

Gebiet erfolgen. Obwohl die Herkunft IUFRO Nr. 2066 aus British Columbia *Inonoaklin* eine Inlandform ist, hat sie sich als gutwüchsig und als widerstandsfähig gegen abiotische Schäden gezeigt. Ferner kommt diese Herkunft aus einem nördlicheren Gebiet vom 49° Breitengrad (Das Saatgut der Herkunft Nr. 2146 aus British Columbia kommt aus der Saatgutzone 1040, das Saatgut der Herkunft Nr. 2150 kommt aus dem gleichen Staat aus der Saatgutzone 1020).

3. Im Osten des Schwarzmeergebietes sollte man in den Höhenlagen zwischen 1200 m bis 2000 m, wo aus verschiedenen Gründen die Aufforstungen keinen Erfolg hatten, trotz allem einer potentiellen Aufforstung mit den oben genannten *Pinus contorta* Herkünften Platz geben, um wenigstens 10% der Holzproduktion für Papier zu decken.

4. Es ist festgestellt worden, daß sich *Pinus contorta* Herkünfte auch über der Baumgrenze von 2500 m im Osten des Schwarzmeer Gebietes anbauen lassen (EYÜBOĞLU, 1986). Nach den Feststellungen von EYÜBOĞLU gab es auf den Flächen über der Baumgrenze beim Wachstum zwischen den Herkünften kaum einen Unterschied. In diesen Gebieten ist es empfehlenswert, sich auf Herkünfte zu stützen, die Überlebenschancen haben und daher hat man die Herkunft Idaho - Fremont empfohlen. Diese Herkunft kommt vom 40° Breitengrad und aus 2300 m Höhe ü. NN. Bei den für Saatguttransfer benutzten Grundregeln geht man von der Hypothese aus, daß für je 100 m Höhenstieg sich der Breitengrad um 1° Grad erhält; davon ausgesehen mußten diese Herkünfte so behandelt werden, als kämen sie aus einem nördlichen Gebiet von 63° Breitengrad. Aus diesem Grunde kann man sie auch außerhalb der Waldgrenze des östlichen Schwarzmeer Gebietes in 2500 m pflanzen. Grundsätzlich kann die *Pinus contorta* unter schlechtesten Bedingungen, sogar als Pionierbaum gepflanzt werden. Im Osten des Schwarzmeergebietes, über der Baumgrenze, wo das nahe dem Wald auf Almen lebende Volk seinen Brennholzbedarf mit Überfällen auf die produktiven Fichtenwälder deckt, in der Absicht diese zu verringern, hat man es für möglich gehalten, die an diese Wälder angrenzende Baumgrenze mit *Pinus contorta* Herkünften aufzuforsten.

5. Bei *Pinus contorta* Aufforstungen ist der Verband zwi-

schen den Pflanzen sehr wichtig, darum muß zuerst der Verband festgestellt werden. Bei *Pinus contorta* Aufforstungen in höheren Lagen hat man einen Verband von 2 × 2,5 m als geeignet angesehen.

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## Genetic Variability of Tolerance to Cold in *Cupressus sempervirens* Progenies

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#### Summary

The Italian cypress (*Cupressus sempervirens* L.), originated in the eastern mediterranean countries, gradually diffused its way northward encountering harsher winters and was subjected to a moderate selective pressure for tolerance to the cold. There is frequent damage to the cypress in Tuscany (Italy) during the winters and early springs. In January, 1985, the temperatures in Florence and the surrounding areas dropped to -20°C for a few days. The severity of the temperature damaged cypress planta-

tions in a number of ways. As a result a study was conducted to determine the genetic variability of the cypress for tolerance to the cold. The study was undertaken on trees derived from controlled crosses which were carried out for research on the cypress' resistance to *Seiridium cardinale* — a common wound pathogen and agent of the cortical canker.

The most important results are:

- the tolerance level of progenies derived from controlled crosses between Italian cypresses naturalized in Tuscany was higher than that of progenies derived from seed collected in natural stands in Greece and in Turkey;
- heritability of tolerance to the cold presents a high value

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(0.94 ± 0.31) and some parents show a good general combining ability.

The high heritability value along with high GCA estimates allows us to develop seed orchards of Italian cypress with the prospect of obtaining progenies which are tolerant to cold and therefore enlarging the area of cultivation northward with regard to this species.

*Key words:* Frost tolerance, Italian cypress progenies, genetic parameters.

### Introduction

The Italian cypress (*Cupressus sempervirens* L.) occurs in natural populations of xeroheiiophilous phytoassociations of the northern hemisphere (Joubert and Burollet, 1934) were the amount of annual rainfall fluctuates from 600 mm to 900 mm with an irregular distribution. In these areas there are long periods of summer drought and mild winters. The Italian cypress is native of the mediterranean basin (Iran, Lebanon, Syria, Israel, and Turkey), Cyprus, some of the islands of Greece, and Libya. From these countries which are characterized by a mediterranean climate, the Italian cypress moved northward. It is possible that it may have been introduced in to Italy by Phoenicians. It was already known by the Etruscans and was spread over the country by the Romans. The Italian cypress can be considered as a naturalized species in Italy and in some localities, especially in Tuscany, it forms pure stands with a natural regeneration. During this adaptation process towards the regions with more rigorous winters, the Italian cypress has suffered damage from recurring cold waves. In Italy, the Italian cypress is considered a quiescent species, in that it quickly moves out of a state of rest when environmental conditions allow (in winter); it is also a dormant species in the summer dry period in that will move out of dormancy only when experiencing a protracted period of favourable photoperiodic or environmental conditions (Gellini and Grossoni, 1980; Lipschitz *et al.*, 1981; Grossoni and Gellini, 1984). For this reason these authors were of the opinion that cold tolerance depends on the tree's physiological condition when the temperatures fall uncharacteristically below certain levels. Dugelay (1957) studied the effects of the February frost of 1956 and found negligible damage on the Italian cypresses grown in the Maritime Alps region. Those cypresses that were grown as windbreaks in the Var region, on the other hand, were highly damaged. In laboratory research Larcher (1975) established that temperatures below -14°C damaged the Italian cypress, whereas Puric (1967) did not find any cold damage on old Italian cypress in Belgrade with protracted temperatures below -20°C. Pavari (1934), Merendi (1961) and other Italian and French silviculturists (personal comm.) noted that Italian cypresses originating from natural stands have been decimated by winter cold in several experimental fields located in Europe. Seedlings raised from seeds collected in naturalized cypress groves in Italy and in France, however, suffered insignificant amounts of damage when they were subjected to rigorous winters.

In Italy during January 1985, temperatures fell below -20°C for many days causing severe damage to the Italian cypress in several localities on the plains and foothills. In Tuscany frost damage caused the death of many Italian cypresses, especially those located at the bottom of a valley or on flat areas. In some cases, damage was reduced or restricted to either the upper part of the crown or limited to the lower part of the crown. In other cases frost damage cut down the seed production drastically. Severe damage

was always observed in the nursery on young seedlings and grafted cypresses. These observations indicated that a variability for tolerance to cold is present among cypress species and individual plants of the same species.

### Materials and Methods

Data used in this study were recorded from an Italian cypress breeding project with the purpose of selecting cypresses resistant against cypress canker disease caused by *Seiridium cardinale* (Wag.) Sutton and Gibson. The rigorous temperatures of January 1985 demonstrated a difference in degree of frost tolerance among the cypresses tested. Consequently, a study on the genetic variability of frost tolerance in Italian cypresses seems possible.

Trees in naturalized stands in Tuscany were selected for their demonstrated resistance level to cypress canker disease following the IUFRO sampling rules. For purposes of the present study, trees can be considered as random members of the population with respect to frost tolerance. Controlled pollinations were made in the spring of 1980 following the mating design North Carolina II suggested by Comstock and Robinson (1952) and the analysis methods reported by Bingham *et al.* (1969) in order to estimate the principal parameters of the trait "cypress canker resistance". Thirty-four different parents (30 trees were used as female and 4 as males) were involved in a total of 120 matings. The mother trees were characterized by three different level of resistance to cypress canker disease as shown in Table 2. Trees are designated 'S', susceptible to the disease; 'I' an intermediate level of resistance; 'R' a high level of resistance. The four testers (trees used as males) were designated 'S', I-1, I-2 and 'R'. Seeds were sown in the spring of 1982 in paperpots (diameter 3 cm, height 15 cm). In March 1983 the seedlings were transplanted in fertipots (diameter 10 cm, height 18 cm) and in the autumn of the same year transplanted in two experimental fields: Monte Morello (Florence) at 600 m asl and Mondeggi (Florence) at around 50 to 100 m asl, both at latitude 43°40' N. The experimental design was a randomized block design at each plantation. The seedlings were planted in row plots of 10 trees for each of the 120 combinations with 4 replications. Rows were 3 m apart and trees were 1 m apart in the rows. The project involved a total of 9 600 seedlings (120 matings × 4 blocks × 10 trees/block × 2 localities). The planting sites were completely cleared and cultivated prior to planting and were weeded and cultivated at least once a year after planting. These conditions are unusual for the cypress and could have influenced the results.

Trees which survived the rigorous temperatures of January 1985 were counted for each plot one year after. The proportion of survivors per plot was calculated as follows:

$$P_{mfk} = \frac{n_{mfk}}{t_{mfk}}$$

where  $n_{mfk}$  is the number of surviving seedlings for the  $m^{\text{th}}$  tester crossed to the  $f^{\text{th}}$  candidate in the  $k^{\text{th}}$  replicate; and  $t_{mfk}$  is the total number of seedlings in the group before the frost.

Since  $t_{mfk}$  was small in this test (10 or fewer seedlings per plot) and the percentages fluctuated widely, adjustments were made as suggested by Bartlett (1947) to make the variances more representative. If the number of survivors was equal to the total number of seedlings, then

$$P_{mfk} = 1 - \frac{1}{4 t_{mfk}}$$

If the number of surviving trees was zero, then

$$P_{mfk} = \frac{1}{4 t_{mfk}}$$

Because the  $P_{mfk}$ 's were binomial, they were transformed to arcsins to stabilize the variances (BARTLETT, 1936) and the homogeneity of variances tested by BARTLETT'S test. In this study  $\chi^2$  was calculated as equal to 53.03, which was not significant. The statistical model assumed that the testers and the candidates were random samples from populations described above and the replications were fixed. The formula for this model is:

$$X_{mfk} = \mu + \alpha_m + \beta_f + (\alpha\beta)_{mf} + R_k + e_{mfk}$$

where  $X_{mfk}$  = the transformed proportion of surviving trees from the cross of the  $m^{\text{th}}$  tester and the  $f^{\text{th}}$  candidate in the  $k^{\text{th}}$  replication;

- $\mu$  = general mean;
- $\alpha_m$  = effect of the  $m^{\text{th}}$  tester ( $m = 1, \dots, 4$ );
- $\beta_f$  = effect of the  $f^{\text{th}}$  candidate ( $f = 1, \dots, 30$ );
- $(\alpha\beta)_{mf}$  = effect of the interaction of the  $m^{\text{th}}$  tester and the  $f^{\text{th}}$  candidate;
- $R_k$  = effect of the  $k^{\text{th}}$  replication ( $k = 1, \dots, 4$ );
- $e_{mfk}$  = effect of the environmental variability and that of the residue of the genetic deviations among fullsibs in the same plot.

COCKFIRHAM (1963) listed five assumptions which must be made when this model is used to estimate the genetic parameters. In this study we consider that the validity of these assumptions is satisfied. Perhaps the assumption that the parents represent a random sample of the population is satisfied only for the cypress population in Tuscany, which has already undergone a natural selection for cold tolerance. Moreover, these candidates and testers can be considered as noninbred because they are more than 50 m apart.

In the locality of Monte Morello (600 m asl) we did not note any consistent damage caused by frost. The analysis of variance was based on the data measured in only one locality.

In Table 1, the calculated variances were given along with the estimates of the variance components and their standard errors obtained using the formula of ANDERSON and BANCROFT (1952). The heritability estimate was based on the combination of variances due to the genetic effects of the tester plants ( $\delta_m^2$ ) and of the mother trees ( $\delta_f^2$ ), and

on the genetic model of the covariances among individuals belonging to the fullsib groups (BECKER, 1984). The standard error of heritability was calculated using the formula suggested by FALCONER (1970).

Estimates of the general combining ability (GCA) and of the specific combining ability (SCA) were determined for the testers and the mother trees applying the formula suggested by ZOBEL and TALBERT (1984).

## Results

Analysis of data variance for the proportion of cypresses surviving frost among the progenies shows that the effects due to the tester plants, mother trees and their interaction were highly significant (Table 1). The variance components and the heritability estimate are reported in the same table.

Value of the GCA of frost tolerance expressed as deviations from the overall mean for both parents are reported in Table 2. A positive GCA value indicates a parent that produce above-average performance of the progeny in relation to the general mean of the tested population for tolerance to frost. Table 2 also shows the values for the SCA, the average performance of the progeny of a cross between two specific parents that are different from what would be expected on the basis of their general combining abilities alone.

The correlation coefficient was found to be  $r = 0.09$ , not significant. It was calculated on the basis of the mean percentage of frost survivors for each mother tree for all testers and the degree of cypress canker resistance of the mother trees (for this calculation we assigned a value of 1 point to resistant trees, 2 points to trees with an intermediate resistance and 3 points to susceptible trees).

## Discussion

### Variability of frost tolerance

The January 1985 frost was very severe and lasted several days with temperatures falling below  $-20^\circ\text{C}$ . In these severe temperatures, Italian cypresses derived from seed imported from natural stands in Greece and Turkey and trees of the following species of *Cupressus* (*C. abramsiana*, *C. atlantica*, *C. cashmeriana*, *C. dupreziana*, *C. goveniana*, *C. lusitanica*, *C. macrocarpa*, *C. pygmaea*) were decimated. By contrast, progenies obtained from controlled crosses between candidates selected in Tuscany exhibited a sufficient tolerance to the frost. Moreover, we must remember that although frost tolerance in the cypress tends to in-

Table 1. — Analysis of variance of adjusted and transformed data for proportion of seedlings surviving frost.

Sources of variation	d.f.	M.S.	F	Expectation M.S.	Variance components and S.E.
Total	mfk-1	479			
Replications	k-1	3			
Testers	m-1	3	11,798.4	152.7**	$\delta_w^2 + k \delta_{mf}^2 + kf \delta_m^2$ $\delta_m^2 = 94.41 \pm 62.18$
Candidates	f-1	29	1,455.7	18.8**	$\delta_w^2 + k \delta_{mf}^2 + km \delta_f^2$ $\delta_f^2 = 61.70 \pm 23.52$
Tester x candidate	(m-1)(f-1)	87	488.5	6.0**	$\delta_w^2 + k \delta_{mf}^2$ $\delta_{mf}^2 = 97.81 \pm 17.61$
Tester-candidate combinations x replications	(mf-1)(k-1)	357	77.3		$\delta_w^2$ $\delta_w^2 = 77.30$

$$V_p = \delta_m^2 + \delta_f^2 + \delta_{mf}^2 + \delta_w^2 = 331.25 \quad h_{m+f}^2 = 2(\delta_m^2 + \delta_f^2)/V_p = 312.22/331.25 = 0.94 \pm 0.31$$

Table 2. — Means, general combining abilities (GCA), and specific combining abilities (SCA) of the candidate trees, expressed in adjusted and transformed data (percent of seedlings surviving frost).

Overall mean = 42.50											
Tester cross performance			I-1	$\bar{x} = 57.11$	GCA = 14.61	I-2	$\bar{x} = 41.44$	GCA = - 1,06	R	$\bar{x} = 36.18$	GCA = - 6.32
			S	$\bar{x} = 35.18$	GCA = - 7.32						
Candidate	Mean	GCA	Tester I-1		Tester R		Tester I-2		Tester S		
			Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	
1 R*	33.93	- 8.57	57.08	8.54	22.88	- 4.73	24.12	- 8.75	31.65	5.04	
2 I	56.03	13.53	70.07	- 0.57	55.27	5.56	56.43	1.46	42.35	- 6.36	
3 S	26.43	-16.07	40.61	- 0.43	20.90	0.79	17.89	- 7.48	26.32	7.21	
4 S	56.21	13.71	51.70	-19.12	56.94	7.05	72.65	17.50	43.55	- 5.34	
5 I	48.68	6.18	58.21	- 5.08	51.70	9.34	41.24	- 6.38	43.55	2.19	
6 R	27.65	-14.85	31.78	-10.48	21.80	0.47	30.48	3.89	26.78	6.45	
7 R	43.80	1.30	45.00	-13.41	49.41	11.93	45.54	2.80	40.27	3.79	
8 S	59.29	16.79	68.58	- 5.32	57.53	4.56	61.66	3.43	49.39	- 2.58	
9 I	39.04	- 3.46	38.18	-15.47	34.78	2.06	34.56	- 3.42	48.69	16.97	
10 R	49.74	7.24	64.34	- 0.01	42.85	- 0.57	42.83	- 5.85	48.94	6.52	
11 I	52.19	9.69	70.77	3.97	55.97	10.10	44.36	- 6.77	37.66	- 7.21	
12 I	39.47	- 3.03	56.46	2.38	35.69	2.54	29.36	- 9.05	36.36	4.21	
13 S	26.09	-16.41	29.67	-11.03	14.40	- 5.37	31.17	6.14	29.14	10.37	
14 R	50.35	7.85	54.64	-10.32	61.18	17.15	32.46	-16.83	53.14	10.11	
15 S	47.22	4.72	76.51	14.68	49.39	8.49	34.56	-11.60	28.44	-11.46	
16 R	44.28	1.78	69.33	10.44	36.64	- 1.32	28.61	-14.61	42.56	5.60	
17 I	30.72	-11.78	49.60	4.27	25.12	0.72	32.46	2.80	15.70	- 7.70	
18 S	47.25	4.75	57.21	- 4.65	36.54	- 4.39	45.00	- 1.19	50.24	10.31	
19 R	43.13	0.63	73.55	15.81	27.32	- 9.49	46.63	4.56	25.81	-10.00	
20 I	56.82	14.31	81.16	9.73	49.39	- 1.11	56.05	0.29	40.67	- 8.83	
21 S	44.50	2.00	61.20	2.09	21.05	-17.13	59.97	16.53	35.78	- 1.40	
22 S	45.08	2.58	61.55	1.86	49.94	11.18	49.32	5.30	19.51	-18.25	
23 R	35.82	- 6.68	46.06	- 4.37	33.30	3.80	47.94	13.18	16.97	-11.53	
24 R	45.06	2.56	75.45	15.78	26.19	-12.55	39.67	- 4.33	38.95	1.21	
25 I	25.77	-16.73	33.75	- 6.63	12.74	- 6.71	31.22	6.51	25.39	6.94	
26 S	43.23	0.73	62.14	4.30	29.08	- 7.03	42.11	- 0.06	39.61	3.70	
27 I	29.07	-13.43	46.36	2.68	22.17	- 0.58	28.76	0.75	19.01	- 2.74	
28 S	30.63	- 3.03	53.58	0.29	16.97	-15.39	45.00	7.38	39.16	7.80	
29 R	38.33	- 4.12	77.59	24.60	14.40	-17.66	48.24	10.92	13.28	-17.73	
30 I	49.02	7.32	52.27	-12.16	53.99	10.49	47.94	- 0.02	45.06	2.56	

\* R trees resistant against cypress canker disease, I trees with intermediate level of resistance, S susceptible trees.

crease with age, the trees in this test were only three years old when the frost occurred and therefore particularly sensitive. For data taken one year after the frost, we did not consider as dead any trees which showed severe damage in the crown but which were nevertheless able to sprout again.

#### Genetic parameters

Heritability of tolerance to frost presents a high value ( $0.94 \pm 0.31$ ). However, since it was calculated with measures done in only one locality and based on percentages of plots, it is of only limited usefulness for an improvement program for frost tolerance of the Italian cypress. The most important goal of this study remains to identify the best genotypes to use as parents for seedling production with a high tolerance to frost. In this connexion, tester I-1 shows a very high GCA, while the following cypress clones No. 8 (GCA = 16.79), No. 20 (GCA = 14.32), No. 4 (GCA = 13.71), No. 2 (GCA = 13.53), and No. 11 (GCA = 9.69) have the highest positive values among the mother trees. It is possible to set up clonal seed orchards with parents which

possess high GCAs. These clonal orchards will yield seed producing seedlings with a higher frost tolerance than seedlings derived from commercial seed.

#### Conclusions

The most important conclusions are the following:

- a high variability of frost tolerance was found among the various cypress species;
- frost tolerance of Italian cypress seedlings derived from seed collected in naturalized populations in Tuscany is higher than that of seedlings from seed collected in natural stands in Greek islands and Turkey;
- the frost tolerance variability among the progenies in this test is high enough to allow the genetic parameters to be estimated;
- some mother trees and the tester I-1 present high GCA values that promise well for establishing clonal seed orchards;
- there is no significant phenotypic correlation between frost tolerance and degree of cypress canker resistance;

— frost tolerance also depends in part on the physiological condition of the tree when the frost occurs. It seems that there is a temperature threshold below which a phenotype with average frost sensitivity dies.

We believe that these results could be important:

- a) for all countries such as France which plan to use the Italian cypress to reforest wide areas or as windbreaks in order to protect crops in localities with frequent severe frost;
- b) for regions such as central and northern Italy, where winter frosts rarely cause the death of cypresses, but can produce several cracks in the trees. These cracks represent a way of penetration into the tree tissues of spores of *S. cardinale*, which is a wound pathogen.

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## Genetics of *Cunninghamia lanceolata* Hook.

### 2. Genetic Variation Within and Between Two Provenance Samples

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#### Abstract

Genetic variation in seed samples of *Cunninghamia lanceolata* from two provenances of the western part of the People's Republic of China were studied. Genotypes of endosperm and corresponding embryo were scored simultaneously at ten enzyme gene loci.

Intrapopulation variation is described by means of the actual and conditional heterozygosities and the genetic diversities. These are given separately for the pool of the female and the male gametic contributions to the embryos as well as the genotypes. The characterization of inter-population variation is based on a statistical comparison, on the genetic distances for alleles and genotypes, and on the measurement of population differentiation. The results clearly indicate considerable amounts of genetic variation within and between the studied provenances. Possible consequences for tree breeding are outlined briefly.

*Key words:* Enzyme gene marker, heterozygosity, genetic diversity, genetic distance, seed provenance, *Cunninghamia lanceolata*.

#### Zusammenfassung

Anhand von Saatgutstichproben wurde die genetische Variation zweier Herkünfte von *Cunninghamia lanceolata* aus dem westlichen Teil der Volksrepublik China untersucht. Genotypen von Endosperm und dazugehörigem Em-

bryo wurden simultan an zehn Enzym-Genorten identifiziert.

Die Variation innerhalb der Populationen wurde anhand der aktuellen und bedingten Heterozygotiegrade sowie der genetischen Diversität beschrieben. Diese Maße wurden getrennt für die weiblichen und männlichen Gametenbeiträge zu den Embryonen sowie für diploide Genotypen berechnet. Die Charakterisierung der Variation zwischen den Populationen basiert auf einem statistischen Vergleich, auf den genetischen Abständen für Allele und Genotypen und auf der Erfassung der Populationsdifferenzierung. Die Ergebnisse zeigen übereinstimmend eine beträchtliche genetische Variation innerhalb und zwischen den untersuchten Provenienzen. Mögliche Konsequenzen für die züchterische Nutzung werden kurz angesprochen.

*Schlagwörter:* Enzym-Genmarker, Heterozygotie, genetische Diversität, genetischer Abstand, Saatgut-Provenienz, *Cunninghamia lanceolata*.

#### Introduction

The maintenance of the capacity of populations to adapt to changing environments is considered to be the most important criterion in the determination of long term survival ability. Such adaptability should in large part be a consequence of genetic variability, which in turn is a function of the genetic variation realized in an actual population and the ability to create genetic variation in subsequent generations (e.g. GREGORIUS *et al.*, 1985). In this way, genetic variation becomes more important the longer populations are exposed to varying environments and the more heterogeneous the spatial environments are. This

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