Lethal Equivalents in Willow, Salix viminalis

By H. KANG[1]), C. HARDNER[2]) and U. GULLBERG[2])

(Received 20th August 1991)

Abstract

The main objective of this paper is to estimate the number of lethal equivalents in the founder population of *Salix viminalis* L. to be used for breeding in Sweden. The technique is to compare survival fractions in material with different degrees of inbreeding. Since *Salix* is a dioecious species, two generations of crossings are required to get inbreeding; the estimate is based on comparing full-sib crossings with outcrosses.

Since the founder population is made up of individuals from different origins (sub-populations), we have to define and estimate mean lethal equivalents based on the lethal equivalents for each subpopulation. We suggest that these means be determined by using the geometric mean of the survival fractions of the subpopulations, and show how they can be estimated.

The probability for mortality in a full-sib crossing given the number of lethal loci in the grandparents is derived. This probability has its maximum when both grandparents have all the lethals in common and its minimum when they have no lethals in common. The range of lethal equivalents among grandparents for different mortalities is calculated.

Because of irregularities in flowering and other biological constraints, the experiment was not well balanced. However, overestimation was opted for whenever possible. The resulting upper estimate of population lethal equivalents (1.99, 3.63) is substantially smaller than that observed in most conifers.

Key words: Lethal equivalents, inbreeding, *Salix viminalis*.

Introduction

Whenever finite number of individuals are selected in a recurrent selection system or when certain inbreeding schemes are deployed in breeding, the level of homozygosity of individuals in the breeding population often increases. Increased homozygosity tends to be accompanied by reduced performance of various traits in the breeding population (FALCONER, 1981; ALLARD, 1960). It is also possible that breeding lines (or populations) could be lost due to deleterious alleles. To forestall such undesirable events, breeders might use crossing schemes designed to avoid inbreeding as long as possible (KIMURA and CROW, 1963; COCKERHAM, 1970; NAMKOONG, 1974), or they might maintain a large population. An opposite strategy is to maintain a set of small populations, and allow inbreeding within each subpopulation (NAMKOONG et al., 1980; LOWE and BUIJTENEN 1981, KANG and NIENSTAEDT 1987).

ERIKSSON et al. (1984), proposed the latter approach for breeding *Salix* spp., and further suggested developing highly inbred lines and purging deleterious alleles from the breeding population by using a regular (full-sib) system of inbreeding. A similar purging process might operate in natural populations (LANDE and SCHEMSKE, 1985). For example, in natural plant (or fern) populations, inbreeders tend to have higher fertility than outbreeders, probably due to a smaller number of deleterious alleles (or lower mutational load) (CHARLESWORTH, 1990; HEDRICK, 1987), HOWEVER, MITCHELL-OLDS and GURIES (1986) failed to show a significant correlation between the level of heterozygosity of tree populations and the number of lethal equivalents.

We do not know the impact of recurrent mutation of deleterious alleles in tree breeding populations. If the impact of recurrent mutation is negligible, then it would be possible to effectively eliminate lethal alleles from tree breeding populations (no alternate mutation-selection equilibrium). NAMKOONG and BISHIR (1987) showed that inbreeding, especially selfing, is far more effective than random mating in purging deleterious (lethal in this case) alleles in a large population. They also showed that the purging rate is c/3, where c is the number of loci that are heterozygous for both parents involved in a crossing. Therefore, in selfing of an individual with n heterozygote loci, n/3 alleles would be purged per generation.

A potential problem with using an inbreeding system, such as full-sib crossing in *Salix*, is that we could lose a large proportion of the breeding population gene pool before the purging is completed. This is possible if the number of lethal equivalents (MORTON et al., 1956; CAVALLI-SFORZA and BODMER, 1971) is high in the initial population or the population size is too small. Thus, it is useful to have information on the number of lethal equivalents in founder breeding populations. This will help to estimate the chances of losing lines or populations due to inbreeding and assist in deciding the desired size of the founder population. The main objective of this paper is to estimate the number of lethal equivalents in the founder population of *Salix viminalis* L. to be used for breeding in Sweden.

Definition of Lethal Equivalents

1) Definition of ε

We will distinguish between the lethal equivalents expected in a randomly chosen gamete (ε) or zygote (2ε) and those in a given individual (E). Lethal equivalent of a random gamete (ε) is defined as "a group of mutant

[1]) North Central Forest Experiment Station, USDA Forest Service, University of Wisconsin-Madison, Dept. of Forestry, 1630 Linden Drive, Madison, WI 53706, USA

[2]) Swedish University of Agricultural Sciences, Dept. of Forest Genetics, Box 7027, S-750 07 Uppsala, Sweden

genes of such number that, if dispersed in different individuals, they would cause on the average one death — e. g., one lethal mutant, or two mutants each with 50% probability of causing death. etc. "(MORTON et al., 1956). The mathematical expression of lethal equivalents (MORTON et al., 1956; CAVALLI-SFORZA and BODMER, 1971) is:

$$[1] \qquad \varepsilon = \sum s_j q_j \qquad 0 \le s_j \le 1,$$

where s_j represents the selection coefficient at the j^{th} locus (or probability of death of an individual due to the deleterious allele at j^{th} locus),

q_j represents the recessive allele frequency at j^{th} locus, and the summation is over all the loci influencing the trait.

If we assume that $s_j = 1$ — i. e., the recessive allele is lethal when homozygous — then we can have more direct definition of ε as the expected number of lethal alleles in a randomly sampled gamete of the reference population ($\varepsilon = \Sigma q$). However, $0 \le s \le 1$, and the number of deleterious (not necessarily lethal) alleles in a random gamete would be greater than or equal to ε. Therefore, ε may be viewed as the minimum expected number of deleterious alleles in a randomly sampled gamete. This quantity may also be viewed as a measure indicating the cumulative effect of the deleterious alleles in a gamete is equivalent to that of ε lethal alleles, provided no interactions among alleles at different loci exist.

2) Definition of $\bar{\varepsilon}$

When two or more populations with different allele frequency are involved in determining lethal equivalents, we may use an average such that

$$[2] \qquad \bar{\varepsilon} = \frac{1}{m} \Sigma\Sigma s_{ij} q_{ij},$$

where m represents the number of populations, and i and j index different populations and loci, respectively. If we assume

$s_{ij} = s_{kj} = s_j$ for all j, then

$\bar{\varepsilon} = \Sigma s_j q_j$

where $q_j = \frac{1}{m} \Sigma q_{ij}$.

3) Definition of E

Lethal equivalents as defined in Eq. 1 depend on the allele frequencies of the population, and are expected values representing the property of the population as a whole. E as frequently defined in forestry (KOSKI, 1971; BRAMLETT and PEPPER, 1974; BISHIR and PEPPER, 1977; BISHIR and NAMKOONG, 1987) represents the estimate of lethal equivalents present in a particular individual chosen in the population, and does not depend on the allele frequencies in the population.

The meaning of E varies depending on the crossing scheme used. When selfings are made, the fraction of survivors (or mortality)of progeny is used to determine the lethal equivalents of the parent. When full-sib crossing is made, the fraction of mortality is used to determine the combined features of grandparents. If both grandparents are heterozygous for k loci, and each grandparent is uniquely heterozygous for l loci, where $l = l_1 + l_2$ and subscripts 1 and 2 distinguish the grandparents, then $n = k + l = k + l_1 + l_2$ defines the lethal equivalents (E). Therefore, E represents the number of common lethals and sum of unique lethals in the grandparents. Only when $k = n$ (or $l = 0$), can E be used as representing lethal equivalents of one grandparent.

With selfing, if many individuals are randomly sampled from the reference population and E is determined for all of them, then the mean of these estimates ($\bar{E}$) may be used to represent 2ε. The same cannot be said for full-sib mating, unless $k = n$ for all individuals. However, $\bar{E}$ may be used as upper estimates of the population lethal equivalents.

Estimation Methods

1) Estimating ε

MORTON et al. (1956) showed that the fraction of survivors (S) in a population is

$$[3] \qquad S \approx \exp[-(A + BF)], \text{ or}$$

$$-\log S = A + BF$$

$$= \Sigma x + A^* + BF$$

$$= \Sigma x + A + (\varepsilon - A^*)F,$$

where Σx represents mortality due to total environmental causes,

$A = \Sigma x + \Sigma q^2 s + 2\Sigma q(1-q)sh$,

$A^* = A - \Sigma x$,

$B = \Sigma qs - \Sigma q^2 s - 2\Sigma q(1-q)sh$,

F = inbreeding coefficient,

q = recessive allele frequency at a locus,

s = selection coefficient at a locus, and

h = degree of dominance.

From Eq. 3 we find

$\varepsilon = A^* + B$.

If we assume that the environmental cause of death is zero ($\Sigma x = 0$), then

$\varepsilon = A + B$.

If we assume that the amount of genetic death due to random mating is negligible (NAMKOONG and BISHIR, 1987), we may let $A^* = 0$, and

$\varepsilon = B$.

If $A^* > 0$, then using B will underestimate ε, while the opposite will hold when $A^* < 0$. We can determine A and B values from Eq. 3, when we know inbreeding coefficients (F) within each of two subgroups in the population and their survivors, and that both groups have the same q, s, and h. Let S_1 and S_2 represent fractions of survivors of groups 1 and 2, respectively; then

$$[4] \qquad B = \frac{-\log(S_1/S_2)}{F_1 - F_2}, \text{ and}$$

$$A = -\log S_1 - f \log(S_1/S_2),$$

where $f = \frac{F_1}{F_1 - F_2}$,

F_1 = inbreeding coefficient of Group 1, and

F_2 = inbreeding coefficient of Group 2.

Let $F_2 = 0$, then

$$[5] \qquad B = \frac{-\log(S_1/S_2)}{F_1}, \text{ and}$$

$$A = -\log S_2,$$

as shown in SORENSEN (1969). Expressions in Eq. 5 can be used as proper estimators if population and sample sizes are same in both subgroups. MORTON et al. (1956) used a weighted regression to accommodate situations with more than two groups with different inbreeding coefficients.

2) Estimating $\bar{\varepsilon}$

When F is identical among different populations, it is necessary to use the geometric mean of survival fractions of different populations to determine the mean lethal equivalents ($\bar{\varepsilon}$) as defined in Eq. 2. Because MORTON et al. (1956) used exponential approximation of survival fractions, the use of the geometric mean will lead to the arithmetic mean ($\bar{\varepsilon}$). If there are m populations, then

$$S_g = [TTS_i]^{-m} \sim \exp[-(\Sigma A_i + \Sigma B_i F)/m]$$
$$= [-(\Sigma x/m + \bar{A}^* + \bar{B}F)]$$

where S_g = geometric mean of survival fractions of different populations,

i indexes different populations,

$\bar{A}^* = \Sigma A^*_i/m$, and $\bar{B} = \Sigma B_i/m$.

then, $\bar{A} + \bar{B} = \frac{1}{m}\Sigma\Sigma s_{ij}q_{ij} = \bar{\varepsilon}$ as defined in Eq. 2.

The mean lethal equivalents are useful when there are subpopulations. The *Salix viminalis* population is made up of individuals from different origins, which are crossed to produce inbred and outcross groups. Therefore, $\bar{\varepsilon}$ will be extensively used in this paper.

3) Estimating E

When environmental causes of death are absent, the number of lethal alleles in a monoecious diploid individual can be estimated by selfing it and comparing the fraction of mortality in the progeny with the plot (*Fig. 1*) of Q (KOSKI, 1971; BRAMLETT and PEPPER, 1974; BISHIR and PEPPER, 1977).

[6] Q = Prob (death of an offspring | n lethal alleles in the parent)

$$= \sum_{r=0}^{n}\binom{n}{r}(1/2)^n(1-2^{-r})^f,$$

where n is the number of lethal alleles in the parent, and f is the number of embryos per ovule.

Equation was derived with the assumption that recessive homozygotes are lethal. Therefore, the parent used for selfing can be either dominant homozygote or heterozygote for all the lethal loci. Since a dominant homozygote cannot produce a lethal allele without mutation, the formulation of Q depends entirely on the heterozygous loci.

With full-sib crossing, the formulation of Q becomes more complex, requiring two generations of crossings and distinction of two initial conditions. Initially, two grandparents are crossed to produce a large parental family. From the parental family two full-sibs are crossed to produce a progeny. With grandparents it is assumed that: (1) Both are heterozygous for k loci, and (2) one parent is heterozygous and the other parent is dominant homozygous for l loci. Assuming independence among loci, the frequency of recessive lethal alleles in the progeny population will be 1/3 (= 1 — α) for k loci (1/3 dominant homozygotes and 2/3 heterozygotes), and 1/4 (= 1 — β) for l loci (1/2 dominant homozygotes and 1/2 heterozygotes). When two individuals are randomly sampled in the parental population to produce a full-sib family, the probability of mortality of a progeny is:

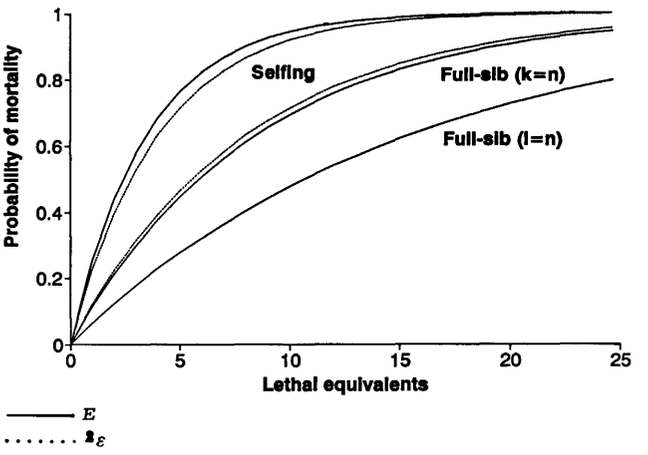

Figure 1. — Probability of mortality and lethal equivalents.

[7] Q = Prob (death of an offspring | k double heterozygotes and l single heterozygotes in grandparents)

$$= \sum_{r=0}^{n}\sum_{i=0}^{r}\binom{k}{i}\binom{l}{j}(1-\alpha)^i(1-\beta)^j\alpha^{k-i}\beta^{l-j}(1-\alpha^i\beta^j)$$

where l = n — k, j = r — i, α = 2/3, and β = 3/4.

The plot of Q (*Fig. 1*) may be used to determine the combined number of lethal loci in the grandparents. In this case we have a range of values for n instead of a single value. For a given probability of mortality, n is maximum when l = n, and minimum when k = n.

4) Removing environmental effects on death

The observed fraction of mortaly (1—S) is usually greater than or equal to Q, because there are nongenetic causes of mortality. BISHIR and NAMKOONG (1987) developed a method to separate maternal nongenetic causes of mortality and genetic mortality. The probability of mortality of an individual sampled from a family involving i^{th} mother and j^{th} father is

$$[8] \qquad c_{ij} = b_i + (1-b_i)Q_{ij},$$

where the progeny is the product of selfing when i = j, and of outcrossing when i ≠ j,

b_i is the probability of mortality due to nongenetic maternal effects, and

Q_{ij} is the probability of genetic mortality .

Assuming $Q_{ij} = 0$ when i ≠ j, the least square estimate of b_i and Q_{ii} are given as (BISHIR and NAMKOONG, 1987):

$$\hat{b}_i = \Sigma_{ji}u_{ij}/t = \bar{u}_i, \text{ and}$$

$$[9] \qquad \hat{Q}_{ii} = (u_{ii} - \hat{b}_i)/(1-\hat{b}_i),$$

where u_{ij} is the observed proportion of mortality in the ij^{th} family ,and t is the number of outcrosses involving i.

For a single full-sib crossing and outcrossings we may require Equation 7 as

$$[10] \qquad c_{ij}^{\gamma} = b_i + (1 - b_i)\,Q_{ij}^{\gamma}, \text{ where}$$

$$\gamma = \begin{cases} 1, \text{ when i and j are full-sibs, and} \\ 0, \text{ when i and j are not related.} \end{cases}$$

In Eq. 10 there is only one j which is a full-sib of i. We may define the least-square estimates of b_i and Q_{ij} in a manner similar to those defined by BISHIR and NAMKOONG (1987).

$$\hat{b}_i = \sum_j u_{ij}^{\gamma=0}/t = \bar{u}_i, \text{ and}$$

$$[11] \quad \hat{Q}_{ij}^{\gamma=1} = (u_{ij}^{\gamma=1} - \hat{b}_i)/(1 - \hat{b}_i).$$

BISHIR and NAMKOONG (1987) showed that this method of eliminating maternal effect is equivalent to the use of survival fraction ratio $\hat{Q} = 1 - \hat{R}$, where $\hat{R} = \bar{S}_{inb}/\bar{S}_{out}$, provided that $\bar{S}_{out}$ was estimated from the same individuals used to determine $\hat{b}_i$.

Materials

Founders of *Salix viminalis* in this experiment originated from many different locations in Sweden, Finland, and France *(Fig. 2)*. Although these founders were sampled in naturally regenerated populations, most of their locations do not belong to the natural range of the species. *Salix viminalis* was introduced to Sweden about 200 years ago from unknown origins (TORSÉN, personal communication). Therefore, there is a good possibility that some of the populations share their origins. Most of these naturally regenerated populations, however, grow in small clumps of 1 to 5 individuals (TORSÉN [3]), personal communication) and could have diversified over 200 years even if they had shared origins.

Cultural Methods

Male and female founders from different populations were pair-mated in 1985 and 1986, producing 10 different parental families *(Fig. 2)*. Seeds were sown immediately after maturation and seedlings were outplanted in the field.

In spring of 1989, shoots possessing developing flowers were collected and placed in buckets of water in a greenhouse to allow the flowers to develop. Prior to pollination the shoots were covered with cotton bags to prevent contamination by foreign pollen. Pollination was undertaken in early April. Generally for each cross only one shoot of female flowers from each female founder was used. Pollination of these flowers was achieved by brushing the male flowers against all the female flowers on the shoot. The shoot was then re-covered with the cloth bag. For most crosses, repollination of each female flower was made at within a week, although for some crossings this may have been limited by the presence of only one male flower on the shoot.

Catkins began breaking in late April and continued until early June. Most catkins were full with what appeared to be seed (approximately 1 mm in size) attached to cotton. Because we thought that viability of seed would decline shortly after release from the catkins, we emptied the bags surrounding the shoots and laid new germination tests with fresh seed each day. We later found, however, that at room temperature there was no difference between germination rates of seeds 0 and 5 days old. In some cases catkins failed to open and dried out. This was thought to be a consequence of drought induced by poor greenhouse treatment. It may be possible that

[3]) Department of Forest Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden

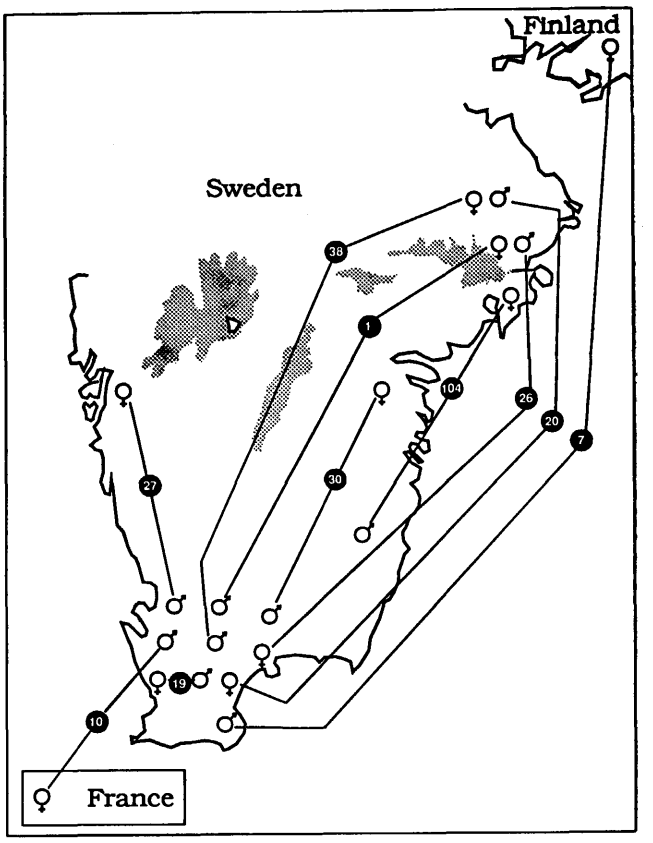

Figure 2. — Origins and crossing patterns of grandparents used in this experiment.

other shoots experienced milder stress that did not result in the death of catkins but that may have affected seed health and development.

The parental families produced between three and five females and males were available and altogether 29 mothers and 33 fathers were crossed in an irregular crossing scheme. The crosses produced 61 inbred (full-sib) and 45 outcrossed families.

To determine germination percent, one to five samples per cross of 20 seeds were placed on saturated filter paper in a petri dish. The petri dishes were kept in a room at 20° C under 24-hour light. Each day water was added so that the filter paper was saturated.

Germination was defined as the rupture of the seed coat by the hypocotyl and extension of the hypocotyl by the third day of sowing. A detailed description of the germination process was given by SIMAK (1982) for *Salix caprea* under similar conditions (although SIMAK included emergence of the cotyledons as part of the germination process). SIMAK also noted that after emergence the germinating seed may exhibit several kinds of abnormal development that can arrest its growth to seedling.

Results and Discussion

1) Mean germination percent and distribution of survival fractions

Overall means of the groups show that inbreds are slightly inferior to outcrosses *(Table 1)*. However, the mean germination percent of inbred was greater than that of corresponding outcross in five parental families. The absolute difference tended to be greater when inbreds were inferior to outcrosses — i. e., 17.3 for mean in-

Table 1. — Mean percent germination of inbred and outcross families.

a) Numbers based on the original set of families

Parental	Inbred			Outcross			Difference
families	#	Mean	(s.d.)	#[1)]	Mean	(s.d.)	
1	2	79.5	(20.5)	6	73.8	(35.7)	5.70
7	11	83.9	(21.9)	10	75.2	(32)	8.70
10	7	36.9	(21.5)	15	65.2	(28.3)	-28.30
19	7	58.8	(36.2)	10	57.5	(29.8)	1.30
20	2	30.0	(35.4)	4	55	(26.4)	-25.00
26	6	57.9	(44.6)	6	63.2	(26.3)	-5.30
27	9	78.4	(18.9)	8	74.3	(28.7)	4.10
30	7	65.6	(14.8)	10	58.3	(32.8)	7.30
38	5	40.1	(29.9)	8	58.9	(27)	-18.80
104	5	69.4	(19.9)	10	78.5	(28.8)	-9.10
Overall	61	63.5		45	66.4		-2.98

b) Numbers after crossings with survival of less than 12% are deleted from data

Parental	Inbred			Outcross			Difference
families	#	Mean	(s.d.)	#	Mean	(s.d.)	
1	2	79.5	(20.5)	5	87.5	(13.3)	-8
7	11	83.9	(21.9)	9	83.0	(21.6)	0.9
10	6	42.7	(16.3)	15	65.2	(28.3)	-22.5
19	7	58.8	(36.2)	9	62.8	(26.2)	-3.97
20	1	55.5	(-)	4	55.0	(26.4)	0.5
26	4	83.4	(26.7)	6	63.2	(26.3)	20.2
27	6	78.4	(18.9)	8	74.3	(28.7)	4.10
30	7	65.6	(14.8)	9	63.6	(29.7)	2
38	4	47.6	(28.7)	8	58.9	(27)	-11.3
104	5	69.4	(19.9)	10	78.5	(28.8)	-9.10
Overall	56	68.6		43	69.3		-0.69

[1)] Most outcross values are counted twice, in rows and columns. The sum of the counts is greater than 45, but less than 90.

breeding depression vs. 5.42 for mean outcrossing depression. The small overall mean difference between the two groups and the presence of outcrossing depression suggest that lethal equivalents of *Salix viminalis* are likely to be smaller than those found in conifers. The outcrossing depression could have been caused by heterozygote inferiority, but the severe asymmetry in the amount of depression suggests otherwise. Other possible explanations are: (1) small numbers of crossings, especially outcrossings, made in the experiment; and (2) use of different source materials. These points will be discussed later.

The distribution of germination fractions of the crosses (*Figure 3*) shows that there are little differences between inbred and outcross. For most classes, inbred tends to have larger actual numbers than outcross (*Figure 3a*), but this is due to the difference in total numbers (61 for inbred vs. 45 outcross); differences between the two groups are not as obvious when frequencies are used (*Figure 3b*). The distributions show no clear central tendency, and we may consider the survival fraction of the crosses as a nonquantitative trait.

2) Estimates of lethal equivalents per crossing (E) and population mean (E_p)

The low overall germination percent of outcross (66%) indicates that large unknown causes of mortality exist. Given such low overall survival percent and the small difference between groups for most classes of survival distribution (*Figure 3*), it is reasonable to assume that the genetic death from outcrossing is negligible (Namkoong and Bishir, 1987). With this assumption we can use Eq. 7 to estimate lethal equivalents (E) of grandparents (parents of crossings made in 1985 and 1986). We can also use 2B as representing the lethal equivalents at the population level (2ε) (third assumption in Eq. 3).

Lethal equivalents (E) for all 61 inbreds were estimated (*Table 2*) using Eqs. 7 and 11 and *Figure 1*. The number of outcrosses used to estimate the fraction of death due to environmental causes b) is shown in the fourth column of *table 2*. In general, the numbers of outcrosses are small. Four outcrosses were available for only three individuals to estimate b. There were 18 inbreds which had no corresponding outcrosses. For these inbreds, b was assumed to be zero, meaning that there are no environmental causes of death. Therefore, E for the 18 crosses are likely to be overestimates. For inbreds with u values smaller than b, $\hat{Q}$ and E were set to be 0, where u represents observed fraction of mortality and $\hat{Q}$ represents estimated fraction of mortality due to genetic causes.

In full-sib crossing, many different values of E are possible for a given Q; E is minimum (E_{min}) when both grandparents are heterozygous for all n loci (k = n), and maximum (E_{max}) when only one grandparent is heterozygous for all n loci (l = n). Therefore, we will use a range notation such as (E) = (E_{min}, E_{max}) to represent lethal equivalents in *Salix viminalis*. The values of (E) shown in *table 2* range between (0,0) and (30,54). There are five crosses with (E) greater than or equal to (12.5, 22.5). Considering the general properties of the experimental population observed in *table 1* and *figure 3*, it is doubtful that these values truly represent lethal equivalents. Furthermore, it is unlikely that an individual

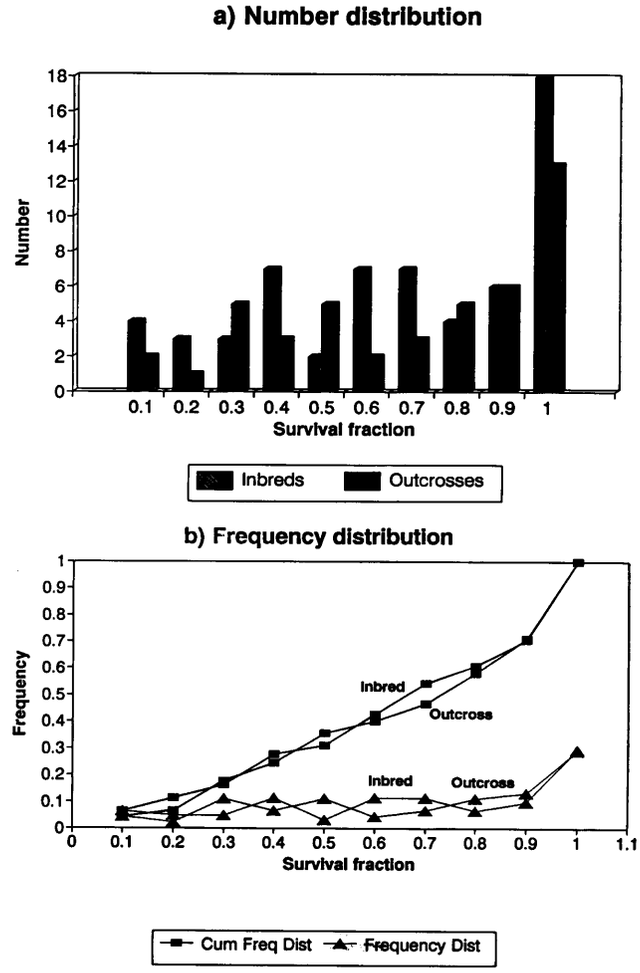

Figure 3. — Distribution of surviving individuals in inbreds and outcrosses.

Table 2. — Estimates of individual lethal equivalents (E).

Parental family	u	$\hat{b}$	#	$\hat{Q}$	E_{min}	E_{max}
1	0.06	0.07	1	0	0	0
	0.35	0.07	1	0.3	3	5.5
7	0.09	0.08	4	0.01	0	0
	0.03	0.08	4	0	0	0
	0	0.08	4	0	0	0
	0.44	0	0	0.44	5	9
	0.11	0	0	0.11	1	1.8
	0.05	0	0	0.05	0.5	0.8
	0.71	0.38	1	0.54	6.5	12
	0.03	0.38	1	0	0	0
	0.16	0.38	1	0	0	0
	0.04	0.06	0	0	0	0
	0.11	0.06	0	0.05	0.5	0.8
10	0.67	0.39	2	0.45	5	9
	0.98	0.39	2	0.97	(30)	(54)
	0.65	0.29	2	0.51	6	11
	0.47	0.29	2	0.25	2.5	4.5
	0.6	0.29	2	0.44	5	9
	0.3	0.51	2	0	0	0
	0.75	0.51	2	0.49	5.5	10.5
19	0.62	0.9	1	0	0	0
	0.74	0.9	1	0	0	0
	0.07	0.33	2	0	0	0
	0	0.33	2	0	0	0
	0.03	0.33	2	0	0	0
	0.81	0.45	3	0.65	9	16
	0.62	0.45	3	0.31	3	5.5
20	0.45	0	0	0.45	5.1	9.25
	0.95	0.64	2	0.86	(16.5)	(30)
26	0.56	0.31	2	0.37	4	7.5
	0	0.31	2	0	0	0
	0.88	0	0	0.88	(18)	(33)
	0.09	0	0	0.09	0.8	1.5
	0.01	0.1	1	0	0	0
	0.98	0.1	1	0.97	(30)	(54)
27	0.15	0	0	0.15	1.4	2.5
	0.58	0	0	0.58	7.3	13.4
	0.09	0	0	0.09	0.8	1.5
	0.35	0.37	2	0	0	0
	0.05	0.37	2	0	0	0
	0.39	0.37	2	0.04	0.5	0.5
	0.23	0	0	0.23	2.1	3.9
	0.03	0	0	0.03	0.3	0.5
	0.07	0	0	0.07	0.7	1.3
30	0.4	0.1	3	0.33	3.5	6
	0.23	0.1	3	0.14	1	2
	0.49	0.1	3	0.43	4.5	8.5
	0.44	0	0	0.44	5	9
	0.07	0	0	0.07	0.7	1.3
	0.45	0	0	0.45	5.1	9.25
	0.33	0.18	1	0.19	1.5	3
38	0.69	0.52	3	0.35	5.5	10
	0.38	0.52	3	0	0	0
	0.9	0.52	3	0.79	(12.5)	(22.5)
	0.83	0.76	1	0.28	2.5	5
	0.2	0	0	0.2	1.9	3.5
104	0.28	0.04	3	0.25	2.5	4.5
	0.11	0.04	3	0.08	0.7	1.3
	0.18	0.04	3	0.15	1.4	2.5
	0.63	0.72	2	0	0	0
	0.33	0.72	2	0	0	0

u = observed fraction of mortality of inbreds; # = number of outcross families used in estimating b; b = estimated maternal effects; and $\hat{Q}$ = estimated probability of mortality of an offspring.

could have (E) = (30, 54) and have survived to reproduction. These values probably originate from large nongenetic causes of death, including experimental errors.

Twenty inbreds had a higher fraction of survival than the corresponding outcrosses — i. e., u is smaller than the corresponding b. For seven of the 20, the difference between inbreds and outcrosses is less than 0.1. In all but one of these cases the survival probability of both inbreds and outcrosses is high and the outcross depressions are likely to be due to sampling errors. At the other extreme, for two inbreds the same single outcross is used to estimate b, and its survival fraction is 0.1 — an unrealistic number. Therefore, for nine of 20 inbred crosses we can reasonably claim that there is no outcrossing depression. Considering the small sample sizes used for estimating b, it is difficult to judge the presence of outcrossing depression for the rest.

Overall means and variances (in parentheses) of E_{min} and E_{max} (*Table 2*) for the population are [3.58 (37.73), 6.5 (123.16)], while the variance-mean ratios are (10.54, 18.95). These ratios are much greater than theoretical expectations. Bishir and Namkoong (1987) showed that the variance of lethal equivalents is expected to be less than or equal to the mean. The ratios between variance and mean in other species are: 0.78 in *Pinus taeda* (Franklin, 1972), 0.5 in *Picea glauca* (Fowler and Park, 1983), 2.81 in *Pseudotsuga menziesii* (Sorensen, 1969), 3.03 in *Pinus virginia* (Bishir and Namkoong, 1987) and 1.45 in *Pinus taeda* (Bishir ad Namkoong, 1987), Bishir and Namkoong (1987) concluded that their ratios were relatively large in their reports because the "observations were drawn from a seed orchard which was probably established over a period of few years from heterogeneous pollen and seed sources." Sorensen (1969) used pollen parents which were located at least 1/4 mile away from the test stand, and this might explain the large ratio in *Pseudotsuga menziesii*. The unusually large ratios in our experiment are partly due to the use of materials with different origins. More importantly, however, the five crossings with unrealistic (E) caused the large ratios. When we take out the five crossings, the means and variances are [1.99 (5.62), 3.63 (18.68)] with ratios of (2.82, 5.15). Further culling from below does not change the ratio as greatly. For example, when bottom one-third — i. e., individual crossings with survival frequencies less than 0.5 — are culled from both inbreds and outcrosses (20 from inbreds and 15 from outcrosses), the means and variances are [1.38 (2.33), 2.66 (8.59)] with ratios of (1.69, 3.23). These reductions are significant, but not as great as those from culling five inbred crossings from the original data.

Considering the pattern of reduction in the variance, we may exclude the five extreme inbred crossings and use (1.99, 3.63) as the population mean $(\bar{E}_p)$ of the lethal equivalents. These numbers may still be overestimates. because 18 inbreds did not have corresponding outcrosses and the environmental causes of mortality were assumed to be absent. The small number of outcrosses used to estimate b influences the estimates of $(\bar{E})$, but its impact on $(\bar{E}_p)$ is likely to be less critical. It is also possible that different origins could bias the estimate of $(\bar{E}_p)$ if the samples of outcrosses are more concentrated in parents of extreme values of (E). The number of times different families are represented in outcrosses varies between four and 15 (*Table 1*, column 5). Family 20, which has the highest $(\bar{E}_f)$ = (5.1, 9.25) was represented four times, and family 10, with the next highest mean lethal equivalents, $(\bar{E}_f)$ = (4, 7.3), was involved in outcrossing 13 times. If we combine these two families, the average would be 8.5. Other families were involved in outcrossing somewhere between six to ten times. We may then conclude that the outcrossings were fairly well distributed among families, and assume that the source heterogeneity has relatively small impact on the estimation of $(\bar{E}_p)$. Therefore, we will use (1.99, 3.63) as the upper estimate of the population mean lethal equivalents.

3) Estimating population lethal equivalents (2ε)

If we assume the experimental population was homogeneous, then we may use the overall group survival fractions (*Table 1*) to estimate the population lethal equivalents as $2\varepsilon = -8\log(63.4/66.4) = 0.37$ (from Ep. 5). When five extreme families with survival fractions less than 0.12 are taken out, $2\varepsilon = 0.08$. Because this experimental population is heterogeneous, this estimate is wrong and we cannot have overall population lethal equivalents (2ε). A reasonable alternative is to use $(\bar{E}_p)$ = (1.99, 3.63) as the population lethal equivalents.

It was stated in the previous section that the heterogeneity of the population is unlikely to have greatly influenced the estimate of $(\bar{E}_p)$. However, no systematic measures of eliminating the heterogeneity effects of the population were used in the estimation process. It is possible to remove source effects in pair-wise combinations of different parental families by estimating 2ε of the families. This is done by taking the geometric mean of the average survival fractions of inbred groups of two parental families, and then comparing this geometric mean with the corresponding average survival fraction of outcross. By taking the overall mean of these 2εs we can obtain $2\varepsilon_p$, and use this as an alternative measure of population lethal equivalents.

To make the results comparable to the estimate of $(\bar{E}_p)$, five inbreds and two outcrosses with survival fractions less than 0.12 were deleted from the data in estimating 2ε. 32 outcross groups (40 individual outcrosses) were compared with their corresponding geometric means of inbreds (*Table 3*). In most cases these groups have only one outcross available. Estimates of 2ε appear to be smaller and more homogeneous than those of E_{min} in *table 2*. Population mean, variance, and variance-mean ratio were also reduced; $2\varepsilon_p = 1.69$, $s^2(2\varepsilon) = 2.45$, and ratio = 1.45. Previously shown minimum estimates are: $\bar{E}_p(min) = 1.99$, $s^2(E_{min}) = 5.62$, and ratio = 2.82. The reduction in the mean appears to be less than that in the variance. It was shown in *figure 1* that 2ε is slightly smaller than $\bar{E}$ for the same probability of mortality. Therefore, it is difficult to say that the use of geometric mean reduced the estimates of lethal equivalents significantly. The reduction in variance, however, appears to have been primarily influenced by eliminating source effects by using the geometric means. A similar trend is observed in *table 4*, whose entries represent family statistics summarized from *tables* 2 (minimum only) and 3. For all the categories — i. e., number of individual estimates representing a family mean, family variance, and the variance-mean ratio — the elimination of source effects shows more even distribution among families than the use of individual data after removing environmental effects.

Table 3. — Estimates of family mean lethal equivalents $(2\bar{\varepsilon})$.

Family identification		Full sib group Average	Survival fraction	Geometric Mean	# of X-ings	Out-X value	R	$2\bar{\varepsilon}$
(1)	(2)	(1)	(2)					
1	7	0.8	0.84	0.82	1	0.93	0.88	1
	10	0.8	0.43	0.58	1	0.66	0.88	0.99
	19	0.8	0.59	0.68	1	0.84	0.81	1.65
	30	0.8	0.66	0.72	1	0.95	0.76	2.19
	104	0.8	0.69	0.74	1	1	0.74	2.38
7	10	0.84	0.43	0.6	1	1	0.6	4.1
	19	0.84	0.59	0.7	2	0.35	2.01	0
	26	0.84	0.69	0.76	1	0.63	1.22	0
	27	0.84	0.78	0.81	1	1	0.81	1.68
	30	0.84	0.66	0.74	1	0.78	0.95	0.42
	38	0.84	0.4	0.58	1	0.89	0.65	3.43
	104	0.84	0.69	0.76	1	0.96	0.79	1.84
10	19	0.43	0.59	0.5	1	0.78	0.65	3.48
	20	0.43	0.55	0.49	1	0.65	0.75	2.34
	26	0.43	0.69	0.54	1	0.9	0.6	4.04
	27	0.43	0.78	0.58	1	0.99	0.59	4.27
	30	0.43	0.66	0.53	2	0.62	0.86	1.21
	38	0.43	0.4	0.41	5	0.43	0.96	0.33
	104	0.43	0.69	0.54	1	0.93	0.59	4.28
19	20	0.59	0.55	0.57	1	0.83	0.69	2.98
	26	0.59	0.69	0.64	1	0.35	1.82	0
	27	0.59	0.78	0.68	1	0.48	1.43	0
	30	0.59	0.66	0.62	1	0.74	0.84	1.42
	104	0.59	0.69	0.64	2	0.65	0.99	0.09
20	26	0.55	0.83	0.68	1	0.53	1.29	0
	27	0.55	0.66	0.6	1	0.2	3	0
26	27	0.58	0.78	0.67	1	0.99	0.68	3.08
	30	0.58	0.66	0.62	1	0.4	1.54	0
27	38	0.78	0.4	0.56	1	0.76	0.73	2.47
	104	0.78	0.69	0.74	1	0.61	1.2	0
30	104	0.66	0.69	0.67	1	0.6	1.12	0
38	104	0.4	0.69	0.53	1	0.9	0.59	4.27

(1) Grandparent crossing from which mother is chosen.
(2) Grandparent crossing from which father is chosen.

Table 4. — Family means and variances of two different lethal equivalents $2\bar{\varepsilon}_f$ and $\bar{E}_f$ (min).

Family	Number of individual estimates		Mean		Var		Var/Mean	
	T3	T2	T3	T2	T3	T2	T3	T2
1	5	2	1.64	1.5	0.42	4.5	0.26	3
7	8	11	1.56	1.23	2.36	5.22	1.51	4.24
10	9	6	2.78	4	2.52	5.3	0.9	1.33
19	8	7	1.2	1.7	2.03	11.57	1.68	6.81
20	4	1	1.33	5.1	2.43	-	1.83	-
26	6	4	1.19	1.2	3.47	3.63	2.92	3.03
27	6	9	1.92	1.46	2.92	5.26	1.53	3.6
30	7	7	0.75	3.04	0.76	3.74	1.01	1.23
38	4	4	2.63	2.48	2.88	5.2	1.1	2.1
104	7	5	1.84	0.92	3.67	1.12	2	1.22

T2: Data obtained from *table 2* $[\bar{E}_f(min)]$
T3: Data obtained from *table 3* $(2\bar{\varepsilon}_f)$.

Although elimination of source effects seems to have significantly reduced population variance, the mean has remained about the same, and we may still use $(\bar{E}_p) = (1.99, 3.63)$ as representing the upper estimate of population lethal equivalents (2ε). We may also choose the median of (E_p) (= 2.8) as a single number to represent the lethal equivalents of our experimental population of *Salix viminalis*.

Concluding Remarks

The samples included in this experimental population of *Salix viminalis* are drawn from the founder breeding population in Sweden. Therefore, the estimate of lethal equivalents in this experiment may be considered as representing that of the breeding founder population. Because of irregularities in flowering and other biological constraints, the experiment was not well balanced. However, overestimation was opted for whenever possible. The resulting upper estimate of population lethal equivalents (1.99, 3.63) is substantially smaller than that observed in most conifers with 8+ lethal equivalents. The lethal equivalents of this experimental population are comparable to those of *Abies procera* (1.8 to 3.4, SORENSEN et al., 1976) and human (3 to 5, MORTON et al., 1956).

The fact that most conifers can carry high lethal equivalents is often explained by the high degree of pollen dispersal and large quantity of seeds they produce. Although most conifers are capable of selfing, they generally produce sufficient quantities of pollen for outcrossing (SARVAS, 1962). Furthermore, the production of large quantities of seed allows the possibility of having many sound seeds after losing selfed seeds. *Salix viminalis* differs from conifers in that it is insect-pollinated and naturally occurs in small clumps. Although it can be clonally propagated, it does not vegetatively propagate by means of root suckers. Therefore, individuals in naturally occuring clumps could have different genotypes. Selfing is not possible due to different sexes, but it is conceivable that inbreeding without concurrent outcrossing is common in *Salix viminalis* and is likely to carry relatively small lethal equivalents.

With the possible exception of families 10 and 38, most families are likely to have lethal equivalents less than 2 (*Table* 4, Column 4). These numbers may increase when the test period is extended to maturity. However, we consider the above numbers to be fairly small, and conclude that the inbreeding program as outlined in ERIKSSON et al. (1984) has a good chance of progressing without losing lines. Early purging of lethal alleles may not be necessary in *Salix viminalis*. It would be, however, desirable to monitor the changes in lethal equivalents as the generations progress.

Acknowledgements

The technical assistance of URBAN PETTERSSON, HARTMUT WEICHELT, and EWA WINKLER is greatly appreciated. We also thank Drs. GÖSTA ERIKSSON, DAG LINDGREN, and FRANK SORENSEN for critically reviewing the manuscript. This study was partly funded by the Swedish Energy Board and by Swedish Council for Forestry and Agricultural Research.

Literature Cited

ALLARD, R. W.: Principles of plant breeding. John Wiley and Sons, New York. 485p. (1960). — BISHIR, J. and NAMKOONG, G.: Unsound seeds in conifers: Estimation of numbers of lethal alleles and of magnitudes of effects associated with the maternal parent. Silvae Genetica 36: 180—185 (1987). — BISHIR, J. and PEPPER, W. D.: Estimation of number of embryonic lethal alleles in conifers: 1. Self-pollinated seed. Silvae Genetica 26: 50—54 (1977). — BRAMLETT, E. L. and PEPPER, W. D.: Seed yield from diallel cross in Virginia pine. In: Seed yield from southern pine seed orchards (Colloquium Proceedings). J. KRAUS (Ed.) (1974). — CAVALLI-SFORZA, L. L. and BODMER, W. F.: The genetics of human populations. W. H. Freeman and Company, San Francisco. 965p. (1971). — CHARLESWORTH, D.: Evolution of low female fertility in plants: Pollen limitation, resource allocation and genetic load. Tree 4: 289-292 (1990). — COCKERHAM, C. C.: Avoidance and rate of inbreeding. In: KOJIMA, K. (Ed.). Mathematical topics in population genetics. Springer-Verlag, New York. pp. 104—127 (1970). — ERIKSSON, G., GULLBERG, U. and KANG, H.: Breeding strategy for short rotation woody species. In: PERTTU, K. (Ed.). Ecology and management of forest biomass production systems. Dept. Ecol. and Environ. Res., Swed. Univ. Agric. Sci. Rep. 15: 199—216 (1984). — FALCONER, D. S.: Introduction to quantitative genetics. Longman, London and New York. 340p. 1981). — FOWLER, D. P. and PARK, Y. S.: Population studies of white spruce. I. Effects of self-pollination. Can. J. For. Res. 13: 1133—1138 (1983). — FRANKLIN, E. C.: Genetic load in loblolly pine. Amer. Nat. 106: 262—265 (1972). — HEDRICK, P. W.: Genetic load and the mating system in homosporous ferns. Evolution 4: 1282—1289 (1987). — KANG, H. and NIENSTAEDT, H.: Managing long-term tree breeding stock. Silvae Genetica 36: 30—38 (1987). — KIMURA, M. and CROW, J. F.: On the maximum avoidance of inbreeding. Genet. Res. Camb. 4: 399—415 (1963). — KOSKI, V.: Embryonic lethals of *Picea abies* and *Pinus sylvestris*. Comm. Inst. For. Fenn. 75: 1—30 (1971). — LANDE, W. J. and SCHEMSKE, D. W.: The evolution of self-fertilization and inbreeding depression in plants. 1. Genetic models. Evolution 39: 24—40 (1985). — LOWE, W. J. and BUIJTENEN, J. P. VAN: Tree improvement philosophy and strategy for the Western Gulf forest tree improvement program. In GURIES, R. and KANG, H. (Eds.): Research needs in tree breeding. Proc. 15th North American quantitative forest genetics group workshop, Coeur d'Alene, Idaho, August 6 to 8, 1981. p. 43—50 (1981). — MITCHELL-OLDS, T. and GURIES R.: Genetic load and heterozygosity in the *Pinaceae*. Can. J. Genet. Cytol. 28: 942—946 (1986). — MORTON, N. E., CROW, J. F., and MÜLLER, H. J.: An estimate of the mutational damage in man from data on consanguineous marriages. Proc. Nat. Acad. Sci. 42: 855—863 (1956). — NAMKOONG, G.: Breeding for future generations. Proceedings of the IUFRO joint meeting of working parties on population and ecological genetics, breeding theory and progeny testing. Pp 29—39 (1974). — NAMKOONG, G., BARNES, R. D. and BURLEY, J.: A philosophy of breeding strategy for tropical forest trees. Unit of Tropical Silviculture, Commonwealth Forestry Institute, University of Oxford. Tropical Forestry papers No. 16. 67p. (1980). — NAMKOONG, G. and BISHIR, J.: The frequency of lethal alleles in forest tree populations. Evolution 41: 1123—1127 (1987). — SARVAS, R.: Investigations of the flowering and seed crop of *Pinus sylvestris* L. Commun. Inst. For. Fenn. 53 (4): 1—198 (1962). — SIMAK, M.: Germination and storage of *Salix caprea* L. and *Populus tremula* L. seeds IUFRO Working party: S2.01.06. International Symposium on forest tree seed storage Petawawa National Forestry Institute, Canada. September 23 to 27, 1980. (1982). — SORENSEN, F.: Embryonic genetic load in coastal Douglas-fir *Pseudotsuga menziesii* var. *menziesii*. Amer. Natur. 103: 389—398 (1969). — SORENSEN, F. C., FRANKLIN, J. F. and WOOLLARD, R.: Self-pollination effects on seed and seedling traits in nobel fir. Forest Science 22: 155—159 (1976).

Peatland and Upland Black Spruce Populations in Alberta, Canada: Isozyme Variation and Seed Germination Ecology

By Z. M. WANG and S. ELLEN MACDONALD[1])

(Received 25th September 1991)

Summary

Seeds from three pairs of peatland and upland black spruce (*Picea mariana* (MILL.) B. S. P.) populations in Alberta were used to study variation in isozymes and seed germination. In the isozyme study, 16 enzyme systems coded by 28 putative genetic loci were investigated. In the germination study, seeds were germinated at 15° C, 25° C, or 35° C and 0, —5, —10 or —15 bars. The data were analyzed by ANOVA and SNK-tests. It was found that isozyme variability was similar among the populations, sites and between habitats. Results of F_{st}-values, X^2-tests, and genetic identities, indicated there is little genetic differentiation between upland and peatland habitats. In the germination study, seeds from upland and peatland habitats did not show expected adaptive responses to temperature, moisture and their interaction, but differentiation among the 6 populations was significant. Overall, seeds germinated best at 25° C, and seeds from peatland populations germinated more slowly and poorly at low temperature than did upland populations. Differences in germination among populations were most likely due to maternal effects and/or locally site-specific selection. Results from both studies indicate that there is little ecotypic differentiation between upland and peatland black spruce populations in Alberta.

Key words: *Picea mariana* (MILL.) B. S. P., isozyme electrophoresis, discriminant analysis, genetic variation, temperature, water potential.

Introduction

Peatland and upland habitats are very different in terms of nutrient availability, and soil structure, temperature, moisture, pH, and aeration (BRADBURY and GRACE, 1983),

[1]) Department of Forest Science, University of Alberta, Edmonton, Alberta, Canada T6G 2H1