

## Graft-take Success in Walnut Under Controlled Conditions and Plant Development in the Nursery

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### Abstract

The production of walnut nursery plants is a complex process, being dependent upon a range of factors that affect graft-take success and behaviour of walnut plants in the nursery. The experiment included two parts. Three different treatments were used in the heated room under controlled air temperature and humidity conditions: Treatment 1-grafting without paraffin treatment of the scion and graft union, coupled with covering the graft with sawdust up to the top of the scion; Treatment 2-grafting involving paraffin treatment of the scion and graft union, coupled with covering the graft with sawdust up to the top of the scion; and Treatment 3-grafting involving paraffin treatment of the scion and graft union, coupled with covering the graft with both sawdust up to the top of the scion and polyethylene foil. The treatments were evaluated for their effect on graft-take success. Graft planting in the nursery was followed by two treatments: treatment without foliar fertilisation and treatment with foliar fertilisation. Under controlled conditions, treatment 3 induced a higher graft-take success as compared to the other two treatments, whereas foliar fertilisation in the nursery had a positive effect on the survival, production of class 1 plants and vegetative growth of walnut plants.

**Keywords:** graft, foliar fertilisation, nursery, walnut nursery plant

### Introduction

Walnut cultivation technology involves a number of organisational and cultural operations including scion and rootstock production, preparation of graft components for grafting, planting and handling of plants in the nursery (Tsurkan, 1990). Grafting success in walnut using the whip-and-tongue technique under controlled conditions is dependent upon choice of cultivars and rootstocks (Mitrović *et al.*, 2008), time of scionwood collection from parent trees (Paunović *et al.*, 2012), time and method of grafting (Solar *et al.*, 2001; Tshering *et al.*, 2006), and air temperature and relative humidity inside the callusing room (Avanzato, 2009; Barut, 2001), whereas nursery plant cultivation is contingent upon environmental conditions, and care and handling operations. Paunović (2010) reports that walnut plant production is a complex process, resulting in an unsatisfactory graft-take success rate as compared to other fruits (60-80%), and the percentage of class 1 nursery plants of as low as 50-60% of the total number of grafted rootstocks. The author also indicates that the use of foliar fertilisation in walnut nursery plant production has been negligible, regardless of its reportedly 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.

The objective of this study was to evaluate the effect of different treatments on the graft-take success under controlled conditions, and observe the effect of foliar fertilisation on the survival rate and vegetative growth of nursery plants.

### Materials and methods

The experiment was conducted at the Fruit Research Institute, Čačak, Serbia, over 2003-2005. One-year-old seedlings of domestic walnut (*Juglans regia* L.) 8-14 mm in thickness were grafted with scions of cvs. 'Seinovo' (control), 'Ovcar', 'Elit', 'G-286' and 'G-139'. Grafting was performed on 4 April 2003 and 2004 by whip-and-tongue grafting in the root crown zone on a sample of 30 grafts per replication, following a randomised block design (5 cultivars × 3 treatments × 4 replications), totalling 1800 grafted rootstocks. In order to stimulate callus formation, the grafts were kept in a heated room under controlled conditions at 26-28°C in 2003, and 28-29°C in 2004. Relative air humidity inside the heated room in 2003 and 2004 ranged from 60 to 70%. The callusing period lasted 28 days. Three treatments were employed in the heated room under controlled conditions: Treatment 1-grafting without paraffin treatment of the scion and graft union, coupled with covering the graft with sawdust up to the top of the scion; Treatment 2-grafting involving paraffin treat-

ment of the scion and graft union (paraffin temperature 60-70°C), coupled with covering the graft with sawdust up to the top of the scion; and Treatment 3-grafting involving paraffin treatment of the scion and graft union, coupled with covering the graft with both sawdust up to the top of the scion and polyethylene foil.

Grafts were planted in the nursery on 20 May 2003 and 25 May 2004. The experiment was laid out in a randomised block design (5 cultivars  $\times$  2 treatments  $\times$  4 replications), with 34 grafts per replication, giving a total of 1360 planted grafts. Two treatments were used in the nursery: Treatment 1-without foliar fertilisation (control); and Treatment 2-with foliar fertilisation. Foliar fertilisation of walnut nursery plants was performed using Humisol containing 15% humic acid, 0.5% N, 2% K<sub>2</sub>O and 2% macro- and micro-nutrients (Ca, S, Mg, Zn, Fe, Cu, B, Mn). During the first year after graft planting in the nursery, two irrigation treatments, each using 0.1% Humisol solution, were applied on 20 May and 10 July 2003 and 25 May and 9 July 2004, and one foliar treatment with 2.5% Humisol solution was conducted on 18 August 2003 and 18 August 2004. During the second growing season, three foliar applications of Humisol were used: on 12 May, 23 June and 22 July 2004, and on 18 May, 20 June and 20 July 2005. Upon shoot emergence during both growing seasons, plant growth was evaluated at twenty-day intervals.

The following parameters were observed during the experiment: 1-graft-take success on day 20 after grafting, 2-graft-take success on day 28 after grafting, 3-survival rate of nursery plants at the end of the first growing season, 4-survival rate of different classes of nursery plants at the end of the second growing season, 5-growth of nursery plants at the end of the first growing season, 6-growth of nursery plants at the end of the second growing season.

The data obtained were subjected to Fisher's model of a three-factor 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 and years as well as interaction means. The results are presented in tabular form.

## Results and discussion

The relative air humidity and air temperature inside the heated room and sawdust temperature in 2003 and 2004 are presented in Tab. 1.

Graft-take success was evaluated on day 20 after grafting. Dunnett's test showed that cv. 'Seinovo' provided a highly significantly higher number of grafts exhibiting callus formation as compared to cvs. 'Ovcar', 'Elit' and 'G-139'. No significant differences were observed between the control cultivar and 'G-286'. The average graft-take success on day 28 after grafting, as determined by Dunnett's test, showed that cv. 'Seinovo' had a highly significantly higher number of successful grafts as compared to the other cultivars. LSD-test revealed a significantly higher number of callused grafts on days 20 and 28 after grafting in the treatment involving the use of both paraffin and foil as compared to the other two treatments. A significant difference was observed between treatment 1 and treatment 2. In terms of years, a significantly lower number of callused grafts were observed in 2003 than in 2004 (Tab. 2).

Dunnett's test showed that at the end of the first growing season cv. 'Seinovo' gave a significantly higher number of survived nursery plants as compared to the other cultivars. At the end of the second growing season, Dunnett's test showed a significantly higher number of class 1 nursery plants in cv. 'Seinovo' than in the other test cultivars. A significant difference was observed in the number of class 2 nursery plants between the control cultivar, 'G-286' and 'Elit', whereas cvs. 'Seinovo', 'Ovcar' and 'G-139' displayed no difference. 'Seinovo' did not exhibit differences in the number of discarded nursery plants as compared to 'Ovcar', 'G-139' and 'Elit', but produced a significantly lower number of discarded nursery plants as compared to 'G-286'. LSD-test revealed that the treatment without foliar fertilisation (control) yielded a markedly lower number of survived nursery plants as compared to the treatment involving foliar fertilisation. A significantly higher number of survived nursery plants were obtained in 2004 than in 2003. At the end of the second growing season, LSD-test showed that foliar treatment gave a significantly higher number of class 1 nursery plants and a significantly lower number of class 2 and discarded nursery plants as compared to the unfertilised treatment (control). A significantly lower number of class 1 nursery plants were obtained in 2004, whereas no significant difference in the number of both class 2 and discarded nursery plants was observed in 2005 (Tab. 3).

At the end of the first growing season, Dunnett's test showed significantly higher vegetative growth in cv. 'Seinovo' as compared to cvs. 'Elit' and 'G-139', significantly lower growth as compared to 'G-286', and no difference between 'Seinovo' and 'Ovcar'. At the end of the second growing season, cv. 'Seinovo' exhibited significant-

Tab. 1. Relative air humidity (%), air temperature inside the heated room and sawdust temperature (°C) in 2003 and 2004

Year	Relative air humidity	Heated room temperature	Sawdust temperature Treatment 1	Sawdust temperature Treatment 2	Sawdust temperature Treatment 3
2003	60.0-70.0	26.0-28.0	22.5-25.5	23.5-26.0	24.5-27.0
2004	60.0-70.0	28.0-29.0	25.0-26.5	25.5-27.0	26.5-28.0

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Tab. 2. Graft-take success on days 20 and 28 after grafting

		Callused grafts on day 20 after grafting (%)	Grafts subjected to further callusing (%)	Discarded grafts (%)	Successful grafts on day28 after grafting (%)
Cultivar (A)	'Ovcar'	49.0±1.07**	48.3±1.03**	2.63±0.22ns	81.7±0.91**
	'Elit'	42.3±1.18**	55.3±1.10**	2.50±0.29ns	79.0±0.67**
	'G-139'	43.0±1.12**	54.3±0.99**	2.63±0.24ns	74.7±1.08**
	'G-286'	60.7± 0.99ns	38.0±0.97 ns	1.37±0.16ns	88.0±0.57**
	'Seinovo'	62.0±1.05	37.3±1.02	0.57±0.10	93.3±0.63
Treatment (B)	Without paraffin	32.3±0.57 c	64.0±0.53 a	3.57±0.22a	72.0 ±0.70 c
	With paraffin	54.0±0.58 b	44.3±0.57 b	1.57±0.15 b	85.3±0.48 b
	Paraffin and foil	67.8±0.65 a	31.4±0.64 c	0.67±0.06 b	92.7 ±0.44 a
Year (C)	2003	47.3±0.73 b	50.4±0.66 b	2.27±0.16a	76.7±0.57 b
	2004	55.5±0.74a	42.8±0.72a	1.60±0.11a	90.0±0.41a
A × B					
'Ovcar'	Without paraffin	28.7	66.3	5.00	72.0 f
	With paraffin	53.0	45.3	1.67	83.0 de
	Paraffin and foil	65.3	33.2	1.23	90.0 bc
'Elit'	Without paraffin	20.4	75.0	4.57	65.7 g
	With paraffin	43.7	53.5	2.90	82.0 de
	Paraffin and foil	62.7	37.3	0.00	89.3 bc
'G-139'	Without paraffin	25.0	70.0	5.00	58.3 h
	With paraffin	49.0	49.2	1.67	78.7 e
	Paraffin and foil	55.0	43.7	1.23	86.7 cd
'G-286'	Without paraffin	43.3	54.7	2.07	79.3 e
	With paraffin	61.3	37.3	1.23	87.7 cd
	Paraffin and foil	77.5	21.7	0.83	97.0 a
'Seinovo'	Without paraffin	44.0	54.7	1.27	84.0 cde
	With paraffin	63.0	36.3	0.40	95.7 ab
	Paraffin and foil	78.7	21.2	0.00	100.0 a
A × C					
'Ovcar'	2003	44.7	52.6	2.50 ab	70.3
	2004	53.3	44.0	2.76 ab	93.0
'Elit'	2003	38.7	56.7	4.67 a	76.7
	2004	45.7	54.0	0.30 b	81.6
'G-139'	2003	37.3	59.7	2.77 ab	64.7
	2004	48.7	48.7	2.50 ab	84.7
'G-286'	2003	57.3	41.7	0.83 b	84.3
	2004	64.0	34.0	1.90 ab	91.6
'Seinovo'	2003	58.0	41.3	0.57 b	87.3
	2004	66.0	33.3	0.57 b	99.3
B × C					
Without paraffin	2003	28.3	67.0 a	4.83	63.7
	2004	36.3	61.0 a	2.30	80.0
With paraffin	2003	50.0	48.3 b	1.67	79.6
	2004	58.0	40.3 c	1.50	91.3
Paraffin and foil	2003	63.6	36.0 c	0.33	86.7
	2004	72.3	26.7 d	1.00	98.7
ANOVA					
Cultivar (A)		**	**	ns	**
Treatment (B)		**	**	**	**
Year (C)		**	**	ns	**
A × B		ns	ns	ns	*
A × C		ns	ns	*	**
B × C		ns	**	ns	ns
A × B × C			** ns ns ns		

A, B, C represent cultivars, treatments and years, respectively. The asterisks in vertical columns represent significant differences between means at  $p \leq 0.05$  and  $p \leq 0.01$  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  $p \leq 0.05$  according to LSD-test

Tab. 3. Survival rate of walnut nursery plants at the end of the first growing season, and nursery plant classes at the end of the second growing season

		Survived plants at the end of the 1st growing season (%)	Class 1 plants at the end of the 2nd growing season (%)	Class 2 plants at the end of the 2nd growing season (%)	Discarded plants at the end of the 2nd growing season (%)
Cultivar (A)	'Ovcar'	77.1±0.92 **	63.5±0.99**	6.44±0.21ns	6.97±0.20 ns
	'Elit'	77.1±0.68 **	60.3±0.81**	9.91±0.26 *	6.97±0.26 ns
	'G-139'	74.1±0.92 **	60.0±1.02**	7.35±0.25ns	6.79±0.20 ns
	'G-286'	84.1±0.64 **	65.9±0.96**	9.56±0.25 *	8.62±0.26**
Treatment (B)	'Seinovo'	88.8±0.58	75.0±0.78	7.53±0.16	6.23±0.24
	Control	75.3±0.44 b	56.8±0.44 b	9.56±0.14 a	8.88±0.13 a
	Fertilisation	85.3±0.53 a	73.2±0.50 a	6.76±0.13 b	5.35±0.10 b
Year (C)	2003	74.1±0.47 b	59.4±0.54 b	7.79±0.15 a	6.91±0.17 a
	2004	86.2±0.43 a	70.6±0.59 a	8.53±0.16 a	7.35±0.13 a
A × B					
'Ovcar'	Control	71.8	55.9	7.35	8.44
	Fertilisation	82.3	71.2	5.50	5.50
'Elit'	Control	71.8	52.0	11.80	8.44
	Nutrition	82.3	68.8	8.09	5.50
'G-139'	Control	70.3	52.6	9.56	8.09
	Fertilisation	78.2	67.6	5.15	5.50
'G-286'	Control	78.5	56.5	10.70	11.40
	Fertilisation	89.7	75.3	8.44	5.88
'Seinovo'	Control	84.1	67.6	8.44	8.09
	Fertilisation	93.8	82.6	6.62	4.41
A × C					
'Ovcar'	2003	68.5 g	55.9 e	5.88	6.62
	2004	85.6 bc	71.2 b	6.97	7.35
'Elit'	2003	72.6 f	57.9 de	8.44	6.23
	2004	81.5 de	62.6 c	11.40	7.70
'G-139'	2003	64.7 g	51.8 f	6.97	5.88
	2004	83.8 cde	68.5 b	7.73	7.70
'G-286'	2003	80.6 e	60.9 cd	10.30	9.18
	2004	87.9 b	70.9 b	8.82	8.09
'Seinovo'	2003	85.0 bcd	70.9 b	7.35	6.62
	2004	92.6 a	79.4 a	7.71	5.88
B × C					
Control	2003	70.3 c	52.3 d	9.26	8.82
	2004	80.3 b	61.7 c	9.85	8.97
Fertilisation	2003	78.5 b	66.8 b	6.32	5.00
	2004	92.0 a	79.4 a	7.21	5.73
ANOVA					
Cultivar (A)		**	**	**	**
Treatment (B)		**	**	**	**
Year (C)		**	**	ns	ns
A × B		ns	ns	ns	ns
A × C		**	**	ns	ns
B × C		**	**	ns	ns
A × B × C		ns	ns	ns	ns

A, B, C represent cultivars, treatments and years, respectively. The asterisks in vertical columns represent significant differences between means at  $p \leq 0.05$  and  $p \leq 0.01$  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  $p \leq 0.05$  according to LSD-test

Tab. 4. Vegetative growth of nursery walnut plants

		Vegetative growth of nursery plants at the end of the first growing season	Vegetative growth of nursery plants at the end of the second growing season
Cultivar (A)	'Ovcar'	15.4±0.69 ns	190.3±6.72 ns
	'Elit'	14.8±0.66 **	183.1±6.82 **
	'G-139'	14.7±0.75 **	182.2±5.58 **
	'G-286'	17.1±0.80 **	195.0±8.52 ns
	'Seinovo'	15.9±0.60	197.7±8.86
Treatment (B)	Control	14.3±0.41 b	171.6±2.35 b
	Fertilisation	16.9±0.36 a	207.8±4.51 a
Year (C)	2003	13.9±0.39 b	
	2004	17.4±0.25 a	207.5±5.09 a
	2005		171.9±2.29 b
A x B			
'Ovcar'	Control	14.5 e	174.5
	Fertilisation	16.3 bc	206.2
'Elit'	Control	13.5 f	165.5
	Fertilisation	16.2 bc	200.8
'G 139'	Control	13.8 ef	166.7
	Fertilisation	15.7 cd	197.8
'G 286'	Control	15.0 de	173.8
	Fertilisation	19.2 a	216.2
'Seinovo'	Control	14.6 e	177.6
	Fertilisation	17.2 b	217.9
A x C			
'Ovcar'	2003	13.5 ef	
	2004	17.3 b	207.2 a
	2005		173.4 c
'Elit'	2003	13.3 ef	
	2004	16.4 bc	199.7 b
	2005		166.6 c
'G 139'	2003	12.4 f	
	2004	17.1 b	194.1 b
	2005		170.4 c
'G 286'	2003	15.7 cd	
	2004	18.6 a	218.1 a
	2005		171.9 c
'Seinovo'	2003	14.4 de	
	2004	17.4 ab	218.3 a
	2005		177.2 bc
B x C			
Control	2003	12.5 d	
	2004	16.2 b	183.4 b
	2005		159.9 c
Fertilisation	2003	15.3 c	
	2004	18.6 a	231.7 a
	2005		184.0 b
ANOVA			
Cultivar (A)		**	**
Treatment (B)		**	**
Year (C)		**	**
A x B		**	ns
A x C		*	**
B x C		**	**
A x B x C		ns	ns

A, B, C represent cultivars, treatments and years, respectively. The asterisks in vertical columns represent significant differences between means at  $p \leq 0.05$  and  $p \leq 0.01$  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  $p \leq 0.05$  according to LSD-test

ly higher growth of nursery plants as compared to 'Elit' and 'G-139', whereas no difference was observed between 'Seinovo', 'Ovcar' and 'G-286'. LSD-test revealed that the treatment without foliar fertilisation (control) stimulated significantly lower vegetative growth during the first and second growing seasons as compared to the foliar treatment. Significantly lower growth of nursery plants was recorded in 2003 and 2005 than in 2004 (Tab. 4).

The differences observed in graft-take success in the experiment were induced by the temperature inside the heated room and sawdust temperature, whereas the fluctuations in air humidity of 10% (60-70%) had no substantial effect, due to the fact that sawdust did not undergo desiccation in deeper layers but only in the top layer.

The highest sawdust temperature and the lowest temperature fluctuations were determined in the treatment involving paraffined foil-covered grafts. In this case, sawdust temperature was 1.5-2°C and 0.5-1°C higher than in the treatment without and with paraffin, respectively. The primary role of paraffin in walnut grafting is to prevent desiccation of the graft union and excessive loss of water from the scion, as the scion has no adequate water supply due to the absence of a connecting water-conducting vessel system between the scion and rootstock. Paraffin also helps in making close contact between the cambial zones of the graft components, thereby fixing them and preventing further movement, particularly upon initiation of multiplication of parenchymal cells at the cross-sections of the graft components. This facilitates meristematic cell division, callus tissue formation and differentiation of water-conducting vessels and other elements, and enhances callus bridge formation at the graft union and efficient adhesion between the rootstock and scion (Hartman *et al.*, 1997). The foil prevented heat and moisture emission in the boxes with grafts, thereby ensuring higher uniformity of sawdust temperature with very few variations observed and preventing sawdust desiccation. Graft-take success on day 20 after grafting in paraffined and foil-covered grafts was 35.5% and 13.8% higher, respectively, as compared to that in the treatment without and with paraffin. Bulatović (1985) recommends covering walnut grafts with foil to ensure higher sawdust temperature, resulting in graft-take success of 65-90%. In the absence of paraffin, the rootstock and the scion developed an insufficiently tight union, which hindered callusing and caused separation of the graft components in many cases. The present results are in agreement with the data obtained by Solar *et al.* (2001). The authors employed treatments with and without paraffin which gave a 83% and 77% graft-take success rate, respectively. The authors recommend the use of paraffin in walnut grafting as a way to retain graft moisture and, accordingly, enhance callus formation. Mitrović and Blagojević (2002) evaluated the effect of different treatments on callusing and graft-take success and obtained the best results in the treatment involving paraffined foil-covered grafts, followed by paraffin-treated grafts and paraffin non-treated grafts.

Foliar fertilisation in the present experiment resulted in a higher survival rate, a higher percentage of class 1 nursery trees and higher vegetative growth of nursery plants at the end of the first and second growing seasons. Paunović *et al.* (2011) underline the effect of foliar fertilisation on the survival and growth of walnut nursery trees. The survival of foliarly treated nursery trees in their study showed an 8.5% and 11.5% increase at the end of the first and second growing seasons, respectively, whereas their growth increase ranged from 1.6 to 3.0 cm at the end of the first growing season, and from 16.2 to 64.5 cm at the end of the second season, depending on cultivar. Solar (2003) achieved increased growth of walnut nursery plants upon foliar treatment. The treated nursery plants reached a height of 142-165 cm, as opposed to untreated plants that attained a significantly lower height of about 96 cm. Salifu *et al.* (2006) and Nicodemus *et al.* (2008) suggested that walnut fertilisation in the nursery increases the nutritive quality of grafted nursery trees, improves photosynthesis and physiological properties of walnut, enhances vegetative growth, and affects nutrient retention in root plugs, being of importance for the subsequent successful establishment of walnut orchards.

## Conclusions

The obtained results suggest the necessity to treat grafts with paraffin and cover them with polyethylene foil during walnut grafting under controlled conditions in order to achieve higher graft-take success. The production of good quality healthy walnut plants under nursery conditions should necessarily involve the use of foliar treatment as an essential plant care practise, particularly during unfavourable weather, in order to reach an increased survival rate and improved vegetative growth of nursery plants.

## Acknowledgements

This work was financially supported by the Ministry of Education and Science of the Republic of Serbia, Project No. 31064: Development and Preservation of Genetic Potential of Temperate Zone Fruits.

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