery plants.

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