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Some Effects of Daylength and Flower Manipulation on the Floral Cycle of Two Cultivars of Avocado (*Persea americana* Mill., Lauraceae), A Species showing Protogynous Dichogamy

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ABSTRACT

Floral cycling of the avocado cultivars Fuerte and Hass was observed under controlled conditions of 25 °C and daylengths of 0 h, 1 h (08.00 h–09.00 h), 6 h (08.00 h–14.00 h), 6 h (13.00 h–19.00 h), 12 h (08.00 h–20.00 h) and 24 h with a photon flux density of 350 μmol m^{-2} s^{-1} (400–700 nm). Normal floral cycling of discrete opening periods of female stage, followed by closed phase, followed by male stage, occurred under all conditions except 24 h in Fuerte, and 0 h and 24 h in Hass. Under continuous light the cycle was disrupted and female and male stages opened throughout the day, and very few Hass flowers opened at all. No male stage Hass flowers opened under continuous darkness. Under the shorter daylengths the floral cycle was condensed, and the timing of the cycle shifted following alteration in the time of start of the light period. Emasculation of flowers in the female stage prevented reopening during the period of opening of the male stage flowers in Fuerte but not in Hass. The effect was localized to the perianth parts adjacent to the removed anther or stamen.

Key words: Flowering; Avocado; Dichogamy.

INTRODUCTION

Flowering in angiosperms is a complex process involving floral induction (Evans, 1971), initiation (Jackson and Sweet, 1972), interaction between environment and genetics (Murfet, 1977), the development of sex expression (Heslop-Harrison, 1972) and in some cases vernalization (Purvis, 1961) and photoperiodism (Vince-Prue, 1975). Recent studies on floral physiology have concentrated on pollination biology (Knox, 1984), and on the role of ethylene and its precursors on corolla senescence (Gilissen and Hoekstra, 1984; Whitehead, Halevy, and Reid, 1984).

Flowers of many species open and close at definite times, often so that the period of flower opening coincides with pollen vector activity (Frankel and Galun, 1977). This is particularly important in outcrossing species where spatial, temporal or biochemical mechanisms may prevent self-fertilization. There have been relatively few studies on the control of the timing and period of flower opening, although an influence of the prevailing light and dark periods is often involved (Matile, 1958; Arnold, 1959; Buggeln, Meeuse, and Klima, 1971; Kaihara and Takimoto, 1981). In this paper we report on the effects of daylength and floral manipulation on the floral cycle of the avocado, an outcrossing species which shows protogynous dichogamy (Robinson and Savage, 1926).

The avocado flower is hermaphrodite (Plate 1A) and consists of three sepals, three petals, nine stamens in three whorls, six nectaries arranged in pairs at the base of each of the inner whorl of stamens, three staminodes which also secrete nectar and a pistil with a single ovule (Reece, 1939). Each flower opens twice. On first opening the flower is in the female stage (Plate 1B). In this stage the pistil is the most conspicuous organ of the flower as the anthers are reflexed against the petals and have not yet dehisced to release their pollen. Pollination of the pistil in this stage results in fertilization and fruit set. After a few hours in the female stage the flower closes completely and reopens the following day in the male stage (Plate 1C). The stamens are now the most conspicuous organs of the flower as the filaments have extended to a more upright position and the anthers have dehisced to release their pollen. The pistil is now obscured by the stamens, and supports reduced pollen tube growth which does not result in fruit set (Sedgley, 1977*b*). The flower then closes again and does not reopen. The nectaries and staminodes secrete in both the female and male stages. There are complementary flowering types to allow for cross-pollination (Plate 1D). In type A cultivars, such as Hass, the female stage flowers open in the morning. They close during the afternoon, night and following morning and reopen in the male stage in the afternoon of the following day. In type B cultivars, such as Fuerte, the female stage flowers open in the afternoon. They close overnight and reopen in the male stage the following morning. As new flowers are opening each day, the type A cultivar is functionally female in the morning and male in the afternoon, and vice versa for a type B cultivar, so pollen transfer can occur between the two. The effects of altered daylength and floral manipulation on flower opening of both flowering types of avocado are reported.

MATERIALS AND METHODS

The experiments were conducted on 15 three-year-old plants of each of the avocado cultivars Fuerte and Hass grafted onto seedling rootstocks from a single parent tree. The plants were approximately one metre tall and trained to three main shoots.

The plants were grown under glasshouse conditions of mean daily maximum temperature $28{\cdot}0 \pm 0{\cdot}5$ °C and minimum $14{\cdot}8 \pm 0{\cdot}3$ °C for the period of the experiment. The daylength was $10{\cdot}9 \pm 0{\cdot}1$ h with a mean photon flux density of $534{\cdot}2 \pm 86{\cdot}5$ μmol m^{-2} s^{-1} (400–700 nm) at midday. Flowers were observed for one week under glasshouse conditions after which the plants were transferred to a growth cabinet with a continuous temperature of 25°C, and variable daylength with a photon flux density of 350 μmol m^{-2} s^{-1} (400–700 nm) from Philips TLMF140 W fluorescent lamps. The daylength treatments were 0 h, 1 h (08.00 h–09.00 h), 6 h (08.00 h–14.00 h), 6 h (13.00 h–19.00 h), 12 h (08.00 h–20.00 h), and 24 h. Two plants of each cultivar were kept under each daylength and flowering was observed for two weeks, except the 0 h treatments where the plants remained for one week only. The plants were then returned to the glasshouse and the flowers observed for a further week. The plants were left for two days to adapt to each new environment before observations were made. Up to 10 open flowers were labelled in the female stage with coloured cotton each day. The flowers were observed each day at 09.00 h, 11.00 h, 13.00 h, 15.00 h and 17.00 h and one day per week at the additional times of 07.00 h, 19.00 h and 21.00 h for subsequent closing and reopening in the male stage.

The remaining three plants of each cultivar were kept in the glasshouse throughout the experiment and observed at 2 h intervals throughout the day. Ten flowers were labelled with coloured cotton in the female stage and stamens, nectaries, staminodes or pistil were removed as shown in Table 2. Flowers were either left unpollinated or pollinated in the female stage by brushing a pollen-bearing valve of an anther of a male stage flower against the stigma. The flowers were observed for subsequent reopening.

RESULTS

In all plants normal floral cycling as shown in Plate 1D was observed under the glasshouse conditions both before and after the growth cabinet treatments. The two trees of each

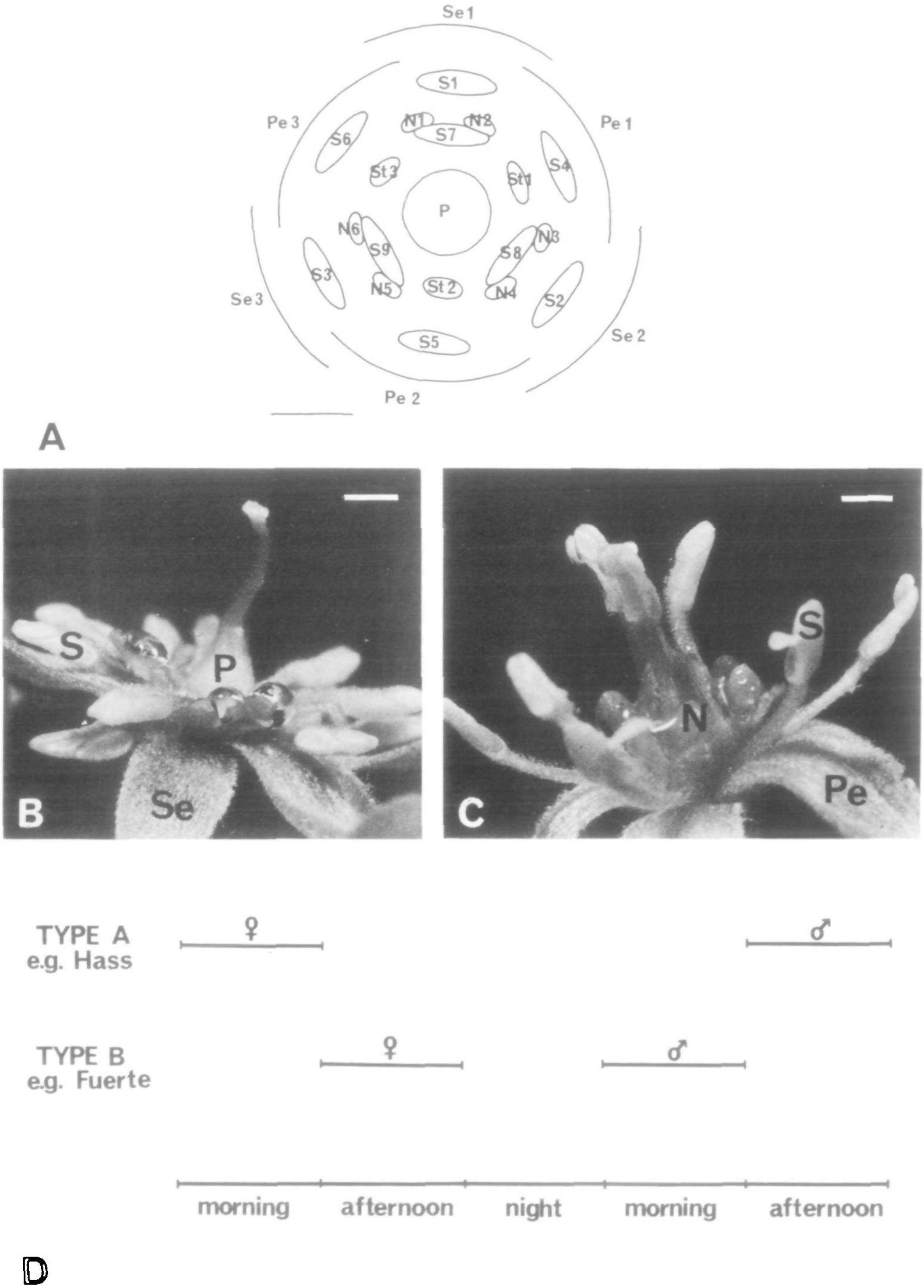

Plate 1. A. Floral diagram of avocado showing sepals (Se), petals (Pe), stamens (S), nectaries (N), staminodes (St) and pistil (P). Bar represents 5·0 mm. B. Avocado flower in female stage showing sepal (Se), stamen (S) and pistil (P). Bar represents 1·0 mm. C. Avocado flower in male stage showing petal (Pe), stamen (S) and nectary (N). Bar represents 1·0 mm. D. Daily floral cycle of avocado cultivars Fuerte and Hass.

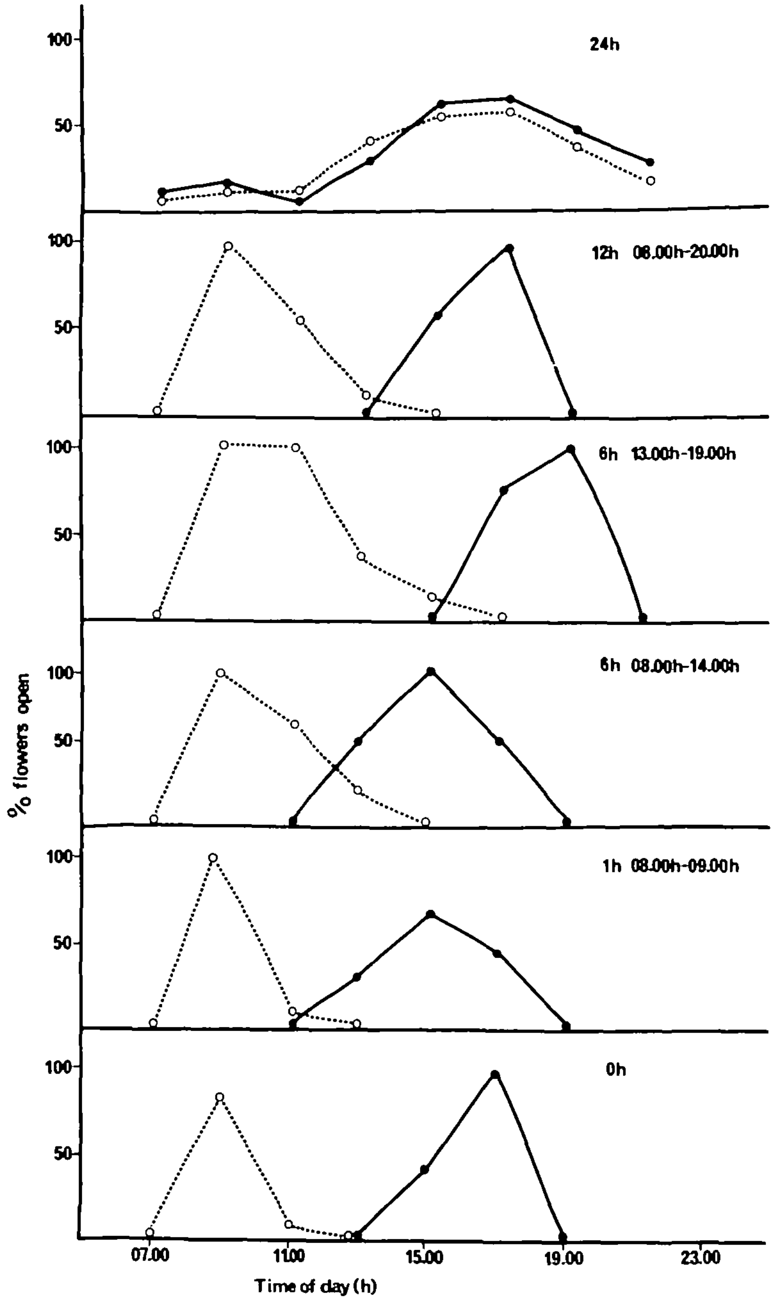

FIG. 1. Daily floral cycle of the avocado cultivar Fuerte under six different daylengths; ●——●, female stage; o-----o, male stage.

cultivar in each of the growth cabinet treatments performed similarly and results have been pooled.

In the cultivar Fuerte, the floral cycle of female stage in the afternoon followed by closed stage followed by male stage the next morning was observed under all daylengths except continuous light (Fig. 1). Under continuous light female and male stage flowers opened throughout the day including late evening and early morning. A minimum of 10 female stage flowers per plant opened each day under all conditions (Table 1), and all were observed to reopen in the male stage, except under continuous light. The peak opening of female stage Fuerte flowers occurred at 17.00 h under 0, 12 and 24 h daylengths and in the glasshouse. The peak opening time was advanced to 15.00 h under the 1 h (08.00–09.00 h) and 6 h (08.00–14.00 h) daylengths but was delayed to 19.00 h in the 6 h (13.00–19.00 h) treatment. The flowers reopened in the male stage between 15 h and 18 h after opening in the female stage, except under continuous light where they reopened 24 h later. The peak of male stage opening was 09.00 h except under 6 h (13.00 h–19.00 h) when the peak was extended to between 09.00 h and 11.00 h and under continuous light where the peak was at 17.00 h.

In the cultivar Hass, the floral cycle of female stage in the morning followed by closed period followed by male stage in the afternoon of the next day was observed under all conditions except continuous darkness and continuous light (Fig. 2). Female stage flowers opened throughout the day at 0 h and 24 h and male stage flowers opened during most of the day at 24 h. Under continuous darkness flowers were not observed to reopen in the male stage. A minimum of 10 female stage flowers per plant opened each day under all conditions except continuous light where very few flowers opened and many unopened buds were shed (Table 1). All flowers were observed to reopen in the male stage, except under continuous darkness and under continuous light. The peak opening of female stage Hass flowers was observed at 09.00 h under all conditions except 6 h (13.00 h–19.00 h) and 24 h. Under the 6 h (13.00 h–19.00 h) daylength peak opening occurred over an extended period of between 09.00 h and 11.00 h. There was no clearly defined peak under continuous light. The flowers opened in the male stage between 27 h and 30 h after opening in the female stage. The peak of male stage opening was at 15.00 h under 12 h and glasshouse conditions. This was advanced to 13.00 h under 1 h and 6 h (08.00 h–14.00 h) and delayed to between 15.00 h and 17.00 h under 6 h (13.00 h–19.00 h). There was some overlap of opening of female and male stage flowers of both cultivars, but particularly of Hass.

Removal of all of the nine stamens or anthers from the female stage flowers of the Fuerte cultivar prevented reopening of the flower during the opening period of male stage flowers (Table 2). Removal of up to six stamens did not prevent reopening provided a continuous whorl was left. If adjacent stamens were removed then the flowers reopened only partly. Removal of five adjacent stamens resulted in the opening of all except one sepal or petal. Removal of six adjacent stamens resulted in all except two perianth parts opening, and removal of seven or eight adjacent stamens in all except three perianth parts opening. Removal of nectaries, staminodes and pistil had no effect on reopening in the male stage. Flowers of the cultivar Hass reopened during the opening period of male stage flowers irrespective of the treatment received during the female stage. The results for both cultivars were the same in pollinated and unpollinated flowers. The ten flowers per treatment all behaved similarly.

DISCUSSION

The floral cycle of the avocado was sensitive to alteration of daylength and to floral manipulation, but the effects varied according to cultivar. The Hass cultivar (type A) was more sensitive to change of daylength whereas Fuerte (type B) was more sensitive to emasculation. Hass flowers failed to reopen in the male stage under continuous darkness and there was a drastic reduction in the number of flowers opening in both stages under continuous light. Fuerte, on the other hand, continued to flower profusely under all conditions. The reduction in the number of male stage flowers of both Fuerte and Hass observed under continuous light may be due to the flowers reopening during the night. Observations were not made

TABLE 1. *Number of flowers and time of peak opening of female and male stages of Fuerte and Hass flowers under six different daylengths*

Daylength (h)	Mean number of flowers scored per plant per day in the female stage		Mean number of flowers scored per plant per day in the male stage		Time of peak opening of female stage after start of light period (h)		Time of peak opening of female stage after start of dark period (h)		Time of peak opening of male stage after peak opening of female stage (h)	
	Fuerte	Hass	Fuerte	Hass	Fuerte	Hass	Fuerte	Hass	Fuerte	Hass
0	10·0	10·0	10·0	0	—	—	—	—	16	—
1 (08.00 h–09.00 h)	10·0	10·0	10·0	10·0	7	1	6	24	18	28
6 (08.00 h–14.00 h)	10·0	10·0	10·0	10·0	7	1	1	19	18	27
6 (13.00 h–19.00 h)	10·0	10·0	10·0	10·0	6	21	24	15	15	30
12 (08.00 h–20.00 h)	10·0	10·0	10·0	10·0	9	1	21	13	16	30
24	10·0	2·9	7·3	1·5	—	—	—	—	24	30
Mean	10·0 ± 0·0	8·8 ± 1·2	9·6 ± 0·5	6·9 ± 2·0	7·3 ± 0·6	6·0 ± 5·0	13·0 ± 5·6	17·8 ± 2·4	17·8 ± 1·3	29·0 ± 0·6

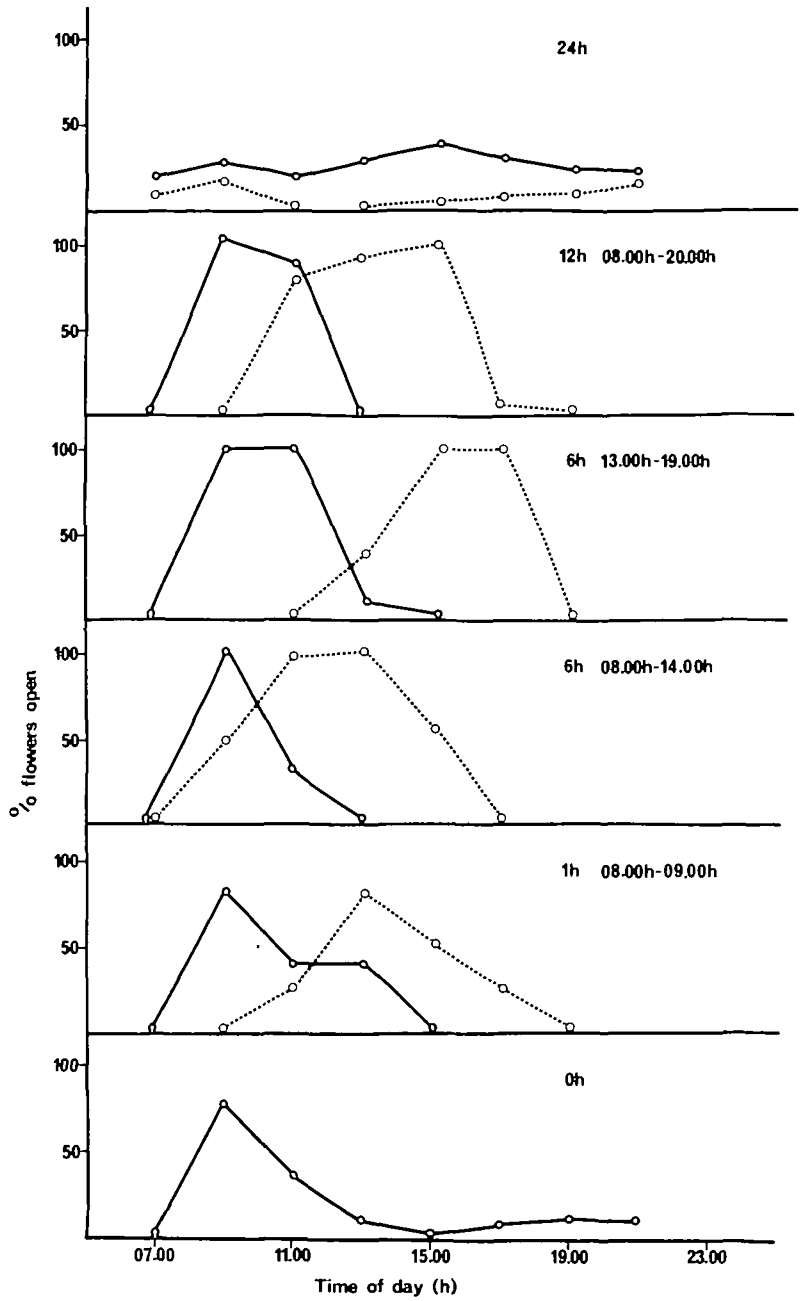

FIG. 2. Daily floral cycle of the avocado cultivar Hass under six different daylengths; o——o, female stage; o-----o, male stage.

TABLE 2. *Effect of manipulation in the female stage on opening during the male stage of the avocado flower*

Flower parts removed in female stage (refer Plate 1A)	S1-9	S7-9 or S1-6	S2-3 and S5 and S8-9	S2-3 and S5-6 and S8-9	S2-6 and S8-9 or S2-9	P or N1-6 or St1-3
Male stage Fuerte flower	closed	open	Pe2 closed rest open	Pe2 and Se3 closed rest open	Pe2 and Se2 & 3 closed rest open	open
Male stage Hass flower	open	open	open	open	open	open

during the night as avocado flowers normally open only during the day (Bergh, 1974). In both cases the disruption was temporary as normal floral cycling was resumed when the plants were returned to the glasshouse conditions.

The avocado is a subtropical species so the 12 h daylength would be similar to that experienced in the native habitat. In addition successful flowering and fruit set have been obtained under similar controlled environment conditions (Sedgley, 1977*a*; Sedgley and Annells, 1981). Under the shorter daylengths of 1 h and 6 h (08.00 h–14.00 h) the floral cycle of both cultivars was condensed somewhat. The peak of female stage opening of Fuerte was advanced by two hours to 15.00 h and the peak of male stage opening of Hass was similarly advanced to 13.00 h. When the 6 h daylength was changed from 08.00 h–14.00 h to 13.00 h–19.00 h the floral of both cultivars shifted accordingly. A similar phenomenon was observed in *Oenothera* by Arnold (1959) where reversal of day and night resulted in a reversal of the period of flower opening. The flowers of both avocado cultivars tended to remain open for longer periods under this treatment. This may be due to the greater alteration in the timing of the start of the light period than under the other conditions. Alternatively it may have been caused by the short periods of light received during the observation periods every two hours during the day. Although observations on plants in dark conditions were kept as short as possible, it may be that this brief exposure to light could have affected the results.

Continuous light disrupted the timing of the floral cycle of both Fuerte and Hass and continuous darkness prevented male stage flowering of Hass. Thus the transition between light and dark appears to be important in the control of anthesis in avocado. Table 1 shows the timing of peak opening of female stage flowers of both cultivars as related to the start of both the light and the dark periods. Female stage opening of the Fuerte cultivar occurred between 6 h and 9 h following the start of the light period whereas the relationship with the start of the dark period was very variable. In the Hass cultivar the opening of the female stage flowers appeared to be more related to the start of the dark period although both relationships were variable. It is possible that there may be a fundamental difference in the nature of the control between the two cultivars. Evidence in the literature varies on which transition may be important in the control of anthesis in other species. Matile (1958) working with *Arum italicum* reported that the transition from darkness to light was the trigger for anthesis. In *Sauromatum guttatum* (Buggeln *et al*., 1971) and *Pharbitis nil* (Kaihara and Takimoto, 1981) the transition from light to dark was found to be effective. Investigations were not conducted into which wavelengths of light were effective in flower opening.

Emasculation of Fuerte flowers in the female stage prevented opening of the flower during the period of male stage opening irrespective of whether or not the flower had been pollinated. The effect was localized to the perianth members adjacent to the removed stamen and removal of other floral parts had no effect on flower opening. It appears that some component of the anther may control the reopening of the flower in the male stage. Gibberellin has been shown to stimulate anthesis in cleistogamous flowers of *Lamium amplexicaula* (Lord and Mayers, 1982) and has been implicated in the induction of maleness in some species (Chailakhyan, 1979). Further work is needed to establish the nature of the control in avocado. Emasculation had no effect on subsequent opening of Hass flowers. The Hass floral cycle is longer than the Fuerte cycle and the period between the closing of the female stage flowers and opening of the male stage flowers is approximately twice as long. It is possible that resynthesis of control factors, or transport from other parts of the plants may have occurred during this period. There was no anatomical difference between the flowers of the two cultivars which could have accounted for the difference in response. There are a number of reports in the literature on cases of altered sex expression due to environmental changes or to mutilation, infection or parasitism of the plant (Heslop-Harrison, 1972). In all cases, however, the effects are relatively long term in that treatment during floral development results in a change at anthesis. In the case of the Fuerte avocado the suppression of the male stage opening is a much shorter term effect which has not been described previously.

The avocado floral cycle is a remarkably precise mechanism but it is also very sensitive to external factors. In addition to the perturbations caused by altered daylength and floral manipulation, the mechanism is disrupted by adverse temperatures. Under day and night temperatures of 17 °C and 12 °C respectively, five out of six type B cultivars studied had few or no female stage flowers, whereas all five type A cultivars had normal but extended floral cycles (Sedgley, 1977*a*; Sedgley and Annells, 1981; Sedgley and Grant, 1983). This difference in response of the two flowering types to a number of external factors suggests a physiological difference in the control of flower opening within the one species.

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