# Variation in Cone Production in a Clonal Seed Orchard of Black Pine

By D. MATZIRIS[1])

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### Summary

Cone production was assessed in 3 successive years (1989, 1990, 1991) in a black pine (*Pinus nigra* ARNOLD) clonal seed orchard. This was established in October 1978 in an area of 11 ha, at Koumani Peloponnesos, Greece and included 52 clones.

The results showed significant variation among clones for the 3 years examined. It was found that 25% of the clones produced 43.3%, 51.0% and 40.0% of the total cones for the years 1989, 1990 and 1991 respectively. Year to year variation was, as expected, highly significant with mean number of cones per tree, 469 for the year 1989, 124 for 1990 and 951 for 1991. Parental balance was influenced by the cone crop size. In good cone production years the genetic base of the seed produced was much broader than from that produced in years of moderate and poor cone crop.

Broad sense heritability values on clone mean basis were found quite high (0.88, 0.82 and 0.86 for the years 1989, 1990 and 1991 respectively). The corresponding value from the analysis combined over years was found to be 0.75. These values indicate that cone production in black pine is under strong genetic control.

Product moment and SPEARMAN rank correlation coefficients were low and insignificant between consecutive years and strong and highly significant between biennial years, indicating carry over effect in cone production.


*Key words:* Black pine, clonal seed orchard, variation, clonal balance, interaction, heritability.

### Introduction

The main objective of a seed orchard is the production of genetically improved seed, for reforestation purposes. In an idealized seed orchard the following requirements are assumed to be fullfiled: a) the orchard is completely isolated from the influence of outside undesirable pollen, b) clones are equally productive in male and female flowers, c) pollen flight and female flower receptivity will coincide, d) crosses among clones will be equally compatible, e) natural self-pollination will occur in insignificant amounts.

The most important objective of seed orchard design is the minimization of undesirable self fertilization and the production of seed of broad genetic base (GIERTYCH, 1975). If genetic diversity of the seed crop is to be maximized all the clones must contribute equally to the seed crop. This ideal situation, however, is rarely met and information is available which indicates that the contribution of clones to the seed crop is variable in many coniferous species (North Carolina State University, 1976; SWEET, 1976; JONSSON, *et al.*, 1976; BHUMIBHAMON, 1978; SCHOEN *et al.*, 1986; EL-KASSABY *et al.*, 1984; REYNOLDS and EL-KASSABY, 1990). Unequal contribution of the clones could be the major factor responsible for the departure from and disturbance of the panmictic equilibrium in the seed orchard. Departure from equal gametic contributions can be also resulted from the absence of flowering synchronization (non-overlapping flowering phenology) of the clones, embryo and gametophytic selection and differential mortality of ramets of certain clones due to grafting incompatibility (SCHOEN *et al.*, 1986).

In the present study the cone production in a black pine seed orchard, comprising 52 clones, is assessed in 3 successive years. The information obtained is very valuable in the management practices of the existing seed orchard and can be also used for better planning of advanced generation seed orchards.

[1]) Forest Research Institute, Terma Alkmanos, 115.28 Athens, Greece

*Table 1.* — Separate and combined-over-years analyses of variance.

| Source | D.F. | Expected Mean Squares |
|---|---|---|
| Separate Analyses | | |
| Replications | $\tau - 1$ | |
| Clones | $c - 1$ | $\sigma^2 + \tau\sigma^2_c$ |
| Error | $(\tau-1)(c-1)$ | $\sigma^2$ |
| Combined Analysis | | |
| Years | $y - 1$ | |
| Replications | $y(\tau-1)$ | |
| Clones | $c - 1$ | $\sigma^2 + \tau\sigma^2_{yc} + y\tau\sigma^2_c$ |
| Clones x years | $(c-1)(y-1)$ | $\sigma^2 + \tau\sigma^2_{yc}$ |
| Error | $y(\tau-1)(c-1)$ | $\sigma^2$ |

$c$ = number of clones = 52
$y$ = number of years = 3
$\tau$ = number of replications = 3
$\sigma^2$ = variance due to error
$\sigma^2_{cy}$ = variance due to interaction of clones x years
$\sigma^2_c$ = variance due to differences among clones which is interpreted as the total genetic variance

## Materials and Methods

### *Plant Materials*

The work reported here was carried out in the 11 ha clonal seed orchard of Koumani, located in the western part of the Peloponnesos, Greece. The orchard was established in October 1978 and comprises 52 clones derived from intensively selected plus trees in the natural forests of the Peloponnesos. Grafts were 2 years old at the time of establishment and were planted at a spacing 6 m × 6 m. Clones (one ramet/clone) were randomly assigned within replications with one only restriction. No grafts of the same clone were planted closer than 30 m. The soil of the area, developed from Tertiary deposits, is a clay loam, moderately acid with adequate supply of metallic cations and organic mater (NAKOS, 1979). A more detailed description of the area is given by MATZIRIS (1989). Commercial cone harvesting started in year 1988 when the trees were 10 years old. Three replications were sampled and the cones, were harvested, and counted separately by tree, in 3 successive years (1989, 1990, 1991).

Design summary: Randomised complete block; 52 clones, one tree per clone per replication; 3 replications sampled.

### *Statistical Evaluation*

Analysis of variance and calculations of the variance components were conducted on the data from each year separately and all years combined. The analyses were done after square-root transformation of the original data to normalize the variance. The form of analysis of variance used is shown in *table 1* (ANDERSON, 1960; COCKERHAM, 1963).

The among clones component of variance ($\sigma^2c$) was interpreted as:

$$\sigma^2_C = C_{OV}(X,Y) = \sigma^2_G + \sigma^2_{CL}$$

where: X, Y, are ramets of the same clone, $\sigma^2_G$ is the total genetic variance within the population and $\sigma^2_{CL}$ is the cloning effect variance due to vegetative propagation (SHELBOURNE, 1969). As the cloning effect variance is confounded with the genetic variance ($\sigma^2_G$), part of the clonal variation may be due to variation in ortet age (WILCOX, 1974). Because the age differences of the selected ortets were small, it is assumed that the cloning effect variance is negligible and can be ignored. However, there may still be "C" effects that are not directly related to chronological age.

Estimates of broad sense heritability (H), were obtained on an individual tree and clone mean basis, each with different phenotypic variance as follows:

$$H = \frac{\sigma^2_C}{\sigma^2_C + \sigma^2} \quad \text{(individual tree basis)}$$

$$H = \frac{\sigma^2_C}{\sigma^2_C + \sigma^2/\tau} \quad \text{(clone mean basis)}$$

For the years combined:

$$H = \frac{\sigma^2_C}{\sigma^2_C + \sigma^2_{yc} + \sigma^2} \quad \text{(individual tree basis)}$$

$$H = \frac{\sigma^2_C}{\sigma^2_C + \frac{\sigma^2_{yc}}{\tau} + \frac{\sigma^2}{\tau c}} \quad \text{(clone mean basis)}$$

The (H) values on clone mean basis are applicable for estimating genetic gain by selecting clones from the specific population and establishing new clonal seed orchards or for roguing the existed.

Product moment and SPEARMAN rank correlations between the combinations of the years were calculated following appropriate procedures (SNEDECOR and COCHRAN, 1968, page 193). It is worth mentioning, that Spearman rank correlation is the best known procedure for studying the degree of relationship between two variables when there is abnormality in both pairs of variables.

## Results and Discussion

The variation among clones in their annual cone production is evident from the analyses of variance (*Table 2*). The differences are very large and statistically highly significant for all years examined, confirming that considerable amount of variation exists among clones. The mean clone values varied from 23 (clone 46) to 1454 cones per tree (clone 6) for the year 1989, with overall mean 469 cones. For 1990 the range was from 15(clone 30) to 431 cones per tree (clone 2) with overall mean 124 and for 1991, which was a very good year, from 114 (clone 46) to 2189 (clone 6) with overall mean 951 cones per tree. One ramet of clone 6, which is one of the best cone producers, in the good year 1991, produced 2508 cones, which was the maximum number recorded in the entire seed orchard. Mean cones per tree for the 3 year means and standard deviations are shown in *table 3*. The average number of seeds per cone was 24 for the poor cone year 1990, 30 for 1989 and 50 for 1991. Considering clean seed production 15 kg/ha 86 kg/ha and 220 kg/ha were produced in 1990, 1989 and 1991, respectively.

In order to estimate each clone's contribution to the yearly cone production, the clones were plotted in decreasing order and the cumulative percentages of each one quarter of the clones were estimated and presented in *figure 1*. In the year 1989 (*Figure 1*) *it may* be seen that 13 (25%) of the most abundantly cone producers, produced 43.3% of the total cones while the 25% of the less producing cones produced only 10.7% of the total cones. Similar trend has been observed and for the years 1990 and 1991, with 25% of the clones producing 51.0% and 40.4% of the total cones respectively. This disproportionate contribution among the orchard clones to the cone crop, which has been found here, is in agreement with a similar ratio reportet in Scots pine seed orchards (JONSSON *et al.*, 1976; BHUMIBHAMON, 1978). A more severe ratio with 20% of the clones producing 80% of the cones, has been reported in *Pinus taeda* (North Carolina State Un., 1976), *Picea sitchensis* (EL-KASSABY *et al.*, 1990) and in *Pinus taeda* (SCHMIDTLING, 1983) seed orchards.

*Table 2.* — Analyses of variance, variance components and broad-sense heritability estimates for cone production by year, and by years combined.

A. Separate Analyses

| Source | D.F. | Mean Squares [1] 1989 | 1990 | 1991 |
|---|---|---|---|---|
| Replications | 2 | 46.2709 | 5.9642 | 118.4102 |
| Clones | 51 | 109.8961 ** | 40.2688 ** | 173.8240 ** |
| Error | 102 | 13.2688 | 6.9511 | 23.5197 |

Variance component and broad sense heritability estimates[2]

| | | | |
|---|---|---|---|
| $\sigma^2_c$ | 32.2091 | 11.1059 | 50.1014 |
| $\sigma^2$ | 13.2688 | 6.9510 | 23.5197 |
| $H_1$ | 0.71 | 0.61 | 0.68 |
| $H_2$ | 0.88 | 0.82 | 0.86 |

B. Combined Analysis

| | | |
|---|---|---|
| Years | 2 | 4712.2581 ** |
| Replications | 6 | 56.8818 |
| Clones | 51 | 72.4225 ** |
| Clones x years | 102 | 18.2384 |
| Error | 306 | 14.5799 |

$\sigma^2_c$ = 6.0204 $H_1$ = 0.28

$\sigma^2_{yc}$ = 1.2194 $H_2$ = 0.75

$\sigma^2$ = 14.5799

[1]) ** Statistically significant at the 0.01 probability level.

[2]) $H_1$, $H_2$ broad sense heritability on individual tree and clone mean basis respectively.

*Table 3.* — Mean number of cones per tree ($\bar{X}$), standard deviations (S. D.) and minimum and maximum tree values.

| Year | Age | $\bar{X}$ | S.D. | Min. | Max. |
|---|---|---|---|---|---|
| 1989 | 11 | 469,48 | 294,46 | 10 | 1732 |
| 1990 | 12 | 124,44 | 99,88 | 2 | 580 |
| 1991 | 13 | 951.05 | 508,73 | 30 | 2508 |

The degree of departure from the ideal situation (equal cone production of all clones) for the three years examined is summarized by the cone yield curves shown in *figure 2*, in which the clones are arranged in descending sequence of number cones. As implied by this method the closer the curves approaches to the straight line, the higher is the possibility that each clone will contribute to the same extent to the gene pool in the seed orchard. It is evident from this figure that the degree of distortion in the parental balance in the seed orchard is influenced by the crop size. In the good cone year (full cone production) 1991, the curve is closer to the ideal situation, that is represented in the figure by the straight line, while in the poorest cone year 1990 the departure from the straight lines is much greater. The curve of the moderate cone year 1989 is lying intermediate. These results indicate that in good cone years the genetic base of the seed produced from the seed orchard is much broader from that produced

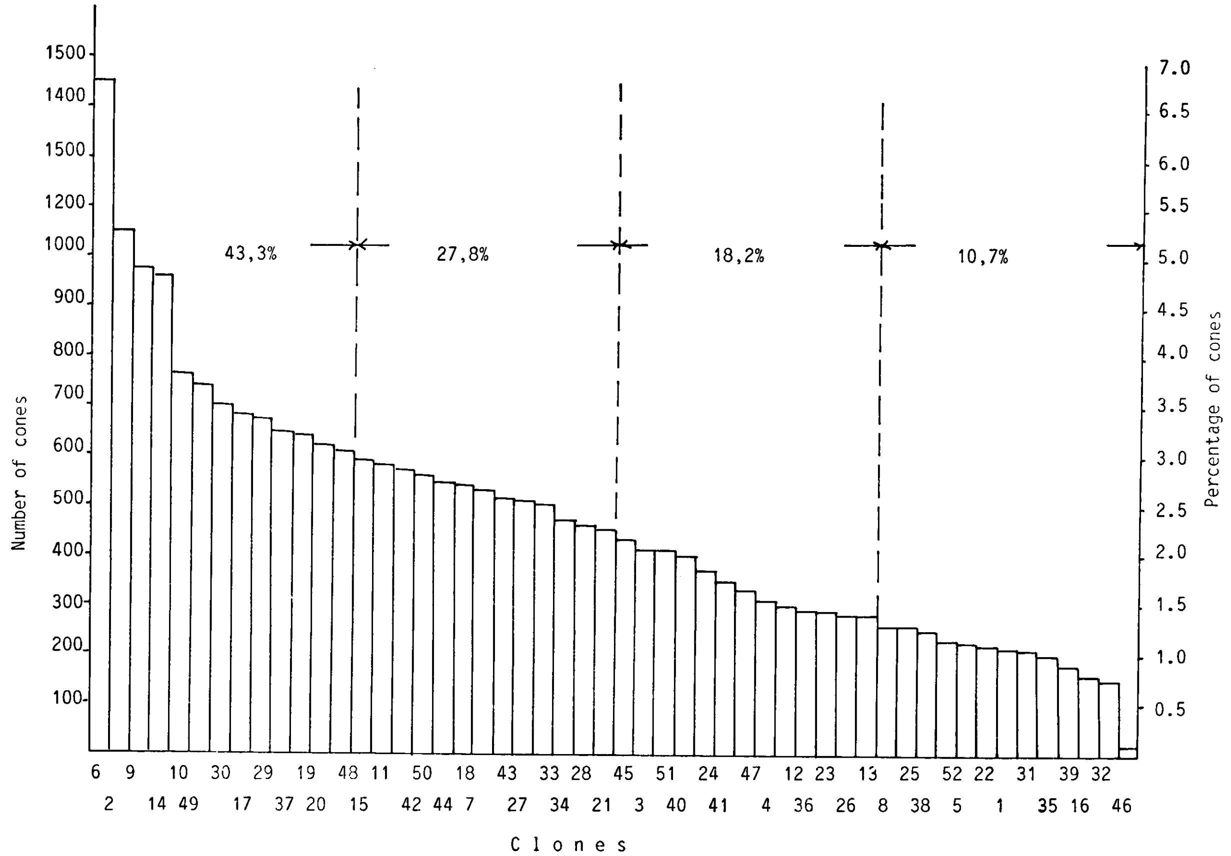


*Figure 1.* — Mean number of cones per clone ramet in a black pine seed orchard (year 1989).

in moderate and poor years. Similar results have been reported in Douglas fir and Sitka spruce seed orchards (EL-KASSABY *et al.*, 1989, 1990).

*Year x Clone Interaction*

In general, clonal variation in cone production followed the yearly trend, i. e., all clones produced more cones in good years, such as 1991, and less is poor years. The combined analysis of variance over years (*Table 2*) showed that the "F" value for the clone x year effect was, although insignificant, close to the critical value (F=0.25, tabular significant value >0.30). The relative ranking of clones change from year to year. The change in ranking is less obvious when the good with the moderate cone years were concerned (*Table 4*). It is evident from the table that the best cone producers (clone 6, 2, 10, 9 and 4) are on top for both years (1991, 1989). A similar trend is shown by the less productive clones (clone 32, 16, 46) which are at the bottom for both years. However intermediate cone producers change in ranking from year to year. This is more obvious when good coners in a poor year and moderate coners in a poor year are examined. SCHMIDTLING (1983) found a similar trend in seed orchards of *Pinus echinata* and *Pinus virginiana* and postulated that the fluctuation in cone production over years has a selective advantage in natural populations by maintaining the insect population low over years. Poor cone years interupt the growth of insect populations so that seed production in the following year is much safer. Year x clone interaction may serve a similar function by shifting reproduction to different genotypes over years.

*Relationship between Years*

The relationship in cone crop between the years studied is shown in *table 5*, in which the product moment and SPEARMAN's rank correlations are listed. The product moment correlation between 1989 and 1990 is 0.27 (NS), between 1990 and 1991 0.33* and between 1989 and 1991 0.82*. The results show that there is no significant relationship between good and poor years, while there is a highly significant correlation between good and moderate years.

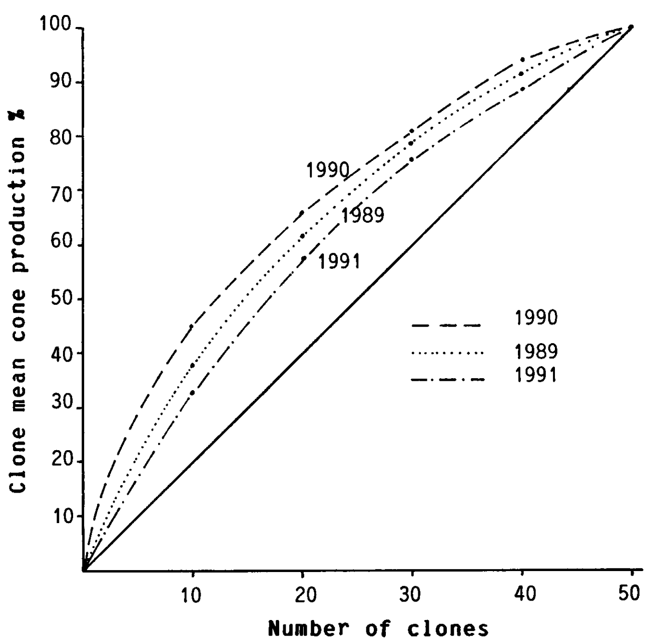


*Figure 2.* — Cumulative cone production curves for clones for a very good cone year (1991), average cone year (1989) and poor cone year (1990). Straight line represents equal contribution. Clones are arranged in order according to the decreasing number of cones.

*Table 4.* — Clone rankings in cone production for the years 1991, 1989.

| Cone production 1991 | | | Cone production 1989 | | |
|---|---|---|---|---|---|
| clone | cones/tr. | rank | rank | cones/tr. | clone |
| 6 | 2189 | 1 | 1 | 1454 | 6 |
| 2 | 1975 | 2 | 2 | 1149 | 2 |
| 10 | 1775 | 3 | 3 | 975 | 9 |
| 9 | 1770 | 4 | 4 | 963 | 14 |
| 14 | 1615 | 5 | 5 | 766 | 10 |
| 15 | 1460 | 6 | 6 | 742 | 49 |
| 48 | 1385 | 7 | 7 | 702 | 30 |
| 20 | 1357 | 8 | 8 | 683 | 17 |
| 34 | 1344 | 9 | 9 | 675 | 29 |
| 17 | 1337 | 10 | 10 | 644 | 37 |
| 30 | 1295 | 11 | 11 | 641 | 19 |
| 11 | 1289 | 12 | 12 | 627 | 20 |
| 49 | 1263 | 13 | 13 | 608 | 48 |
| 29 | 1208 | 14 | 14 | 588 | 15 |
| 7 | 1199 | 15 | 15 | 583 | 11 |
| 40 | 1154 | 16 | 16 | 572 | 42 |
| 43 | 1149 | 17 | 17 | 566 | 50 |
| 42 | 1138 | 18 | 18 | 546 | 44 |
| 19 | 1133 | 19 | 19 | 543 | 18 |
| 52 | 1087 | 20 | 20 | 533 | 7 |
| 18 | 1038 | 21 | 21 | 516 | 43 |
| 37 | 1015 | 22 | 22 | 507 | 27 |
| 50 | 1010 | 23 | 23 | 504 | 33 |
| 44 | 998 | 24 | 24 | 472 | 34 |
| 51 | 968 | 25 | 25 | 457 | 28 |
| 28 | 955 | 26 | 26 | 453 | 21 |
| 23 | 894 | 27 | 27 | 433 | 45 |
| 24 | 891 | 28 | 28 | 408 | 3 |
| 36 | 871 | 29 | 29 | 408 | 51 |
| 13 | 844 | 30 | 30 | 404 | 40 |
| 38 | 775 | 31 | 31 | 373 | 24 |
| 41 | 761 | 32 | 32 | 354 | 41 |
| 3 | 760 | 33 | 33 | 334 | 47 |
| 33 | 720 | 34 | 34 | 306 | 4 |
| 4 | 707 | 35 | 35 | 296 | 12 |
| 21 | 703 | 36 | 36 | 292 | 36 |
| 27 | 693 | 37 | 37 | 291 | 23 |
| 22 | 646 | 38 | 38 | 282 | 26 |
| 47 | 643 | 39 | 39 | 281 | 13 |
| 25 | 629 | 40 | 40 | 269 | 8 |
| 31 | 620 | 41 | 41 | 266 | 25 |
| 45 | 606 | 42 | 42 | 256 | 38 |
| 35 | 579 | 43 | 43 | 236 | 52 |
| 26 | 473 | 44 | 44 | 230 | 5 |
| 39 | 462 | 45 | 45 | 223 | 22 |
| 12 | 426 | 46 | 46 | 216 | 1 |
| 1 | 411 | 47 | 47 | 212 | 31 |
| 5 | 410 | 48 | 48 | 206 | 35 |
| 8 | 357 | 49 | 49 | 185 | 39 |
| 32 | 327 | 50 | 50 | 159 | 16 |
| 16 | 183 | 51 | 51 | 154 | 32 |
| 46 | 114 | 52 | 52 | 23 | 46 |

*Table 5.* — Product moment (above diagonal line) and Spearman rank (below diagonal line) coefficients for cone production, in a clonal seed orchard of black pine.

| Characteristic | | | $X_1$ | $X_2$ | $X_3$ |
|---|---|---|---|---|---|
| Cones | 1989 | ($X_1$) | | 0.27 | 0.82** |
| Cones | 1990 | ($X_2$) | 0.12 | | 0.33* |
| Cones | 1991 | ($X_3$) | 0.87** | 0.24 | |

*), **) values are statistically significant at the 0.05 and 0.01 probability level respectively.

SPEARMAN's rank correlations had similar values. The correlation coefficient between good (1991) and moderate (1989) years was found highly significant (r = 0.87**), indicating that the observed change in ranking of clones is highly significant or expressing it in other way, that there are no significant changes between clones in cone production in these two years. The rank correlation between good (1991) and poor (1990) as well as between moderate (1989) and poor (1990) years, were found insignificant and very low, with values 0.12, and 0.24 respectively, indicating that the changes in ranking of clones in these years are significant. The results show that year to year correlations in cone production are not significant between consecutive years (1989 x 1990, 1990 x 1991), while it is highly significant between biennial years (1989 x 1991), indicating the presence of carry over-effect.

*Variance Components and Heritability Estimates*

The variance components and the broad sense heritability estimates have been presented in *table 2*. The estimates of clone variances from single years are larger from the value estimated from the combined-over-years analysis. This is due to year x clone interaction component of variance, which, in the single analyses by year, is confounded with the genetic component of variance. The clone by year interaction component of variance could arise for 2 reasons, namely differing overall clone means and components of variance in the 3 years and changes of clone rankings over the 3 years. In examining the estimates of variances (*Table 2*), it is apparent that the estimates are higher in year 1991, followed by year 1989 and 1990. These differences suggest that the variances are not independent of the means and that scaling effect is present, besides the transformation made.

Broad sense heritability values on individual tree bases were ranked from 0.61 (1990) to 0.71 (1969), while the values on clone mean basis were, as expected, higher (H = 0.82 for 1990, and H = 0.88 for 1989). The values estimated from the combined over years analyses were 0.28 and 0.75 on single tree and clone mean basis respectively.

The higher heritability values from the single year analyses were expected, because the clone x year interaction component of variance is confounded with the clone variance (Becker, 1984). The high heritability values indicate that cone production in black pine is under strong genetic control. Heritability values estimated in slash pine (Varnell *et al.*, 1967) were much lower, suggesting that this parameter is a property of the species age and siting of the material to which it may referred.

### Conclusions

From a study of cone production in a black pine clonal seed orchard, over three successive years, 11, 12 and 13 years, the following conclusions were drawn:

1. There is a significant amount of genetic (clonal) variation in cone production. The clones do not contribute equally to the next generation since more of the cones are produced by only a few clones; 25% of the clones produced 43%, 51% and 40% of the total cones in moderate, poor and good cone years respectively.

2. Crop size influences the degree of parental balance (equal contribution of all clones), with the good cone crop years being closer to the ideal situation.

3. Year to year clone mean correlations in number of cones per tree are positive and very strong between good and moderate cone years and insignificant between good X poor and moderate X poor cone years.

4. Cone production is under strong genetic control, indicating that considerable gain can be expected from roguing or by selecting good cone producing clones and establishing new clonal seed orchards.

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# Genetic Variation in Growth and Wood Specific Gravity and its Utility in the Improvement of Interior Spruce in British Columbia

By A. D. Yanchuk[1]) and G. K. Kiss[2])

(Received 27th November 1992)

[1]) Research Branch, B. C. Ministry of Forests, 1450 Government Street, Victoria, B. C., Canada V8W 3E7

[2]) Kalamalka Forestry Center, B. C. Ministry of Forests, 3401 Reservoir Road, Vernon, B. C., Canada V1B 2C7

### Summary

Juvenile wood specific gravity (SG) from increment cores was assessed by the maximum-moisture content