Genetic Variation and Realized Genetic Gain from Aleppo Pine Tree Improvement

By D. I. MATZIRIS[1])

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Summary

In January 1987 a 10 ha clonal seed orchard of Aleppo pine (*Pinus halepensis* MILL.) was established in the area Amphilochia, Greece. The orchard comprises 76 clones derived from intensively selected trees in the natural forests of Euboia island. In November 1986, two open pollinated progeny tests were established in central west Greece proximal to Nafpaktos and Stamna. Seedlings from 63 to 70 families, including a commercial check, were planted at the Stamna and Nafpaktos locations, respectively. One year later (SPRING, 1988) an additional planting including 32 families plus the commercial check was established in the area of Limni on Euboia island. Assessments were made in 1997 when trees were 10 years (Nafpaktos and Stamna) and 9 years (Limni) from planting and the following results were obtained.

Variation among families for growth (height, DBH, volume), bark thickness, branch angle, cone production, straightness, and crown form were considerable. The mean annual height increment was greater at Stamna (75 cm) followed by Nafpaktos (46 cm) and Limni (43 cm). The mean annual growth at Stamna (75 cm) is considered very satisfactory and indicates the high potentiality of Aleppo pine when planted in relative good sites.

Narrow sense heritability estimates on individual tree basis (h^2) were variable depending on the characteristic and the location of the experimental planting. Heritability values for height were 0.45, 0.43 and 0.42 for Nafpaktos, Stamna and Limni plantings, respectively. The corresponding h^2 values for branch length, branch thickness and branch angle were 0.22, 0.26 and 0.42. Cone production was the stronger inherited trait with $h^2 = 0.68$ for Limni and $h^2 = 0.57$ for Stamna.

Realized genetic gains were calculated for growth characteristics (height, DBH and volume) using the pooled data of the two (Nafpaktos and Stamna) experimental plantings; Limni site was not considered due to low number of families (32) included in the planting. The performances of the improved materials were compared to unimproved materials (commercial check). From the selection made in natural stands the realized gain was 5.2% for height, 7.68% for DBH and 13.0% for volume over the commercial check. When 20% of the total number of clones with the lower breeding value are removed (rogued) from the seed orchard an additional gain of 2.7% for height, 2.71% for DBH and 8.19% for volume over the unrogued seed orchard is resulted. Thus, the total realized genetic gain for first generation, genetically tested, seed orchard of Aleppo pine is summed to 7.9% for height, 10.38% for DBH and 21.25% for volume. These results clearly show that selection of Aleppo pine followed by clonal seed orchard establishment and progeny testing is very profitable.

Key words: Realized genetic gain, breeding value, heritability, rogued seed orchard, *Pinus halepensis.*

Introduction

Aleppo pine (*Pinus halepensis* MILL.) is a serotinous species well adapted to dry mild Mediterranean ecosystems. Sexual reproduction starts at a very young age with pronounced precocity in cone and seed production (MATZIRIS, 1997, 1998). Early reproductive maturity provides a high selection advantage in situations where fires tend to destroy forest tree stands (HEYBROEK, 1974). Early reproduction coupled with serotiny make the species highly competitive with other woody vegetation in fire-sensitive Mediterranean ecosystems. When present in an undisturbed landscape, Aleppo pine becomes the dominate species.

In Greece Aleppo pine occurs along the coast and in some islands (PAPAIOANNOU, 1935; PANETSOS, 1975). The geographic distribution is very discontinuous, as it has been strongly affected by past geological changes and human interference. Since Aleppo pine forests are found along the coast and at low elevations, they were accessible by man from antiquity. Dysgenic selection has been practised over a long period resulting in the genetic erosion of extant populations, as indicated by an allopatric distribution and poor stem form. However, there still exists stands that have well adapted phenotypes with good growth and straight stems. Additionally, introgression of genes from *Pinus brutia* TEN. (a closely related species) into the Greek populations of Aleppo pine has resulted not only in higher heterozygosity but also in better growth, and stem form and resistance to *Matsucoccus joshephy* BODENHEIMER et HARPAZ (SCHILLER et al., 1986).

A wide network of Aleppo pine provenance trials covering nearly the whole geographic distribution of the species have been established in Greece, as well as, in many other parts of the world (PALMBERG, 1975; PANETSOS, 1981; ECCHER et al., 1982; FUSARO, 1986; WEINSTEIN, 1989; TULUKCU et al., 1989). The results of these tests have shown that the provenance from the island of Euboia was superior in Greece and also in many other countries within and outside of the Mediterranean region. Based on these results, a selection program was initiated in Greece within the best populations on the Euboia island. In 1987, a 10 ha clonal seed orchard, including 76 clones and a total number of 2630 grafts was established in the area of Amphilochia, west Greece. Progeny testing of clones in the orchard was the second phase of the improvement program. Data obtained through these genetic tests are necessary for the management of the already established seed orchard as well also advanced generation breeding.

The objective of the present investigation is to study genetic variation in growth and quality characteristic of Aleppo pine, identify clones with relatively low breeding values for roguing the seed orchard and estimate realized genetic gain from the first generation clonal seed orchard.

Material and Methods

Progeny Test Establishment

Open pollinated (OP) cones were harvested from all selected trees at the time scions were collected for grafting to form the Amphilochia orchard (SPRING, 1986). Seed were extracted by mother tree, and sown in the nursery in paper pots, filled with

[1]) National Agricultural Research Foundation, Forest Research Institute, Terma Alkmanos, Athens 11528, Greece

a sterilized mixture of peat moss and perlite (2:1). In November 1986, two experimental plantings were established in central west Greece proximal to Nafpaktos and Stamna. Seedlings from 63 and 70 families, including a commercial check (CC), were planted at the Stamna and Nafpaktos locations, respectively. The CC was comprised of seedlings originating from selected seed stand No. 24 in north Euboia, that produces high quality progenies (MATZIRIS, 1989). One year later (SPRING, 1988), an additional planting of 32 families plus the CC was established in the area of Limni on Euboia island. All families were treated alike in the nursery as well as at the sites. The characteristics of the planting sites are shown in *table 1.* All planting sites were well prepared by bulldozer before establishment.

A randomized complete block design was selected for the experimental design. Ten tree row plots were used for the first two plantings (Nafpaktos, Stamna) and 8 tree row plots were used at the Limni site. Four, 2 and 3 replications were planted at the Nafpaktos, Stamna and Limni sites, respectively. Trees were planted at a spacing of 2 m x 3 m at each site.

Measurements

The progeny tests were measured for height (HT) and DBH (at 1.3 m) after 10 years at the Stamna and Nafpaktos sites and 9 years at the Limni site. Stem volume (VOL) was calculated using the formula VOL= 3.14/3 X $(DBH)^2/4$ X HT. Cone production and bark thickness (at DBH) were measured at the Limni and Stamna plantings; at Limni all cones were counted while at Stamna, due to large number, they were evaluated using a scale of 1 (up to 10 cones) to 5 (more than 40 cones). Stem straightness and crown form also were assessed at these two sites, using a scale of 1 (=best) to 6 (=worst).

Branching characteristics were evaluated only at the Limni site on the largest branch proximal to the DBH measurement height. Branch length, thickness (at juncture with the trunk), and angle were measured.

Statistical Analyses and Genetic Interpretations

Analyses of variance were conducted on all characteristics in two stages due to missing trees within plots and the need for the estimation of the within plot variance (SNEDECOR and COCHRAM, 1968; NAMKOONG, 1979; FALCONER, 1970; BECKER, 1985). Initially, the mean squares within plot variances (σ_w^2) were estimated from individual tree data. Then, ignoring the differences in the number of trees per plot, analyses based on plot means were made. Mean squares were equated to expected mean square values and variance components were derived.

The narrow sense heritability on individual tree basis (h^2) was estimated as the ratio of additive genetic variance to total phenotypic variance (σ^2_{ph}).

$$h^2 = \sigma^2_A / \sigma^2_{ph} = 4\,\sigma^2_f / (\sigma^2_f + \sigma^2_{rf} + \sigma^2_w)$$

Where, the variance between the half sib families (σ^2_f) is interpreted as the covariance between individuals within the half sib families, that is one quarter of the additive genetic variance

$$\sigma_f^{\,2} = \text{Cov. H. S.} = 1/4\,\sigma^2_A$$

Some full sibling progeny may be present within the families and correspondingly would increase the additive genetic variance. However, since the seeds for the progeny tests were collected from the original selected trees, that are widely separated from each other, this influence should be minimal and practically of no importance (RUOTSALAINEN and LINDGREN, 1998).

Correlation coefficients between all combinations of the characteristics studied were estimated using appropriate procedures (SNEDECOR and COCHRAN, 1968).

Estimate of Realized Genetic Gain

Realized genetic gain was estimated for the unrogued and rogued clonal orchard at Amphilochia. The gain for the first stage of selection was computed by subtracting the mean of the commercial check (CC) from the overall mean of the families of the selected trees included in the seed orchard; the difference was subsequently expressed as percentage of the commercial check. The gain from the second stage of selection, resulting from roguing the seed orchard on the basis of progeny testing was derived by subtracting the overall mean of the progeny tests (excluding CC) from the mean of the families, which were proven to be genetically superior on the basis of volume production and are maintained in the seed orchard; this was also expressed as percentage gain on the basis of unrogued progeny mean. Thus, the total realized genetic gain from rogued seed orchard was computed by the addition of gains from the two stages of selection (mass selection plus progeny testing).

Results and Discussion

The overall means of the characteristics studied along with ranges among family are shown in *table 2.*

Table 1. – Site characteristics of the experimental plantings.

Characteristic	Nafpaktos	Stamna	Limni
Latitude	38° 32	38° 24	38° 47
Longitude	21° 18	21° 46	23° 18
Elevation	180	200	230
Year of establishment	Nov. 1986	Nov. 1986	March, 1988
Soil parent material	Flysch	Tertiary deposits	Calcareous marl
Rainfall / year, mm	983	792	813
Summer rainfall, mm	51	32.5	56
Natural vegetation	*Arbutus spp.* *Erica* spp.	*Arbutus spp.* *Erica spp.* *Guercus ilex*	Opening within natural stands of Aleppo pine

Table 2. – Overall means of the characteristics studied at the age of 10 (Nafpaktos and Stamna) and 9 (Limni) years progeny tests of Aleppo pine.

Characteristic	mean	Range	S. D.
NAFPAKTOS			
Height (m)	4.23	2.99–5.23	0.683
DBH (cm)	7.66	5.64–9.60	0.952
Volume (m ^{3}x100)	0.738	0.27–1.313	0.254
STAMNA			
Height (m)	7.50	6.07–8.87	0.683
DBH (cm)	11.16	9.11–12.62	0.899
Volume (m ^{3}x100)	2.706	1.61–3.89	0.571
Bark thickness (mm)	8.06	5.90–9.23	0.688
Straightness	2.74	1.50–3.50	0.407
Crown form	2.96	1.80–3.90	0.414
Cones	3.08	1.8–4.69	0.674
LIMNI			
Height (m)	4.13	3.51–4.82	0.300
DBH (cm)	5.82	3.96–6.96	0.758
Volume (m ^{3}x100)	0.445	0.159–0.603	0.1419
Bark thickness (mm)	4.21	2.93–5.20	0.906
Branch length (m)	1.54	1.50–1.77	0.175
Branch thickness (mm)	20.93	14.99–24.10	3.496
Branch angle (degrees)	61.2	50.6–70.1	6.520
Straightness	2.76	2.3–3.2	0.2825
Crown form	2.85	2.1–3.4	0.3330
Cones	4.0	1–5.8	1.7880

Growth Characteristics

At the Stamna planting the family means for total tree height at the age of 10 years varied from 6.07 m to 8.87 m. The overall mean was 7.5 m, with a standard deviation of 0.683. A mean annual height growth of 75 cm for the first decade is considered very satisfactory and indicates that Aleppo pine has a high production potential when planted in relative good sites. Some individual trees approached 13.0 m, in height, almost double the plantation mean. Diameter was variable among families ranking from 4.11 cm to 12.62 cm with an overall mean of all families 11.66 cm. Volume had an overall mean 2.206 m^3 X 100 and ranged among families from 1.61 m^3 X 100 to 3.89 m^3 X 100.

The Nafpactos plantation lies at the southern end of very extensive flysch deposits in western Greece. There were significant differences among families for all growth characteristics (height, DBH, and volume). The overall mean height was 4.23 m with range among families from 2.9 m (family 62) to 5.23 m (family 32). Family means for height, DBH and volume for this planting were 4.23 m, 7.66 cm and 0.736 m^3 X 100, respectively. This growth is comparatively slower than the Stamna site due to lower site quality and the stronger competition of natural vegetation developed after planting.

At Limni the differences among families were statistically significant for all (except branch length and branch thickness) characteristics studied. Mean height at the age of nine years was 4.13 m, which correspods to an annual increment of 46 cm, that is little higher from the Nafpaktos (43 cm) and much lower from that at Stamna (75 cm). Diameter and volume were also lower at this site (overall means 5.82 for DBH and 0.445 m^3 X 100 for volume).

Branching Characteristics Straightness and Crown Form

Branching characteristics are economically important because they affect wood quality. Branch length, branch diameter and branch angle were measured only at Limni planting. The analyses of variance showed significant differences among family means only for branch angle. The branches of Aleppo pine are not formed in whorls; instead they are arranged in irregular positions around and along the stem. The mean length of the largest branch at DBH was 1.54 m and ranged among families from 1.34 m (family 13) to 1.77 m (family 33). Branch angle significantly varied among families from 50.6 to 70.1 degrees, with an overall mean of 61.2 degrees. These results parallel previous studies in young progeny tests of Scots pine (*Pinus sylvestris* L.) (EHREMBERG, 1963). In Aleppo pine, apical dominance is often lost in the terminal leader at a young age, and a lateral bud grows upward to replace the terminal leader. Many factors determine the time (age) when apical dominance is lost such as vigour, competition and reproductive patterns (ZIMMERMANN and BROWN, 1971). The new leader is replaced the following year by the shoot of another competing lateral bud, and this process is repeated year after year and influences the straightness of the stem. The phenomenon is very common in areas where moisture is a limiting factor for tree growth. Under certain extreme environmental conditions, growth is completely suppressed, as all the newly develop leaders are dried year after year. Eventually these trees may become dwarves in appearance. A few trees of this type were detected within the first replication at Nafpaktos progeny test, but not the other tests.

Allepo pine is relatively a fast growing species, but it has less desirable straightness and crown form than any other coniferous species naturally grown in Greece. The differences among families for straightness were statistically significant for both plantings, while differences in crown form were significant only at Limni planting. At Stamna straightness ranged among families from 1.5 to 3.5 score units with overall mean 2.74, while the corresponding values for crown form ranged from 1.8 to 3.9. Similar variation was also observed among families at Limi planting.

Bark Thickness

The differences among families were statistically significant at Limni planting, but not at Stamna due to high within plot variance. At Stamna planting, the mean annual increment in bark thickness was 0.81 mm with a range among families from 0.59 to 0.923 mm. At Limni, the family mean values ranged from 0.32 mm to 0.58 mm with an overall mean 0.47 mm. Variation in bark thickness may has some effect on the total wood volume of the trees. Trees with the same DBH may produce different quantities of wood volume depending on the thickness of the bark. Bark is largely a waste product in utilization, but an important protective coating in living trees. Thicker bark also may be advantageous for fire tolerance or resistance to diseases and insects (PEDERICK, 1970). A strong correlation existed between DBH and bark thickness (r=0.85**); therefore, selection for change in bark thickness will result in indirect selection for diameter. At present, however, selection for bark thickness in Aleppo pine is rather impractical, since there are many other characteristics of higher economic importance.

Cone Production

Variation among families in cone production was evident from the analyses of variance. The differences were very large and statistically significant for both plantings (Stamna and Limni). At Limni planting, the average number of cones among the various families ranged from 1 for the poorest family to 5.8 for the heaviest producing family, with an overall mean 4. At Stamna, where trees grew much faster (*Table 2*), the estimated mean number of cones per tree was 30. Although direct comparison for the two sites cannot be made because of different ages of the plantings (Limni 9 years, Stamna 10 years), the influence of the site quality on the cones produced is very obvious. An evaluation made in a previous study (MATZIRIS, 1997) in the seed orchard of Amphilochia at the age of 10 years has shown that the cones produced per tree varied among clones from 40 to 343 with an overall mean 155. The larger number of cones at this seed orchard was expected since the different in nature materials in the seed orchard (clones) grow in larger spacing (6 m x 6 m) and under intensive culture.

Variance Components and Heritability Estimates

The variance components for all characteristics studied and the heritability estimates are presented in *table 3*. In all cases, tree to tree variation (the within plot variance) was the predominant source of variation. Narrow sense heritability estimates on individual tree basis (h^2) were variable depending on the content and the location of the experimental planting. The h^2 for tree height were found 0.45, 0.43, and 0.42 for Nafpaktos, Stamna and Limni progeny tests, respectively. These values are in close agreement with the value 0.45 reported by PANETSOS (1981) in an eight year old open pollinated progeny test of the same species grown in Greece. Cone production was the stronger inherited characteristic, with h^2 values 0.68 at the Limni planting and 0.54 at the Stamna planting. Heritability values of branch length, branch thickness and branch angle were 0.22, 0.26, and 0.42, respectively. These values are comparable with those reported by SCHRUM and GERHALD (1970) in six-year-old Scots pine progeny tests. They estimated higher h^2 value for branch angle (0.55) and lower for branch length (0.37). The h^2 values for straightness and crown form were 0.32 and 0.35, respectively, at the Stamna planting and 0.41 and 0.57 at the Limni planting, respectively.

Interrelationship Among Characteristics

As expected, growth characteristics were strongly correlated. Branch length was moderately correlated with height and DBH with r values 0.56** and 0.57**. The correlation between cone production and height was 0.23** and cone production and DBH 0.22** at the Limni planting. The corresponding values for Stamna planting were much higher (0.44** and 0.42** respectively). The positive correlation coefficients between cone production and height growth were expected, as there is a positive correlation between vegetative and reproductive fitness in forest trees (HEYBROEK, 1974; NAMKOONG, 1979). Natural selection favours trees with high reproductive capacity and growth. Cone production and growth were also found to be strongly correlated in progeny tests of *Pinus sylvestris* L. as well as in grafted materials of Aleppo pine (MATZIRIS, 1997). The correlation coefficients between straightness and crown form were statistically significant with r values 0.77** for Stamna and 0.60** for Limni plantings.

Realized Genetic Gain

The estimated gain must be applicable over a range of sites where orchard seedlings are used. Therefore, overall mean values of the progeny families, common to two location (Stamna and Nafpaktos), for total tree height, DBH and volume were ranked. The Limni site was not included in the ranking since only 32 families were included in this planting. The family mean values for height, DBH, and volume ranged from 5.00 m to 6.87 m (mean=5.87 m), 7.86 cm to 10.9 cm (mean=9.40 cm), and 0.01136 m^3 to 0.02440 m^3 (mean=0.01722 m^3), respectively. The corresponding means of the commercial check were 5.58 for height, 8.73 cm for DBH and 0.01523 m^3 for volume. Thus, the realized genetic gain from the selection made in natural stands is 5.2% for height, 7.67% for DBH and 13.06% for volume over the control (*Table 4*). When 20%, of the total number of clones, with the lower breeding value (i. e., clones 2, 10, 16, 21, 27, 34, 35, 50, 60, 64, 67, 80) are rogued from the seed orchard an additional gain of 2.7% for height, 2.71% for DBH and 8.19% for volume, over the unrogued seed orchard, is realized. Therefore, the total gain realized from the first generation rogued seed orchard of Aleppo pine is 7.9% for height, 10.38% for DBH and 21.25% for volume (*Table 4*). When the two locations (Stamna and Nafpaktos) were examined separately the estimated gains for volume, from the selection made in natural stands, were 13.8% and 10.64% for the Stamna and Nafpaktos plantings, respectively; the corresponding gains from roguing the seed orchard were 7.2% for the first and 5.6% for the second planting, respectively. Thus the total gain is higher (21.2%) at the better site of Stamna and lower (16.24%) at the slower growing planting of Nafpaktos. This is in ageement with the results reported by DHAKAL et al. (1996) in slash pine (*Pinus elliottii* ENGELM. var. *elliottii*) progeny tests including 2051 control pollinated families grown in 175 tests. They found that superior families for tree volume express increased gain (on a percent basis) compared to unimproved materials on better quality sites. They concluded that genetic differences among genetic groups are greater on better sites and that the group containing the best control pollinated families based on parental breeding values produced 22% more volume than the unimproved check.

At the Stamna planting, where straightness and crown form were evaluated the gains from selection made in natural stands were 5.5% for straightness and 3.1% for crown form, compaired to unimproved materials (CC). It is worth mentioning that straightness and crown form were strongly emphasized in the initial selection of the Plus trees, parallel to growth characteristics; for roguing the seed orchard emphasis was given to growth characteristics, while straightness and crown form were only slightly considered (straightness and crown form of the rogued clones were below the average of the progenies mean).

The selection intensity of the second stage of selection (80% of the clones were selected) is quite low and higher gains should be expected if this intensity increases. However, this will provide progeny with a broad genetic base that is essential, due to the long rotation period of Aleppo pine and the necessary flexibility to respond to future changing ecological and economic conditions.

Similar studies in the literature suggest that the gains are reasonable, although direct comparisons cannot be made due to differences in species, selection intensities etc.. PEDERICK and GRIFFING (1977) working with Radiata pine (*Pinus radiata* D.

Table 3. – Component of variances[1]) and narrow sense heritability (h^2) on individual tree basis, at the age of 10 (Nafpaktos and Stamna) and 9 (Limni) years of Aleppo pine.

Characteristic	σ_f^2	σ_{rf}^2	σ_w^2	h^2
NAFPAKTOS				
Height	0.971**	0.363	1.1811	0.57
DBH	0.4795**	0.729	6.1669	0.26
Volume	0.0327**	0.0906	0.2668	0.33
STAMNA				
Height	0.1771*	0.4399	1.040	0.45
DBH	Negative	1.051	4.450	not estimated
Volume	Negative	0.536	1.3990	not estimated
Bark thickness	0.112	0.400	2.496	0.15
Straightness	0.0665*	1.1070	0.6480	0.32
Crown form	0.0695	0.1106	0.615	0.35
Cone number	0.2605*	0.1966	1.464	0.57
LIMNI				
Height	0.0626**	0.0630	0.4690	0.42
DBH	0.3620**	0.2570	2.381	0.48
Volume	0.0080**	0.0099	0.0380	0.58
Bark thickness	0.0827**	0.4940	0.8030	0.24
Branch legth	0.0050**	0.0135	0.0720	0.22
Branch thickness	1.9623**	6.4910	21.1970	0.26
Branch angle	11.343**	15.955	80.4070	0.42
Straightness	0.0343**	0.1150	0.1820	0.41
Crown form	0.052**	0.1159	0.1940	0.57
Cone number	1.8310**	3.2240	5.7400	0.68

[1]) σ_f^2, σ_{rf}^2, σ_w^2, are variance component estimates for families, reps x families and within plot, respectively

Table 4. – Overall family means and ranges for height, DBH and volume, over the two locations (Stamna and Nafpaktos) and realized genetic gains.

Characteristic	Height, m	DBH, cm	Volume, m^3x100
Minimum	5.00	7.86	1.136
Maximum	6.87	10.90	2.440
S deviation	0.458	0.694	0.331
Overall mean	5.87	9.40	1.722
Commercial check	5.58	8.73	1.523
Real. gain from selection made in natural stands (ΔG_1), %	5.20	7.67	13.06
Progeny means after roguing 20% of the inferior clones	6.03	9.655	1.863
Real. gain from roguing 20% of the inferior clones (ΔG_2), %	2.70	2.71	8.19
Total genetic gain from rogued seed orchard ($\Delta G_1 + \Delta G_2$), %	7.9	10.38	21.25

DON) found that orchard stock grew faster in two out of three trials in comparison with control, and the removal of 20% of the genetically inferior clones resulted in an additional gain in volume of 4%. Progeny tests of selected clones of Radiata pine grown in Australia (ELDRIDGE, 1974) showed an average superiority over the commercial check of 4% for height and 5% for DBH, corresponding to 11% superiority in volume. Open pollinated progeny tests of Scots pine (*Pinus sylvestris* L.) established in different parts of Hungary (MATYAS, 1974) from seed of selected clones, at the age of ten years were 10% superior in volume over the commercial check. All results indicate that a 10% improvement in volume for the first stage of selection (initial selection of Plus trees) plus an additional 10% from roguing the seed orchard after progeny testing. Limited selection by culling the poorest families has been also recommenced, for ponderosa and western white pines, to begin at the age of 10 years (STEINHOFF, 1974). The evaluation of the progeny tests have been made in relative young age (10 years), and the estimated gain may be different than realized gain at the end of the rotation and further evaluations have to be made as the materials become older.

Literature

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The Impact of Extraction and Storage Conditions on the Viability of Radiata Pine Pollen

By I. Z. SIREGAR[1]) and G. B. SWEET[2])

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Abstract

The impact of extraction temperature, moisture content and storage temperature on *in vitro* pollen germination and tube growth, was determined in a sequence of experiments.

Pollen extracted at 25 °C, dried to 10% moisture content and stored in a refrigerator at 4 °C for almost a year retained a germination level of 84%, compared with its level of 93% immediately after extraction.

Pollen which was extracted or stored under conditions different from the above lost a greater amount of viability. There were significant interactions between extraction temperature, moisture content and type of storage: while pollen in less than optimal condition lost viability very rapidly under poor storage modalities, pollen extracted and dried as above was able to tolerate poor storage relatively well.

Key words: Pinus radiata, seed orchard, pollen viability and germination, thawing, rewarming and freezing.

Introduction

Developments in New Zealand seed orchard management over the last decade have led to the introduction of controlled-

[1]) Faculty of Forestry, Bogor Agricultural University, Kampus IPB Darmaga, PO Box 168, Bogor, Indonesia

[2]) School of Forestry, University of Canterbury, Private Bag 4800, Christchurch, New Zealand