stand structure caused by the actual thinning, we cannot be sure how the additive and phenotypic variances would have behaved if the actual thinning had not taken place. Genes which affect competitive ability would gradually become important as between-tree competition increased before thinning. Thinning presumably reduces the amount of between-tree competition for resources so the genes affecting competitive ability could then become relatively less important than before the thinning. The extremely high genetic correlations between data obtained before thinning and data obtained several years after thinning suggests that this change in relative importance of genes affecting competitive ability is not very great. But the effect of this change on genetic variances is unknown. The next question is; how do these results affect the inferences drawn from progeny tests?

Progeny tests usually supply information about the parents both as individuals and as a population. Neither form of thinning affects the relative rankings of the parents with respect to their breeding values. The information provided about the performance of the parents as individuals is largely unaffected by thinning operations. However, the estimates of parental population parameters are considerably affected by selective thinning, although they are not by random thinning. So some account must be taken of thinning procedures when making inferences about the parental population as a whole.

Heritability estimates taken from an unthinned progeny test should not be used to estimate genetic change if breeding is to be done with trees chosen in selectively thinned tests unless due allowance is made. Similarly, estimates from selectively thinned tests should be used only with allowance for thinning if unthinned or randomly thinned tests are the basis of the breeding program. Presumably, the level of selection at thinning will also have an effect on the heritability estimates. Consequently, selections should be made from trials which have faced exactly the same thinning treatment as those from which the heritability estimate was obtained. If this provides too stringent a condition on the application of the estimates, then it is probably better not to thin the progeny trials selectively at all.

The problem of selecting individuals within a plot for measuring expensive or difficult traits is similar to the problem of selective thinning. If the data are to be used only for rankings and not for parameter estimation then selection of trees for measurement should have little ef-

fect. This contention is supported by the extremely high genetic correlation between diameter before thinning and 3 or 5 years after thinning at both sites. It is also in agreement with Lee's (1974) results in a nursery trial of P. ponderosa, and with Kung's (1977) more theoretical results. Selecting individuals to measure difficult traits has a similar effect on heritability estimates as selective thinning if the selection is based on some character which is genetically related to the difficult trait. If diameter is measured only in what appear to be the fattest trees, then the heritability estimate will be inflated. If height is measured only in the fattest trees, the heritability will be inflated by an amount proportional to the correlation between diameter and height. It follows that if trees must be selected for measurement, then some inflation of heritability estimates must be accepted, although rankings of families are unlikely to be affected. Kung (1977) states that it is easier to choose the tallest tree in a plot than to choose randomly. If the trees are so tall that it is not possible to measure them all, then it is probably too difficult to be sure that the tallest one and not the second tallest one has been chosen. The effect of such mistakes on Kung's results are unknown. For making estimates of genetic variances, measurements of less than a whole plot should be made at random, although for ranking families some systematic selection of trees within a plot for measurement is acceptable.

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Identification of Hybridity of Casuarinas grown in Taiwan¹)

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Abstract

Seven morphological traits, branchlet anatomic structure of Casuarina glauca, C. equisetifolia and their putative

hybrids and growth characters of their seedlings were evaluated to compare the differences within and among the four taxa grown at Yun-lin County, Taiwan. Hybrid index scales based on morphological and growth traits were constructed for parent trees and open-pollinated progenies to illustrate the occurrence of introgressive hybridization during the several decades of composite planting. The evidence of introgression can be verified by the variability of number and shape of rib as well as the significant correlation among traits. The theoretical reasoning

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of natural hybridization of two *Casuarina* species grown in Taiwan was discussed.

Key words: Casuarina glauca, C. equisetifolia, hybrid index, introgressive hybridization, phenotypic correlation.

Zusammenfassung

Sieben morphologische Merkmale und die anatomische Zweigstruktur von Casuarina glauca, C. equisetifolia und ihrer vermeintlichen Hybriden sowie die Wachstumseigenschaften ihrer Sämlinge werden untersucht, um die Unterschiede innerhalb und zwischen den vier in Yun-lin-County, Taiwan, vorkommenden Arten herauszufinden. Hybrid-Index-Skalen, die auf morphologischen Merkmalen und Wachstumseigenschaften basieren, wurden ermittelt. Für die Elternarten und die frei abgeblühten Nachkommenschaften wurden Hybrid-Index-Maßstäbe konstruiert, um das Auftreten von introgressiver Hybridisierung während verschiedener Dekaden bei gemischter Auspflanzung darzustellen. Der Beweis einer Introgression kann durch die Variabilität bei Anzahl und Form der Rippen an den Trieben, genauso wie anhand signifikanter Korrelationen zwischen den Merkmalen erkannt (verifiziert) werden. Die theoretische Begründung einer natürlichen Hybridisierung von zwei Casuarina-Arten, die in Taiwan vorkommen, wird diskutiert.

Introduction

The establishment of coastal windbreaks in Taiwan is of great importance because the island often suffered greatly from monsoon and typhoon almost every year. At present, 83.9% of the coastal sandhill has been planted with exotic *Casuarina* species which exhibited superior growth, high degree of drought resistance as well as salt tolerance. The introduction and initial planting of *Casuarina* trees in Taiwan dated back to 1897. At that time, more than ten species were introduced, but only a few have survived owing to the differential adaptability to Taiwan environments (Chung, 1940; Lin *et al.*, 1956; Sheng and Kang, 1961).

Since 1904, seeds for afforestation and reforestation programs have been collected locally on the island. Through decades of composite planting of Casuarina species, natural hybridization may have occurred. In 1967 Dr. L. A. S. Johnson of Royal Botanic Gardens of Sydney, Australia, examined morphologically 103 specimens which were collected in Taiwan, and grouped them into three Taxa: Casuarina equisetifolia ssp. equisetifolia*), C. glauca and C. equisetifolia ssp. equisetifolia \times C. glauca**). The third one is the natural hybrid of the former two species.

In this study, we examined seven morphological characters and anatomic structure of branchlets from sampled trees grown in Yun-lin County of Taiwan. In addition, seedling growths were also compared among the seedlots collected from the same trees. The objectives are to further confirm Johnson's findings and to provide a basis for species or variety selection for the plantings of Taiwan coastal shelterbelts.

Material and Methods

From the areas of Pei-kang, Yuan-chang and Shih-hu of Yun-lin County, 11 trees with the age of thirty were sampled and classified into 4 groups (A.B.C.D.) by subjective evaluation of gross morphology according to the extension brochure written by Kan and Chang (1954). Two

trees in A and three trees in B belong to the species of *Casuarina glauca* and *C. equisetifolia* respectively. Groups C and D, consisted of 2 and 4 trees respectively but their species name could not be assured.

Cones and twenty branches of about 30 cm long were collected from each sampled tree, and packed in a polyethylene bag. Four replications of 25 branchlets each were randomly collected from the branches in each of the 11 bags. For each branchlet, we measured the total length and the length of ten internodes of the median portion. Number of sheath teeth was counted for each internode.

During the time of tree sampling, ten 5-mm long segments of 1-year-old branchlets were taken from each sampled tree and, then, put into F.A.A. fixing solution immediately for anatomical study. Paraffin method was used for cross section and permanent slicemaking. The differences of internal structure of the branchlets among trees were compared by observing the slices under light microscope.

Four replications of 25 cones each were randomly taken from each of the same 11 polyethylene bags and weighed. In addition, the length and diameter of cones were also measured. Seeds were extracted by hand. Four replications of 300 seeds each from every lot were sampled and weighed. Germination was conducted in a growth chamber at 25°C during 8-hour photoperiod and at 20°C in the dark. One week later, germinants with radicle longer than 5 mm were transplanted in polyethylene tubes (diameter 7 cm, height 16 cm) in which the medium is composed of sandy soil from Tao-yuan coastal Casuarina forest, clay loam, and buffalo stool compost in the ratio of 3:1:1 by volume, All the transplants were grown in a greenhouse by using randomized complete block design with three replication. Twenty transplants from each seedlot were arranged on a bi-row plot. When the seedlings were seven months old, their heights and root-collar diameters were measured.

Data were subjected to analysis of variance to test the significance of differences between or among parent trees as well as seedling families within group and among groups. Calculation of hybrid index scores followed the methods adopted by Hicks (1974) and Bhat and Hicks (1976).

Results

Morphological and Growth Characters

A. Comparison between or among trees within group The means and their standard errors of seven morphological measurements and seedling growth data are shown in *Table 1*. It is apparent that for most traits, the average values of groups C and D are intermediate between A and B except root-collar diameter for which the overall means of the former two groups fell slightly (not significantly) outside the span of the latter two group means. In addition, the group D means for internode length and cone diameter are also considerably out of the range from A to B means. The small values of standard errors for most characters may reflect the low within-tree sampling variance, and, hence, indicate that the characters chosen in this study are appropriate for taxon identification.

As indicated in *Table 2*, no significant difference between two parent trees of group A was found in branchlet length, internode length, fresh weight, length and diameter of cones, seed weight, height growth and root-collar diameter of progeny seedlings. The only significant difference was seen in the number of sheath teeth. From field survey and laboratory observation, we found that these two trees

^{*)} The scientific name of this taxon is C. equisetifolia var. equisetifolia as used by Doran and Hall (1983).

^{**)} Personal communication with Dr. Huang, Botany Department of National Taiwan University.

Root-collar diameter at 7th month (mm) 90 3.76±0.00 3.33±0.30 .78±0.16 3.80±0.02 3.82±0.19 3.41±0.34 3.36±0.04 3.90±0.23 3.85±0.34 3.34±0.21 3,88±0,0 Table 1. — Means and standard errors of seven morphological measurements of parental groups and two growth data of their progeny seedlings. at 7th month (cm) Seedling height 74.30±4.54 73.73±1.80 74.66±9.89 44.72±2.16 44.60±4.17 44.56±0.18 74.07±9.76 74.66±0.01 44.37±4.05 63.56±7.21 63,57±9,99 63.57±0.01 Seed weight seeds) 0.181±0.009 0.340±0.014 0.359±0.060 0.263±0.005 014 0.172±0.003 0.177±0.006 0.387±0.005 0.459±0.012 0.170±0.004 0.175±0.008 0.173±0.004 0.249±0.006 0.261±0.006 0.282±0,003 0.264±0. (300 diameter (cm) 1.44±0.12 1.53±0.09 1.40±1.30 1.14±0.01 1.34±0.02 1.32±0.04 1.32±0.03 1.18±0.02 1.39±0.11 1.22±0.12 1.33±0.01 1.13±0.01 1.16±0.04 Cone Cone length (cm) 1.80±0.17 1.21±0.03 1.76±0.18 1.1840.04 02 90 1.73±0.07 1.72±0.07 03 .04 1.23±0.03 1.24±0.02 1.46 ± 0.17 1.65±0.17 1.20±0. 1.71±0. 1.25±0. 1.67±0 Fresh weight cones) 17.52±1.16 31.57±1.70 31.81±1.92 31.09±1.72 31.49±0.37 24.17±0.53 27.10±1.12 79±0.60 28.50±1.82 83±1,02 31±3,38 18.83±0.91 18.18±0.93 24.54±0.97 23.79±0.71 (25 34. Sheath teeth (number) 05 46 13.7740.98 15.30±0.77 14.54±1.08 60 6.85±0.07 6.86±0.04 02 10.11±0.08 10.11±0.01 10.51±0.49 46 28 37 9.73±0. 10.21±0. .06±0. 6.85±0. 10.10±0 9.77+0 9 length (cm) 0.97±0.04 Internode 03 05 05 9 0.94±0.03 0 0.70±0.03 0.69±0.02 0 1.10±0.04 0.95±0.02 0.93±0.06 1.08±0.0 0.95±0. 0.68±0. 0.69±0. 1.01±0. 1.04±0. length (cm) .21 42.57±1.37 41.43±1.62 16.57±1.23 16.27±0.78 17.05±1.25 16.63±0.39 36.36±1.22 39.50±2.30 37.93±2.22 28.46±1.70 27.97±2.17 29.07±1.78 .90 Branchlet 24.80±1. 27.57±1. .28±2. 60. code 10 13 12 equisetifolia glauca Group υ i. ٠ ان

possessed a number of morphological features in common, i.e. dense conical crown, straight and round trunk, rugged bark with deep grooves, upright upper branches, pendulous lower branches, bluish-green branchlets of 30—60 cm long, internode about 10 mm long, number of sheath teeth 12—17, globular cone with flat top and 1.1—1.5 cm in length as well as 1.0—1.3 cm in diameter, and small pale-brown seeds. These features resemble closely to those of *Casuarina glauca* described by Kan and Chang (1954), Badran et al. (1976), Bentham (1873) and Ewart (1930). All these find-

Table 2. — Results of variance analysis of morphological measurement of parent trees and growth data of progeny seedlings.

	Group A	Group B	Group C	Group D		
Branchlet length	ns	ns	ns	*		
Internode length	ns	ns	ns	ns		
Sheath teeth number	*	ns	ns	ns		
Cone fresh weight	ns	ns	ns	**		
Cone length	ns	ns	**	ns		
Cone diameter	ns	ns	ns	*		
Seed weight	ns	**	ns	**		
Seedling height	ns	ns	ns	ns		
Root-collar diameter	ns	ns	ns	ns		

ns -- Statistically non-significant at 5% level.

ings may confirm that trees in group A are of the species of C. alauca.

Three trees in group B exhibited non-significant difference in branchlet length, internode length, number of sheath teeth, fresh weight, length and diameter of cones, height and root-collar diameter of progenies, but not in seed weight. The common appearance for the three trees are large and open crown, trunk with longitudinal gully or furrow-like but not round surface, rough bark, spreading branches, bright-green branchlets of 13—20 cm long, internode 4—8 mm long, number of sheath teeth 6—8, long ellipsoid cones with 1.4—2.0 cm in length and 1.1—1.5 cm in diameter, reddish-brown seeds with 5.5 mm in length. These features closely resemble those of *C. equisetifolia* which was described by Kan and Chang (1954), Lin et al. (1956), Badran et al. (1976) and Bentham (1873). Therefore, trees in group B should be of the species of *C. equisetifolia*.

Two trees in group C did not exhibit significant variations in eight out of the nine morphological and growth characteristics examined. Therefore, they may be coinsidered as the members in the same group.

The result of variance analysis for group D indicated that among the four trees, no significant difference was found in internode length, number of sheath teeth, cone length, height and root-collar diameter of progeny seedlings, but the differences in branchlet length, cone diameter, cone fresh weight, and seed weight are significant at 5% or 1% level. However, the difference in cone fresh weight and seed weight may be partly due to different genetic combinations in different parent trees, but not entirely to the inherent factors.

B. Comparison among Groups

The results of analysis of variance for the nine traits are summarized in *Table 3*. The highly significant difference among four groups for every trait can be seen from the calculated F values. When we further look at the ranked means in the Duncan's test, it is apparent that group A and B are significantly different from each other for all traits examined, and, hence, they can be assured as distinct species. The mean values for most characters of group C were closer to *C. glauca* (group A) and were significantly different from those for *C. equisetifolia* (group B). Group D is somewhat closer to *C. equisetifolia* than to *C. glauca* with respect to the means of cone fresh weight and cone length. However, when height growth and rootcollar diameter of the 7-month-old progeny seedlings were considered, group D is much closer to *C. glauca*. As for C

^{* --} Statistically significant at 5% level.

^{** --} Statistically significant at 1% level.

Table 3. — The calculated F values and Duncan's new multiple range test from variance analysis on group means.

Trait	Calculated "F"	Duncan's new multiple range test (a)					
		Ranked means					
Branchlet length	296.77**	A C D B 41.42 37.93 27.57 16.63					
Internode length	166.93**	D A C B 1.06 <u>0.95 0.95</u> 0.96					
Sheath teeth number	285.15**	A C D B 14.54 10.11 10.06 6.85					
Cone fresh weight	73.32**	B D C A 31.49 30.31 24.17 18.18					
Cone length	42.59**	B D C A 1.71 1.67 1.24 1.20					
Cone diameter	19.83**	D B C A 1.40 1.33 <u>1.16 1.13</u>					
Seed weight	134.23**	B D A C 0.40 0.26 <u>0.18 0.17</u>					
Seedling height at 7th month	48.25**	A D C B 74.66 73.73 63.57 44.56					
Root-collar diameter at 7th month	6.20**	D C A B 3.88 3.80 3.76 3.36					

^{** --} Calculated F indicates a significant difference in the means at the 1% level of probability.

vs. D, the means for seven out of nine traits differed significantly from each other except for number of sheath teeth and root-collar diameter.

The above-mentioned facts can be clearly demonstrated by the hybrid index scores (Fig. 1). The hybrid indices of parent trees were constructed from seven morphological data while those of seedling families were from two growth measurements. The index values for parent trees exhibited that both groups C and D were located within the range from A to B, wherein group C approximated group A, and group D was more or less intermediate between A and B.

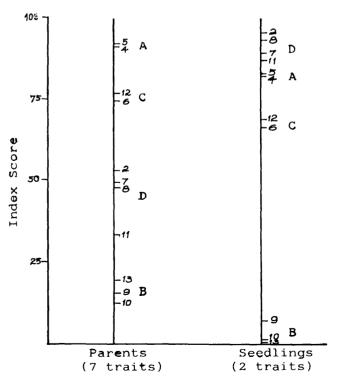


Fig. 1. — Two hybrid indices of parent trees and their seedling families.

From the index ranking of seedling families, we could see that group C was still situated in the A-to-B range and consistently in close with A, whereas group D fell slightly outside the span of A and B indices. The jump of group D over A on the index ranking of seedling families may result from the possible heterosis of growth characters.

Anatomic Structure of Branchlet

The cross section of 1-year-old branchlet was taken from internode portion. As shown in $Fig.\ 2A$, the number of ribs of $C.\ glauca$ in group A ranges from 12 to 17, and tightly arranged around the outer layer of branchlet. Thus, the longitudinal between-rib furrows on the surface of branchlet are narrow and shallow. There are only a few sclerenchymatous cells between the two masses of chlorenchyma which are located just below the epidermis of the ribs. The cauline vascular bundles are not clearly visible.

Fig. 2B indicates that the number of ribs of *C. equisetifolia* in group B is mostly 7. The ribs are not tightly arranged, so that the furrows between them are wider and deeper. There are two masses of well-developed chlorenchyma between which the T-shaped sclerenchyma is located. The cauline vascular bundles are separated in 7 portions by broad medullary rays.

The rib numbers of the trees in group C are from 10 to 11, and arranged somewhat similar to that of *C. glauca* in group A. The chlorenchyma is situated inside the epidermis of each rib. The sclerenchyma between the two masses of chlorenchyma is arranged in a narrow band. The cauline vascular bundles are separated by broad medullary rays (Fig. 2C).

Trees in group D exhibit similar numbers and arrangement of ribs as that of *C. equisetifolia* in group B. The sclerenchyma develops very well and extends to the leaf-trace, and, consequently, divides the chlorenchyma in 2 symmetrical masses. The xylem of cauline vascular bundles form an almost continuous ring which, in turn. is traversed by narrow rays (*Fig. 2D*).

Table 4 is a 9×9 correlation matrix which was constructed by using tree-means of the various traits for calculating

⁽a) -- means underscored by a common line are not significantly different at the 5% level of probability.

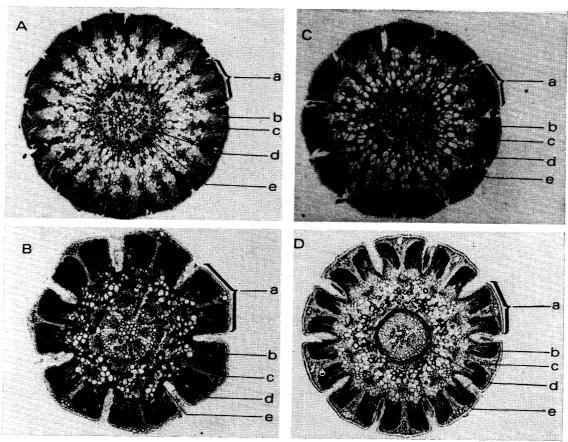


Fig. 2. — Cross sections of 1-year-old branchlet of each group.

A. Group A (C. glauca) C. Group C

B. Group B (C. equisetifolia) D. Group D

(a. rib b. chlorenchyma c. scelerenchyma d. cauline vascular bundle e. pith)

correlation coefficients. Most traits were significantly correlated except internode length and cone diameter, which may indicating possible heterosis.

Discussion and Conclusion

The interspecific hybridization between Casuarina glauca and C. equisetifolia var. equisetifolia in Australian native habitat has not been reported in the literature previously. This may be due to the fact that C. equisetifolia var. equisetifolia is not sympatric with C. glauca (Doran and Hall, 1983). The occurrence of natural hybridization in Taiwan may be interpreted as that the introduction of these two

species into a new environment might have induced them to interbreed by removing or modifying certain reproductive isolation barriers which initially existed in their native habitats. Another evidence of natural hybridization of $C.\ cunninghamiana \times C.\ glauca$ was found in Egypt (Badran et al. 1976). Stebbins (1950) and Grant (1971) mentioned that if natural hybridization takes place in a region that provides new habitats to the segregating progenies, many of these segregants may survive and contribute to a certain extent to the evolution of the population concerned.

According to Hicks (1973), characters which reflect genetic differences between taxa should, therefore, possess ge-

 ${\it Table~4.} \hspace{0.1in} \textbf{--} \hspace{0.1in} \textbf{Simple correlation coefficients among morphological and growth traits.} \\$

	Α	В	С	D	E	F	G	н	I
Branchlet length (A)		0.585 ^{ns}	0.883**	0.869**	0.892**	0.700*	0.932**	0.716*	0.655*
Internode length (B)			0.573 ^{ns}	0.199 ^{ns}	0.245 ^{ns}	0.022 ^{ns}	0.685*	0.924**	0.981**
Sheath teeth No. (C)				0.811**	0.751**	0.550 ^{ns}	0.794**	0.799**	0.611*
Cone fresh weight (D)					0.914**	0.754**	0.716*	0.437 ^{ns}	0.295 ^{ns}
Cone length (E)						0.740**	0.784**	0.426 ^{ns}	0.359 ^{ns}
Cone diameter (F)									0.039 ^{ns}
Seed weight (G)								0.766**	0.745**
Seedling height at 7th month (H)									0.930**
Root-collar diameter at 7th month (I)								

ns -- Statistically non-significant at 5% level.

^{* --} Statistically significant at 5 % level.

^{** --} Statistically significant at 1% level.

netic correlations that would be reflected in the phenotype. Simple phenotypic correlation coefficient was calculated for parent and progeny hybrid index scores and a significant value ($\mathbf{r}=0.681^*$) was obtained when all groups were combined. This may indicate that the traits used in this study were under genetic control and relatively diagnostic for taxon identification or classification. Bhat and Hicks (1976) also obtained a similar result for selected shortleaf pine, loblolly pine and putative hybrids.

Anderson (1949) has pointed out that introgression could cause genetic correlation among morphological characters. In the present study, significant phenotypic correlations were fairly frequent among traits as mentioned above. This condition is compatible with introgression too.

The distribution patterns of four groups on both hybrid index scales (Fig. 1) may imply that most characteristics of groups C and D are intermediate between C. glauca and ${\it C. equisetifolia}, \ {\it but phylogenetically closer to C. glauca.}$ Such a situation could reflect introgressive hybridization in which C. glauca is the predominant backcross species. In addition, the rib shape of trees in groups A, C and D were round without ridge, while that of trees in C. equisetifolia possessed protrudent ridges. Such anatomic features can also support the evidence of introgression. Introgressive hybridization has also occurred in areas of the overlapping range where Picea rubens and P. mariana are in contact or association, and consequently, many of the hybrid characters are intermediate which made traditionally taxonomic confusion. Under the circumstances, Manley (1971) successfully employed the hybrid index technique to discriminate among individuals growing in introgressed popula-

There is no question that trees in group B belong to C. equisetifolia since their morphological characters coincided with those previously described by Bentham (1873), Ewart

(1930), Kan and Chang (1954), and Badran $et\ al.$ (1976). But whether they are of $C.\ equisetifolia\ ssp.\ equisetifolia\ needs further investigation.$

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Effets de la température de germination et de la stratification sur la germination des semences de Pinus halepensis Mill.

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Résumé

La germination des semences de 9 provenances différentes de l'espèce *Pinus halepensis* Mill., (représentant la distribution naturelle de l'espèce) a été étudiée en fonction de la température de germination (15°, 18°, 21°, 24° C) et de la stratification (0, 30, 60 jours à 3—4° C).

On a mis en évidence l'absence d'obstacles sérieux à la germination chez *P. halepensis* et la relative homogénéité du comportement de germination des diverses provenances à l'intérieur de l'intervalle thermique testé.

La stratification abaisse la capacité germinative de certaines provenances, favorise la germination aux températures les plus élevées chez d'autres et provoque une augmentation de la vitesse de germination et par conséquent de la valeur germinative (GV) chez toutes les provenances. Les différences observées lors de cet essai et les diverses capacités de résister au stress hydriques des semences (mises en évidence dans des expériences précédentes) sont l'objet d'une discussion sur l'étalement de la germination de l'espèce, sur l'importance des différentes époques de germination des provenances étudiées et sur la valeur adaptative de ces modifications.

Mots clés: Pinus halepensis, germination, variation géographique.

Summary

Seed germination of 9 provenances of *Pinus halepensis* $M_{ILL.}$, (representative of the natural distribution of the species) has been studied in relation to germination temperature (15°, 18°, 21°, 24° C) and to stratification (0, 30, 60 days to 3—4° C).

P. halepensis seeds germinate without serous obstacles and different provenances show relatively homogeneous germination patterns.

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