Pecan Pollen Stored Over a Decade Retains Viability

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Abstract. In vitro germination of freshly collected pollen and pollen stored 1, 10, 11, 12, and 13 years in liquid nitrogen was examined for ‘Desirable’ pecan [Carya illinoinensis (Wangen.) C. Koch]. Viability of pollen stored in liquid nitrogen for 1, 10, 11, 12, and 13 years was not diminished in comparison to that of fresh pollen. Morphology of stored pollen grains and the germ tube was normal. Thus, liquid nitrogen may offer a means of haploid preservation of pecan.

Pecan pollen viability has been the subject of research for about 85 years. Early studies indicated that pecan pollen was short-lived and viable for only a few days (Smith and Romberg, 1932; Stuckey, 1916; Wetzstein and Sparks, 1985; Woodroof, 1930). Studies later proved that ‘Desirable’ pecan pollen stored for nearly 2 years at –80 °C and –196 °C retained germination capacity equal to freshly collected pollen provided pollen was rehydrated before in vitro assay for germination (Yates and Sparks, 1989). Shortly thereafter, ‘Desirable’ pollen stored at –80 or –196 °C for up to 3 years was demonstrated to effect fruit set (Yates and Sparks, 1990). Dried pollen (cv. Stuart) could be stored in moisture proof bags for at least 2 years at –12 °C, and at least 2 months at 5 °C, without losing viability (Yates et al., 1991), thus facilitating economical storage of large amounts of pollen for supplemental pollination in commercial orchards.

A viability decay curve derived by mathematical modeling (Yates and Sparks, 1989) and viability following ultra-cold temperature storage (Yates and Sparks, 1989, 1990) suggested cryopreservation of pollen might be a means for long-term storage of pecan pollen. The current research was designed to evaluate the merits of pecan pollen cryogenics by determining if pecan pollen: 1) could be stored for longer than 3 years, 2) viability decreased with increasing years of storage, and 3) viability was dependent on the year of collection.

Materials and Methods

Pollen was collected from four or more ‘Desirable’ pecan trees growing near Athens, Ga., during Spring 1986, 1987, 1988, and 1989. The trees were randomly selected each year. For each collection year, pollen from all trees was bulked and stored in Nunc cryotubes (Rupp and Bowman, Atlanta, Ga.) at –196 °C in liquid nitrogen (Yates and Sparks, 1990) for up to 13 years. Viability of pollen was determined by in vitro germination, using the methods of Yates and Sparks (1989). Briefly, pollen was sub-sampled from the bulked inventory, rehydrated at 25 °C for 4 h, incubated overnight at 25 °C, and pollen germination determined microscopically by counting grains where tube length equaled or exceeded grain diameter. Sub-sampling from all stored pollen treatments was done on the same date. At least 800 pollen grains from each assay of four assay tubes were counted per pollen treatment. Pollen germination was expressed as percentage of intact grains forming germ tubes.

Pollen germination was analyzed as a completely randomized design (Snedecor and Cochran, 1967) with four observations per treatment. Results from one assay tube constituted the basic experimental unit. Germination of fresh pollen was compared to pollen stored in liquid nitrogen for 1, 10, 11, 12, and 13 years that was collected during the growing season from 1986 through 1989. Mean separation was by Duncan’s multiple range test.

Morphology of rehydrated viable and nonviable pollen grains after storage in liquid nitrogen for 11 years (Fig. 2A and B) did not appear to differ from fresh pollen (Yates and Sparks, 1992) or from pollen stored in liquid nitrogen for ≈2 years (Yates and Sparks, 1989). Cytoplasmic contents were evenly distributed throughout the grain, extending to the outer circumference of the pollen grain wall in unfixed, intact pollen grains (Fig. 2A) or as a cross section of a fixed pollen grain (Fig. 2B). Also present were trans-

Results and Discussion

Germination of pecan pollen stored for up to 13 years in liquid nitrogen was significantly higher than that of freshly collected pollen (Table 1) and pollen viability did not decline with time in storage up to 13 years. The year of collection did not affect pollen germination following cryogenic storage. Pollen stored for 11 years in liquid nitrogen produced normal pollen tubes (Fig. 1).

Germination of 1986 and 1987 fresh pollen was determined before our discovery that rehydration of pecan pollen prior to suspension in germination medium is essential for repeatable results (Yates and Sparks, 1989). Thus, low germination rate of 1986 fresh pollen (12%) may be attributable to more indistinct environmental conditions (from the severe 1985–86 drought) during pollen collection in 1986 than in 1987, and supports the necessity for rehydration of fresh pollen prior to examination (Yates and Sparks, 1989).

Germination rates of fresh pollen collected in 1988 and 1989 were lower than those of cryogenically stored pollen for these years. Specifically, the pollen grains (regenerated with weak or malformed cell walls) do not withstand the rigors of liquid nitrogen freezing and instead deteriorate. Only pollen grains with intact cell walls, not pollen fragments, were assessed for the presence or absence of a germ tube. Deterioration of weak grains would account for higher germination of cryogenically stored pollen. However, disintegration of pollen grains on exposure to cryogenic conditions would not explain the decreased viability of 1986 pollen stored for 1 year compared to 13 years. A justification for these results is not readily apparent based on current knowledge of pecan pollen biology.

Morphological characteristics of viable pollen stored in liquid nitrogen for 11 years (Fig. 2A and B) did not appear to differ from fresh pollen (Yates and Sparks, 1992) or from pollen stored in liquid nitrogen for ≈2 years (Yates and Sparks, 1989). Cytoplasmic contents were evenly distributed throughout the grain, extending to the outer circumference of the pollen grain wall in unfixed, intact pollen grains (Fig. 2A) or as a cross section of a fixed pollen grain (Fig. 2B). Also present were trans-

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Table 1. In vitro germination of fresh and stored ‘Desirable’ pecan pollen.

<table>
<thead>
<tr>
<th>Year pollen collected</th>
<th>Years stored</th>
<th>Pollen germination</th>
</tr>
</thead>
<tbody>
<tr>
<td>1986</td>
<td>0 years</td>
<td>12 ± c</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>51 ± b</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>60 ± a</td>
</tr>
<tr>
<td>1987</td>
<td>0 years</td>
<td>46 ± b</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>61 ± a</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>65 ± a</td>
</tr>
<tr>
<td>1988</td>
<td>0 years</td>
<td>49 ± b</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>65 ± a</td>
</tr>
<tr>
<td>1989</td>
<td>0 years</td>
<td>47 ± b</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>65 ± a</td>
</tr>
</tbody>
</table>

1Pollen germination as percentage of intact grains forming germ tubes. Means separated by Duncan’s multiple range test, P ≤ 0.05.

2Fresh pollen examined without rehydration.

3Fresh pollen examined following rehydration.

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Bulges forming at the pores appeared intact (Fig. 2 A and B). In nonviable pollen grains cytoplasm was unevenly distributed in both intact grains (Fig. 2C) and cross sections of fixed pollen grains (Fig. 2D), a distinctly different appearance from that of viable pollen. In intact dead cells, areas apparently devoid of cytoplasmic constituents resulted from the collapse of the cytoplasmic membrane. Some areas of the protoplasm had a crystalline-like appearance displaying refractive qualities. The discrete translucent and opaque particles visible in live pollen grains (2B) appeared to coalesce to form much larger amorphous shapes in cross-sections of dead pollen grains (Fig. 2D). Usually, bulges did not form at the pores of dead grains, but, when present, appeared irregular in shape and density (Fig. 2D).

In summary, the results of germination (Table 1) and morphological analyses (Fig. 2) show that pecan pollen stored in liquid nitrogen for at least 13 years is viable, that pollen vigor does not decline with time in storage, and that pollen viability is independent of collection year. The longevity of ‘Desirable’ pecan pollen predicted from a viability decay curve (Yates and Sparks, 1989) is validated by the current results. Pollen was removed from liquid nitrogen storage and held for 59 d at ambient laboratory conditions. Pollen germination declined rapidly within the first 10 d, but was much slower for the remaining 49 d. Mathematical modeling of this decay curve predicted that pollen treated in this manner should remain viable for 10 years. Even though the pollen in the current study was stored continuously in liquid nitrogen, the results demonstrate pollen was viable for 13 years. Thus, pecan pollen can be established as a long-lived species, rather than short-lived pollen as concluded by earlier workers. Besides providing for shorter-term storage for breeding purposes, cryogenic pollen storage may also be an effective means of germplasm preservation for pecan.

Literature Cited


