tree variation of monoterpene hydrocarbon composition of slash pine oleoresin. Phytchemistry 9: 809—815 (1970). —— Rockwood, D. L.: Cortical monoterpene and fusiform rust resistance relationships in slash pine. Phytopathology 64: 976—979 (1974). —— Rudloff, E. von and Rehfeldt, G. E.: Chemosystematic studies in the genus *Pseudotsuga*. IV. Inheritance and geographical variation in the leaf oil terpenes of Douglas-fir from the Pacific Northwest. Can. J. of Botany 58: 546—556 (1980). —— SAS Institute Inc.: SAS User's Guide: Statistics 5 ed.. SAS Institute, Cary, NC. 956 pp. (1985). —— Schiller, G.: Variation in resin composition of the Italian cypress (*Cupressus sempervirens* L.) grown in Israel. Silvae Genetica 39: 89—95 (1990). —— Schiller, G. and Madar, Z.: Variation in foliage resin composition within te Italian cypress (*Cupressus sempervirens* L.) species complex and its relation to canker diseases. Eur. J. For. Path. 21: 179—184 (1991). —— Schuck, H. J.: Monoterpenes and resistance of conifers to fungi. *In*: Resistance to diseases and pests in forest trees. Ed. by H. M. Heybroek, B. R. Stephan and K. von Weissenberg, pp 161—167 Centre Agric. Publ. Document, Wageningen, The Netherlands (1982). —— Squillace, A. E.: Analysis of monoterpenes by gas-liquid chromatography. *In:* Miksche, J. P. (ed.). Modern Methods in Forest Genetics. pp. 120—157. Springer Verlag, Berlin (1976). —— Stephens, M. A.: Use of the von Mises distribution to analyse continuous proportions. Biometrika 69 (1): 197—203 (1982). —— Zohar, Y.: Autecology of *Cupressus sempervirens* L.. In: Scientific Activities 1980 to 1984. Department of Forestry. Special Publication No. 228, Department of Scientific Publications, The Volcani Center, Bet Dagan, Israel (1984).

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

---

[1]) This manuscript represents a portion of the senior author's Ph. D. dissertation.

[2]) Faculty of Forestry, University of British Columbia, Vancouver, B.C., V6T 1Z4, Canada.

[3]) Natural Resources Canada, Pacific Forestry Centre, 506 West Burnside Rd., Victoria, B.C., V8Z 1M5, Canada.

[4]) Canadian Pacific Forest Products Ltd., Saanich Forestry Centre, 8067 East Saanich Rd., R. R.# 1, Saanichton, B. C., VOS 1MO, Canada.

[5]) Permanent address: ASEAN-Canada Forest Tree Seed Centre, Muak-Lek, Saruburi 18180, Thailand.

[6]) To whom reprint requests should be addressed.

seed storage on the genetic diversity of Sitka spruce seedlots and the vigour of orchard-produced seeds.

Materials and Methods

Seed Source

Pacific Forest Products Ltd. provided the seeds for this study from the Sitka spruce seed orchard located in Saanichton, British Columbia (latitude 48°35'N, longitude 123°24'W). The orchard consists of 139 clones (averaging 9.3 ramets per clone) selected from elevations between 0 m and 415 m on western Vancouver Island, Washington and Oregon. The orchard was established in 1971 in a random single-tree mix.

In September 1990, wind-pollinated seeds from 6 clones were collected for the study. The clonal identity of the seeds was maintained during seed extraction, after which the seeds were stored at 2° C until used. They were then subjected to accelerated aging according to the AOSA's seed-vigour-testing procedure (Association Official Seed Analysts, 1983).

In this study, it is assumed that (1) the processes of seed deterioration under accelerated aging conditions are similar to those under natural conditions — only the rate of deterioration is increased (Delouche and Baskin, 1973), and (2) the decline in germination after accelerated aging is associated with the initial degree of deterioration of the seedlots, i.e., high-vigour lots will show little decline in germination, whereas low-vigour lots will show a marked decline in germination (Baskin, 1977).

Accelerated Aging

A pilot study conducted on a single clone under 4 temperatures: 35° C, 37.5°C, 40° C and 45° C (data not shown) indicated that 37.5° C was the appropriate temperature for accelerated aging in Sitka spruce. This temperature is lower than that prescribed for accelerated aging (Association of Official Seed Analysts, 1983). Blanche et al. (1988) also recommended the use of lower temperatures when accelerated aging is applied to tree seeds.

In this study, accelerated aging was applied at 37.5° C for 8 aging periods from 0 to 21 days at 3-day intervals. Each test consisted of 10 samples of 100 seeds. Each sample was placed separately on a wire-mesh screen (Hoffman Mfg. Co.) in a tightly-closed incubation box containing 50 ml. of water. The distance between the seeds and the water surface was 1.4 cm. Samples were obtained in succession so that the samples for the longest accelerated-aging treatment (21 days) were placed in the incubator first and the shortest-aged samples (3 days) were the last. At the end of the test all samples were removed and 2 samples from each accelerated aging treatment were used for moisture-content determination (International Seed Testing Association, 1985). The remaining samples (8) were kept at room temperature overnight to reduce the effect of rapid temperature change on seed germination, as recommended by Bourland and Ibrahim (1982). All the seed samples (moldy and non-moldy seeds) were rinsed under running, tepid, water for 2 minutes prior to germination testing.

Germination Test

All germination tests were conducted under ISTA rules (International Seed Testing Association, 1985). Following the rinsing, 4 samples were germinated immediately without stratification. The remaining four were soaked in water for 24 hr, drained, placed in plastic bags, and stratified at 2° C for 21 days before germination. For germination, each sample was spread in a tightly-lidded, clear plastic germination box lined with moistened cellulose wadding (Kimpak) and filter paper, then placed in a germinator set at 30° C for an 8 hr day, and 20° C for a 16 hr night. Light, at approximately 1000 lux, was provided during the day by means of cool-white fluorescent tubes. Germinants were counted every day for 21 days and classified as normal or abnormal (International Seed Testing Association, 1985).

The germination data were calculated and expressed as germination capacity (GC), the percentage of seeds that had germinated at the end of the test; peak value (PV), the maximum quotient derived by dividing daily the accumulated number of germinants by the corresponding number of days (which is the mean daily germination of the most-vigorous component of the seedlot, a mathemetical expression of the tangent drawn through the origin of the sigmoid curve representing a typical course of germination) (Czabator, 1962); and germination value (GV), the combination of both the speed and completeness of germination into a single value (Czabator, 1962).

Statistical Analysis

The analysis of variance used was based on the following nested-factorial, additive, linear model:

$$Y_{ijkl} = \mu + C_i + P_j + CP_{ij} + T/C_{(i)k} + PT/C_{(i)jk} + \epsilon_{(ijk)l}$$

where μ = overall mean,

C_i = the effect of ith clone, i = 1 to 6 (random effect),

P_j = the effect of jth seed pretreatment, j = 1 to 2 (fixed effect),

CP_{ij} = the effect of interaction between clones and seed pretreatments,

$T/C_{(i)k}$ = the effect of kth aging time within clones, k = 1 to 8 (fixed effect),

$PT/C_{(i)jk}$ = the effect of interaction between seed pretreatments and aging times within clone,

and $\epsilon_{(ijk)l}$ = residual term, l = 1 to 4.

Data transformations were conducted using an ad-hoc procedure for finding appropriate transformation to normalize the calculated response variables and achieve homogeneity of variances.

Results and Discussion

The variation in the effect of accelerated aging due to clonal differences is highly significant ($P < 0.01$) (*Table 1*). Clonal variation in germination parameters ranges between 30.96% (GC) and 41.22% (GV) of total variation. Variation due to seed pretreatment is not significant for PV and GV, but is significant ($P < 0.05$) for GC, and accounts for 2.46% to 18.26% of total variation (*Table 1*). Variation due to the length of the accelerated-aging period is highly significant, accounting for the largest proportion of the total variation, ranging from 46.47% in GC to 50.23% in GV. Clonal response to the various treatments (pretreatment, accelerated aging and their interaction) was consistend as expressed by the low residual term (i.e., replication-to-replication variation) (*Table 1*).

It appears that 3 to 6 days of accelerated aging enhance germination of unstratified and stratified seeds in some clones (*Figure 1* and *2*), but germination gradually declines with longer treatment. The germination patterns obtained for stratified seeds after the aging treatments produced

reduced levels of variation than in unstratified seeds (*Figure 1* and *2*). Similar results were obtained from a standard germination test conducted on the same species, indicating that stratification treatment was essential to overcome seed dormancy (CHAISURISRI et al., 1992). Germination enhancement due to short-time accelerated aging might be of operational importance. The relation between germination rate and accelerated aging treatment followed that of germination capacity (*Figure 3* and *4*). As expected germination rates for stratified seeds were generally higher than that of unstratified ones, confirming the presence of seed dormancy in this species (CHAISURISRI et

Table 1. — Variation in germination of 6 Sitka spruce clones following accelerated aging (AA) and seed pretreatment (values are percentages of the total mean squares).

Source of Variation	Degrees of Freedom	Expected Mean Squares[1]	Germination Parameters[2]		
			GC	PV	GV
Clone (C)	(C-1) = 5	$\sigma_e^2 + 64\sigma_c^2$	30.96^{xx}	34.70^{xx}	41.22^{xx}
Pretreatment (P)	(P-1) = 1	$\sigma_e^2 + 32\sigma_{cp}^2 + 192\phi_p$	18.26^{x}	12.17^{ns}	2.46^{ns}
C x P	(C-1)(P-1) = 5	$\sigma_e^2 + 32\sigma_{cp}^2$	2.07^{xx}	3.14^{xx}	3.65^{xx}
AA-Time within Clone (T/C)	C(T-1) = 42	$\sigma_e^2 + 8\sigma_{t/c}^2$	46.47^{xx}	48.09^{xx}	50.23^{xx}
P x T/C	C(T-1)(P-1) = 42	$\sigma_e^2 + 4\sigma_{p1/c}^2$	1.70^{xx}	1.20^{xx}	1.54^{xx}
Residual	CPT(N-1) = 288	σ_e^2	0.54	0.70	0.90

ns) Non significant
*) Significant at $P < 0.05$.
**) Significant at $P < 0.01$.
1) Expected mean squares computed from the nested-factorial experimental design.
2) GC = Germination capacity; the percentage of seeds that had germinated at the end of the test (Arcsin).
PV = Peak value; a mathematical expression of the break of a sigmoid curve representing a typical course of germination (1—1/(X+1)).
GV = Germination value, (1—1/(X+1)).

al., 1992). It is most interesting to note that the decline in germination rate due to the aging treatment varied among clones.

Seed moisture content is increased at 3 days of accelerated aging (on average from 6.7% to 33%, based on dry weight) and then remains relatively stable to day 12 (*Figure 5*). Seed moisture content increases after 12 days of treatment, probably because cell membranes become severely damaged, and viability is subsequently lost. This decline in germination is associated with the increase in moisture content (*Figure 6*). Correlations between average GC and moisture content are negative and highly significant ($P < 0.01$) for stratified ($r = -0.94$) and unstratified ($r = -0.94$) seeds.

The period when accelerated aging enhances seed germination has been termed the "conditioned stage" by Bird and Reyes (1967), while the term "deteriorated stage" was

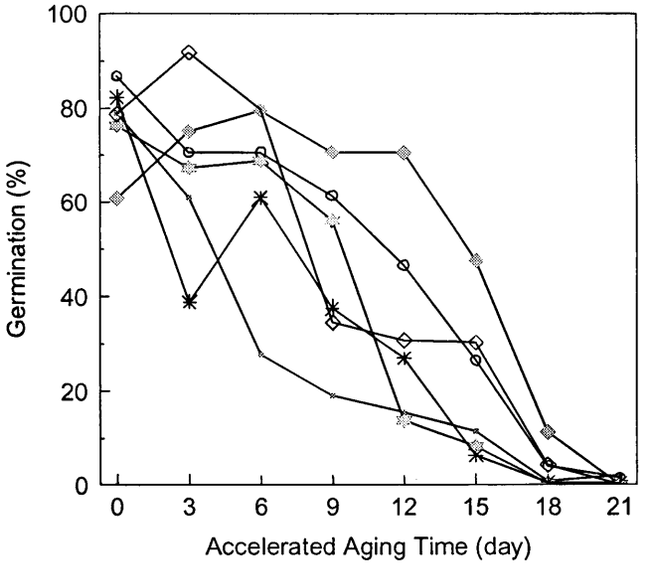

Figure 1. — Average germination capacity (GC) of unstratified seeds of 6 Sitka spruce clones after accelerated aging. Clone 24 (circles), 44 (squares), 61 (closed dimonds), 92 (stars), 416 (open dimonds), and 421 (asterisks).

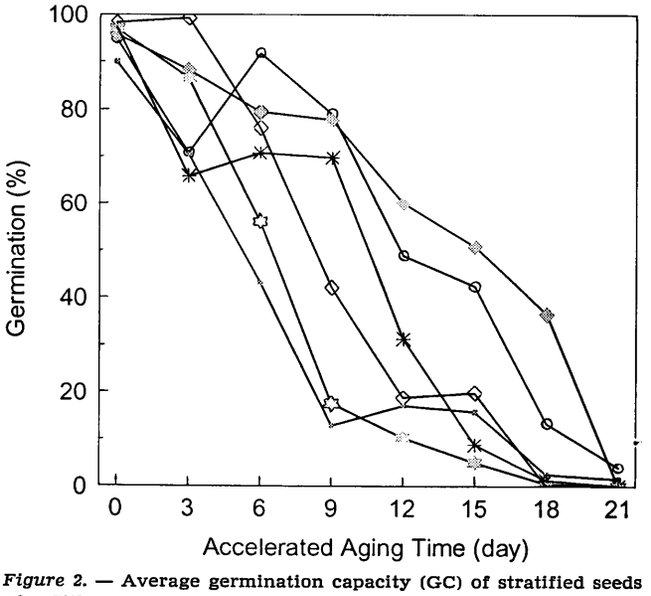

Figure 2. — Average germination capacity (GC) of stratified seeds of 6 Sitka spruce clones after accelerated aging (see *figure 1* for clonal identification).

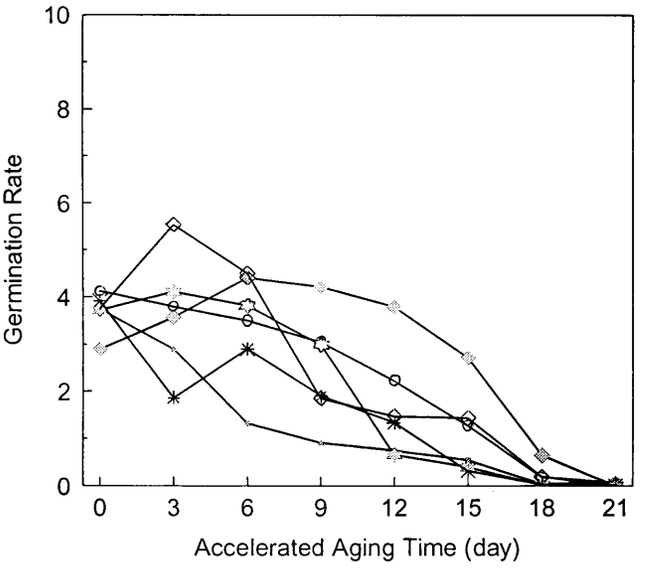

Figure 3. — Average germination rate (PV) of unstratified seeds of 6 Sitka spruce clones after accelerated aging (see *figure 1* for clonal identification).

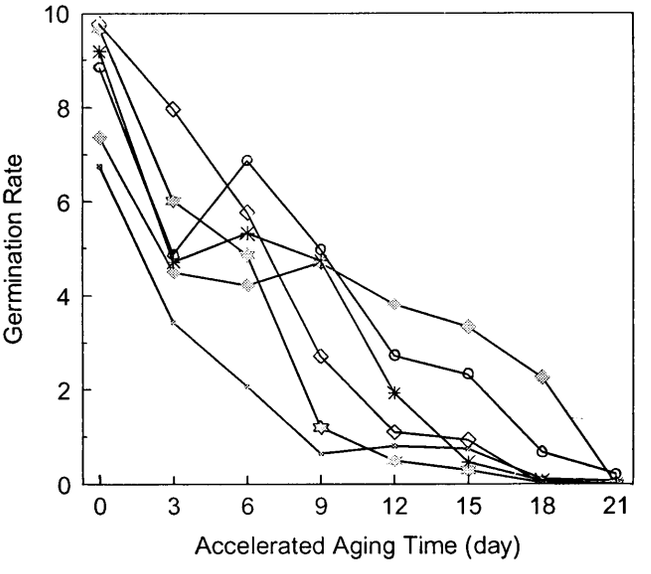

Figure 4. — Average germination rate of stratified seeds of 6 Sitka spruce clones after accelerated aging (see *figure 1* for clonal identification).

used for the period when germination decreases until the seed dies. The germination trends after accelerated aging observed in this study fit the two stages described above. Enhancement of seed germination due to a short period of accelerated aging was observed in water oak (*Quercus nigra L.*) Blanche et al., 1990). Such enhancement is normal for all annual seed plants (Bourland and Ibrahim, 1982), and is due to the increase in moisture content that brings the level of hydration closer to the minimal requirement for seed germination. The breakdown of polymeric storage compounds also accounts for this enhancement (Blanche et al., 1988, 1990; Pitel, 1980). However, the extent of germination enhancement in response to accelerated aging is also influenced by the initial seed quality (Blanche et al., 1990), including moisture content (McDonald, 1977; Toa 1979).

The first event during the "deteriorated stage" is believed to be caused by a loss of membrane integrity (Basa-

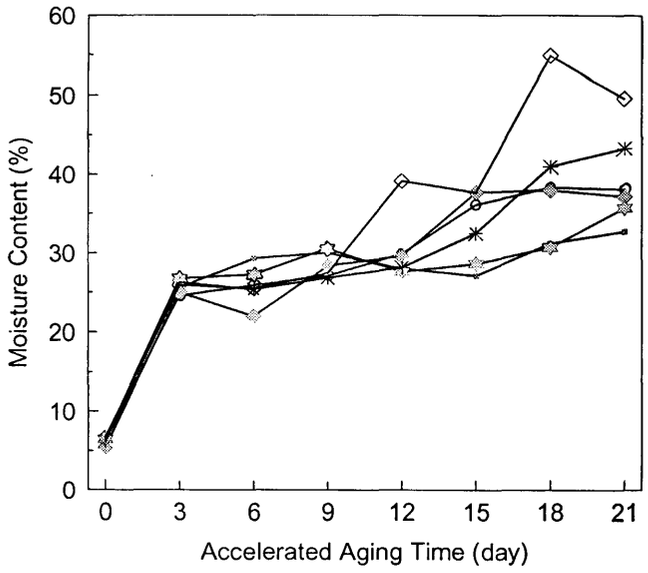

Figure 5. — Moisture content of seeds of 6 Sitka spruce clones after accelerated aging (see *figure 1* for clonal identification).

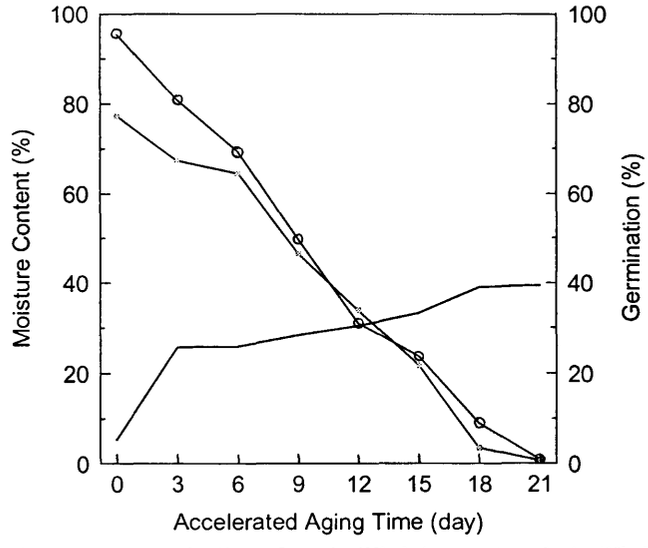

Figure 6. — Germination of unstratified (squares) and stratified (circles) seeds, and their moisture content, after accelerated aging; average for 6 clones.

VARAJAPPA et al., 1991); cell permeability is increased, allowing large quantities of cellular components to diffuse out when the seed is placed in water (SCHNATHORST and PRESLEY, 1963). The increase in membrane permeability in accelerated-aged seeds is possibly due to changes in the molecular structure of the membrane (KOOSTRA, 1973; PITEL, 1980; SIMOLA, 1974). The increase in metabolism during aging depletes food reserves and, subsequently, seed vigour declines (BLANCHE et al., 1990). The loss of seed viability and differential survival of biotypes due to accelerated aging was found to be associated with selection and genetic shifts in germplasm accessions of wheat (STOYANOVA, 1991).

The response of seeds to accelerated aging varies according to species BENNETT-LARTEY, 1991; BLANCHE et al., 1988), population (MILBY and JOHNSON, 1989), family (BOURLAND and IBRAHIM, 1982) and individual seeds (DELOUCHE and BASKIN, 1973). In Sitka spruce, the observed germination differences of treated seeds clearly indicate that different clones will age/deteriorate at different rates during storage. In annuals, seed storability and seed vigour have been found to be related (DELOUCHE and BASKIN, 1973; Association of Official Seed Analysts, 1983). While it is not yet known if vigour and storability are related in Sitka spruce seeds, it is interesting to speculate that the data presented here suggest that accelarated aging may be used as a method of indexing seed vigour in this species.

In practice, when seeds collected from different orchard clones are bulked into a single seedlot, long-term storage of this seedlot might reduce its genetic diversity. Since the contribution of clones to seed production in this orchard is known to be unbalanced (EL-KASSABY and REYNOLDS, 1990), then the seed contribution of some clones might be eliminated and/or reduced by long term-storage.

For agricultural crops, germination following accelerated aging and periods of storage are closely associated, i.e., those lots that have high survival after accelerated aging stored well, while lots that were severely reduced in their germination by accelerated aging declined rapidly in storage (DELOUCHE and BASKN, 1973). To-date, there are no such data available for tree seeds.

Acknowledgements

The authors thank M. D. MEAGHER for reviewing the manuscript and D. W. TAYLOR for technical assistance. This study is supported in part by ASEAN-Canada Forest Tree Seed Centre (CIDA) grant to K. C., Pacific Forest Products Ltd., and Natural Resources Canada, Pacific Forestry Centre.

References

Association of Official Seed Analysts: Seed vigor testing handbook. Contribution No. 32, Association of Official Seed Analysts (1983). — BASAVARAJAPPA, B. S., SHETTY, H. S., and PRAKASH, H. S.: Membrane deterioration and other biochemical changes associated with accelerated ageing of maize seeds. Seed Sci. and Technol. **19**: 279—286 (1991). — BASKIN, C. C.: Vigor test methods — Accelerated aging. Assoc. Off. Seed Anal. Newslett. **51**: 42—52 (1977). — BENETT-LARTEY, S. O.: The longevity of pea, sunflower and groundnut seeds under controlled temperature and moisture content conditions. Trop. Sci. **31**: 9—19 (1991). — BIRD, L. S. and REYES, A. A.: Effects of cotton seed quality on seed and seedling characteristics. In: Proc. Beltwide Cotton Prod. Res. Conf.. pp. 199—206. National Cotton Council, Memphis, Tennessee (1967). — BLANCHE, C. A., ELAM, W. W. and HODGES, J. D.: Accelerated aging of *Quercus nigra* seed: biochemical changes and applicability as a vigor test. Can. J. For. Res. **20**: 1611—1615 (1990). — BLANCHE, C. A., ELAM, W. W., HODGES, J. D.: BONNER, F. T. and MARQUEZ, A. C.: Accelerated aging of selected tree seeds. In: Proc. 10th North American forest biology workshop. Eds. J. WORRALL, J. LOO-DINKINS and D. P. LESTER. pp. 327—334. Vancouver, British Columbia (1988). — BOURLAND, F. M. and IBRAHIM, A. A. L.: Effects of accelerated aging treatments on six cotton cultivars. Crop Sci. **22**: 637—640 (1982). — CHAISURISRI, K., EDWARDS, D. G. W. and EL-KASSABY, Y. A.: Genetic control of seed size and germination in Sitka spruce. Silvae Genet. **41**: 348—354 (1992). — CZABATOR, F. J.: Germination value: An index combining speed and completeness of pine seed germination. For. Sci. **8**: 386—396 (1962). — DELOUCHE, J. C. and BASKIN, C. C.: Accelerated aging techniques for predicting the relative storability of seed lots. Seed Sci. and Technol. **1**: 427—452 (1973). — DICKSON, M. H.: Genetic aspects of seed quality. HortSci. **15**: 771—774 (1980). — EDWARDS, D. G. W.: Maturity and quality of tree seeds — a state-of-the-art review. Seed Sci. and Technol. **8**: 625—657 (1980). — EL-KASSABY, Y. A.: Domestication and genetic diversity — should we be concerned? For. Chron. **68**: 687—700 (1992). — EL-KASSABY, Y. A. and REYNOLDS, S.: Reproductive phenology, parental balance, and supplemental mass pollination in a Sitka-spruce seed-orchard. For. Ecol. Manage **31**: 45—54 (1990). — International Seed Testing Association: International rules for seed testing. Seed Sci. and Technol. **13**: 299—513 (1985). — KNEEBONE, W. R.: Some genetic aspects of seed vigor. J. Seed Technol. **1**: 86—97 (1976). — KOOSTRA, P.: Changes in seed ultrastructure during senescence. Seed Sci. and Technol **1**: 417—425 (1973). — KUENEMAN, E. A.: Genetic control of seed longevity in soybeans. Crop Sci **23**: 5—8 (1983). — MARQUEZ-MILLANO, A.,

ELAM, W. W. and BLANCHE, C. A.: Influence of accelerated aging on fatty acid composition of slash pine (*Pinus ellioyyii* ENGELM. var. *elliotti*) seeds. J. Seed Technol. **15**: 29—41 (1991). — MCDANIEL, R. G.: Genetic factors influencing seed vigor: Biochemistry of heterosis. Seed Sci. and Technol. **1**: 25—50 (1973). — MCDONALD, M. B., Jr.: The influence of seed moisture on the accelerated aging seed vigor test. J. Seed Technol. **2**: 18—28 (1977). — MCDONALD, M. B., Jr.: Assessment of seed quality. HortSci. **15**: 184—188 (1980). — MCWILLIAM, J. R. and GRIFFING, B.: Temperature-dependent heterosis in maize. Aust. J. Biol. Sci. **18**: 569—583 (1965). — MILBY, T. H., and JOHNSON, F. L.: Variation in response to stratification and ageing in gamagrass seeds from different geographic locations. Seed Sci. and Technol. **17**: 413—419 (1989). — MINOR, H. C. and PASCAL, E. C.: Variation in storability of soybeans under simulated tropical conditions. Seed Sci. and Technol. **10**: 131—139 (1982). — NDIMANDE, B. N., WEIN, H. C. and KUENEMAN, E. A.: Soybean seed deterioration in the tropics. The role physiological factors and fungal pathogens. Field Crops Res. **4**: 113—121 (1981). PASCAL, E. H. II and ELLIS, M. A.: Variation in seed quality characteristics of tropically grown soybeans. Crop. Sci. **18**: 837—840 (1978). — PITEL, J. A.: Accelerated aging studies of seeds of jack pine (*Pinus banksiana* LAMB.) and red oak (*Quercus rubra* L.). In: Proc. International Symposium on Forest tree seed storage IUFRO Working party on Seed Problems. Eds. B.S.P. WANG and J. A. PITEL, pp. 40—54. Petawawa National Forestry Institute, Chalk River, Ontario (1980). — POTTS, H. C., DUANGPATRA, J., HAIRSTON, W. G. and DELOUCHE, J. C.: Some influences of hardseededness on soybean seed quality. Crop. Sci. **18**: 221—224 (1978). — ROBERTS, E. H.: CYTOLOGICAL, genetical and metabolic changes associated with loss of viability. In: Viability of seeds. Ed. E. H. ROBERTS. pp. 253—306. Chapman and Hall, London (1972). — SCHNATHORST, W. C. and PRESLEY, J. T.: Proneness of deteriorated cottonseed to decay and seedling diseases related to physicobiochemical factors. In: Proc. Beltwide Cotton Prod. Res. Conf. pp. 57—58. National Cotton Council, Memphis, Tennessee (1963). — SIMOLA, L. K.: Ultrastructure changes in the seeds of *Pinus sylvestris* L. during senescence. Stud. For. Suec **119**: 1—22 (1974). — STOYANOVA, S. D.: Genetics shifts and variations of gliadins induced by seed ageing. Seed Sci and Technol. **19**: 363—371 (1991). — TOA, K. J.: An evaluation of alternative methods of accelerated aging seed vigor test for soybeans. J. Seed Technol. **3**: 30—40 (1979). — WEIN, H. C. and KNUENEMAN, E. A.: Soybean seed deterioration in the tropics. Varietal differences and techniques for screening. Field Crops Res. **4**: 123—132 (1981).

In Vitro Propagation of Salix caprea L. by Single Node Explants

By H. NEUNER and R. BEIDERBECK

Botanisches Institut der Universität Heidelberg,
Im Neuenheimer Feld 360, D-69120 Heidelberg, Germany

(Received 21st December 1992)

Summary

Salix caprea was propagated *in vitro* from single node explants of field-grown plants. Two media were used for the induction of shoot growth: SCHENCK-HILDEBRANDT'S and AHUJA'S medium. The newly formed shoots were rooted on 1/50 strength AHUJA medium. Plantlets were acclimatized to soil and greenhouse conditions under waterlogging conditions. In the greenhouse the plants grew to a height of 1 m within 5 months.

Key words: Salix caprea, goat willow, micropropagation, tissue culture.

Introduction

The techniques of micropropagation may promote breeding programs for difficult-to-root forest species by facilitating clonal propagation and plant selection. Several reviews on micropropagation of woody plants are available (BONGA, 1982; BROWN and SOMMER, 1982), however publications on the genus *Salix* are very infrequent (READ et al., 1989; GARTON et al., 1983), possibly because of the ease by which most willow species can be propagated from cuttings. However, the pioneer shrub and excellent pollen donor, *Salix caprea* L., cannot be propagated by hardwood cuttings (NEUMANN, 1981; CHEMLAR and MEUSEL, 1986) and propagation by green softwood cuttings under mist is subject to considerable seasonal variations (LATTKE, 1965). This study was initiated to develop an *in vitro* method for an year-round propagation.

Material and Methods

Plant source

Young shoots ($\leq$ 4 mm diameter) were harvested throughout the year from 9 individual plants of *Salix caprea* L. growing along roadsides in Nußloch/Baden. They were defoliated and cut into segments of $\leq$ 6 cm length. Segments with large catkin buds were discarded.

Shoot formation on explants

Shoot segments were washed with running tap water for 15 min and, generally, surface sterilized for 30 min by stirring in solutions of 1% to 10% NaOCl with some drops of Tween 20 as a surfactant. Subsequently the segments were washed with sterile water (3 x 10 min). Under a laminar flow cabinet shoot segments were cut into single node explants (a node with an internode segment of 4 mm length at its base) and placed in test tubes (2.5 cm diameter) with 20 ml solidified nutrient medium, leaving the buds above the medium. The tubes were closed with Caputs (Bellco R) and incubated under culture conditions: 25° C, 12:12 hours light-dark regime with 2000 Lux fluorescent white light (Sylvania cool white, GTE).

Two media with some variations were used:

1. SH medium (SCHENCK and HILDEBRANDT, 1972) consisting of SH salts and vitamin mixture (Sigma Chemicals, Deisenhofen) supplemented with 20 g/L sucrose, 2.5 g/L gelrite and varying amounts of benzylamino purine (BAP) or kinetin; before autoclaving pH was adjusted to 5.7.

2. ACM medium consisting of the macro- and microelements, vitamins and organic compounds as described by AHUJA (1983) supplemented with 20 g/l sucrose, 2.5 g/l gelrite and varying amounts of BAP or kinetin; before autoclaving pH was adjusted to 5.5 to 5.6.

Rooting

After buds had grown into young shoots *in vitro* for 4 weeks to 5 weeks the shoots were cut off the original explants, transferred into new test tubes (2.5 cm diameter)