radiata. FAO, Forestry and Forestry Products Studies, no. 14, 328 pp. (1960). — SHELBOURNE, C.: New Zealand's Radiata Pine Breeding Programme. Progress, Problems, and Future Plans. Silvicultura **VIII**(32): 738–740 (1983). — SHELBOURNE, C. and THULIN, I.: Early results from a clonal selection and testing programme with radiata pine. N. Z. J. For. **4**(2): 387–398 (1974). — SNEDECOR, G. and COCHRAN, W.: Statistical Methods. The Iowa a State Univ., Press, USA. 593 pp. (1967). — WILCOX, M.: Genetic variation and inheritance of resistance to Dothistroma needle blight in *Pinus radiata*. N. Z. J. F. Sci. **2**: 14–35 (1982). — WILKINSON, L.: SYSTAT: The system for Statistics. Evanston, IL. SYSTAT, Inc., MGLH, 126 pp. (1986).

Pollen Sizing in Jack Pine (*Pinus banksiana* Lamb.) with a Hemocytometer

By G.-É. CARON[1]) and G. R. POWELL[2])

(Received 19th December 1994)

Abstract

Evaluation of pollen viability and pollen size variation is instrumental in polycross testing. Polymixes used for polycross should be adjusted so that they are composed of equal numbers of viable pollen grains from each male. In this study, yearly and clonal variation in jack pine (*Pinus banksiana* LAMB.) pollen size is evaluated for pollen collected from 15 clones and 25 clones in 1992 and 1993, respectively; 13 clones were common to both years. *In vitro* pollen viability was assessed by using agar with 5% sucrose. Pollen sizing was achieved with a hemocytometer, commonly used by medical technologists to count various blood cells. Pollen viability averaged 70%, 86%, and 90% for 1992 freshly collected pollen, 1992 pollen stored for 1 year, and 1993 freshly collected pollen, respectively. Clonal viability varied between 54% and 90%, 67% and 95%, and 75% and 98% for 1992 freshly collected pollen, 1992 pollen stored for 1 year, and 1993 freshly collected pollen, respectively. The number of pollen grains per mg (MG) or ml (ML) of dry pollen was quite variable between years and among clones. ML averaged 16.4 million and 17.7 million in 1992 and 1993, respectively. The highest clonal ML value in 1992 and 1993 was 1.8 times and 1.7 times that of the smallest value, respectively. No pollen morphometric measure was valid in estimating reliably ML or MG. Adjustments to obtain equal numbers of viable pollen grains for each male parent in a polymix are recommended.

Key words: Pinus banksiana, pollen viability, pollen size, hemocytometer, polymix, polycross.

FDC: 161.6; 164.6; 165.53; 181.521; 174.7 *Pinus banksiana.*

Introduction

A strategy for the genetic improvement of jack pine (*Pinus banksiana* LAMB.) suggests the use of polycross tests (FOWLER, 1986). In the polycross mating design, each selected tree is crossed with a mix of pollens (polymix) from a number of unrelated trees. The polycross test is used to assess general combining ability (GCA) of selected trees and, in turn, to identify full-sib families having high GCA parents (FOWLER, 1986; FOWLER and WISELOGEL, 1993). If the polycross is to provide an accurate estimate of additive genetic variance and GCA, the pollen parents used in the polymix must be equally successful in the pollination-fertilization process (FOWLER and WISELOGEL, 1993). Random mating occurs in polymix pollinations of loblolly pine (*Pinus taeda* L.) (WISELOGEL and VAN BUIJTENEN, 1988). However, differential male reproductive success has been indicated in other species (see MORAN and GRIFFIN, 1985; CHELIAK *et al.,* 1987; MULCAHY and MULCAHY, 1987; APSIT *et al.,* 1989). Hence, some controversy exists over the use of polymixes (see FOWLER, 1987), and this is related to whether the reproductive bias is real and can result in serious distortion of the genetic parameters being estimated.

Polymixes used for the polycross should be adjusted so that they are composed of equal numbers of viable pollen grains from each male. Pollen viability and pollen size variation must therefore be considered. Various tests are available to evaluate *in vitro* pollen viability (see GODDARD and MATTHEWS, 1981; JETT *et al.,* 1993, etc). Pollen sizing has previously been determined by weighing and counting a standard volume (POHL, 1937), with a microscope (SZIKLAI, 1963), or with electronic particle counters (HO and OWENS, 1974; ADAMS, 1982; LUTIER and VAISSIÈRE, 1993). Pollen sizing can also be achieved using a hemocytometer. The hemocytometer, herein proposed for forestry application, is commonly used by hospital laboratory technologists to count various blood cells (PLATT, 1969; HUDSON and HAY, 1980; SIEVERD, 1983).

In the present study, yearly and clonal variation in pollen size was evaluated with the use of a hemocytometer for pollen collected from 15 and 25 jack pine clones in 1992 and 1993, respectively; 13 clones were common to both years. *In vitro* pollen viability was assessed. Adjustments required to obtain equal numbers of viable pollen grains for each male parent in a polycross will be discussed.

Materials and Methods

Pollen collection and storage

Pollen was collected and extracted by J. D. Irving Ltd. in late May of 1992 and 1993 at their clonal seed orchard situated 45

[1]) École de sciences forestières, Université de Moncton, 165 Boulevard Hébert, Edmundston, New Brunswick, Canada E3V 2S8

[2]) Faculty of Forestry and Environmental Management, University of New Brunswick, Bag Service No. 44555, Fredericton, New Brunswick, Canada E3B 6C2

km north east of Sussex, New Brunswick. There were 15 and 25 clones collected in 1992 and 1993, respectively, and 13 clones were common to both years. Pollen from these clones is used in the formulation of the New Brunswick Tree Improvement Council polymix which is used in controlled pollination for testing GCA of second generation selections in the jack pine improvement program. The pollen cones were hand picked, placed in paper pollen-proof bags and sealed. The bags were laid on their sides so that the pollen cones were distributed over as large a surface area as possible and the bag gussets were expanded to facilitate air movement and more rapid drying. The bags were placed in a forced air seed kiln at ambient temperature so that drying resulted from removal of moist air rather than from high temperatures. After 24 h to 48 h, the bags were removed from the kiln and the contents sieved beneath a vertical laminar flow hood to minimize contamination. The pollen was decanted into glass vials and sealed with cotton plugs before refrigeration in a plastic container (10 vials to 20 vials per container) with some desiccant. Pollen processed in this manner normally has less than 10% moisture content and appears to flow smoothly, with no clumping. In 1992, pollen was shipped after extraction; that of 1993 was shipped by mid-July (stored at –15 °C until shipment). For shipment to the École de sciences forestières of the Université de Moncton, vials were placed in loose cotton stoppered vials in a desiccator. Upon arrival, pollen was stored at 4 °C to 5 °C until testing.

Longer-term pollen storage was as follows: i) dry pollen (< 10% MC) was placed in a desiccator for 24 h at room temperature, ii) the desiccator was placed at 4 °C to 5 °C for 1 week; iii) the desiccator with the cork screw stoppered vials was transferred to a freezer (–30 °C) (see MATTHEWS and KRAUS, 1981). When removed from the freezer, desiccator-stored pollen was warmed in 2 stages: i) 24 h at 4 °C to 5 °C, and ii) 3 h to 4 h at room temperature.

Pollen viability tests

Pollen viability tests were conducted in July following pollen collection in both 1992 and 1993, and a second time in July 1993 for the 1992 collection. Dry pollen was rehydrated before testing according to the technique of GODDARD and MATTHEWS (1981). A petri dish with pollen was placed in a seed germination box over cellulose paper saturated with water. The sealed germination box was then placed in complete darkness in an incubator at 21 °C for 16 h. Pollen viability was tested by i) 5% sucrose incorporated into an agar medium (GODDARD and MATTHEWS, 1981) and ii) the BREWBAKER and KWACK (1963) procedure. For simplicity, only the agar/5% sucrose tests will be herein reported. Results obtained with the BREWBAKER and KWACK (1963) procedure were similar to, and did not contradict, those obtained with the agar/5% sucrose method.

Dry Difco Bacto Agar (10 g) was mixed gradually with 2000 ml of deionized water (0.5% agar) and dissolved sucrose (5%). For each pollen lot, pollen was deposited on cooled agar in 3 pre-sterilized petri dishes. Care was taken to disperse pollen grains evenly. Petri dishes were placed in germination boxes with a small quantity of water under a supporting grill to maintain high relative humidity. Pollen was incubated at 28 °C for 63 h. For each replicate, a minimum of 200 grains was observed under a compound microscope (100x). Pollen grains with pollen tube length equal to or exceeding grain diameter were tallied as viable. Viability (%) was estimated for each replicate.

Pollen sizing

Pollen-size variation among clones was determined using a hemocytometer. Hemocytometer slides with improved NEUBAUER ruling are commonly used by medical technologists to count various blood cells such as erythrocytes (red blood cells) and leukocytes (white blood cells) (PLATT, 1969; SEIVERD, 1983). In medical laboratories, the number of cells in small volumes taken at random is counted to pre-determined rules with the use of the hemocytometer, and this value is adjusted to give an estimate of the total population (HAY and HUDSON, 1989). Although pollen grains differ morphologically and chemically from blood cells, the theory behind the hemocytometer slide can be applied to determine the number of pollen grains in a known volume of liquid.

Solution preparation

Viable pollen grains typically float in water and therefore a homogeneous suspension would be difficult to obtain. To overcome this, pollen buoyancy must be eliminated. GREENWOOD (1986) achieved this by placing pollen grains in absolute methanol (MeOH), thus rupturing the pollen's sacci. Ruptured pollen grains sink in water. This method was effective with jack pine and the morphology of the grains was not affected.

For each clone, 3 solutions were prepared. In a vial, 10 mg of pollen, 0.30 ml of MeOH, and 2 ml of deionized H_2O were added. The quantity of MeOH used permitted all pollen to sink, after which H_2O was added. For this study, the dilution factor (DF) of the solution was established as follows:

$$DF = \frac{\text{mass of pollen used (mg)}}{\text{volume of liquid used in the solution (ml of } H_2O \text{ and MeOH)}} \quad [1]$$

Generally the amount of deionized water added to a solution varies with pollen size. Through experimentation, it was determined that the amount of deionized water needed to maintain a count of between 20 and 50 pollen grains per large square on the hemocytometer was 2 ml. In medical practice, an average of 20 cells to 50 cells per large square is recommended (PLATT, 1969; SEIVARD, 1983). A homogeneous suspension is required to ensure that the random samples are representative of the whole solution. The mixture was agitated by vigorous pipetting (for 2 minutes to 3 minutes) to obtain a homogeneous dispersal of the grains in the solution. Three samples were sequentially taken from each vial with a micropipette for hemocytometer observation. Between samples, solutions were agitated to maintain homogeneity.

Hemocytometer measurement

A tiny ruled area is located in each of 2 counting chambers on the hemocytometer. Both ruled areas are identical and are separated by an empty space (moat) between the chambers. With a cover-slip in place, a micropipette was used to transfer a small amount of the solution to both chambers of the hemocytometer by carefully touching the edge of the cover-slip with the pipette tip and allowing each chamber to fill by capillary action. Controlling the flow with the index finger, the first 4 or 5 drops were discarded. Solutions in both counting chambers don't mix because of the moat. If the counting chamber is flooded or contained air bubbles, the coverglass and counting chamber were cleaned and the counting chamber was charged once again with a small portion of the solution.

The counting adopted in this study is similar to that adopted by medical technologists for leukocytes (PRATT, 1969; SIEVERT, 1983). Starting with 1 chamber, all grains in the central large square and the 4 corner squares were counted. Pollen grains touching the middle line of the upper and left perimeters of each square were counted. Grains touching the middle line of the lower and right perimeters were not counted. The same

procedure was repeated for chamber 2. Pollen counts for each counting chamber should agree within a certain range. Formula [2] was used to check the acceptable difference between the 2 chambers:

$$2 \times \sqrt{\#\text{ of grains of side 1} + \#\text{ of grains of side 2}} \quad [2]$$

The difference should be less than the results obtained from [2].

Each large square of the hemocytometer, with cover-slip in place, represents a total volume of 0.1 mm^3. Since 1 cm^3 is equivalent to approximately 1 ml, the subsequent pollen concentration per ml of solution is determined using formula [3] (see Sigma Chemical Company, 1991):

$$\text{Number of pollen grains per ml of solution (Hml)} = \frac{\text{Number of pollen grains counted}}{\text{Number of large ruled squares}} \times 10^4 \quad [3]$$

where 1 large ruled square represents 0.1 mm^3 of solution. The factor 10^4 is to convert counts to a per cm^3 or per 1 ml (= Hml) basis.

Estimate of pollen grains

Pollen quantity is normally expressed in volume (or weight). The number of pollen grains per milligram of pollen (MG) used to prepare a solution can be derived from [4]:

$$\text{Number of pollen grains per mg of pollen (MG)} = \frac{\text{Hml}}{\text{DF}} = \frac{[3]}{[1]} = \frac{\#\text{ grains/ml of solution}}{\text{mg of pollen/ml of solution}} \quad [4]$$

The number of pollen grains per unit of volume based on the hemocytometer measurements can be estimated according to the following 2 steps: i) first the ratio (RATIO) of water volume displacement is determined for a certain mass of pollen:

$$\text{RATIO} = \frac{\text{volume of dry pollen (ml)}}{\text{mass of dry pollen (mg)}} \quad [5]$$

and ii) then, the number of grains per millilitre of dry pollen (ML) is estimated:

$$\text{Number of grains/ml of pollen (ML)} = \frac{\text{MG}}{\text{RATIO}} = \frac{[4]}{[5]} \quad [6]$$

Pollen measurement

Seven morphometric measures of pollen dimension were obtain under a compound microscope (at 400X; in µm) for 10 pollen grains from each of the 13 common clones in both 1992 and 1993. These are: front view – pollen width (PW), corpus width (CW), pollen height (PH1), and corpus height (CH); side view – pollen height (PH2), corpus length (CL), and saccus length (SL). Measures taken from the front and side were not from the same grains.

Statistical analysis

Pearson product-moment correlations were performed using SAS Institute Inc. (1990a) for i) pollen viability tests done in 1992 and 1993 and ii) pollen viability between years using the 13 common clones. Analyses of variance and variance component analyses (SAS Institute Inc., 1990b) were performed only for: i) pollen tests from 1992 freshly collected pollen and after 1 year of storage (n = 15 clones), ii) pollen tests with freshly collected pollen in 1992 and 1993 (n = 13 clones), and iii) concomitant tests of pollen of 1992 stored for 1 year and freshly collected pollen in 1993 (n = 13 clones). All factors were considered random. Before the various analyses, pollen viability data were arcsine transformed. Analyses of variance and variance component analyses were also performed for MG and ML using the 13 clones common to both 1992 and 1993. All factors were considered random. Finally, Pearson product-moment correlations were calculated for i) the 7 variables of pollen dimension (PW, CW, PH1, CH, PH2, CL, SL), ML, and MG, and ii) between (arcsine transformed) pollen viability and the seven variables of pollen dimension. Analyses of variance were conducted for each of the seven variables using the 13 common clones.

Results

Pollen viability

Viability tests conducted in 1992 after pollen collection indicated about 70% pollen viability (*Table 1*). Among clones, viability ranged from 90.0% ± 1.3% (Clone 117; mean ± S.E.) to 54.1% ± 2.6% (Clone 120; *Fig. 1A*). After 1 year of storage, viability was higher than before storage (*Table 1*) and difference in viability among clones was smaller (max. of 95.0% ± 0.7% and min. of 67.2% ± 7.0%; *Fig. 1B*). Fresh pollen in 1993 had higher variability than 1992 fresh pollen and stored pollen (*Table 1*). Again, variation in viability among clones was small (max. of 98.0% ± 0.2% and min. of 74.9% ± 5.2%; *Fig. 1C*). Pearson product-moment correlations indicated no relationship between tests of freshly collected pollen in 1992 and 1993, and 1992 stored pollen (*Table 2*). Over 54% of variation in pollen viability after collection and 1-year storage was associated with Treatment (*Table 3A*). Fresh pollen tests (*Table 3B*), and 1992 stored pollen compared to 1993 freshly collected pollen (*Table 3C*), indicated that Year and the interaction Treatment x clone explained the largest variation in pollen viability, respectively. The factor Clone explained less than 9% of the variation in each variance component analysis (*Table 3A, B, C*). No significant correlation in clonal pollen viability was found between the 2 collections (1992 and 1993) and for pre- and post-storage viability of 1992 pollen ($p > 0.40$).

Table 1. – Jack pine pollen viability (%; mean ± S. E.) using agar with 5% sucrose for freshly collected pollen in 1992 and 1993, and for pollen of 1992 stored for 1 year.

Pollen collection year	Time of viability testing	n	Pollen viability
1992	After collection	15	70.2 ± 11.5
	After storage	15	86.4 ± 7.3
1993	After collection	25	89.8 ± 5.9

Pollen sizing

Number of grains per mg (MG)

MG averaged 63 819 ± 2 831 in 1992 (n = 15 clones) compared to 73 401 ± 1 901 in 1993 (n = 25 clones). For the 13 common clones, the mean values were 63 513 ± 2 045 and 78 463 ± 1 407 in 1992 and 1993, respectively. Factors Year and Clone were significant (*Table 4*), with Year explaining 47% of data variability (*Table 4*). Ranking of some clones changed between 1992 and 1993 (*Figs. 2A* and *B*) resulting in a significant Year x clone interaction (*Table 4*). Clone 166 had the highest value in both 1992 and 1993 (86 020 ± 3 374 and 94 709 ± 3 028, respectively). Clone 162 in 1992 and clone 1103 in 1993 had the

Table 2. – PEARSON correlation matrix (with p-value) of jack pine pollen viability (%). The viability tests were conducted with freshly collected pollen in 1992 and 1993, and 1992 pollen stored for 1 year.

Variable	1	2	3
1. After collection in 1992	1.0000	0.1432	0.2328
	(0.0)	(0.6405)	(0.4037)
	15	13	15
2. After collection in 1993		1.0000	0.0086
		(0.0)	(0.9777)
		25	13
3. 1992 stored pollen			1.0000
			(0.0)
			15

smallest value, respectively (45 693 ± 310 and 52 082 ± 1 408, respectively). In 1992, the smallest MG value among clones was 53.1% that of the largest; in 1993, it was 55.0%.

Number of pollen grains per ml (ML)

Larger pollen grains were recorded for 1992 resulting in lower ML values. ML averaged 16 409 720 ± 482 265 (n = 15 clones) in 1992 and 17 738 642 ± 274 105 (n = 25 clones) in 1993. For the 13 common clones, ML was 16 467 224 ± 514 256 in 1992 compared to 18 645 169 ± 386 748 in 1992. Factors Year, Clone, and Year x clone interaction were significant and explained 20%, 32%, and 21% of the variability, respectively (*Table 5*). Clone 166 had the highest ML value (smallest pollen grains) in both 1992 and 1993 (21 881 916 ± 858 409 in 1992 and 23 219 224 ± 742 406 in 1993; *Fig. 3A* and *B*). The smallest value in each year was: in 1992, clone 162 with 12 020 449 ± 81 510; in 1993, clone 1103 with 13 800 637 ± 373 179 pollen grains (*Fig. 3A* and *B*).

Pollen dimension

Seven morphometric measures of pollen dimensions were obtained from each of the 13 common clones (*Table 6*). Only

Table 3. – Analysis of variance and variance components (%) of jack pine pollen viability tests.

Source of variation	df	Mean square	F-value	p-value	Variance component
A. 1992 pollen, after collection vs after storage					
Model	29	0.0696	20.96	< 0.0001	
Treatment	1	0.8828	265.92	< 0.0001	54.76
Clone	14	0.0497	14.96	< 0.0001	8.64
Treatment x clone	14	0.0314	9.47	< 0.0001	27.33
Error	57	0.0033			9.27
B. 1992 and 1993 pollen after collection					
Model	25	0.0931	28.72	< 0.0001	
Year	1	1.3126	404.65	< 0.0001	66.88
Clone	12	0.5773	14.83	< 0.0001	3.80
Year x clone	12	0.0366	11.29	< 0.0001	22.86
Error	50	0.0032			6.46
C. 1992 pollen after storage vs 1993 pollen after collection (Concomitant testing)					
Model	25	0.0347	8.54		
Treatment	1	0.1102	27.10	< 0.0001	12.76
Clone	12	0.0306	7.51	< 0.0001	0.00
Treatment x clone	12	0.0326	8.03	< 0.0001	61.42
Error	51	0.0041			25.82

Table 4. – Analyses of variance and variance components (%) for the number of pollen grains per mg of dry pollen for 13 jack pine clones common to 1992 and 1993 collections.

Source of variation	df	Mean square	F-value	p-value	Variance component
Model	25	4.516×10^8	10.68	< 0.0001	
Year	1	4.358×10^9	52.88	< 0.0001	46.83
Clone	12	4.952×10^8	6.01	0.0020	29.39
Year x clone	12	8.241×10^7	1.95	0.0493	5.71
Error	52	4.229×10^7			18.06

PH1 was correlated with MG and ML (*Table 7*). Although PH1 was correlated with PH2, the latter was not correlated with MG or ML (*Table 7*). Of the 4 front view measures (see *Table 6*), PW was significantly correlated ($p < 0.05$) with CW and PH1 (*Table 7*). Of the 3 sideview measures, a highly significant correlation was noted between CL and SL ($p < 0.0001$). From the 7 measures, the Year was significant only for CH ($p = 0.0341$). Conversely, the Clone was significant for CW ($p = 0.0275$), CL ($p = 0.0005$), and SL ($p = 0.0061$).

Little correlation was found between pollen viability and the 7 pollen measures (*Table 8*). Only PH1, CL, and SL obtained from 1992 stored pollen were correlated with pollen viability after storage (*Table 8*).

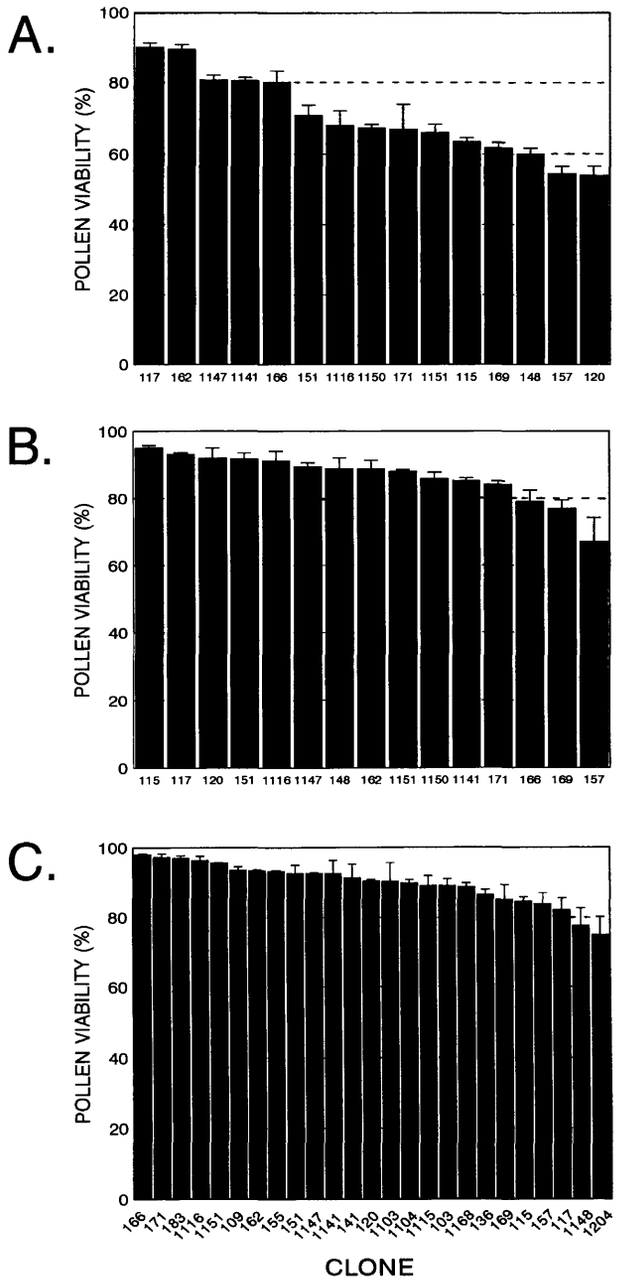

Figure 1. – Pollen viability using agar with 5% sucrose for A. freshly collected pollen in 1992, B. 1992 pollen stored for 1 year, and C. freshly collected pollen in 1993. Thirteen clones are common to 1992 and 1993 collections.

Discussion

The hemocytometer has herein been shown to be an effective mean of counting pollen grains in the preparation of polymixes for polycross studies. The hemocytometer has been used for some time by medical technologists to count various types of blood cells. The major advantage of the hemocytometer is its low investment cost for use with small numbers of samples to be evaluated. Laboratories wishing to count pollen grains for polycross should consider using the hemocytometer: it is likely to be more cost-effective than electronic particle counters.

The hemocytometer technique used in this study to evaluate pollen grains in a fixed volume (ML) or weight (MG) is similar to that described previously for counting blood cells per unit of blood (see PLATT, 1969; SEIVERD, 1983). In this study, some modifications were made owing to differences between blood cells and pollen grains (see Material and Methods). Some of these changes are: rupturing of the pollen's sacci with methanol to eliminate pollen buoyancy and to obtain an homogeneous suspension, the dilution factor (DF), and the Ratio of water volume displacement for a certain mass of pollen.

This study has shown that considerable variation in ML exists for jack pine pollen between collection years and among clones. Pollen grains were much larger in 1992 than in 1993 resulting in lower ML values in 1992 (16 467 224 vs 18 645 169). Clonal variation explained the largest variability in ML (32%). Clone 166 with the smallest pollen grains in 1992 had 1.82

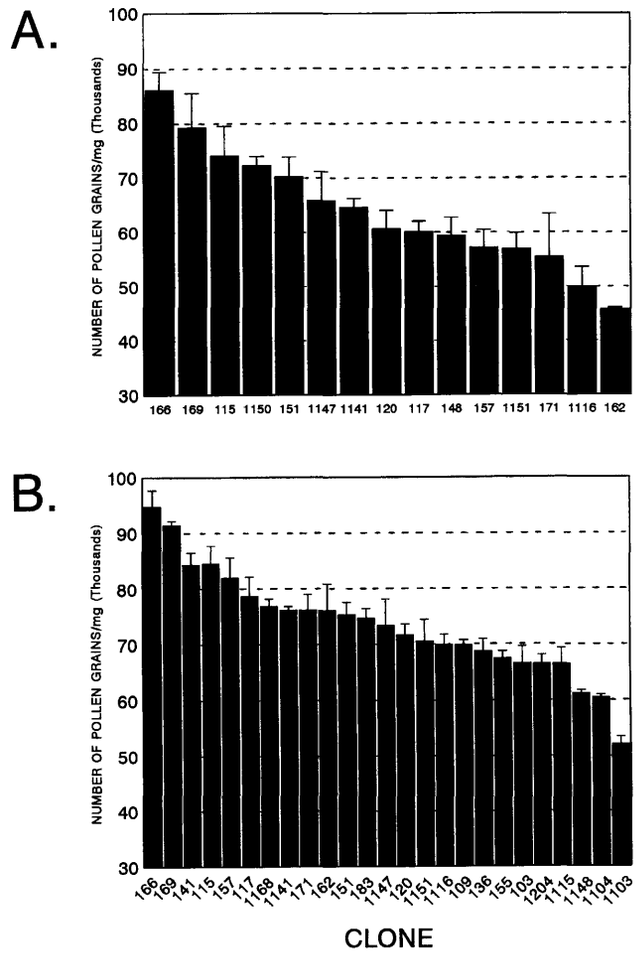

Figure 2. – Number of pollen grains per mg of dry pollen for jack pine clones in A. 1992 and B. 1993. Thirteen clones are common to 1992 and 1993 collections.

Table 5. – Analyses of variance and variance components (%) for the number of pollen grains per ml of dry pollen for 13 jack pine clones common to 1992 and 1993 collections.

Source of variation	df	Mean square	F-value	p-value	Variance component
Model	25	2.251×10^{13}	8.16	< 0.0001	
Year	1	9.250×10^{13}	9.88	0.0085	20.30
Clone	12	2.983×10^{13}	3.19	0.0277	32.49
Year x clone	12	9.358×10^{12}	3.39	0.0011	20.95
Error	52	2.757×10^{12}			26.26

times more grains than Clone 162 with the largest grains. Similarly in 1993, 1.68 times more grains were noted for the clone with the largest vs the clone with the smallest ML values. LUKE (1992) found that the clone with the largest ML had 2.74 and 2.20 more grains than the clone with the smallest ML for white spruce (*Picea glauca* (MOENCH.) VOSS) and black spruce (*Picea mariana* (MILL.) B.S.P.), respectively. Because polymixes for jack pine are based on ML, volume adjustments are needed. Results further indicated that some "Year x clone" interaction was present. Hence, pollen sizing evaluation must be conducted after each collection, even when the same clones are used continuously. However, some clones appeared to be quite stable (Clones 166, 169) while others varied greatly between years (Clones 157, 1151). Additional clones in 1993 had generally lower ML values, thus with larger grains, on average, to clones common to both years (*Fig. 2A* and *B*). This further indicate the need to evaluate ML for polycross studies.

Unlike for ML, the "Collection Year" explained the largest variability in MG (47%). Variability associated with "Clone" was 29% and the interaction, although significant, minimal (6%). MG averaged 70 988 pollen grains for the 13 common clones, with a higher value in 1993 than in 1992 (78 463 vs 63 513). The largest clone mean was 1.88 times that of the smallest in 1992, and 1.82 in 1993. These findings differ from ADAMS (1982) who found only a factor of 1.34 times between the largest and smallest MG values for Douglas-fir (*Pseudotsuga menziesii* (MIRB.) FRANCO). Jack pine polymixes based on MG must therefore be adjusted for differential clonal values.

ADAMS (1982) suggested measuring pollen diameter of Douglas-fir (grain being spherical) as an indication of pollen size owing to a strong negative correlation with MG. In this study, no correlation was found between ML and MG with 6 of the 7 morphometric measures of pollen size. Only PH1 had a significant correlation with ML and MG, but since PH2 was not correlated with ML or MG, it is doubtful that pollen height could be used for predicting these parameters. Hence, no morphometric measure of pollen dimension appears reliable in predicting ML or MG. This predictive difficulty may, in part, relate to the shape of jack pine pollen grain (RICHARD, 1970; ADAMS and MORTON, 1972; OWENS and SIMPSON, n.d.).

A quick indication of ML can however be obtained with PW. Of the 7 morphometric measures, PW appears to better estimate ML but caution must be practiced as a poor correlation was

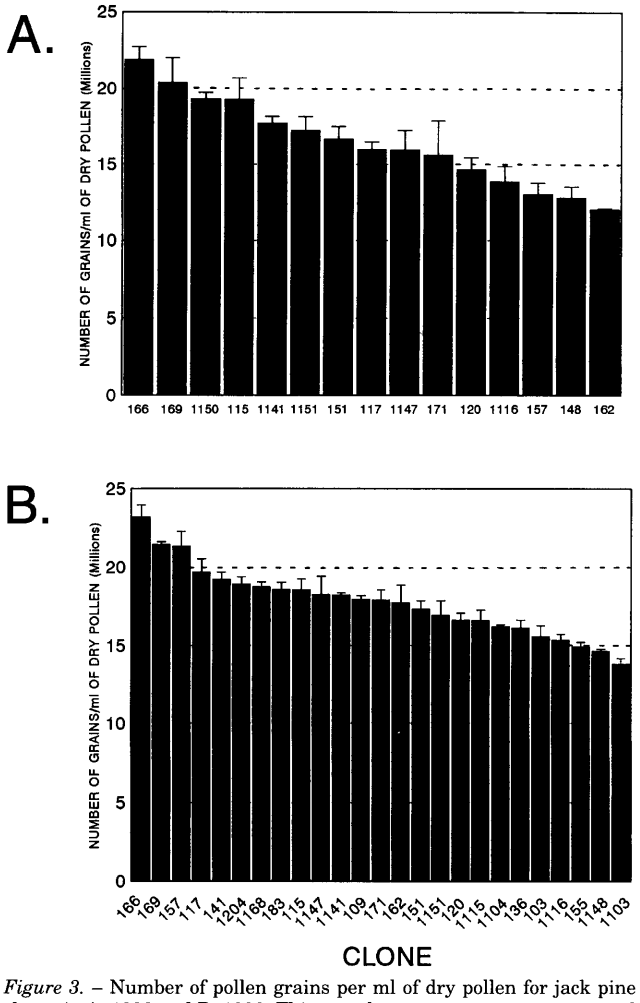

Figure 3. – Number of pollen grains per ml of dry pollen for jack pine clones in A. 1992 and B. 1993. Thirteen clones are common to 1992 and 1993 collections.

found between the 2 variables. To obtain that estimate, 2 assumptions are needed: i) the jack pine pollen grains are spherical, and ii) the "random close-packing" of hard sphere structures (see FINNEY, 1983) occurs. Results of FINNEY (1983) led to the characterization of a „random close-packed" structure with an upper limiting density of 0.6366 ± 0.0004. For Clone 166 in 1992 and 1993, PW was 38.4 µm and 40.4 µm, respectively. Assuming that the pollen is a sphere, then the volume of a grain (V_g) for Clone 166 in 1992 and 1993 would be equal to 29 648 µm³ and 34 450 µm³, respectively ($V_g = 4/3 \times \pi \times (\text{width}/2)^3$). The next step would be to estimate the number of grains in a "close-packed" environment – or when grains are randomly scattered in a vial. Hence, a total of 21 471 937 and 18 478 955 grains could be estimated for ML using the sphere and close-packing assumptions. These two values are close estimates to those with the hemocytometer, namely, 21 881 916 and 23 219 224, respectively. For the smallest values amongst the 13 common clones, ML with the hemocytometer was 12 020 449 for Clone 162 in 1992 and 15 572 788 for Clone 1114 in 1993. With PW of 42.9 µm and 42.7 µm, ML under the sphere and close-packing assumptions was 15 399 126 and 15 616 525 for Clones 162 and 1114, respectively. Other morphometric measures of pollen dimension tended to either underestimate or overestimate ML.

Jack pine pollen, in this study, averaged 41.6 µm for PW and 39.6 µm for CL. PW is much less than that previously reported by RICHARD (1970; 49 µm ± 4 µm), ADAMS and MORTON (1972; 51 µm), and OWENS and SIMPSON (n.d.; 50 µm). Similarly, CL differed from that reported by OWENS and MOLDER (n.d.; 39.6 vs 33, respectively). However, CW and CH are comparable (35.4 µm and 15.3 µm in this study vs 37 µm and 16 µm in OWENS and SIMPSON, n.d.). Differences in PW and CL could be related, in part, to genetic variation among provenances, sampling technique and intensity, time of pollen measurement after collection, and/or pollen preparation and examination techniques used.

Table 6. – Mean values (± S.E.) for 7 pollen morphometric measures (µm) from 13 jack pine clones common to 1992 and 1993 collections.

		Front view				Side view		
Year	n	Pollen width PW	Corpus width CW	Pollen height PH1	Corpus height CH	Pollen height PH2	Corpus length CL	Saccus length SL
1992	130	41.53	35.30	32.92	14.92	32.54	39.45	31.77
		(0.29)	(0.30)	(0.25)	(0.16)	(0.31)	(0.26)	(0.33)
1993	130	41.71	35.57	32.39	15.63	32.35	39.71	32.26
		(0.29)	(0.26)	(0.19)	(0.19)	(0.31)	(0.27)	(0.31)
Mean	260	41.62	35.43	32.66	15.27	32.45	39.58	32.02
		(0.21)	(0.20)	(0.16)	(0.13)	(0.22)	(0.19)	(0.22)

Table 7. – PEARSON correlation matrix (with p-value) of the number of pollen grains per ml of dry pollen, number of pollen grains per mg of dry pollen, and of 7 morphometric measures of pollen collected from 13 jack pine clones common to 1992 and 1993 collections.

Variable	1	2	3	4	5	6	7	8	9
1. Number of grains/1 ml of dry pollen (ML)	1.0000 (0.0)	0.9040 (< 0.0001)	-0.3372 (0.0920)	-0.1560 (0.4466)	-0.5743 (0.0022)	-0.1420 (0.4889)	-0.2035 (0.3188)	-0.1125 (0.5842)	0.0342 (0.8684)
2. Number of grains/1 mg of dry pollen (MG)		1.0000 (0.0)	-0.2921 (0.1477)	-0.1476 (0.4718)	-0.5232 (0.0061)	-0.0235 (0.9091)	-0.0305 (0.8824)	-0.1130 (0.5827)	-0.0214 (0.9173)
3. Pollen width (PW)			1.0000 (0.0)	0.4073 (0.0389)	0.5566 (0.0031)	0.1456 (0.4777)	0.1408 (0.4926)	0.2840 (0.1596)	0.0741 (0.7191)
4. Corpus width (CW)				1.0000 (0.0)	-0.0074 (0.9713)	0.1290 (0.5298)	-0.4673 (0.0161)	0.2938 (0.1452)	0.2458 (0.2358)
5. Pollen height (front view, PH1)					1.0000 (0.0)	0.1173 (0.5682)	0.5166 (0.0069)	-0.2485 (0.2210)	-0.4003 (0.0427)
6. Corpus height (CH)						1.0000 (0.0)	0.1455 (0.4782)	0.0729 (0.7236)	0.0773 (0.7075)
7. Pollen height (side view, PH2)							1.0000 (0.0)	-0.1454 (0.4785)	-0.3391 (0.0902)
8. Corpus length (CL)								1.0000 (0.0)	0.8785 (<0.0001)
9. Saccus length (SL)									1.0000 (0.0)

Table 8. – PEARSON correlation matrix (with p-value) of pollen viability and 7 pollen morphometric measures from 13 jack pine clones common to 1992 and 1993 collections. Pollen measures were obtained in 1993 for both 1992 and 1993 pollen collections.

	Front view				Side view		
Viability test	Pollen width PW	Corpus width CW	Pollen height PH1	Corpus height CH	Pollen height PH2	Corpus length CL	Saccus length SL
After collection in 1992	0.2762	0.1552	0.1438	0.4784	0.0303	-0.0822	-0.2821
	(0.3610)	(0.6126)	(0.6393)	(0.0982)	(0.9218)	(0.7894)	(0.3504)
After collection in 1993	0.0193	0.4296	0.0319	-0.2091	-0.2756	-0.1429	-0.2235
	(0.9501)	(0.1429)	(0.9176)	(0.493)	(0.3620)	(0.6413)	(0.4629)
1992 stored pollen	0.3986	-0.1739	0.6559	0.2820	0.4695	-0.6552	-0.6430
	(0.1773)	(0.5698)	(0.0149)	(0.3506)	(0.1055)	(0.0151)	(0.0177)

The present study indicates considerable variation in pollen viability and pollen size. In the Maritimes, a minimum of 20 trees is used for all polycrosses, with each male parent represented by an equal volume of pollen (FOWLER, 1986). Polymixes used for the polycross should be adjusted so that they are composed of equal numbers of viable pollen grains from each male. Volume adjustment for difference in viability among males, is simply the product of volume required and the reciprocal of the proportion of viable pollen, as determined by a viability test. Final volume adjustment is obtained as the product of (viable) pollen volume and ML (or MG). By making these 2 adjustments, the chance that any one pollen can dominate in the cross is reduced considerably. Other factors such as competitive ability of pollen (MORAN and GRIFFIN, 1985; CHELIAK *et al.*, 1987) and/or differential vigour of the successful embryo (MULCAHY and MULCAHY, 1987) may also influence fertilization success of clones in polycross studies.

Acknowledgements

Funding for this project was provided by the Research Committee of the École de sciences forestières with additional support through the MENTOR Program of the New Brunswick Provincial Government for student employment. The authors thank Mr. G. ADAMS of J. D. Irving Ltd. for collecting the pollen, Mrs. A. Émond, Edmundston Regional Hospital, for her technical guidance, and Miss J. LAFOREST and Mrs. V. BLIER-BOSSÉ for their technical assistance. Special thanks to Mr. J. D. SIMPSON and Dr. D. P. FOWLER, Canadian Forest Service – Maritimes, for their critical review of the manuscript.

References

ADAMS, W. T.: Clonal variation in pollen-related characteristics of Douglas-fir. Can. J. For. Res. **12**: 403–408 (1982). — ADAMS, R. J. and MORTON, J. K.: An Atlas of pollen of the trees and shrubs of Eastern Canada and adjacent United States. Univ. Waterloo, Biological Series No. 8, 52 pp. (1972). — APSIT, V. J., NAKAMURA, R. R. and WHEELER, N. C.: Differential male reproductive success in Douglas-fir. Theor. and Applied Genet. **77**: 681–684 (1989). — BREWBAKER, J. L. and KWACK, B. H.: The essential role of calcium ion in pollen germination and tube growth. Amer. J. Bot. **50**: 859–865 (1963). — CHELIAK, W. M., SCROPPA, T. and PITEL, J. A.: Genetics of the polycross. 1. Experimental results from Norway spruce. Theoretical and Applied Genetics **73**: 321–329 (1987). — FINNEY, J. L.: Structure and properties of granular materials: Guidelines from modelling studies of liquids and amorphous solids. *In*: Advances in the mechanics and the flow of granular materials. Volume I. Ed. M. SHAHINPOOR. Gulf Publ. Co., Houston, U.S.A. pp. 19–39 (1983). — FOWLER, D. P.: Strategies for the genetic improvement of important tree species in the Maritimes. Inf. Rep. M-X-156. Forestry Canada – Maritimes Region (1986). — FOWLER, D. P.: In defense of the polycross. Can. J. For. Res. **17**: 1624–1627 (1987). — FOWLER, D. P. and WISELOGEL, A. E.: Pollen polymixes. *In*: Advances in Pollen Management. Eds. D. L. BRAMLETT, G. R. ASKEW, T. D. BLUSH, F. E. BRIDGWATER and J. B. JETT. U.S.D.A. Agric. Handbook 698, pp. 79–82 (1993). — GODDARD, R. E. and MATTHEWS, F. R.: Pollen testing. *In*: Pollen management handbook. Ed. E. C. FRANKLIN. U.S.D.A. Agric. Handbook 587, pp. 40–43 (1981). — GREENWOOD, M. S.: Gene exchange in loblolly pine: the relation between pollination mechanism, female receptivity and pollen availability. Am. J. Bot. **73**: 1443–1451 (1986). — HO, R. H. and OWENS, J. N.: Microstrobili of Douglas-fir. Can. J. For. Res. **4**: 561–562 (1974). — HUDSON, L. and HAY, F. C.: Practical immunology. Second Edition. Blackwell Scientific Publ., Oxford (1980). — JETT, J. B., BRAMLETT, D. L., WEBBER, J. E. and ERICKSSON, U.: Pollen collection, storage, and testing. *In*: Advances in pollen management. U.S.D.A. Agric. Handbook 698, pp. 41–46 (1993). — LUKE, A. B.: Variation in size and viability of black spruce and white spruce polymix pollen. B.Sc.F. thesis. University of New Brunswick, Fredericton, N.B. (1992). — LUTIER, P. M. and VAISSIÈRE, B. E.: An improved method for pollen analysis of honey. Rev. Palaeobot. Palynol. **78**: 129–144 (1993). — MATTHEWS, F. R. and KRAUS, J. F.: Pollen storage. *In*: Pollen management handbook. Ed. E. C. FRANKLIN. U.S.D.A. Agric. Handbook 587, pp. 37–39 (1981). — MORAN, G. F. and GRIFFIN, A. R.: Non-random contribution of pollen in polymix crosses of *Pinus radiata* D. DON. Silvae Genet. **34**: 117–121 (1985). — MULCAHY, D. L. and MULCAHY, G. B.: The effect of pollen competition. Am. Scien. **75**: 44–50 (1987). — OWENS, J. N. and SIMPSON, S. J.: Manual of Conifer Pollen from British Columbia Conifers. B.C. Ministry of For. 39 pp. (n.d.). — PLATT, W. R.: Color atlas and textbook of hematology. J. B. Lippincott Co., Philadelphia (1969). — Pohl, F.: Die Pollenkorngewichte einiger windblütiger Pflanzen und ihre Ökologische Bedeutung. Beih. z. Bot. Centralbl. **57**A: 112–172 (1937). — Richard, P.: Atlas pollinique des arbres et de quelques arbustes indigènes du Québec. Nat. can. **97**: 1–34 (1970). — SAS Institute Inc.: SAS Procedures Guide. Version 6, 3rd edition. SAS Institute Inc. (1990a). — SAS Institute Inc.: SAS/STAT User's Guide. Volume 2. GLM-VARCOMP. Version 6, 4th edition. SAS Institute Inc. (1990b). — SIEVERD, C. E.: Hematology for medical technologists. Lea and Febiger, Philadelphia (1983). — Sigma Chemical Company: Biochemicals, organic compounds for research and diagnostic reagents. (1991). — SZIKLAI, O.: Variation and inheritance of some physiological and morphological traits in *Pseudotsuga mensziesii* (MIRB.) FRANCO. Ph.D. thesis, Univ. of British Columbia, Vancouver, B.C. (1963). — WISELOGEL, A. E. and VAN BUIJTENEN, J. P.: Probability of equal mating in polymix pollination of loblolly pine (*Pinus taeda* L.). Silvae Genet. **37**: 184–187 (1988).