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Research Note

Seed size and chilling affect germination of *Larix decidua Mill.* seeds

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Summary

We tested whether seed size and cold chilling impact on seed germination for seven populations of the conifer species *Larix decidua. For* all seven populations, seed germinability was positively related to seed mass: larger seeds germinated to a higher percentage. In addition, cold chilling also had a positive affect on germination for all seven populations. Our data suggest that the selection of the larger seeds in the population and cold chilling are likely to be of benefit for seedling production of this species.

Experimental and discussion

Larix decidua Mill. is the only deciduous conifer that occurs naturally in Europe. It is predominantly a species of alpine, mountainous regions and is often the woody species that reaches the highest altitudes. It is also highly adaptable in terms of soil and climatic conditions. In Italy it is present only in the Alps from the Veneto to the Piedmont region. *L. decidua* is an important species for watershed management and water quality, as well as having other values such as amenity and forestry (Pignatti, 1998).

Unlike many conifers e.g. *Pinus sylvestris,* cone and seed production takes place during the same year as flowering, with manual harvesting of cones occurring from October to February. However, seed production can be irregular, for example, as a result of climatic extremes; late frosts can "burn" spring flowerings and summer droughts can hinder cone maturation. In addition, embryo abortion can result in a high proportion of empty seeds (Tigabu and Oden, 2004; Slobodnik and Guttenberger, 2005).

Among European tree species, larch seeds are one of the most complex and difficult to process, as the cone is highly lignified and compact and the seeds within are typically very

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heterogeneous in size. Following harvest, the standard method in Italian forestry is to dry cones in kilns: first at room temperature then in an oven c. 48°C, somewhat higher than the temperature used for Norway spruce *(Picea abies)* or Scotch pine *(Pinus sylvestris)* (*c.* 42°C). The seed is then extracted from the cone using a cylinder (trommel), which rotates on a slightly inclined horizontal axis, in which there are hundreds of rotating blades. The cone scales are sheared off and the seeds dislodged from the cone.

During processing, the seedlot is sorted based on seed size resulting in six batches differing in seed size. Two of the batches contain almost no seed and are composed of ligneous material e.g. cone scales. These two batches are usually discarded and not considered valid for commercial purposes. After processing, the remaining four samples are mixed together and either put on the market or stored.

Within a species, seed size can impact on a range of seed quality traits, including germination and dormancy, seed longevity and the level of desiccation tolerance. For example, in the trees *Aesculus hippocastanum* and *Acer pseudoplatanus,* the mean population level seed mass is positively related to germinability and the level of desiccation tolerance (Daws *et al.,* 2004, 2006a). Similarly for herbs, even among seeds produced by the same parent plant, larger seeds often have higher levels of and faster germination than smaller seeds (Baloch *et al.,* 2001; Van Molken *et al.,* 2005).

Since for commercial production, *L. decidua* seeds are routinely sorted into four different sized categories we tested the impact of seed mass on seed germination. Furthermore, to assess whether such patterns apply widely within the species we assessed the impact of seed size on germination for seven distinct populations growing within the Italian range of the species (Daiano 46°18'N 1°W; Livigno 46°33N 2°20'W; Pragelato 45°N 5°30'W; Sozzine 46°15'N 1°55'W; Travignolo 46°19'N 0°45'W; Vallelunga 46°49'N 1°50'W; Veda 46°22'N 0°15'W). Seeds were collected in the 2004-05 season from a minimum of 50 trees per population. Each seedlot consisted of 4 of the 6 portions obtained through mechanical selection by the calibrating cylinder and portions 5 and 6, composed predominantly of dwarf shoots and scale bits, were eliminated.

1000-seed weight was determined, for each of the four seed size classes per provenance and on each whole commercial seed lot (i.e. portions 1 to 4) (ISTA, 2006). For germination, newly processed fresh seeds, were sown on filter paper at 30/20°C with light applied during the 8h elevated temperature period. In addition, the effect of chilling on germination was tested by sealing seeds at 7% moisture content within plastic bags followed by storage at 2°C for 100 days, before sowing as above.

Germination data was analysed using binary logistic regression implemented in Minitab 13 (Minitab Inc., Pennsylvania, USA) to test for an effect of seed source (seven categories, 1 to 7 based on population), chilling (two categories, 0 and 1) and seed mass (1000-seed weight in g). This approach assumes that each individual seed in the population is a statistically independent unit (since each individual seed can either germinate or not) and the goodness of fit of these models was assessed using Wald tests (Tabachnick and Fidell, 2001).

For the seven provenances of *L. decidua,* average 1000-seed weight of the four size sorted portions was c. 3.5 to 7.1 g (figure 1). These differences in mass significantly affected both pre- and post-chilling germination levels; larger seeds consistently germinated to higher levels than smaller seeds (figures 1 and 2). This effect was significant when both averaging 1000-seed weight for each portion across the seven populations (Two-way Anova, $F_{3,55}$ = 10.9, P < 0.001, figure 1) and when using the actual 1000-seed weight for each individual seed portion / population combination (Binary logistic regression, Wald test statistic = 12.9, P < 0.001, figure 2).

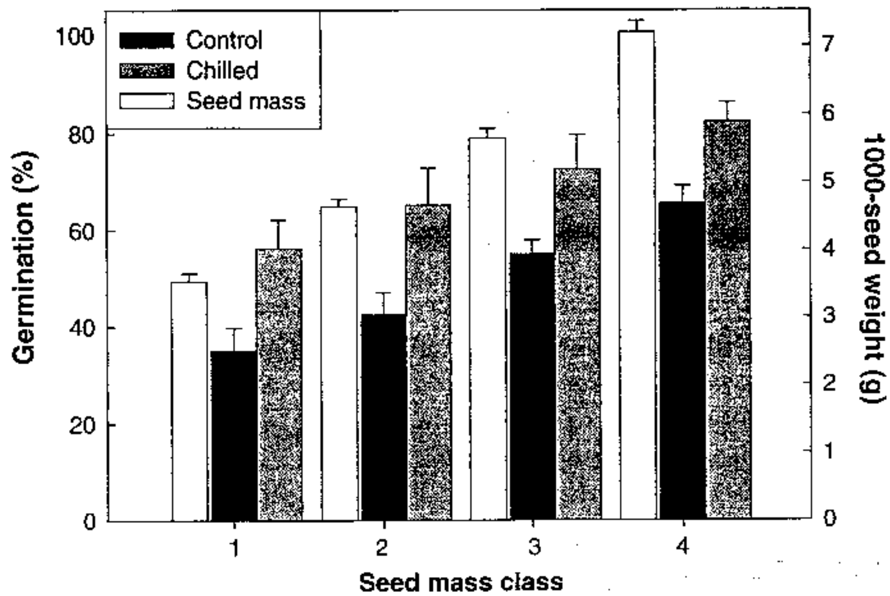

Figure 1. 1000-seed weight (open bars — right y-axis) and percentage germination (shaded bars — left y-axis), both pre- and post-storage, of seeds of *Larix decidua,* in each of the four seed weight classes used in the germination studies. The data presented (+1SE of the mean) represents mean values across the seven populations used.

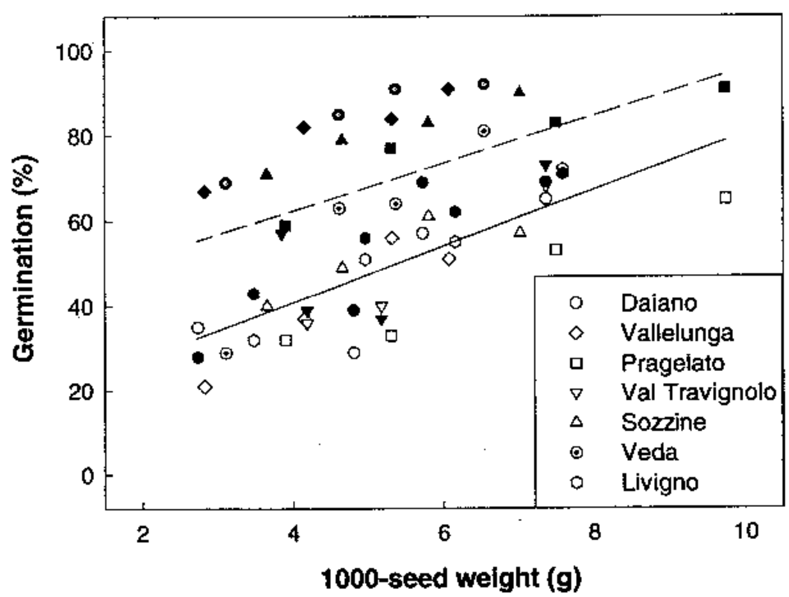

Figure 2. The relationship between 1000-seed weight and germination percentage both pre- (open symbols) and post- (closed symbols) storage for each of the four seed mass classes for each of the seven *Larix decidua* populations. Populations are denoted on the graph using different symbols (see legend). To facilitate interpretation, linear regressions lines fitted to the data for all seven populations (with and without chilling) are presented.

The effect of cold chilling on germination was highly significant with germination improving by c. 2.4 times following chilling (Binary logistic regression, Wald test statistic = 4.4, $P < 0.001$). In addition, all sizes of seed responded positively and in a similar way to chilling; the interaction between seed mass and chilling was not significant (Binary logistic regression, Wald test statistic = 0.1, $P > 0.05$). We also found that there was a highly significant effect of seedlot on the germination response for three populations (Veda, Sozzine and Vallelunga; Binary logistic regression, Wald test statistic > 6.82, $P < 0.001$). For these three populations, germination level was at least two times higher than in the other four populations (Odds-ratios of 2.1 to 3.2).

Irrespective of provenance, we found that larger seeds had a significantly higher germination level. Such an effect on seed mass has been observed previously in a wide range of species and may reflect either the fact that larger seeds are more mature at dispersal (Daws *et al.,* 2004; Wagner and Mitterhoffer, 1998) or that the proportion of empty seeds is lower in the larger seed size classes. A beneficial effect of seed size on germination has also been reported in the conifers *Larix kaempferi* and *Pinus strobilus* where larger seed size was related to faster emergence and greater initial seedling size (Logan and Polland, 1981; Parker *et al.,* 2006). Consequently, for *L. decidua* and potentially other tree species, the larger seeded portion(s) may be of higher value for seedling production. This proposition is also supported by the finding that seedling survival from larger seeds is also higher in many species; larger seeds have greater reserves which enables a greater tolerance to a range of hazards, including shade, drought and physical damage (Crawley and Nachapong, 1985; Armstrong and Westoby, 1993; Leishman and Westoby, 1994; Harms and Dalling, 1997; Leishman *et al.,* 2000). Nonetheless, one potential concern using size-sorted seeds is that genetic diversity may be reduced. This is of most concern if individual trees either produce large or small seeds, rather than the entire size range. However, at least for the tropical tree *Warburgia salutaris,* Daws *et al.* (2003) found that within a population, the major determinant of seed size was fruit size suggesting that this may not be a major concern.

Three of the populations of *L. decidua* germinated to a higher level than the others. Although this may be as a result of climate conditions during seed development (Daws *et al.,* 2004, 2006a) there are no obvious differences between the sites in terms of annual temperature and rainfall (data not shown). Nonetheless, annual means may not actually capture potentially important micro-climatic differences between sites. In addition, since these differences may also be genetic it reinforces the need to collect seeds from multiple sites to ensure capture of as much genetic variation as possible. A positive effect of chilling on germination has also been observed in a number of temperate trees including both conifers and non-conifers *(e.g. Acer pseudoplatanus, Aesculus hippocastanum, Pinus coulteri, P. lambertiana* and *P. taeda;* Cooke *et al.,* 2002; Daws *et al.,* 2004, 2006ab) and can be interpreted as an adaptation to facilitate germination in spring (Steadman and Pritchard, 2004). Similarly in larch, seeds are dispersed from the parent tree on windy and sunny days during the winter and are buried in the snow. For such seeds, a cold moist chilling requirement reduces the likelihood of germination in winter which would almost certainly result in seedling mortality from frost, whilst facilitating rapid germination when snow melts in spring.

In conclusion we have found that seed size variation, cold chilling and population effects all have a significant impact on germination of seeds of *L. decidua.* In particular, the effects of seed size on germination suggest that the larger seed size samples may be of greater value in terms of seedling production and establishment although the potential risk of genetic selection needs to be addressed.

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