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Avocado Breeding Research in Australia

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The CSIRO Division of Horticultural Research has been breeding avocados on a small scale for about 10 years *(Sedgley& Alexander 1978, Sedgley, Alexander & Skene 1980).* The aim is to produce improved varieties of both scions and rootstocks for the wide range of climates encountered in Australia. There are a number of characters under assessment. At present, the major selection criterion is time of fruit maturity, as there is a need for year-round availability of fruit to maintain consumer interest. Other scion characters of importance are precocity yield, and consistency of bearing plus various fruit characteristics including overall size and shape, color and ease of peeling of the skin seed size and separation from the flesh, and flesh flavor, quality, and oil content Frost tolerance and time of flowering are also important characters' particularly in southern Australia. In addition, we are interested in" varieties which will tolerate the high temperatures of northern Australia Desirable rootstock characteristics include tolerance to salinity, *Phytophthora cinnamomi,* and frost, plus ease of propagation and effects on vigor, tree shape, and tree size.

Methods used in avocado breeding have been described by Bergh (1975) At the CSIRO we are using the three major techniques for avocado variety improvement which are variety introduction, open pollination, and controlled pollination, all followed by field selection for desirable horticultural characteristics.

Variety introduction involves the importation of avocado cultivars developed overseas and their assessment under Australian conditions. There is much more to be done here as importation has been very slow over recent years due to the problems with indexing for avocado sunblotch viroid. The recent acceptance of the DNA probe technique which gives results within weeks *(Palukaitis et. al. 1981)* should improve considerably the flow of imported material. Quarantine for one year is currently required for observation for the Black Streak problem. In particular, there is a deficiency in Australia of West Indian avocado germplasm. There are many excellent West Indian-type varieties in Florida which could form the basis of an industry in northern Australia, as well as providing a source of salt tolerance for southern areas. In effect, we in Australia are currently lacking approximately a third of the available avocado germplasm. Cultivars recently released from CSIRO quarantine include AKKO J7 and AKKO F3 from Israel, Espanta and Haes 7315 from Hawaii, Fortuna from Brazil, and Jim and Marshelline from California.

Avocado flowers are functionally dichogamous and there are complementary flowering types A and B. The floral cycle of female stage followed by closed stage followed by male stage is very sensitive to temperature. In the variety Fuerte, the cycle operates well at a daytime temperature of 25°C; but at 33°C, vegetative growth appears to be stimulated at the expense of reproductive development, and flowers and young fruit are shed *(Sedgley 1977a).* At a daytime temperature of 17°C, there are very few female stage flowers, as the majority of flowers open once only, in the male stage. This is particularly disturbing as the flower must be pollinated in the female stage for successful fruit set *(Sedgley 1977b).* Fuerte is effectively female sterile at a temperature of 17°C. The Hass variety is much more tolerant than Fuerte of adverse temperatures during flowering *(Sedgley& Annells 1981).* There is less flower and fruit shed at 33°C, and the flowers have a female stage at 17°C. However, the length of the floral cycle is doubled at 17°C, and there is poorer pollen tube and embryo growth than at 25°C.

Observations from the south Queensland coast area that Sharwil may show an adverse response to low temperature *(A. Whiley, personal communications)* stimulated research into a wider range of avocado varieties grown in Australia *(Sedgley & Grant 1983).* Five type A and six type B varieties have now been investigated. The type A varieties Hass, Reed, Wurtz, Rincón, and Jalna all show a similar response to low temperature during flowering (Fig. 1). All have some female-stage flowers and all have an extended floral cycle. However, most of the female-stage flowers open during the night, so the chances of insect pollination are reduced. Ants have been observed visiting avocado flowers at night and night-flying moths may also visit, but honeybees are generally accepted to be the major pollinators of avocado flowers *(McGregor 1976).* The six type B varieties studied are Fuerte, Bacon, Ryan, Edranol, Sharwil, and Hazzard (Fig. 2). Bacon is the only variety with reasonable numbers of female-stage flowers at 17°C. Fuerte has less than 10% of the flowers with a female stage, and the other varieties have none. As with the type A varieties, the length of the floral cycle is considerably lengthened at 17°C as compared with 25°C. Pollen tube growth is poor in all varieties at 17°C, but only in those with reasonable numbers of female stage flowers were the ovules penetrated by a pollen tube (Table 1). The varieties Fuerte, Ryan, Edranol, Sharwil, and Hazzard were effectively female-sterile. Peterson (*1956)* observed that type B varieties tend to be less productive under cool flowering conditions than type A cultivars, and this is one of the reasons for a changeover from Fuerte to Hass in California *(Tourney 1981).* The data suggest that there may be some linkage between the type B genotype and disruption of the floral cycle by low temperature. This is a character which needs to be considered in the breeding program for varieties for southern Australia.

Because of the sensitivity to temperature of the avocado floral cycle and the need to exclude pollinating insects, the hand pollinations for the breeding program are carried out in a temperature-controlled glasshouse. Our plants are all in pots, which has the advantage of mobility and flexibility for manipulation of plants. The facility to have trees rooted in the ground within the glasshouse would have the advantage of higher fruit production but reduced flexibility. Flowers are labelled with colored cotton, and a few pollinated each day during the flowering period as they develop. The flowers are not emasculated or bagged as the pollen does not become airborne, and the dichogamy mechanism prevents self pollination. Anthers are removed from the prospective male parent with fine forceps, and pollen is transferred to the stigma of the female by gently brushing a recurved pollen-bearing valve of the anther against the stigma. Pollen from one anther valve is sufficient to effect successful pollination, so many female-stage flowers can be pollinated from the 9 anthers of one male-stage flower. All varieties tested so far are cross- and self-compatible as measured by pollen tube growth and ovule penetration (Table 2), *(Sedgley 1979a).* There is evidence, however, that the Fuerte variety has a higher proportion of defective embryo sacs when compared with the Hass variety (Table 3), *(Sedgley 1979b).* This results in a lower proportion of fertilized embryo sacs following pollination.

Avocado cultivars do not all flower at the same time *(Alexander 1975),* so a grafting technique has been developed to ensure synchronous flowering. Budwood which has differentiated floral buds is collected from field-grown trees before the late-spring flush. It can be stored at 4°C for up to four months and is then bottle-grafted (approach grafted with about 15 cm of graft union) when required to mature stock plants which have been previously disbudded and topped to produce a build-up of carbohydrate. These grafts will flower after a few weeks and, because of the maturity of the stock and the large graft area, may carry fruit to maturity when pollinated. Thus early and late-flowering varieties can be manipulated to flower synchronously for crossing, and the pollination season can also be extended. In the temperature-controlled glasshouse, the avocado plants flower during June to August. An additional method for extending the period over which pollinations can be carried out is to keep some plants outside over winter and then bring them into the glasshouse for pollination during the natural flowering period in southern Australia of August to October. This period can be further extended to January using the bottle-grafting technique. Thus, it is possible to pollinate avocados over 6 months of the year. A further strategy for breeding is pollen storage. Avocado pollen has a very low viability and requires refrigeration and desiccation for storage even for one month *(Sedgley 1981).* However, the pollen can be stored from one year to the next in liquid nitrogen, although its viability is reduced. It is advisable to use fresh pollen wherever possible.

One of the major problems in hand pollination of avocado is the low flower to fruit ratio. Millions of flowers may be produced by an avocado tree, but a good crop is measured in thousands of fruit. Flowering and fruit set can be improved by girdling (*Trochoulias& O'Neill 1976)* but still, most of the hand-pollinated fruit are shed before fruit and seed maturity. Research has shown that all of the fruits shed one month after flowering have a normal embryo and endosperm and there are no anatomical abnormalities *(Sedgley 1980).* To increase the yield of hybrids, an *in vitro* culture method has been developed so that the shed immature embryos can be saved *(Skene & Barlass 1983).* Embryos removed from fruits after six weeks post-pollination are placed on a medium which stimulates shoot production from the embryonic axis. These shoots are then micrografted to a stock plant and will subsequently grow as a normal graft, after protection for a few weeks by the upper leaves of the stock enclosed in a small plastic bag. The plants are kept shaded. The immature embryos can also be grown on their own roots, but the induction of roots is inconsistent and the micrografted plants grow much more quickly.

The hybrid avocado progeny are maintained in a temperature-controlled glasshouse. Material is grafted onto viroid-tested seedling stocks and planted in the field for assessment. In addition, some mature trees have been topworked to the progeny in an effort to induce earlier fruiting and to economize on field space. So far, we have a total of 130 hybrid progeny of known parentage involving the varieties Fuerte, Hass, Jalna, Edranol, Bacon, Hall, Reed, Zutano, Rincon, Ryan, Hazzard, Wurtz, a Guatamalan-type seedling, and two early-maturing Mexican-type seedlings. Some of the earliest hybrids have flowered; but due to the recent hot summers and cold winter, they have not produced any fruit for assessment so far. The severe frosts experienced in the Mildura region during the 1982 winter killed many of the grafted progeny, so the material will have to be regrafted and replanted and assessment will be delayed. Some of the hybrid progeny survived the frost, and the severity of damage has been recorded (Table 4). The results are difficult to compare, as some progeny were small trees having been planted for varying lengths of time, and some were topworked and so much higher from the ground. Nevertheless, the results give an indication of frost tolerance. Most of the unaffected or slightly damaged hybrids had parents which have a reasonably high level of frost tolerance such as Bacon, Edranol, Fuerte, Ryan, Hass, and Jalna *(Toohill & Alexander 1979).* However, two of the parents, Reed and Hall, have low frost tolerance, so the tolerance of the hybrids must have been inherited from the other parents, Jalna and Bacon. This observation is of particular interest, as one of the aims of the breeding program is to combine the frost tolerance of the Mexican race with the salinity tolerance of the West Indian race for rootstocks for southern Australia. Ultimately, the hybrids will be assessed for all characters of interest.

We believe that the prospects for avocado breeding in Australia are good. The main disadvantage is that our numbers of progeny are low. This is because we have restrictions on the availability of land and manpower. However, some overseas varieties, such as Hass, were selected from quite small populations. The avocado is a relatively unselected crop species as it is new to large-scale cultivation, and we feel that the variability in fruit characteristics observed in the progeny assessed so far indicates plenty of scope for further selection. In addition, the production by controlled hand-pollination of families of avocado plants of known parentage offers Australia a unique opportunity to refine the rather hit-and-miss process of avocado breeding. The inheritance of characters of interest can be followed from the parents through subsequent generations, so that future crosses can be made on a more rational basis. We also hope to use the hybrid progeny to look for possible vegetative markers, so that we can select for desirable fruit characters without having to wait for the progeny to flower and fruit. One way of doing this is to use iso-enzyme analysis by starch gel-electrophoresis. The electrophoresis techniques have already been applied to avocado in the USA *(Torres & Bergh 1978a & b, 1980, Torres et al. 1978)* and we hope to use them on our progeny.

The success of the avocado breeding program depends upon the support of the Australian avocado industry. We are interested hi developing new varieties for all areas of Australia, but we have only limited facilities for the testing of advanced selections. We hope that growers and Research Stations around Australia will co-operate with us in the testing program so that any potential new Australian varieties have the best chance of development.

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REFERENCES

Alexander, D.McE. (1975) — Flowering times of avocados in the Murray Valley. Journal of the Australian Institute of Agricultural Science 41: 264-267.

Bergh, B.O. (1975) — Avocados in 'Advances in Fruit Breeding' ed. J. Janick and J. N. Moore. (Purdue University Press - Indiana) pp. 541-567.

Bergh, B.O. and Whitsell, R.H. (1974) — Self-pollinated Hass seedlings. California Avocado Society Yearbook 57: 118-126.

Bergh, B.O. and Whitsell, R.H. (1975) — Self-pollinated Fuerte seedlings. California Avocado Society Yearbook 58: 128-134.

McGregor, S. E. (1976) — Insect pollination of cultivated crop plants. U.S.D.A. handbook No. 496.

Palukaitis, P., Rakowski, A.G., Alexander, D.McE.,and Symons, R.H. (1981) — Rapid indexing of the sunblotch disease of avocados using a complementary DNA probe to avocado sunblotch viroid. Annals of Applied Biology 98: 439-449.

Peterson, P.A. (1956) — Flowering types in the avocado with relation to fruit production. California Avocado Society Yearbook 1956: 174-179.

Sedgley, M. (1977a) — The effect of temperature on floral behaviour, pollen tube growth, and fruit set in the avocado. Journal of Horticultural Science 52:135-141.

Sedgley, M. (1977b) — Reduced pollen tube growth and the presence of callose in the pistil of the male floral stage of the avocado. Scientia Horticulturae 7: 27-36.

Sedgley, M. (1979a) — Inter-varietal pollen tube growth and ovule penetration in the avocado. Euphytica 28: 25-35.

Sedgley, M. (1979b) —Light microscope study of pollen tube growth, fertilization, and early embryo and endosperm development in the avocado varieties Fuerte and Hass. Annals of Botany 44: 353-359.

Sedgley, M. (1980) — Anatomical investigation of abscissed avocado flowers and fruitlets. Annals of Botany 46: 771-777.

Sedgley, M. (1981) —Storage of avocado pollen. Euphytica 30: 595-599.

Sedgley, M. and Alexander, D.McE. (1978) — Breeding avocados in Australia. Fruit Varieties Journal 32: 7-8.

Sedgley, M. and Annells, C.M. (1981) — Flowering and fruit-set response to temperature in the avocado cultivar 'Hass'. Scientia Horticulturae 14: 27-33.

Sedgley, M. and Grant, W.J.R. (1983) — Effect of low temperatures during flowering on floral cycle and pollen tube growth in nine avocado cultivars. Scientia Horticulturae 18: 207-213.
Sedgley, M., Alexander, D.McE., and Skene, K.G.M. (1980) — Methods used in avocado breeding. International Plant Propagators' Society Proceedings 30: 575-578.
Skene, K.G.M. and Barlass, M. (1983) — *In vitro* culture of abscissed immature avocado embryos. Annals of Botany in press.
Toohill, B.C. and Alexander, D.McE. (1979) — Frost tolerance of 19 avocado cultivars in the Mildura region of the Murray valley. Journal of the Australian Institute of Agricultural Science 45: 196-199.
Torres, A.M. and Bergh, B.O. (1978a) — Isozymes as indicators of outcrossing among 'Pinkerton' seedlings. California Avocado Society Yearbook 63: 103-110.
Torres, A.M. and Bergh, B.O. (1978b) — Isozymes of 'Duke' and its derivatives. California Avocado Society Yearbook 63: 111-117. Torres, A.M. and Bergh, B.O. (1980) — Fruit and leaf isozymes as genetic markers in avocado. Journal of the American Society for Horticultural Science 105: 614-619.
Torres, A.M., Diedenhofen, U., Bergh, B.0.,and Knight, R.J. (1978) — Enzyme polymorphisms as genetic markers in the avocado. American Journal of Botany 65: 134-139.
Tourney, J. (1981) — Once popular Fuerte hits bottom. Avocado Grower 5(6): 11-12.
Trochoulias, T. and O'Neill, G.M. (1976) — Girdling of 'Fuerte' avocado in subtropical Australia. Scientia Horticulturae 5: 239-242.

TABLE 1. Percentage of ovules penetrated by a pollen tube in eleven avocado cultivars at 17°C day and 12°C night.

Flowering type	Cultivar	Percentage of ovules penetrated by a pollen tube
A	Hass	32
A	Reed	8
A	Wurtz	23
A	Rincón	3
A	Jalna	1
B	Fuerte	0
B	Ryan	0
B	Edranol	0
B	Sharwil	0
B	Hazzard	0
B	Bacon	1

TABLE 2. Some compatible combinations of avocado cultivars as measured by pollen tube growth.

	Pollen Source								
	Edranol	Ryan	Fuerte	Bacon	Reed	Talbot	Jalna	Hass	Sharwil
Edranol	X	X	X	X	X	X	X	X	NT
Fuerte	X	X	X	X	X	X	X	X	NT
Bacon	X	NT	X	X	NT	X	NT	X	NT
Reed	X	X	X	X	X	X	NT	NT	NT
Jalna	X	NT	X	X	NT	X	X	X	X
Hass	X	X	X	X	X	X	X	X	NT

TABLE 3. Fertilization of Fuerte and Hass embryo sacs up to 48 h after pollination.

	Fuerte	Hass
Percentage of ovaries with an embryo sac penetrated by a pollen tube	18	64
Percentage of fertilized embryo sacs	20	75
Percentage of disorganized embryo sacs	60	25

TABLE 4. AVOCADO HYBRID PROGENY FROST DAMAGE — Assessed December 1982

No Damage	Number of hybrids	Some leaf damage	Number of hybrids
JALNA X FUERTE	4	JALNA X FUERTE	6
JALNA X BACON	1	JALNA X BACON	1
JALNA X HALL	1	EDRANOL X FUERTE	3
EDRANOL X FUERTE	1	EDRANOL X HASS	1
TALBOT X FUERTE	1	RYAN X HASS	2
		BACON X REED	1
		BACON X HASS	1
		BACON X JALNA	1
		BACON X HALL	1
		HASS X FUERTE	1

FIGURE 1

Floral cycle of the type A avocado cultivars Hass, Reed, Wurtz, Rincon, and Jalna at 25°C and 17°C.

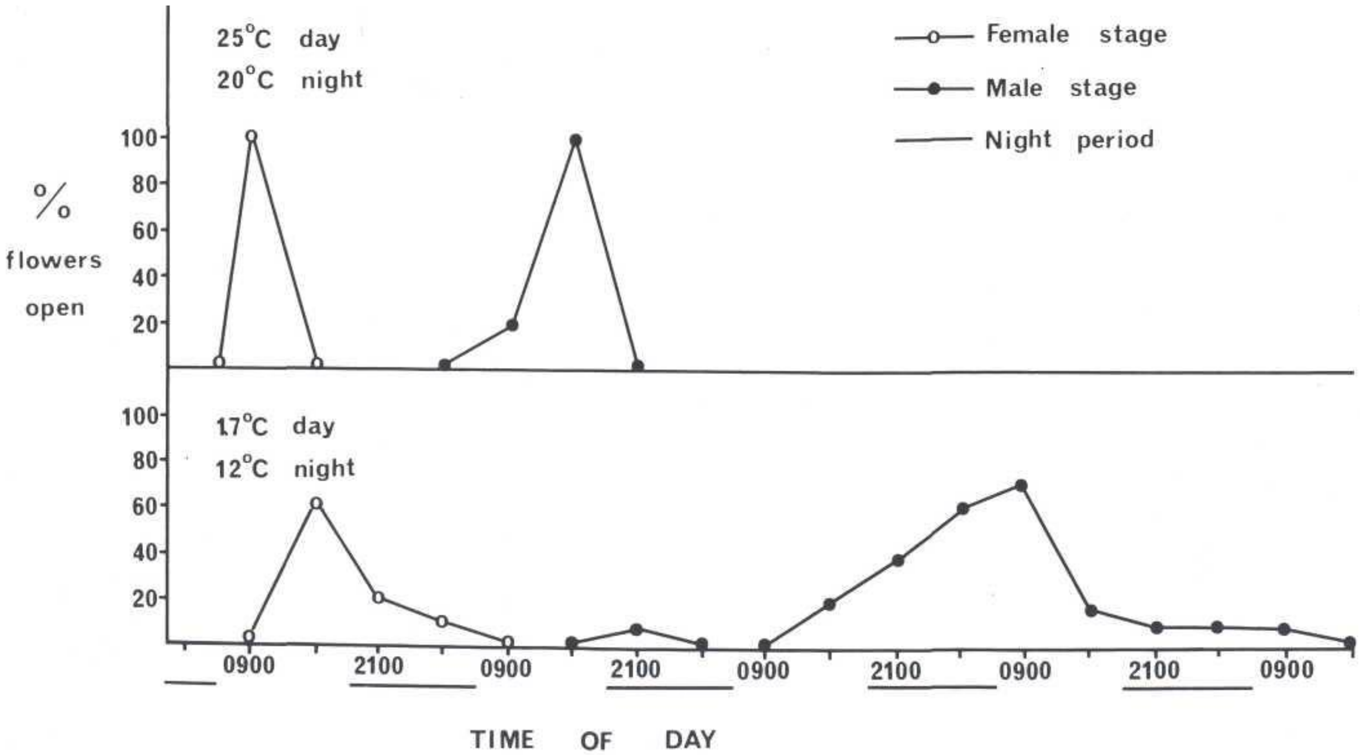

FIGURE 2
Floral cycle of the type B avocado cultivars Fuerte, Bacon, Ryan, Edranol, Sharwil, and Hazzard at 25°C and 17°C.