

# SELECTION OF THE BEST METHOD FOR VEGETATIVE PROPAGATION OF MATURE *ALNUS GLUTINOSA* (L.) GAERTN. TREES RESISTANT TO *PHYTOPHTHORA ALNI*

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## Abstract

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Although alder is readily propagated by seeds our objective was to examine the feasibility of propagating mature *Alnus glutinosa* (L.) trees by vegetative means that could be used to propagate trees resistant to *Phytophthora alni*. Both softwood and hardwood cuttings were taken. In the case of hardwood cuttings, two different treatments, based on differences in temperature, two growth stimulators (NAA, IBA) and rooting conditions, were tested. Rooting success rate was low, with only 1.3 to 5% of treated cuttings rooting, in comparison with 0 to 1.3% of the untreated control cuttings. In the case of softwood cuttings, two treatments were used which differed in their use of growth stimulators (NAA, IBA) and the dates when material was collected. In contrast to the hardwood cuttings, the softwood cuttings rooted better in both treatments. The cuttings collected at the later date rooted better; 30 to 42.5% of the treated cuttings rooted when compared to 15% in the control treatment. Softwood cuttings collected in the middle of July and then treated with 1% IBA rooted the best of all, with 42.5% of cuttings rooting successfully.

black alder, hardwood cuttings, softwood cuttings, vegetative propagation

Black alder, *Alnus glutinosa* (L.) Gaertn., also called European alder or European black alder, is one of about 30 species of alder found mainly in moist, sunny habitats in the Northern Hemisphere. Its native range is in Europe, Asia, and North Africa (Steiner, 1983).

Alders are easily propagated by seeds if large numbers of young plants are required, and this method of propagation has been strongly recommended by some (Faria *et al.*, 2008). However, because of the inherent variation resulting from sexual reproduction, using seeds as a means of propagation is not appropriate when many identical individuals are urgently required. In seeking to rapidly propagate plants possessing a rare but highly desirable character, in this case disease resistance, it is more appropriate to use vegetative means.

Alders can be vegetatively propagated through layering (Wilson and Jewett, 1986), air layering (Sattoo, 1963), grafting (Robinson *et al.*, 1979), budding (Buraczyk and Kaźmierczak, 2003) and the rooting of either softwood or hardwood cuttings (Obdržálek and Pinc, 1997; Ayan *et al.*, 2006; Králík and Šebánek, 1983). The lowest success rates were reported for budding and grafting (Robinson *et al.*, 1979; Buraczyk and Kaźmierczak, 2003) while up to 100% success has been observed using air layering (Sattoo, 1963). The rooting success of softwood and hardwood cuttings has been reported as ranging from 0 to 68% (Schrader and Graves, 2000; Ayan *et al.*, 2006; Faria *et al.*, 2008) and from 0 to 85% (Holloway and Zasada, 1979; Králík and Šebánek, 1983; Java and Everett, 1992; Faria *et al.*, 2008). These widely differing success rates may be the result of differences in the particular alder species used, the

methods employed and the dates on which material was collected.

In our situation, the use of softwood and hardwood cuttings seemed to be the most suitable method to employ for propagating mature alder trees. The treatments employed, such as growth regulator concentration and method of application, were chosen on the basis of published results of cuttings taken from young trees, since there is little specific information about the vegetative propagation of adult alder trees. The highest success rates for hardwood cuttings have been reported by Králík and Šebánek (1983) and Java and Everett (1992), and therefore their methods for propagating hardwood cuttings were employed, and the standard approach to propagating ornamental alder using softwood cuttings (Obdržálek and Pinc, 1997; Obdržálek, personal communication) was also used. The goal of this study was to identify the best method for propagating mature trees of *A. glutinosa*, in order to produce trees resistant to the black alder pathogen, *Phytophthora alni*.

## MATERIAL AND METHODS

### Hardwood cuttings

The methods used for rooting hardwood cuttings are summarized in Tab. I. One-year old shoots from naturally growing adult *A. glutinosa* trees (> 40 years old) were collected at the beginning of March (9<sup>th</sup> March) and the shoots growing directly off the trunk were preferred. Cuttings were prepared from the basal parts of these shoots. Their length was about 20 cm (3 to 4 internodes) and consisted of at least 4 viable buds; the basal cut was made just below the lowermost bud and the apical cut just above the uppermost bud. Twenty cuttings, with 4 replications for each treatment, were used in this experiment.

The first group of cuttings was dipped in a 0.2% IBA (Indole-3-butyric acid, DUCHEFA) and 0.1% NAA (1-Naphthaleneacetic acid, DUCHEFA) aqueous solution for 24 hours (Java and Everett, 1992). The control cuttings were placed in tap water for an equal length of time. Afterwards both the treated and untreated cuttings were put in a cooling chamber where they were stored for one month at a temperature of 4 °C. After one month, the treated and untreated cuttings were planted into boxes with

a rooting substrate (raised bog peat and perlite, 7:3 by volume; with limestone 2 kg.m<sup>-3</sup>, pH<sub>H<sub>2</sub>O</sub>=5.2) and the boxes placed outside. The cuttings were watered with tap water when necessary. After 8 weeks the number of successfully rooted cuttings was counted and the percentage of success calculated.

The second group of cuttings was treated by dipping in a 0.02% IBA aqueous solution for 24 hours (Králík and Šebánek, 1983). The control cuttings were dipped in tap water for an equal period of time. The treated and control cuttings were placed in beakers of tap water at room temperature. The water in the beakers was changed every 3 to 4 days. The percentage success in rooting was evaluated after 8 weeks.

### Softwood cuttings

Standard hormone treatments for ornamental alders (Obdržálek and Pinc, 1997; Obdržálek, personal communication) were used for softwood cuttings. The treatments used for rooting softwood cuttings are summarized in Tab. I.

Annual shoots from naturally growing adult *A. glutinosa* trees (the same as for hardwood cuttings) were collected on two dates in July (7<sup>th</sup> July and 19<sup>th</sup> July). The semi-ripe parts of shoots arising on the trunk were used for the softwood cuttings, which were approximately two internodes in length. A slightly-inclined cut was made about 3 cm under the lowermost bud and the apical cut just above the uppermost bud. The leaves growing from the lower nodes were removed and the leaves from the uppermost nodes were cut in half to reduce water loss through transpiration. The cuttings were rinsed in a light pink solution of potassium permanganate (KMnO<sub>4</sub>) for a few minutes, to sterilize them and prevent any possible contamination of neighbouring plants. Each treatment was replicated 20 times.

The cuttings were treated with two rooting hormones in powder form. The first treatment contained 0.1% NAA and the second 1% IBA, applied to the basal ends of the cuttings. The control cuttings received no hormone treatment. The cuttings were planted into multipots with a rooting substrate (raised bog peat and perlite, 7:3 by volume; with limestone 2 kg.m<sup>-3</sup>, pH<sub>H<sub>2</sub>O</sub>=5.2), and placed in a greenhouse at a temperature of about 22 °C. There was light mist irrigation with automatic control

I: Summary of treatments

Cuttings	Treatment	Rooting conditions
Hardwood	1	24 hours in 0.2% IBA and 0.1% NAA, then one month at 4°C
		control (24 hours in water, then one month at 4°C)
	2	24 hours in 0.02% IBA
		control (24 hours in water)
Softwood	1	0.1% NAA
	2	1.0% IBA
		control (without hormone treatment)

of temperature and humidity. The cuttings were carefully taken out of the multipots 8 weeks later and the number which had successfully rooted was counted and the percentage calculated.

### Data analysis

The data were statistically analysed using the software package Statistica 9.0 (Statsoft, Inc., Tulsa, OK). The associations between two variables (treatment and rooting rate) were analysed using *Chi*-square tests. The cuttings treated with a growth stimulator and the corresponding untreated ones were used in association analyses. Where there was a significant association, its degree was assessed using the *Phi* coefficient of association. Differences in rooting success between the two dates for taking cuttings were tested using the sign test.

## RESULTS AND DISCUSSION

### Hardwood cuttings

No significant association ( $p > 0.05$ ) between any of the treatments and rooting success was found for hardwood cuttings (Tab. II). 1.3% of the hardwood cuttings rooted after being treated with 0.2% IBA and 0.1% NAA and stored in the cold for one month, compared to 0% in the control. This treatment was reported by Java and Everett (1992) as the best hormone treatment to achieve rooting of cuttings. Interestingly, they (Java and Everett, 1992) achieved 76% success in rooting cuttings of *Alnus incana* but had no success at all with *Alnus viridis* subsp. *sinuata*, even though they used the same combination of hormone treatments. This striking difference suggests that in this respect there are significant differences between alder species.

Holloway and Zasada (1979) reported very low rates of rooting success, 0.3% for *A. crispa* and 0% for *A. incana*, even though the cuttings were treated with IBA rooting hormones. In our trial, the cuttings treated with 0.02% IBA for 24 hours and then soaked in water for two months had the highest percentage rooting success (5%), compared to the control (1.3%), in which cuttings were soaked in plain water for the same time. However, Králík and Šebánek (1983) achieved a much higher success rate (60% compared to 15% in the untreated control) using the same method (basal parts of one-year-old shoots for cuttings, sampling dates and treatments), and even better results were reported by Psota

II: Rooting success of hardwood cuttings (sampling 9<sup>th</sup> April 2010)

Treatment	N	Rooted cuttings	
			%
1 0.2% IBA, 0.1% NAA	80	1	1.3
Control	80	0	0.0
2 0.02% IBA	80	4	5.0
Control	78	1	1.3

N = number of cuttings

(1987) who achieved 100% natural rhizogenesis in January (using IAA, NAA and IBA). The observed differences in the results could be related to the age of the trees from which the cuttings are taken. Králík and Šebánek (1983) took cuttings from two-year-old seedlings, Psota (1987) took them from one- or two-year old seedlings, but in this work the cuttings were taken from adult trees. Radwan *et al.* (1989) also reported better quality of roots on cuttings taken from young trees compared to adult trees.

### Softwood cuttings

An association between treatments and rooting success was observed for both dates when cuttings were taken (Tab. III). The positive effect of 1% IBA was significant ( $p < 0.01$ ), both at the beginning and in the middle of July, whereas the 0.1% NAA treatment was significant ( $p < 0.05$ ) only in the middle of July. However, the observed associations were weak ( $\Phi = 0.25$ ,  $\Phi = 0.30$  and  $\Phi = 0.18$ , respectively).

From the methods used, the variant without any hormone treatment (control) had the smallest number of rooted cuttings on both dates, as expected. Better rooting success was observed in the variant using 0.1% NAA, but the best results were for the cuttings treated with 1% IBA. Almost half of the cuttings collected at the later date rooted successfully. The best results achieved here are slightly lower than those reported by Ayan *et al.* (2006) and Schrader and Graves (2000), who respectively tested the rooting abilities of *Alnus glutinosa* subsp. *barbata* and *Alnus maritima*.

The date on which cuttings are taken has a strong influence on rooting success rates and this is linked to natural rhizogenesis and the activity of endogenous growth hormones in black alder cuttings during the year (Psota, 1987; Šebánek, 2008). In *A. glutinosa* there are two periods of increased natural rhizogenesis. The first period,

III: Rooting success of softwood cuttings

Treatment	7. 7. 2010			19. 7. 2010		
	N	Rooted cuttings		N	Rooted cuttings	
			%			%
1 0.1% NAA	80	6	7.5	80	24	30.0
2 1% IBA	80	19	23.8	80	34	42.5
Control	80	5	6.3	80	12	15.0

N = number of cuttings

connected with the breakdown of endogenous dormancy of the mother plant, is from December to February and the second one, before the onset of dormancy, is from July to September (Psota, 1987). Therefore it is widely recommended to take either hardwood cuttings from January to April (Psota, 1987) or softwood cuttings in June or July (Obdržálek and Pinc, 1997; Schrader and Graves, 2000; Ayan *et al.*, 2006). Comparing the dates in our trial, significant differences ( $p < 0.05$ ) in rooting were observed between the 1<sup>st</sup> and 2<sup>nd</sup> dates in both groups of treated cuttings whereas no differences were observed in the control cuttings. Cuttings prepared in the second half of July rooted more successfully under both treatments, even though Ayan *et al.* (2006) recommend collecting shoots of *Alnus glutinosa* subsp. *barbata* in the middle of June rather than at the beginning of July. The apparent disagreement here might be explained by the different climates in which the source trees were growing and where the studies were conducted.

It is to be expected that the quality of cuttings will be strongly affected by both seasonal changes and differences between growing seasons.

## CONCLUSIONS

The feasibility of vegetatively propagating adult alder trees has been demonstrated. Rooting success was highly dependant on the type of cuttings and treatments used. The success in rooting hardwood cuttings was extremely low for all treatments, and so hardwood cuttings can not be recommended for vegetatively propagating mature black alder trees. In contrast, the softwood cuttings were more successful, although the number of rooted cuttings obtained was dependant on both the treatment used and the date when cuttings were collected. In conclusion, it can be said that softwood cuttings collected in mid-July and treated with 1% IBA can be recommended as a method for propagating adult alder trees resistant to *Phytophthora alni*.

## SUMMARY

The aim of this trial was to identify a suitable method for the vegetative propagation of adult *Alnus glutinosa* trees resistant to *Phytophthora alni*.

Two different hormone treatments were used for the hardwood cuttings whereas two different treatments and two collection dates were used for the softwood cuttings. The hardwood cuttings were either dipped for 24 hours in a 0.2% IBA and 0.1% NAA aqueous solution, and then stored in the cold for one month before planting out, or they were dipped for 24 hours in a 0.02% IBA aqueous solution and then left to stand in water at room temperature. The softwood cuttings were treated with either 0.1% NAA or 1% IBA rooting hormone in powder form, and then planted out in a greenhouse. Cuttings treated similarly but without growth hormones were used as controls. The percentages of rooted cuttings were observed 8 weeks following the hormone treatments. Only 1.3 to 5% of the hardwood cuttings rooted in comparison with 0 to 1.3% in the controls. In contrast, the softwood cuttings rooted better in all cases, and a significant difference was observed between the different dates on which cuttings were taken. Those taken at the later date rooted better, with a 30 to 42.5% success rate compared to the control with 15%.

Softwood cuttings collected in the middle of July and treated with 1% IBA, an approach which gave a 42.5% success in rooting, can be recommended as a practical method for vegetatively propagating adult black alder trees resistant to *Phytophthora alni*.

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