Methods to Improve the Rooting of Hardwood Cuttings of the 'Gisela 5' Cherry Rootstock

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SUMMARY. The effect of external factors on the rooting of hardwood cuttings of the 'Gisela 5' cherry rootstock (*Prunus cerasus* × *Prunus canescens*) was studied. Experiments were conducted with cuttings of different lengths (15, 20, 25, and 30 cm) and diameters (6 to 8, 9 to 11, and 12 to 14 mm) and with different indole-3-butyric acid (IBA) concentrations (0, 1, 2, 3, 4, and 6 g·L⁻¹). Significantly improved rooting was achieved using cuttings 20 cm long and 9 to 11 mm in diameter collected in November or March from the upper part of the parent shoot and subsequently quick-dipped in 1 g·L⁻¹ IBA solution. Cuttings placed vertically in perlite:peat (1:1) or perlite with bottom heat gave the best rooting percentage. No effect on callusing or rooting of cuttings was found after incubation of cutting in suspensions of *Agrobacterium rubi*. If the optimal combination of factors is selected, 'Gisela 5' can be propagated from hardwood cuttings with a rooting efficiency of ≈50%.

'Gisela 5' is a triploid hybrid developed at the University of Giessen, Germany, and is among the best dwarfing [≈50% of 'Mazzard' sweet cherry (*Prunus avium*)], precocious and productive rootstocks for modern, intensive sweet cherry growing (Bassi, 2005; Liu et al., 2005; Sitarek et al., 2005; Walter and Franken, 1998; Whiting and Ophardt, 2005; Yang et al., 2005; Zimmermann, 1994). It has good grafting compatibility, tolerance to ilarviruses, and adaptation to various edaphoclimatic conditions (Lugli et al., 2005; Walter and Franken, 1998). This rootstock has shown very good adaptability in Greek soil-climate conditions and has replaced 'Mazzard' rootstocks in many cherry orchards.

Hardwood cuttings are most often used in propagation of deciduous woody plants (Hartmann et al., 2002) as one of the cheapest and easiest methods of vegetative propagation. They are easily handled, are not readily perishable, and may be shipped safely over long distances.

Typically, they require little or no special equipment during rooting, which can be readily performed in commercial nurseries. Previous work has demonstrated the propagation of 'Gisela 5' cherry rootstock from leaf, greenwood, and soft cuttings (Stefancic et al., 2006; Trobec et al., 2004), but no data are available for the propagation of this rootstock by hardwood cuttings.

Many external factors such as cutting length and diameter, applied indole-3-butyric acid (IBA) concentration, date of cutting collection, and preheating can affect and increase rooting of hardwood cuttings of many species (Gill and Chitkara, 1990; Loach, 1988; Tofanelli et al., 2003) by influencing the endogenous auxin and carbohydrate contents of the tissues (Hartmann et al., 2002). Some bacteria such as those belonging to the genus *Agrobacterium* release auxin, can also have a positive effect on the rooting of cuttings (Sezai et al., 2003a, 2003b). Furthermore, a correlation among polarity, root

differentiation, and auxin movement can be made (Hartmann et al., 2002).

This study investigated the effect of several external factors (cutting length, thickness, IBA concentration, orientation, rooting substrate, position of cutting on the shoot, and application of *Agrobacterium rubi*) in an attempt to improve the rooting of hardwood cuttings of the 'Gisela 5' cherry rootstock for use in the Greek horticultural industry.

Materials and methods

PLANT MATERIAL. Mother trees (1-year-old) were established at the Pomology Institute (NAGREF), Naoussa, Greece, where the climate is temperate. As part of standard maintenance, irrigation was provided five to six times in a vegetative season, Bordeaux mixture was sprayed at the leaf fall stage, and nitrogen (ammonium sulphate at 120 kg·ha⁻¹ N) was applied each year in the middle of February. The mother trees were ≈1.5 m high when the cuttings were collected.

Cuttings were taken from the previous season's growth, 60 to 80 cm in two consecutive years (2003 and 2004), and percentage rooting and percentage callusing (includes cuttings with callus and those with callus plus roots) were recorded 2 months after treatment. Cuttings were inserted to a depth of 10 cm in rooting substrate with bottom heat. The rooting substrate was 3:1 river sand (total porosity 35%, waterholding capacity 25%) and perlite (total porosity 75%, waterholding capacity 20%; Argyrometalevmaton and Varytins, Mylos, Greece). The temperature at the base of cuttings was maintained at 18 ± 2 °C. Cuttings were held in a glass house (10 × 12 × 4 m) with natural light (≈11 h per day) and a relative humidity that fluctuated between 70% and 90%. Cuttings were irrigated once per week.

PREPARATION OF INDOLE-3-BUTYRIC ACID SOLUTIONS. Indole-3-butyric acid was first dissolved in 3 mL

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Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
29.5735	fl oz	mL	0.0338
0.3048	ft	m	3.2808
2.54	inch(es)	cm	0.3937
25.4	inch(es)	mm	0.0394
1.1209	lb/acre	kg·ha ⁻¹	0.8922
0.001	ppm	g·L ⁻¹	1000
(°F - 32) ÷ 1.8	°F	°C	(1.8 × °C) + 32

absolute ethanol and subsequently diluted to a concentration of 50% v/v ethanol, which was subsequently increased to 500 mL (by adding absolute ethanol) with simultaneous stirring, and then another 500 mL of distilled water was added. The control solution was 50% v/v ethanol.

AGROBACTERIUM RUBI TREATMENTS. Two isolates of *A. rubi* (B63 and B149; LMG Bacterial Collection, Laboratorium voor Microbiologie Universiteit Gent, Gent, Belgium) were used and grown for 5 d on nutrient agar with beef extract (0.3% w/v), peptone (0.5% w/v), and agar (1.5% w/v). When required, the bacterial growth was scraped aseptically from the plate surface with a loop and suspended in sterile distilled water. The concentrations of the suspensions are provided in Table 1.

Cuttings (20 cm long, 1.2 to 1.4 cm in diameter) were collected on 30 Nov. and first dipped to 1 cm in the IBA solution (1 g·L⁻¹) for 15 s and left to dry at room temperature for 1 h. This avoids problems that result from exposing the bacterial cells to the relatively high alcohol concentration. The choice of IBA level was based on an unpublished study in which rooting of peach (*Prunus persica*) rootstock cuttings was improved by *A. rhizogenes* treatment. Subsequently, the cutting bases were dipped in the *A. rubi* suspension for 30 min (Table 1). Cuttings were set in the 3:1 sand:perlite rooting substrate with bottom heat as described previously.

The experiment was designed as a completely randomized block with

two bacteria strains × two IBA concentrations × five replicates of 20 cuttings.

CUTTING LENGTH. Cuttings of 9 to 11 mm diameter were taken on 30 Nov., trimmed to a length of 15, 20, 25, or 30 cm, and dipped to 1 cm in a 1 g·L⁻¹ IBA solution for 15 s. After surface drying, the bases were treated with a powder containing 50% w/v talcum powder and 50% w/v captan (Captan 75W; Euthimiadis, Sindos, Greece) as a protective fungicide against a variety of soil pathogens. Cuttings were set in the 3:1 sand:perlite rooting substrate with bottom heat as described previously.

The experiment was designed as a completely randomized block with four cutting lengths × five replicates of 20 cuttings.

INDOLE-3-BUTYRIC ACID CONCENTRATION AND CUTTING BASE DIAMETER. Cuttings were taken on 30 Nov. and trimmed to 20 cm in length with diameters of 6 to 8, 9 to 11, or 12 to 14 mm measured at the base of each cutting. These were dipped to 1 cm for 15 s in IBA solutions (1, 2, 3, or 6 g·L⁻¹). Cuttings were set in the 3:1 sand:perlite rooting substrate with bottom heat as described previously.

The experiment was designed as a completely randomized block with three diameters × six IBA levels × five replicates of 20 cuttings.

CUTTING POSITION WITHIN THE PARENT SHOOT AND COLLECTION DATE. Cuttings of 20 cm length and 9 to 11 mm in diameter were taken on 15 Nov., 5 Dec., and 1

Mar. from different positions on the parent shoot (upper, middle, and lower). The choice of dates was based on a previous study (C. Tsipouridis, unpublished data). Cuttings were treated with 1 g·L⁻¹ IBA and set in the 3:1 sand:perlite rooting substrate with bottom heat as described previously.

The experiment was designed as a completely randomized block with three collecting dates × three positions × five replicates of 20 cuttings.

DIFFERENT SUBSTRATES. Cuttings of 20 cm length and 9 to 11 mm diameter were taken on 30 Nov. and treated with 1 g·L⁻¹ IBA as described previously. Six different rooting substrates were used: peat, perlite, peat and perlite 1:1, sand, coarse vermiculite, and fine vermiculite. The sand and perlite were described previously. The peat (Klasmann-Deilmann, Geeste, Germany) had a total porosity of 70% and a waterholding capacity of 35%. Peat and perlite 1:1 had a total porosity of 70% and a waterholding capacity of 30%. Fine vermiculite (total porosity 50%, waterholding capacity 55%) and coarse vermiculite (total porosity 60%, waterholding capacity 40%) were supplied by Mathios Pefractories (Athens, Greece). Cuttings were treated and set in the appropriate rooting substrate with bottom heat as described previously.

The experiment was designed as a completely randomized block with six rooting substrates × five replicates of 20 cuttings.

CUTTING ORIENTATION. The correlation of polarity of root differentiation with auxin movement is known and also the polarity in auxin varies in intensity among different tissues (Hartmann et al., 2002). These experiments were conducted to confirm that the orientation affects the rooting of the 'Gisela 5' cherry rootstock hardwood cuttings.

Cuttings of 20 cm long and 9 to 11 mm diameter were collected on 5 Dec., stored at 0 to 3 °C for 17 d, and then prepared and treated with IBA as described previously. The cuttings were set vertically in the conventional orientation, horizontally, inverted, and at 45° in the sand:perlite rooting substrate with bottom heat as described previously.

The experiment was designed as a completely randomized block with four cutting orientations × five replicates of 20 cuttings.

Table 1. Effect of the bacterium *Agrobacterium rubi* on percentage of callusing (cuttings with callus + cuttings with roots) and rooting of the 'Gisela 5' cherry rootstock hardwood cuttings 20 cm (7.9 inches) long and 12 to 14 mm (0.47 to 0.55 inch) in diameter.

Treatment	<i>A. rubi</i> cells (10,000/mL) ^z	IBA concn (g·L ⁻¹) ^y	Callusing (%)	Rooting (%)		
			Mean	Year 1	Year 2	Mean
Control	—	—	92 a ^w	8 a	11 a	10 a
B149 ^x	712	—	96 a	0 b	0 b	0 b
B149	8,860	—	90 a	0 b	0 b	0 b
B149 + IBA	712	1	72 c	0 b	0 b	0 b
B149 + IBA	8,860	1	80 bc	0 b	0 b	0 b
B63 ^x	915	—	92 a	0 b	0 b	0 b
B63	7,900	—	88 ab	6 a	10 a	8 a
IBA + B63	915	1	76 c	0 b	0 b	0 b
IBA + B63	7,900	1	72 c	0 b	0 b	0 b

^z1 cell/mL = 29.5735 cells/fl oz.

^yIBA = indole-3-butyric acid; 1 g·L⁻¹ = 1000 ppm.

^xB149 and B63 = strains of *A. rubi*.

^wMeans are calculated from five replicates of 20 cuttings each. Values in the same column followed by different letters are significantly different (*P* < 0.05) according to Wald test.

STATISTICAL ANALYSIS. In all experiments, years and blocks were taken as random effect factors and treatments as fixed effect factors. To test significant differences between treatments and interactions, the Generalized Linear Models (SPSS Grad Pack 16 for Windows; SPSS, Chicago, IL) were applied at $P = 0.05$.

In all experiments, statistical analyses of the data were carried out after angular transformation. Significant differences between treatments were tested by Wald test at $P = 0.05$.

Results

AGROBACTERIUM RUBI TREATMENTS. *Agrobacterium rubi* did not improve the callusing or rooting performance of the 'Gisela 5' cherry rootstocks (Table 1) and in some treatments, the percentage of callusing and rooting was reduced significantly below control levels.

CUTTING LENGTH. The highest rooting percentage was found on 20-cm cuttings and the lowest on those 30 cm in length (Table 2). Cuttings of 25 cm exhibited a higher rooting percentage than those of 15 and 30 cm in both years. In both years, 15-cm cuttings did not show a significant difference in rooting from those of 30 cm.

INDOLE-3-BUTYRIC ACID CONCENTRATION AND CUTTING BASE DIAMETER. Cuttings without IBA treatment and those dipped in 2 g·L⁻¹ IBA had a relatively high callusing percentage, but significantly less than those dipped in 1 g·L⁻¹ IBA (Table 3). As IBA concentration increased, the callusing percentage was reduced. Cuttings with a 6 to 8 mm diameter and treated

with 1 g·L⁻¹ IBA solution showed the highest callusing percentage. A significant difference in callusing percentage was found between cuttings with 9- to 11-mm and 12- to 14-mm diameters. Interactions between thickness and IBA concentration for callusing were significant.

Cuttings dipped in 1 g·L⁻¹ IBA solution showed the highest percentage of rooting (Table 3) and a relatively high rooting percentage was also found on cuttings with 2 g·L⁻¹ or without IBA treatment. IBA at 3 g·L⁻¹ significantly reduced the rooting of cuttings and no rooting was seen on cuttings dipped in 4 or 6 g·L⁻¹ IBA. Cuttings with diameters of 6 to 8 mm and 9 to 11 mm showed the highest rooting percentage, whereas no rooting was observed from those with 12- to 14-mm diameter. Interactions between diameter and IBA concentration for rooting were significant (Table 3).

CUTTING POSITION WITHIN THE PARENT SHOOT AND COLLECTION DATE. Cuttings taken from the middle or upper part of shoots had higher callusing percentages compared with those taken from the lower part (Table 4). Higher callusing was observed on

the cuttings taken on 15 Nov. or 2 Mar. than those taken on 1 Dec. Cuttings collected on 15 Nov. and 2 Mar. had similar callusing percentages (Table 4) independent from their position on the parent shoot. No significant difference in callusing was found between cuttings taken from the middle and upper shoot regions collected on 1 Dec., whereas no callusing was found on cuttings taken from the base of the parent shoots. Interactions between cutting position and time of setting for callusing were significant (Table 4).

A higher percentage of rooting was found for cuttings collected from the middle or the upper shoot parts in comparison with those taken from the lower region. Cuttings taken on 15 Nov. or 2 Mar. showed higher rooting than those taken on 1 Dec. Interactions between cutting position and time of setting for rooting were significant (Table 4). The highest rooting percentage was found on cuttings taken from the base or the upper region of shoots on 15 Nov. or 2 Mar. and cuttings taken on 1 Dec. from the middle of shoots. Cuttings taken from the base of parent shoots showed no rooting.

Table 2. The effect of cutting length on rooting percentage of hardwood cuttings of the 'Gisela 5' cherry rootstock hardwood cuttings 9 to 11 mm (0.35 to 0.43 inch) in diameter.

Cutting length (cm) ^z	Rooting (%)		
	First year	Second year	Mean
15	13 c ^y	14 c	14 c
20	44 a	42 a	43 a
25	33 b	31 b	32 b
30	6 c	17 c	12 c

^z1 cm = 0.3937 inch.

^yMeans are calculated from five replicates of 20 cuttings each. Values in the same column followed by different letters are significantly different ($P < 0.05$) according to Wald test.

Table 3. The effect of indole-3-butyric acid (IBA) concentration and cutting diameter on callusing (cuttings with callus and cuttings with roots) and rooting of hardwood cuttings of the 'Gisela 5' cherry rootstock 20 cm (7.9 inches) long.

IBA concn (g·L ⁻¹) ^z	Callusing (%)					
	Diam (mm) ^z			Mean of three diameters (%)	Year 1	Year 2
	6 to 8	9 to 11	12 to 14			
0	71 b ^y	31 d	0 g	34 b	36 b	32 b
1	86 a	70 b	21 e	59 a	61 a	56 a
2	58 c	35 d	6 f	33 b	35 b	29 c
3	26 de	12 f	9 f	16 c	18 c	12 d
4	11 f	8 f	0 g	6 d	6 d	6 e
6	4 fg	0 g	0 g	1 e	1 e	1 f
Mean IBA concn (%)	43 a	26 b	6 c			

IBA concn (g·L ⁻¹)	Rooting (%)					
	Diam (mm)			Mean of three diameters (%)	Year 1	Year 2
	6 to 8	9 to 11	12 to 14			
0	26 b	9 c	0 d	12 c	13 c	10 c
1	29 b	52 a	0 d	27 a	28 a	25 a
2	24 b	25 b	0 d	16 b	18 b	14 b
3	8 c	0 d	0 d	2 d	4 d	1 d
4	0 d	0 d	0 d	0 d	0 e	0 d
6	0 d	0 d	0 d	0 d	0 e	0 d
Mean IBA concn (%)	15 a	14 a	0 b			

^z1 g·L⁻¹ = 1000 ppm; 1 mm = 0.0394 inch.

^yMeans are calculated from five replicates of 20 cuttings each. Values followed by different letters are significantly different ($P < 0.05$) according to Wald test.

Table 4. Effect of cutting position within the parent shoot and collection date on the percentage of callusing (cuttings with callus + cuttings also with roots), and rooting of hardwood cuttings of the ‘Gisela 5’ cherry rootstock [20 cm (7.9 inches) long and 9 to 11 mm (0.35 to 0.43 inch) in diameter].

Position within the parent shoot	Callusing (%)			Mean of three dates (%)
	15 Nov.	1 Dec.	2 Mar.	
Base	79 a ^z	0 d	75 a	51 b
Middle	68 a	43 c	75 a	62 a
Top	78 a	40 c	73 a	64 a
Mean of three positions (%)	75 a	28 b	74 a	

Position within the parent shoot	Rooting (%)			Mean of three dates (%)
	15 Nov.	1 Dec.	2 Mar.	
Base	35 a	0 c	33 a	23 b
Middle	29 b	33 a	30 b	31 a
Top	31 ab	27 b	35 a	31 a
Mean of three positions (%)	32 a	20 b	33 a	

^zMeans are calculated from five replicates of 20 cuttings each. Values followed by different letters are significantly different ($P < 0.05$) according to Wald test.

DIFFERENT SUBSTRATES. Cuttings placed in sand had the highest percentage of callusing with the lowest on cuttings placed into perlite, peat:perlite (1:1), and vermiculite (fine) (Table 5). The callusing percentage of cuttings placed into peat, perlite, peat:perlite (1:1), and vermiculite (fine) were statistically similar with no significant difference in the percentage of callusing between perlite and vermiculite (coarse).

The cuttings in peat:perlite (1:1) and perlite gave the highest rooting with no significant difference found

Table 5. Effect of different rooting substrates on percentage of callusing (cuttings with callus + cuttings also with roots) and rooting of the ‘Gisela 5’ cherry rootstock hardwood cuttings 20 cm (7.9 inches) long and 9 to 11 mm (0.35 to 0.43 inch) in diameter.

Rooting substrates	Callusing (%)	Rooting (%)
Sand	92 a ^z	28 bc
Peat	61 c	26 bc
Perlite	70 bc	47 a
Peat and perlite 1:1	67 c	53 a
Vermiculite (coarse)	79 b	33 bc
Vermiculite (fine)	62 c	23 c

^zMeans are calculated from five replicates of 20 cuttings each. Values in the same column followed by different letters are significantly different ($P < 0.05$) according to Wald test.

among vermiculite (coarse), peat, sand, and vermiculite (fine).

CUTTING ORIENTATION. Cuttings placed vertically gave the highest percentage of callusing, whereas those placed at 45° gave the lowest in both years (Table 6). The percentage of callusing on inverted cuttings was significantly lower than that on vertical cuttings. Inverted cuttings showed a significantly higher percentage of callusing than those placed horizontally.

Cuttings placed vertically gave the highest rooting percentage in both years (Table 6) with no significant difference in percentage rooting between vertical and angled cuttings in Year 1 but a significantly lower level in Year 2. Cuttings placed horizontally, or inverted, gave practically no rooting (one inverted was rooted).

Discussion

As a relatively new cherry rootstock, ‘Gisela 5’ has not yet been subjected to extensive scientific study, resulting in limited knowledge of its vegetative propagation from hardwood cuttings. Vegetative propagation is gaining in importance as the economical life of cherry orchards is becoming shortened as a result of innovations in planting layout and selection of new cultivars with better fruit quality. Consequently, increasing quantities of planting material are required.

In comparison with other types of cuttings (semihardwood, softwood, and so on), hardwood cuttings are

Table 6. The effect of cutting orientation on percentage callusing (cuttings with callus + cuttings also with roots) and rooting of hardwood cuttings of the ‘Gisela 5’ cherry rootstock [20 cm (7.9 inches) long and 9 to 11 mm (0.35 to 0.43 inch) in diameter].

Cutting orientation ^z	Callusing (%)		
	Year 1	Year 2	Mean
Vertical	82 a ^y	77 a	80 a
Angle	44 d	42 d	43 d
Inverted	65 b	72 b	69 b
Horizontal	56 c	53 c	55 c

Cutting orientation ^z	Rooting (%)		
	Year 1	Year 2	Mean
Vertical	16 a	24 a	20 a
Angle	11 a	9 b	10 b
Inverted	0 b	1 c	1 c
Horizontal	0 b	0 c	0 c

^zOrientation of cuttings by which they were placed in bottom heat. The cuttings were set vertically in the conventional orientation, horizontally, inverted and at 45°.

^yMeans are calculated from five replicates of 20 cuttings each. Values in the same column followed by different letters are significantly ($P < 0.05$) according to Wald test.

easy to take, handle, and store, which allows for flexibility in the preparation of the cutting and, in general, less precision than cuttings that include actively growing tissue (Hartmann et al., 2002). There is less chance of cutting desiccation or mechanical damage during all stages of handling, storage, preparation, and placement in the greenhouse. Hardwood cuttings are taken at a time of year when field responsibilities are limited and there is little need for special treatment, other than cold storage, before the cuttings are dealt with in the greenhouse. Helpfully, more dormant deciduous cuttings than summer cuttings can be stored per unit area because of the absence of foliage (Hartmann et al., 2002). Hartmann et al. (2002) also reported that the juvenility of cuttings plays an important role in rooting and factors such as cutting length and diameter, collection date, and position of cuttings on the parent shoots affect the rooting of hardwood cuttings.

The present study also indicated that the second half of November and the first days of March are better for collecting cuttings than early December, in line with earlier studies that indicated November as the best month to collect hardwood cuttings

for propagation of peach (Gill and Chitkara, 1990; Tsipouridis et al., 2005). This 'Gisela 5' study suggests that 20-cm long cuttings with 9 to 11 mm diameter collected from the middle of the parent shoots and treated with 1 g·L⁻¹ IBA gave the best rooting. Abdullah et al. (1988) reported that the best rooting of hardwood cuttings of american sycamore (*Platanus occidentalis*) was obtained with 25-cm long cuttings cut 1 m from the shoot base of the 8-year-old tree. Hardwood cuttings taken from more basal or middle cane segments of the 'Norton' grapevine rootstock (*Vitis aestivalis*) rooted better than cuttings from the apical region (Keeley et al., 2004) and Marini (1983) found that cuttings of peach 5 mm in diameter rooted 100% better than those of 2 mm.

Synthetic auxins such as IBA have a strong promotional action on rooting of cuttings, other than hardwood, of the 'Gisela 5' cherry rootstock (Stefancic et al., 2006; Wang et al., 2005). The optimal IBA concentration for rooting hardwood cuttings varies between species (Ozkan et al., 2005; Pirlak, 2000; Rosier et al., 2004; Tsipouridis et al., 2004, 2005) and in this study, 1 g·L⁻¹ produced the best results.

The characteristics of rooting substrates are very important for rooting success (Pokorny and Austin, 1982). A good rooting substrate will have a high waterholding capacity combined with a high degree of aeration. Verdonck et al. (1983) have attempted to quantify these requirements for optimal rooting and suggest that 20% of the volume of an ideal rooting substrate should be air space and 20% to 30% readily available for water. The results of this study indicated that perlite:peat (total porosity 70%, waterholding capacity 30%) and perlite (total porosity 75%, waterholding capacity 20%) are optimal for 'Gisela 5' hardwood cuttings. Tofanelli et al. (2003) tested six rooting substrates (sand, carbonized rice husk, vermiculite, sand + carbonized rice husk, sand + vermiculite, and carbonized rice husk + vermiculite) for rooting of peach hardwood cuttings and found that the highest rooting frequency was obtained with vermiculite, independent of thickness. Denny and Arnold (2001) found the perlite substrate the best for the rooting of cuttings of

american smoketree (*Cotinus obovatus*), whereas Ercisli et al. (2002) found the highest rooting percentage for kiwifruit (*Actinidia chinensis*) hardwood cuttings was obtained in peat + perlite and peat + sawdust substrates. Tsipouridis et al. (2005) found that perlite was the best rooting substrate for peach hardwood cuttings.

Sinnott (1960) states that polarity is the first step in differentiation and this is inherent in the tissues themselves and not dependent on gravity. Rooting of cuttings involves such a gradient of growth regulator within the stem, and it is a consequence of the polarity of the tissues and not the cause of this (Wareing and Phillips, 1975). The results of this study showed that vertically placed hardwood cuttings gave the highest rooting percentage with similar results reported by Tsipouridis et al. (2005). Polarity reversal can result in a drastic decrease (60%) in rooting percentage of hardwood cuttings of 'Kober 5BB' grapevine rootstock (*Vitis berlandieri* × *Vitis riparia*) (Cristoferi et al., 1988). One inverted cutting did produce roots during this investigation, but no further information was collected from it.

Some bacteria such as those belonging to the genus *Agrobacterium* can release auxin and affect positively the rooting of cuttings (Sezai et al., 2003a, 2003b), but the results of this study showed no effect, or a negative effect, of *A. rubi* on callusing and rooting of cuttings. The relatively low percentage of rooting in the control cuttings for this part of the investigation was probably related to the diameter of cuttings used as it was shown in Expt. 1 with the cuttings 12 to 14 mm in diameter. In previous research, *A. rubi* and *A. rhizogenes* have been shown to promote the rooting of cuttings in different plants (Chen et al., 2004; Felker et al., 2005; Li and Leung, 2003; Sezai et al., 2003a, 2003b). Ercisli et al. (2004) reported three strains of *A. rubi* (A1, A16, A18) to stimulate adventitious root formation on hardwood stem cuttings of two rose (*Rosa* sp.) genotypes. It would appear that the strains of *A. rubi* used in this study were ineffective in improving the rooting of cuttings probably because of reduced quantities of auxin produced by these strains, but further

work is needed to investigate the effectiveness of the infection process.

The study has shown improved rooting can be achieved for 'Gisela 5' using cuttings of 9 to 11 mm diameter and 20 cm length quick-dipped in 1000 mg·L⁻¹ IBA solution. In addition, the cuttings should be collected at 15 Nov. or 2 Mar. instead of the first days of December and placed vertically into a perlite:peat (1:1) or perlite rooting substrate. In practical terms, it appears this hardwood rootstock can be propagated by cuttings with a rooting percentage of ≈50%.

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