The influence of moisture content of forest tree pollen on its reponse to different viability tests

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Influence of the moisture content of forest tree pollen on its response to different viability tests

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Introduction

Pollen management has become an integral part of intensive tree improvement, especially for facilitating breeding programs and improving both orchard yields and genetic efficiency. To handle pollen successfully, particularly its storage, drying to moisture contents of less than 10% is required (Webber, 1987; and unpublished data). However, in certain angiosperms, dehydration can have a detrimental effect on pollen germination and vigor which is only restored by rehydration (Shivanna and Heslop-Harrison, 1981).

The cause of this reduction in germination and viability with dehydration is not fully understood but loss of membrane integrity may be involved (Shivanna and Heslop-Harrison, 1981). Certainly, it is a principal factor in the loss of vigor in corn (Kerhoas, *et al.*, 1987).

In this paper, data from previous experiments are summarized to show the factors affecting reactivation of pollen vigor, and data from recent tests, which demonstrate the effect of rehydration atmosphere (humidity, temperature and exposure time) on *in vitro* viability response, are shown.

Materials and Methods

Procedures for testing *in vitro* viability have been described by Charpentier and Bonnet-Masimbert (1983) and Webber (1987). The effect of 2 relative humidities (70 and 100% RH) and 3 temperatures (10, 20 and 30°C) on rehydration of Douglas fir *(Pseudotsuga menziesii)* pollen and their effects on germination and conductivity over 9 exposure times (0, 3, 6, 16, 24, 48, 72, 96 and 120 h) were tested. The 70% RH was generated by controlling wet-bulb/dry-bulb temperatures using a water bath *(T1)* and an incubator *(T2)*. The 100% RH was created by saturating air in a closed container maintained at the incubator temperature *T2*.

Germination of Douglas fir pollen was assessed for class 1 grains (defined as elongation more than twice the original diameter of the grain) after 48 h of incubation and expressed as a percentage of the total number of pollen grains observed. Conductivity was determined using 25 mg of pollen in 7.5 ml of ultra-pure H_2O and measured after 1 h at 25°C (cold leachate).

Results

Correlation analysis for Douglas fir pollen

Table I summarizes the correlation coefficients between 3 *in vitro* assays (respiration, conductivity and germination) and percent filled seeds per cone (% *Fspc*) for Douglas fir pollen. It is apparent that respiration is less sensitive to the moisture content or hydration state. However, conductivity and most certainly germination are very sensitive, showing significant improvement inc correlation coefficients with either increasing initial moisture content (MC) or hydration state.

Hydration effects on assay responses of Douglas fir pollen

Fig. 1 shows the average response of 3 Douglas fir pollen lots to conductivity and germination tests after hydration in 2 humidities at 10, 20 and 30°C. At 70% RH, average MC was about 11%, whereas, at 100% RH, MC increased to about 48%. Conductivity was affected by both humidity and temperature. At 70% RH, conductivity values increased (particularly at 30°C), suggesting that membrane reorganization was incomplete. However, at 100% RH, membranes appeared to stabilize early and conductivity values remained relatively stable, at least up to 72 h, after which deterioration was apparent (again, more prominent at 30°C).

Of particular interest was the differential response by pollen lots (data not shown). Our best lot, determined by assay response, remained stable and showed the least deterioration in conductivity over time when compared to our poorest lot. It is also interesting to note that conductivity values at 10°C were unaffected by exposure time and, in fact, at 100% RH actually improved (lower conductivity values indicate more stable membranes). Values for 20°C showed an intermediate response.

Germination results led to similar conclusions. In addition to an ageing effect, which was accelerated by 30°C and 100°C RH, there was also a shock effect which was prevalent in the early stages of hydration and most severe for 30°C. It was less severe for 10°C and not observed at all for 20°C. Again, the poorest lot seemed to be more sensitive than the best one.

Table I. Correlation coefficients between 3 *in vivo* viability assays and *in vivo* seed yields (% *Fspc*) for Douglas fir pollen comparing the effect of 4 moisture contents (MC) and 2 hydration (hyd) levels.

Assays	*Correlation coefficients*[a]					
	effect of % MC				*Prehydration*[b]	
	4%	*8%*	*12%*	*16%*	*no hyd*	*hyd*
Resp x % *Fspc*	0.393	0.547	0.758	0.565	0.911	0.865
% Cond x % *Fspc*	–0.137	–0.654	–0.848	–0.703	–0.595	–0.841
% Germ x % *Fspc*	0.087	0.328	0.843	0.768	0.284	0.766

[a] Data from unpublished results.
[b] Hydration of pollen at 100% RH for 16 h at 25°C.

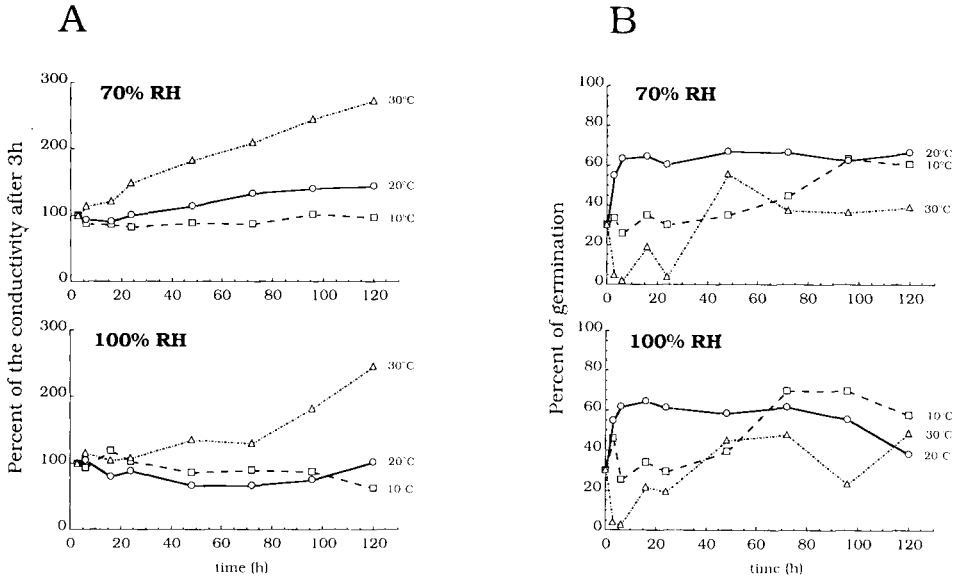

Fig. 1. Effect of air humidity, temperature and hydration duration on (**A**) conductivity (expressed as the % of the measurement after 3 h under these conditions) and (**B**) germination (expressed as the % of class 1 grains).

Conclusion

Correlating the response of a particular pollen lot to *in vivo* fertility is largely dependent upon its moisture content. Since most lots in storage are dehydrated to less than 10% MC, prehydration usually improves the viability response. This is particularly true for germination and conductivity but apparently not for respiration.

Regarding the effects of hydration on germination, results from pollen hydrated at 70% RH suggest that membranes may be fully reactivated compared to 100%. However, the negative impact of 30°C (at 70% RH) is observed immediately and affects the poorer lots more. Hydration at 10°C appears to be best for stabilizing and, indeed, improving pollen membranes (lower conductivity), but germination results are not as good as for 20°C. It is also apparent that the germination response is highly variable, suggesting that the source of this variation should be determined.

Data collected from this and previous tests suggest that prehydrating Douglas fir pollen before *in vivo* testing is essential and that current procedures (100% RH/16 h/20°C) are still acceptable. In white spruce *(Picea glauca),* however, these hydration conditions did improve the correlation between germination and yield but had little or even negative effects on other assay responses (Webber, unpublished data). Work must continue to evaluate the effect of hydration on viability responses for all species and, in particular, investigate the effect of hydration at low temperature on *in vivo* fertility (Mellerowicz and Bonnet-Masimbert, 1986).

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