pallasiana var. pyramidata (YÜCEL, 1999). In this study, in accordance with the literature, the germination was inhibited by  ${\rm H_2SO_4}$  in all concentrations and concentrations of 1% and grater hindered the germination completely. HCl was seen to have inhibited germination at all concentrations, and stopping at 3% concentrations.

Among origins there was difference in terms of sensitivity towards NaCl, KNO $_3$  H $_2$ SO $_4$  and HCl (*Table 10*). Whether the seed and cone weight have effect on seed germination percentages was tested through regression analysis. According to this analysis, these is no significant relation between 1000 grain seed weight and seed germination percentage ( $r^2 = 0.07$ ). In relation to y = 0.1319 x + 94.4416 regression equation and as it is observed from F = 0.2276 and p = 0.6659 values, 1000 grain seed weight justifies the seed germination percentages. Similarly, according to y = 0.0001 x + 96.0699 regression equation, the cone weight does not justify the germination percentage (F = 0.1592, p = 0.7166,  $r^2 = 0.05$ ).

Preserving the plant gene resources is of great importance both for the continuation of biological variety and for economy. For this reason, Ebe black pine, which is under extinction, should be urgently put under protection through a program.

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# Factors Influencing Rooting in Cutting Propagation of Cypress (Cupressus sempervirens L.)

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## Abstract

Some aspects related to the rooting of cuttings of *Cupressus* sempervirens were examined in this work. They are of both scientific and practical relevance, dealing with the genetic variability of the rooting ability, the expression of this character in different periods of the year and the efficiency of the modality of hormone supplying.

A greater efficiency of indole-butyric acid given to the cuttings in the form of either potassium salt solution or talcum dispersion were found, compared to the alcoholic solution. The

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different treatments did non affect the number of adventitious roots per cutting and the mean root length.

A strong variability in the rootability of cuttings was found among different genotypes. In an experiment performed on April with 20 selected clones, the rooting percentage of the K-IBA-treated cuttings ranged between 0% and 88%, with most of the clones showing a rooting potential below 30%; in the same trial the best-rooting clone gave 34% rooting of nontreated cuttings. In another experiment performed on December the rooting was very poor for all the clones tested, whether for the treated or for the untreated cuttings.

With three clones characterised by a different rooting potential, the rootability of cuttings was detected, along the best rooting season, making a series of tests in which cuttings were collected at short intervals. A characteristic trend in the ability to root, in terms of natural and induced root potential and, particularly, in the expression of this potential along the period studied was observed in the clones tested.

Key words: Cypress, cutting, rooting, auxin, clone.

## Introduction

The selection of genotypes of Common Cypress (Cupressus sempervirens L.) resistant to Seiridium cardinale (Wag.) Sutt. & Gibs., a pathogen that has seriously damaged this species in the latest decades, is an important goal to achieve. It founds its bases on the exploitation of a wide inter- and intra-specific variation in resistance to the pathogen that has been detected in recent years (Andréoli, 1979; Raddi, 1979; Xenopoulos, 1990, 1991; Teissier du Cros et al., 1991). For this aim it is necessary to dispose of efficient propagation methods. These include both traditional techniques, as cuttings (Capuana and Lambardi, 1995; Stankova and Panetsos, 1997; Spanos et al., 1999) and new techniques from tissue culture (Fossi et al., 1981; Lambardi et al., 1995; Capuana and Giannini, 1997; Spanos et al., 1997).

The propagation of C. sempervirens by rooting of cuttings, however, is not routinely used as a means of fast nursery production, because of the lack of some important requisites that hinder an advantageous utilization of this method at a commercial scale. The unstable responsiveness to hormonal treatments, the different rooting capacity among clones, the difficulties of assessing the most suitable period for the collection of cuttings, are the main factors that strongly limit the diffusion of this propagation technique (LORENZI and CECCARELLI, 1981; HARTMANN et al., 1990; SINISCALCO and PAVOLETTONI, 1990; CAPUANA and LAMBARDI, 1995). The necessity of enhancing our knowledge on practical aspects of cutting propagation in cypress is at the origin of this study, whose principal aims were to assess the variability of both natural and induced rooting capacities of different genotypes as well as to identify the most favourable period for collecting cuttings and the most suitable hormone treatment.

A first experiment was performed during spring in order to evaluate the efficiency of three different types of hormone treatment. Subsequently the rootability of a large number of clones was tested. This experiment was repeated during winter to verify the influence of the period of collection (i.e. the vegetative state of the plant) on the rootability of cuttings. Finally, with three clones characterized by different rooting potentials, the rootability of cuttings was detected, along the best rooting season, making a series of tests in which cuttings were collected at short intervals.

## **Materials and Methods**

Cuttings were collected from the lowest third of the crown of 18-year-old grafted plants growing in a clonal orchard where a collection of clones were under selection for resistance to Seiridium cardinale.

Experiment 1 – On the last week of January, February, March, and April 1995, cuttings were randomly taken from different clones which had previously displayed a similar rooting attitude (data not presented). After collection, the cuttings were stored in plastic bags at 4°C for three hours. After they have been shortened to a 10 cm to 12 cm length and after removing leaves in their basal portion, the cuttings were subjected to three different indole-3-butyric acid (IBA) treatments, at two different concentrations (5 g l $^{-1}$  and 15 g l $^{-1}$ ). Preliminary experiments on IBA treatments had previously given indications on the choice of the hormone concentrations (data not shown). Treatments consisted of:

- IBA dispersion in talcum powder (cuttings were moistened with distilled water and the basal 2 cm were dipped in the powder; powder in excess was then gently shaken off);
- IBA in 35% alcohol solution (the basal 2 cm of the cuttings were dipped for 10 sec.);
- IBA -potassium salt water solution (K-IBA) (the basal 2 cm of the cuttings were dipped for 10 sec.).

Three replications of twelve cuttings per treatment per clone (plus one series of non-treated cuttings, as control) were realized. The cuttings were inserted at 5 cm x 5 cm spacing and 4 cm deep in a bed of moist perlite on a glasshouse bottom-heated bench and subjected to an intermittent mist system, whose frequency was related to the light intensity and allowed to keep cuttings visibly wet during the entire experiment. The temperature of the heated medium was fixed at  $23\pm2\,^{\circ}\mathrm{C}$  and the bench was covered, but not tightly sealed, with transparent polyethylene film. The cuttings received natural daylight during the rooting period, and the ambient temperature of the glasshouse was kept at a minimum of  $20\,^{\circ}\mathrm{C}$ . After 4 months, the cuttings were removed from the bench and evaluated for rooting. Cuttings were classified as rooted when at least one root longer than 1 mm was present.

Experiment 2 – On April, cuttings were taken from twenty different clones whether resistant or susceptible to the fungus. After collection, the cuttings were divided in two samples. A first series of cuttings was treated for 10 seconds with a 0.5% potassium salt solution of indole-butyric acid (K-IBA) and a second was left untreated.

The experiment was repeated on December 1995. The cuttings were collected from the same 20 clones tested on April and treated with the procedure previously described.

Each group of treated or non-treated cuttings consisting of 3 replications and 15 cuttings, a total of 1800 cuttings was prepared for each experiment.

Experiment 3 – In 1996, a new series of rooting tests was carried out, collecting cuttings at weekly intervals, during the most favourable period for rooting (late March to late April) from three clones (namely 771, 366, and 47) characterised by a different rooting potential (low, medium, high, respectively). Six collections of cuttings were performed, following the same procedure previously stated. For each collection, a first series of cuttings was treated for 10 seconds with a 0.5% potassium salt solution of indole-butyric acid (K-IBA) and a second was left untreated. Each sample of cuttings consisted of 3 replications x 15 cuttings.

In all experiments cuttings were taken from a similar range of types, sizes and positions of shoots. Fully randomised block designs were used in all experiments.

## Statistical analyses

Experiments 1 and 3: ANOVA analysis was performed on data after transformation of percentage (arcsin  $\sqrt{x}$ ) and sub-

sequent verification of their normal distribution. Significant differences were tested by Tukey test at  $p \le 0.05$ .

Experiment 2: rooting percentage data were evaluated by the Chi-square test for homogeneity of proportions.

#### Results

In Experiment 1, K-IBA and IBA in talcum dispersion resulted generally the best performing treatments, 0.5% K-IBA giving in all the months tested the highest rooting (significantly different from the control and from the IBA-alcohol treatments, in each month). The IBA-alcohol solution, on the contrary, induced the poorest rooting, especially at the highest concentration. The best rooting was obtained with the cuttings collected at the end of March, while the poorest was recorded on February (Figure 1). No interactions were found between treatments and collection's period (data from ANOVA analysis not reported). The number of adventitious roots per rooted cutting was significantly higher in the IBA-treated, compared with the untreated cuttings, while different treatments did non significantly affect this result, that, for the treated cuttings, was ranging from 2.1 to 2.6. No significant differences were found among different treatments for the mean root length, resulting between 41 mm and 52 mm (Table 1).

In Experiment 2, performed on April with 20 selected clones, non-treated cuttings from six clones rooted in a range between 12% and 34% (*Figure 2*). Rooting of the K-IBA-treated cuttings

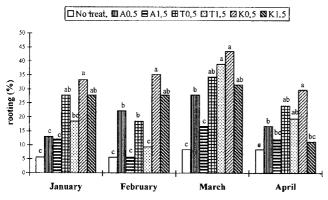


Figure 1. — Rooting of cuttings collected from January to April and treated with IBA, supplied, at 0.5% and 1.5%, by three different methods: alcohol solution (A), talcum dispersion (T), and potassium salt water solution (K). Tukey's test was carried out for each monthly collection at p=0.05.

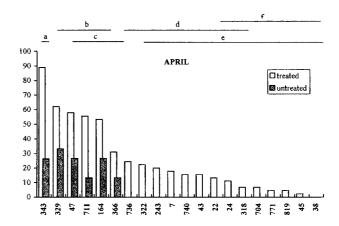
 $\it Table~1.$  – Number and length of roots of the IBA-treated cuttings of cypress.

IBA Treatment 1	Roots per rooted cutting	Root length
	(mean n° ± SE) <sup>2</sup>	(mean cm ± SE)2
No treatment	1.45 ± 1.8 a	4.08 ± 3.4 a
A 0.5	$2.58 \pm 1.4 \text{ b}$	$5.21 \pm 2.7$ a
A 1.5	$2.30 \pm 1.4 b$	$4.27 \pm 2.0 a$
T 0.5	$2.55 \pm 1.7 b$	$4.76 \pm 2.7 a$
T 1.5	$2.10 \pm 1.4 b$	$4.84 \pm 2.5 \text{ a}$
K 0.5	$2.08 \pm 1.4 \text{ b}$	$5.02 \pm 3.2 a$
K 1.5	2.28 ± 1.4 b	4.37 ± 2.4 a

<sup>&</sup>lt;sup>1</sup>) A = IBA (%) solution in 35% ethanol; T = IBA (%) in talcum powder; K = IBA-potassium salt (%) water solution.

ranged between 0% and 88%, with 5 out of the 20 clones achieving >50% rooting with 0.5% K-IBA; furthermore, the six clones which rooted without IBA were the six which rooted best with IBA. The rooting of cuttings collected on December from the same clones was very poor. Only few clones showed a small rooting capacity, always below 10%, whether for the treated or for the untreated cuttings.

In Experiment 3 on the weekly collections performed in spring, the three clones tested displayed a different behaviour.



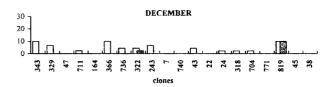


Figure 2. — Rooting of cuttings collected on April and December from 20 clones. Bars on the top refer to the differences among the IBA-treated clones in April (Chi-square analysis with mean separation at p=0.05).

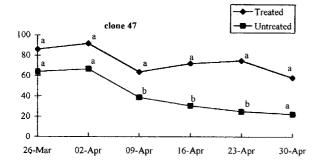
Clone 771 confirmed to have a low natural rooting potential, below 18%; the IBA-treated cuttings of this clone rooted consistently better when collected in the period from March 26 to April 9, reaching a peak of 80% rooting on April 2. In the clone 366 the existence of a certain rooting ability was stated in the whole period tested, ranging from 20% to 40%. All along this period the natural rooting capacity was improved by the hormone treatment, allowing from 45% to 75% rooting. The supposed best clone for rooting, number 47, showed a very high natural rooting capacity in the first two weeks considered; this capacity was further on improved by the hormone treatment leading to rooting higher than 90% for the collection of April 2. Even in the following weeks the rooting of cuttings was significantly improved after the hormone treatment (Figure 3).

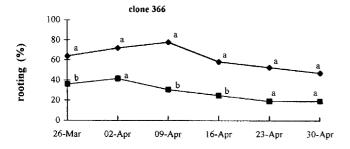
Cuttings generally developed a functional root system which allowed a normal growth of the plants after transfer to pots in a shaded area of the nursery.

## **Discussion and Conclusions**

The effectiveness of the hormone applications in promoting the adventitious rooting of cuttings is commonly accepted, even if the reproducibility of results is not easily achieved (LOACH, 1988). Most of the literature on the auxin treatments for adventitious rooting is focused on testing the efficiency of different types and concentrations of plant growth regulators, nevertheless, it must be stressed that the method used for

<sup>2)</sup> Different letters indicate significant differences after TUKEY test at p=0.05.





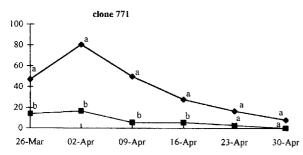


Figure 3. – Rooting of cuttings collected from three clones at weekly intervals in the period late March/late April. Letters on the lines indicate differences between treated and non-treated cuttings within each collection date, after Tukey's test at p=0.05.

their supplying can also affect the success of rooting. As an example, on a variety of species, as *Juniperus* and *Thuja* spp., and some deciduous shrubs, K-IBA proved to have a better effect than the IBA-alcohol treatment (CARPENTER, 1991). In our study, IBA supplied as potassium salt solution or in talcum dispersion showed a better effect on rooting than the treatment with alcoholic IBA-solution. On the other hand, it must be considered that the more pronounced decrease in rooting at the highest concentration of IBA in alcoholic solution raises the possibility that the lower concentration tested is also above optimum for the alcoholic formulation which is likely to penetrate cells at the base of the cutting more effectively than the aqueous solution (K-IBA).

It is well known that many factors concerning different aspects of the donor tree and the modalities of collecting cuttings can influence their rootability: for instance, the growth stage (hardwood, semi-hardwood, and softwood), and the type of cutting (distal and proximal portions of the stem), studied in *Cupressus arizonica* var. *glabra* by STUBBS *et al.* (1997), or the original position of the cuttings (superior or inferior part of the crown), observed in *C. sempervirens* by CAPUANA and LAMBARDI (1995) and the hedging of the donor plants (STANKOVA and PANETSOS, 1997). Above all, there is relatively strong genetic

control over rootability of cuttings (Farmer *et al.*, 1992), and a large variability of this character emerged among *C. sempervirens* clones (Siniscalco and Pavolettoni, 1990; Farmer *et al.*, 1992; Capuana and Lambardi, 1995; Stankova and Panetsos, 1997).

In our experiments, both natural (untreated cuttings) and induced (treated cuttings) rooting capacity displayed a variation related to genotype. In the clones characterised by a poor spontaneous rooting capacity, the hormonal treatment was not able to improve this tendency to levels of practical value, while for those clones displaying a higher natural rooting potential, the treatment strongly increased the percentage of rooted cuttings.

According to previous studies, the period of spring growth gave best rooting of Cupressus sempervirens softwood cuttings (LORENZI and CECCARELLI, 1981; SINISCALCO and PAVOLETTONI, 1990; CAPUANA and LAMBARDI, 1995). COUVILLON (1988) found that the period of active growth and starting of lignification corresponding to late spring-early summer has to be recommended. Nevertheless other authors stated that the best results for rooting of several Cupressus species could be achieved from late fall to late winter (Hartmann et al., 1990; Browse, 1985). With C. sempervirens, in particular, Stankova and PANETSOS (1997) obtained the highest rooting with cuttings collected in December from 4-year-old plants. It cannot be excluded that, in this latest work, the age of the ortet had an influence on the expression of the rooting potential along the year, nor that the lower air temperature and light conditions of December had, in our case, led to slower rooting.

We can also hypothesize that, within the most favourable period for rooting (to be intended as a physiological state of the mother plant), each genotype has a specific "rooting window", strongly variable in length, during which the cutting, as a consequence of the most favourable physiological condition, displays the greatest capacity to develop adventitious roots and/or is more responsive to the hormonal rooting treatment.

It must be finally remarked that further investigations, with the use of a larger number of cuttings per variant, are advisable in order to obtain more precise information on this latest aspect of the rooting process and its implications in the commercial exploitation of the cutting propagation of cypress.

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## Maximizing Gain at Restricted Group Coancestry in Selection from Populations with a Hierarchical Structure

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#### Abstract

A general model was derived to find a set of optimal family contributions within a single cycle of selection from populations with a strictly hierarchical structure. The model maximized genetic gain at restricted selection proportion and group coancestry, or minimized group coancestry at restricted selection proportion and genetic gain. Populations generated from single-pair/open-pollinated and nested mating designs, as special cases of hierarchical populations, were considered in order to exemplify optimal selection through numerical analyses and simulations. Numerical analyses were made with the assumption that family numbers were finite, while family sizes were infinitely large. Monte Carlo simulations generated breeding populations of finite family number and size. The contribution of a full-sib family was a function of within-family variation, the breeding values of the different types of families involved, and the constraints considered in optimization. Results concerning the optimal solutions were discussed in terms of selection intensity, group coancestry, heritability and

Key words: Breeding population, optimal selection, family contributions, gain, group coancestry, effective size.

## Introduction

Selection to improve mass performance involves two basic considerations. The first is how best to increase the expected genetic gain in the breeding population; various selection methods for this purpose have been proposed (e.g. Falconer and Mackay, 1996). Genetic gain increases when information on relatives is used to identify individuals with the highest breeding values (Lush, 1947; Osborne, 1957; Henderson, 1984; Falconer and Mackay, 1996). It is well established that BLUP is the best selection method in a single cycle of selection.

The second consideration is the increase in the level of group coancestry (Cockerham, 1967; hereafter, referred to as coances-

try for short unless otherwise noted) in the breeding population. This increase can hinder the realization of the expected gain in production populations and of the long-term breeding goals due to increased probability of inbreeding (depression) and the reduction of genetic variation for further selection. This issue is of particular significance for outcrossing species like forest trees. It is inevitable that selection increases coancestry or reduces genetic variability (BULMER, 1971; BURROWS, 1984; SANTIAGO and CABALLERO, 1995; WEI, 1995). In fact, the maximum gain by using BLUP is obtained at the expense of available genetic variances for later generations of breeding. There are selection methods that result in low or minimum coancestry. For instance, within-family selection leads to minimum coancestry (e.g. Wei, 1995; Wei and Lindgren, 1995). In addition to gain and coancestry, selection intensity is also often considered as an important factor in selection.

There are many studies on the effects of selection on genetic gain or coancestry alone (e.g. Lush, 1947; Osborne, 1957; Robertson, 1970; Bulmer, 1971; James, 1972; Burrows, 1984; Santiago and Caballero, 1995). Most of the practical applications of selection emphasized gain but gave little or no attention to the resultant increase in coancestry. Only recently have some studies compared selection alternatives, and developed selection methodology, to take account of both gain and coancestry (Toro and Perez-Enciso, 1990; Quinton et al., 1992; Wray and Goddard, 1994; Wei, 1995; Wei and Lindgren, 1995; Brisbane and Gibson, 1995; Villanueva and Woolliams, 1997; Meuwissen, 1997; Rosvall and Andersson, 1999). Coancestry consideration differs from situation to situation (e.g. among species). A method that allows breeders effectively and flexibly

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