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EFFECT OF PARAFFIN TREATMENT ON WALNUT GRAFTS UNDER BENCH GRAFTING

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Abstract

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The effect of paraffin on the onset of bud break, onset of callus formation and graft-take percentage in walnut under bench grafting using the whip-and-tongue method was evaluated in this study. The experiment involved the following cultivars: Šeinovo, Ovčar, G-286, G-139 and Elit. Two treatments were employed: Treatment 1 – grafting involving immersion of the rootstock-scion union into paraffin, and Treatment 2 – grafting without immersion of the rootstock-scion union into paraffin. Sawdust temperature in the treatment with paraffin was 0.5-1°C higher than that in the treatment without paraffin. This induced earlier bud break, earlier callus formation and higher graft-take percentage in walnut.

Key words: walnut, cultivar, grafting, paraffin

Introduction

Walnuts are nut crops of high importance. The increase in the standard of living in Serbia has led to an increasing demand for walnuts, given their incomparable high nutritional value unseen in any other fruit. Current walnut production in Serbia is insufficient to meet the country's needs, as it supplies less than 50% of national requirements (Mitrovic, 2002). In order to achieve improvement, expansion projects have been undertaken through the cultivation of grafted walnut plants. The best and most effective results under continental climate conditions have been attained by bench grafting using the whip-and-tongue grafting method. Grafting success is dependent upon a multitude of factors, including choice of cultivars and rootstocks (Mitrovic et al., 2008), time of scionwood collection from parent trees (Mitrovic, 1995), time and method of grafting (Tshering et al., 2006), air temperature and humidity inside the heated room (Barut, 2001; Gandev, 2007; Paunovic et al., 2010). Walnut grafting has been dealt with by a large number of authors, but only few of them have evaluated the effect of graft treatment with paraffin under bench grafting conditions. Given the above, the objective of this study was to assess the effect of paraffin on the onset of bud break, onset of callus formation and graft-take percentage under bench grafting using the whip and tongue method.

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Materials and Methods

The experimental research was conducted at the Fruit Research Institute, Čačak, Serbia in 2004 and 2005.

One-year-old seedlings of domestic walnut (*Juglans regia* L.) 8-14 mm in thickness were grafted with scions of cvs. Šeinovo (control), Ovčar, Elit, G-286 and G-139. The scionwood of the test cultivars was collected during winter dormancy from 15-year-old parent trees of the Fruit Research Institute. Grafting was performed on 4 April 2004 and 7 April 2005 using whip-and-tongue grafting in the root crown zone. A randomized block design was used (5 cultivars x 2 treatments x 4 replications).

In order to stimulate callus formation, the grafted plants were stored for 28 days under controlled temperature and humidity conditions. The temperature inside the heated room was 27-28°C in 2004 and 28-29°C in 2005. Relative air humidity in 2004 and 2005 ranged from 60 to 70%. On day 20 after grafting, all shoots growing from the grafted rootstocks were removed. Fresh conifer sawdust was used as the substrate.

Air temperature and relative humidity inside the heated room were measured by a Wilh Lambrecht KG Gottingen 252 thermohygrograph, and sawdust temperature by an NTOS M- 1718-80 soil depth thermometer having a measurement range of -20°C to +50°C.

The experiment involved two treatments:

- Treatment 1 – grafting involving immersion of the rootstock-scion union into paraffin (paraffin temperature 60-70°C);
- Treatment 2 – grafting without immersion of the rootstock-scion union into paraffin.

The following parameters were evaluated during the experiment:

- onset of bud break;
- onset of callus formation;
- graft-take percentage on day 20 after grafting;
- graft-take percentage on day 28 after grafting.

The data obtained were statistically analyzed using Fisher's model of three-factorial analysis of variance – ANOVA. The significance of 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. The LSD test was performed at $P \leq 0.05$ to test the significance of differences between treatments, years as well as interaction means. The results are presented in both figure and tabular forms.

Results

The air temperature inside the heated room in 2004 was within the range of 27.0-28.0°C. Sawdust temperature in the treatment involving paraffined and non-paraffined grafts ranged from 24.0-26.0°C and 23.0-25.5°C, respectively. In 2005, the heated room temperature was higher – 28.0-29.0°C. The increasing temperature inside the heated room induced an increase in sawdust temperature, which was 24.5-26.5°C in the treatment without paraffin and 25.5-27.0°C in that with paraffin. Relative air humidity in 2004 and 2005 varied from 60 to 70% (Table 1).

Bud break during 2004 occurred seven days after grafting in paraffin-treated grafts and nine days after grafting in untreated grafts. In 2005, bud break commenced six days after grafting in paraffined grafts and seven days after grafting in non-paraffined grafts. The difference in the onset of bud break between paraffined and non-paraffined grafts was two days in 2004 and one day in 2005 (Figures 1 and 2).

The cultivars showed differences in the onset of bud break. Bud break first occurred in cv. Šeinovo, a day later in Ovčar and G-286, four days later in G-139 and seven days later in Elit.

In 2004, callus formation started nine days after grafting in paraffin-treated grafts and eleven days after grafting in untreated grafts. In 2005, the onset of callus formation was seven days after grafting in paraffined grafts and eight days after grafting in non-paraffined grafts. The difference in the onset of callus formation between paraffin-treated and untreated grafts was two days in 2004 and one day in 2005 (Figures 3 and 4).

In 2004, the onset of callus formation was simultaneous in cvs. Šeinovo, Ovčar and G-286, but occurred three days later in G-139 and four days later in Elit. In 2005, callus forma-

Table 1
Relative air humidity (%), air temperature inside the heated room and sawdust temperature (°C) in 2004 and 2005

Year	Relative air humidity	Temperature inside the heated room	Sawdust temperature	
			Non-paraffined grafts	Paraffined grafts
2004	60.0-70.0	27.0-28.0	23.0-25.5	24.0-26.0
2005	60.0-70.0	28.0-29.0	24.5-26.5	25.5-27.0

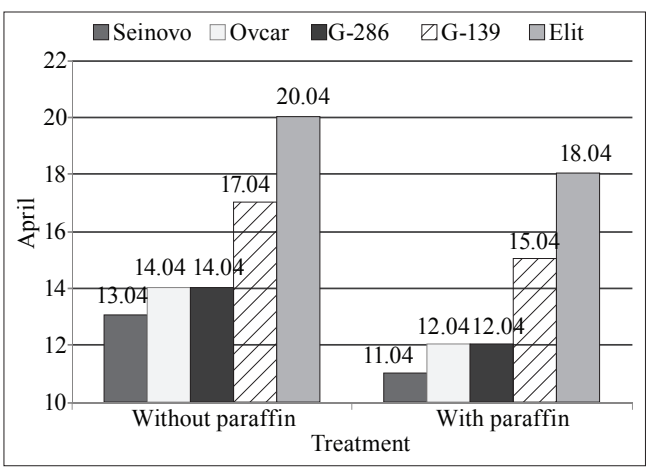

Fig. 1. Onset of bud break in 2004

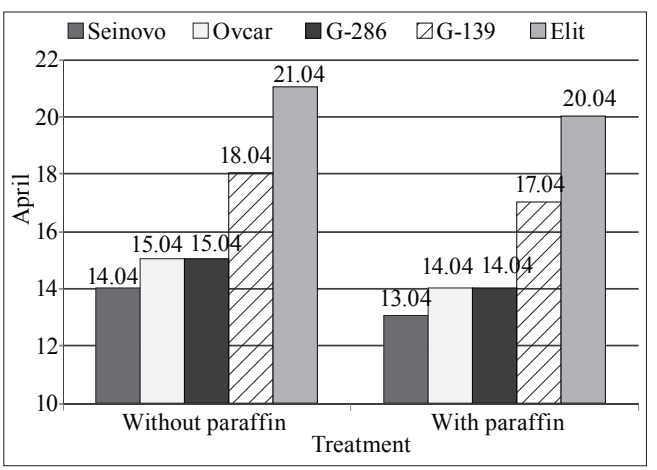

Fig. 2. Onset of bud break in 2005

tion was simultaneous in cvs. Šeinovo, Ovčar and G-286, but started two days later in G-139 and three days later in Elit.

The 10% fluctuation in relative air humidity during 2004 and 2005 (60-70%) had no significant effect on bud break and callus formation due to the fact that sawdust did not undergo desiccation in deeper layers but only in the top layer.

The first evaluation of graft-take percentage was performed twenty days after grafting

Table 2 presents graft-take percentage across cultivars, years and treatments on day 20 after grafting.

Table 3 gives results on the average graft-take percentage in grafts exhibiting callus formation, grafts subjected to fur-

Table 2
Graft-take percentage on day 20 after grafting

Cultivar	Year	Treatment	Percentage of callus formation	Percentage of grafts subjected to further callusing	Percentage of unsuccessful grafts
Ovčar	2004	with paraffin	62.5	35.6	1.90
	2004	without paraffin	45.8	50.0	4.17
	2005	with paraffin	70.0	27.5	2.50
	2005	without paraffin	58.3	37.9	3.83
Elit	2004	with paraffin	56.7	39.8	3.50
	2004	without paraffin	47.5	48.3	4.15
	2005	with paraffin	62.4	34.4	3.17
	2005	without paraffin	50.2	44.5	5.30
G -139	2004	with paraffin	57.5	39.0	3.50
	2004	without paraffin	48.3	47.5	4.20
	2005	with paraffin	67.5	29.9	2.60
	2005	without paraffin	53.3	43.3	3.35
G -286	2004	with paraffin	69.2	28.9	1.90
	2004	without paraffin	52.1	45.4	2.50
	2005	with paraffin	70.4	28.8	0.80
	2005	without paraffin	59.1	39.2	1.67
Šeinovo	2004	with paraffin	70.9	28.3	0.83
	2004	without paraffin	54.2	44.1	1.67
	2005	with paraffin	71.7	27.4	0.90
	2005	without paraffin	60.8	37.7	1.50

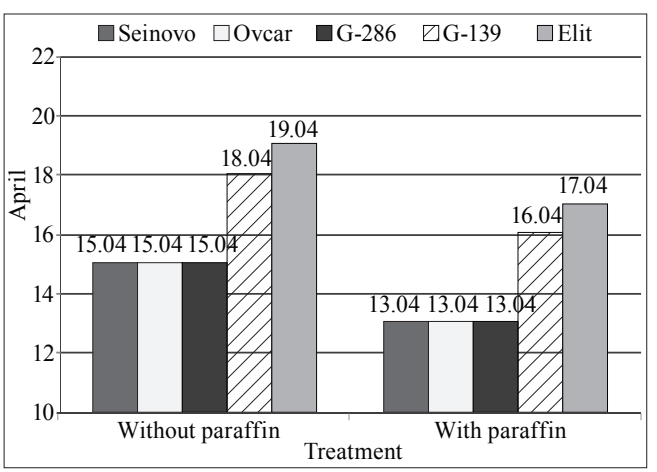

Fig. 3. Onset of callus formation in 2004

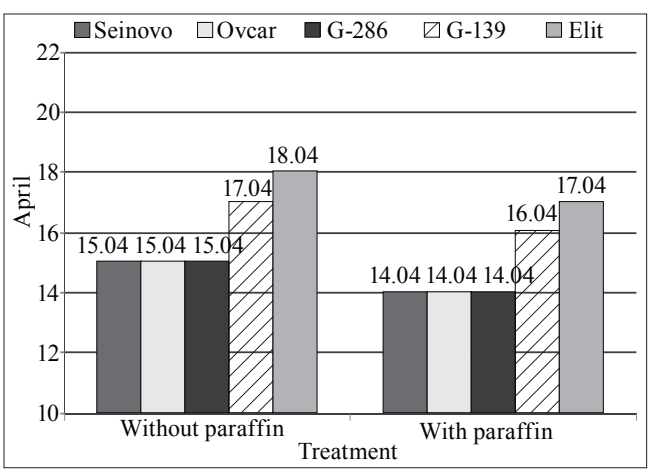

Fig. 4. Onest of callus formation in 2005

ther callusing and grafts showing no callus development on day 20 after grafting for all cultivars, treatments and years.

Dunnett's test (P≤0.01 and P≤0.05) showed that cv. Jbeinovo had a highly significantly higher percentage of callus formation as compared to cvs. Ovčar, Elit and G-139. No significant differences were observed between the control cultivar and G-286.

LSD-test (P≤0.05) revealed a highly significantly higher percentage of callus formation in the treatment involving paraffining as compared to that without the use of paraffin.

LSD-test (P≤0.05) showed that a highly significantly lower percentage of grafts exhibiting callus formation was recorded in 2004 than in 2005.

Graft-take percentage across cultivars, years and treatments on day 28 after grafting is outlined in Table 4.

Table 5 presents the average percentage of grafts showing successful and unsuccessful graft-take on day 28 after grafting for all cultivars, treatments and years.

Dunnett's test (P≤0.01 and P≤0.05) showed a highly significantly higher grafting success in cv. Šeinovo as compared to cvs. Ovčar, Elit, G-139 and G-286.

LSD-test (P≤0.05) revealed that grafting success was highly significantly higher in paraffin-treated grafts than in untreated grafts. The test also showed that a highly significantly lower number of grafts was obtained in 2004 as compared to 2005.

Discussion

The results obtained suggest that cultivars, years and treatments showed differences in the onset of bud break, onset of callus formation and graft-take percentage in walnut.

In 2004 and 2005, sawdust temperature was 0.5-1°C higher in the treatment involving paraffined grafts than in the treatment without paraffin. The higher sawdust temperature in the treatment involving paraffined grafts most likely resulted from the use of paraffin. The primary role of paraffin is to prevent desiccation of the union of the 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

Table 3
Average graft-take percentage in walnut on day 20 after grafting

		Grafts showing callus formation, %	Grafts subjected to further callusing, %	Grafts showing no callus formation, %
Cultivar (A)	Ovčar	59.1±1.07**	37.7±1.03**	3.10±0.22**
	Elit	54.2±1.18**	41.7±1.10**	4.03±0.29**
	G-139	56.6±1.12**	39.9±0.99**	3.41±0.24**
	G-286	62.7±0.99ns	35.6±0.97ns	1.70±0.16ns
	Šeinovo	64.4±1.05	34.4±1.02	1.22±0.10
Treatment (B)	Without paraffin	53.0±0.57 b	43.8±0.53 a	3.23±0.22 a
	With paraffin	65.9±0.58 a	32.0±0.57 b	2.16±0.15 b
Year (C)	2004	56.5±0.73 b	40.8±0.66 a	2.83±0.16 a
	2005	62.4±0.74 a	35.1±0.72 b	2.56±0.11 a
ANOVA				
Cultivar		**	**	**
Treatment		**	**	**
Year		**	**	ns
A x B		ns	ns	ns
A x C		ns	ns	ns
B x C		ns	ns	ns
A x B x C		ns	ns	ns

- A, B and C represent cultivars, treatments and years, respectively
- The asterisks in vertical columns represent significant differences between means at P≤0.05 and P≤0.01 according to Dunnett's test and ANOVA (F-test) results; ns- non-significant
- The values within treatment, year and interaction mean columns designated with the same small letters do not differ significantly at P≤0.05 according to LSD-test

Table 4
Graft-take percentage on day 28 after grafting across cultivars, years and treatments

Cultivar	Year	Treatment	Percentage of successful grafts	Percentage of unsuccessful grafts
Ovčar	2004	with paraffin	69.3	30.7
	2004	without paraffin	57.5	42.3
	2005	with paraffin	78.3	21.7
	2005	without paraffin	64.6	35.4
Elit	2004	with paraffin	65.1	34.9
	2004	without paraffin	51.7	48.3
	2005	with paraffin	70.4	29.6
	2005	without paraffin	61.2	38.8
G -139	2004	with paraffin	66.4	33.6
	2004	without paraffin	54.5	45.5
	2005	with paraffin	73.2	26.8
	2005	without paraffin	60.8	39.2
G -286	2004	with paraffin	80.0	20.0
	2004	without paraffin	69.2	30.8
	2005	with paraffin	82.5	17.5
	2005	without paraffin	75.8	24.2
Šeinovo	2004	with paraffin	85.5	14.5
	2004	without paraffin	72.5	27.5
	2005	with paraffin	95.8	4.20
	2005	without paraffin	83.6	16.4

Table 5
Average graft-take percentage in walnut on day 28 after grafting

		Percentage of successful grafts	Percentage of unsuccessful l grafts
Cultivar (A)	Ovčar	67.4±0.91**	32.5±0.91**
	Elit	62.1±0.67**	37.9±0.67**
	G-139	63.7±1.08**	36.3±1.08**
	G-286	76.9±0.57**	23.1±0.57**
	Šeinovo	84.3±0.63	15.6±0.63
Treatment (B)	Without paraffin	65.2±0.70 b	34.8±0.70 a
	With paraffin	76.6±0.48 a	23.4±0.48 b
Year (C)	2004	67.2±0.57 b	32.8±0.57 a
	2005	74.6±0.41 a	25.4±0.41 b
ANOVA			
Cultivar (A)		**	**
Treatment (B)		**	**
Year (C)		**	**
A x B		**	**
A x C		**	**
B x C		ns	ns
A x B x C		**	**

- A, B, C represent cultivars, treatments and years, respectively
- The asterisks in vertical columns represent significant differences between means at P≤0.05 and P≤0.01 according to Dunnett's test and ANOVA (F-test) results; ns- non-significant
- The values within treatment, year and interaction mean columns designated with the same small letters do not differ significantly at P≤0.05 according to LSD-test

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. The poorer results achieved in the treatment without paraffining (later bud break, later onset of callus formation and lower graft-take percentage) were in agreement with the literature data. Solar et al. (2001) employed omega bench grafting with and without the use of paraffin, resulting in the percent graft-take of 83% and 77%, respectively. These authors recommend the use of paraffin in walnut grafting as a way to retain graft moisture for a longer period of time and, accordingly, enhance callus formation. Mitrovic and Blagojevic (2002) studied the effect of paraffin treatment on callus formation and graft-take percentage in walnut, and obtained better results in paraffin-treated grafted plants as compared to untreated grafts, with graft-take percentage being highest in cv. Šeinovo, followed by Ovčar, G-139 and Elit. These results complied with the present findings.

The observed difference in graft-take between years, regardless of treatment, was induced by both air temperature inside the heated room and sawdust temperature. The temperature inside the heated room (28.0-29.0°C) in 2005 was higher than in 2004 (27.0-28.0°C). The higher temperature inside the heated room led to a 1-1.5°C increase in sawdust temperature in 2005 in both treatments as compared to 2004. This induced earlier bud break and callus formation, as well as a 5.90% and 7.40% increase in average graft-take percentage on day 20 and day 28 after grafting, respectively, in 2005 as compared to 2004.

Length of graft storage under controlled air temperature and humidity conditions was found to affect graft-take. In 2005, graft-take percentage after graft storage for 28 days under controlled conditions was 12.2% and 10.7% higher in the treatment without and with the use of paraffin, respectively, as compared to the graft-take percentage achieved after 20 days of storage.

Stanisavljevic and Mitrovic (1997) achieved the percent graft-take of 81.9% in walnut kept for three weeks under controlled conditions. The graft-take obtained by Achim and Botu (2001) was 63.0% after a period of 25-28 days, and that by Paunovic et al. (2010) ranged from 65.3%-86.0% in grafts callusing over a period of four weeks.

Cultivar-specific characteristics also became evident in the experiment. Medium early-season cultivars (Šeinovo, G-286 and Ovčar) showed earlier bud break, earlier callus formation and a higher graft-take percentage than the medium late cultivar G-139 and the late cultivar Elit. Similar findings were reported by Stanisavljevic and Mitrovic (1997), Mitrovic and Blagojevic (2002) and Mitrovic et al. (2008).

Generally, the results obtained in this study comply with those reported by other authors. Lantos (1990) observed that callus formation at 26-28°C started 6-8 days after grafting, lasting for two to three weeks until complete callus formation, with the average percent graft-take being 64%. In whip-and-tongue grafting, Korac et al. (1997) recommend that temperature inside the heated room should be 30-32°C during the first several days and maintained thereafter at 27-28°C at the graft union, due to the requirement of a much higher temperature for optimal callus formation in walnut than in other fruit species. At a temperature of 26-28°C, graft-take percentage was 80%, with the first signs of callus formation being observed on day 7 after grafting and extensive callusing on day 10 after grafting. Ninkovski (2005) reports the minimum temperature requirement of at least 12°C for callus formation. At this temperature, callus formation is a long slow process, but at 20°C callusing rate is faster. The optimal temperature range for callus formation is 25-28°C. Tsurkan (1990) shows that callus formation at 24-26°C starts 10-12 days after grafting and terminates within 16-18 days. Ozkan and Gumus (2001) employed whip-and-tongue grafting at 27°C and obtained a 66 to 70% graft-take percentage depending on the cultivar, with the onset of callus formation occurring 6-7 days after grafting. Tshering et al. (2006) achieved a 62.5% graft-take percentage. Tshering et al. (2006) cited the results obtained by Sitton (1931) who reported late callus initiation and a low amount of callus formed at a temperature below 20°C, callus damage at temperatures above 30°C and no callus formation at 40°C in grafted black walnut (*Juglans nigra* L.) plants.

Conclusions

Walnut grafting requires the immersion of grafts into paraffin due to the fact that paraffin enables better adhesion of the rootstock and the scion, prevents water loss from both the scion and the graft union, enhances callus bridge formation and contributes to the fusion of the graft components. The use of paraffin helps maintain a favorable thermal regime of the substrate, thereby positively affecting bud break, callus formation and graft-take percentage in walnut.

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