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Changes in Water Soluble Polyuronides in the Pulp Tissue of Ripening 'Bosc' Pears following Cold Storage in Air or in 1% Oxygen

P.M. Chen[1] and D.M. Borgic[2]

Mid-Columbia Experiment Station, Oregon State University, Hood River, OR 97031

Additional index words. *Pyrus communis*, flesh firmness, extractable juice, controlled atmosphere storage, hydroscopic binding capacity

Abstract. **'Bosc' pears (*Pyrus communis* L.) harvested at an optimum maturity, based on flesh firmness (about 62 N), were stored either in air or 1% O_2 (plus <0.03% CO_2) at −1°C. Fruit stored in air for 1 to 3 months softened rapidly after 2 days of ripening at 20°C and reached ripeness with flesh firmness of 20 N or lower by the 9th day. Ripening was associated with a reduction in extractable juice (EJ) and an apparent increase in water soluble polyuronides (WSP). Fruit stored in air for 4 to 5 months also softened rapidly after 2 days of ripening, but flesh firmness was still between 26 and 30 N after 9 days; however, EJ and WSP of fruit did not change appreciably during 9 days of ripening. The WSP content in fruit stored in either air or 1% O_2 increased substantially during 6 months of storage at −1°C. Increased WSP content during storage did not affect the quantity of EJ. Fruit stored at 1% O_2 showed a reduction in EJ and an increase in WSP during the 9-day ripening period, whereas, in long-term air-stored fruit, EJ did not decline while WSP was degraded. Correlation of EJ and WSP during each ripening period provided an estimation of storage life. Increased WSP after ripening might be responsible for the increase in hygroscopic binding capacity of the ripened pulp tissue.**

Winter pears stored in air at −1°C for a proper period of time (usually 3 and 5 months for 'Bosc' and 'd'Anjou' pears, respectively) or in low-O_2 atmosphere for 6 to 8 months, respectively, are capable of ripening at 20° with desirable dessert quality upon removal from cold storage (5, 6, 15). Pear fruit with this quality have good flavor and a juicy, buttery texture. However, fruit stored in air at −1° for a prolonged period of time (usually 4 months for 'Bosc' and 6 months for 'd'Anjou) tend to have a dry, coarse texture and poor flavor upon ripening (5, 6, 15). Development of the juicy, buttery texture is associated with a reduction in extractable juice, which probably results from an increase in solubility of pectic substances in the pulp (8). Polyuronide is the major component of pectic substances in fruit pulp (16); changes in its solubility are related closely to the texture modifications. The main purpose of this study was to investigate the changes in flesh firmness, extractable juice (EJ), and water soluble polyuronides (WSP) in 'Bosc' pear fruit during a 9-day ripening period after monthly removal of fruit from a 5-month air-storage, or after 6 months of storage in 1% O_2 at −1°C.

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[1]Associate Professor.
[2]Biological Technician.

Materials and Methods

Plant material, types of storage, and sampling periods. 'Bosc' pears at optimum maturity with an average flesh firmness of 62 N (14 lbf) were harvested from 3 separate uniform, mature trees in an orchard at the Mid-Columbia Experiment Station. After harvest, all fruit were drenched with benomyl (600 ug · liter^{-1}).

Fifty benomyl-treated fruit from each tree were divided into 5-fruit lots which were placed into perforated polyethylene snack bags. Ten bags of fruit from each tree were placed into a 19-liter wide-mouth glass jar in a cold room at −1°C. Each jar was equipped with a gas-tight lid having inlet and outlet ports fitted with Tygon tubing. After sealing, each jar was flushed with prepurified N_2 gas (about 99.995% purity, with C_2H_4 and C_2H_2 below 0.05 ppm) for 24 hr. One percent O_2 in each jar was obtained by mixing synthetic air (which is made of 79% N_2 and 21% O_2) and prepurified N_2 with the aid of 2 flowmeters and a mixing tube. The flow rate was maintained at about 50 ml · min^{-1}. The CO_2 concentration in each jar was maintained below 0.03% by the use of 5 packages of 100 gL of hydrated lime in each jar. The O_2 level was maintained at 1.0% ± 0.2% throughout the 6 months of storage. Another set of 50 fruit/tree received synthetic air at about 50 ml · min^{-1}, provided as air storage under an identical flow-through system. The relative humidity (RH) in each jar was not monitored.

The remainder of the fruit were transferred at random into 20-kg wooden boxes with perforated poly liners and stored in air at −1°C until used. One box of fruit (about 100 fruit) per tree was removed from air storage at monthly intervals for 5

months and ripened for 9 days at 20° ± 1°. About 10 liters of water were poured onto the floor of the ripening room each day during ripening period. With the air circulated by 2 fans and with the closure of the door, the RH in the ripening room could be maintained between 92% and 96% as monitored by a calibrated hygrothermograph (model 5-594, Belfort Instrument Company). Decayed fruit were discarded. Ten fruit per tree constituted an experimental unit, and were used for determinations of flesh firmness, EJ, and WSP on days 0, 2, 4, 7, and 9 of each ripening period. Fruit stored in 1% O_2 or air in the flow-through system were removed from jars after 6 months of storage and also ripened under the same conditions. Five fruit per tree were used for determinations of flesh firmness, EJ, and WSP during ripening.

Measurement of flesh firmness and EJ. Flesh firmness of fruit was determined by a UC pressure tester (9). A Hunter spring force gauge (Model #L-30 lb) with an 8-mm diameter plunger was mounted on a stand for operation like a drill press (13). Each fruit was penetrated to a depth of 9 mm at 3 pared spots located on the sector not exposed to direct sunlight.

After determining flesh firmness, each fruit was peeled and 4 wedge-shaped sectors were cut from the pulp. Mixed sectors from each experimental unit were weighed and passed through a 1-speed juicerator (Acme Model #6001), equipped with a uniform strip of milk filter, at 3000 × *g* for 3 min. The amount of EJ was measured and expressed as ml juice · 100 g^{-1} fresh weight.

Preparation of freeze-dried pulp powder and determination of WSP. Another 4 sectors of peeled pulp tissue were cut from the fruit from each experimental unit and diced into cubes about 1 cm^3. To reduce oxidative browning, cubes were transferred immediately into aluminum trays and covered with cloth wetted with 0.5% sodium bisulfite. Cubes then were put into a 120-ml plastic cups, covered with lids, and frozen at −20°C for 48 hr. The frozen samples were freeze-dried, ground into fine powder (200 mesh), and stored at −20°C until analyzed.

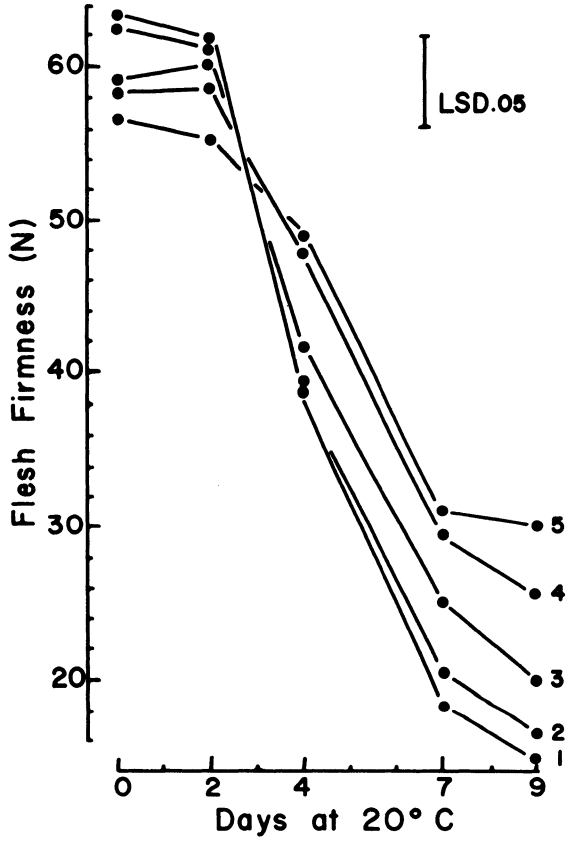

Fig. 1. Fruit softening patterns of 'Bosc' pears during a 9-day ripening period at 20°C following cold storage for 1–5 months at −1°C in air. The numbers at the end of each curve indicate the month(s) of storage.

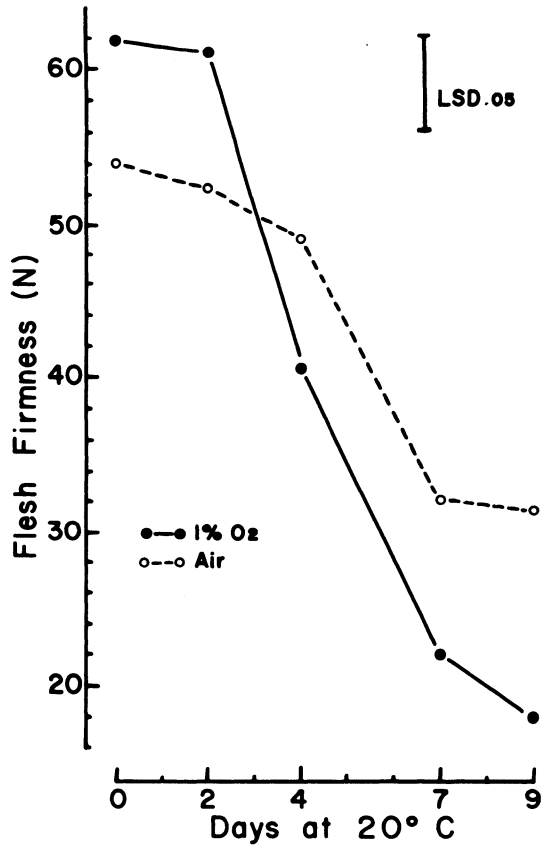

Fig. 2. Fruit softening patterns of 'Bosc' pears during a 9-day ripening period at 20°C following 6 months of cold storage at −1°C either in 1% O_2 (plus 0.03% CO_2) or in air.

Procedures preparing cell wall materials and extracting the water soluble fraction were modified from the method of Jermyn and Isherwood (12). One g of freeze-dried powder was boiled with constant stirring in 30 ml 80% ethanol for 3 min and filtered (Whatman #1). The ethanol-insoluble residue (EIR) was washed 12 times with 5 ml boiling 80% ethanol, oven-dried at 65°C for 24 hr, and weighed. The yield of EIR was between 130 and 150 mg · g^{-1} freeze-dried powder. According to Jermyn and Isherwood (12), the cell wall material prepared by this method contains all the polysaccharides, and most of the pectic substances originally present in the cell sap. The EIR was placed in a beaker and resuspended by adding 1 ml 80% ETOH followed by 45 ml boiling distilled water. The EIR suspension was incubated in a water bath at 85°C with a constant shaking for 24 hr and filtered through Whatman #4 filter paper with 19 washings with about 10 ml boiling water. The filtrate was collected into a 250-ml volumetric flask. After cooling to room temperature, the volume was adjusted to 250 ml with distilled water.

Soluble polyuronides in the hot water extract were assayed from a 0.4 ml aliquot of the preparation plus 0.2 ml distilled water by the method of Blumenkrantz and Asboe-Hansen (3) as modified by Ahmed and Labavitch (1). Since the RH in the air

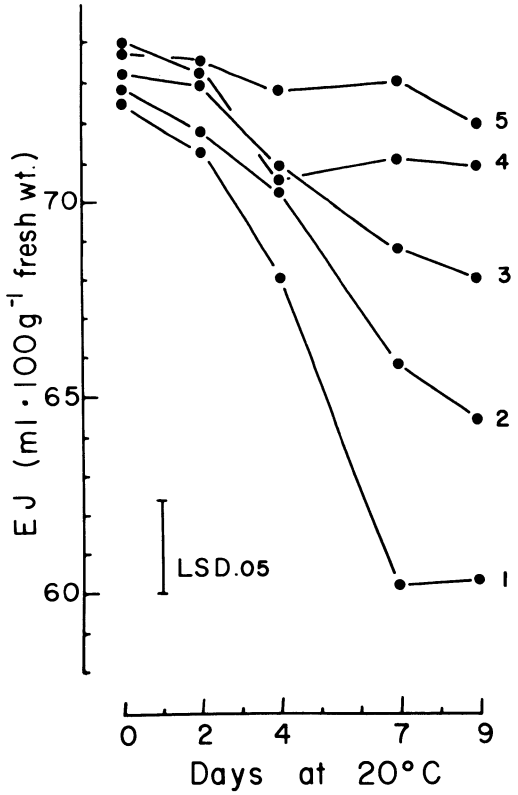

Fig. 3. Changes in the amount of extractable juice (EJ) of 'Bosc' pears during a 9-day ripening period at 20°C following cold storage for 1 to 5 months at −1°C in air. The numbers at the end of each curve indicate the month(s) of storage.

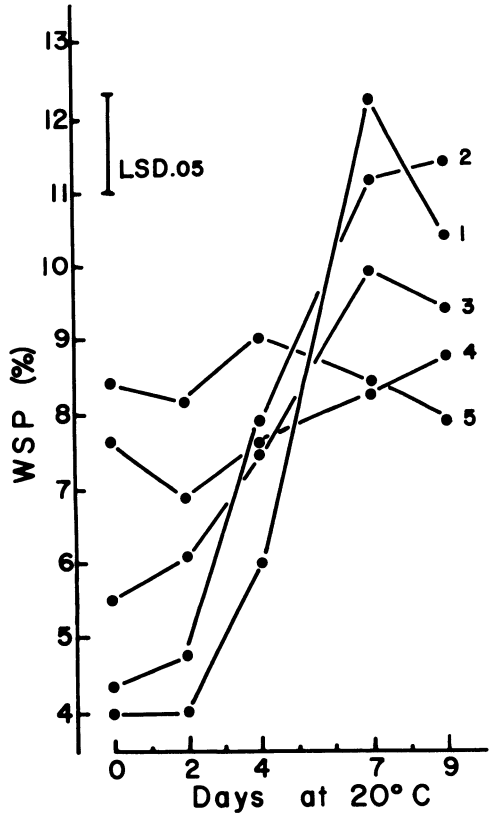

Fig. 5. Changes in water soluble polyuronides (WSP) of 'Bosc' pears during a 9-day ripening period at 20°C following cold storage for 1–5 months at −1°C in air. The numbers at the end of each curve indicate the month(s) of storage.

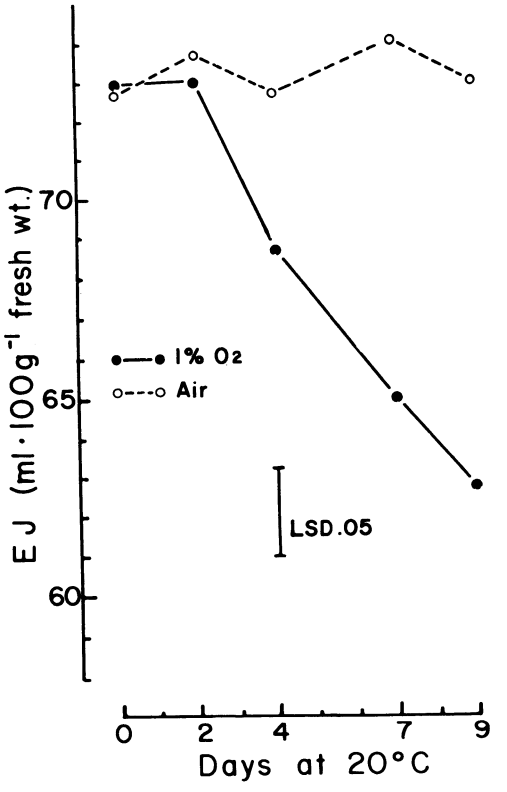

Fig. 4. Changes in the amount of extractable juice (EJ) of 'Bosc' pears during a 9-day ripening period at 20°C following 6 months of cold storage at −1° either in 1% O_2 (plus 0.03% CO_2) or in air.

storage and in each jar was not controlled and monitored the amount of water loss in fruit during the storage period was not known. Furthermore, the loss of titratable acids and the variability of soluble solids in pear fruit during storage period has been reported (5, 7). In postharvest fruit, the increase in one chemical fraction could be due simply to the loss of the other fraction(s) and thus might not be a true increase in such fraction. Therefore, the amount the WSP was expressed as the percentage of EIR in order to show the relative changes of polyuronides from water-insoluble form to water-soluble form during each ripening period, since neither water-insoluble nor soluble polyuronides were soluble in 80% ethanol.

Results and Discussion

Fruit stored in air at −1°C for 1 to 3 months softened rapidly after 2 days of ripening at 20° and reached 20 N or lower on day 9 (Fig. 1). These fruit developed a juicy, buttery texture upon ripening (data not presented). Fruit stored in air for 4 to 5 months also softened during ripening, but to a lesser extent, reaching 26–30 N after 9 days (Fig. 1). They had a dry, coarse texture (data not presented). Fruit stored in 1% O_2 at −1°C for 6 months softened, and ripening was similar to that of fruit stored in air for 3 months or less, whereas fruit stored in air at −1°C for 6 months softened and ripened similar to those stored in air for 4 to 5 months (Fig. 2).

The EJ of fruit stored in air for 3 months or less or in 1% O_2 for 6 months declined markedly during ripening (Fig. 3 and 4). The reduction of EJ suggested an increase in the hygroscopic binding capacity of the pulp tissue in the ripened fruit. The EJ

Table 1. Correlation coefficients (r) between extractable juice (EJ) and water soluble polyuronides (WSP) of 'Bosc' pears during a 9-day ripening period at 20°C. Fruit had been stored either in air or in 1% O_2 (plus 0.03% CO_2) at −1° or different periods of time.

Types of storage	Months of storage					
	1	2	3	4	5	6
Air	−0.932**[z]	−0.901**	−0.837**	+0.017NS	−0.185NS	−0.055NS
1% O_2 (0.03% CO_2)	---	---	---	---	---	−0.863**

[z]Significantly correlated at the 1% level (**), and not significantly correlated (NS). The measurements of EJ and WSP on each sampling day (i.e., day 0, 2, 4, 7, and 9) during each ripening period are treated as a pair of observation. Therefore, the total number of observations (n) for each ripening period is 15.

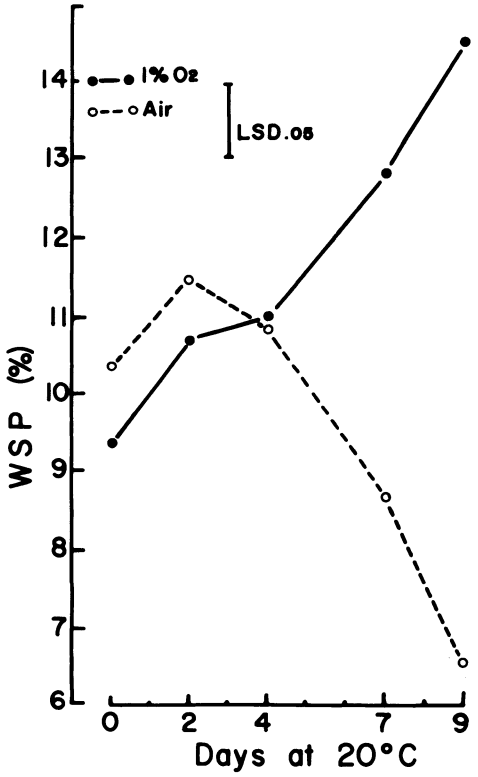

Fig. 6. Changes in water soluble polyuronides (WSP) of 'Bosc' pears during a 9-day ripening period at 20°C following 6 months of cold storage at −1° either in 1% O_2 (plus 0.03% CO_2) or in air.

of fruit stored in air for 4 months or longer did not decrease significantly during ripening, suggesting that the pulp tissue had lost its hygroscopic binding capacity, resulting in a dry, coarse texture (Fig. 3 and 4).

Regardless of storage atmospheres, WSP in fruit at day 0 increased from 4% after 1 month of storage at −1°C to 10% after 6 months (Fig. 5 and 6). The increase of WSP during cold storage did not affect initial EJ which remained relatively constant at 73–74 ml · 100 g^{-1} fresh weight throughout 6 months of storage (Fig. 3 and 4).

Fruit stored in air for 1 to 3 months showed an apparent increase in WSP during each 9-day ripening period (Fig. 5). This phenomenon agreed with the normal ripening and softening behavior of a number of fruit species (2, 4, 10, 11, 14). The increased WSP was negatively correlated with EJ during each ripening period (Table 1). The results are consistent with the idea that polyuronides, solubilized from the cell wall during ripening, are responsible for the reduction of EJ and, therefore, for the increase of hygroscopic binding capacity of the ripening pulp tissues.

Fruit stored in air for 4 or 5 months showed little or no change in WSP during ripening (Fig. 5), suggesting either that there was no further release of polyuronides from the cell wall or that the release of polyuronides from the cell wall had reached an equilibrium with the degradation of WSP into the uronic acid monomer. Ben-Arie et al. (4) reported that the rapid decline in the insoluble pectic substances and increase in water soluble pectin in ripening 'Spadona' pear also were accompanied by a rapid rise in free galacturonic acid monomer.

Although WSP in fruit stored in 1% O_2 for 6 months had increased to 9.4%, it continued increasing to 14.5% during 9 days of ripening (Fig. 6). The increased WSP values also were negatively correlated with EJ during the ripening period (Table 1). Since WSP increased during cold storage but did not affect EJ, it was possible that the polyuronides solubilized during storage were qualitatively different from that released during ripening.

The WSP in fruit stored in air for 6 months was 10.3% at day 0, increasing slightly to 11.5% on day 2, and then rapidly decreasing to 6.5% on day 9 (Fig. 6). The results suggested that a rapid degradation of WSP into uronic acid monomer might have occurred in these fruit during the ripening period, and the liberation of polyuronides from the cell wall might become quite limited.

Since WSP in fruit stored in air for 4 months or longer was no longer correlated with EJ during ripening (Table 1), the development of dry, coarse texture apparently was related to the failure of the cell wall to release WSP, combined with a rapid degradation of WSP into uronic acid monomer during ripening which, in turn, resulted in the loss of hygroscopic binding capacity of the ripened pulp tissue.

Based on the correlation coefficient between EJ and WSP (Table 1), the storage life of 'Bosc' pears at −1°C could be estimated to be less than 4 months in air but over 6 months in 1% O_2.

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Factors Affecting in Vitro Germination and Storage of Jojoba Pollen

Chin Won Lee[1], John C. Thomas[2], and Stephen L. Buchmann[3]
Department of Plant Sciences, University of Arizona, Tucson, AZ 85721

Additional index words. xerophyte, cryogenic storage, *Simmondsia chinensis*

Abstract. **Conditions for in vitro germination of jojoba [*Simmondsia chinensis* (Link) Schneider] pollen were optimized in order to study the influence of storage temperature on viability. A medium consisting of 300 mg·liter^{-1} $CaCl_2 \cdot 2H_2O$, 100 mg·liter^{-1} KNO_3, 10 mg·liter^{-1} H_3BO_3, 20% sucrose, and 4% to 5% Difco Bacto-agar was optimal for germinating both fresh and stored pollen. Pollen germinated readily in media with a pH range of 4 to 8. The optimum incubation temperature range for pollen germination was 25° to 30°C. When stored at room temperature (22° to 25°), the initial pollen viability was decreased to 50% in 3 weeks and to 0% after 10 weeks, as determined by in vitro germination. Pollen stored at 4° maintained its initial viability for 10 weeks, followed by a gradual decrease in germination to 70% in 17 weeks and 0% after 22 weeks. Pollen stored at −196° in liquid nitrogen for 2 years retained a germination percentage as high as that of fresh pollen. The cryogenically stored pollen, when used in controlled pollinations, produced normal fruit set comparable to that with fresh pollen.**

The unique properties of the liquid wax contained in the seed of jojoba have stimulated much interest in recent years, leading to domestication of the plant as a new oil crop for arid regions. Now, more than 15,000 ha of commercial jojoba plantations have been established in the dry, warm areas of the southwestern United States, Mexico, and other countries (3). In Arizona, the dioecious jojoba is wind pollinated during February and March (2). At this time, the availability of pollen and stigmatal receptivity must be coordinated for successful fruit set (7). This is especially true in commercial jojoba plantations where the numbers of male plants are few. One way to ensure pollen availability when stigmatic receptivity is at a maximum is to store and later disperse the pollen at the optimum time; a practice which requires the ability to store pollen in a viable state.

Favorable conditions for pollen storage and maintenance of pollen viability have been investigated for many agronomic and horticultural crops (4, 6). Generally, low temperatures (4° to −20°C) combined with low humidity are known to lengthen the storage life of the pollen. However, the optimum moisture requirements and the maximum longevity of pollen viability under low temperature conditions vary greatly, depending on plant species. The viability of fresh and stored pollen is best determined by germination tests. Whereas some plants require simply water or sugar source for pollen germination, pollen grains of many other plants demand a more complete medium with sugar, agar, boron, and Ca in certain pH and temperature ranges for germination (4, 6, 8). The purpose of this study was to optimize a jojoba pollen germination medium and monitor the viability of the pollen after storage at different temperatures.

Materials and Methods

All jojoba pollen used in this study was collected from native stands of male jojoba in the Tucson Mountains and the Santa Catalina foothills near Tucson, Ariz. The staminate inflorescences were collected at anthesis on a sheet of aluminum foil

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[1]Assistant Professor. Present address: Dept. of Hort., Colorado State Univ., Fort Collins, CO 80523.
[2]Graduate Assistant
[3]Research Scientist, ARS/USDA, Carl Hayden Bee Research Center, 2000 E. Allen Road, Tucson, AZ 85719.