of ethylene from the fruit, both of which are inversely related to the pressure (1, 5, 7). Reducing the oxygen partial pressure affects fruit metabolism by lowering the rate of respiration (3, 6). Under such conditions the rate of production and action of ethylene are lowered (5); in addition, the constant ventilation of the commodity prevents the accumulation of ethylene in the storage chamber, which might hasten ripening even at a low temperature (14). The low rate of weight losses under subatmospheric pressure during prolonged storage was probably due to the fact that the chamber was ventilated with water-saturated air, eliminating the water vapor gradient between the commodity and the atmosphere.

Although ripening was markedly retarded under subatmospheric conditions all fruits ripened normally several days after being transferred to atmospheric pressure and 14°C (Figs. 3, 4, 5, 6). This indicates that there was no damage to the fruit tissue by the treatment, and that the fruit retained the ability to undergo normal ripening processes and develop proper texture and taste.

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Influence of Pollen Grain Stage at the Time of Hand Pollination as a Factor on Fruit Set of Cherimoya[1]

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Additional index words. Annona cherimola

Abstract. **Preliminary research to investigate additional factors to increase results obtained by hand pollination in cherimoya (*Annona cherimola* Mill.) revealed that the first flowers to open gave a poorer fruit set with hand pollination than later ones. Flowers that opened earliest had the majority of the pollen grains in the tetrad stage with thick walls, full of starch and did not germinate. Flowers that opened later had a greater proportion of individual pollen grains, no starch grains, and greater cytoplasmic streaming. Many grains germinated after a few hours.**

The protogynous character of the flowers of some *Annona* spp. accounts for poor fruit set. The later is also affected by the morphology of the flowers and adverse climatic conditions (5, 7, 8). Insects rarely visit cherimoya flowers in Chile, which would suggest that entomophilous pollination only plays a secondary role.

Hand pollination has been used to improve set and obtain well formed fruits (3, 4, 5, 6). Various factors influence successful artificial pollination, mainly viability of the pollen, receptivity of the pistils, climatic conditions, internal conditions of the plant (5, 6). Artificial pollination has been used in some commercial orchards in Chile. The success of hand pollination in increasing fruit set is variable and uncertain, however. Preliminary research to investigate some factors to increase the results obtained by hand pollination is reported here.

Maximum bloom of the cherimoya normally occurs in Jan.-Feb. in Chile. Two treatments were established on Jan. 2, 1972 at La Cruz. Flowers of 'Concha Lisa' were collected and stored as the pollen source (4, 5). Ten flowers in each of 5 trees were pollinated the next morning when the pistils were receptive with pollen liberated by means of a small brush (4, 5) and the other treatment consisted of 20 flowers in each of 4 trees treated with a pollen suspension in a 10% sucrose solution.

Neither of these procedures induced fruit set. Microscopic observation of pollen suspended in Kwack's medium (2) and 22 variations with different sucrose, boric acid, Ca and K concentrations indicated that the pollen was immature and unable to germinate. This medium has been used successfully with *Annona reticulata* L. pollen (1). The majority of the cherimoya pollen grains were in tetrad stage, with thick walls, full of starch, as illustrated in Fig. 1. Cytoplasmic streaming was occasionally observed in localized zones where the starch was already hydrolized. Pollen of the 'Terciopelo' was in a similar stage on the same date. Germination of some pollen grains of 'Concha Picuda' was observed.

A second trial was set on Jan. 11, 1973. Twenty flowers of 'Concha Lisa' were marked in each of 4 trees. Half of them were sprayed with water during anthesis, and the others were left untreated to observe natural fruit set. A remarkable improvement in fruit set was observed 21 days later in flowers treated with water (Table 1). However, only 1 fruit remained on the tree of all of the fruits which had set after 59 days, indicating that the number of carpels fertilized was still insufficient to carry the fruit until maturity.

Flowering of this species occurs at the time of year when atmospheric humidity is low. A drop of water is deposited in the flower at the time of pollination in Egypt to prevent dehydration (5). It has been reported that 'Concha Lisa' is the cultivar with the lowest level of stigmatic secretion (6). These results confirm the necessity of an increase in available moisture at the flower level to increase fruit set.

In another experiment, pollen was collected in bottles when naturally shed by the tree at 18:00 to 19:00 hours and used the next day. Flowers were hand

Table 1. Effect of water sprayed to cherimoya flowers at anthesis on fruit set.

Tree	No. flowers treated	No. fruit set 21 days after anthesis	
		Natural	Water sprayed
A	10	0	8
B	10	0	5
C	10	2	4
D	10	1	2

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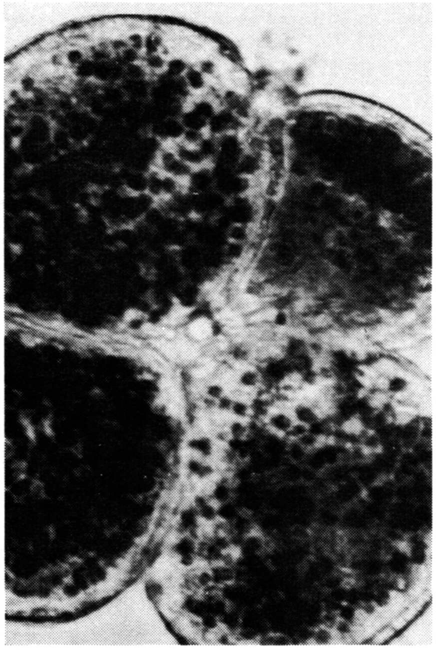

Fig. 1. Pollen grains at anthesis (Jan. 2) showing tetrad stage with thick walls and full of starch. Stained with KI•I. (×940).

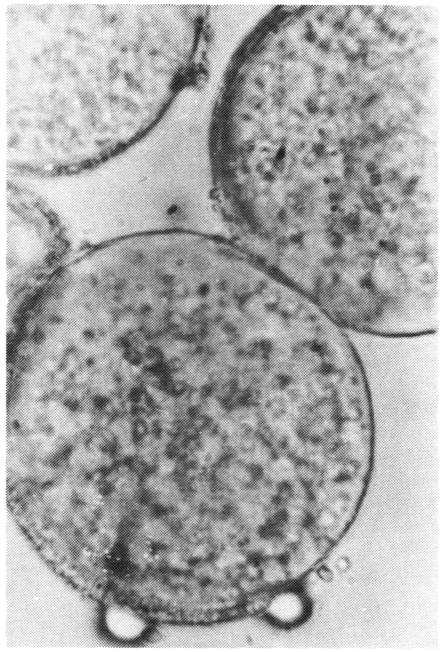

Fig. 2. Pollen grains at anthesis on Feb. 1, showing thin walls and no starch grains. Stained with KI•I. (×940).

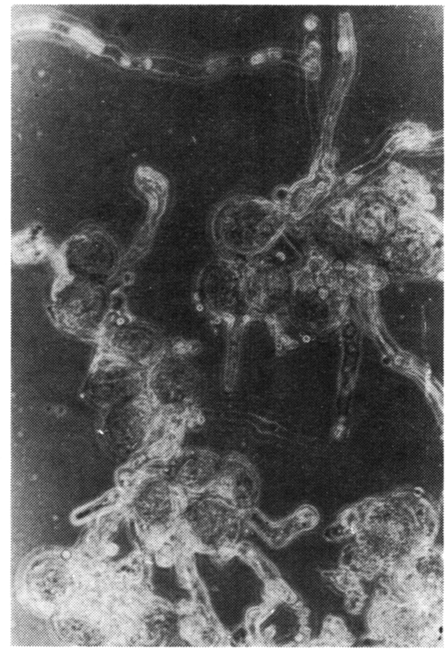

Fig. 3. Germinated pollen grains 2 hr after incubation on Kwack's medium. (Phase contrast. ×128).

pollinated and immediately sprayed with water on Jan. 19, 25 and Feb. 1, and fruit sets of 43.7%, 57.0% and 94.4%, respectively, were obtained. The fruit remained in the tree until maturity, and produced, in general, well-conformed sizable fruits.

Pollen used on Feb. 1 had a greater proportion of individual grains with thin walls, no starch grains (Fig. 2) and greater cytoplasmic streaming than those of the first date. Many grains germinated after a few hours (Fig. 3).

Even though the first flowers to open gave a poor fruit set with hand pollination, their setting was actually very good when treated with growth regulators (over 90%). Furthermore, later fruit growth was also found to be excellent (unpublished results).

Therefore it is proposed that the vegetative condition of the tree may influence ripening and viability of the pollen rather than development of the ovaries and the availability of organic and inorganic nutrients necessary to maintain the development of the fruit.

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Seasonal Susceptibility of Grapefruit to Chilling Injury as Modified by Certain Growth Regulators[1]

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Additonal index words. 2,4-D, gibberellic acid, benzyladenine, *Citrus paradisi*, cold storage

Abstract. **Changes in susceptibility to chilling injury of grapefruit (*Citrus paradisi* MacF.) were found to vary directly with the growth activity of the trees. Exogenous growth regulators were applied to test the hypothesis that they may be involved in seasonal variations in susceptibility to chilling injury. Benzyladenine, gibberellic acid and 2,4-dichlorophenoxyacetic acid applied postharvest and benzyladenine and 2,4-D applied preharvest significantly altered susceptibility to chilling injury although the direction and extent of the changes were neither consistent nor predictable.**

Grapefruit and lime (*C. aurantifolia* Swing.) are the most susceptible among citrus species to the low temperature disorder known as chilling injury (CI). Affected fruit develop discolored rind pitting between 12.8°C (55°F) and 0°C (32°F), which becomes necrotic (Fig. 1) and results in subsequent fruit decay. Lyons (2) hypothesized a pathway of events leading to CI in which mitochondrial membranes are the initial site at which this degenerative process begins. Grierson (1) postulated that susceptibility of grapefruit to CI throughout the season is related to the general growth condition of the tree, probably via its growth regulator (GR) levels at time of harvest; i.e., fruit from actively growing trees are more susceptible to CI than those from inactive ones. Schiffmann-Nadel et al. (3) have drawn attention to the analogy between reduction of CI by thiabendazole (TBZ) and the inhi-

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