Protocol for rating seed orchard seedlots in British Columbia: quantifying genetic gain and diversity

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Summary

Seed orchards are the principal delivery method for genetically selected materials to operational reforestation programmes in British Columbia, Canada. Orchard parents are assigned breeding values for the improved trait (e.g. stem volume growth). The genetic quality of orchard seedlots is rated annually by weighting the breeding value of each orchard parent by its proportional gamete contribution (male and female) to the seedlot. Parental gamete contributions are also used to calculate an effective population size to quantify genetic diversity in a seedlot. Adaptation and genetic representation of populations are carefully considered at orchard establishment to ensure minimum seedlot standards are met. The calculation of genetic worth also considers the gamete contribution of non-orchard pollen parents, and is lowered for the negative effects of contaminating pollen and raised for the beneficial effects of supplemental pollen. The protocols for collecting information required to rate seedlots vary by species and location, but the calculations for estimating genetic worth, pollen contamination and genetic diversity are the same. Both the protocols and the formulae used to calculate the genetic quality of orchard seed lots are discussed.

Introduction

British Columbia (BC), Canada, currently supplies about 40 per cent of its annual seed needs from seed orchards, with an average genetic gain of about 10 per cent (FGC, 2002). Established goals are for these values to rise to about 75 per cent and 12 per cent, respectively, by the year 2007, averaged over 12 species and 36 seed planning units. For these species, seed orchards are very cost-effective for delivery of improved traits (genetic gain), such as wood volume and insect or disease resistance. However, the economics of certain species in various seed production units do not warrant a tree improvement programme due to low planting demand, low site indices, or low wood value.

Three important benefits are realized from

orchard seed produced from parents selected for increased growth. First and foremost, is increased stand volume production. Figure 1 shows the difference in volume growth for an orchard seedlot with 20 per cent gain compared with a natural-stand seedlot with no gain. This genetic gain is based on breeding values forecast to an index (rotation) age by including the correlation in growth between the age of selection and the index age (Xie and Yanchuk, 2003). Secondly, plantations reach the free-to-grow stage earlier, often reducing brushing and weeding costs. Figure 2 shows the initial superior growth of orchard seed whereby plantations reach the free-to-grow stage earlier. A plantation reaches its free-to-grow stage when crown height is above and free of competing vegetation. On publicly owned forest land in BC, this has important
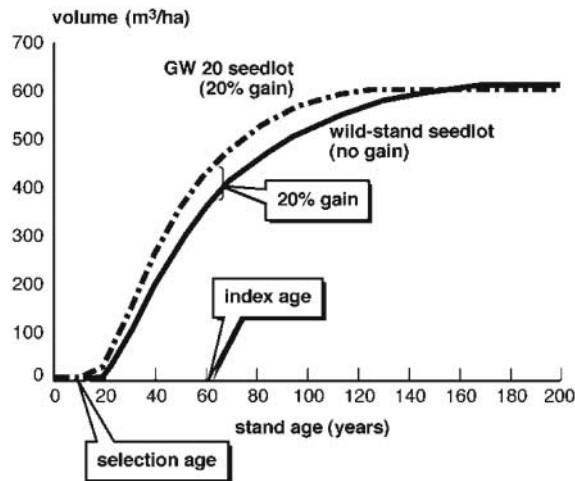

Figure 1. A schematic representation of stand volume production over time for an orchard seedlot of GW = 20 and a natural stand (not selected) seedlot (from Tanz, 2001).
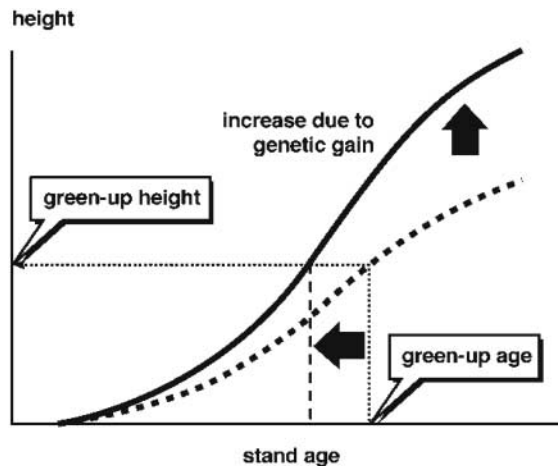

Figure 2. A schematic representation of early height growth for orchard and wild seed, showing earlier achievement of the green-up stage (from Tanz, 2001).

implications, as adjacent timber stands cannot be accessed until the plantation has reached the free-to-grow stage. Lastly, the added growth realized by using seed orchard seed may be incorporated in timber supply analyses, with a resulting increase in timber flow over time. In some situations, this increase in timber flow may impact harvest expectations within as little as two decades. Therefore, it is important to have reliable information about the genetic quality (expected gain) of seed orchard seed used for reforestation. For this purpose we developed a seedlot rating protocol (Woods *et al.*, 1996) to be used for all seedlots that are registered for reforestation on public lands.

Delivering genetic gain

Seed orchards will be our most economical tool for delivery of genetic gain in the foreseeable future, despite a potential increase in vegetative propagation for selected material with special traits (i.e. insect resistance). Orchard mating dynamics, the effects of domestication, and seed orchard management procedures can all affect the genetic potential of a seedlot (El-Kassaby, 1995). Gains may be reduced due to selfing and pollen contamination. However, orchard management techniques have developed substantially over the past few years (Webber, 1995; Webber and Painter, 1995), with the objectives of balancing parental contribution and enhancing gain. Because of orchard dynamics and the cost associated with protecting and improving genetic gain, we must have a reliable quantifiable tool for estimating the genetic worth of orchard seedlots.

Seedlot rating

In BC, orchard seedlots must be registered to ensure their genetic quality and diversity before seedlings may be planted on public land. Woods *et al.* (1996) described such an evaluation protocol for BC, which now forms the basis for annual seedlot rating. To register a seedlot, a seed orchard manager must evaluate two components:

1 Genetic worth: the level of gain for commercial trait(s)

2 Genetic diversity: distribution of parental gametic contributions

Genetic worth is an estimate of a seedlot's average level of improvement for the commercial trait of interest at the index age. Breeding values are weighted by clonal contribution to a seedlot, and lowered for the amount and genetic quality of non-orchard pollen contribution (contamination) and raised for the genetic quality and efficacy of added (supplemental) pollen. Genetic diversity is assessed using the concept of effective population size, or effective number of clones in a seed orchard.

Adaptability, the fitness of orchard progeny in the target seed zone of the orchard, is also important. This is considered at the time of orchard establishment, when choosing appropriate orchard parents (clones) based on the performance of their offspring in progeny tests. Based on these progeny tests, each orchard is assigned a seed planning unit, which is a geographic area within a specific elevational band.

Data collection

Male and female gamete contributions for each parent clone in an orchard must be determined prior to the registration of seedlots. Pollen contribution can only be estimated under some assumptions. Our assumptions are that male contribution is relative to the volume of pollen produced in the orchard cloud, and is adjusted for other pollen sources (i.e. contamination and supplemental pollen). We further assume that all pollen parents are equally available to all receptive females. This assumption, of course, is seldom realized, but when the number of parents is high (>40) we believe that seedlots will closely represent the average genetic value of the orchard. However, for high gain orchards with fewer parents (<30), the phenology of pollen shed and female receptivity may play a more important role.

Generally, seed orchard managers decide how male gamete contribution is best estimated. The following methods are acceptable. (1) Survey each ramet in the orchard to estimate the number of pollen bud clusters for each tree and assign a crop intensity rating for each tree. This categorical rating (e.g. very heavy, heavy, medium, light, very light) is then converted into a quantitative

measure depending on the size (age) of the tree. (2) Estimate the number of pollen-bud clusters on a subset of ramets for each clone and multiply that number by the number of ramets per clone. Both approaches have worked well for most BC orchards.

Female gamete contributions from parent clones are also estimated as a proportion of all contributing parents. This is done by either measuring the volume of cones harvested per clone, or the weight of cones produced per clone. Kjær and Wellendorf (1997) showed that the phenotypic correlation between cone volume harvested per clone and number of viable seeds per clone produced was high ($r = 0.91$). Thus, while the number of (filled) seed collected from each ramet of each contributing clone would seem to be a more precise and direct estimator of female gamete contribution, the added cost and effort required to collect these data are not warranted.

Estimating the amount of pollen contamination is difficult and can only be approximated. Both the timing of contamination and its potential impact are estimated using pollen monitoring. There are many procedures for monitoring pollen cloud density but we chose 7-day recorders because of their ease of operation over an extended period (see Webber and Painter, 1995). Pollen monitors are established both external to the orchard (regional monitoring) and within the orchards (orchard monitoring). The level of contamination for any particular clone is then estimated as the ratio of external counts to orchard counts summed over the receptivity period of the orchard clones, and adjusted for monitor efficiency based on pre-orchard pollen flight capture levels. Pollen charts are counted for a 24-h period and then expressed as pollen capture mm^{-2} 24 h^{-1}. Details of the sampling strategy are very important, thus the number of monitors to be used and how to locate them must be carefully determined for each orchard site. Furthermore, external orchard monitoring sites must be sufficiently removed from the orchard as to not be significantly affected by the orchard pollen cloud. Otherwise, estimates for contamination will be inflated.

The period of clonal receptivity can be determined on either all individuals (ramets) or a subsample. Receptivity period for all orchard species is defined as starting on the date when at least 20 per cent of the flowers are receptive, and ending when 80 per cent of the cones are past receptivity. Supplemental mass pollination (SMP) can also be factored into the calculation of an orchard's genetic worth. However, its actual efficacy is difficult to quantify. To accurately determine SMP efficacy requires paternity analysis using either isozyme or DNA fingerprinting methods (Wheeler and Jech, 1992; Stoehr et al., 1998). Neither technique lends itself to routine analyses so BC has chosen to use a flat rate of 25 per cent for supplemental pollination efficacy under all orchard conditions. This rate was based on two field trials in a mature Douglas-fir orchard (see El-Kassaby et al., 1993; Webber, 1995), and is only applicable if strict pollen management procedures (collection, storing and testing) (Webber, 1995) are used. SMP efficacy varies under different orchard pollen cloud conditions. In mature orchards where pollen clouds are seldom limiting, the 25 per cent rate is reasonable; however, in young orchards where pollen clouds can vary by both year and phenology, 25 per cent is likely underestimating SMP efficacy.

Genetic worth calculations

Calculating the genetic worth of seedlots with no supplemental pollen added is as follows:

$$GW = \Sigma\{(PC)(BVC) + BV_i[(1 - PC) M_i + F_i]\}/2 \tag{1}$$

where GW is seedlot genetic worth; PC is proportion of contaminant pollen; BVC is breeding value of contaminant pollen; BV_i is breeding value for clone i; M_i and F_i are male and female proportional gamete contributions, respectively, of clone i.

In the absence of pollen contamination, equation (1) simplifies to:

$$GW = \Sigma BV_i (M_i + F_i)/2 \tag{2}$$

When SMP is factored into the calculation of genetic worth, the following is used:

$$GW = \Sigma\{[PSMP_i BVSMP + (1 - PSMP_i) \\ (PC BVC + (1 - PC) BV_i M_i)] + BV_i F_i\}/2 \tag{3}$$

where GW is seedlot genetic worth; $PSMP_i$ is proportion of SMP success in clone i; BVSMP is

mean BV for SMP mix; PC is proportion of contaminant pollen; BVC is breeding value of contaminant pollen; BV_i is breeding value for clone i; M_i and F_i are male and female proportional gamete contributions, respectively, of clone i.

When calculating the genetic worth of seed orchard seedlots, it is important to know the errors around all parameters of the model. Errors associated with calculating breeding values and estimating the gamete contribution (cones or seed and pollen) are two of the principal factors that can introduce errors in GW. M. Stoehr (unpublished) compared three combinations of calculating GW; weighted BV only, weighted cones per clone only (i.e. considering only female gamete contributions), and weighted cones and pollen per clone. Variation between the three methods did occur, but the differences were seldom large enough to warrant concern and no bias was introduced to estimates. Rounded BV values to the nearest 0.1, 3 and 5 were also used to calculate GW and again the model was relatively insensitive to the precision of the BV value used due to the relatively large number of orchard parents.

Genetic diversity

Genetic diversity is also considered at orchard establishment and is affected by the number of orchard parents, relatedness of orchard parents (if any), orchard design and orchard management. A requirement for an established minimum level of diversity is based on the effective population size (N_e) described by Robertson (1961) or the status number *sensu* Lindgren. Effective population size is also based on the proportional gamete contribution of each non-related orchard parent (both female and male):

$$N_e = 1/\Sigma(P_i^2) \qquad (4)$$

where $P_i = (F_i + M_i)/2$ and F_i and M_i are the proportional female and male gamete contributions of orchard parent i, respectively.

In orchards where there are relationships of various levels among seed orchard parents, the N_e calculations are modified according to Lindgren and Mullin (1998):

$$N_e = 0.5/\Sigma(P_iP_jc_{ij}) \qquad (5)$$

where P_i is the gametic contribution of parent i; P_j is the gametic contribution of parent j and c_{ij} is coefficient of relatedness (0.125 for half-sibs, 0.25 for full-sibs and parent–offspring relationships).

In BC, there are still a few untested, un-rogued, low-gain orchards for which there is no requirement to collect gamete contribution data. Thus, an N_e calculation as described above is not possible. In these cases, an effective number of clones is calculated according to Kang *et al.* (2001), where the P_i in equation (4) is the proportion of ramets of clone i in the orchard.

Pollen contamination increases N_e by a factor of $1/(1 - M)^2$, where $M = 1/2$ of the pollen contamination rate (Lindgren and Mullin, 1998). For example, if a seedlot has an $N_e = 20$ and there is 10 per cent pollen contamination, the N_e would be boosted to 22. However, no 'credit' is given to this increase in N_e as seed orchard managers are encouraged to prevent, or at least minimize, pollen contamination.

In BC, we are concerned that seedlots have an N_e greater than a minimum threshold, not with the absolute measure of N_e. This minimum threshold is set at $N_e = 10$, and all seedlots must exceed this threshold to be used for public land reforestation. An N_e of 10 captures 95 per cent of the population's genetic diversity (diversity = $1 - 1/2N_e$) (Nei, 1973). There is no danger of a further reduction of N_e in the next generation since plantations established from orchard seed will not be used for seed collections. Table 1 shows the average N_e of the seedlots of BC's commercial seed orchard species and their range over the last two years. The vast majority of seedlots have an N_e far exceeding 10.

There is also interest in how an orchard seedlot's level of genetic diversity compares with natural stands. Stoehr and El-Kassaby (1997) compared three levels of domestication (breeding zone with seed orchards, seed orchard parents with seed crop, and seedlot with plantation). Isozymes were used to calculate four measures of genetic diversity: total number of alleles per population, average number of alleles per locus, percentage of polymorphic loci, and observed and expected heterozygosity. While the seed orchard parents, seedlot and plantation had fewer alleles per population and alleles per locus, they showed higher values than the

Table 1: Average effective population sizes (N_e) for registered seedlots from British Columbia seed orchards harvested in 2001 and 2002

Species	Average N_e	Range of N_e	No. of seedlots registered
Thuja plicata	34.4	11.8–83.8	13
Pseudotsuga menziesii			
var. *glauca*	26.8	9.8–86.6	33
var. *menziesii*	24.3	21.5–27.0	2
Tsuga heterophylla	21.6	2.5–31.1	5
Larix occidentalis	35.9	20.3–50.7	4
Pinus contorta	32.3	14.6–58.0	37
Pinus monticola	53.0	18.9–110.6	7
Picea glauca × *engelmannii*	32.6	10.0–64.5	23

natural stand populations for percentage of polymorphic loci and both heterozygosity estimates. These data show that the genetic diversity of plantations is not lowered through seed orchard production.

Conclusions

The rating scheme described by Woods *et al.* (1996) provides a reliable, consistent procedure for assessing the genetic quality of orchard seedlots. In this paper, we included modifications to the effective population size calculations to reflect potential relatedness among orchard parents in advanced generation orchards. This effective population size estimate provides the seed user, and ultimately the marketplace, with assurance that genetic diversity is not lost through the genetic selection and domestication of forest trees. The rating protocol also provides a quantifiable tool for evaluating the economic return of orchard activities to both protect and improve the genetic value of seedlots. The anticipated genetic gains through the use of orchard seed, expressed as GW, are incorporated into many timber supply analysis models in support of determinations of harvest levels. Finally, the common protocol and language provided by rating seedlots for GW and N_e provide both seed producers and seed buyers with common terms and measures for producing, cataloguing and buying orchard seed.

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