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Foliage Resin Composition of Cupressus sempervirens L. as Affected by Environmental Factors[1])

By G. SCHILLER[2])

(Received 7th October 1992)

Abstract

Effects of some environmental factors on the relative amounts (%) of *Cupressus sempervirens* L. foliage resin compounds were examined in a partial factorial experiment. The results presented show that environmental factors, such as soil, water availability and radiation intensity, affect to a certain degree the foliage resin composition of clonal plants of *Cupressus sempervirens* L. cv. *stricta* and var. *horizontalis*.

Key words: Cupressus sempervirens, Italian cypress, monoterpenes.

Introduction

In earlier studies of the foliage resin composition of *Cupressus sempervirens* L. (SCHILLER, 1990; SCHILLER and MADAR, 1991) significant differences were detected in different environments between populations and between diseased and visually nondiseased trees. Differences in the development of cankers caused by the fungi *Seiridium* sp. or *Diploida* sp. were attributed to differences in soil properties (MADAR, personal communication) and water availability (MADAR *et al.*, 1989). Autecological studies of cypress have shown differences in growth patterns on different soils of *Cupressus sempervirens* cv. *stricta* and var. *horizontalis* (ZOHAR, 1984). Numerous studies, *e.g.*, BRIDGEN and HANOVER (1982), ENNOS and SWALES (1988), FRANICH *et al.* (1982), MICHELOZZI et al. (1990), ROCKWOOD (1974), SCHUCK (1982), have shown relations between resin composition and resistance to fungi.

The hypothesis that underlays the use of coniferous resin composition as a genetic indicator is that the composition is strongly inherited and subject to only minor environmental influences (SQUILLACE, 1976; VON RUDOLFF and REHFELDT, 1980). This premise was critically reviewed by BIRKS and KANOWSKY (1988) who concluded that the evidence for this assumption is not conclusive.

The present study was undertaken to determine whether, and to what extend, environmental factors affect the foliage resin composition of *Cupressus sempervirens* L. and hence, the reliability of the technique as a genetic indicator and as an indicator for disease resistance.

Materials and Methods

a) Plant material

Cuttings were taken from 2 different 70-year-old trees of *C. sempervirens* L. (*Fig. 1*) growing at the Volcani Center at Bet Dagan, Israel (32°00' lat. N; 34°49' long. E.). Tree *A* was of var. *horizontalis* (MILL.) GORDON, and tree *B* was of cv. *stricta* (var. *stricta* AIT. = var. *pyramidalis* NYMAN). Both trees are not irrigated and grow on a calcareous sandstone at 50 m' a.s.l. In October 1989, 5-cm-long cuttings of the 2 trees were taken from a few main branches at a similar height above ground. Cuttings were rooted using the method developed by CHEMLA (1986). In December, the rooted cuttings were transplanted into 5-liter plastic nursery bags.

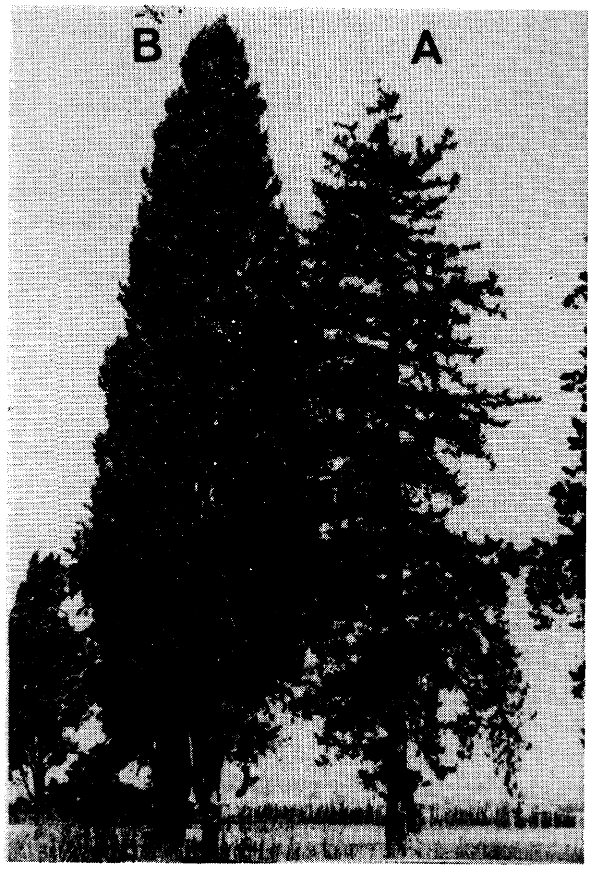

Figure 1. — Italian cypress (*Cupressus sempervirens* L.) trees at Bet Dagan.

(A) Var. *horizontalis* (MILL.) GORDON;
(B) cv. *stricta* AIT. (=var. stricta AIT. = var. pyramidalis NYMAN).

b) Experimental design

The treatments applied were as follow:

[1]) Contribution from the Agricultural Research Organization, The Volcani Center, Bet Dagan 50250, Israel. No. 3392-E, 1991 series.

[2]) Department of Natural Resources, Agricultural Research Organization, The Volcani Center, P.O. Box 6, Bet Dagan 50250, Israel.

Table 1. — Environmental factors and treatment combinations.

radiation regime	Soil type	cv. stricta		var. horizontalis	
		Irrigation regime			
		1	2	1	2
full light	rendzina	a	d	- -	- -
	terra rossa	b	e	b	e
	grumusol		- -	c	f
65% light	rendzina	g	j	- -	- -
	terra rossa	h	k	h	k
	grumusol	i	- -	i	- -

The letters indicate the different treatments in *Figur 4*.

a.- *Soil types*: (1)terra rossa; (2) rendzina; (3) basaltic grumusol.
b.- *Light*; (1) full light, (2) about 65% of full light.
c.- *Irrigation*; (1) once a week, (2) once every 3 weeks to field capacity.

The layout of the experiment was a partial factorial design (*Table 1*), differences in resin composition between cv. *stricta* and var. *horizontalis* had been established earlier (SCHILLER, 1990). Each treatment consisted of 5 to 11 cuttings of each clone. In some cases not enough cuttings were available to test all treatments.

c. Resin analysis

After 1 year in the nursery, the rooted cuttings grew to about 1 m in height and were bearing enough foliage for resin extraction. Also 11 foliage samples were collected from each of the 2 original trees, from branches facing the same direction and at the same height above ground, to minimize within-tree variation in resin composition (FRANKLIN, 1976; ROBERTS, 1970). The resin was extracted in November, before the new flush of growth, to avoid mixing previous year's and new sprays (ADAMS and HAGERMAN, 1976). Between 0.05 ml and 0.2 ml resin were extracted from the foliage of each sample, using steam distillation, of which 2.0 μl were used for analysis.

Resin was analyzed using a Packard 7400 gas liquid chromatograph, fitted with a flame ionization detector, and a 4-m-long glass column of 2 mm in diameter packed with OV-101 (100% methyl fluid) 8% on chromosorb W-60/80. Operating conditions were 250^0 C at the injector and the detector, and 50^0 C to 230^0 C at the column, with temperatures increasing at the rate of 5^0 C/min. Nitrogen was used as carrier gas at a flow rate of 25 ml/min.

Peak areas representing different compounds were calculated as component percentages of the sum total of all peak areas, using a 3390-A Hewlett-Packard integrator. For statistical analysis percentages were transformed by means of the natural logarithm transformation $Y_i = \ln(x_i)$ where x_i is the percentage of the amount at the i-th peak as recommended by KUNG (1988). The data obtained were analyzed by the General Linear Model and discriminant procedures (SAS, 1988). Only 29 compounds out of 44 with an area percentage above 0.5% were subjected to statistical analysis. Several peaks, *i.e.*, resin compounds, could be identified with the help of pure standards as indicated in *figure 2*.

Results

Off the 3 environmental factors applied to the saplings, only 2 factors *i.e.*, light and water availability had a marked affect on saplings growth of the 2 taxon *i.e.*, cv. *stricta* and var. *horizontalis*; whereas soil type has not, under nursery conditions, affected the growth of the saplings. Under full light, reduction of water availability has lowered foliage dry meter accumulation from an overall average of 109.6 g ± 47.6 g per sapling to 67.7 g ± 16.9 g at the end of the year; whereas, reduction of light intensity by 35% with or without reduction of water avail-

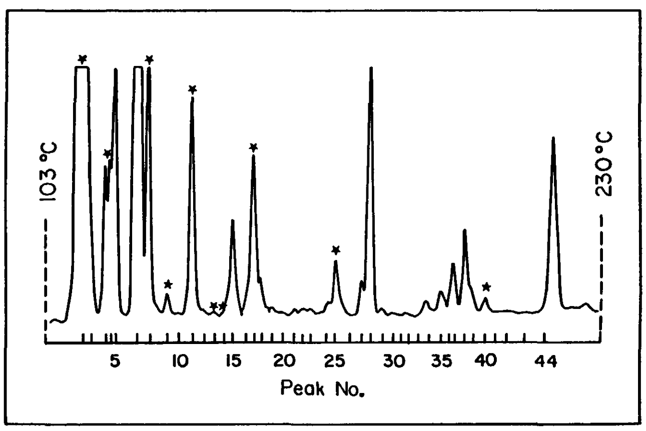

Figure 2. — Typical integrator chromatogram of branchlet resin composition of *Cupressus sempervirns* L. cv. stricta. Peaks marked with an astrisk are compounds used to discriminat between the varieties.
(Peak 1 = α-pinene; 3 = β-pinene; 5 = myrcene: 6 = Δ^3-carene; 28 = caryophyllene).

Table 2. — Statistical parameters of compounds selected by stepwise discriminant analysis (major compounds are marked with an astrisk).

Com -pound	F	Prob. > F
1*		0.0087
4	9.129	0.0031
7*	21.535	0.0001
11*	18.270	0.0001
13	10.401	0.0016
14	4.401	0.0380
17*	5.017	0.0269
25*	20.064	0.0001
28*	3.452	0.0656
40	6.259	0.0137

ability has not reduced dry meter production and the overall saplings average dry wight was 116.7 g ± 29.2 g and 106.9 g ± 19.5 g, respectively.

Discrimination between clones in their resin composition

Stepwise backward discriminant analysis was used, to reduce the number of variables (compounds) from 29, to maintain only those that contributed significantly to the separation between clones. Ten compounds, (7 major and 3 trace compounds, marked in *figure 2*, were selected to create the discriminant vector. The F value and probability of F of each of these compounds is presented in *table 2*. The overall R-square of this set of variables was 0.551.

Canonical discriminant analysis, using these 10 compounds, was applied to 133 measurements of the 10 variables and 2 classes (clones): 82 measurements of cv. *stricta* and 51 of var. *horizontalis*. Results presented in *table 3* show that more than 83% of the observations (clones) were assigned correctly.

Table 3. — Efficiency of discrimination between cv. stricta and var. *horizontalis*.

From clone	assigned to clones:		
	stricta	horizontalis	total
stricta	68 82.9%	14 17.1%	82 100.0%
horizont-alis	4 7.8%	47 92.2%	51 100.0%

Principal component analysis, using initial factor and varimax rotation methods, was conducted for each of the 2 clones on the vector created by the 10 variables. Factors 1 and 2 explain more than 51%, and the first 4 factors — more than 75% of the variance, in each of the 2 clones. The results presented in *figure 3* show the patterns of the selected resin compounds. In the 2 clones the compound 11, 17 and 28 are grouped together. In var. *horizontalis* (B), the compounds 4, 14, 25 are grouped together, whereas in cv. *stricta* (A), compound 25 is placed differently. The compounds 7 and 13 differ also in their position in the 2 clones.

Thus, data presented so far demonstrate significant differences between trees of cv. *stricta* and var. *horizontalis* in resin composition, thereby strengthening results gained in an earlier study (Schiller, 1990).

Effect of environmental factors on foliage resin composition within clones

ANOVA procedure was applied to identify the impact of single and combined factors on the relative quantities (%) of the 29 resin compounds in each of the two clones. *Table 4* shows that in cv. *stricta* the relative quantities of 11, out of the 29, compounds were affected significantly by single or combined factors (the probability of F for

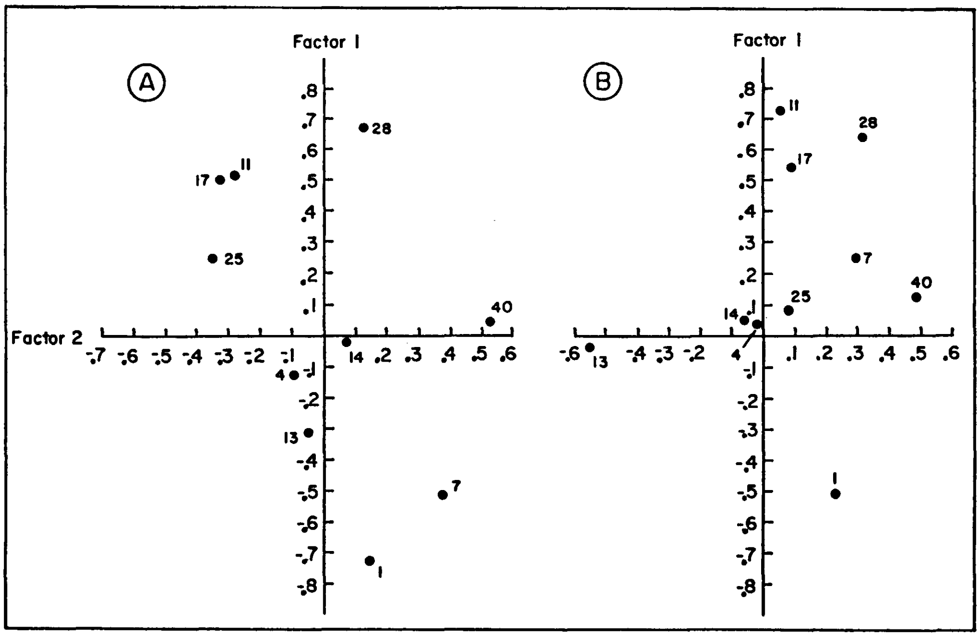

Figure 3. — Position of compounds selected to create the discriminant vector in: (A) cv. *stricta*, and (B) var. *horizontalis*, using principal component analysis.

Table 4. — Results of ANOVA to identify sources of variation (major compounds are marked with an astrisk).

Com-pounds	F	Source of Variation Prob.> F				
		soil	radi-ation	irri-gation.	irri.-X - soil	irri.-X - radi.
			cv. stricta			
1*	8.44	---	0.0001	---	---	---
5	6.49	0.0001	0.0200	---	---	---
7*	34.40	0.0001	0.0001	---	0.0067	---
11*	16.49	0.0221	0.0001	0.0219	---	0.0003
15	5.08	0.0008	0.0001	---	---	---
17*	8.90	---	0.0001	0.0001	---	---
22	15.57	---	0.0001	0.0040	0.0075	---
23	9.81	---	0.0001	---	---	---
25*	6.46	---	0.0001	---	---	---
37	12.21	---	0.0001	0.0127	---	0.0087
44*	22.28	0.0001	0.0039	0.0001	0.0005	---
			var. horizontalis			
1*	4.05	---	---	---	0.0261	0.0266
5	4.84	---	---	---	---	0.0423
7*	5.73	0.0133	---	0.0041	---	0.0082
8	10.56	0.0245	0.0034	0.0010	---	0.0173
13	3.82	0.0850	---	---	---	---
15	13.50	0.0060	---	---	---	---
17*	9.29	0.0002	0.0036	---	---	0.0422
22	10.28	0.0005	0.0023	---	---	0.0091
23	4.30	0.0030	0.0006	---	---	---
24	17.81	0.0470	0.0151	---	---	---
28*	10.49	0.0030	0.0181	---	---	---
34	11.52	0.0076	0.0122	---	---	0.0013
38	7.22	0.0430	---	---	---	---
44*	16.23	0.0001	0.0001	---	---	0.0002

the model is < 6.46). Of the 11 compounds 8 are major ones and 3 are trace compounds (*Fig. 2*). Five of these 11 compounds (1, 7, 11, 17, 25) were included in the vector used to discriminate between clones. All 11 compounds were affected by light intensity; of these, 5 compounds were also affected by soil, and 5 by the irrigation regime. Only 2 compounds were affected by all 3 factors: -soil, moisture, and light.

In var. *horizontalis,* 14, out of the 29, compounds were affected significantly in their relative amounts (probability of F for the model < 3.82;); of these 7 compounds are major ones and 7 are trace compounds (*Fig. 2*). Twelve compounds were affected by soil, 8 by radiation, and 2 by irrigation. Only 1 compound was affected significantly by all 3 factors. Thus, there is evidence of a effect on some resin compounds of environmental conditions.

To gain some insight into the interactions among environmental factors and the resin composition, a backward stepwise discriminant analysis among classes (treatment combinations) was applied, for each clone, to retain for further statistical analysis, an assemblage of compounds that best discriminate between treatment combinations. The results presented in *table 5* show that in cv. *stricta,* 8 compounds, of them 5 major and 3 trace compounds, were found to be sufficient; whereas in var. *horizontalis* 14 compounds, of them 8 major and 6 trace compounds, were needed. The overall squared canonical correlation coefficient, for discriminating between treatments within cv. *stricta,* was 0.334; and within var. *horizontalis* —0.626.

Canonical discriminant analysis, using the compounds selected by backward discriminant analysis, was applied to analyze changes in the resin composition, as affected by environmental factors, of the cuttings. For cv. *stricta,* 71 measurements of the 8 variables in 9 classes (combinations of environmental factors were analyzed, whereas for var. *horizontalis,* 38 measurements of 14 variables in 7

Table 5. — Results of backward stepwise discriminant analysis (major compound are marked with an astrisk).

Com-pounds	Partial R-square	F	Prob.>.F
	cv. stricta		
1*	0.459	5.849	0.0001
6	0.300	2.953	0.0080
7*	0.513	7.234	0.0001
11*	0.623	11.387	0.0001
23	0.321	3.249	0.0042
24	0.271	2.558	0.0190
29	0.323	3.287	0.0039
37	0.617	11.087	0.0001
	var. horizontalis		
1*	0.790	11.311	0.0001
4	0.753	9.131	0.0001
5	0.796	11.675	0.0001
7*	0.692	6.727	0.0007
8	0.790	11.309	0.0001
11*	0.863	18.945	0.0001
22	0.805	12.395	0.0001
23	0.898	26.531	0.0001
24	0.787	11.058	0.0001
25*	0.617	4.824	0.0042
35	0.681	6.415	0.0009
39	0.893	24.969	0.0001
40	0.698	6.936	0.0006
44*	0.812	12.942	0.0001

classes were tested. In cv. *stricta*, the first 3 canonical coefficients out of 8, and in var. *horizontalis*, out of 6, can explain more than 92% of the variance. In the cv. stricta, the first canonical coefficient was loaded heavily by compounds 1, 7, and 11, and the second coefficient by compounds 1, 11, and 37. In var. *horizontalis* the first canonical coefficient was loaded heavily by the compounds 1, 11, and 23, and the second coefficient by the compounds 8, 11, and 39. *Figure 4* shows the class means on the canonical variables in the 2 clones.

In cv. *stricta* (A) there was no significant difference in resin composition between cuttings grown on rendzina and those on terra rossa under full light and with improved water availability (points *a* and *b*, respectively). Water stress (irrigation once every 3 weeks) caused changes in resin composition symbolized by the points *d* and *e*; the direction of the change from the points a and b to *d* and *e*, respectively, was similar. Furthermore, independent of soil type and/or water application, reduction of radiation caused a change of resin composition in a different direction, and all cuttings grown under these conditions had a relatively similar resin composition (from the points *a* and *b* to *g*, *h*, *i*, *j*, and *k*, respectively).

In var. *horizontalis* (B) there was also a significant difference in resin composition between cuttings grown on different soils under full light and with water availability (points *b* and *c*). Changes in ecological conditions caused a different change in resin composition in comparison with those of cv. *stricta*. Whereas changes in light intensity and /or water stress caused only small changes in the resin composition of cuttings raised on basaltic grumusol (from points *c* to *f* and *i*, respectively), relatively significant changes occurred under these conditions in the resin composition of cuttings grown on terra rossa soil (from point *b* to points *e* and *h*).

Discussion and Conclusion

One of the rationales for the use of resin composition as a genetic marker, is the assumption, that pathways of

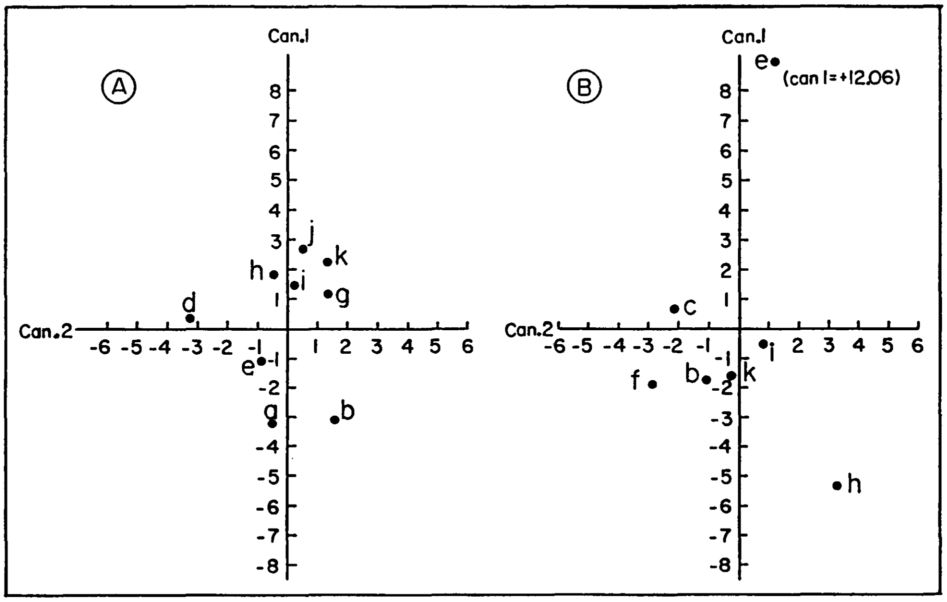

Figure 4. — Changes in resin composition of clonal material of: (A) cv. *stricta* and. (B) var. *horizontalis*, due to environmental factors described by class means of canonical variables (for explanations see *table 1*).

resin compounds synthesis are stable and insensitive, to changes in the environment. However, only few of the numerous studies, dealing with the chemosystematics and/or resin composition of conifers, were conducted to determine the effects of changes in the environmental factors on resin composition of clonal material. Results of studies on clones of *Pinus monticola* DOUGL. have shown negligible differences in monoterpene levels due to effects of adverse growing conditions (HANOVER, 1966), but water stress was found to strongly affect the xylem resin composition of *Pinus taeda* L. (HODGES and LARIO, 1975; GILMORE, 1977). Only small differences in resin composition due to changes in environmental factors were found in the leguminous tree *Hymenaea courbaril* (LANGENHEIM *et al.*, 1979). Nutritional status caused undoubtedly some variation in terpene composition of *Abies grandis* (DOUGL.) LINDL. (MUZIKA *et al.*, 1989).

Our study has shown that environmental factors affect, to some extent, the foliage resin composition of clonal material of *Cupressus sempervirens* L. The results show that in cv. *stricta*, *radiation* had the largest impact on resin composition, more then soil type and soil moisture availability or a combination of these factors. In var. *horizontalis*, soil type had the strongest effect, more then radiation and soil moistre; water availability and radiation significantly affected resin composition.

The results in themselves are of interest in spite of the limitations of the scope of the investigation. First, no attempt was made to determine the range of variation among clones of the same taxon. In a previous study (SCHILLER, 1990) the foliage resin composition was investigated of a total of 493 trees of cv. *stricta* and var. *horizontalis* (and intermediate phenotypes) from 26 populations growing under widely differing environmental conditions; the occurrence was revealed of several chemotypes, part of which could be attributed to different seed sources. In the light of our present findings, these results would need to be somewhat revised, since contrary to (at the time) current assumptions the effect of site factors on resin composition is larger than expected. The existence of differences in resin compositions within clones could be due to the random occurrence of minor biochemical mutations in some branchlets of the trees from which the cuttings were taken; an example of such locally limited mutations is the well-known aurea mutation (which is, however, lethal by causing the death of affected springs due to absence of chlorophyll) (POHLHEIM, 1971). Another cause of within-clone variation may rise due to small differences which cannot be eliminated in nursery trials such as variation in soil, microclimate interactions between neighboring plants affecting access to light, etc. Nevertheless, it is believed that the present study is adequate to measure the relative proportions between genetic and environmental variation, since chemical analysis discriminated correctly in 87% of the material examined between cv. *stricta* and var. *horizontalis*.

Results obtained regarding effects of, and interactions between, environmental factors are difficult to interpret at this stage. The 3 soils under study are known to differ in many characters such as alkalinity, mechanical composition, contents and availability of major nutrients and trace elements, etc. (DAN and RAZ, 1970); to the best of our knowledge there is no specific information on their effects on photosynthesis and metabolite partitioning in plants. Reduction of light by shading is liable to modify not only light intensity but also to have wide-ranging effects on microclimate-air, soil and plant temperatures, relative humidity, etc.-and, hence, on physiological processes such as transpiration, gas exchange and photosynthesis. With present knowledge it would be spurious to assign to each of these factors a defined role on resin composition. Finally, under the climatic conditions prevailing in Israel, increased water supply will primarily affect vegetative growth by increased absorption of soil nutrients and improved plant water relation; whereas, reduction of the very high insolation in summer will reduce the distraction of chlorophyll. To elucidate all these and other questions regarding the effect on resin composition of the complex of environmental factors would require comprehensive research.

Nevertheless, it is believed that our conclusions are in spite of their preliminary character, of value by demonstrating that foliage resin composition of *Cupressus sempervirens* L. is sensitive, to some extent, to environmental factors. This finding calls for caution when resin composition of this taxa, *i.e.*, cv. *stricta* and var. *horizontalis* from different environments is used as a genetic marker.

Acknowledgements

The author express his thanks to the Forest Department of the Jewish National Fund for partly supporting this project 274-0005

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Accelerated Aging of Sitka Spruce Seeds[1])

By K. CHAISURISRI[2])[5]), D. G. W. EDWARDS[3]) and Y. A. EL-KASSABY[2])[4])[6])

(Received 13th October 1992)

Abstract

An accelerated aging test was conducted on seed orchard-produced Sitka spruce seeds from 6 clones. Seeds were aged at 100% RH and 37.5° C for 0 to 21 days at 3-day intervals and a paired (stratified and unstratified) germination test was conducted. Germination parameters (germination capacity, germination value, and peak value) increased in seeds acceleratedly-aged for 3 to 6 days (the conditioned stage), then declined (the deteriorated stage) thereafter. Seed moisture content and average germination capacity were negatively and highly-significantly correlated, for both stratified and unstratified seeds. Significant clonal differences were observed for the germination parameters, indicating the degree of deterioration is clone-specific. The impact of these differences on the genetic diversity of stored, bulked seedlots is discussed.

Key words: Sitka spruce, seed germination, accelerated aging, gene conservation.

Introduction

Germination tests are usually conducted as part of seed-quality testing. Whereas the standard germination test is based on estimating the maximal potential for seed viability, or the ability of a seed to produce a normal plant under favorable conditions, it is not adequate for assessing field emergence (MCDONALD, 1980). A vigour test based on stress conditions is more appropriate for testing seed emergence since it implies the ability of the seed to germinate under both favourable and unfavourable conditions (KNEEBONE, 1976). Possible causes of variation in the level of seed vigour include (1) genetic constitution, (2) environment and nutrition of the mother plant, (3) stage of maturity at harvest, (4) seed size, weight or specific gravity, (5) mechanical integrity, (6) deterioration and aging, and (7) pathogens (Association of Official Seed Analysts, 1983).

Viability in seeds has been found to be highest at the time of physiological maturity, and to decline with age (EDWARDS, 1980). DELOUCHE and BASKIN(1973) have described seed deterioration as encompassing initial membrane degradation and ending with loss of germinability. The symptoms of this deterioration may include decrease in metabolic activity, susceptibility to stress, impaired rate of germination and seedling growth, storability, plant development and yield, emergence potential, and increase in seedling abnormalities. The processes of seed deterioration in a population are independent among the individual seeds, and the time course for deterioration ranges from days to years. Thus, the germination percentage of a seedlot decreases with time in proportion to the number of individual seeds that have become no longer germinable (DELOUCHE and BASKIN, 1973). Differences in the degree of seed deterioration can be revealed through a vigour test that can pinpoint whether the differences stem from seed processing or are genotype-specific (Association of Official Seed Analysts, 1983).

Seed vigour has been demonstrated to be heritable (DICKSON, 1980; KNEEBONE, 1976; MCDANIEL, 1973), and varies according to field weathering (PASCAL and ELLIS, 1978; POTTS et al., 1978; NDIMANDE et al., 1981) and storage conditions (WEIN and KUENEMAN, 1981; MINOR and PASCAL, 1982). In some plants, this trait is inherited maternally (KUENEMAN, 1983). When seeds age, not only does their physiological activity change, but also their chromosomal structure changes (ROBERTS, 1972; PITEL, 1980).

Accelerated aging, a method included in stress tests, has been effectively used to estimate seed vigour and storability in annuals (DELOUCHE and BASKIN, 1975; Association of Official Seed Analysts, 1983). Recently, the accelerated aging technique, which has utility as a seed vigour test of agronomic crops has attracted the attention of tree seed researchers as a means for evaluating the efficacy of *ex-situ* gene conservation method (BLANCHE et al., 1988, 1990; PITEL, 1980; MARQUEZ-MILLANO et al., 1991; EL-KASSABY, 1992). This study employed the standard procedure developed for agricultural seeds to determine if accelerated aging could be used to estimate the impact of long-term

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