mittent temperatures also affected the rate of softening if the simulated transit temperature was above 1.7°C. These results indicate that transit temperatures near or above 7.2°C may cause rapid softening of fruit within 3 weeks and are unfavorable for 'long-period' shipment. Pears exposed to 3 days of intermittent temperatures above 7.2°C must be held at –1.1°C to avoid excessive softening during shipment. Pears held for 3 days intermittent temperature at 1.7°C or above, plus 4 weeks at transit temperatures of –1.1°or 1.7°C, softened rapidly during 16 days in a ripening environment of 20°C. This suggests that those fruit have an undesirably short shelf life during further distribution.

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Effects of Harvest Date on Ripening Capacity and Postharvest Life of 'd'Anjou' Pears[1]

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Additional index words. Pyrus communis, flesh firmness, respiration, ethylene production, internal ethylene, dessert quality, ethanol insoluble matters, titratable acids, soluble solids

Abstract. **'D'Anjou' pears (*Pyrus communis* L.) were harvested at weekly intervals for a 3-week period beginning at the start of commercial harvest in the Hood River Valley, Oregon. Late-harvested fruit at flesh firmness of 5.9 to 5.4 kg ripened with fair to good quality following 30 days storage at –1.1°C. Fruit harvested at optimum flesh firmness of 6.4 to 6.1 kg required 60 days of postharvest chilling to ripen with quality. The development of ripening capacity corresponded to the increase in internal ethylene to 1.5–2.0 ppm during cold storage. Dessert quality of late-harvested fruit declined after 90 days of storage while quality of optimum-harvested fruit continued to improve until 150 days in storage. Flesh firmness and ethanol-soluble matters indicated that fruit harvested over the 3-week period were of different maturities. Concentrations of titratable acids and soluble solids varied among different harvest groups.**

Optimum maturity of 'd'Anjou' pears is usually judged to be when flesh firmness is between 6.8 and 5.9 kg (15 and 13 lb.) (4). Commercial harvest may last as long as 3 weeks which implies that pears are picked at different degrees of maturation. These pears must be stored at low temperature, or treated with ethylene, before they will ripen. Premature 'd'Anjou' pears ripen slower than the fully mature fruits when they are treated with exogenous ethylene (17). Hansen and Mellenthin (4) warned that winter pears harvested at an advanced stage of maturity tended to develop coarser texture and have shorter storage life. We observed that late-harvested 'd'Anjou' pears had developed the capacity to ripen much earlier than fruit harvested at optimum maturity. The main purpose of this study was to investigate the relationship between the harvest date, the ripening capacity, and the dessert quality of 'd'Anjou' pears after cold storage.

Initiation of ripening activities of climacteric fruit is controlled by the threshold level of internal ethylene concentration (3, 11, 13), but dessert quality of a ripe fruit may be related to organic substances such as acids, sugars, and cell wall materials maintained in the fruit during storage as well as to enzyme activities during ripening (12, 14). The second objective was to study the effect of harvest date on changes in internal ethylene and organic substances of 'd'Anjou' pears during cold storage.

Materials and Methods

Preparation of fruit samples. Eight uniform and mature 'd'Anjou' trees from the same location at the Mid-Columbia Experiment Station, Hood River, Ore., were randomly divided into 4

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units of 2 trees each. Fruit were harvested from 1 unit at each harvest date to eliminate the possible influence of changes in the fruit-to-leaf ratio. Samples were picked at weekly intervals over a 22-day period beginning Sept. 4, 1979, the first day of commercial harvest. Fruit harvested on these 4 dates were subsequently referred to as Harvests I, II, III, and IV, respectively. All samples were treated with a drench of benomyl fungicide (600 ppm) and ethoxiquin scald inhibitor (2,600 ppm) immediately after harvest, randomly transferred into 20-kg wooden boxes with perforated polyliners, and stored at –1.1°C until use. Flesh firmness, ripening capacity, internal ethylene, dessert quality, and certain biochemical constituents of fruit were determined at each harvest date and after 10, 20, 30, 45, 60, 90, 120, and 150 days of cold storage.

Flesh firmness. Flesh firmness of fruit was determined by a UC pressure tester (2) with an 8-mm plunger and 3 pared punches per fruit. Ten pears from each harvest group were measured at each sampling period.

Ripening capacity. Ripening capacity of fruit was determined by measuring the rates of respiration and ethylene production daily as described previously (16), except that the temperatures of the gas chromatograph used for ethylene measurement were maintained at 70°C for the column and 100°C for both injector and detector. A 1-ml air sample was injected for each measurement. Four pears from each harvest group were put in 1 chamber for the measurement of CO_2 and C_2H_4 production and each harvest group was replicated 3 times at each sampling period.

The rates of CO_2 and C_2H_4 production at each sampling period were expressed as the daily average rate (DAR) of CO_2 and C_2H_4 production which was the sum of daily CO_2 or C_2H_4 production rates divided by the number of days to a climacteric peak. If no climacteric peak was found within 20 days, as in the early sampling periods, the DAR is a mean of 20 days' production rates.

Internal ethylene. The intercellular gas in the fruit was extracted (1) under reduced pressure of 600 mm Hg for 20–25 sec. One ml of extracted gas was immediately injected into the gas chromatograph to determine the ethylene concentration. Three pears from each harvest group were measured at each sampling period. A preliminary experiment revealed that the internal ethylene concentration in a fruit stabilized between 2 and 5 hr after the fruit was removed from the cold storage and placed at 20°C. Therefore, internal ethylene measurements were made after 2 hr at 20°C.

Dessert quality. About 20 fruits from each harvest group at each sampling period were ripened at 20°C and 5 fruits were randomly evaluated for dessert quality (6).

Ethanol insoluble matters. Ethanol-insoluble matters were obtained by the ethanol extraction method described previously (6). Triplicated 3-fruit samples from each harvest group were used for each extraction. The dry weight of the ethanol-insoluble matters was determined gravimetrically after being oven dried at 70°C for 24 hrs and expressed as g per 100 g fresh pulp.

Titratable acids and soluble solids. Three fruits from each harvest group were peeled, sliced, and juiced in a juicerator (Acme Model 6001). Titratable acids were determined by titrating 25 ml of juice to pH 7.2 using 0.1 N NaOH, and calculated as milliequivalent (meq) acids per 100 ml juice. Soluble solids were measured by a hand refractometer and expressed as g soluble solids per 100 ml juice. Each harvest group was also replicated 3 times per sampling period.

Results and Discussion

Flesh firmness. Firmness decreased sequentially with harvest from 6.4 kg (14.1 lb.) for Harvest I to 5.5 kg (12.2 lb.) for Harvest IV (Fig. 1). Harvest IV had a firmness at harvest below the optimum range (6.8 to 5.9 kg) (4) and thus was considered as late harvested fruit. Harvests I and II were harvested at optimum maturity, while Harvest III was marginal.

During cold storage, the firmness of Harvest I decreased from 6.4 kg to 5.9 kg within 30 days, remained at the level until 120 days, and then decreased to 5.5 kg after 150 days. The firmness of Harvest II also decreased rapidly from 6.2 kg to 5.8 kg in 30 days, had little decline from 30 to 60 days, but then dropped again steadily to 5.2 kg after 150 days. The firmness of Harvest III decreased rather linearly from 5.9 kg after 20 days storage to 4.8 kg (10.5 lb.) after 150 days. The firmness of Harvest IV declined from 5.5 kg at harvest to 4.8 kg after only 45 days storage, but then decreased very gradually to 4.5 kg (10 lb.) after 150 days. 'D'Anjou' pears are considered (10) to be at end of commercial storage life when they soften to a firmness of 4.5 kg (10 lb.); thus, Harvests III and IV had limited postharvest life.

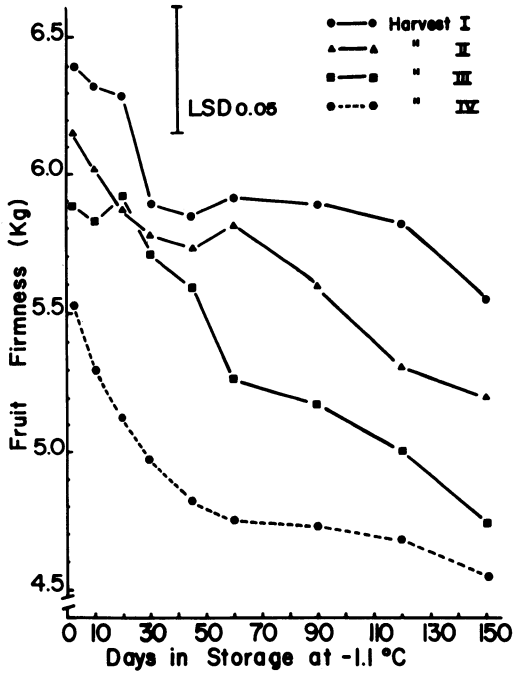

Fig. 1. Changes in flesh firmness during storage at –1.1°C of 'd'Anjou' pears harvested at 4 dates.

Ripening capacity, internal ethylene, and dessert quality. Daily average rates (DAR) of CO_2 production were 4.5–6.5 mg/hr-kg for all harvest dates at harvest and increased little until they had been stored for 20 days (Fig. 2). The DAR of C_2H_4 production were below detection limits (0.007 ppm) for Harvests I and II until 45 days of storage and for Harvests III and IV until 20 days of storage. The DAR of C_2H_4 production in Harvests I and II increased to 0.3–0.8 µl/hr-kg after 60 days of storage, while those in Harvests III and IV increased to about 1 µl/hr-kg after 30 days of storage. If the DAR of C_2H_4 production was undetectably low during the "ripening" period at 20°C, there was also no climacteric rise in respiration, and the fruit softened to about 3.5 kg (7–8 lb.) without developing a desirable dessert quality. Late-harvested fruit (i.e., Harvests III and IV) required a shorter period of postharvest chilling (3, 10, 15) to develop the ripening capacity as compared with the fruit harvested at optimum maturity (i.e., Harvests I and II) (Fig. 2). The development of ripening capacity

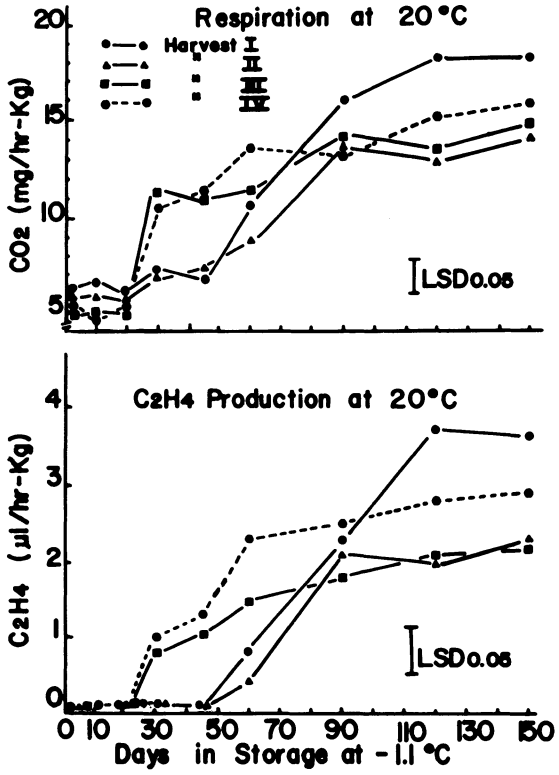

Fig. 2. Changes in daily average rates of CO_2 and C_2H_4 of 4 harvest dates of 'd'Anjou' pears after being stored at –1.1°C for 9 intervals and ripened at 20°C.

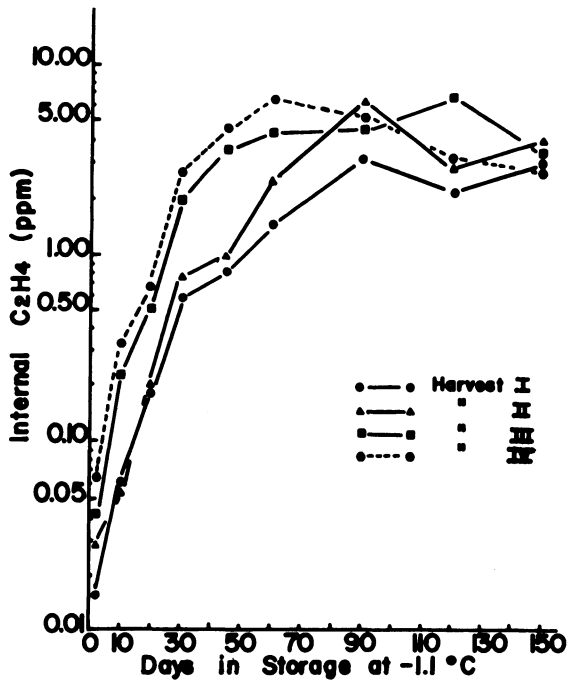

Fig. 3. Changes in internal ethylene concentration during storage at –1.1°C of 'd'Anjou' pears harvested at 4 dates; LSD 5% = 1.13 ppm.

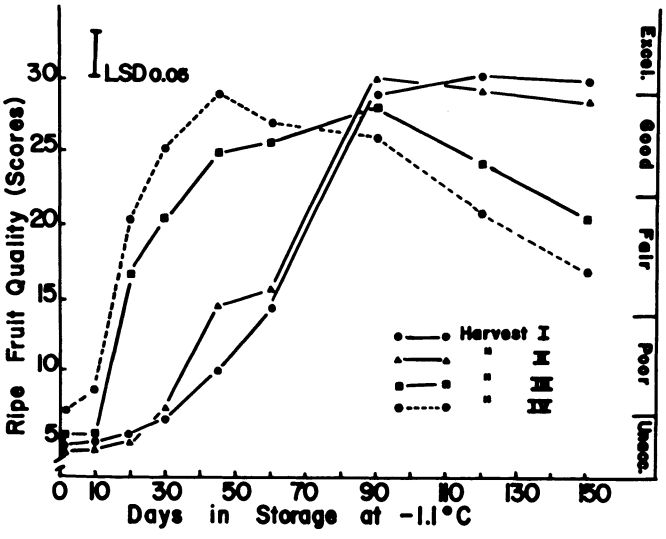

Fig. 4. Changes in dessert quality of 'd'Anjou' pears harvested at 4 dates after being stored at –1.1°C for 9 intervals and ripened at 20°C. (Excel. = Excellent; Unacc. = Unacceptable).

was directly related to the accumulation of internal C_2H_4 in the fruit during the storage period. Internal C_2H_4 concentration in the fruit increased exponentially to 3–5 ppm during 30–60 days at –1.1°C and was constant thereafter (Fig. 3). When the internal C_2H_4 concentration reached 1.5–2.0 ppm (Fig. 3), the fruits were capable of ripening normally at 20°C (Fig. 2). After this ripening capacity had been developed there was no longer a consistent relationship between harvest date and either DAR of CO_2 or C_2H_4, or internal C_2H_4 concentrations (Figs. 2 and 3).

Earlier development of the ripening capacity in Harvests III and IV was also associated with a faster increase in dessert quality as compared with Harvests I and II (Fig. 4). After 30 days of storage, fruit from Harvests III and IV developed a buttery texture, moderate juiciness, and a good flavor upon ripening.

Although the fruit of Harvests I and II also developed ripening capacity after 60 days of storage, the dessert quality ratings were much lower than those of Harvests III and IV (Fig. 4) because ripe fruit of Harvests I and II remained firm in texture and lacked in flavor. The dessert quality of the fruit of Harvests III and IV, ripened after 90 days of storage, declined rapidly because of a rapid development of soft and coarse texture, while the quality of Harvest I and II ripened to excellent quality ratings after 90 to 150 days of storage (Fig. 4).

Since the late-harvested fruit require less chilling than optimum-harvested fruit to develop the ripening capacity for good dessert quality, they offer a potential for early marketing. However, their rapid decline in dessert quality after 90 days' storage indicated that they cannot be stored for the extended periods.

Ethanol insoluble matters, titratable acids, and soluble solids. Ethanol insoluble matters (EIM) decreased from 3.2% fresh weight for Harvest I to 1.9% for Harvest IV at harvest (Fig. 5) with differences among harvests being greater than corresponding firmness differences (Fig. 1). During storage, EIM of Harvest I and II rapidly declined to the level of 2.1–2.2% within 30 days while EIM of Harvests III and IV decreased to the level of 1.8–1.9% within 20 days at –1.1°C.

The decrease in EIM content during the harvest period might be due to a rapid cell enlargement and a decrease in cell wall thickness which correlate with a decrease in flesh firmness (8, 9). However, a decrease in the starch content during the harvesting period might also account for the decrease in EIM content. Since EIM content of Harvests I and II declined about 0.7–1.8% as comparted to a decrease of 0.1–0.3% in Harvests III and IV within 30 days of storage (Fig. 5), starch-to-sugar conversion in 'd'Anjous' might have occurred early in storage. Therefore, the higher EIM content in fruits of Harvest I and II at harvest might be due to a higher starch content in those fruits. Li (5) reported that starch in 'd'Anjou' was not detectable by the iodine method after 30 days in storage.

Titratable acids (TA) content at harvest was decreased from 5.5 for Harvest I to about 4.5 meq/100 ml juice for Harvests II, III, and IV with little relationship to the different harvest dates (Fig. 6). During the 150-day storage period, TA of Harvests I and IV declined with time so that later harvested fruit tended to have less TA after a given length of storage.

Soluble solids increased from 12.1 g/100 ml for Harvest I to about 13.1 g/100 ml juice for Harvests III and IV at harvest, then rapidly stabilized at about 13.2 g/100 ml juice for all harvest dates within 45 days in storage; they remained at that level with little change throughout the 150-day storage period (Fig. 7).

The results demonstrated that EIM and flesh firmness are 2 useful indicators of fruit maturity at harvest and during the storage period. TA and soluble solids, on the other hand, varied among the harvested groups and therefore were not a reliable measurement of the fruit maturity. However, TA and soluble solids in the fruit vary with season, location, and preharvest temperatures (7). 'd'Anjou' with high acid and sugar contents at harvest have a better postharvest quality than those with low acid and sugar contents (7). The relationship between the organic substances in 'd'Anjou' and their postharvest quality merits further study.

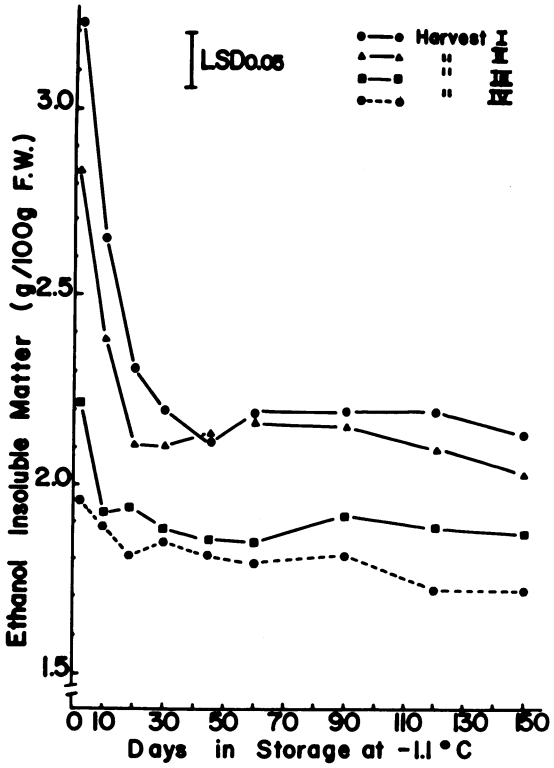

Fig. 5. Changes in ethanol insoluble matters during storage at −1.1°C of 'd'Anjou' pears harvested at 4 dates.

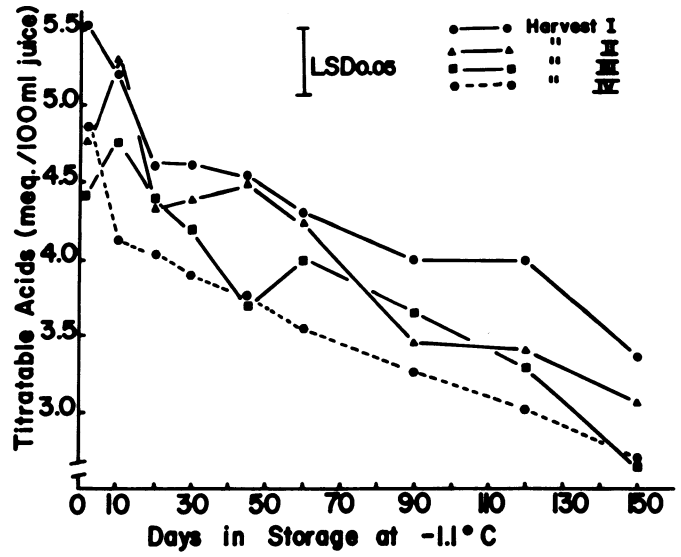

Fig. 6. Changes in titratable acids during storage at −1.1°C of 'd'Anjou' pears harvested at 4 dates.

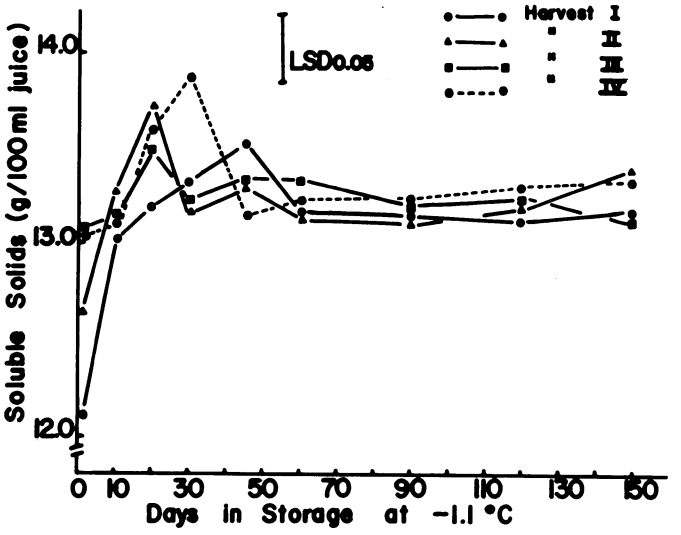

Fig. 7. Changes in soluble solids during storage at −1.1°C of 'd'Anjou' pears harvested at 4 dates.

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Regeneration of Plants from Callus-derived Protoplasts of *Salpiglossis*[1]

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Additional index words. Salpiglossis sinuata, tissue culture

Abstract. **Callus cells were used as the cell source for isolating protoplasts of *Salpiglossis sinuata* L. Callus was initiated on the cut edges of leaf sections cultured on Uchimiya and Murashige (UM) medium under 24 $\mu Em^{-2}s^{-1}$ at 30°C. Friable callus from subcultures on UM was subjected to an enzymatic solution of 1% Driselase, 0.75% Pectinase, 2% Cellulase R-10, and 8% mannitol in CPW salts, and incubated at 50 rpm, for 4–5 hr to release protoplasts. Following washing, counting, and dilution, protoplasts were plated in liquid Murashige and Skoog medium (MS) modified by deleting the ammonium ions and adding (mg/liter): 250 L-glutamine, 0.1 L-serine, 2.0 thiamine, 0.5 indoleacetic acid (IAA), 1.0 2,4-D, 0.5 6-benzylamino purine (BA) and 9% mannitol. Within 2–5 days, cell wall synthesis and the first cell division occurred. Green-yellowish colonies, 2 to 3 mm in diameter, formed in 2.5 months and were transferred to MS + 1.0 mg/liter N^6-isopentenyladenine (2iP), where shoot primordia were evident within 21 days. After full development and elongation of shoots, they were dipped in 1000 ppm indolebutyric acid (IBA) and placed in MS + 0.001 mg/liter 2,4-dichlorophenoxyacetic acid (2,4-D) to initiate roots. Rooting was carried out at 22±2°C under 80 $\mu Em^{-2}s^{-1}$ Cool White fluorescent tubes. Nine of 10 regenerated plants examined cytologically were $2n = 44$, normal for the species, and 1 plant was $2n = 88$.**

The potential for crop improvement through protoplast technology has been widely discussed (5,20,23), and significant advances have been made in the last decade. These advances in *in vitro* methodology and their eventual application are intended to make significant contributions in plant breeding.

To date, the majority of plant species that have been successfully regenerated from isolated protoplasts are food, drug, and ornamental crops in the Solanaceae. These include *Atropa, Datura, Lycopersicon, Nicotiana, Petunia* and *Solanum* (20). A summary of all plant genera regenerated from protoplasts is tabulated in the recent review by Thomas et al. (20).

Leaf explants of *Salpiglossis sinuata* are highly morphogenetic (15), and callus regenerates on an extensive range of hormones (2). Our preliminary results on regeneration of *Salpiglossis* from protoplasts have been reported (3), and herein are presented the complete details on the methodology, cytology of regenerated plants, and the potential use of *Salpiglossis* in somatic hybridization.

Materials and Methods

Seedling cultures. Plants to provide leaves as a cell source for protoplasts were obtained from seeds sown at weekly intervals on V.S.P. (Bay Houston Towing Co.) and maintained at 21±2°C, 64 $\mu Em^{-2}s^{-1}$ (G.E. Cool White fluorescent tubes) for 16 hr per day. The seedlings were transplanted into plastic cell packs, and vegetative growth continued in the greenhouse under natural photoperiod supplemented with 550 $\mu Em^{-2}s^{-1}$ for 16 hr daily. A minimum night temperature of 22±2°C was maintained; daytime temperature fluctuated with the season. The plants were fertilized 3 times weekly at a rate of 200 ppm N from 20N–8.6P–16.6K. Diseases and insects were controlled according to standard cultural procedures. Fully expanded leaves were obtained from the lower portion of 60- to 90-day-old seedlings. These were surface-sterilized with a 7% Clorox solution (5.25% NaOCl) for 30 min followed by 6 separate rinses with sterile distilled water.

Detached leaves were allowed to become flaccid after sterilization,

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