EFFECT OF CALLUSING CONDITIONS ON GRAFTING SUCCESS IN WALNUT (*Juglans regia* L.)

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(Received May 31, 2011/Accepted November 22, 2011)

ABSTRACT

This experimental study was conducted at the Fruit Research Institute, Cacak, Serbia, to determine bud break and callus formation dates, the degree of callus formation, grafting success on day 20 after grafting and overall grafting success on day 28 after grafting in walnut (*Juglans regia* L.) under different treatments and bench grafting conditions in cultivar Seinovo (control) and the selections 'Ovcar', 'G-286', 'Elit' and 'G-139'. Three treatments were applied: Treatment 1 – covering the graft with sawdust up to the top of the scion without dipping the scion and graft union in paraffin; Treatment 2 – covering the graft with sawdust up to the top of the scion and dipping the scion and graft union in paraffin; and Treatment 3 – covering the graft with both sawdust, up to the top of the scion, and polyethylene foil, and dipping the scion and graft union in paraffin. The best results of the parameters tested were obtained in the treatment involving dipping the graft in paraffin and covering it with both sawdust, up to the scion, and polyethylene foil. The treatment without the use of paraffin, involving only the covering with conifer sawdust up to the top of the scion yielded the poorest results.

Key words: walnut, cultivar and selection, bud, callus, paraffin, scion

INTRODUCTION

Serbia has favourable environmental conditions for intensive walnut production, but current production does not satisfy market demand for this fruit species, the reason for this being the generative propagation of walnut from natural populations over the last several decades, resulting in the presence of populations with a pronounced biotype-specific polymorphism. One of the methods used to improve walnut production in Serbia is grafting. The best and most effective results under continental climate conditions are produced by bench grafting using the whip and tongue grafting technique. Bugarcic and Mitrovic (1985) stress the complexity of walnut grafting technology and report an unsatisfactory percentage of grafting success of 60 to 80%. Tsurkan (1990) describes walnut production as a series of organisational and technical operations including scion and rootstock production and storage until used for grafting, as well as preliminary preparation of graft components for grafting. Grafting success in walnut is affected by a multitude of factors such as the choice of cultivars and selections, rootstock quality, time of scion wood collection from parent trees, time and method of grafting, air temperature and relative air humidity inside the graft storage room (Karadeniz and Kazankaya, 1997; Barut, 2001; Tshering et al., 2006; Gandev, 2007; Avanzato, 2009).

The objective of this study was to determine the effect of different treatments on bud break date, callus formation date, the degree of callus formation, grafting success on day 20 after grafting and overall grafting success on day 28 after grafting.

MATERIAL AND METHODS

The experimental study was conducted at the Fruit Research Institute, Cacak, Serbia in 2003 and 2004. One-year-old seedlings of domestic walnut (*Juglans regia* L.) were grafted with scions of cv. Seinovo (control) and the selections 'Ovcar', 'Elit', 'G-286' and 'G-139'. The scion wood of both the cultivar and selections was collected during winter dormancy from parent trees at the Institute. The grafting operation was performed on 4 April 2003 and 2004 using whip-and-tongue grafting at the crown of the rootstock. A randomised block design (5 cultivars x 3 treatments x 4 replications) was employed, giving a total of 1.800 grafted rootstocks.

The grafts were stored indoors for 28 days under controlled conditions at a temperature of 27-29 ^{o}C and relative air humidity of 60-70%. On day 20 after grafting, shoots were removed from the grafted rootstocks. Fresh conifer sawdust was used as the substrate.

The experiment included three treatments:

- 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);
- 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 graft storage room were measured with a Wilh Lambrecht KG Gottingen 252 thermohygrograph, and sawdust temperature with an NTOS M- 1718-80 soil depth thermometer with a measuring range of -20 °C to +50 °C.

The following parameters were determined during the experiment:

- date of bud break (across the treatments and cultivars);
- date of callus formation (across the treatments and cultivars);
- degree of callus formation at the graft union, evaluated visually on a scale of 2 to 5, where 5 (excellent) – complete callus formation at the graft union, 4 (very good) – ¼ of the graft union did not develop callus, 3 (good) – ½ of the graft union did not develop callus, and 2 – no callus formation at the graft union;
- percentage of grafting success on day 20th after grafting;
- percentage of grafting success on day 28th after grafting.

The data obtained were statistically analysed using Fisher's model of two-factorial analysis of variance – ANOVA (Fisher, 1953). The significance of the differences between the means for the control cultivar and those for the other selections at $p \leq 0.01$ and $p \leq 0.05$ was defined using Dunnett's one- and two-sided comparison test (Dunnett, 1955). The LSD test was performed at $p \leq 0.05$ to test the significance of the differences between the treatments as well as that of the interaction means. The results are presented in both graphical and tabular forms.

RESULTS

The air temperature inside the graft storage room in 2003 and 2004 ranged from 27 °C to 29 °C and relative air humidity from 60 to 70%. Sawdust temperature was 24.5-26.5 °C in Treatment 1, 25.0-27.0 °C in Treatment 2 and 26.0-28.0 °C in Treatment 3 (Tab. 1).

Bud break (Fig. 1) in 2003 and 2004 occurred six days after grafting in Treatment 3, eight days after grafting in Treatment 2 and nine days after grafting in Treatment 1. In terms of the test cultivar and selections, bud break first occurred in 'Seinovo', a day later in 'Ovcar' and 'G-286', four days later in 'G-139', and seven days later in 'Elit', in all the treatments and both years.

Callus formation (Fig. 2) started seven days after grafting in Treatment 3, nine days after grafting in Treatment 2 and ten days after grafting in Treatment 1. Callus development was observed to be simultaneous in 'Seinovo', 'Ovcar' and 'G-286', but was initiated two days later in G-139 and three days later in 'Elit', in all the treatments and both years.

Table 1. Relative air humidity [%], air temperature inside the graft storage room and sawdust temperature [°C] in 2003 and 2004

Relative air humidity	Temperature inside the graft storage room	Sawdust temperature Treatment 1	Sawdust temperature Treatment 2	Sawdust temperature Treatment 3
60.0-70.0	27.0-29.0	24.5-26.5	25.5-27.0	26.0-28.0

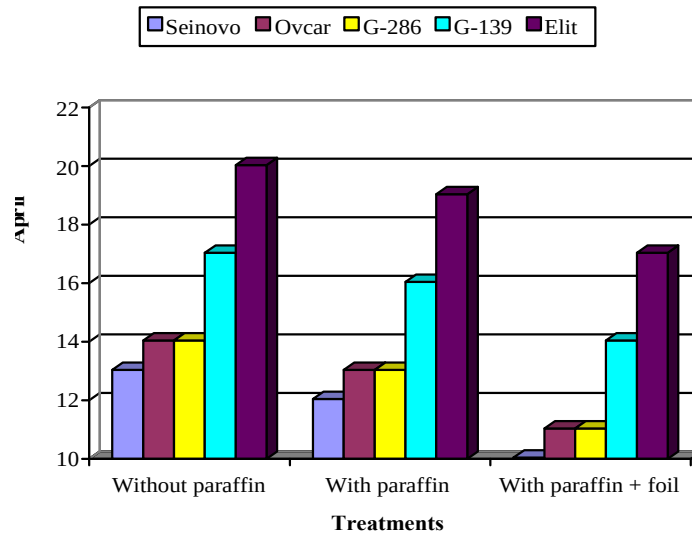

Figure 1. Date of bud break in 2003 and 2004

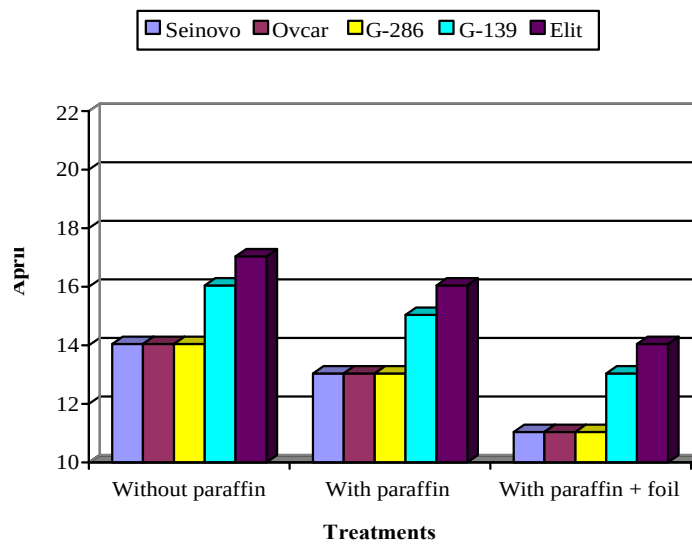

Figure 2. Date of callus formation in 2003 and 2004

The degree of callus formation and percentage of grafting success were assessed on day 20 after grafting.

Dunnett's test ($p \leq 0.01$ and $p \leq 0.05$) showed that 'Seinovo' had a significantly higher percentage of complete callus formation (55.3%) as compared to the selections 'Ovcar' (42.3%), 'Elit' (35.4%) and 'G-139' (36.3%). No significant differences were observed between the control cultivar and the 'G-286' selection (54.0%).

The LSD test ($p \leq 0.05$) revealed a significantly higher percentage of complete callus formation (61.2%) in Treatment 3 than in Treatment 1 (25.7%) and Treatment 2 (47.3%). A significant difference was also observed between Treatments 1 and 2 (Tab. 2).

In terms of the degree of callus formation, Dunnett's test ($p \leq 0.01$ and $p \leq 0.05$) showed that it was significantly higher in 'Seinovo' (4.11) than in Ovcar (3.79), 'Elit' (3.69) and 'G-139' (3.78), with no significant differences found between the control and 'G-286' (3.94).

The LSD testing of the differences between the treatments ($p \leq 0.05$) indicated that Treatment 1 resulted in a significantly lower degree of callus formation (3.68) as compared to Treatment 2 (3.88) and Treatment 3 (4.03). The differences between Treatments 2 and 3 were not significant (Tab. 2).

On day 28 after grafting, the overall percentage of grafting success was evaluated. Dunnett's test ($p \leq 0.01$ and $p \leq 0.05$) showed a significantly higher percentage of grafting success in 'Seinovo' (86.7%) as compared to 'Ovcar' (75.0%), 'Elit' (68.0%), 'G-139' (72.3%) and 'G-286' (81.3%).

The LSD test ($p \leq 0.05$) revealed that grafting success was significantly lower in Treatment 1 (65.3%) than in Treatments 2 and 3 (78.7% and 86.0%, respectively). A significant difference was found between Treatments 2 and 3 (Tab. 3).

DISCUSSION

The results obtained suggest that the differences between the treatments in terms of the parameters tested were induced by both air temperature inside the graft storage room and sawdust temperature. The 10% fluctuation in relative air humidity (60-70%) had no significant effect on the results obtained due to the fact that the sawdust did not undergo desiccation in deeper layers, but only in the top 2-3 cm layer.

Mitrovic and Blagojevic (2002) evaluated the effect of different treatments on callus formation and grafting success and reported the best performance in the treatment involving the use of paraffin and plastic foil, followed by the treatments with paraffin and without the use of paraffin. Those results are in agreement with the results of the present study.

In this experiment, the lowest sawdust temperature was in Treatment 1 (24.5-26.5 ^{o}C), being 1.5 ^{o}C and 0.5-1 ^{o}C lower as compared to that in Treatment 3 and Treatment 2, respectively. The lower sawdust temperature led to a later onset of

Table 2. Assessment of the degree of callus formation and grafting success on day 20 after grafting

Cultivar and treatment		Percentage of complete callus formation	Percentage of grafts subjected to further callusing	Percentage of un-successful grafts	Degree of callus formation
Cultivar (A)	Ovcar	42.3±1.07**	55.0±1.03**	2.63±0.22ns	3.79±0.10**
	G-139	36.3±1.12**	61.0±1.10**	2.50±0.29ns	3.78±0.11**
	Elit	35.4±1.18**	62.0±0.99**	2.59±0.24ns	3.69±0.10**
	G-286	54.0±0.99ns	44.7±0.97ns	1.28±0.16ns	3.94±0.09ns
	Seinovo	55.3±1.05	44.0±1.02	0.60±0.10	4.11±0.07
Treatment (B)	without paraffin	25.7±0.57 c	70.7±0.53 a	3.57±0.22 a	3.68±0.07 c
	with paraffin	47.3±0.58 b	51.2±0.57 b	1.51±0.15 b	3.88±0.07 ab
	with both paraffin and foil	61.2±0.65 a	38.0±0.64 c	0.69±0.06 b	4.03±0.07 a
ANOVA					
Cultivar (A)		**	**	ns	**
Treatment (B)		**	**	**	**
A x B		ns	ns	ns	ns

A and B represent cultivars and treatments, respectively
Asterisks in vertical columns represent significant differences between the means at $p \leq 0.05$ and $p \leq 0.01$ according to Dunnett's test and ANOVA (F-test); ns – non-significant
The values within treatment and interaction mean columns designated with the same small letters do not differ significantly at $p \leq 0.05$ according to LSD-test

Table 3. Grafting success in walnut on day 28 after grafting

Cultivar and treatment		Percentage of successful grafts	Percentage of unsuccessful grafts
Cultivar (A)	Ovcar	75.0±0.91**	25.0±0.91**
	G-139	72.3±0.67**	27.7±0.67**
	Elit	68.0±1.08**	32.0±1.08**
	G-286	81.3±0.57**	18.7±0.57**
	Seinovo	86.7±0.63	13.3±0.63
Treatment (B)	without paraffin	65.3±0.70 c	34.7±0.70 a
	with paraffin	78.7±0.48 b	21.3±0.48 b
	with both paraffin and foil	86.0±0.44 a	14.0±0.44 c
ANOVA			
Cultivar (A)		**	**
Treatment (B)		**	**
A x B		**	**

A and B represent cultivar and treatments, respectively
Asterisks in vertical columns represent significant differences between the means at $p \leq 0.05$ and $p \leq 0.01$ according to Dunnett's test and ANOVA (F-test); ns – non-significant
The values within treatment and interaction mean columns designated with the same small letters do not differ significantly at $p \leq 0.05$ according to LSD-test

bud break (nine days after grafting) and a later date of callus formation (ten days after grafting). Treatment 1 did not involve the use of paraffin. In bench grafting, using the whip and tongue method, the role of paraffin is to prevent desiccation of the graft union (graft components) and excessive loss of water from the scion, which occurs due to inadequate water supply to the scion resulting from the absence of a common vessel system with the rootstock. In addition, paraffin helps in making a close contact between the cambial zones of the rootstock and scion, thus fixing them and preventing any movement, particularly upon the initiation of multiplication of parenhymal cells at the cross-sections of the graft components. This enables successful fusion between the rootstock and scion. Without paraffin in Treatment 1, the union between the rootstock and scion was insufficiently firm, which led to frequent separation of the graft components. This had a direct effect on callus formation (3.68), grafting success on day 20 after grafting (25.7%) and the overall grafting success on day 28 after grafting (65.3%). Paraffin was used for grafts in Treatment 2, providing more effective adhesion between the rootstock and scion, faster callus bridge formation at the graft union and faster fusion between the graft components. The higher sawdust temperature (25.5-27.0 °C) as compared to Treatment 1 and the use of paraf-

fin are the main reasons for an earlier bud break (eight days after grafting), earlier date of callus formation (nine days after grafting), higher degree of callus formation (3.88), higher grafting success on day 20 (47.3%) and higher overall grafting success on day 28 after grafting (78.7%). Solar et al. (2001) carried out omega bench grafting in 'Elit' with and without the use of paraffin. Grafting success was 83% and 77% in the treatments with and without the use of paraffin, respectively. The authors recommend the use of paraffin in walnut grafting as a method of retaining graft moisture for a longer period of time and, accordingly, enhancing callus formation. The highest sawdust temperature (26.0-27.0 ^{o}C) was determined in Treatment 3. Apart from paraffin, the use of polyethylene foil was another important factor that contributed to the increase in sawdust temperature of 1.5 ^{o}C and 0.5-1 ^{o}C as compared to sawdust temperature in Treatments 1 and 2, respectively. The foil prevented heat loss and helped to retain moisture in the sawdust, thus contributing to obtaining the earliest dates of bud break (six days after grafting) and callus development (seven days after grafting), the highest degree of callus formation (4.03%), the highest percentage of grafting success on day 20 (61.2%) and the highest overall grafting success on day 28 after grafting (86.0%). Bulatovic (1985) recommends covering the grafts with foil in whip-and-tongue grafting of walnut because of the foil's ability to provide a more uniform and higher sawdust temperature. The grafting success obtained ranged from 65 to 90%.

In this experiment, the cultivar and the selections tested exhibited their cultivar-specific characteristics. An earlier bud break and earlier callus formation were observed in the medium-early 'Seinovo' and the selections 'Ovcar' and 'G-286', and later in the medium-late 'G-139' and the late 'Elit'. As for the percentage of grafting success, it was the highest in 'Seinovo' (86.7%), followed by 'G-286' (81.3%) and 'Ovcar' (75.0%), and the lowest in G-139 (72.3%) and 'Elit' (68.0%), which was likely a result of their later onset of the growing season. Stanisavljevic and Mitrovic (1997) performed whip-and-tongue grafting at a temperature of 26-28 ^{o}C and obtained the best results in 'Seinovo', followed by 'G-286' and 'Ovcar', and the poorest performance in 'G-139' and 'Elit'. Similar results were reported by Mitrovic and Blagojevic (2002) and Mitrovic et al. (2008), who obtained the lowest percentage of well-callused grafts and the lowest grafting success in 'G-139' and 'Elit' in all their studies as compared to 'Seinovo' and the selections 'G-286' and 'Ovcar', which is in agreement with the results presented here.

The results obtained in this study suggest a dependence of bud break and callus formation dates, the degree of callus formation and grafting success on the air temperature inside the graft storage room and the sawdust temperature. The best results were produced by the treatment in-

volving dipping the grafts in paraffin and covering them with sawdust up to the scion and polyethylene foil because of the highest sawdust temperature. The use of paraffin and polyethylene foil played an important role in this treatment. The paraffin enabled better adhesion between the rootstock and scion, prevented water loss from the scion and, consequently, desiccation of the graft union, and enhanced the formation of the callus bridge, thus contributing to successful fusion of the graft components. The polyethylene foil prevented heat loss and provided longer retention of moisture in the sawdust, thereby leading to an increased sawdust temperature and positively affecting callus formation and the overall grafting success.

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WPŁYW WARUNKÓW KALUSOWANIA NA SKUTECZNOŚĆ SZCZEPIENIA ODMIAN ORZECHA WŁOSKIEGO (*Juglans regia* L.)

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S T R E S Z C Z E N I E

Celem doświadczenia przeprowadzonego w Instytucie Sadownictwa w Cacak w Serbii było określenia terminu pękania pąków i powstawania kalusa, stopnia wytworzenia kalusa, skuteczności szczepienia 20 dni po szczepieniu i całkowitej skuteczności szczepienia 28 dni po szczepieniu w zależności od warunków traktowania i szczepienia w ręku odmian orzecha włoskiego (*Juglans regia* L.) 'Seinovo' (kontrola) oraz 'Ovcar', 'G-286', 'Elit' i 'G-139'. Zastosowano trzy traktowania: Traktowanie 1 – przykrycie szczepu trocinami aż do wierzchołka zrazu; Traktowanie 2 – przykrycie szczepu trocinami aż do wierzchołka zrazu i zanurzenie zrazu i miejsca zespolenia w parafinie i Traktowanie 3 – przykrycie szczepu trocinami aż do wierzchołka zrazu i folią polietylenową, i zanurzenie zrazu i miejsca zespolenia w parafinie. Najlepsze wyniki badanych parametrów uzyskano w traktowaniu obejmującym zanurzenie szczepu w parafinie i przykrycie go trocinami i folią polietylenową. Najgorsze wyniki dało traktowanie bez użycia parafiny, polegające tylko na przykryciu szczepu trocinami drzew iglastych aż do wierzchołka zrazu.

Słowa kluczowe: orzech włoski, odmiana, pączek, kalus, parafina, zraz