



Guide to handling of tropical and subtropical forest seed

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GENETIC IMPLICATIONS OF SEED HANDLING

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Other Chapters of the book Guide to Handling of Tropical and Sub-Tropical Forest Seed by Lars Schmidt soon available on www.dfsc.dk

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GENETIC IMPLICATIONS OF SEED HANDLING

12.1 Introduction

Seeds carry in their genes the potential performance of the trees that will develop from them. A seed with a genotype for fast growth may produce a fast growing seedling if the environment is favourable. A seed with poor genotype will produce poor progeny no matter how the environment is. This is why collection from the right seed sources is important. In addition to genetic superiority of the seed source, it is important that seeds are collected from a sufficient number of seed trees in order to avoid inbreeding in future generations. Inbreeding depression is known from several species e.g. exotics where the first accession had a very narrow genetic base, often of just a few mother trees. Therefore, for most planting purposes it is advised to collect an approximately equal amount of seeds from not less than 25 distant (and hence alleged unrelated) seed trees (chapter 3.3). Each of the 25 trees should thus contribute approximately 4% of the seed in the lot. Where seed is collected for the establishment of conservation stands or other genetic purposes, it is important to consider the number of mother trees and the distance between them as well as equal representation of families in the seed lot (Kitzmilller 1990).

When seeds from individual families (mother trees) are bulked (mixed) in the field, control of family composition of the seed lot is lost. Subsequent handling may then change the proportion of various families in a seed lot and in some cases even eliminate families. For example, a family with relatively small seed may lose a relatively high proportion of its seed during processing or grading if small seeds are selectively eliminated. Such elimination or imbalance between parents may in practice be unimportant in seed lots with a large number of parent trees. However, where the number of families is small, e.g. smaller than 10, further narrowing of the genetic base during handling may have genetic implications.

Accidental elimination or change of the relative proportion between different families in a seed lot may occur at any step in the seed handling process. Since seeds differ in physical, physiological and morphological characters and many of these characters vary according to family, each step in the processing and handling procedure may influence seeds of individual families differently, e.g. eliminate a higher proportion of one family than another. Consequently, part

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of or whole families may be lost during processing and handling, and the ratio between families in the final bulked seed lot or plants in the nursery may be different from the ratio between families in the seed lot before processing (Lauridsen 1995).

This chapter will look into possible causes of deliberate or unintentional selective removal of seeds during various procedures of seed handling. Documentation of the genetic impact of seed handling is scarce and mostly from temperate species. Although the principles hold for tropical and subtropical species as well, more research is necessary in order to quantify the possible genetic impact.

Equal representation of all families in a seed lot is never possible in practice unless family identity is maintained by keeping the seeds separate throughout all procedures from collection to planting in the field. Whenever the seeds are bulked, the proportion of each family in the seed lot will be changed due to handling and processing procedures. Any change in the balance implies over-representation of some families and under-representation of others.

Unequal proportions of families in the seed lot or planting population may be due to unequal proportions in the original seed lot or to a change occurring during handling and nursery practice. Some situations are summarised below which will be further evaluated in section 12.2.1-5.

1. Seed collection. Number of viable seeds of different families at the time of collection may differ due to disproportionate number of seeds or fruits collected, different number of seeds in the fruits or different viability due to stage of maturity, insect predation, infection or number of filled seeds.
2. Processing. Processing such as threshing, depulping, purification or grading may eliminate viable seeds, either through mechanical damage or elimination of seeds with a particular size or morphology.
3. Storage. Variation in viability may eliminate seeds of low relative viability.
4. Pretreatment. Pretreatment may affect seeds differently, e.g. over- or under-treatment of seeds.
5. Germination. Germination rate may differ. This may be closely connected to storage and pretreatment.
6. Nursery practice. The development of seedlings may differ, for example in size and health. Often the smaller size seedlings are not planted.

The impact factors may interact in various ways and some factors are correlated. For example, a relatively thin seed-coat in legumes may enhance susceptibility to mechanical damage during processing, decrease storability by reduced resistance to pests, and make the seed more susceptible to damage during scarification. Factors may also be negatively correlated. For example, very thick-coated seeds may be better protected against damage during processing and storage

but suffer delayed germination due to impermeability and under-treatment during scarification and consequently be culled during seedling stage. Eventually, deleterious effects may accumulate during the handling process. Mechanical damage may increase susceptibility to pest and diseases, which causes reduced storability and leads to reduced vigour of the seedlings.

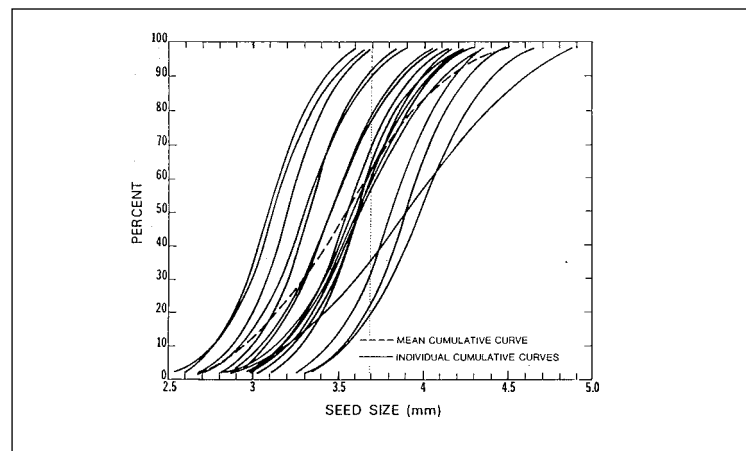
Most handling procedures are designed for the average seed or seedling with adjustment to take account of variation within the seed lot. The closer the seed or seedling is to average in any character influenced by handling, the smaller the likelihood that the seed may be unintentionally eliminated through processing and handling.

For most characters the elimination risk is one sided e.g. elimination of seeds under a certain size or viability, while only few are two sided, e.g. over- and under-treatment of dormant seeds during pretreatment. Some handling procedures have a general influence on seeds while others are specific for particular types.

Random elimination occurs if the probability of a seed being eliminated is independent of its physical or physiological features. For example, if storability of large and small seeds or of different families is the same, storage condition will not influence the genetic composition of the seed lot even if viability of the seed lot goes down.

Systematic elimination occurs if a procedure tends to eliminate a particular portion of the seeds. Since the genes of the mother plant determine physiological and especially physical characters of the seeds, there is often a correlation between handling elimination and specific families. For example, seed size often varies according to family and elimination of small seeds usually leads to unintentional elimination of a larger portion of some families than of others (see fig. 12.1). Hence, the extent to which systematic elimination of seeds and seedlings affects particular families is determined by the genetic influence on the character on which the process works. Only systematic elimination may change the balance between families in the seed lot.

Figure 12.1. Cumulative curves for seed size of 18 Douglas fir (*Pseudotsuga menziesii*) seed lots and mean cumulative curve for the same 18 seed lots. If e.g. seeds smaller than 3.7 mm diam. (vertical dotted line) are removed, the three families on the left are eliminated altogether, while families with larger seeds have various proportions of their seed removed, depending on size distribution. See discussion section 12.2.2 (from Silen and Osterhaus 1979).



12.2.1

Seed processing

The implication of handling upon genetic diversity of seeds and seedlings can be summarised in the following points.

1. Any procedure or condition that tends to eliminate viable seeds or seedlings at any stage implies the risk of changing the balance between families in the seed lot or seedling population.
2. If the distribution of seed or seedling characters of individual families differ from the whole lot, then any handling procedure that implies elimination of seeds or seedlings for that particular character will change the balance between genotypes.
3. The earlier in the process bulking takes place, the higher the risk of changing the balance during subsequent handling.
4. The smaller the numbers of families in the seed lot, the greater the genetic implications of seed handling.
5. The higher the number of seeds eliminated by any one procedure, the higher the risk that families will be lost.

In the following sections the individual handling procedures will be discussed in relation to their effect on directional selection.

Seed processing aims at balancing maximum effectivity in terms of number of seeds extracted, high purity etc. with a minimum of damage to the seeds. In practice, processing always implies a risk of damage or injury to some seeds (chapter 6.6). Some frequent types and causes of damage are the following:

1. **Mechanical damage.** Usually on the seed-coats but occasionally on the embryos with large cotyledons. Damage may occur during any mechanical handling such as extraction, cleaning and dewinging.
2. **Heat damage.** Usually occurring at high kiln temperature for extracting seeds from cones. Can also occur by fermentation of fruit pulp prior to washing.
3. **Chemical damage.** Sometimes occurring during purification by flotation in organic liquids. Other potential sources are fungicides or insecticides.
4. **Moisture damage.** For example by partial imbibition during washing/cleaning pulpy fruits or anoxia during soaking.

The degree of potential damage depends on handling procedure and seed type:

1. The more fragile the seed, the more sensitive it is to damage. Seeds with thin seed-coats or large cotyledons without or with little enclosing endosperm are easily damaged by some processing methods.
2. The more forcible the process, the higher the potential damage. E.g. threshing and beating indehiscent pods carries a risk of damage.

Mild damage e.g. on seed-coats may, however, have a beneficial impact on germination by scarifying the seed-coat. A study of processing effects on seed quality of various conifers was carried out by Allen (1958). In a series of experiments it was shown that procedures such as dewinging, kiln drying, tetramine treatment (a fungicide) and other factors had a detrimental effect on the overall seed quality.

Large variations were found between different seed lots in terms of injuries caused by dewinging which suggests genetic variation. Damage during dewinging may occur when the seeds are tumbled together with debris. Great damage was encountered in seeds of *Abies lasiocarpa* where up to 50% of the seeds was lost. Excessive dewinging caused dull, dusty seed-coats, susceptible to mould and producing weak seedlings (Edwards 1981).

Temperature damage of conifer seeds during extraction in the kiln has been widely reported. Edwards (1981) states that high kiln temperature especially in the early phases may damage seeds and that the effect is especially serious for immature seeds. Wang *et al.* (1992) found a substantial loss of viability of *Pinus contorta* var. *latifolia* seeds by exposing the cones to a scorching temperature of 220°C for 20 min followed by a 60°C kiln temperature for 20 min. Short scorching time (<1.5 min) had no damaging effect.

A few investigations suggest that seed damage may occur during chemical treatment. Fumigation with carbon disulfide or hydrocyanic acid for killing *Megastigmus* spp. in conifers affected viability (Sweeney *et al.* 1991). Some alcohols used for flotation (sorting empty from filled seeds) had negative effect on viability of *Pinus* spp. seeds; others caused no loss in viability (Hodgson 1977).

Several studies have been made on the effect of damage to seeds of crop species. On maize (*Zea mays*) varying degrees of damage on seed-coat, endosperm and embryo could be detected in 89% of the seeds after processing (Jahufer and Borovoi 1992). The injuries affected germination, seedling development, susceptibility to diseases, plant growth and development and grain yield.

12.2.2 Seed grading

Seed grading is a continuation of seed cleaning/purification in which the seeds are graded according to size or weight. The purpose of grading is to improve the physiological quality of the seed lot by eliminating small, empty and underdeveloped seeds (chapter 6). The discarded small seeds are believed to include, apart from empty seeds, seeds of low vigour e.g. developed after self-pollination. Sometimes a larger fraction of small yet viable seeds are deliberately eliminated from the seed lot based on the assumption that seed size and vigour are correlated. Grading can change the genetic constitution of the seed lot by eliminating part of or whole families with relatively small seeds. The degree to which that is the case depends on the systematic variation in seed size among families; this variation may be caused by heredity, or by environmental or developmental factors.

Variation and inheritance of seed size have been documented for a number of species, e.g. *Picea glauca* (Hellum 1976), *Acacia holosericea* (Hellum 1990), *Pseudotsuga menziesii* (Clair and Adams 1991), *Pinus sylvestris* (Lindgren 1982), *Santalum album* (Bagchi and Sharma 1989) and *Acacia* spp. (Bagchi *et al.* 1990).

Seed weight is mainly influenced by maternal factors and is under strong genetic control (Lindgren 1982, Tyson 1989). Several studies have been carried out to examine the correlation between seed size and seedling size/vigour (Chaisurisri *et al.* 1994, Dunlap and Barnett 1984, Fowells 1953, Griffin 1972, Sorensen and Campbell 1993). Although Chaisurisri *et al.* found no statistically significant correlation between two size classes (large and small) and 8 month old seedlings in *Picea sitchensis*, the general conclusion to be drawn from all the studies is that large seeds generally germinate faster and produce larger and more vigorous seedlings. The difference is usually pronounced during the early stages of seedling development but tends to become smaller and may disappear after one or more growth seasons. This is in harmony with the assumption that large seeds provide a physiological advantage primarily in terms of a large nutrient supply for the germinating seeds. Large seeds may secure rapid and vigorous germination and seedling growth, but are not necessarily correlated with the genetic growth potential of the embryo.

The genetic implication of seed grading according to size/weight has been demonstrated. Hellum (1976) found large variation in seed-weight distribution among 9 families of *Picea glauca*, and large variation in seed weight in two consecutive years. The relative distribution of seed weight tended to be the same in the two years. The general practice is to use only the heaviest seeds for container plants, and the author demonstrated that this practice would eliminate a large part of the genetic variation in the seed lot. Even more extreme results were obtained by Silen and Osterhaus (1979) for *Pseudotsuga menziesii* (fig. 12.1). They showed that elimination of the lightest one third of a seed lot would affect 16 out of 18 families to varying degrees. Six would lose more than 50% of their seeds. Among those six were two of the top five for two-year progeny height. Using only the heaviest one third of the seeds in their experiment would totally change the balance between families, moreover excluding much of the seeds of the top families in the progeny height test. Lindgren (1982) showed variation in seed weight between 34 clones of *Pinus sylvestris*. As for the other two referred studies there was almost no overlap in seed weight between the clone with the heaviest seed and the clone with the lightest seeds. Statistically significant differences were found between clones as well as between ramets of the same clone.

The conclusion from these studies is that grading with elimination of the lightest seeds will influence the genetic composition of the seed lot, both by changing the relative proportion of the families and, with a larger fraction discarded, by eliminating whole families. Hence, what may be won in physiological quality by getting more vigorous seedlings from the heavy seed fraction may be lost in genetic quality and diversity.

12.2.3 Seed storage

Seeds in storage are exposed to natural physiological deterioration or ageing, which will ultimately lead to loss of viability. In addition, stored seeds may be exposed to damage by e.g. insects and fungi. The speed by which seeds deteriorate depends on genotype (species, variety etc.) and the physiological condition (stage of maturity, moisture content etc.) of the seeds as well as the storage conditions (chapter 8).

Orthodox seeds have long physiological storage potential and proper storage during periods within normal forest seed operation, i.e. a few years, is unlikely to reduce viability of a seed lot significantly. Consequently short to medium term storage has no, or minimal, impact on genetic constitution of seed lots.

Genetic change during storage can occur in the following instances:

1. Long term storage of orthodox seeds in seed banks.
2. Short term storage of recalcitrant seeds.
3. Medium term storage of orthodox seeds under adverse storage conditions, e.g. high temperature and humidity and/or exposed to fungal infections.

Events and conditions prior to storage can have great influence on storability. For example, immature seeds, seeds damaged by pests and fungi and seeds with high moisture contents have reduced storability. Mechanical injuries to seeds during processing may indirectly affect storability by making them more susceptible to infection and loss of moisture during storage (cf. section 12.2.1). Non-lethal deterioration during storage may affect seed vigour (rate of germination and seedling growth), and thereby affect selection of seedlings in the nursery (cf. section 12.2.5).

Genotype has a very strong influence on storability. If overall viability of the seed lot decreases significantly during storage, e.g. to 50%, the genetic constitution of the seed lot is likely to be narrowed.

Barnett and McLemore (1970) found that storability of *Pinus palustris* seed varied between trees within individual seed crops but were unable to find statistically significant individual tree correlation between two seed crops. That indicates that other environmental factors, such as variation in maturity, may overshadow the genetic variation. Emmanuel and Dharmaswamy (1991) found a large variation in storability of *Tectona grandis* seed from different provenances but within-provenance variation (bulked seed) was not investigated.

Genetic implications of seed storage are discussed by Chaisurisri *et al.* (1993). They found significant clonal variation in germination capacity as well as in germination rate for six *Picea sitchensis* clones exposed to accelerated ageing at 37.5°C for 3-21 days (after 21 days viability was 0). They concluded that since 'different clones age/deteriorate at different rates during storage, long term storage of bulked seedlots might be reduced in genetic diversity'.

12.2.4 Pretreatment

Most pretreatment methods are closely connected to dormancy (chapter 9). Pretreatment includes e.g. scarification (physical dormancy), stratification (physiological dormancy) and leaching (inhibitory compounds).

Dormancy may cause elimination of seeds and consequently influence genetic constitution of seed lots if the degree of dormancy varies within the seed lot and a certain portion of the seeds fail to germinate due to inappropriate pretreatment. Germination failure may be due to over- or under-treatment of the seeds. Under-treatment means that dormancy has not been broken, over-treatment that seeds are damaged by the treatment. Over-treatment or under-treatment may cause complete failure of germination or it may cause delayed germination and reduced germination speed.

Variation in dormancy (partly in the seed-coat, partly in the embryo) was investigated for 20 families of *Pinus monticola* (Hoff 1987); 7% showed no dormancy at all, the remaining 93% showed various degrees of dormancy. A small portion of the dormancy could be ascribed to the hard outer layer of the seed-coat. The main factors determining dormancy were located in the inner layer of the seed-coat (a papery membrane) and in the gametophyte-embryo. Owing to variation in degree of dormancy and its location, different families responded differently to pretreatment. 21 days of pretreatment was sufficient to overcome dormancy caused by the hard outer seed-coat, 42-90 days of stratification was sufficient to overcome physiological dormancy in the embryo while the maximum of 105 days in the experiment was insufficient to fully overcome dormancy caused by the inner impermeable membrane.

Genetic variation in dormancy has also been documented for *Pseudotsuga menziesii* by Edwards and El-Kassaby (1995), who concluded that a stratification period of less than 5 weeks resulted in delayed germination and large variation in germination of different genotypes.

If a seed lot contains seeds with different degrees of dormancy (whether directly genetic or due to different degrees of maturity, processing influence etc. which in turn may be genetically influenced (see previous sections), then a pretreatment which fails to release seeds from dormancy (under-treatment) or causes damage to seeds (over-treatment) may influence the genetic constitution of the seed lot. The factors resulting in over-treatment of hard seed are discussed in chapter 9. Compared to scarification most other pretreatments are probably unlikely to cause major damage by over-treatment. Too long a stratification period may cause indirect damage to seeds by exposing them to infection during pretreatment. Seed may also be lost to germination after dormancy has been overcome if the conditions are conducive to germination. Long exposure to water, either during leaching to remove inhibitors, or during soaking to overcome hard seed dormancy, may harm the seeds, either by restricting respiration, by fungal infection or by imbibition. Genetic implications of over-treatment have not been investigated in any of the above situations.

12.2.5 Germination and seedling size

Any deleterious factor caused by events during seed handling will ultimately be manifested during seed germination and early seedling growth. In addition, germination and growth may be directly influenced by genotype. However, it is usually difficult to separate the direct genetic effects from those related to previous factors such as storability or dormancy.

In *Pseudotsuga menziesii*, El-Kassaby *et al.* (1992), found strong maternal control of germination speed. The maternal influence may be especially pronounced in gymnosperms in which the 'endosperm' (megagametophyte) is of entirely maternal origin.

In addition to the genetically determined speed of germination, genotypes may also vary genetically in terms of optimal germination requirements and tolerances. Under sub-optimal germination conditions such as moisture or temperature stress, germination and seedling behaviour are likely to be different from those under optimal conditions, and the variation is likely to be larger (Campbell and Sorensen 1994). Hence, the genetic composition of the culled nursery population is likely to vary according to environmental conditions.

Intentional grading of seedlings, equivalent to that of seeds, is a routine procedure in many nurseries. The selection usually favours large seedlings, discarding those under a certain size limit. For direct sowing of container plants, growers usually sow more than one seed per cavity, then remove the smaller germinants after germination (El-Kassaby *et al.* 1992). This practice implies that seed of genotypes with deep dormancy, slow germination rate and slow initial growth may unintentionally be removed. Among the many factors that can lead to directional selection, Campbell and Sorensen (1984) listed seed-size grading, stratification period, sowing date and culling as the most important ones.

Pre-germination deterioration and culling often interact. Low germination leads to more scattered seedling emergence, which on the other hand may mean that plants grow better because of less competition, and culling rate may be lower because all seedlings are needed. On the other hand, high germination rate often implies high seedling density and competition, which may ultimately result in higher number of suppressed seedlings. In addition, the higher number of seedlings may in itself tend to lead to a higher culling rate (Campbell and Sorensen 1984).

HANDLING PROCEDURE	METHOD	POSSIBLE INJURIES	FACTORS AFFECTING GENETIC CONSTITUTION OF THE SEED LOT
Collection	Hand or machine collection, from the trees or from the ground	Few; possible injuries on embryos by throwing fruits or sacks of fruits	Variation in maturity, number of filled seeds, number of seeds per fruit and infestation/predation rate Variation in susceptibility to injuries
Extraction	Drying/heating Threshing Depulping	High kiln temperature Mechanical injuries on seed-coat and embryo.	Variation in susceptibility to injuries
Cleaning/ Purification	Sifting Winnowing etc.	Few; possible injuries on seed-coat and embryo	Variation in susceptibility to injuries Removal of viable seeds with debris
Seed grading	Sorting seeds by size/weight	Few or none	Removal of specific fraction of the seeds e.g. small/light
Storage	Usually cool and dry, sometimes with insecticides or fungicides	Physiological deterioration/ ageing Infestation by insects or fungi	Variation in physiological storability Variation in susceptibility to infestation Variation in damage by pesticides
Pretreatment	Scarification of hard seeds/fruits Stratification Leaching	Damage of vital parts of the embryo	Variation in dormancy rate and susceptibility to pretreatment Under- and over-treatment of seed
Germination	Species specific	Suboptimal germination conditions	Variation in germination rate Variation in susceptibility to seedling pests and diseases
Grading of seedlings	Usually largest and healthiest seedlings selected	None	Selective removal of the smallest seedlings

Table 12.1.
Summary of seed handling impact on genetic constitution of seed lots.

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