Low Temperature Storage of Almond Pollen

P. Martínez-Gómez and T.M. Gradziel¹

Department of Pomology, University of California, Davis, CA 95616

E. Ortega and F. Dicenta

Departamento de Mejora y Patología Vegetal. Centro de Edafología y Biología Aplicada del Seguro-Consejo Superior de Investigaciones Científicas, P.O. Box 4195, E-30080 Murcia, Spain

Additional index words. *Prunus dulcis*, pollen, viability, pollen germination, cryopreservation, breeding

Abstract. Pollen of the California almond cultivars Nonpareil, Ne Plus Ultra, Sonora, and Peerless was evaluated for viability following storage over 12 months at 4, 0, –20, and –80 °C. The proportion of viable pollen exceeded 80% for all cultivars and for all temperatures evaluated after 2 months of storage. Following 12 months of storage at 4 °C, germination decreased to 8% for ‘Nonpareil’, 10% for ‘Ne Plus Ultra’, 50% for ‘Sonora’, with no germination observed for ‘Peerless’. Storage at sub-freezing temperatures maintained pollen viability above 70% in ‘Nonpareil’, ‘Ne Plus Ultra’, and ‘Sonora’ and above 40% in ‘Peerless’. Cultivars differed significantly in their tolerance to low temperature pollen storage. Within cultivars, differences in pollen germination following storage at 0, –20, or –80 °C were nonsignificant.

The genetic improvement of almond [*Prunus dulcis* (Mill.) D.A. Webb] cultivars frequently require pollen storage because of the asynchronous flowering among genotypes. Hybridization between late and early-flowering genotypes requires short-term pollen storage since the quality of almond pollen deteriorates rapidly at moderate temperatures and humidities (Martínez-Gómez et al., 2000). In addition, the pollination of early-flowering cultivars with pollen from late-flowering cultivars, or the use of pollen from other countries with different climates, may require long-term storage of up to 1 year.

Low temperatures have been reported effective for long-term preservation of pollen from nut trees. Cryopreservation at temperatures to –196 °C has been studied. However, a comparison with long-term storage temperatures near 0 °C were not included (Barnabás and Kovács, 1997; Jiang and Gao, 1989; Luza and Polito, 1985, 1988; Parfitt and Almehdhi, 1984; Polito and Luza, 1988). The viability of almond pollen can be assessed by in vitro germination on a sucrose and agar medium (García and Egea, 1979; Hill et al., 1985; Parfitt and Almehdhi, 1984; Weinbaum et al., 1984). The results of these assays correlated well with in vivo results obtained through fruit set determinations (Klungness et al., 1983; Loreti et al., 1979). The objective of this study was to evaluate pollen viability for four almond cultivars following storage for up to 12 months at 4, 0, –20 and –80 °C.

Received for publication 14 June 2001. Accepted for publication 10 Aug. 2001. We thank the support of Spanish Ministry of Education and Culture in the stay of Pedro Martínez-Gómez and Encarnación Ortega in the United States.

¹E-mail address: tmgradziel@ucdavis.edu.

Materials and Methods

Four California almond cultivars, ‘Nonpareil’, ‘Ne Plus Ultra’, ‘Sonora’ and ‘Peerless’, were used. Pollen was collected from flowers at the popcorn stage of development and then dehydrated for 48 h at 22 °C in a silica gel desiccator with a relative humidity (RH) of <5% as measured by a Mannix model PTH8708 hygrometer (Mannix Testing & Measurement, Lynbrook, N.Y.). Samples of 10 mg were stored for up to 12 months in sealed 1.5-mL eppendorf tubes at 4, 0, –20, and –80 °C in controlled temperature chambers at the Univ. of California Fruit and Nut

Postharvest Research Lab., Dept. of Pomology, Davis, Calif. Pollen viability was tested at 2-month intervals using germination and growth on an artificial medium. Prior to germination tests, eppendorf tubes were gradually warmed to 24 °C then placed for 1 h in a rehydration chamber at ≈ 96% RH to avoid damage from moisture imbibition when pollen is exposed to the culture medium (Hoekstra, 1984; Barnabás and Kovács, 1997). Pollen was then dusted onto petri dishes containing 25 mL of culture medium [15% sucrose, 1.5% agar, 100 ppm H₃BO₃, 300 ppm Ca(NO₃)₂·4H₂O] (Brewbaker and Kwack, 1963; García and Egea, 1979; Parfitt and Almehdhi, 1984; Weinbaum et al., 1984). Pollen on media was incubated at 24 °C for 6 h, which was near optimum conditions for the in vitro germination and growth of almond pollen tubes (García and Egea, 1979; Weinbaum et al., 1984). Germination counts were made at ×40 magnification (STEMI-SV8 Zeiss microscope; Carl Zeiss, New York). Pollen was scored as germinated when the length of the tube exceeded the pollen diameter (Ducon, 1968).

Four different fields of vision, each with an area of 12.5 mm² and including ≈250–300 grains, were examined per treatment. Results were analyzed by two-way analysis of variance (SAS Institute, 1989). Means were separated by Tukey’s Studentized range test at *P* < 0.05.

Results and Discussion

Germination of freshly collected pollen was ≥95% for all the cultivars (Fig. 1) and was generally higher than reported by others (García and Egea, 1979; Hill et al., 1985; Klungness et al., 1983; Loreti et al., 1979; Weinbaum et al., 1984). The decline in pollen viability with time was similar among cultivars (Fig. 1). Martínez-Gómez et al. (2000) reported comparable trends for short-term storage of al-

Fig. 1. Pollen germination of four almond cultivars stored up to 12 months at four temperatures. (Bars show SD about mean.)

mond pollen at 4 °C. Interestingly, after storage at 4 °C from 6 to 10 months, increases of germination percentages were consistently observed. Similar increases in germination of stored pollen have been observed in our earlier testing of different germination media (unpublished data) and may represent the deterioration of a sporopollenin based germination inhibitor, as suggested by the work of Pimienta (1980).

Pollen viability decreased slightly to ~90% after 2 months at 0, -20, and -80 °C, and to 80% at 4 °C in the four cultivars assayed. These results are consistent with Martínez-Gómez et al. (2000) who suggested 4 °C is suitable for almond pollen storage [20% relative humidity (RH)] for up to 2 months. The viability of pollen stored at 4 °C decreased gradually to 8% in 'Nonpareil', 10% in 'Ne Plus Ultra', 50% in 'Sonora' and 0% in 'Peerless' by the end of the storage period. Pollen viability at the other three storage tempera-

tures remained above 70% in 'Nonpareil', 'Ne Plus Ultra', and 'Sonora' cultivars and near 40% in 'Peerless' (Fig. 1, Table 1). After 12 months, 'Peerless' had the lowest average germination (32%) followed by 'Nonpareil' (55%), 'Ne Plus Ultra' (59%), and 'Sonora' (69%) (Table 1). Differences among the cultivars and storage temperatures were statistically significant, but no significant interactions were identified.

Pollen viability following storage at -80 °C was consistently lower (though not statistically separable) than that at 0 and -20 °C for the four cultivars assayed (Table 1). Polito and Luza (1988) observed a negative effect of cryopreservation of pollen in some pistachio cultivars at -196 °C vs. -20 °C after 12 months of storage, possibly due to cellular lesions produced during freezing and thawing at the former temperature. Parfitt and Almehdi (1984) and Luza and Polito (1985), however, did not find a significant loss of viability following cryopreservation of pollen of almond and walnut cultivars in liquid nitrogen at -196 °C though final germination rates were variable.

In conclusion, pollen longevity following cold storage varied with almond genotype. Mechanisms responsible for these genetic differences remain poorly understood and are currently being investigated. Storage at 4 °C did not significantly decrease the pollen viability after two months. Successful pollen storage for longer periods of up to 1 year required temperatures at or below 0 °C. Temperatures of 0, -20, and -80 °C, gave similar results after 1 year. The extra cost of storage at -80 °C, including the extra precautions required for warming and rehydrating stored pollen, may make storage temperatures of 0 to -20 °C more convenient for longer storage of 2-12 months.

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Table 1. In vitro germination of pollen of four almond cultivars after 12 months storage at four temperatures.

Cultivar	Temp (°C)	Pollen germination (%) ^a
Nonpareil	4	8 f
	0	76 ab
	-20	77 ab
	-80	65 bc
Pooled means		55 b
Ne Plus Ultra	4	10 f
	0	82 a
	-20	76 ab
	-80	71 ab
Pooled means		59 b
Sonora	4	50 cd
	0	81 a
	-20	77 ab
	-80	70 ab
Pooled means		69 a
Peerless	4	0 f
	0	45 de
	-20	46 de
	-80	36 e
Pooled means		32 c

^aMeans in columns with the same letter are not significantly different ($P < 0.05$).