The Effect of Fertiliser and Shading Treatments on Rooting Efficiency in Cuttings of the Cupressaceae

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(Received 19th July 1999)

Abstract

The effects of different fertiliser applications on rooting of cuttings taken from glasshouse-grown stock plants of *Cupressus sempervirens, C. torulosa, C. arizonica,* and *Chamaecyparis lawsoniana* was examined.

The response to fertilising of stock plants with macronutrients (NPK) or macronutrients plus microelements varied with species. Stock plant fertilising significantly reduced rooting rates in *C. sempervirens* cuttings, but significantly increased rooting in *C. torulosa, C. arizonica* and *C. macrocarpa* cuttings treated with rooting powder, in comparison to control stock plants treated with tap water. Fertilising of *Chamaecyparis lawsoniana* stock plants had no effect on rooting of cuttings.

Treatment of stock plants with increasing concentrations of nitrogen fertiliser ($(NH_4)_2SO_4$ in tap water) significantly affected rooting of most tested species. Nitrogen treatments, however, had no significant effect on rooting of *C. sempervirens* and *Chamaecyparis lawsoniana* cuttings. In cuttings of *C. torulosa, C. arizonica* and *C. macrocarpa,* however, the high nitrogen concentration (800 mg $N.l^{-1}$) significantly reduced rooting rates, whereas the lowest nitrogen concentration (100 $mg.l^{-1}$) induced maximum rooting.

Significant variability was found in rooting abilities of cuttings taken from stock plants of different *C. sempervirens* seed families. Shading of *C. sempervirens* stock plants significantly reduced rooting rates in certain families, but had no effect in other families.

Application of rooting powder (NAA-Captan mixture) to cuttings significantly increased rooting rates, and in *Chamaecyparis lawsoniana* significantly increased the number of roots per cutting, but had no effect on root length.

Key words: Cupressus, Chamaecyparis, nitrogen fertilisers, light conditions, vegetative propagation, cuttings, rooting, rooting powder.

Introduction

The Mediterranean Cypress, *Cupressus sempervirens* L., is of great importance in its native range, valued for both timber production and its importance as a landscape species. In recent years, however, populations of *C. sempervirens* have been severely damaged by cypress canker caused by *Seiridium cardinale* (WAG.) SUTT. & GIBS.. Both inter- and intraspecific variation in resistance to the pathogen has been reported in the Cupressaceae (ANDREOLI, 1979; GRASSO and PONCHET, 1979; RADDI, 1979; PONCHET and ANDREOLI, 1979; XENOPOULOS, 1990, 1991; TESSIER DU CROS *et al.,* 1991), exploitation of which may allow stands severely damaged by canker to be replaced. Proposed methods for increasing resistant genotypes selected from different provenances of *Cupressus* species have included both cuttings (CAPUANA and LAMBARDI, 1995; STANKOVA and PANETSOS, 1997) and tissue culture/micropropagation (THOMAS *et al.,* 1977; FOSSI *et al.,* 1981; LIPUCCI *et al.,* 1987; LAMBARDI *et al.,* 1995; CAPUANA and GIANNINI, 1997; SPANOS *et al.,* 1997a).

Propagation by cuttings is an inexpensive, rapid and simple method for increasing desirable genotypes of plants, and does not require the special techniques necessary in grafting, layering, budding or micropropagation. A successful method of rooting, although more expensive for some difficult-to-root species, is particularly useful for studies on broadsense heritability (LIBBY, 1974), enabling the establishment of clone banks or seed orchards (DORMAN, 1976) and testing the importance and effects of soil conditions, fertilisation regimes, growing space, and other environmental factors on the field performance of clones (LIBBY, 1974; CLARK and SLEE, 1984; ZOBEL and TALBERT, 1984). Such propagation methods often involve certain technical inputs and problems such as the need for an artificial environment, the potential susceptibility of rooted cuttings to pests and diseases, and abnormal growth in some species. Another serious problem in the rooting of cuttings is dependency on age of the stock plant. Cuttings from young trees will often root readily, but cuttings taken from mature trees may be almost impossible to root (WRITE, 1976; ZOBEL and TALBERT, 1984). 'Rejuvenation' of *Cupressus* stock plants by hedging or serial grafting has been shown to halt maturation or at least slow its rate (CALVANESE *et al.,* 1991). *C. sempervirens* has been micropropagated successfully from 150 year old trees, but rooting efficiency was very low compared with material from 5 week old or 15 years old trees (CAPUANA and GIANNINI, 1997).

Many factors are known to affect the success of propagation by cuttings. The physiological status of the stock plants may be manipulated by altering the watering and temperature regimes, use of supplementary lighting, use of carbon dioxide enrichment, application of mineral nutrients, girdling or hard pruning of stock plants, or critical timing of removal of cuttings (MACDONALD, 1986; HARTMANN *et al.,* 1990). Treatment of the cuttings following removal from stock plants is also of importance, including methods and length of storage, wounding of cuttings, application of growth regulator preparations, mineral nutrition during striking and leaching of nutrients from the substrate. Environmental conditions during rooting, such as humidity, temperature, light intensity and quality, and composition of the substrate, each also exert a major influence on rooting (MACDONALD, 1986; HARTMANN *et al.*, 1990).

This paper reports the effects of application of rooting powder, stock plant mineral nutrition and stock plant shading on the rooting capacity of stem cuttings taken from 8-month-old seedlings of *Cupressus sempervirens, C. torulosa, C. arizonica, C. macrocarpa* and *Chamaecyparis lawsoniana* as a part of the cypress breeding programme for resistance to *Seiridium* canker. Methods developed for these seedlings may be transferred to older plant material, or used to propagate more resistant individuals selected using advanced testing procedures (TONON,

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1994; TONON *et al.,* 1995; SPANOS and WOODWARD, 1997; SPANOS *et al.,* 1997b).

Material and Methods

Plant Material

Seed of *Cupressus sempervirens, C. torulosa, C. macrocarpa* and *C. arizonica* were obtained from Sandeman Seeds, West Sussex, England. *Chamaecyparis lawsoniana* seed was from stocks held in the Forestry Department, University of Aberdeen. Seed from different families of *Cupressus sempervirens* were supplied by the Forest District Office of Thessaloniki, Greece, and the Forest Research Institute in Athens, Greece: K1, K2 and K6 were taken from 3 individual trees showing phenotypic resistance to *Seiridium cardinale* from the Chania provenance, Krete; G1 were taken from a healthy tree in a mixed *Pinus brutia-C. sempervirens* stand in the Forest Park of Thessaloniki.

Seed were imbibed for 24 hours in tap water with a few drops of Tween 20 added as a wetting agent, soaked in 9% (w/v, 30% v/v) H_2O_2 for 30 minutes, rinsed in three changes of sterile distilled water and stratified in moistened peat in polythene bags for four weeks at 4 °C. After four weeks cold stratification seed were sown in 21.5 cm x 15 cm seed trays containing pure perlite and watered daily. Three weeks after sowing, seedlings were transplanted to 0.3 l plastic pots containing a loam/peat/sand mixture (7:2:3 v/v/v). Seedlings were protected from damping-off by watering with a tap water solution of Cheshunt compound (4 $g.l^{-1}$). After five weeks growth, seedlings were transplanted to either 0.5 l or 3.5 l plastic pots with the same potting medium.

Stock Plant Fertiliser Treatments

Two different stock plant fertiliser treatments were applied (*Table 1*). In the first treatment, stock plants in 3.5 l pots were supplied with one of two compound fertilisers, in 300 ml volumes per pot, at 7-day intervals over 3 months (September, October, November). Fertiliser 1 (F1) contained ($mg.l^{-1}$): N – 156; P – 69; K – 150. Fertiliser 2 (F2) was made by dissolving 1.5 g Phostrogen ™ in each litre of water, giving final concentrations of ($mg.l^{-1}$): N – 156; P – 69; K – 350; Mg – 20.3; Fe – 6.2; Mn – 0.3. Control plants were watered with 300 ml tap water.

In the second fertiliser treatment, plants growing in 0.5 l pots were each given 50 ml liquid N fertiliser at 7-day intervals over a 3 month period, as detailed above. N was applied as $(NH_4)_2SO_4$ (ammoniacal N: 21%) in 6 concentrations: 0, 50, 100, 200, 400 and 800 $mg.l^{-1}$ N (*Table 3*).

Preparation of Cuttings

Stem cuttings of 5 cm to 12 cm in length, with a basal diameter of 5 mm to 2 mm were taken in December 1992, from shoots arising from the lower half of the seedlings, using a sharp knife. Cuttings were rinsed in running cold tap water, the leaves from the lower 2 cm of the stem removed and discarded, and the cutting base briefly dipped (1 min) in 50% ethanol solution. Following rinsing three times in tap water, cuttings were dipped to a basal length of 1 cm in a commercial rooting powder ('Strike', May and Baker Ltd., Dagenham, England) containing naphthalene acetic acid (NAA) and Captan; control cutting were not treated with rooting powder.

Propagation Bench Conditions

Cuttings were inserted at 3 cm x 3 cm spacing directly into a peat-sand (1:1 v/v) mixture on a mist propagation bench in the glasshouse. Prior to insertion, and at 7-day intervals thereafter, the substrate was drenched with a proprietary formulation of benomyl. Air temperature on the mist bench ranged from 15 °C to 20 °C during the day and 10 °C to 15 °C at

Table 1. – Percentage rooting in cuttings of *Cupressus sempervirens, C. torulosa, C. arizonica, C. macrocarpa,* and *Chamaecyparis lawsoniana* from stock plants subjected to three fertiliser treatments. Cuttings were either treated (RP) or not treated (NT) with rooting powder containing naphthalene acetic acid mixed with captan, and were assessed for rooting 3 months after sticking.

	Rooting Rate (%)[1]					
	Fo[2]		F1		F2	
Species	**NT[3]**	**RP**	**NT**	**RP**	**NT**	**RP**
Cupressus sempervirens	40.5±13.3	58.1±4.2	8.3±0.0	29.8±3.2	16.7±2.4	38.1±2.4
Cupressus torulosa	33.3±9.6	38.9±5.6	22.2±10.0	66.7±12.7	19.5±2.8	80.6±12.1
Cupressus arizonica	11.1±2.8	33.3±4.8	25.0±0.0	69.4±15.5	13.9±2.8	38.9±2.8
Cupressus macrocarpa	13.9±2.8	38.9±13.9	27.8±10.0	72.2±5.6	27.8±2.8	58.3±4.8
Chamaecyparis lawsoniana	97.2±2.8	97.2±2.8	77.8±11.1	88.9±5.6	91.7±8.3	83.3±4.8

ANOVA for the rooting percentage of cypress cuttings.

Source of variation	DF	SS	MS	F	P[4]
Block	2	1347.1	673.6	4.52	0.015 *
Tree species	4	41607.9	10402.0	69.78	0.000 ***
Fertilizing	2	107.3	53.7	0.36	0.699 n.s.
Auxin	1	13396.8	13396.8	89.87	0.000 ***
Species x fertilising	8	7935.8	992.0	6.65	0.000 ***
Species x auxin	4	3809.0	952.3	6.39	0.000 ***
Fertilis. x auxin	2	1394.4	697.2	4.68	0.013 *
Spec. x fert. x aux.	8	2084.4	260.5	1.75	0.107 n.s.
Error	58	8646.2	149.1		
Total	89	80328.8			

[1]) Data presented are the means of three blocks ± the standard errors. LSD (0.05) for means = 19.94.

[2]) Fertiliser regimes. Fo = Stock plants watered with tap water only; F1 = ($mg.l^{-1}$): N - 156; P - 69; K - 150; F2 = ($mg.l^{-1}$): N - 156; P - 69; K - 350; Mg - 20.3; Fe - 6.2; Mn - 0.3.

[3]) NT = Non-treated cuttings; RP = cuttings treated with rooting powder containing naphthalene acetic acid and captan.

[4]) *: $p<0.05$; ***: $p<0.001$; n.s.: $p>0.05$.

Table 2. – Number and length of roots formed on *Chamaecyparis lawsoniana* cuttings from stock plants subjected to three fertiliser treatments. Cuttings were either treated (RP) or not treated (NT) with rooting powder containing naphthalene acetic acid mixed with captan, and were assessed for rooting 3 months after sticking.

	Number and length of roots[1]					
	Fo[2]		F1		F2	
	NT[3]	RP	NT	RP	NT	RP
Number of roots per cutting[4]	4.1±0.2	6.6±0.9	5.9±0.7	7.7±0.6	5.2±0.5	7.6±0.8
Root length (mm)	62.8±3.9	65.3±8.9	60.9±5.9	59.4±6.0	59.1±6.1	66.3±2.3

ANOVA for the mean number of roots per rooted cutting of *Chamaecyparis lawsoniana.*

Source of variation	DF	SS	MS	F	P[5]
Block	2	0.141	0.071	0.05	0.956 n.s.
Fertilizing	2	6.522	3.261	2.09	0.175 n.s.
Auxin	1	22.826	22.826	14.62	0.003 **
Fertiliz. x auxin.	2	0.296	0.148	0.09	0.910 n.s.
Error	10	15.617	1.562		
Total	17	45.403			

Analysis of variance for the mean root length of *Chamaecyparis lawsoniana* rooted cuttings.

Source of variation	DF	SS	MS	F	P[5]
Block	2	2.396	1.198	1.18	0.345 n.s.
Fertilizing	2	0.474	0.237	0.23	0.795 n.s.
Auxin	1	0.344	0.344	0.34	0.572 n.s.
Fertiliz. x auxin.	2	0.570	0.285	0.28	0.760 n.s.
Error	10	10.117	1.012		
Total	17	13.902			

[1]) Means are based on the number of cuttings forming roots in three blocks ± the standard errors.

[2]) Fertiliser regimes. Fo = Stock plants watered with tap water only; F1 = (mg.l^{-1}): N - 156; P - 69; K - 150; F2 = (mg.l^{-1}): N - 156; P - 69; K - 350; Mg - 20.3; Fe - 6.2; Mn - 0.3.

[3]) NT = Non-treated cuttings; RP = cuttings treated with rooting powder containing naphthalene acetic acid and captan.

[4]) LSD (0.05) for mean number of roots per cutting = 2.28.

[5]) **: p<0.01; n.s.: p>0.05.

Table 3. – Percentage rooting in cuttings of *Cupressus sempervirens, C. torulosa, C. arizonica, C. macrocarpa* and *Chamaecyparis lawsoniana* from stock plants treated with increasing concentrations of N-fertiliser. Cuttings were either treated (RP) or not treated (NT) with rooting powder containing naphthalene acetic acid mixed with captan, and were assessed for rooting 3 months after sticking.

	Rooting Rate (%)[1]											
	0 mg.l^{-1} N[2]		50 mg.l^{-1} N		100 mg.l^{-1} N		200 mg.l^{-1} N		400 mg.l^{-1} N		800 mg.l^{-1} N	
Species	NT[3]	RP	NT	RP	NT	RP	NT	RP	NT	RP	NT	RP
Cupressus sempervirens	20.0±0.0	40.0±0.0	26.7±12.0	40.0±10.0	13.3±3.3	40.0±5.8	16.7±3.3	56.7±3.3	10.0±0.0	43.3±6.7	10.0±0.0	60.0±10.0
Cupressus torulosa	46.7±3.3	66.7±8.8	63.3±8.8	60.0±23.1	63.3±8.8	60.0±23.1	33.3±14.5	56.7±8.8	10.0±0.0	60.0±10.0	16.7±6.7	36.7±8.8
Cupressus arizonica	33.3±3.3	46.7±16.7	26.7±8.8	80.0±10.0	43.3±3.3	80.0±10.0	26.7±3.3	83.3±3.3	33.3±3.3	76.7±3.3	26.7±3.3	30.0±5.8
Cupressus macrocarpa	43.3±12.0	60.0±5.8	33.3±8.8	73.3±14.5	40.0±15.3	90.0±5.8	20.0±5.8	56.7±8.8	26.7±12.0	60.0±5.8	13.3±3.3	16.7±3.3
Chamaecyparis lawsoniana	76.7±8.8	86.7±8.8	86.7±3.3	90.0±5.8	63.3±17.6	93.3±3.3	70.0±5.8	83.3±12.0	73.3±12.0	90.0±5.8	73.3±8.8	83.3±6.7

ANOVA for the rooting percentage of cypress cuttings.

Source of variation	DF	SS	MS	F	P[4]
Block	2	567.8	283.9	1.29	0.279 n.s.
Tree species	4	48552.2	12138.1	55.16	0.000 ***
Nitrogen	5	8637.8	1727.6	7.85	0.000 ***
Auxin	1	30942.2	30942.2	140.62	0.000 ***
Species x nitrog.	20	10901.1	545.1	2.48	0.001 **
Species x auxin	4	2418.9	604.7	2.75	0.031 *
Nitrogen x auxin	5	2477.8	495.6	2.25	0.054 *
Spec. x nitr. x aux.	20	7327.8	366.4	1.67	0.049 *
Error	118	25965.6	220.0		
Total	179	137791.1			

[1]) Data presented are the means of three blocks ± standard errors. LSD (0.05) for means = 23.74.

[2]) Stock plants growing in 0.5 l pots were treated with 50 ml tap water (0 mg.l^{-1}) or 50 ml tap water containing an amount of $(NH_4)_2SO_4$ to give the indicated concentrations of N in solution.

[3]) NT = Non-treated cuttings; RP = cuttings treated with rooting powder containing naphthalene acetic acid and captan.

[4]) *: p<0.05; **: p<0.01; ***: p<0.001; n.s.: p>0.05.

night. Under-soil heating cables maintained a constant 25 ± 3 °C throughout the rooting period. Supplementary lighting was provided by two 40 W fluorescent lamps, giving an average light intensity of 4 W.m^{-2} bench, with a 24 hour photoperiod.

Cuttings were examined for rooting 3 months after sticking, by washing under cold tap water to remove adhering substrate. Rooting was defined as the emergence of one or more roots of 5 mm or greater in length from the basal 1 cm of the cutting. Numbers of cuttings with roots were recorded for all species tested. As cuttings of *Chamaecyparis lawsoniana* rooted readily, the number of roots per cutting, and root length were recorded in addition to percentage cuttings forming roots.

Effects of Shading on Rooting of Cupressus sempervirens Cuttings

Stock plants of *C. sempervirens* var. *horizontalis* seedlings K1, K2 and K6, raised from the 3 Chania provenance (Krete) trees, and tree G1, from the Forest Park of Thessaloniki, growing in Jiffy 7 peat pellets were divided into two groups containing equal numbers of plants and subjected to two different lighting regimes for three months. One group was maintained on the glasshouse bench, in full light (average daylight intensity = 6 $W.m^{-2}$ to 7 $W.m^{-2}$). The second group of seedlings was grown under shading (S) provided by wooden slats, giving an average light intensity of 0.6 $W.m^{-2}$ to 0.9 $W.m^{-2}$. At the end of the three month period, cuttings were removed from the seedlings, as described above, treated or not treated with rooting powder and inserted into rooting compost. Cuttings were examined for rooting 3 months after sticking, as described above.

Experimental Design

Factorial randomised block designs were used in all experiments. In the NPK fertiliser experiments, the five tree species, three fertilising treatments and two auxin (rooting powder) treatments were replicated randomly in three blocks, giving a total of 90 (5 x 3 x 2 x 3) plot treatments, with 12 cuttings per plot. For the experiments using different N application rates, five tree species, six nitrogen treatments and two auxin treatments were replicated randomly in three blocks, giving a total of 180 (5 x 6 x 2 x 3) plot treatments with 10 cuttings per plot. The experiments investigating the effects of stock plant shading had four stock plant families of *C. sempervirens,* two illumination treatments and two auxin treatments, repeated in three blocks, giving a total of 48 (4 x 2 x 2 x 3) plot treatments, with 30 cuttings per plot.

Data were analysed using ANOVA. Paired comparisons were made by testing for least significant difference (LSD).

Results

Effect of NPK Fertilising of Stock Plants on Rooting of Cuttings

The application of the two NPK fertiliser regimes to stock plants resulted in significantly different rooting rates ($P<0.001$) between species (*Table 1*). Application of rooting powder to cuttings significantly increased ($P<0.001$) the percentage rooting of cuttings in all species tested, with the exception of *Chamaecyparis lawsoniana,* where the treatment had no significant effect.

Highest rooting rates for *C. sempervirens* were obtained in cuttings from stock plants watered with tap water only, regardless of rooting powder application. With *C. torulosa, C. arizonica* and *C. macrocarpa,* however, treatment of stock plants with fertilisers, coupled with application of rooting powder to cuttings, resulted in more than twice as many cuttings rooting for each species. This effect was most striking with cuttings of *C. torulosa* from stock-plant fertiliser treatment F2 (Phostrogen), where large increases in rooting rates, from 19.5% in untreated to 80.6% in rooting powder-treated cuttings, occurred.

In *Chamaecyparis lawsoniana,* treatment of stock plants with fertilisers prior to removal of cuttings caused decreases in the number of cuttings forming roots ($P>0.05$). Maximum rates of rooting (97.2%) were obtained in cuttings from tap water-treated stock plants, whereas fertiliser treatments suppressed rooting rates. In most cases, *Chamaecyparis lawsoniana* cuttings rooted significantly more readily ($P<0.05$) than *Cupressus* cuttings.

Application of NAA-Captan rooting powder to cuttings of *Cupressus* species resulted in highly significant increases in rooting rates compared with non-treated cuttings, regardless of stock-plant fertiliser treatment ($P<0.001$). Treatment of cuttings from all stock-plant fertiliser treatments with rooting powder resulted in much higher rates of rooting for all *Cupressus* species tested, compared with control cuttings from each fertiliser treatment (*Table 1*). In contrast, with *Chamaecyparis lawsoniana* application of rooting powder did not increase the number of cuttings developing roots. Moreover, in this species, stock-plant treatment F2 decreased the number of cuttings developing roots when rooting powder was applied ($P>0.05$).

Fertilising stock plants with NPK had no significant effect on the number of roots per cutting and root extension in *Chamaecyparis lawsoniana* cuttings ($P>0.05$; *Table 2*). However, treatment of cuttings with rooting powder increased significantly the number of roots per cutting ($P<0.01$; overall mean 7.30±0.42), compared with control cuttings (overall mean 5.04±0.37) (*Table 2*). In all stock plant treatments cuttings treated with rooting powder developed more roots (Control and F2: $P<0.05$, F1: $P>0.05$) than control cuttings. No significant increases in root length were observed in *Chamaecyparis lawsoniana* cuttings treated with rooting powder.

Effect of Increasing Concentrations of N Fertilising of Stock Plants on Rooting of Cuttings

The effects of applying increasing N concentrations to stock plants on rooting of cuttings varied between the 5 species tested (*Table 3*). Cuttings of *Cham. lawsoniana* showed the most consistent and highest rates of rooting of the 5 species, regardless of N concentrations applied to stock plants.

Application of rooting powder to cuttings of *Cupressus* spp. significantly increased rooting rates, irrespective of stock-plant nitrogen fertiliser treatment ($P<0.001$).

In the absence of rooting powder application, *C. sempervirens* cuttings taken from stock plants treated with 50 $mg.l^{-1}$ N showed maximum rooting. In cuttings treated with rooting powder, however, highest rates of rooting were obtained when stock plants were treated with 200 $mg.l^{-1}$ N or 800 $mg.l^{-1}$ N. In *C. torulosa* without rooting powder treatment, cuttings from stock plants treated with 100 $mg.l^{-1}$ N gave maximum rates of rooting (63.3%) whereas the higher concentrations of N, 400 $mg.l^{-1}$ and 800 $mg.l^{-1}$, resulted in the lowest rates of rooting, 10.0% and 16.7%, respectively. Where *C. torulosa* cuttings were treated with rooting powder equal rooting rates ($P>0.05$) were found with 0, 50, 100, 200 and 400 $mg.l^{-1}$ N, whereas cuttings from the 800 $mg.l^{-1}$ treatment had the lowest rooting rate (36.7%). Cuttings of *C. arizonica* from stock plants treated with 50 $mg.l^{-1}$ N or 100 $mg.l^{-1}$ N and dipped in rooting powder had the highest rooting rates for this species. Application of rooting powder to cuttings of *C. macrocarpa* significantly increased rooting rates in all N-treatments, including 0 $mg.l^{-1}$ N, with the exception of cuttings from stock plants treated with 800 $mg.l^{-1}$ N.

Application of increasing concentrations of N to *Cham. lawsoniana* stock plants had no significant effects on rooting rates, irrespective of treatment with rooting powder (*Table 3*; $P>0.05$). In the absence of rooting powder treatment, increasing concentrations of N caused a decrease in the numbers of cuttings rooting in *Cham. lawsoniana.* When rooting powder was applied to cuttings of this species, the highest rates of rooting were obtained from stock plants treated with 100 $mg.l^{-1}$ N. Rooting rates for cuttings of *Cham. lawsoniana* were significantly higher than for those of the four *Cupressus* species ($P<0.05$).

Concentration of N applied to stock plants had no significant effect on the mean number of roots formed, or the length of roots produced on cuttings of *Cham. lawsoniana* (*Table 4*). Sig-

nificantly more roots were produced, however, on cuttings of this species treated with rooting powder (P<0.05). This effect was particularly marked in cuttings from stock plants treated with 100 mg.l^{-1} N.

Effects of Shading on Rooting of Cupressus sempervirens Cuttings

There were highly significant differences (P<0.001) in rates of rooting between the different *C. sempervirens* families, irrespective of stock plant shading treatment (*Table 5*). Of the four families tested, cuttings of the family K2 rooted most readily (P<0.01), followed by family G1, K1 and K6. Responses to stock plant shading varied within the different families. Although rooting rates increased slightly in families K1 and K6 from shaded stock plants, when cuttings were not treated with rooting powder, this increase was not significant (P>0.05). The decrease in rooting of K1 and K6 cuttings from shaded stock plants treated with rooting powder, compared with cuttings of the same families from stock plants grown in full illumination also was not significant (P>0.05). In contrast, stock plant shading decreased significantly (P<0.05) the rooting of K2 and G1 cuttings in comparison with cuttings from stock plants grown in full illumination. The highest rooting rates of K2 and G1 cuttings were obtained with rooting powder-treated cuttings from stock plants grown in full illumination (82.2% and 74.5%, respectively).

Application of rooting powder to the cuttings resulted in highly significant increases (P<0.001) in rooting rates of all *C. sempervirens* families, regardless of stock plant illumination.

Discussion

Differences in the abilities of stem cuttings from forest trees to form roots have been reported to be strongly related to species (ZOBEL and TALBERT, 1984; HARTMANN *et al.*, 1990). Cuttings of some species root very easily, for example *Populus* and *Salix* spp., whereas cuttings from other species are very difficult to root, for example *Abies* and *Pinus* spp.. Clonal differences in the ability to form roots on cuttings of *Pinus nigra* have also been reported (SPANOS, 1992).

Differences in rooting percentage between the five cypress species tested in the current study were highly significant, irrespective of stock plant treatment. Of the five species tested, cuttings of *Chamaecyparis lawsoniana* formed roots most readily. In most stock plant treatments, cuttings of *C. sempervirens* rooted less readily than cuttings of the other *Cupressus* species. Highly significant differences in rooting rates were also found between the four *Cupressus sempervirens* families tested. Moreover, in the same provenance (Krete island) of *Cupressus sempervirens* stock plants, significant differences were recorded between seed families in relation to rooting ability of cuttings.

Responses to stock plant fertiliser treatments of the tested tree species varied. Application of NPK fertiliser, with or without micronutrients, to *C. sempervirens* stock plants significantly decreased rooting rates in comparison to tap water-treated stock plants. In contrast, application of NPK plus micronutrients fertilisers to *C. torulosa, C. arizonica* and *C. macrocarpa* stock plants significantly increased the rooting of cuttings treated with rooting powder. With *Chamaecyparis lawsoniana,* treatment of stock plants with NPK fertiliser had no effect on rooting rates in comparison to the control treatment. In this species, stock plant fertilising had no significant effects on the number of roots per cutting or length of roots formed. HARTMANN *et al.* (1990) reported that stock plants of woody species grown under mineral deficiencies (e.g. P, K, Mg, Ca, Zn) usually produce cuttings with poor root systems. The small effect of stock plant fertilising on the overall rooting may have resulted from the cultivation of stock plants in a substrate apparently without mineral deficiencies, at least at the time of potting.

Fertilising stock plants with nitrogen alone significantly affected the rates of rooting of cuttings in *C. torulosa, C. arizonica* and *C. macrocarpa,* but not in *C. sempervirens* and *Cham. lawsoniana.* The highest nitrogen concentration treatment, 800 mg.l^{-1}, significantly decreased rooting rates in *C. torulosa, C. arizonica* and *C. macrocarpa* cuttings in comparison to the other treatments. Treatment of stock plants with 100 mg.l^{-1} nitrogen gave the highest rates of rooting, particularly when combined with dipping of cuttings in rooting powder. Stock

Table 4. – Number and length of roots formed on *Chamaecyparis lawsoniana* cuttings from stock plants treated with increasing concentrations of N-fertiliser. Cuttings were either treated (RP) or not treated (NT) with rooting powder containing naphthalene acetic acid mixed with captan, and were assessed for rooting 3 months after sticking.

	Number and length of roots[1]											
	0 mg.l^{-1} N[2]		50 mg.l^{-1} N		100 mg.l^{-1} N		200 mg.l^{-1} N		400 mg.l^{-1} N		800 mg.l^{-1} N	
	NT[3]	RP	NT	RP	NT	RP	NT	RP	NT	RP	NT	RP
Number of roots per cutting[4]	4.4±0.8	5.5±0.3	3.7±0.2	4.9±0.5	4.0±0.7	5.6±0.8	3.8±0.2	4.2±0.2	4.4±0.1	4.9±0.7	4.0±0.3	4.7±0.8
Root length (mm)	4.2±1.1	4.9±0.7	5.4±0.9	5.8±0.3	4.6±0.8	5.2±0.5	4.9±0.4	5.8±0.8	5.2±0.7	5.0±0.7	4.2±1.2	4.8±0.7

ANOVA for the mean number of roots per rooted cutting of *Chamaecyparis lawsoniana.*

Source of variation	DF	SS	MS	F	P[5]
Block	2	0.6494	0.3247	0.36	0.705 n.s.
Nitrogen	5	4.0573	0.8115	0.89	0.506 n.s.
Auxin	1	7.2810	7.2810	7.97	0.010 *
Nitrogen x auxin	5	1.5089	0.3018	0.33	0.889 n.s.
Error	22	20.0991	0.9136		
Total	35	33.5957			

ANOVA for the mean root length of *Chamaecyparis lawsoniana* cuttings.

Source of variation	DF	SS	MS	F	P[5]
Block	2	7.745	3.872	2.36	0.118 n.s.
Nitrogen	5	5.781	1.156	0.71	0.626 n.s.
Auxin	1	2.641	2.641	1.61	0.218 n.s.
Nitrogen x auxin	5	1.117	0.223	0.14	0.982 n.s.
Error	22	36.069	1.639		
Total	35	53.352			

[1]) Means are based on the number of cuttings forming roots in three blocks ± the standard errors.

[2]) Stock plants growing in 0.5 l pots were treated with 50 ml tap water (0 mg.l^{-1}) or 50 ml tap water containing an amount of $(NH_4)_2SO_4$ to give the indicated concentrations of N in solution.

[3]) NT = Non-treated cuttings; RP = cuttings treated with rooting powder containing naphthalene acetic acid and captan.

[4]) LSD (0.05) for mean number of roots per cutting = 1.69.

[5]) *: p<0.05; n.s.: p>0.05.

plant nitrogen – fertiliser treatments did not affect significantly the number of roots per cutting or length of roots formed in *Cham. lawsoniana* cuttings. The results of this work support the findings of HAISSIG (1974, 1989) and MASON (1989) who suggested that moderate nitrogen and adequate carbohydrate levels in the stock plants present the best nutritional status for the optimum rooting of gymnosperm cuttings. In general, the high nitrogen stock plant treatment, 800 mg.l^{-1}, had a negative effect on rooting capacity in most cypress species, whereas lower nitrogen concentrations resulted in higher rates of rooting. Increased nitrogen content in the tissues of stock plants used for cuttings may have decreased the carbon-nitrogen ratio, an important factor in the rooting of cuttings (HAISSIG, 1974, 1989; HARTMANN *et al.,* 1990).

Heavy shading of stock plants caused a significant decrease in rooting of cuttings from seed families K2 and G1, but had no significant effects on rates of rooting in cuttings of families K1 and K6. Exclusion of light during stock-plant growth is known to increase rooting of cuttings in some difficult to root woody species (HARTMANN *et al.,* 1990). Exclusion of light or short days (8 hours to 10 hours daylight) influences the levels of phenolic compounds and gibberellin-like substances which may act as rooting co-factors or inhibitors of natural auxin production in the plant (NANTA *et al.,* 1974; WHITEHILL and SCHWABE, 1975). In the experiments reported in this paper, these compounds may have acted as rooting inhibitors and reduced the rooting capacity of cuttings from seed families K2 and G1 of *C. sempervirens,* although it is not clear why this effect was significant only in these two families. Moreover, compared to cuttings taken from stock-plants growing under shade, the carbohydrate content of cuttings taken from stock-plants growing in full light would have been higher, because of increased photosynthesis leading to a higher carbon-nitrogen ratio, more favourable to rooting (HAISSIG, 1974c, 1989; HARTMANN *et al.,* 1990).

Treatment of cuttings from *Cupressus* species with rooting powder containing NAA plus Captan significantly increased the rates of rooting. Treatment of *Cham. lawsoniana* cuttings with rooting powder, however, did not affect significantly rooting rates, but did significantly increase the number of roots per cutting. The results of these experiments support the findings of several other authors. Plant growth regulators of the auxin group, such as indole-butyric acid (IBA) and NAA are known to increase rooting rates, numbers of roots per cutting and root length in cuttings of gymnosperms (LIBBY and CONKLE, 1966; GIROUARD, 1974; MAYHEAD and OFESI, 1989; SPANOS, 1992). Generally, NAA is more effective than IBA at low concentrations in rooting of gymnosperms cuttings (HARTMANN *et al.,* 1990). Addition of fungicides such as captan or benlate to rooting powder preparations of auxin in talcum powder increased the rooting percentage of *Pinus strobus* cuttings (THIELGES and HÓITINK, 1972). In these experiments the mixture of NAA and captan proved to be highly effective in promoting rooting of cypress cuttings.

Other measures not tested in this work may also improve rooting rates of cuttings. Extending the period for rooting from 3 months to 4 months has recently been reported to increase rates of rooting in *C. sempervirens* (STANKOVA and PANETSOS, 1997), although these authors used IBA as the auxin treatment, rather than NAA as used here. Moreover, the season in which *C. sempervirens* cuttings were taken had a significant effect on the success of rooting, with shoots taken from older trees in winter rooting more readily than similar material collected in spring (STANKOVA and PANETSOS, 1997).

Results presented in earlier publications from this work (SPANOS, 1995; SPANOS and WOODWARD, 1997; SPANOS *et al.,* 1997a, b), coupled with those described in this paper, suggest that young seedlings of *Cupressus* species may be screened for their resistance to *Seiridium* at less than two years of age using glasshouse (SPANOS, 1995) and *in vitro* techniques

Table 5. – Effects of stock plant shading on percentage rooting rates of cuttings from four different seed families of *Cupressus sempervirens.* Families K1, K2 and K6 were from trees in the Chania provenance (Krete); G1 was from a tree growing in the Thessaloniki Forest Park. Cuttings were assessed for rooting 3 months after sticking.

		Rooting Rate (%)[1]	
Seed Family	**Stock plant treatment[2]**	**NT[3]**	**RP**
K1	Open bench	26.7±3.9	52.2±7.8
	Shaded	32.2±4.0	41.1±4.8
K2	Open bench	63.3±6.9	82.2±9.1
	Shaded	43.3±1.9	61.1±6.2
K6	Open bench	15.6±1.1	43.3±6.9
	Shaded	25.6±4.0	38.9±2.9
G1	Open bench	40.0±3.3	74.5±6.2
	Shaded	26.7±1.9	57.8±9.5

ANOVA for rooting of *Cupressus sempervirens* families.

Source of variation	DF	SS	MS	F	P[4]
Block	2	1209.12	604.56	9.96	0.000 ***
Seed family	3	6926.20	2308.73	38.04	0.000 ***
Illumination	1	948.30	948.30	15.63	0.000 ***
Auxin	1	5926.30	5926.30	97.65	0.000 ***
Family x illumin.	3	1040.54	346.85	5.72	0.003 **
Family x auxin	3	463.23	154.41	2.54	0.075 n.s.
Illumin. x auxin	1	237.14	237.14	3.91	0.057 n.s.
Fam. x illumin. x aux.	3	137.01	45.67	0.75	0.530 n.s.
Error	30	1820.72	60.69		
Total	47	18708.55			

[1]) Data presented are the means of three blocks ± standard errors. LSD (0.05) for means = 12.90.

[2]) Stock plant treatments: open bench in the glasshouse, light intensity = 6 W.m^{-2} to 7 W.m^{-2}; shading (below wooden slat, light intensity = 0.6 W.m^{-2} to 0.9 W.m^{-2} s). Cuttings were removed after 3 months of treatment.

[3]) NT = Non-treated cuttings; RP = cuttings treated with rooting powder containing naphthalene acetic acid and captan.

[4]) **: $p<0.01$; ***: $p<0.001$; n.s.: $p>0.05$.

(TONON, 1994; TONON *et al.,* 1995; SPANOS and WOODWARD, 1997; SPANOS *et al.,* 1997b), and that the more resistant genotypes can be vegetatively propagated using either *in vitro* (SPANOS *et al.,* 1997a) or glasshouse-based techniques, depending on the facilities and technical expertise available. In addition, further refinement of both the *in vitro* and *ex vitro* vegetative propagation protocols may enable more efficient propagation from resistant genotypes.

Acknowledgements

K.A. SPANOS was supported by a scholarship from the Greek Government. The technical assistance of JANIS BRODIE and DOUGLAS PROUDFOOT is gratefully acknowledged.

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