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Vegetative Propagation of *Cupressus sempervirens* L. of Cretan Origin by Softwood Stem Cuttings[1])

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(Received 21st February 1997)

Abstract

One winter and 2 spring experiments were conducted testing the influence of genotype, auxin treatment, duration of the rooting period, time of cuttings collection and preliminary hedging on the rooting percentage and root quality of *Cupressus sempervirens* softwood stem cuttings. In total, 15 hedged and 7 nonhedged 4-year-old seedlings from 7 Cretan provenances of the species were used as cutting donors. The duration of the rooting period (3 months vs. 4 months) significantly affected the rooting and callus formation percentages. One month extension of the rooting period resulted in a 41.73% increase of rooted cuttings. The genotype was a major factor influencing the percentage of rooting, number of roots and length of the main root. Neither the treatment application, nor the time extension were able to offset the variation among the genotypes. The auxin treatment enhanced rooting and its positive effect was more pronounced during shorter rooting periods. However, the optimal IBA concentration for rooting differed among the genotypes and treatment x rooting duration interaction was present. The comparative results between the effect of the season and the preliminary rejuvenation showed that the cuttings obtained from slowly growing shoots (either from the lower part of nonhedged donors or those collected in winter) demonstrate higher capability to survive and to root than those from intensively growing shoots (from hedged donors, collected during the spring). Statistically significant genotype x season interaction was also found.

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Key words: *Cupressus sempervirens*, cutting propagation, genotype, indole-3-butyric acid, duration of rooting, time of cutting collection, hedging.

FDC: 232.328.1; 165.3; 165.44; 161.4; 181.36; 174.7 *Cupressus sempervirens*; (495.9).

Introduction

The Mediterranean cypress (*Cupressus sempervirens* L.), which is an irreplaceable part of the Mediterranean region, has been widely used since ancient times. It is a tree well adapted to calcareous, dry and poor sites, possessing valuable biological properties and exceptional timber quality. Extensive exploitation for millennia, the effect of recent pests like the cypress cancer caused by *Seiridium cardinale*, accompanied by the fact that *Cupressus sempervirens* has limited natural distribution compared with the other common softwoods, have imposed the necessity for the development of a breeding programme for this species.

The use of vegetative propagation is of vital importance to tree improvement and has become one of the most important tools of the tree improvement forester (ZOBEL and TALBERT, 1984). It can provide new clones of *Cupressus sempervirens* with superior growth, adapted to adverse ecological conditions and resistant or tolerant to *Seiridium cardinale,* which are needed for environmental protection, preservation of the traditional Mediterranean landscape, timber production and crop protection as windbreaks.

Natural cypress stands on the island of Crete have not suffered from mass infection by *Seiridium cardinale* yet (PAPAGEORGIOU, 1994) and their use in breeding for wood quality is recommended (PARASKEVOPOULOU, 1987). The present study investigates the variation in the cuttings rootability among different genotypes of the species of Cretan origin. The influence on the rooting success of other exogenous and endogenous factors, such as: auxin treatment, season of cutting collection, duration of rooting and preliminary rejuvenation of the donor plants, are studied as well.

Materials and Methods

The experiments were carried out in a rooting bed equipped with bottom heat and mist system in a greenhouse. Paper pots (5 cm/5 cm/10 cm) were used to insert the rooting material. They were filled with substrate, consisting of peat and perlite in 1:1 v/v. During the winter experiment, ground temperature of 22 °C ± 4 °C and ambient temperature of 21°C ± 5 °C were maintained. The spring experiments were performed at 24 °C ± 4 °C ground temperature and 25 °C ± 5 °C ambient temperature. The mist system was adjusted to maintain 95 ± 5% air humidity. A photoperiod of 18 hours was provided with a high pressure lamp (HPL-N, 400 W) installed at 1.5 m above the rooting bed.

The cutting donors were chosen among 4-year-old cypress seedlings grown in the nursery of the Mediterranean Agronomic Institute of Chania. Seedlot for the establishment of the plantation was collected from 9 natural provenances of *Cupressus sempervirens* from Western Crete. The seedlings were either from open-pollinated families (established in progeny test) or only their provenance origin was known (included in provenance test). The 220 tallest trees from the nursery plantation were exposed to preliminary rejuvenation by hedging twice, in December 1993 and then again in September 1994.

In Experiment 1, 15 genotypes (denoted as G1 to G15 – *Table 1*) from 6 provenances were used as cutting donors. They were chosen among the hedged seedlings considering as a criterion the possibility to provide sufficient number of good quality softwood stem cuttings for propagation. In Experiment 2, 6 of the 15 genotypes in Experiment 1 (1 per provenance) were repeated as ortets (*Table 1*). In Experiment 3, the rooting material was obtained from the lower part of 7 nonhedged seedlings (1.5 m to 2 m in height). The ortets (denoted as N1 to N7 – *Table 1*) were selected from different provenances, having intensive primary growth and providing sufficient number of cuttings.

Table 1. – Genotypes used in the rooting experiments.

Genotype	Provenance	Family abbreviation	Experiments in which the genotype was used
G1	Fress	U 2	1
G2	Zourva	Z 14	1
G3	Agia Erini	*	1, 2
G4	Fress	*	1, 2
G5	Prasses	D 2	1
G6	Prasses	D 10	1
G7	Prasses	D 15	1, 2
G8	Zourva	Z 5	1, 2
G9	Fress	U 11	1
G10	Omalos	H 5	1, 2
G11	Askifou	B 6	1, 2
G12	Askifou	B 10	1
G13	Agia Erini	*	1
G14	Omalos	H 4	1
G15	Fress	*	1
N1	Agia Erini	*	3
N2	Fress	F 26	3
N3	Prasses	D 17	3
N4	Zourva	Z 2	3
N5	Omalos	H 17	3
N6	Askifou	B 6	3
N7	Kalergi	*	3

* Only the provenance origin of the cutting donor is known

Experiment 1 was conducted during the winter (starting on 16 December 1994). The rooting ability of the 15 chosen genotypes of *Cupressus sempervirens* was studied at different concentrations of IBA and durations of rooting. Six of the genotypes (those which were included in Experiment 2) were also used in the study of the seasonal changes in the rootability. Experiments 2 and 3 were carried out during the spring (starting on 17 April 1995). The rooting ability of the genotypes at different concentrations of IBA was examined for the influence of the time of the cuttings collection (Experiments 1 and 2) and the type of the donor plant, either hedged or non-hedged ortets (Experiments 2 and 3).

Homogeneous softwood cuttings 12 cm to 15 cm in length and 2 mm to 4 mm in diameter were prepared. The cuttings from each genotype were grouped in three bundles, one of which was left as a control while the other 2 were treated by basal dip in concentrated solution (2000 ppm or 4000 ppm) of indole-3-butyric acid (IBA) for 5 seconds.

The bundles were left to dry for 2 minutes under cover and all 3 groups were swirled in captan powder (25% captan) before being placed in the rooting medium. During the period of the experiment the cuttings were sprayed with water solution of captan (1.5 g/l) at 2-week intervals. The application of combined NPK (20:5:30) fertilizer (2 g/l) at 10-day intervals started one and a half months after the initiation of the experiment and continued until the end.

The experiments continued for 3 (Experiments 2 and 3) to 4 (Experiment 1) months. By the end of the third month, all cuttings were examined for rooting and the percentage of rooting, percentage of callus formation, number of roots and length of the main root were recorded. The non-rooted cuttings of Experiment 1 were left in the rooting bed and checked for rooting and callus formation 1 month later.

The experiments were performed in split-plot design with 3 replications. The main plots-genotypes, arranged randomly

in each replication, were divided into sub-plots, randomly assigned to the 3 different treatments. The small size of the donor plants did not allow more than 3 cuttings per sub-plot to be used.

The data for Experiment 3 and for each rooting period of Experiment 1 (3 months and 4 months, respectively) were subjected separately to Analysis of variance (ANOVA) for split-plot design (STEEL and TORRIE, 1980; SNEDECOR and COCHRAN, 1989), in order to study the effect of genotype and auxin treatment. When significant, it was followed by DUNCAN's multiple range test at $p \leq 0.05$ for comparison of the means. Before the analysis of variance and DUNCAN's multiple range test application, the data concerning the variables rooting and callus percentages were subjected to arcsin squire root transformation (SNEDECOR and COCHRAN, 1989) to improve the normality. The small sample size used, suggested that the nonparametric statistical tests might be more appropriate. For each factor separately (genotype and treatment), WILCOXON rank-sum tests were performed. However, since the parametric and the nonparametric tests produced virtually identical results, only the results from ANOVA are presented and discussed. Variance component analysis was performed to locate and apportion the total variation.

WILCOXON rank-sum test was used for the comparative analyses studying the influence of the season and the preliminary rejuvenation on the rooting success. Analysis of variance for a split plot design in space and time (STEEL and TORRIE, 1980) was performed to study the effect of the duration of rooting period on the rooting and callus percentages (Experiment 1) and to check for interactions between the season and the other 2 factors (genotype and treatment) (Experiments 1 and 2).

All statistical analyses were performed using SAS 6.08.

Results

Experiment 1

As can be seen from *table 2*, the genotypes that had shown high rooting rates by the end of the third month, did not have a sufficient number of non-rooted cuttings left to contribute to any significant additional rooting 1 month later. Despite this fact, all genotypes studied exhibited an increase of rooted cuttings during the fourth month. The overall rooting and callus percentages appeared to be significantly affected by the duration of the rooting period (*Table 3*). During the fourth month, the non-treated cuttings showed 108% increase in rooting, while the cuttings treated by 2000 ppm and 4000 ppm IBA showed 17.24% and 37.5% increase, respectively. The overall increase in the rooting percentage was 41.73%.

Table 2. – Rooting rates (%) per genotype, treatment and rooting period (Experiment 1).

Genotype	Period of rooting											
	3 months						4 months					
	Treatment						Treatment					
	control		2000ppm		4000ppm		control		2000ppm		4000ppm	
G1	11	B[1]	56	AB	89	A	67	AB	56	ABC	89	AB
G2	11	B	22	B	22	BC	22	ABC	22	BC	22	CD
G3	33	AB	22	B	22	BC	44	ABC	22	BC	78	ABC
G4	67	A	89	A	89	A	78	A	89	A	100	A
G5	0	B	56	AB	33	ABC	11	BC	78	AB	56	ABCD
G6	44	AB	89	A	78	AB	67	AB	89	A	89	AB
G7	44	AB	56	AB	56	ABC	78	A	56	ABC	67	ABC
G8	0	B	33	AB	33	ABC	11	BC	56	ABC	44	ABCD
G9	22	AB	44	AB	33	ABC	33	ABC	44	ABC	33	BCD
G10	11	B	22	B	44	ABC	11	BC	33	ABC	56	ABCD
G11	22	AB	56	AB	33	ABC	33	ABC	67	ABC	44	ABCD
G12	0	B	22	B	67	AB	22	ABC	22	BC	78	ABC
G13	0	B	22	B	0	C	0	C	44	ABC	11	D
G14	0	B	56	AB	0	C	56	ABC	67	ABC	44	ABCD
G15	11	B	0	B	22	BC	44	ABC	11	C	44	ABCD
Average	18.52	A[2]	42.96	B	41.48	B	38.52	A	50.37	AB	57.04	B

[1] – The letters present the results from the DUNCAN's Multiple Range test ($\alpha = 0.05$) by rooting period and treatments (vertically). Means followed by the same letter are not significantly different.
[2] – The letters present the results from the DUNCAN's Multiple Range test ($\alpha = 0.05$) by rooting periods (horizontally). Means followed by the same letter are not significantly different.

Table 3. – F-test significance of Analysis of variance for repeated measurements for percentages of rooting and callussing (Experiment 1).

Dependent Variable	Source of Variation							
	R[1]	G[1]	Tr[2]	G*Tr[2]	T[3]	G*T[4]	Tr*T[5]	G*Tr*T[5]
Rooting (%)	ns	***	***	ns	*	ns	*	ns
Callusing (%)	ns	ns	ns	ns	*	ns	ns	ns

R - Replication; G - Genotype; Tr - Treatment; T - Time;
ns = $P > 0.05$; * = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$
[1] Error term = R*G
[2] Error term = R*G*Tr
[3] Error term = R*T
[4] Error term = R*G*T
[5] Error term = R*G*Tr*T

Some of the genotypes (G3, G14) changed conspicuously ranking of the rooting rates with the time. However, most of the genotypes were consistent and the interaction time x genotype as well as the triple interaction time x genotype x treatment were not significant. The time x treatment interaction affected the rooting percentage (*Table 3*) which was demonstrated by a change in ranking of the treatments at the end of the 4 month period (*Table 2*). At the end of the third month, 2000 ppm IBA treated cuttings rooted slightly better than those treated with 4000 ppm IBA, while for the longer period, 4000 ppm of IBA treatment showed higher rooting rates.

The analysis of variance applied to 3 and 4 months data separately, proved that the genotype is a major factor influencing the rooting (*Table 4*). Genotypes G4, G6 and G7 showed the highest rooting percentages for both time periods and were among the best rooted in all treatments (*Table 2*). The effect of treatment was also significant, but less pronounced for the longer rooting period (*Table 4*). The hormone-treated cuttings rooted significantly better than the nontreated ones at the end of the third month, while at the end of the fourth month significant difference in rooting was proved only between the nontreated cuttings and those treated with 4000 ppm IBA (*Table 2*). A change in the ranking position of some genotypes according to the different treatments was present for both time periods (*Table 1*). However, this change was not enough for significant genotype x treatment interaction (*Table 4*).

Table 4. – F-test significance of Analysis of variance for the studied variables at 3 and 4 month periods (Experiment 1).

Source of variation	3 month period				4 month period	
	Rooting (%)	Callus (%)	Number of roots	Length of the main root	Rooting (%)	Callus (%)
Replication [1]	ns	*	*	ns	ns	ns
Genotype [1]	**	*	***	***	**	ns
Treatment [2]	***	ns	***	***	*	ns
Genotype*Treatment [2]	ns	ns	ns	ns	ns	ns

ns = $P > 0.05$; * = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$
[1] Error term = Replication*Genotype
[2] Error term = Replication*Genotype*Treatment

The genotype accounted for 24.57% of the total variation of rooting at the end of the third month and for 22.21% of the variation at the end of the fourth month. The hormone application contributed by 12.73% and 4.87% to the variation at 3- and 4-month periods, respectively.

None of the studied factors affected the callus formation at the end of the fourth month, while only the genotype and the

replication showed significant effect on the callus percentage at the three month period (*Table 4*).

The genotype and the treatment were the major factors determining the number of roots and the length of the main root (*Table 4*), recorded by the end of the third month. The mean values of these 2 variables varied among the genotypes from 2 to 10 roots and from 3.7 cm to 8.1 cm main root. A higher number of roots was recorded for the hormone treated cuttings of almost all the genotypes.

The genotype accounted for 19.59% of the variation in the number of roots and for 35.64% of the variation in the length of the main root. The auxin treatment contributed to the variation in the root number and root length by 13.78% and 8.42%, respectively.

Experiment 2

Some of the cuttings started wilting and top-drying 3 weeks after the beginning of the experiment. Although infection by fungi was not found, at the end of the third month 75% of the propagules had dried. The final results recorded 1.85% rooted cuttings and 3.7% cuttings with callus (*Table 5*). Because of the unsatisfactory rooting, the collected data were used only for the comparative analyses, studying the influence of season and preliminary hedging on the rooting success.

Table 5. – Percentages of rooting and callus formation during the winter and spring experiments by treatments.

Treatment	Percentage of rooting		Percentage of callus	
	Winter (Exp.1)	Spring (Exp.2)	Winter (Exp.1)	Spring (Exp.2)
Control	29.63 *** [1]	0	7.40 ns	3.70
2000 ppm IBA	46.30 ***	0	7.40 ns	1.85
4000 ppm IBA	46.30 ***	5.56	11.11 ns	5.56
Average	40.74	1.85	8.64	3.70

1 – Wilcoxon rank-sum test for the variable season
ns = Z > 0.05; * = Z ≤ 0.05; ** = Z ≤ 0.01; *** = Z ≤ 0.001

Experiment 3

The percentages of rooting and callus formation for the different genotypes and treatments are shown in *table 6*. The genotype was the most important factor for rooting and callus formation (*Table 7*). The rooting percentage varied from 0% to 44.44% among the genotypes and N4 showed significantly higher rooting rate than the rest (*Table 6*).

Table 6. – Rooting rates (%) per genotype and treatment for Experiment 3.

Genotype	Treatment							
	control		2000 ppm		4000 ppm		Average	
N1	0	A[1]	56	A	11	B	22.22	B
N2	0	A	11	AB	33	AB	14.81	BCD
N3	0	A	22	AB	33	AB	18.52	BC
N4	11	A	56	A	67	A	44.45	A
N5	0	A	11	AB	11	B	7.41	BCD
N6	0	A	0	B	11	B	3.7	CD
N7	0	A	0	B	0	B	0	D
Average	1.59	A[2]	22.22	B	23.81	B	15.87	

[1] – The letters present the results from the DUNCAN's Multiple Range test (α = 0.05) by rooting period and treatments (vertically). Means followed by the same letter are not significantly different.
[2] – The letters present the results from the DUNCAN's Multiple range test (α = 0.05) by rooting periods (horizontally). Means followed by the same letter are not significantly different.

The auxin application improved rooting and the IBA-treated cuttings rooted significantly better than the nontreated ones (*Table 6*). Only N1 changed its ranking position according to the different treatments, and the genotype x treatment interaction was not statistically significant. All genotypes (except N1) had a maximum rooting rate at 4000 ppm of IBA treatment (*Table 6*).

The genotype accounted for 19.75% and the treatment for 14.19% of the total variation in the rooting percentage.

The factors genotype and treatment influenced the number of roots and the length of the main root (*Table 7*). The genotype contributed to the total variation in the number of roots and length of main root by 10.88% and 11.12%, respectively. The hormone treatment accounted for 7.67% of the variation in root number and 11.06% of the root length, respectively. The variable number of roots was also affected by the genotype x treatment interaction which contributed to its total variation by 26.63%. The average number of the roots varied from 1 to 3.3 among the genotypes and from 1 to 2.1 among the treatments. The mean length of the main root ranged from 4.6 cm to 6.3 cm among the genotypes and from 4.5 cm to 5.8 cm among the treatments

Table 7. – F-test significance of Analysis of variance for the studied variables (Experiment 3).

Dependent Variable	Source of Variation			
	R[1]	G[1]	Tr[2]	G*Tr[2]
Rooting (%)	*	***	**	ns
Callusing (%)	ns	*	ns	ns
Number of roots	ns	**	**	*
Length of the main root	*	*	*	ns

R - Replication; G - Genotype; Tr - Treatment;
T - Time;
ns = P > 0.05; * = P ≤ 0.05; ** = P ≤ 0.01;
*** = P ≤ 0.001
[1] Error term = R*G
[2] Error term = R*G*Tr

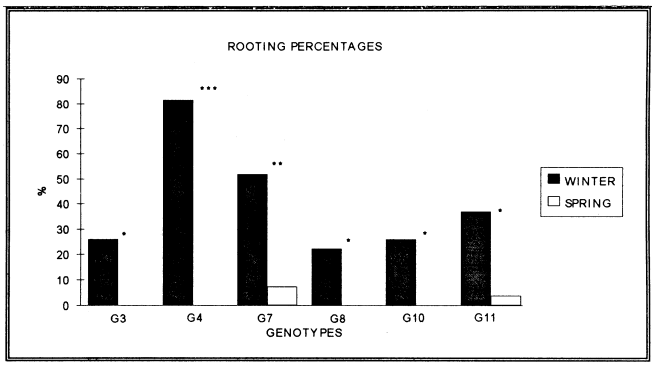
Comparative results

Season

The winter cutting collection was done when the cypresses were growing slowly, almost 1 month after the autumn peak of primary growth in November. The collection in spring took place during the period of intensive primary growth, before the spring peak, from succulent, fast developing shoots. The average percentages of rooting and callus obtained from the winter and spring experiments are presented in *table 5*.

The nonparametric statistical test showed that the cuttings collected during the winter (mid-December) rooted significantly better than those from the spring collection (mid-April) (Probability > |Z| = 0.0001). The overall rooting percentage from the winter experiment was 40.74%, while the spring experiment was 1.85% (*Table 5*).

Since the same genotypes were examined in both seasons, comparison at the genotype level was performed. All the



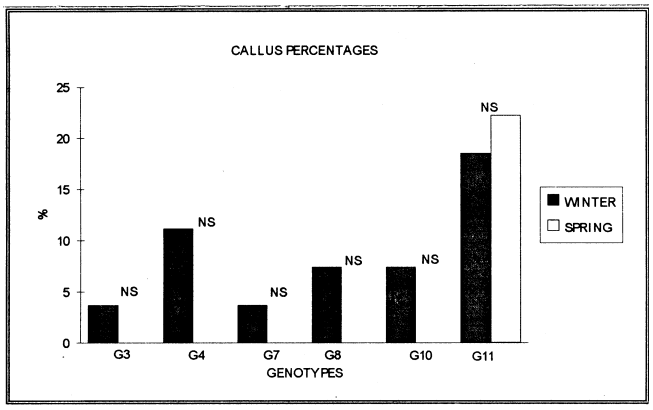
NS Z > 0.05; *Z < 0.05; **Z < 0.01; ***Z < 0.001

Figure 1. – Differences in the rooting rates between the winter and spring cutting collections at genotype level (Experiments 1 and 2).

genotypes exhibited significantly higher rooting rates during the winter, and for 2 of them (G4, G7) the difference was highly significant (*Figure 1*). Significantly higher rooting during the winter was present for all treatments (*Table 4*).

The analysis of variance revealed the presence of season x genotype interaction ($Pr > F = 0.0129$). Genotype 4 which had shown highest rooting rate during the winter, did not give any rooted cuttings in spring. On the other hand, G7 and G11 rooted well during both seasons.

The percentages of callus for the whole cutting material in the 2 seasons were significantly different, being higher during the winter ($Pr > |Z| = 0.006$). However, at treatment and genotype levels, the season did not seem to influence the formation of calli (*Table 5* and *Figure 2*).



NS Z > 0.05; *Z < 0.05; **Z < 0.01; ***Z < 0.001

Figure 2. – Differences in the callus percentages between the winter and spring cutting collections at genotype level (Experiments 1 and 2).

Preliminary hedging

The cuttings from nonhedged donors were obtained from the lower part of the crown. Under the shadow of the upper crown and the neighbouring trees the shoots were not growing intensively and had dark-green leaves. The hedged ortets were exposed to full sunlight, exhibiting fast growing, light-green, succulent shoots.

The statistical test showed significant difference in the rooting ($Pr > |Z| = 0.0003$) and callus formation ($Pr > |Z| = 0.0079$) between the cuttings from hedged and nonhedged donors. The propagation material from the nonhedged ortets resulted in 15.87% of rooted cuttings, while that from the hedged ortets only in 1.85%.

The nontreated cuttings from both categories donor plants rooted poorly and their rooting percentage did not differ significantly (*Table 8*). The auxin treated cuttings had significantly higher rooting rates when obtained from nonhedged ortets and only the cuttings from hedged donors treated with the higher auxin concentration showed some rooting (*Table 8*). Only the 2000 ppm IBA treated cuttings from nonhedged ortets formed a significantly higher number of calli in comparison with the other category (*Table 8*).

Table 8. – Percentages of rooting and callus by treatments for hedged and nonhedged donors.

Treatment	Percentage of rooting		Percentage of callus	
	Hedged (Exp.2)	Non-hedged (Exp.3)	Hedged (Exp.2)	Non-hedged (Exp.3)
Control	0 ns1	1.59	3.70 ns	7.94
2000 ppm IBA	0 **	22.22	1.85 *	15.87
4000 ppm IBA	5.56 *	23.81	5.56 ns	15.87
Average	1.85	15.87	3.70	13.23

1 – Wilcoxon rank-sum test for the variable hedging.
ns = Z > 0.05; * = Z ≤ 0.05; ** = Z ≤ 0.01; *** = Z ≤ 0.001

Discussion

Duration of rooting period

The periods usually used for rooting of *Cupressus sempervirens* cuttings are 3 (PANETSOS, 1993) to 4 months (CAPUANA and LAMBARDI, 1994). However, no experiments have been conducted to study the optimal duration of rooting required for this species. Some research work on this subject has been done for *Chamaecyparis* selected cultivars (OSIECKA, 1991). The experiment by JORDANOV (1992), who obtained up to 60% rooting of *Cupressus sempervirens* cuttings from 15 year old donor plants, keeping them in the rooting bed for 220 days, suggested that the duration of the rooting period could be of key importance for the species. Although the first experiment did not aim to study this problem in detail, it proved that the extension of the rooting period by one month could give 40% more rooted cuttings, which is a very high number for the difficult-to-root species such as *Cupressus sempervirens*. It could be noticed that the non-treated cuttings demonstrated a remarkable increase in rooting during the fourth month, which can be ascribed to delayed expression of the potential (inherited) genotype rootability. The results agree with the conclusions by JOHNSEN (1986) and OSIECKA (1991) that the effect of the root-promoting substances on final rooting is particularly pronounced when rooting period is short, since their action is primarily in the acceleration of rooting. In the present study this was especially the case with the lower hormone concentration (2000 ppm), while the higher (4000 ppm) showed a more prolonged effect.

All the genotypes showed an increase in rooting during the fourth month and may be of interest for future experiments. However, they could be classified in several categories according to the results obtained by the end of the fourth month. The well rooting genotypes (G1, G4, G6, G7) for which the profit from prolongation of the rooting period by one month is questionable, constitute the first category. The second category contains genotypes that rooted satisfactorily during the first 3 months and showed increase during the extension period. The genotypes that rooted poorly during the 3-month period and exhibited pronounced increase during the fourth month, constitute the third category. The poorly rooted genotypes, which were indifferent to time are classified in the fourth category. For the first category it could be recommended a new experiment limited to the time periods already examined, be aimed at evaluating the potential amount of rooting increase. The second and third categories require trials with more prolonged time for rooting, while with the fourth category should try higher auxin concentrations and treatment-time combinations.

Along with the quantitative change for time, a qualitative change was also noticed in some genotypes. In this sense, the genotypes could be divided into 2 main groups: 1) genotypes which showed time x treatment interaction and 2) genotypes which did not. Those genotypes that exhibited time x treatment interaction were unpredictable, especially in the cases when all the treatments showed some increase of the rooting percentage during the extension period. In this case, it could be recommended that the rooting period in the new experiments be extended, and the percentage of rooting for each treatment should be evaluated at 2 to 3 week periods (OSIECKA, 1991). It would also be useful to evaluate the potential anticipated increase by examining a higher number of cuttings, especially for the well rooting genotypes. Some of the genotypes (G5, G9, G11, G13, G14) were consistent, regardless of time and developed maximum rooting at 2000 ppm IBA treatment. Genotype G10 showed rooting percentages proportional to the

auxin concentrations and its response to concentrations above 4000 ppm IBA may be of interest.

Genotype

The results from the experiments showed conclusively that the basic rooting potential is determined by the genotype of the donor plant. The genotype effect was substantial for all investigated variables in agreement with previous findings on *Cupressus sempervirens* (CHEMLA, 1986; SINISCALCO and PAVOLETTONI, 1990; PANETSOS, 1993; CAPUANA and LAMBARDI, 1994) and other species (DAOUST *et al*., 1987; KIDOH *et al*., 1989; HENRY *et al*., 1992). The rooting percentages among the genotypes of Experiment 1 ranged from 7.41% to 81.48% (third month data), while those of Experiment 3, ranged from 0% to 44.44%. This was overcome neither by the applied treatments, in agreement with the study by CAPUANA and LAMBARDI (1994) (Experiments 1 and 3), nor by the extended rooting period (Experiment 1). The best rooting genotypes (G1, G4, G6, G7 – Experiment 1; N4 – Experiment 3) could be used for mass propagation, which is worthily especially for those originated by seeds, collected from the provenances Prasses (G6, G7 – Experiment 1) and Zourva (N4 – Experiment 3). The parental trees of their provenances grow well and form straight stems (PAPAGEORGIOU, 1994) having at the same time poor seed germinability (PAPAGEORGIOU, 1994). Considering the fact that the donor plants were chosen among the tallest, best growing trees in the nursery, further selection of trees combining good rootability and growth potential can be performed.

Auxin treatment

The results showed that hormone treatment influences the rooting rates significantly for some genotypes. This is in agreement with findings by other investigators (CHEMLA, 1986; SINISCALCO and PAVOLETTONI, 1990; PANETSOS, 1993; CAPUANA and LAMBARDI, 1994). IBA application increased the number of roots per cutting, as was found by CAPUANA and LAMBARDI (1994) and JORDANOV (1992). According to the experiments, the length of the main root also seems to be affected by the auxin treatment which disagrees with the conclusion by CAPUANA and LAMBARDI (1994) that the length of the roots is indifferent to the hormone stimulator. Two thousand ppm IBA treatment showed highest rooting rates in the 3-month rooting period of Experiment 1. Although this agrees with the study by PANETSOS (1993), the difference between the 2 auxin concentrations was not significant. For the 4 month period of Experiment 1 and for Experiment 3 (3 months rooting period), the rooting percentages appeared to be generally correlated with the IBA concentrations, as was also stated by CAPUANA and LAMBARDI (1994). However, there is always a strong effect of a particular genotype of the donor plant, as noted by SINISCALCO and PAVOLETTONI (1990) and CAPUANA and LAMBARDI (1994), and some of the genotypes rooted better at the lower auxin concentration.

Higher IBA concentrations [8000 ppm IBA (CHEMLA, 1986; BLYTHE, 1989); 10000 ppm and 15000 ppm IBA (CAPUANA and LAMBARDI, 1994)] have been examined and recommended in other investigations on *Cupressus sempervirens* cutting propagation. In all our experiments, satisfactory rooting (56% to 100%) was obtained for at least one of the treatments for more than half of the genotypes. However, the overall rooting percentages achieved (*Tables 2* and *6*) and the nonsatisfactory rooting of the other half of the genotypes, suggest that higher hormone levels could be tried, especially if mass propagation is needed.

It needs to be pointed out that the data for the variables number of roots and length of main root used in the analyses were correlated to the rooting percentages. Separate analyses using the values only for the rooted cuttings could not be performed because the relatively low rooting percentage would suppose highly unbalanced design, inappropriate for analyses. More correct and firm conclusions about the variation of these 2 variables characterising the root quality could be drawn, if they are studied through a specific design, as was done in the study by JOHNSEN (1986) on *Picea abies*. In the present experiments the best rooted genotypes do not always have the highest quality root system (data not shown).

Time of cutting collection

According to HARTMANN and KESTER (1983), when rooting narrow-leaved evergreens, best results may be expected if the cuttings are taken during the period from late fall to late winter. BROWSE (1985) recommended the same period of year particularly for the genus *Cupressus* and LAMB KELLY and BOWBRICK (1985) even specified February as the best time for cypress cutting propagation. The present study showed that the cuttings collected in December had higher survival and rooting percentages than those from the spring collection (April). The results are in agreement with the previously cited authors and with the finding by CHEMLA (1986) that the optimal seasons for rooting cypress were December and July, during the dormant or cessation periods of growth.

According to HARTMANN and KESTER (1983), the rank-growing, succulent tissues are likely to have low carbohydrate storage and high nitrogen content which is unfavourable for rooting. Such rapidly growing shoots may also be low in other components necessary for rooting. The authors recommended that shoots in which the growth has decreased and carbohydrates have accumulated should be selected for cutting material. RAUTER (1982) pointed out that since active shoot growth and foliage expansion often occur simultaneously with rooting, there is competition for the available carbohydrates. It should also be noted that the newly developing leaves are intensively transpiring and can remove the moisture from the cuttings before they have the opportunity to form roots, leading easily to death. Despite the mist system, the relatively higher medium and ambient temperatures during the spring experiment also promoted high transpiration rates and following desiccation of the propagules.

The above considerations seem to be a possible explanation for the results achieved in the present study. The winter collection of cuttings was done in a period of slow growth and when lignification had already begun, while the spring collection was from succulent, intensively growing shoots. The explanation of the results agrees with the findings on *Abies cephalonica* by PANETSOS et al. (1990). They found that the succulent and soft cuttings collected from growing shoots deteriorated before rooting, while cuttings collected 2 to 3 weeks after the end of shoot elongation exhibited the highest percentage of rooting. RAUTER (1982) and COUVILON (1988) also recommended late spring or early summer as appropriate times for cuttings collection as growth extension is complete and lignification is just starting.

According to the studies by LORENZI and CECCARELLI (1981), SINISCALCO and PAVOLETONNI (1990), and CAPUANA and LAMBARDI (1994), the period of spring growth, just after its initiation, gave best rooting of *Cupressus sempervirens* softwood cuttings. However, none of the cited authors has conducted experiments in mid-December. Also, the cutting material in their experiments originated from the lower parts of grafted plants, while in the present experiments the cuttings were collected from the upper part of hedged donors. In this

sense, WISE and CALDWELL (1992) stated that although hedging eliminates mature upper crowns that would produce cuttings with reduced rooting capacity, the resulting juvenile shoots may still be subject to seasonal fluctuations. The authors cite experiments with slash pine cuttings collected from hedged and nonhedged donors in which the results concerning the best time for cutting collection are contradictory.

The preconditioning of the ortets in the present experiments is more comparable with the research by PANETSOS (1993) on *Cupressus sempervirens* where hedged donors were used. The results from his study showed that the cuttings collected in early summer rooted better than those from the spring collection. The advantage of late summer cutting collection as opposed to the early spring one was shown also for *Chamaecyparis* selected cultivars (OSIECKA, 1991).

The results are further complicated by the presence of genotype x season interaction. The genotype which rooted best during the winter, did not root at all in the spring experiment. At the same time, despite the mass drying of the cuttings during the spring experiment, one of the genotypes (G11) kept most of its cuttings alive without rooting. Since different prerequisites, such as high food reserves, C:N ratio, higher auxin levels to low cytokinins, and gibberellins have to be satisfied in order to achieve good rooting, it is logical that the optimal condition for each particular genotype will be reached at a different moment.

Finally, it can be concluded that the time of year at which cuttings are collected is of key importance for the success of rooting. However, the optimum season for rooting is more related to the physiological condition of the plant than to any given calendar date. In this sense, the environmental preconditioning of individual donor plants (an effect referred to as common or "C" effect) must also be considered when the experiments are performed and conclusions are drawn. The results from the present study and the recommendations of the other investigators imply that a new trial should be done with a late spring cutting collection from the same donor plants. It would verify the reasons for the poor rooting achieved during the spring and would clarify the best time for cuttings collection in the particular conditions.

Preliminary rejuvenation of the donor plant

Hedging has for long time been recognised as a method to maintain juvenility of plants. However, this technique seems to cause some physiological changes in the ortets (WISE and CALDWELL, 1992), adversely affecting the rooting (PANETSOS, unpublished).

In the present study, the material from nonhedged donors showed significantly higher rooting percentage than the cuttings from the hedged ortets (15.87% vs. 1.85%). Despite this fact, firm conclusions can not be drawn about the advantages of nonhedged over hedged donors concerning the rooting rate, because the genotypes used from the 2 categories were not the same. However, the different morphological characteristics, the successive changes during the rooting, and the survival rate of the cuttings originated from the 2 type ortets, were striking. The cuttings from hedged donors were succulent, possessed characteristics of sun shoots and had fewer leaves. Soon after their insertion into the rooting medium they started drying from the top and at the end of the rooting period, there was 75% mortality. The cuttings from the nonhedged donors had a higher number of dark-green leaves. Only 25% of the cuttings had dried at the end of the third month.

Besides the effect of topophysis, ZOBEL and TALBERT (1984) recognised another cause of variation among the propagules obtained from a single donor plant and connected with their location on it. They called it periphysis, which refers to locations in different environments on an individual tree, such as shade and sun shoots. Even though the present rooting material was not obtained from the same plant, the shoots collected from the hedged donors could be classified as sun shoots and those from the nonhedged as shade shoots. In the present case, there were fast growing, succulent, low in carbohydrates shoots from hedged ortets on the one hand, and slowly growing, with high carbohydrate: nitrogen ratio shoots from nonhedged donors, on the other hand. As the nonhedged stock plants were positioned very closely, natural etiolation of the basal part of the crown may also have played a role in enhancing adventitious root formation (CAPUANA and LAMBARDI, 1994). It could be concluded that the cuttings from hedged donors exhibit different growth patterns than those from the lower shaded part of the nonhedged donors. Although they are thought to be more juvenile in character, they appeared to be more susceptible to and more dependent on the environmental conditions. In this case, the risk of getting lower rooting percentage (from the non-rejuvenated donors) is more justified than the risk of losing the whole cutting material. However, the question of whether the cuttings from hedged donors possess higher rootability than those from the young lower third of nonhedged donors has not been answered. The fact that cuttings from hedged ortets rooted only when treated with the higher IBA concentration casts doubt on the subject. Therefore, an experiment should be conducted with rooting material from the same genotypes but coming from both rejuvenated and nonrejuvenated ramets.

Acknowledgments

We are grateful to the Mediterranean Agronomic Institute of Chania where this research was conducted with the financial support of the EU Contract No AIR 3-CT 93-1675.

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Outcrossing Rates in Two Stands of Noble Fir (*Abies procera* REHD.) in Denmark

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(Received 4th March 1997)

Summary

The outcrossing rates in 2 stands of noble fir (*Abies procera* REHD.) in Denmark were determined from the segregation at 3 polymorphic allozyme loci in 20 progenies from 18 trees in each stand. The outcrossing rates were found to be slightly smaller than one: 0.98 and 0.90, of which only the latter value differed significantly from one. Noble fir therefore resembles other coniferous species by reproducing according to a mixed mating system, although with low level of selfing. The differentiation among the populations was relatively low, as commonly observed in wind-pollinated trees: the estimated F_{ST} value was 0.029.

Key words: Abies procera, mating systems, allozymes, gene diversity, population differentiation.

FDC: 165.4; 174.7 *Abies procera*; (489).

Introduction

Noble fir (*Abies procera* REHD.) has its natural distribution area in North America, mainly in the high elevations of the Cascade range in Oregon and Washington. It was introduced to Denmark in a small scale from the 1850s (LANGE, 1994; LARSEN, 1983). Since the 1950s, it has been planted widely in the Danish forestry, where it is used for production of Christmas greenery.

Noble fir has been reported to have relative low inbreeding depression in seed set and seedling survival (SORENSEN et al., 1976), but inbreeding depression, in vigor at a level commonly found in conifers (SORENSEN and MILES, 1982). A potential for natural inbreeding thus exists, and this concern initiated a study of selfing rates in a Danish seed orchard based on 100 clones (SIEGISMUND et al., 1996), because the mating system of this species had not been investigated previously. This study concluded that the progenies from the seed orchard clones where 100 % outcrossed.

Seed orchards are managed differently from traditional stands. One could expect the selfing rates to be higher in older and denser stands due to limited pollen movement compared to the widely spaced seed orchard with trees of lesser sizes. For this reason, the selfing rates in 2 traditional stands are estimated in the present study.

Materials and Methods

The stands

Two stands were selected for this study. One 52 year old stand (F587, [Danish seed stand approval number]) located within Ulborg State Forest District, and one 44 year old stand (F443) from Klosterheden State Forest District. Both stands are dense, but the Ulborg stand grows on a windy site in contrast to the Klosterheden stand, which grows on a more protected, gentle tract. The stands have known origin in older Danish noble fir stands from Buderupholm and Esrum State Forest Districts, respectively.

Seed collection and treatment

Cones were collected from 18 trees in each of the investigated stands in the fall of 1993, which was a year with a very dense cone crop and abundant flowering. The seeds were extracted from the cones and were stratified moist for about 6 weeks at 5 °C upon which they were germinated at room temperature. After 3 to 5 weeks the germinating seeds were partitioned into embryo and megagametophyte, which both were stored at −80 °C. From each tree 20 embryos and 10 megagametophytes were analyzed with enzyme electrophoresis. The genotypes of the megagametophytes were used to determine the genotype of the mother tree.

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