

# 6 Reproductive Biology

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Avocado, like all vascular plants, has two distinct developmental phases, juvenile and adult. In the juvenile phase, floral inductive signals fail to initiate floral development. The plant is incompetent with regard to sexual reproduction and restricted to vegetative growth. The juvenile phase of avocado can exceed 15 years before seedling trees flower. However, breeding programmes have produced progeny that flower and set fruit in dramatically shorter periods of time. For example, progeny of 'Pinkerton' and 'Gwen' have flowered and set fruit within 2 years of seed planting (Bergh and Lahav, 1996). Cues that trigger the transition from the juvenile to the adult phase in avocado remain elusive. This problem is circumvented in the establishment of commercial orchards with trees propagated by grafting scion buds from adult trees on to seedling rootstocks. Such trees are fully responsive to internal and external signals that initiate floral development after a short vegetative adult phase of 2–5 years, depending on the cultivar. Despite the fact that without flowers there are no fruit, regulation of the annual transition of the shoot apical meristem (SAM) from vegetative to floral development resulting in inflorescence and flower formation in apical and axillary (lateral) buds is poorly understood in avocado. Major advances in deciphering the genetic regulation of flowering in the herbaceous model plant *Arabidopsis thaliana* have made possible the identification of key genes involved in avocado

floral development pathways (Chanderbali *et al.*, 2006, 2009), and have set the stage for rapid progress in elucidating the internal and external signals regulating flowering in avocado.

Avocado trees flower profusely, producing 100,000 to >1,000,000 flowers (Bergh, 1986). Even with many flowers, yield is typically low. Many developmental events must successfully occur between flower formation and fruit harvest – pollination, pollen germination, pollen tube growth through the mid-stylar region to the ovule, syngamy, embryo development and seed formation. The fruit must survive three naturally occurring abscission periods, the vagaries of climate and short-comings in cultural management practices, which interact to affect every step in the process. The observation of Goldschmidt and Monselise (1977) for citrus aptly applies to avocado – the number of fruit persisting to harvest is so small relative to the number of flowers produced that in essence the entire flower population abscises except for a negligible portion. Indeed, avocado fruit set ranges between 0.001 and 0.23%, depending on cultivar and year (Cameron *et al.*, 1952; Bergh, 1986; Inoue and Takahashi, 1990). Thus, avocado has earned the reputation as a shy and erratic bearer. Annual production remains woefully below its theoretical potential yield of 32.5 t ha<sup>-1</sup> (Wolstenholme, 1987) in all avocado-producing countries. Even excellent growers are unable to sustain this level of production and yields have averaged less than

10 t ha<sup>-1</sup> for the past decade in most countries (Garner and Lovatt, 2008). Still, as a general rule, avocado yield is proportional to floral intensity, making the capacity to regulate flowering an important factor in managing productivity (Garner and Lovatt, 2008).

Changes in global climate have already impacted avocado production worldwide. Avocado industries have suffered from winter pre-bloom freezes with increased frequency, droughts of greater duration, record flooding and excessively high temperatures during fruit set. In many instances, the loss of the crop initiated alternate bearing, production of a light, low yield 'off' crop followed by a heavy, high yield 'on' crop 1–2 years later, depending on how quickly the trees recovered from the initial stress. Once initiated, alternate bearing cycles become entrained by endogenous tree factors that ultimately impact floral intensity and, hence, yield. Alternate bearing has become a problem of sufficient magnitude that a renewed research effort is underway in several avocado-producing countries to obtain basic information that will lead to a practical solution. The results of this research have and will continue to contribute to our understanding of floral development in avocado, in relationship to fruit development and avocado tree phenology under the varied climatic conditions in which the avocado is grown commercially.

In many countries, climate change and population growth increasingly jeopardize the availability of high quality irrigation water at an affordable price. Fertilizer prices have steadily risen worldwide. The costs of land and labour have also increased for many growers. It is imperative that growers reduce the cost of inputs and/or increase yield per hectare on an annual basis to increase and stabilize grower income and industry sustainability. Knowledge of avocado reproductive biology at every step in the process is fundamental to increasing flowering, fruit set and yield, and for mitigating alternate bearing to achieve these goals.

## Flowering

### Environmental factors that promote flowering

The physiology of flowering in perennial fruit trees has received relatively little attention.

According to Mullins *et al.* (1989), the reasons are clear. First, the large size and morphological complexity of trees makes them difficult subjects for experimental work in controlled environments. Second, the rate of experimentation in the field is slow: seedlings of most woody perennials have a protracted juvenile phase during which they are incapable of flowering, and adult sexually mature (reproductively competent) individuals normally produce flowers only once a year. Third, most temperate, subtropical and tropical tree crops do not conform to the well-established models for regulation of flowering; they are neither photoperiodic nor thermoperiodic and environmental responses, when they occur, are far from clear-cut.

Floral induction is where an environmental stimulus, most commonly photoperiod or temperature, leads to floral initiation. Often, interactions between environmental stimuli and endogenous developmental cues exert some control over floral initiation (Wilkie *et al.*, 2008). Different environmental factors are considered to be perceived by different parts of the plant. Photoperiod and irradiance are perceived mainly by mature leaves. Temperature is perceived by all plant parts, although low temperature (vernalization) is mainly perceived by the shoot apex (Bernier *et al.*, 1993). Water availability is perceived by the root system (Davies and Zhang, 1991). Since the different factors that promote flowering are perceived by different parts of the plant, it is implied that the fate of the apical meristem is controlled by an array of long-distance signals that are integrated within the intact plant to initiate a common sequence of events leading to commitment to flowering (Bernier *et al.*, 1993).

There is evidence that low temperature and short day length stimulate flowering in avocado. A growth chamber study done by Buttrose and Alexander (1978) with 'Fuerte' avocado demonstrated that the absence of high temperature (e.g. > 20°C) rather than the actual low temperature was responsible for inflorescence initiation. In their study, the effect of short day length was not qualitatively associated with floral initiation but it hastened the time to flowering.

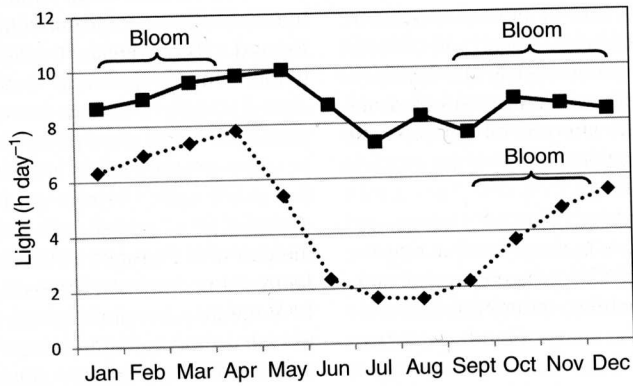
The response of 'Hass' avocado to day length imposed under controlled environment conditions in a growth chamber, as described

above, appears to be different from the response attained in nature. For example, in San Vicente de Cañete, Lima, Peru ( $13^{\circ}05' S$ ), due to coastal fog, the number of clear, sunny days during winter-spring is reduced (less than 3 h of light per day; Fig. 6.1), causing an intense and extended period of bloom, composed mostly of determinate axillary inflorescences (Fig. 6.2). In contrast, in many avocado producing regions of Mexico, such as Uruapan, Michoacán ( $19^{\circ}25' N$ ), the common type of inflorescence is indeterminate, probably a result of longer day lengths (Fig. 6.1).

Davenport (1986) mentioned that for avocado cultivars better adapted to warmer climates (West Indian-Guatemalan race hybrids), day length might play a more important role in inducing flowering than low temperature. However, recent results support the association between cool temperatures ( $\leq 20^{\circ}C$ ) and flowering in the 'Choquette' and 'Booth-8' cultivars

in the semi-warm climate of Nayarit, Mexico (Cossio-Vargas *et al.*, 2007; Salazar-García *et al.*, 2008).

In another study, Nevin and Lovatt (1989) used low-temperature stress to promote flowering in 'Hass' avocado. They subjected the trees to a low-temperature treatment of 8-h days (photosynthetically active radiation =  $500 \mu E m^{-2} s^{-1}$ ) at  $15-18^{\circ}C$ ; and 16-h nights at  $10-13^{\circ}C$ . Flowering intensity was the same for 'Hass' trees receiving 4 or 8 weeks of low-temperature treatment and inflorescence development, followed the same timetable with the exception of flower opening, which was 1 week later in trees receiving 8 weeks of low-temperature treatment. This study and a later one carried out in controlled conditions confirmed that 'Hass' avocado requires no more than four weeks of low-temperature stress to induce flowering (Salazar-García *et al.*, 1999).



**Fig. 6.1.** Average number of hours with sunlight per day by month in Uruapan, Michoacán, Mexico (Northern Hemisphere; solid line) and Cañete Valley, Lima, Peru (Southern Hemisphere; dotted line).



**Fig. 6.2.** Intense production of determinate inflorescences in 'Hass' avocado is favoured by short day length in the Cañete Valley, Lima, Peru.

Studies using controlled environmental conditions, like those mentioned above, are useful to explain physiological responses found under field conditions. A 2-year study done in commercial 'Hass' avocado orchards in the semi-warm climate of Nayarit, Mexico, confirmed that accumulation of 27.5 days at temperatures  $\leq 19^{\circ}\text{C}$  resulted in flowering of apical buds on winter and summer flush shoots (Salazar-García *et al.*, 2006). This amount of 'chilling' was equivalent to the 4-week low-temperature treatment previously reported to cause flowering of 'Hass' avocado in growth chambers (Nevin and Lovatt, 1989; Salazar-García *et al.*, 1999).

'Hass' is the most cultivated avocado worldwide (see Crane *et al.*, Chapter 8, this volume) and new orchards are being established in areas with no previous existence of this crop. Presence of environmental conditions that ensure flowering is a challenge in new areas. Data from avocado-producing areas of the American Continent indicate that a decrease in temperature is associated with the successful transition to flowering of 'Hass' avocado at different latitudes (Fig. 6.3). Differences in temperature patterns during the year between the Northern and Southern Hemispheres are evident. Monthly maximum temperatures in the Northern Hemisphere fluctuate from  $17.3^{\circ}\text{C}$  (San Luis Obispo, California) to  $32.1^{\circ}\text{C}$  (Peribán, Michoacán, Mexico). For the minimum temperatures, averages in most regions vary between  $5$  and  $15^{\circ}\text{C}$ , except for Xalisco, Nayarit, Mexico, where minimum temperatures are between  $15$  and  $20^{\circ}\text{C}$  in the summer (June to September) (Fig. 6.3a). For the Southern Hemisphere, average monthly maximum temperatures range from  $17^{\circ}\text{C}$  (Quillota, Chile) to  $32.9^{\circ}\text{C}$  (Tucumán, Argentina), which are very similar to the Northern Hemisphere (Fig. 6.3b).

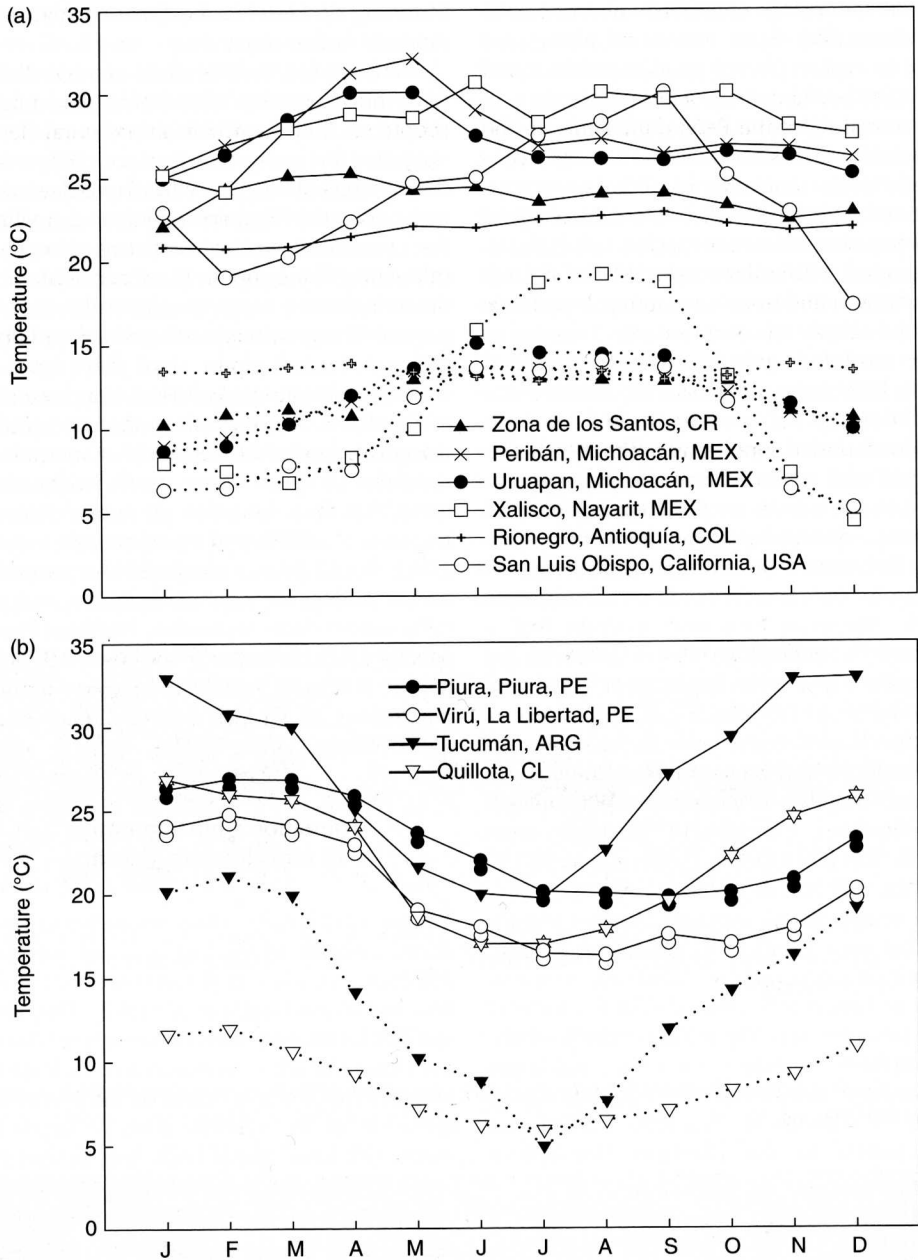
There are two new avocado-producing regions in the coastal desert of Northwest Peru, Virú ( $8^{\circ}28'$  S) and Piura ( $5^{\circ}12'$  S), which have 'unusually' warm temperatures for 'Hass' avocado trees. In these regions, monthly averages for both maximum and minimum temperatures vary less than  $1^{\circ}\text{C}$ . Monthly averages for minimum and maximum temperatures throughout the year fluctuate from  $15.9$  to  $24.7^{\circ}\text{C}$  (Virú) and  $19.2$  to  $26.9^{\circ}\text{C}$  (Piura) (Fig. 6.3b). The physiology and phenology of

flowering of 'Hass' under these environments deserves further research.

Information on the effect of water-deficit stress on promotion of avocado flowering is limited. A study by Chaikiattiyos *et al.* (1994) suggested that neither constant nor cyclic water-deficit stress during 12 weeks (pre-dawn leaf water potential ( $\Psi_{\text{leaf}}$ ) decreased to  $-2.1$  MPa in the constant stress treatment) was effective in stimulating flowering in 'Hass' avocado scions on an unknown rootstock. Water-deficit stress prevented vegetative growth and delayed flowering of treated plants until they were re-watered. Subsequent severe leaf drop, browning of shoot tips, and necrosis of the leaf tips and margins indicated the sensitivity of avocado to water-deficit stress. These results make sense given that floral initiation in 'Fuerte' (Osuna-Enciso *et al.*, 1985) and 'Hass' (Salazar-García, 2000; Rocha-Arroyo *et al.*, 2011a) avocados occurs during the summer and early autumn rainy season (June–September, Northern Hemisphere) and culminates in the so-called 'crazy bloom' in August–September in several producing regions of Mexico (Salazar-García *et al.*, 2005; Salazar-García, 2007).

### Transition from vegetative to reproductive growth

The period of transition from vegetative to reproductive growth in the primary axis meristem (PAM) of avocado was documented for the first time by Salazar-García *et al.* (1998). They used apical buds from summer flush shoots of 10-year-old 'Hass' avocado trees in a commercial orchard in southern California. Close to the end of the extension of the summer flush of vegetative shoots (23 July), apical buds had an external pointed shape and had a convex PAM and one or two young secondary axis inflorescence meristems (SAM) in the axils of bracts. By the end of July, the buds were pointed and closed. At this stage, a low convex shoot apical meristem with separated bracts was evident plus one to three SAMs. By 30 August, bud scales started to abscise. The PAM was convex again and four secondary axis meristems were present in the axils of inflorescence bracts. A decreased rate of SAM production was observed at this stage. Salazar-García *et al.* (1998) mentioned that the



**Fig. 6.3.** Average monthly temperatures in several 'Hass' avocado-producing regions in the Northern (a) and Southern (b) Hemispheres of the American Continent.

presence of a convex PAM indicated an active apex, producing either inflorescence bracts or leaf primordia. Secondary axis inflorescence meristems formed in the axils of bracts, just below

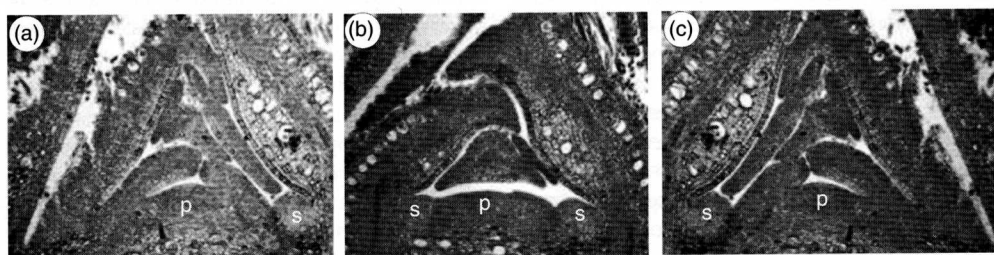
the PAM. These axillary meristems typically formed inflorescences. But, as reported for other species (Bernier *et al.*, 1981a), flowering in avocado is not obligatory at this point, but depends

on environmental conditions. After initiation of these secondary axis meristems the PAM becomes flattened and its activity decreases, but at no time was dormancy detected.

Flattening of the PAM during this period is associated with the potential for full transition from vegetative to reproductive development. A flattened meristem is associated with the transition to the reproductive condition in many species (Bernier *et al.*, 1981a). If environmental conditions promoting vegetative growth had prevailed, the growth of the apical two SAMs formed prior to the transition phase would have been suppressed by the production of new leaf primordia on the PAM. However, under conditions optimal for flowering, these meristems developed into the basal lateral cymes of the inflorescence and the other cymes were produced by the new activity of the PAM (Salazar-García *et al.*,

1998). The period of transition from the vegetative to reproductive stage was identified as S-1 (convex PAM), S-2 (low convex PAM) and S-3 (convex PAM) of the macro- and microscopic scales designed to assess floral development in 'Hass' avocado (Fig. 6.4) (Salazar-García *et al.*, 1998). The subsequent formation of additional secondary axis inflorescence meristems is consistent with commitment of the primary axis meristem to flowering.

In 'Hass' avocado the transition phase occurs early in the process of shoot development. It may take from 2 weeks to more than 4 months (Salazar-García *et al.*, 1998, 2006; Rocha-Arroyo *et al.*, 2010). In addition to orchard management practices, geographical factors, such as latitude and climatic conditions affect the time of emergence of vegetative flushes. Table 6.1 illustrates the periods of



**Fig. 6.4.** Transition from vegetative (a) to reproductive phase (c) in apical buds of 'Hass' avocado. Note a low convex primary axis meristem in b. Abbreviations: p, primary axis meristem; s, secondary axis inflorescence meristem.

**Table 6.1.** Time of emergence of vegetative flushes in several 'Hass' avocado-producing regions located at different latitudes.

Regions	J	F	M	A	M	J	J	A	S	O	N	D
Cambria, California, USA (35°37' N)												
Corona, California, USA (32°52' N)												
Tepic, Nayarit, Mexico (21°30' N)												
Michoacán, Mexico (18°45' -20°6' N)												
Zona de los Santos, Costa Rica (9°44' -9°32' N)												
Virú, La Libertad, Peru (8°28' S)												
Tucumán, Argentina (26°56' S)												
Quillota, Chile (32°54' S)												

Vertical cells, major flush (winter or spring), usually during or after bloom. Horizontal and dotted cells, secondary flushes.

occurrence of vegetative flushes in several avocado-producing countries of the American Continent.

In cultivars of warm climates the transition phase may take longer than in 'Hass'. In Nayarit, Mexico (21°28' N; 440 m), this developmental stage required 6 (April–October) and 3 months (July–October) for spring and summer shoots of 'Choquette', respectively. In the case of summer shoots of 'Booth-8', it took 4 months (July–November) (Cossio-Vargas, *et al.*, 2008a; Salazar-García *et al.*, 2008).

### Commitment to flowering

At a certain point after the transition from the vegetative to reproductive condition, the process is no longer reversible and the PAM is committed to flowering (McDaniel, 1994). In the avocado, only some apices flower; others continue the vegetative growth of the tree; thus, the primary axis meristem plays two roles, one is to produce inflorescence bracts and the other is to produce leaf primordia.

The study of early stages of avocado flowering is critical to the development of orchard management strategies to increase floral intensity or, alternatively, to promote vegetative shoot growth and decrease flowering. For such strategies to be successful, it is essential to know the time when shoot apical buds reach irreversible commitment to flowering (ICF), or floral determination. To prevent an inadvertent increase in vegetative shoot growth at the expense of flowering, cultural practices that can prevent flowering, like nitrogen fertilization, canopy pruning (hedging, topping), or application of some plant growth regulators, must be carried out after ICF.

Some years ago it was assumed that a PAM had reached commitment to flowering when the two apical bracts (microscopic level) were extended over the secondary axis inflorescence meristem, which is indicative of the initiation of the terminal flower in the triplet (Thorp *et al.*, 1994). However, the results of Salazar-García *et al.* (1998) showed that this stage occurs very late in the development of the avocado inflorescence, whereas commitment to flowering is an early event.

Low temperature (LT) is a factor known to inhibit or enhance floral initiation in avocado that can be used to identify an anatomical change

associated with commitment to flowering. Container-grown 'Hass' avocado trees were exposed to LT treatments of 10/7°C (day/night) to stimulate flowering and warm temperatures 25/20°C (day/night) to stop the process (Salazar-García *et al.*, 1999). This controlled environment study showed that control trees maintained under the warm temperatures and trees receiving 1 or 2 weeks of LT treatment did not flower; all growth was vegetative. Four weeks at 10/7°C (10-h day/14-h night) were sufficient for apical buds of the 'Hass' avocado to become committed to flowering; they produced 83% inflorescences and no vegetative shoot growth, the remaining buds remaining inactive. Anatomical sections of the apical buds at time zero revealed a convex PAM that had produced a secondary axis inflorescence meristem in the axil of each of two inflorescence bracts. At this time the secondary axis inflorescence meristems did not have apical bracts. During the 4 weeks of exposure to LT, no anatomical changes were observed in the primary axis meristem, despite the effects of the different lengths of the LT treatment on the developmental fate of the apical bud.

Macroscopic analysis of apical buds at the beginning and end of the LT treatments revealed that the buds had not visibly changed and remained pointed with closed scales (Salazar-García *et al.*, 1999). Thus, no anatomical differences were found between apical buds committed to flowering and those that were not. Axillary buds were less developed than the apical bud. At the start of the LT treatment, axillary buds bore no secondary axis inflorescence meristems in the axils of their inflorescence bracts, whereas apical buds had two (apical bracts were absent). After 4 weeks of LT treatment, axillary buds had produced one pair of secondary axis inflorescence meristems (with no apical bracts). Thus, after 4 weeks of LT treatment, axillary buds had caught up with apical buds both anatomically and developmentally since axillary buds were also committed to flowering at this time. Hence, based on their anatomy, it is possible to distinguish axillary buds that are fully committed to flowering from those that are not by the presence or absence of a single pair of secondary axis inflorescence meristems (no apical bracts).

Differences in avocado cultivar, latitude, climate and shoot age are factors that influence

tree phenology and affect the time and stage of development when apical buds become committed to flowering. In a 2-year study in Corona, California, apical buds of summer shoots of 'Hass' avocado became committed to flowering by 15 October (Salazar-García *et al.*, 1998). Partial senescence, abscission, and separation of the outermost bud scales of apical and axillary buds were the visual cue associated with the stage of inflorescence development when three or more secondary axis inflorescence meristems were formed (Fig. 6.5). This visual cue corresponds to Stage 3 of the visual scale developed by Salazar-García *et al.* (1998) and can be used in a commercial avocado orchard to estimate the proportion of buds committed to flowering versus those that are not.

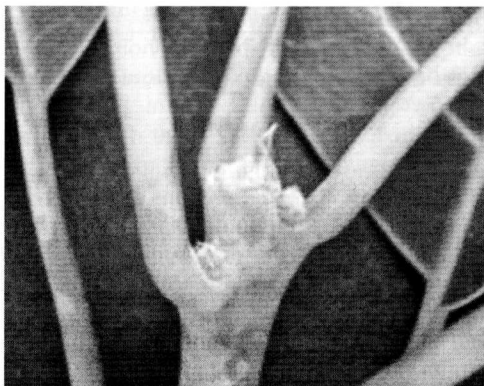
Shoot defoliation, alone or in combination with girdling, has also been used to study the time of commitment to flowering in avocado. Leaves maintain buds in a paradormant condition. Defoliation removes this growth-inhibiting factor, forcing rapid bud growth; hence its utility as a tool for imposing timed responses. The main effect of girdling is to prevent the influx of phloem-translocated, floral-stimulating signals (Bernier *et al.*, 1981b; Bernier, 1988) produced in other portions of the tree into experimental branches. In a branch that has been both defoliated and girdled, buds are forced to grow and express their developmental state without the influence of incoming developmental signals. Thus, defoliation and girdling can serve as convenient tools to determine the time of ICF in avocado under field conditions. If flowering occurs on defoliated

and girdled shoots, buds would have been irreversibly committed to flowering at the time of treatment, that is, capable of initiating floral morphogenesis using their own 'developmental programme'.

A shoot defoliation study conducted at two locations in California showed differences in the date that autumn flush shoots emerged and reached ICF. In the Irvine orchard (south of Los Angeles) autumn shoots emerged 1 month earlier than in Somis (north of Los Angeles). However, in Irvine, apical buds needed 45 days more to have 30% of buds committed to flowering, compared to Somis, which required only 15 days (Table 6.2). Cooler temperatures in Somis (annual average maximum/minimum temperature = 24.2/7.3°C) than in Irvine (annual average maximum/minimum temperature = 24.2/10.3°C) could partially explain these differences, as discussed by Rocha-Arroyo *et al.* (2010). Buds that had reached ICF in both locations of California were at Stage 3 of the visual scale of Salazar-García *et al.* (1998).

Shoot defoliation and girdling were used to determine the time when apical buds borne on winter and summer shoots of non-irrigated 'Hass' avocado orchards under the subhumid, semi-warm climate of Nayarit, Mexico (annual average maximum/minimum temperature = 28.7/14.3°C) reached ICF (Salazar-García *et al.*, 2006). Both types of shoots were defoliated (Year 1) or defoliated and girdled (Year 2) at different stages of bud development from September to January in each case. Irrespective of the time of treatment or shoot age, ICF of apical buds occurred by 15 October and in both years this stage was associated with an average of 27.5 days at temperatures  $\leq 19^\circ\text{C}$ . Buds irreversibly committed to flowering were graded at Stage 3 of the Salazar-García *et al.* (1998) scale that corresponds to closed and pointed buds with partial senescence of bud scales.

In another study, shoot defoliation and girdling were used to determine the influence of climate, irrigation and shoot age (winter, spring and summer flushes) on the date apical buds of 'Hass' avocado reached ICF in Michoacán, Mexico (Rocha-Arroyo *et al.*, 2010). Climate influenced the date of ICF on shoots from the three flushes studied, which occurred earlier in cooler climates (Table 6.3). Winter shoots (oldest) required more time after emerging to achieve ICF, compared to spring and summer



**Fig. 6.5.** External characteristics of 'Hass' avocado buds irreversibly committed to flowering.



**Table 6.2.** Type of growth produced by autumn shoots of 'Hass' avocado trees in response to shoot defoliation in two orchards in California. Evaluations were done at full bloom: Irvine (17 April 2004) and Somis (2 April 2004). (S. Salazar-García, and C.J. Lovatt, unpublished).

Location	Defoliation date (2003)	Bud development <sup>b</sup>	Type of growth (% of total shoots) <sup>a</sup>		
			Floral <sup>c</sup>	Vegetative	Inactive
Somis, Ventura County (34°16'55.78"N, 119°2'44.52"W)	22 Oct.	1.0	0c <sup>d</sup>	100a	0b
	6 Nov.	2.8	40b	60b	0b
	21 Nov.	3.8	80a	0d	20a
	Control		70ab	30c	0b
Irvine, Orange County (33°43'10.84"N, 117°44'26.18"W)	19 Sept.	1.0	0b	100a	0a
	4 Oct.	2.0	0b	90a	10a
	19 Oct.	2.5	10b	90a	0a
	3 Nov.	2.7	10b	90a	0a
	18 Nov.	2.9	30ab	70ab	0a
	3 Dec.	4.0	50a	50b	0a
	Control		50a	50b	0a

<sup>a</sup>Average of 10 tree replications for each defoliation date and 20 tree replications for the control.

<sup>b</sup>According to the scale of Salazar-García *et al.* (1998).

<sup>c</sup>All floral shoots were indeterminate.

<sup>d</sup>Mean separation within columns for each location by Duncan's multiple range test,  $P = 0.05$ .

**Table 6.3.** Irreversible commitment to flowering (ICF) in 'Hass' avocado as affected by climate and shoot age in Michoacán, Mexico. (Adapted from Rocha-Arroyo *et al.*, 2010.)

Climate / avg. temp.	Type of shoot								
	Winter			Spring			Summer		
	DOE <sup>a</sup>	ICF <sup>b</sup>	BD <sup>c</sup>	DOE	ICF	BD	DOE	ICF	BD
Subhumid warm (21.4°C)	9 Jan.	23 July (195)	3.6	14 Apr.	26 Sept. (165)	5.0	24 June	7 Sept. (75)	4.1
Subhumid semi-warm (19.8°C)	15 Jan.	29 June (165)	3.6	13 Apr.	28 May (45)	2.9	26 July	9 Sept. (45)	3.0
Humid semi-warm (16.6°C)	9 Jan.	23 June (165)	3.5	24 Apr.	7 Aug. (105)	4.6	23 July	6 Sept. (45)	2.3
Subhumid temperate (17.1°C)	15 Jan.	30 May (135)	3.6	12 Apr.	26 June (75)	3.4	13 July	28 July (15)	1.4

<sup>a</sup>Date of emergence.

<sup>b</sup>Days to irreversible commitment to flowering. Numbers in brackets are average shoot age (days) to beginning of ICF.

<sup>c</sup>Stage of bud development according to the visual scale of Salazar-García *et al.* (1998).

shoots (youngest). Shoots from the three vegetative flushes, attained ICF 29–42 days earlier in non-irrigated (rainfed) than in irrigated orchards. Although affected by shoot age, the predominant stage of bud development at the time of ICF was between 3 and 4 on the visual scale of Salazar-García *et al.* (1998). However, for summer flush shoots it was evident that some buds reached ICF at Stages 1 or 2

(Table 6.3). Anatomical sections of these buds showed the same developmental stages characteristic of the transition from vegetative to reproductive development illustrated in Figure 6.4, but this transition occurred much faster and buds looked visually smaller which resulted in a lower number on the visual scale.

There is little information on ICF for avocado in warm climates. A recent study used

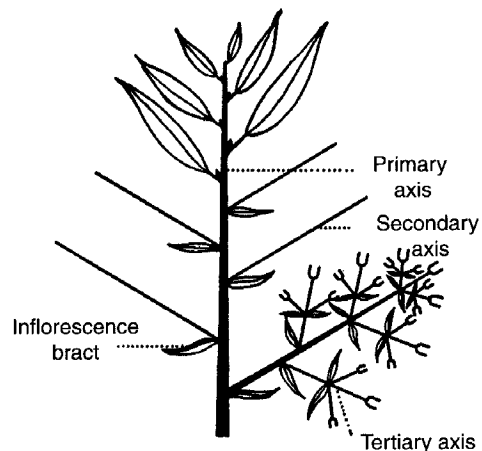
shoot defoliation and girdling to establish the date when ICF occurred on apical buds of spring and summer shoots of 'Choquette' and 'Booth-8' avocados cultivated under rainfed conditions (annual rain 1237 mm) (Salazar-García *et al.*, 2008). The study was done in the subhumid, warm climate (average temperature maximum/minimum 30.1/15.4°C; 442 m above sea level) of Jalcocotán, Nayarit, Mexico (21°28' N; 105°5' W). The date of ICF for the 'Choquette' avocado was 15 November and there were no differences due to shoot age (spring or summer shoots). For 'Booth-8', ICF of summer shoots occurred by 30 November. Macroscopic characteristics of apical buds irreversibly committed to flowering were closed and pointed buds with partial senescence of bud scales with averages of 3.0 to 3.4 of the Salazar-García *et al.* (1998) visual scale, which agreed with previous studies of the 'Hass' avocado.

### Floral development

To assess the process of floral development in a fast and reliable way, Salazar-García *et al.* (1998) developed a scale depicting both macroscopic (external) and microscopic (anatomical) features of both bud and floral shoot development, from the vegetative stage (Stage 1) to anthesis (Stage 11). This information was integrated for use in the field by avocado researchers and orchard managers (see Colour Plate 9). Stages 1 to 3 correspond to the transition from the vegetative to reproductive phase. Floral initiation starts at S-4 and buds are characterized by separation of bud scales revealing the expanding inflorescence bracts; almost all secondary axis inflorescence meristems can be present, with the basal secondary axes showing bracts. Initiation of the flower perianth occurs at S-5, first in the terminal flowers on secondary and tertiary axes. Buds at S-6 have a round shape and expansion of inflorescence bracts is evident. At this stage, the secondary axes of the inflorescence are totally formed and each one bears several cymes of flowers (tertiary axis of the inflorescence). Additional features of this stage are that terminal flowers on both secondary and tertiary axes have a

complete perianth; anthers and gynoecium are at early formation. At S-7, bud break occurs; the flowers are more developed although ovule and pollen grains are not yet fully formed. In S-8 ('cauliflower stage'; Lovatt, 1994), the elongation of the secondary axes of the inflorescence occurs. Pollen and ovule formation is still ongoing. At S-9, the elongation of the tertiary axes (cymes) of the inflorescence is observed. Pollen and ovule development is complete. In indeterminate floral shoots, the vegetative bud emerging at the tip of the inflorescence can be visible. At S-10, flowers are fully differentiated but unopened. Sexual organs are mature and ready for anthesis. S-11 corresponded to the flower at anthesis where the stigma is receptive and pollen may be shed. Depending on tree and environmental conditions, bud break at the apex of indeterminate inflorescences can start the vegetative flush.

Avocado produces two types of floral shoots: determinate, in which the primary axis develops into a terminal flower (Schroeder, 1944) and indeterminate, in which a bud forms on the primary axis that continues the vegetative growth of the shoot (Fig. 6.6) (Reece, 1942). The indeterminate type of inflorescence is more abundantly produced (Schroeder, 1944; Salazar-García *et al.*, 1998); however, the determinate type is common on stressed trees. Both types of floral shoots consist of secondary axes (lateral panicles), producing tertiary axes (cymes),



**Fig. 6.6.** Diagram of an avocado floral shoot (after Reece, 1942).

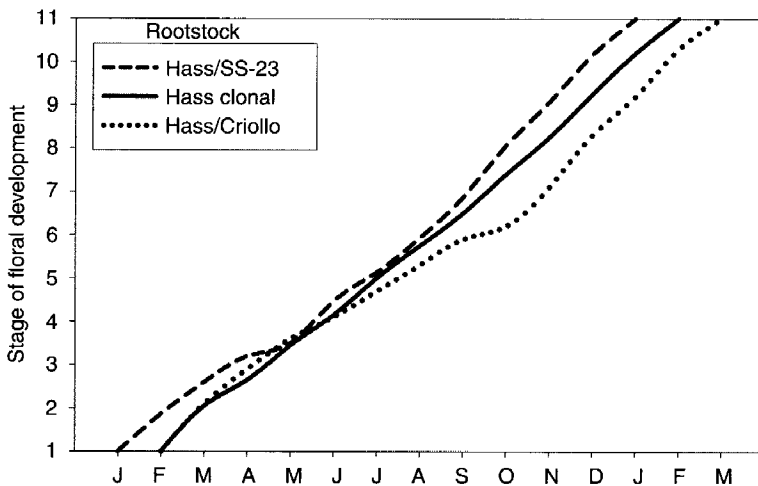
which bear a terminal flower and two lateral flowers (Reece, 1942; Salazar-García *et al.*, 1998). Development of the secondary axes within the bud proceeds in an acropetal fashion, so a developmental range occurs in a single floral shoot, with the most advanced secondary axes (i.e. at perianth differentiation) at the base and the youngest (i.e. at initial elongation of the meristem) just below the primary axis meristem (Salazar-García *et al.*, 1998). However, on an individual secondary axis, development is basipetal, with the terminal flowers (lacking subtending bracts) differentiating and maturing first, as is typical in a cyme (Salazar-García *et al.*, 1998). The fate of the apex of the primary axis meristem of the floral shoot becomes determined after the secondary and tertiary axes have reached irreversible commitment to flowering (Salazar-García and Lovatt, 1999).

Fully open flowers are ~1.0 cm in width and 6–7 mm in length (Davenport, 1986). They are perfect, hypogynous, regular and trimerous (Schroeder, 1952). The perianth is formed by six tepals (Blanke and Lovatt, 1993). Inside the perianth are three whorls of stamens and one of staminodes (Bergh, 1986). Aligned with each tepal is one stamen and one nectar-secreting, yellow staminode. Similarly, two stamens are aligned with each tepal, the interior one having a pair of nectaries at its base. Therefore, each flower normally has a total of nine stamens

(Bergh, 1986). The simple pistil is located in the centre. It has a superior ovary with one anatropous ovule (Schroeder, 1952; see also Chanderbali *et al.*, Chapter 3, this volume).

#### *Rootstock and scion effect on floral development*

Genetic characteristics of avocado influence floral development as well as bloom time and fruit maturity, either as rootstock, scion or their interaction. However, expression of this effect can be hidden or modified by orchard management and environmental conditions. Generally, it is accepted that some genotypes of Mexican, Guatemalan and West Indian rootstocks may cause early, intermediate and late bloom, respectively. Results from a rootstock trial of 'Hass' on several rootstocks in the subhumid, semi-warm climate of Tepic, Nayarit, Mexico, showed that the Mexican race rootstock caused bloom to occur 4 and 8 weeks before the 'Hass' clonal (Guatemalan × Mexican) on its own roots and 'Hass' on West Indian seedlings, respectively (Fig. 6.7). However, the 8-week difference in time of bloom advanced fruit maturity (21.5% pulp dry matter) by only 3 weeks. This was because fruit set during late flowering experienced warmer temperatures after ovule fertilization compared to fruit set earlier.



**Fig. 6.7.** Floral development of winter shoots of 'Hass' avocado, from vegetative bud (Stage 1) through anthesis (Stage 11), grafted on SS-23 clonal rootstock (Mexican), Criollo seedlings (West Indian) and 'Hass' clonal (on its own roots) in Nayarit, Mexico. (S. Salazar-García, unpublished.)

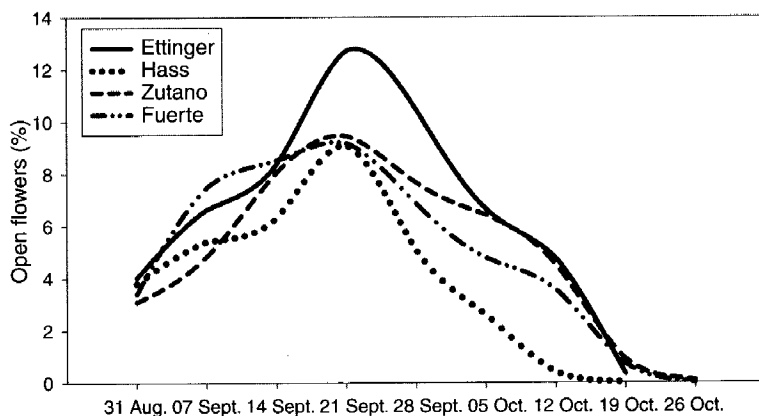
Field observations in Queensland, Australia, showed that cultivars grafted to Mexican race rootstocks flower earlier than when grafted to Guatemalan rootstocks (Young, 1992). This influence caused earlier fruit maturity, between 3–5 weeks and 8–12 weeks, respectively, for 'Fuerte' and 'Hass' grafted to Mexican race seedling rootstocks ('Mexicola' seedling and 'Duke' 7 clonal) in comparison to local Guatemalan × Mexican race hybrid seedling rootstocks ('Plowman' and 'Velvick'). The performance of Mexican rootstocks was consistent in a subtropical climate, intermediate in a cool temperate climate, and not different from Guatemalan rootstocks in hot, humid tropical areas in northern Queensland (Young, 1992). This is probably because, after fruit set, high temperatures during the spring and summer nullify the influence of rootstocks by speeding up fruit development.

Root growth is affected by orchard management practices; however, the genetic background of the rootstock is important. Observations by Salazar-García (personal communication) indicate that in warm environments (like on the Peruvian coast), root growth of Mexican race rootstocks is almost nil during the summer due to constant high upper soil temperature of 25 to >34°C (night/day). In comparison, Guatemalan and West Indian rootstocks maintain full root activity. Decreased root activity in the Mexican rootstocks causes cessation of shoot growth. This is conducive to early transition to floral development that results in very intense and

profuse flowering, composed predominantly of determinate inflorescences.

Interaction of rootstocks is important when managing pollinizers to ensure bloom overlap. In the warm climate of Virú, Peru (8°32' S; 78°40' W), there was almost a perfect synchrony between 'Hass' bloom and that of pollinizers 'Ettinger', 'Zutano' and 'Fuerte' when grafted to 'Topa Topa' seedling rootstocks (Fig. 6.8). However, lack of full overlap may occur if scions of either 'Hass' or the pollinizers are grafted to rootstocks of different races. Under these conditions, bloom usually starts first on scions grafted to Mexican rootstocks, followed by Guatemalan and West Indian, although the last two may show no practical differences.

Time of bloom is related to the genetic makeup of the cultivar as well as climatic conditions. Time of flowering is important for having a profitable crop; thus, cultivar selection is of major relevance. If bloom occurs early in the winter-spring, low temperatures may negatively affect pollen development and/or fruit set. However, late in the spring or early in the summer, reduced or no fruit set may result due to excessively high temperatures. Depending on the latitude, rains may occur in the winter, summer or year round, affecting both flowering phenology and insect activity, including insects required for pollination and fruit set; the occurrence of diseases that attack flowers and young fruit is an additional issue influenced by rain.



**Fig. 6.8.** Progression of flower opening of several avocado cultivars on 'Topa Topa' seedling rootstocks in Chao, Virú, Peru. (Cristiam Calderón and Juan Huanca, 2010, personal communication.)

In most avocado producing regions, scion cultivars of Mexican race and Mexican × Guatemalan hybrids have been reported to bloom early (Schroeder, 1951). In California, generally the Mexican and the Mexican × Guatemalan hybrids are the earliest to begin bloom (January). West Indian cultivars start to flower in mid-January–February. The pure Guatemalan race cultivars start blooming from mid-February to mid-March (Bergh, 1986). In Florida, Reece (1942) and Davenport (1982) reported that the earliest evidence of floral bud development occurred in the West Indian cultivars ('Pollock' and 'Simmonds') and in their hybrid ('Lula') from late November to early January. In pure Guatemalan race cultivars ('Nabal' and 'Itzamna'), flower development only became evident in January.

For many years, researchers and growers have been searching for cultivars that might be more productive than 'Hass' or extend the harvest season. Some cultivars have been tested, like 'Pinkerton', 'Shepard', 'Reed', 'Gwen', 'Whitsell' and 'Esther', mainly in the USA, Australia and South Africa (Whiley *et al.*, 1990). However, none fully passed all the criteria imposed by growers, packers, shippers, marketers, industry and consumers. In countries growing Mexican and Guatemalan race avocados, two cultivars are of major economic importance, 'Fuerte' and 'Hass'. These two have become the industry standards and form most of the international trade of avocados.

As the consumer market demands 'Hass' avocado, 'Hass'-like varieties are a must. 'Lamb Hass' (see description in Crane *et al.*, Chapter 8, this volume) is a promising cultivar for some avocado growing areas where it has been evaluated (California, Australia, New Zealand, South Africa, among others). However, this cultivar faces low industry interest (Anonymous, 2000). In addition, it may require a different protocol for harvest and postharvest handling (Dixon *et al.*, 2008a). However, in some countries, 'Lamb Hass' is of great interest for extending the harvest season since it is harvested much later than 'Hass'.

An extended flowering season has been documented for 'Hass' in the state of Michoacán, Mexico. This is due to variation in climatic conditions across the biggest avocado-producing region in the world. In some climates, a single tree may produce up to four

bloom flushes from July to April the next year, named, respectively, 'Loca' (Crazy), Advanced, Normal and Marceña (from March). The different bloom flushes overlap across the different altitude zones, making it possible to harvest 'Hass' avocado throughout the year. However, a regional crop is composed of fruit set from the different blooms in the following proportions: Crazy, 5–20%; Advanced, 10–25%; Normal, 60–80%; and Marceña, 15–30% (Salazar-García *et al.*, 2005). Origin of the name 'crazy bloom' refers to the fact that this bloom occurs in some climates, in some years, on some trees and on some vegetative flushes (Rocha-Arroyo *et al.*, 2011a). Fruit set from this bloom is harvested during a period of high demand (June to early September) and reduced domestic supply and sells at an extremely high price. Fruit set by the crazy bloom is reduced due to summer rains. Also, if the crazy bloom sets fruit, it is common for the more developed fruit from earlier blooms (i.e. Normal or Marceña) to abscise.

In the late 1980s, Carlos Méndez detected a distinct avocado tree in Uruapan, Michoacán, Mexico. This tree consistently exhibited the crazy bloom (July–September), compared with surrounding 'Hass' trees. Fruit from this bloom coincided with that of the crazy bloom crop of 'Hass'. This 'Hass'-like avocado was locally named 'Hass-Mendez' and now it is trademarked 'Hass Carmen'<sup>®</sup> (Illsley-Granich *et al.*, 2011; see Crane *et al.*, Chapter 8, this volume for a description of the cultivar). The fruit of 'Hass Carmen' is not morphologically different from the range of fruit morphologies found for conventional 'Hass' in Mexico. This new avocado cultivar is widely grown in Mexico and it is either in early commercial development or commercial trial in the major avocado producing and exporting countries (Illsley-Granich *et al.*, 2011). These authors speculate that the 'off' season producing habit of 'Hass Carmen' will be more pronounced in semitropical climates that experience wet summers and dry, mild winters; while in more extreme Mediterranean climates with cold winters, the bloom will be more synchronized and occur during the traditional bloom period. Based on the observations of Salazar-García (personal communication), in warm climates, the erratic occurrence of cool night temperatures in the

early part of the summer in the Northern Hemisphere stimulates vegetative shoot growth at the expense of the crazy bloom.

### **Phenology and modeling of floral development**

#### *Phenology of floral development*

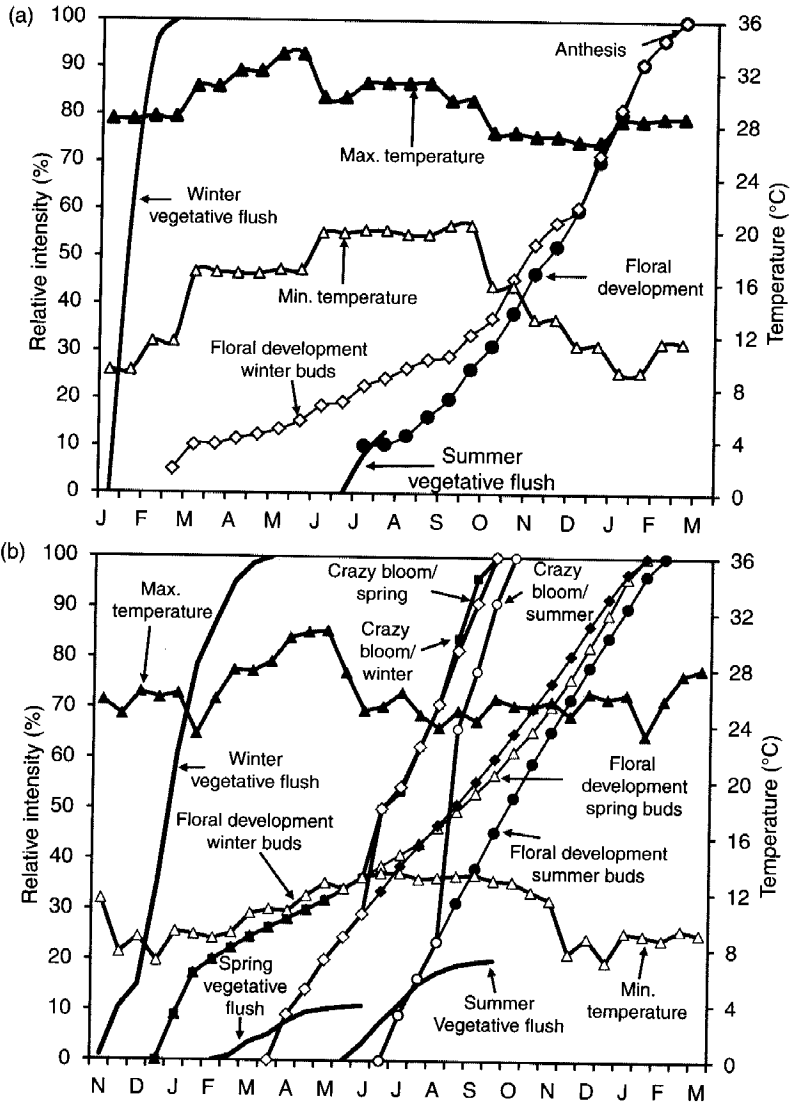
Floral bud development may start at the end of shoot elongation if tree and/or environmental conditions are conducive to floral development; if not, the buds continue vegetative shoot growth or remain inactive. Once the buds are committed to floral development, the length of time to reach anthesis depends on climatic conditions, with temperature being the driving factor (Salazar-García *et al.*, 1998, 2007a,b). Results from controlled environment and field research confirm that irrespective of the cultivar, a decrease in temperature is necessary for buds to become committed to reproductive growth (Buttrose and Alexander, 1978; Nevin and Lovatt, 1989; Cossio-Vargas *et al.*, 2007; Rocha-Arroyo *et al.*, 2010). This means that buds on later developing vegetative shoots (summer and autumn) will become committed to flowering faster as they will approach the normal flowering time (cooler temperatures) in a shorter period of time. Buds on vegetative shoots that developed earlier (winter and spring) will remain vegetative during the upcoming period of warm temperatures. According to this, the elapsed time from the end of shoot elongation to anthesis (S-1 to S-11 on the floral development scale of Salazar-García *et al.* (1998)) may vary from 4 to more than 12 months. A field example of this performance for the 'Hass' avocado in the states of Nayarit and Michoacán, Mexico is given in Figure 6.9. Winter shoots required more time to complete the process of floral development than summer and autumn shoots.

When establishing differences in avocado floral development due to natural or induced conditions, a good strategy is to use a 'biofix'. The end of shoot elongation has been useful for this purpose. Comparison of the progress of floral development on winter shoots in several climates of Michoacán, Mexico showed differences in this process; however, they

disappeared at the time of anthesis (S-11) (Fig. 6.10). Similar results were obtained for apical buds on summer shoots in five locations of California; however, Cambria (coldest location) reached anthesis (S-11) two weeks ahead of the warmer locations.

Under normal conditions, the fate of the buds produced by the different vegetative flushes during the year is influenced by tree crop load and environmental conditions present during the first weeks of shoot growth. Buds on vegetative shoots may produce floral or vegetative growth or remain inactive. Irrespective of time of vegetative flushing, at bloom flowering shoots look very similar. An experienced eye may easily recognize shoot age: oldest shoots have a wider diameter and stiffer leaves, and larger production of axillary floral shoots, which in prolonged winters become the determinate type. At bloom, it is common for winter, spring and summer shoots to exhibit similar vigour. A key feature to distinguish winter, spring and summer shoots from autumn shoots is that autumn shoots produce only terminal floral shoots; axillary buds do not undergo bud break.

Little information is available on the importance of the different vegetative flushes to the production of floral shoots. In most avocado-producing areas, flowering is produced largely by winter or spring shoots, which emerge during or after the major bloom; or spring and summer flush shoots in the humid subtropics (Whiley *et al.*, 1988). However, flowering on summer or autumn shoots is important in some regions. For example, in coastal areas of Peru, more than 90% of flowering shoots of 'Hass' avocado is produced by spring flush shoots that developed 10–12 months earlier. A similar situation occurs in New Zealand (Dixon *et al.*, 2008b). In Nayarit, Mexico, contributions to flowering of winter and summer shoots were very similar (close to 50%); however, the number of winter shoots was three- to four-fold more than the number of summer shoots (Salazar-García *et al.*, 2006). A very descriptive illustration about the contribution of different vegetative flushes to flowering in several climates of Michoacán, Mexico is shown in Colour Plate 10. In the subhumid, warm climate (0.12% of total avocado land), winter, spring and summer flushes only



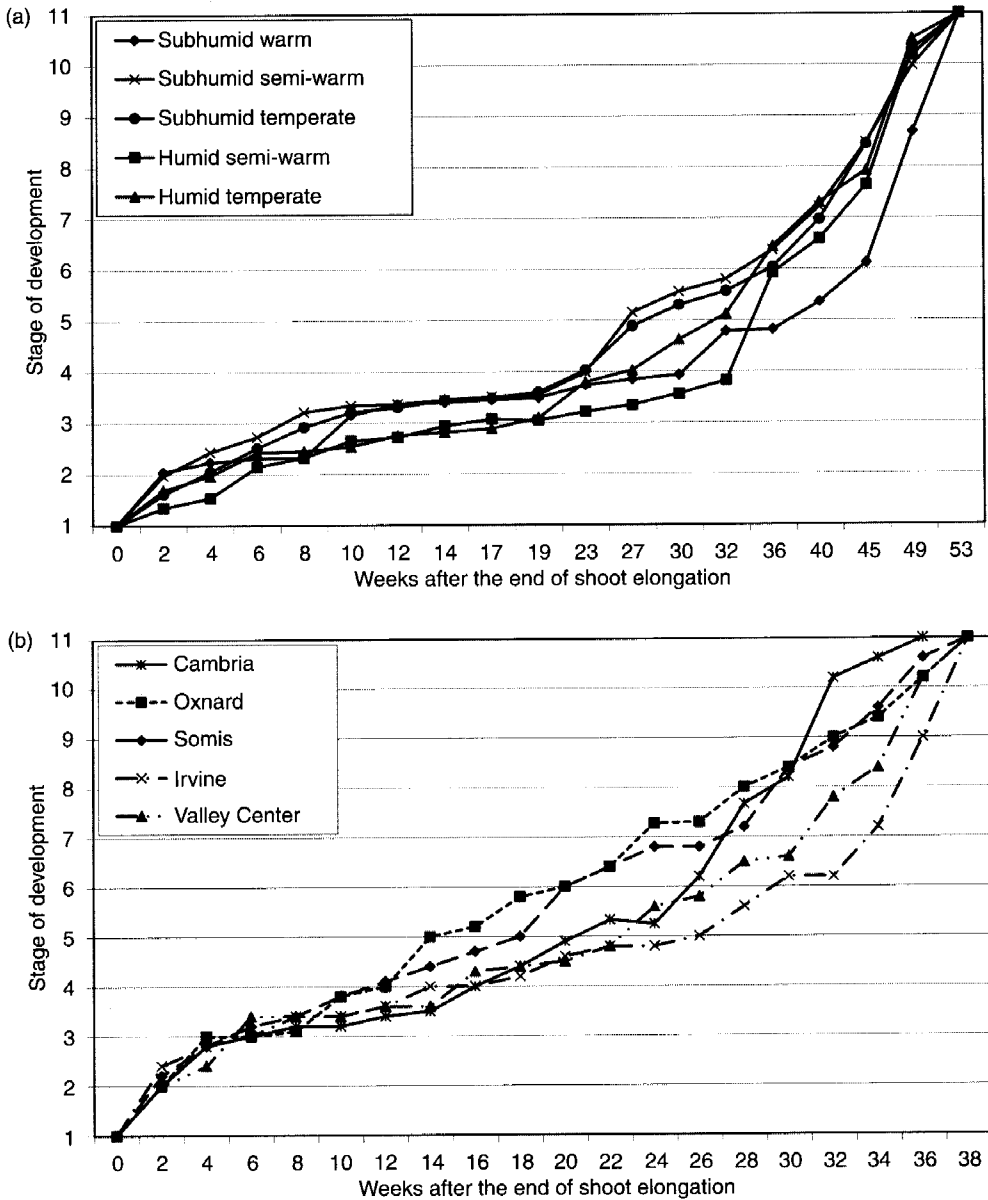
**Fig. 6.9.** Air temperatures in two 'Hass' avocado producing areas of Mexico and time of emergence of vegetative flushes and their respective floral development. (a) Tepic, Nayarit: subhumid semi-warm climate; (b) Uruapan, Michoacán: subhumid, semi-warm climate (most humid). (Adapted from Cossio-Vargas *et al.*, 2008b; Rocha-Arroyo *et al.*, 2011a.)

produced floral shoots during the Normal and Marceña (from March) bloom times. However, in the subhumid, semi-warm (most humid) and subhumid temperate areas, which account for 57 and 26% of total avocado land, respectively, different proportions of Crazy, Advanced, Normal and Marceña blooms are produced. This information opens the potential to test treatments to alter the

time of vegetative shoot flushes or to increase the production of shoots of a particular flush in order to have more floral shoots at a desired time.

*Modelling floral development*

In preceding sections of this chapter, the effect of temperature as a promoter of avocado floral



**Fig. 6.10.** Floral development of 'Hass' avocado: (a) in several climates of Michoacán, Mexico (winter shoots emerged during Dec. 2005–Jan. 2006) and (b) at six locations from south (Valley Center) to North (Cambria) of California (summer shoots were born during Jul.–Aug. 2005). (S. Salazar-García, unpublished.)

development was described. This fact provided the opportunity to develop models to predict when key floral developmental events would occur. Using ambient temperature records and field floral development data obtained from

1998 to 2006, the effect of ambient temperature on floral development of 'Hass' avocado in the semi-warm climate of Nayarit, Mexico was quantified (Salazar-García *et al.*, 2007a,b,c). The complete process of floral



development (from the end of shoot elongation to anthesis) was related to ambient temperature and mathematically modeled. Floral development was correlated to accumulated chilling days (ACD) and a floral development prediction model was obtained for winter shoots,  $winter_{ACD \leq 21}$  ( $R^2 = 0.99$ ). In the case of summer shoots two prediction models were developed,  $summer_{ACD \leq 19}$ ,  $summer_{ACD \leq 20}$ , both of them with  $R^2 = 0.99$ . These models were validated in the same 'Hass' producing region for their capacity to predict when buds or floral shoots reached S-8 (cauliflower stage) or S-11 (anthesis), resulting in  $R^2 = 0.99$ .

In another study, the prediction models generated by Salazar-García *et al.* (2007b) for 'Hass' avocado in Nayarit were evaluated for their capacity to predict the floral bud development on winter, spring and summer shoots of trees growing in several climates of Michoacán, Mexico. Effectiveness of the Nayarit prediction models was affected by climate and shoot age (Salazar-García *et al.*, 2009). Only the model  $summer_{DFA \leq 19}$  showed a strong capacity to predict floral development of summer shoots ( $R^2 = 0.94$ ) using a data set from four climates of Michoacán, one subhumid warm, two subhumid semi-warm and one subhumid temperate, which account for 85% of the 'Hass' avocado-producing area. These results justified the development of floral development prediction models for Michoacán, which were obtained in 2009 (Salazar-García *et al.*, unpublished).

The 'Hass' avocado floral development prediction models already described have been used to construct simulation programmes currently available on the internet. These applications predict the date of occurrence of key floral developmental events based on daily temperature information provided by automatic weather stations situated by the 'Hass' producing regions of Nayarit (<http://www.cesix.inifap.gob.mx/desarrollofloral.php>) and Michoacán (<http://www.cesix.inifap.gob.mx/desarrollofloralmichoacan.php>). Floral development simulators are helpful for proper and effective timing of foliar sprays to control pests and diseases that coincide with anthesis and early vegetative shoot growth, increasing their benefit to cost ratio. More examples of the use of the prediction models are related to the timing of: (i) pruning;

(ii) fertilization (especially soil-applied nitrogen); (iii) boron sprays at the cauliflower stage; and (iv) placement of beehives at the optimal time for pollination, etc.

## Manipulating flowering

### Growth retardants

Physiology of avocado flowering is often thought to be similar to other woody evergreen fruit trees, such as citrus and mango (Bower *et al.*, 1990). However, the most common factor is that a cessation of vegetative growth must occur in order to start the floral development process. Thus in avocado, absence of vegetative growth is conducive to flowering and vice versa. There are several chemical and/or cultural treatments that alter floral development. Some of them advance bloom, others may cause a delay and some others inhibit floral initiation.

The use of growth retardants, such as paclobutrazol  $\{(R^*,R^*)\text{-}\beta\text{-}[(4\text{-chlorophenyl)methyl]\text{-}\alpha\text{-}(1,1\text{-dimethylethyl})\text{-}1H\text{-}1,2,4\text{-triazole}\text{-}1\text{-ethanol}\}$ , (Cultar<sup>®</sup>; Syngenta Crop Protection, UK Limited), a triazole plant growth regulator that inhibits gibberellin biosynthesis (Davis *et al.*, 1988), or uniconazole-P (Sunny<sup>®</sup>; Aquamarine B.V.) has been focused on controlling tree vigour. Early studies on the use of paclobutrazol resulted in profuse flowering. This was the case for potted (Symons and Wolstenholme, 1989) or field-grown 'Hass' (Köhne and Kremer-Köhne, 1990) avocado trees. Soil (drench), foliar or trunk injections caused similar effects but they were dose dependant (Köhne and Kremer-Köhne, 1990).

Regarding the duration of the effect of growth retardant treatments, Köhne and Kremer-Köhne (1990) showed that for 'Hass' avocado the effect of paclobutrazol foliar sprays lasted 4–6 weeks after application, whereas the shoot growth suppression through soil drench application lasted 6 months. Adato (1990) reported no residual or cumulative effect of 4% Cultar<sup>®</sup> (paclobutrazol) sprays in two consecutive years of treatment, for tree vigour, general appearance, yield or other factors.

The initial purpose of using paclobutrazol or uniconazole in avocado was to control tree

size; the most current use is for delaying and reducing shoot growth at the time of bloom during spring. This is achieved by foliar sprays before or during anthesis with the goal of favouring fruit set and initial fruit growth by limiting the competition for tree resources in these critical stages of phenology (Köhne and Kremer-Köhne, 1987; Adato, 1990; Wolstenholme *et al.*, 1990). However, careful attention must be taken with regard to growing conditions, current alternate bearing cycle, doses, reapplications and stage of tree phenology. In addition, orchard management practices, such as nitrogen fertilization or pruning, which stimulate excessive vegetative growth during the spring flush, must be avoided.

### *Gibberellic acid*

Gibberellins have a different effect than growth retardants as they stimulate growth and shoot elongation. Although not intensive, some research has been done to develop strategies using foliar-applied gibberellic acid ( $GA_3$ ) to regulate inflorescence phenology and intensity of flowering in order to increase yield and reduce alternate bearing of 'Hass' avocado.

$GA_3$  foliar sprays affected the type of growth produced by apical buds. A study done in California in an alternate bearing orchard resulted in higher production of vegetative shoots at the expense of inflorescence production for control trees carrying an 'on' crop. The opposite occurred for the 'off' crop year, resulting in greater production of inflorescences (Salazar-García and Lovatt, 2000). This research was the first to use  $GA_3$  to alter this relationship.  $GA_3$  (100 mg l<sup>-1</sup>) applied in September, before ICF (buds at Stage 3; Colour Plate 9) of any cropping year effectively reduced inflorescence production and increased vegetative shoot production.  $GA_3$  (25 or 100 mg l<sup>-1</sup>) applied in November (buds at Stage 4) prior to the 'on' bloom year reduced inflorescence production and increased the production of vegetative shoots, compared to the control.  $GA_3$  sprays in November, when buds had only one to three secondary axis inflorescence meristems without apical bracts during the year of this study, also resulted in production of partially formed inflorescences (with less than 10 secondary axes) (Salazar-García and Lovatt,

1998), which reduced flowering intensity. Stimulation of the growth of the vegetative primary axis meristem by  $GA_3$  arrested the growth of secondary axis meristems adjacent to it; the response was stronger at higher  $GA_3$  concentrations. However, flowering was not fully inhibited, demonstrating that  $GA_3$  did not revert secondary axis meristems into vegetative ones. Aborted meristems could be observed after inflorescence elongation as scars with small inflorescence bracts (Salazar-García and Lovatt, 1998).  $GA_3$  (25 mg l<sup>-1</sup>) applied in November decreased yield (50%) in the 'on' bloom year.  $GA_3$  treatment in January (buds at Stage 5) or in March (buds at the cauliflower stage; Stage 8) had no effect on the number of inflorescences produced in either the 'on' or 'off' bloom year due to the high proportion of buds at an advanced stage of development at the time of treatment. Monthly applications of  $GA_3$  (25 mg l<sup>-1</sup>) during the process of inflorescence bud development (September to January) reduced production of inflorescences more than 65% in both 'off' and 'on' bloom years; however, there was no related effect on yield. Similar inhibitory effects of  $GA_3$  (250 mg l<sup>-1</sup>) were obtained with repeated sprays (February + March + April + two sprays in May; Southern Hemisphere) to young potted 'Hass', 'Fuerte' and 'Ryan' trees (Rossouw *et al.*, 2000). Non-treated control trees produced more floral than vegetative shoots at spring bloom.

Floral shoot phenology can be altered by  $GA_3$  treatments. Different from what had been reported for other fruit tree species,  $GA_3$  sprays never delayed flowering, but hastened floral shoot development of 'Hass' avocado. Even at a low concentration (50 mg l<sup>-1</sup>), when  $GA_3$  was applied in December or January, 50% of the inflorescences reached the cauliflower stage 23 days earlier than the controls. Higher  $GA_3$  concentrations applied to the foliage (100 or 1000 mg l<sup>-1</sup>) and later application dates further accelerated the rate at which buds reached the cauliflower stage; however, the date of anthesis was not significantly affected (Salazar-García and Lovatt, 1998). This behaviour seems to be a natural mechanism of avocado to avoid anthesis when low temperatures are prevailing.

Competition between the vegetative shoot (while still a 'sink') and the flowers of indeterminate floral shoots at the time of fruit set is still

a hypothesis to explain low fruit set in avocado (Zilkah *et al.*, 1987; Cutting and Bower, 1990; Whiley, 1990; Bower and Cutting, 1992). In California, vegetative growth and leaf expansion of indeterminate floral shoots of 'Hass' avocado is usually delayed relative to the elongation of the secondary axes of the inflorescence. GA<sub>3</sub> sprays dramatically increased the rate of development of the vegetative shoot at the apex of indeterminate floral shoots (Salazar-García and Lovatt, 1998, 2000). Precocious vegetative shoot development of indeterminate floral shoots caused by GA<sub>3</sub> treatments might result in more successful inflorescences in terms of fruit set and yield by eliminating the competition between reproductive and vegetative growth. Leaves of the precocious vegetative shoot of indeterminate inflorescences treated with GA<sub>3</sub> were sufficiently mature to be sources (based on net CO<sub>2</sub> assimilation rates) at the time of fruit set (Blanke and Lovatt, 1998). However, a 2-year field experiment showed no effect of GA<sub>3</sub> (0, 25 and 100 mg l<sup>-1</sup>) foliar sprays at different stages of inflorescence development on fruit yield (Salazar-García and Lovatt, 2000). Nevertheless, GA<sub>3</sub> (25 mg l<sup>-1</sup>) applied in March of an 'off' bloom year increased (twofold) the production of commercially valuable fruit (213–269 g per fruit) and delayed skin blackening.

Application of GA<sub>3</sub> also alters floral shoot morphology. Determinate inflorescences were the most abundant type produced by young 'Hass' avocado trees growing in a glasshouse. Trunk injections of GA<sub>3</sub> (25 or 50 mg per tree) at Stage 5 of inflorescence development significantly increased the production of indeterminate inflorescences at the expense of determinate inflorescences (Salazar-García and Lovatt, 1999). Inflorescences from both control and GA<sub>3</sub>-treated trees had 10 secondary axes, the normal number for a fully formed inflorescence (Salazar-García *et al.*, 1998). However, GA<sub>3</sub>-treated trees produced inflorescences with longer secondary axes than control trees. Secondary axes of control inflorescences ranged from 5–20 cm in length, whereas those of GA<sub>3</sub>-treated trees were 30–50 cm long. GA<sub>3</sub> injected at Stage 5 of inflorescence development in this study caused precocious development of the vegetative shoot apex of indeterminate floral shoots that developed

successfully. Stimulating vegetative growth at the inflorescence apex did not inhibit inflorescence development. Whereas the full number of secondary axis meristems of the inflorescence is already formed at Stage 5, tertiary axes are still being initiated (Salazar-García *et al.*, 1998). The results provided additional evidence that secondary and tertiary axes are committed to flowering at this stage, consistent with the presence of the perianth on these axes, but that the final fate of the floral shoot apex is determined much later. Thus, potential determinate inflorescences were redirected to indeterminate floral shoots by the GA<sub>3</sub> treatments.

No visual signs of toxicity have been observed for any GA<sub>3</sub> spray concentration evaluated on avocado (Salazar-García *et al.*, 1998). GA<sub>3</sub> (50 and 100 mg l<sup>-1</sup>) had no negative morphological effects. However, GA<sub>3</sub> (1000 mg l<sup>-1</sup>) applied on all treatment dates caused a remarkable elongation of inflorescence axes that were too weak to support setting fruit. A similar response was observed in young and mature trees trunk-injected with GA<sub>3</sub> (Salazar-García and Lovatt, 1999).

### Pruning

In modern avocado production, pruning must be a routine orchard management practice. There are several pruning styles or techniques for young, mature or old (overcrowded) orchards. However, growers do not always utilize this practice due to the potential loss of crop and income that usually results. Lack of bloom during the pruning year or the next year is the common cause of reduced yield. However, pruning in the proper way and time can help to minimize crop losses due to lack of bloom.

In regions where a single, highly synchronized bloom occurs and harvest of mature (non-overcrowded) trees is performed about 2–3 months before bloom, light pruning to stimulate production of new shoots should be done immediately after harvest. New (developed after pruning) and old vegetative shoots develop into floral shoots producing a regular (not excessive) but early bloom. When fruit is on the tree, size picking and light selective pruning help to obtain a regular and early bloom.

In old and overcrowded orchards, the practice of stumping and top-working (see Ernst *et al.*, Chapter 9, this volume, for a description of top-working) with 'Hass' has worked to effectively rejuvenate trees and get them back into flowering and production. A good description and a proposed timetable for the stump-top-work rejuvenation method are described by Hofshi *et al.* (2010).

The best time to stump or cutback selected branches and limbs of giant, overcrowded trees is after harvest of an 'on' crop year. This will allow the trees to produce a crop in the next cropping year. In intensive avocado orchards, a combination of selective side and top pruning combined with plant growth retardants results in regular bloom and good yields (Gardiazábal *et al.*, 2011).

When use of plant growth retardants is not an option, it is recommended that limb or branch pruning be done after buds have reached irreversible commitment to flowering. This will ensure that limbs and branches that were not pruned will flower in the same season.

Flower pruning in 'Hass' avocado has been practised when the trees were entering an 'on' year. This resulted in less alternate bearing and larger fruit size. Flower pruning at inflorescence stages before full bloom gave the best results (Roe and Morudu, 2000). All pruning techniques require the growers to take a close look at the stage of tree phenology and the 'on'/'off' status of the trees in their orchard. This will help to avoid non-productive years. Further details on pruning are provided by Whiley *et al.*, Chapter 12, this volume.

## The Mature Flower

### Flower development

Flowers are borne on either determinate or indeterminate floral shoots. Each floral shoot bears an inflorescence with multiple secondary and tertiary axes (Fig. 6.6) (Reece, 1942). Determinate shoots have a terminal flower, whereas the apical meristem of indeterminate floral shoots continues to grow vegetatively (Schroeder, 1944). The majority of floral shoots produce indeterminate inflorescences (Salazar-

García and Lovatt, 1998; Schroeder, 1944; Thorp *et al.*, 1994) though the ratio of indeterminate to determinate flowering shoots appears to be dependent on cultivar, growing region and floral shoot age. Approximately 100 flowers are typically produced per inflorescence (Salazar-García and Lovatt, 1998; Garner, 2004). In a study by Salazar-García and Lovatt (1998), the number of flowers per inflorescence was not significantly different between the two flowering shoot types, but Garner (2004) found that determinate inflorescences produced during 'off' crop blooms had significantly more flowers than indeterminate inflorescences, whereas determinate inflorescences produced during 'on' blooms had significantly fewer flowers than indeterminate inflorescences.

### Bloom phenology

After buds become committed to flowering in the late summer to early autumn (Salazar-García *et al.*, 1998), bloom occurs during the following spring. Bloom is typically protracted, and has been documented to last for as little as 18 days for 'Harvest' in Spain (Alcaraz and Hormaza, 2009) and as long as 149 days for 'Hass' in California (Garner, 2004). To measure and compare avocado bloom length, studies have been conducted in numerous growing regions, including California (Winslow and Enderud, 1955; Robinson *et al.*, 2002; Garner, 2004), Brazil (Falcão *et al.*, 2001), Mexico (Cossio-Vargas *et al.*, 2008b; Rocha-Arroyo *et al.*, 2011a), Israel (Levin, 1981), China (Lu *et al.*, 2004), South Africa (Kaiser and Wolstenholme, 1993) and the Mediterranean (Papademetriou, 1976; Loupassaki *et al.*, 1995; Demirkol, 2002; Alcaraz and Hormaza, 2009). It should be noted that the criteria used to determine the beginning and end of bloom within these studies, when provided, varied in inclusiveness, from those that excluded periods when few flowers were at anthesis to those that included the period from the first flowers at anthesis until no unopened flowers were observed. Generalizations regarding the length of bloom are possible despite variations in the timing and intensity of reproductive development associated with cultivar, location and seasonal

weather conditions. For the majority of cultivars and growing regions, the bulk of bloom occurred over a period of 1–3 months. Bloom length is cultivar dependent and for a given cultivar, is often highly variable from year to year, likely due to seasonal weather differences (Winslow and Enderud, 1955; Levin, 1981; Garner, 2004). Though a relationship between crop load and the duration and timing of bloom has been suggested (Robinson *et al.*, 2002), studies including multiple cultivars do not demonstrate consistent relationships between crop load and bloom length (Winslow and Enderud, 1955; Garner, 2004).

### Anthesis

Avocado flowers are complete and perfect. Although flowers lacking a complete gynoecium have been reported for 'Zutano', 'Bacon' and 'Fuerte', the rate of occurrence is extremely low (< 0.01%) (Inoue and Takahashi, 1990). Avocado is characterized by a rare flowering mechanism called synchronous protogynous dichogamy (Stout, 1923). The functionality of the male and female organs is typically temporally separated. Each flower opens twice. During the first opening, the flowers are functionally female with a receptive stigma and anthers that have not dehisced. At the second opening, the flowers are functionally male with a typically desiccated and unreceptive stigma and anthers that dehisce 1–2 h after opening. During the first opening, both self-pollen and cross-cultivar avocado pollen can adhere, hydrate and germinate successfully on avocado stigmas (Papademetriou, 1974; Sedgley, 1979a), though typically no more than one pollen tube reaches the ovary (Sedgley, 1976). Studies in humid, semi-tropical Florida suggest that the stigma could continue to be receptive during the second opening, possibly due to high humidity (Davenport *et al.*, 1994). A study by Davenport (2000) demonstrated that pollen tubes can reach the egg apparatus within 48 h of pollination of male stage flowers, but evidence of fertilization or gametophyte viability after this extended period was not demonstrated. Several studies have demonstrated that despite the fact that pollen grain

germination occurs in avocado flowers that were hand-pollinated during the functionally male stage, pollen tube growth is limited and few pollen tubes reach the ovary within 24 h (Sedgley, 1977b; Shoval, 1987), possibly due to callose deposition within the aging style (Sedgley, 1977b, 1979c).

Avocado cultivars that are clonally propagated can be divided into two types based on the typical timing of flower openings (Stout, 1923). Type A cultivars (such as 'Hass') open as functionally female in the morning of the first day of anthesis, and as functionally male in the afternoon of the second day of anthesis. Type B cultivars (such as 'Bacon', 'Fuerte' and 'Zutano') open first as functionally female in the afternoon and then as functionally male on the morning of the second day of anthesis. Under optimal environmental conditions, this provides an elegant system for cross-pollination because when anther dehiscence occurs in one flowering type, only the other flowering type is receptive.

### Pollination

For pollination to take place there must be a temporal overlap in the opening of functionally male and female flowers. In the 1920s, growers were advised to interplant complementary (type A and type B) cultivars within their orchards to increase pollination and fruit set because opportunities for self-pollination (pollen movement from anther to stigma within a flower, or between flowers of the same cultivar ('close pollination')) were thought to be almost zero due to the temporal separation of functional male and female flower organs (Stout, 1923). Later work revealed that environmental conditions have a substantial effect on the overlap of functionally male and female flowers. At low temperatures of 17/12°C (day/night) flower openings in the female stage of type A trees were delayed (Sedgley and Annells, 1981). There is a significant negative linear relationship between mean daily temperature and the hour of day of flower openings (Ish-Am and Eisikowitch, 1992). Therefore, frequent or erratic changes in temperature can result in 'irregular' blooming and overlap of female and

male stages within the same flowering type. These effects should allow pollen transfer to occur within inflorescences, among inflorescences of the same tree or among trees of the same cultivar (Lesley and Bringham, 1951). This overlap of functionally male and female flower openings has been demonstrated not only by observational studies of the flowers (Continella *et al.*, 1992; Ish-Am and Eisikowitch, 1992; Loupassaki *et al.*, 1995; Alcaraz and Hormaza, 2009), but also by the successful set of fruit in large-scale monocultures (Hodgson, 1947). Furthermore, high rates of outcrossing between cultivars of the same flowering type have been reported (Violi *et al.*, 2009). In the case of type B avocado cultivars, low temperatures of 17/12°C (day/night) can cause the female stage of flowering to be omitted altogether, thus reducing pollination rates (Sedgley, 1977a; Sedgley and Grant, 1983).

Davenport *et al.* (2007) hypothesized that fruit that are the result of self-pollination must be the result of male stage self-pollination, not close pollination, because they rarely observed extended periods of overlap of female and male stages within a cultivar. However, the length of the overlap between female and male stages can be more than ample for pollination to occur. For example, Alcaraz and Hormaza (2009) found that the female and male stages of floral development overlapped for at least 2 h for seven of the ten cultivars studied. Furthermore, prolonged overlap is not necessarily required for pollen transfer to occur, as bees or other insects could carry pollen for an extended period.

Rates of pollination are affected by the movement of pollen through the orchard. Avocado pollen has been described as sticky (Stout, 1933), requiring a large, flying pollinator. Several efficient, putative native pollinators of avocado have been identified in Mexico, including stingless bee species (Apidae, Meliponinae) (Ish-Am *et al.*, 1999; Can-Alonzo *et al.*, 2005) and the Mexican honey wasp (*Brachygastra mellifica* Say) (Ish-Am *et al.*, 1999). Honeybees (*Apis mellifera* L.) are considered the primary pollinator of avocado in the majority of commercial growing regions, including those in Mexico (Ish-Am *et al.*, 1999), California (Visscher, 1997), Australia (Vithanage, 1990), South Africa (Eardley and Mansell, 1996) and Israel (Ish-Am and Eisikowitch,

1993). However, honeybees are not very effective pollinators of avocado. They are easily attracted to more fragrant flowers, such as citrus (*Citrus* spp.) and wild flowers (Ish-Am and Eisikowitch, 1993, 1998). Avocado nectar is unattractive to honeybees (Afik *et al.*, 2006b), probably due to its high potassium and phosphorus content (Afik *et al.*, 2006a). Furthermore, the activity of honeybees is severely limited in cool, overcast or rainy weather (reviewed in Free, 1970).

Through a series of studies conducted in Florida or California, Ying *et al.* (2009a) demonstrated that avocado pollen can dry and become windborne. The authors also reported that avocado trees caged to prevent pollinator access had yields equal to those with access to pollinators, leading the authors to conclude that honeybees do not contribute significantly to avocado yields, and that wind pollination plays a substantial role in avocado production in Florida and California. However, evidence of how widespread wind pollination of avocado is and its impact on pollen viability, pollination and fertilization rates, and fruit set was not demonstrated definitively in these studies. In a 7-year, five-cultivar field study in Israel, Ish-Am and Lahav (2011) found that the rate of bee visitations to avocado trees was positively correlated with pollination rates, whereas wind velocity was not significantly related to pollination rates. Other researchers have found that avocado pollination and/or fruit set are typically greatly reduced when pollinators are prevented from accessing avocado inflorescences (Peterson, 1955; Papademetriou, 1976; Gazit, 1977; Vithanage, 1990; Johannsmeier and Morudu, 1999; Malerbo-Souza *et al.*, 2000; Can-Alonzo *et al.*, 2005), suggesting that honeybees and other pollinators contribute significantly to avocado pollination, and ultimately to yield. The results of these studies are consistent with Scholefield's (1982) conclusion that the nectaries and small stigmatic surface of an avocado flower are inconsistent with the typical morphology of wind-pollinated flowers. In a recent study of 'Hass' avocado trees in California by Hoddle *et al.* (2010), open-pollinated trees sprayed with water (spray control) and caged trees accessible to honeybees had equally high levels of fruit set. Trees caged to exclude large flying insects, including

honeybees, but accessible to small insects such as flower thrips (*Frankliniella* spp.) and wind-borne pollen had very low fruit set. Caged trees sprayed repeatedly with insecticides to eliminate even small insects so that fruit set could result only from wind pollination had no fruit. These results substantiate the very limited contribution that self-pollination, small insects and wind make to pollination and yield of the avocado.

### Fertilization

The avocado is self-compatible (Continella *et al.*, 1992; Sedgley, 1979a) and fertilization is generally not limited by the number of pollen grains on the stigma (Shoval, 1987), or by selectivity of the pollen grain or the stigma surface for germination (Schroeder, 1942). Pollen grain germination and pollen tube growth are sensitive to temperature. Temperatures within the range of 20–30°C proved optimal for *in vitro* pollen germination (Loupassaki *et al.*, 1997). Pollen tubes grow abnormally or fail to reach the ovule when exposed to temperature regimes of 33/28°C (day/night) or 17/12°C (day/night), respectively (Sedgley, 1977a; Sedgley and Annells, 1981). Ovule growth and viability are also inhibited at these two temperature regimes (Sedgley and Annells, 1981). Analysis of 'Hass' avocado flowers revealed that growth of many pollen tubes stopped mid-style with only one pollen tube continuing to grow to the ovule (Jaganath, 1993). Based on these data, Lovatt (1997) hypothesized that 'Hass' pollen tube growth might be stopped by a mid-style barrier that breaks down in the absence of outcrossing. Although pollen tubes are capable of travelling the length of the stigma and style within 1–3 h (Schroeder, 1942; Papademetriou, 1974; Sedgley, 1977a), Sedgley (1979b) noted that ovule penetration does not occur until 18–24 h after pollination. Fertilization of the polar nuclei and egg cells occurs within 48 h of pollination (Sedgley, 1979b). Even when a pollen tube penetrates the ovule, fertilization might still fail to occur, a phenomenon that was observed more frequently in 'Fuerte' than in 'Hass' ovules (Sedgley, 1979b).

In addition to temperature, several other factors affect avocado flower abscission

including, but not limited to, the growth of fungi on pistils (Thomas *et al.*, 1994) and nutrient availability. Even when trees were supplied with nutrients in amounts considered adequate for production and had leaf nutrient concentrations in the optimal range, foliar applications of boron (B) and nitrogen (N as urea) at the cauliflower stage of inflorescence development increased yield of 'Hass' avocado (Lovatt, 1994), consistent with the demonstrated effects of these nutrients on pollen tube growth and ovule longevity, respectively (Jaganath, 1993). Although one Australian study indicated that N availability was not limited during flower development on vigorous shoots (Thorp *et al.*, 1993), carefully timed soil applications of N in California during bloom and early fruit set increased 4-year cumulative yield of 'Hass' (Lovatt, 2001). These results indicate that increased B and N are utilized by the tree during flower and early fruit set. In a 3-year study comparing foliar  $\text{KH}_2\text{PO}_3$  and  $\text{KH}_2\text{PO}_4$  applied at the cauliflower stage of inflorescence development with a control receiving soil-applied  $\text{KH}_2\text{PO}_4$ , foliar-applied  $\text{KH}_2\text{PO}_3$  significantly increased 3-year cumulative yield of 'Hass' avocado (Gonzalez *et al.*, 2010).

### Outcrossing

Commercial scions of avocado are vegetatively propagated and although avocado monocultures can be productive (Hodgson, 1947), a study in 1966 concluded that close planting of different avocado cultivars increased yields (Bergh *et al.*, 1966). In 1978, isozyme analysis was first used to demonstrate that outcrossing was occurring between neighboring avocado cultivars in California (Torres and Bergh, 1978).

In a series of studies conducted in Israel, isozyme analysis of avocado embryos demonstrated that fruit which are the product of self-fertilization (as a result of self- or close-pollination) abscised at a much higher rate than fruit that were the product of outcrossing (Degani *et al.*, 1986, 1989, 1997). High outcrossing rates have also been reported among progeny of avocado trees interplanted with or near pollinizer cultivars in Spain (Alcaraz and Hormaza, 2011), Australia (Sulaiman *et al.*, 2004), California (Vrecenar-Gadus and Ellstrand,

1985; Chen *et al.*, 2007; Garner *et al.*, 2008; Schnell *et al.*, 2009; Ying *et al.*, 2009a,b) and Florida (Borrone *et al.*, 2008; Violi *et al.*, 2009). The success of the pollen parent in promoting fruit retention is cultivar dependent (Gazit and Gafni, 1986; Goldring *et al.*, 1987; Degani *et al.*, 1989, 1990, 1997; Sulaiman *et al.*, 2004). The contribution of outcrossing to fruit set must be due to selective abscission of selfed fruit, superior resource allocation and/or hormone content of outcrossed fruit, or a combination of these factors. It has been hypothesized that offspring fitness of many angiosperms is increased by selective abortion of certain developing fruit (reviewed in Lee, 1984; Stephenson and Winsor, 1986). It has been suggested that inbreeding depression results in decreased embryo vigour, thus promoting the abscission of avocado fruit with embryos that are the product of self-pollination (Degani *et al.*, 1997). Inbreeding depression has also been demonstrated in studies of avocado seedlings, in which progeny that are the result of self-fertilization were found to be more susceptible to *Phytophthora cinnamomi* infection (Violi *et al.*, 2009) or more likely to have lethal mutations (Schnell *et al.*, 2009) than those that were the result of outcrossing. Though Alcaraz and Hormaza (2011) found that abscised fruit were typically the result of self-fertilization, they suggested that these fruit were likely produced late in bloom, when bloom overlap within the orchard was limited, and these late-season fruit were unable to compete with larger, faster-growing fruit that set earlier in the season.

Despite the fact that the selective retention of outcrossed fruit is typically highly significant, significant relationships between outcrossing rates and yields have only been established in a few of the Israeli studies, and were often weak or cultivar dependent (Degani *et al.*, 1989, 1990, 1997). Researchers have speculated that the initial rate of outcrossing and its effect on fruit set and yield might be dependent, in part, on climate (Goldring *et al.*, 1987). Under mesic (non-stressful) temperate growing conditions, only weak correlations between outcrossing rates and yields have been obtained. In an isozyme study, a significant relationship between outcrossing and yield was detectable only when data from all replications and orchards were pooled and

even this relationship was extremely weak ( $R^2 = 0.11$ ) (Vrecenar-Gadus and Ellstrand, 1985). In another study of 'Hass' orchards at three different California locations, outcrossing rates, determined by random amplified polymorphic DNA (RAPDs), were only significantly correlated with yield at one location ( $R^2 = 0.50$ ) (Kobayashi *et al.*, 2000). In an alternate bearing orchard in California, outcrossing rates determined by microsatellites were not related to the number or kilograms of fruit harvested for either crop year or for both crop years combined ( $P > 0.05$  and  $R^2 < 0.01$  for number and kilograms harvested), and the degree of alternate bearing was not significantly correlated with outcrossing rate (Garner *et al.*, 2008). In Spain, Alcaraz and Hormaza (2011) found that the relationship between 'Hass' yields and outcrossing rates as determined by microsatellites was not significant ( $P = 0.86$  and  $P = 0.70$  for the 2 years of the study).

Even if interplanting of pollen donor trees contributes to increased fruit set and yield, the practice may not be horticulturally and economically practical in all growing regions. In areas where cool air temperatures often delay flower openings, substantial yields are attainable in orchards with high rates of close pollination. Outcrossing rates and the potential contribution of outcrossing to fruit persistence and yield could also be limited by other factors, including bloom overlap with an effective pollinizer and the distance to the nearest pollinizer. In the few studies of avocado outcrossing rates in which the timing of bloom was reported, overlap between cultivars was typically extensive but incomplete (Degani *et al.*, 1997; Garner *et al.*, 2008; Alcaraz and Hormaza, 2011), suggesting that high outcrossing rates could require more than one pollinizer cultivar to guarantee consistent bloom overlap.

Though outcrossing has been detected among 'Hass' up to 80 m from the nearest pollinizer tree (Vrecenar-Gadus and Ellstrand, 1985), there is typically a negative correlation between outcrossing rate and distance to a pollinizer cultivar (Vrecenar-Gadus and Ellstrand, 1985; Degani *et al.*, 1989, 1990, 1997; Kobayashi *et al.*, 2000; Ying *et al.*, 2009a; Alcaraz and Hormaza, 2011). That



higher outcrossing rates require close proximity of pollinizer trees is likely due to the foraging habits of honeybees (*Apis mellifera* L.) within avocado orchards. The movement of honeybees to adjacent rows decreases hyperbolically (Ish-Am and Eisikowitch, 1998). Ying *et al.* (2009a) have suggested that the relationship between outcrossing rates and distance from a pollinizer is also consistent with wind pollination. Garner *et al.* (2008) found that high outcrossing rates in 'Hass' trees in California were only possible when each 'Hass' tree was adjacent to a pollinizer tree, and hypothesized that high densities of pollinizer trees (a minimum of 16% of trees in an orchard planted on the square) would be required for high rates of outcrossing throughout an orchard. They suggested that because the financial return of pollinizer cultivars in California is extremely low in comparison with 'Hass', that the cost of interplanting pollinizer cultivars could far exceed the monetary benefits of any potential increases in 'Hass' yields that outcrossing might provide, especially during years when environmental conditions during bloom decrease the likelihood of outcrossing (Garner *et al.*, 2008).

### Fruit development

Pollination without fertilization can briefly stimulate ovary enlargement. The resulting parthenocarpic fruit enlarge to approximately 2–3 mm or less in diameter (Sedgley, 1980) and approximately 4 mm or less in length (Tomer and Gazit, 1979) before abscising 2–4 weeks after flowering.

The development of larger seedless fruit, typically referred to as 'cukes', is not the result of parthenocarpic growth, but is instead the product of fertilization (Tomer *et al.*, 1980). After fertilization, the embryo and endosperm of a 'cuke' fail to develop properly, resulting in embryo abortion (Tomer *et al.*, 1980; Steyn *et al.*, 1993). The persistence of these seedless fruit is rare in most avocado cultivars, though it is relatively common for 'Fuerte' (Blumenfeld and Gazit, 1974) and on girdled tree branches of 'Hass' (Garner, 2004). Seedless fruit abscission is thought to be due to the limited sink strength and availability of growth promoting hormones

typically provided by the large seed of the avocado (Blumenfeld and Gazit, 1974).

Basic anatomical studies of avocado embryo and endosperm development have been conducted (Schroeder, 1952; Sedgley, 1979b; Tomer and Gazit, 1979). With successful fertilization, fruit enlargement occurs rapidly as a result of high rates of cell division and expansion, but unlike other fruit, cell division continues throughout fruit development (Schroeder, 1953). This increase in fruit size has generally been described as sigmoidal, but the accuracy of such a description is dependent on both cultivar and method of measurement (Piper and Gardner, 1943; Valmayor, 1967; Blumenfeld and Gazit, 1974; Mougheith *et al.*, 1978).

It has been suggested that early increases in fruit size can be attributed to seed growth (Mougheith *et al.*, 1978). Basic seed development was described by Piper and Gardner (1943). They reported that embryo and endosperm are surrounded by thick 'integuments', and the embryo grows within the gelatinous nucellus or endosperm, which is thought to nourish the developing embryo. As the seed matures, the embryo is surrounded by large cotyledons sheathed by continuously thinning 'integuments'. However, this integumentary interpretation of the avocado seed coat is no longer accepted. Steyn *et al.* (1993) noted that the integuments represent only a very small non-vascularized portion of the seed coat, which is therefore not a testa. The vascularized portion is actually pachychalazal, in common with its ancient basal lineage (the magnoliid clade) in the Lauraceae (see Chanderbali *et al.*, Chapter 3, this volume). At maturity, the membranous pachychalazal seed coat is brown and papery. The seed coat provides the only vascular connection between the seed and mesocarp (Blumenfeld and Gazit, 1970b). Anatomical observations of abscising avocado fruit are often characterized by premature deterioration of the nucellus (Davenport and Manners, 1982; Garner, 2004) and the seed coat (Davenport and Manners, 1982; Cowan *et al.*, 1997; Garner, 2004). Whether deterioration of these tissues is the cause of fruit abscission or a symptom thereof remains to be determined.

The second stage of avocado fruit growth is dominated by mesocarp growth (Mougheith

*et al.*, 1978). The seed is surrounded by an actively dividing mesocarp that accumulates carbohydrates and proteins (Davenport and Ellis, 1959; Mougheith and Abdel-Hamid, 1978) and fatty acids, especially oleic acid (Mougheith and Abdel-Hamid, 1978; Eaks, 1990). During fruit growth, mesocarp water content decreases as oil content increases as a percentage of dry matter (Church and Chace, 1922; Davenport and Ellis, 1959; Eaks, 1990). Avocado fruit are unique relative to other fruit tree crops in that they accumulate high concentrations of protein (Church and Chace, 1922; Davenport and Ellis, 1959) and seven carbon ( $C_7$ ) sugars (Davenport and Ellis, 1959; Liu *et al.*, 1999b, 2002; Cowan, 2004). The  $C_7$  sugar, D-manno-heptulose, might be responsible for continued sugar uptake and growth of avocado fruit by inhibiting respiration, hexokinase activity and/or damage by reactive oxygen species (Cowan, 2004; Bertling and Bower, 2005, 2006; Bertling *et al.*, 2007). Avocado fruit development is slower than that of most other fruit tree crops. For example, developing 'Hass' fruit reach maturity 14–18 months after the start of bloom in some cool avocado-growing regions (Bergh, 1975). Therefore, mature fruit are present on the tree during the spring bloom and early fruit set of the next crop year. However, the fruit of most avocado cultivars, including 'Hass', do not ripen until harvested or until their pedicels are girdled (reviewed in Tingwa and Young, 1975), and therefore can be left to 'hang' on the tree for 18–19 months from the initial bloom date. The  $C_7$  sugars might play a role in the unusual ripening habit of avocado fruit (Robinson *et al.*, 2002; Cowan, 2004). As the phloem-transported  $C_7$  sugar content of avocado fruit decreases after both harvesting and pedicel girdling, it has been suggested that  $C_7$  sugars might prevent ripening when the fruit are still attached to the tree (Liu *et al.*, 1999b, 2002). However, other biochemical changes, including changes in fruit polyamine concentrations (Winer and Apelbaum, 1986) and hormone concentrations of fruit or stems, have also been suggested as possible ripening signals (Tingwa and Young, 1975). The putative role of  $C_7$  sugars in ripening and their known antioxidant activity have led to studies of the role of  $C_7$  sugars in post-harvest

fruit quality (Bertling and Bower, 2005; Bertling *et al.*, 2007).

### Assimilate partitioning and reproductive development

Inter- and intra-seasonal competition for limited carbohydrate resources have often been suggested as major factors restricting fruit production in avocado. Several studies of alternate bearing trees demonstrated correlations between starch reserves in branches and yields the following year (Scholefield *et al.*, 1985; van der Walt *et al.*, 1993; Whiley *et al.*, 1996a, 1996b). At the end of an 'off' year, starch reserves were high and at the end of an 'on' year, starch reserves were low, with the following years' yields highly positively correlated to the size of these reserves. Although the correlations were highly significant in all of these studies, there was no evidence of causation in that there was no indication that carbohydrate availability limited yields in 'off' years.

Competition for assimilates also occurs every spring and summer during reproductive and vegetative development. This competition is thought to be especially acute within indeterminate floral shoots. The leaves of indeterminate floral shoots are sinks during at least some period of early fruit set (Whiley, 1990; Finazzo *et al.*, 1994). Thus, vegetative and reproductive sinks are developing contemporaneously and in close spatial proximity. Indeterminate floral shoots have less fruit set than determinate floral shoots (Buchholz, 1986; Lovatt *et al.*, 1994; Thorp *et al.*, 1994; Salazar-García and Lovatt, 1998). The removal of the vegetative portion of indeterminate floral shoots (Biran, 1979; Zilkah *et al.*, 1987; Bower and Cutting, 1992) and the inhibition of vegetative growth with foliar applications of paclobutrazol (Cultar®) (Wolstenholme *et al.*, 1990; Zilkah and David, 1993) have been shown to increase fruit set. It has therefore been suggested that the reduced competition within determinate floral shoots, which do not have a vegetative component, is responsible for their higher fruit set. However, other researchers argue that carbohydrate export from developing indeterminate floral shoots to other tree structures provides evidence that carbohydrate production does not limit fruit set on those shoots (Thorp *et al.*, 1993; Finazzo *et al.*, 1994).

Furthermore, Finazzo *et al.* (1994) noted that fruit abscission continued after this period of competition, suggesting that fruit abscission from indeterminate floral shoots cannot be due solely to competition between vegetative and reproductive sinks.

In attempts to determine if and how carbohydrates limit avocado fruit set, numerous studies have examined the cycling of carbohydrate reserves in avocado trees throughout their phenology. Stem reserves are thought to be the only carbohydrate reserves utilized during spring reproductive and vegetative growth, as trunk and root supplies are not depleted at this time (Liu *et al.*, 1999a). In studies of 'Hass' and 'Fuerte' trees in Australia and of 'Hass' in California, branch starch concentrations were maximum just prior to flowering and then decreased during flowering, summer vegetative shoot growth and fruit development, with starch concentrations then increasing gradually during the winter until the next flowering period (Scholefield *et al.*, 1985; Whiley *et al.*, 1996a,b; Liu *et al.*, 1999a). Total soluble sugar (TSS) cycling was similar to that of starch, except that TSS concentrations peaked 2 months prior to flowering and again in the summer as flowering was ending and early fruit set was beginning (Liu *et al.*, 1999a). The authors noted that photoassimilates produced during the summer appeared to be utilized during summer growth, since storage reserves did not increase at this time. They hypothesized that photosynthate produced during the vegetative and reproductive growth of the summer might be adequate to sustain such growth (Liu *et al.*, 1999a). This suggests that limitations to fruit production are due to resource allocation, not resource availability. The idea that carbohydrates might not be limiting to yields has been hypothesized for other fruit tree crops (Bangerth, 2000). This has led many researchers to investigate the roles of endogenous hormones in the allocation of reserves to fruit and competing sinks, and the use of exogenous plant growth regulators to increase fruit growth by manipulating sink strength.

### **Endogenous hormones and fruit development**

The seed of developing avocado fruit contains the highest concentrations of hormones analyzed,

but as the fruit nears maturity and the seed coat becomes desiccated, concentrations of hormones decrease dramatically. In avocado fruit grown in Israel, researchers found that numerous cultivars had a high concentration of auxin in the seed with higher concentrations found in fruit with faster mesocarp growth, suggesting that auxins influence sink strength of growing fruit (Gazit and Blumenfeld, 1972). GA synthesis might also influence sink strength of fruit, as GA-like activity in the seed coat decreases over time as tissue growth rates decrease (Blumenfeld and Gazit, 1972). Cytokinin (CK) concentrations are also high in avocado seeds and seed coats, and decrease as fruit mature (Blumenfeld and Gazit, 1970a). Other researchers have found that concentrations of isopentenyladenine (IPA), a CK, are high in the developing seed coat and decrease with fruit maturity, becoming undetectable once the seed coat is dry (Cutting *et al.*, 1986). The concentration of the polyamines putrescine, spermidine and spermine are high during early fruit development, suggesting that polyamines might play a vital role in early fruit growth, possibly by promoting cell division (Winer and Apelbaum, 1986; Kushad *et al.*, 1988). Polyamine concentrations are highest in the seed coat and decrease with maturity (Winer and Apelbaum, 1986). Thus the large seed, or more specifically its seed coat, is thought to provide the sink strength for avocado fruit. This might help to explain the low rate of persistence of seedless avocado fruit.

Phenotypically small 'Hass' fruit (< 200 g) have been used as a model system to investigate the synergistic and antagonistic nature of hormones in avocado fruit growth. In 1993, Cutting referred to the production of this small fruit variant in the warmest avocado-growing regions of South Africa, and the 'rescuing' of these fruit by CK applications. The small fruit variant has since been used by the Cowan laboratory to model the role(s) of hormones in avocado fruit development, and the interrelationship of the hormones produced by the isoprenoid pathway, which generates, among other bioactive compounds, CKs, abscisic acid (ABA) and GAs. Compared to normal fruit, the small fruit are characterized by early degeneration of the seed coat, decreased cell numbers, high mesocarp ABA concentrations, low CK

concentrations and a 70% decrease in the activity of the rate limiting enzyme early in the isoprenoid pathway, 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) (Cowan *et al.*, 1997). HMGR activity and/or expression in developing fruit are hypothesized to be regulated by sugar and ABA content (Richings *et al.*, 2000). Initially, it was the ratio of CKs to ABA that was thought to be critical for proper fruit development (Cowan *et al.*, 1997). Moore-Gordon *et al.* (1998) confirmed that as IPA concentrations decreased, ABA concentrations increased and a significant positive linear relationship exists between the IPA to ABA ratio and fruit size. Further work by the Cowan laboratory using the small fruit variant model demonstrated that though seed coat ABA content increases transiently during the onset of the small fruit phenotype, CK-like activity is not different between the normal and small fruit types during fruit development (Taylor and Cowan, 2001; Cowan *et al.*, 2005). However, indole-3-acetic acid (IAA) content within the developing seed was significantly higher in normal fruit compared to small fruit (Cowan *et al.*, 2005). Decreased ABA biosynthesis and increased IAA biosynthesis are correlated with decreased xanthine dehydrogenase (XDH) activity (Taylor and Cowan, 2004). Cowan *et al.* (2005) hypothesize that stress might decrease the activity of XDH, increasing the seed ABA to IAA ratio, ultimately resulting in reduced fruit growth. In addition to being a model for the role of hormones in avocado fruit development, differences in the expression of genes thought to be responsible for the regulation of cell division have also been demonstrated between the normal and small fruit phenotypes (Dahan *et al.*, 2010).

Ethylene is produced by developing avocado fruit, with the majority of it being produced in the seed coat (Sitrit *et al.*, 1987). An increase in ethylene production by developing avocado fruit precedes their abscission and is correlated positively with fruit abscission rates and inversely with fruit size (Adato and Gazit, 1977a). However, ethylene evolution is thought to be one of the last steps in the changes preceding abscission and not the underlying cause of fruit abscission (reviewed in Bangerth, 2000). The seed coat is also the site of high levels of pre-climacteric ethylene synthesis in

harvested fruit, though this ethylene production only results in the ripening of mature fruit (Adato and Gazit, 1977b).

### Flower and fruit abscission

In contrast to agronomic fruit tree species such as apple (*Malus domestica* Borkh.) and pear (*Pyrus communis* L.), which often require thinning to reduce fruit set and increase the production of fruit of marketable size and quality (reviewed in Williams and Edgerton, 1981; Looney, 1998; Bangerth, 2000), the avocado has extremely low fruit set (< 0.1%) even in healthy, well-managed orchards (Whiley and Schaffer, 1994; Garner and Lovatt, 2008). The avocado and many other subtropical fruit tree crops, including mango (*Mangifera indica* L.) (Singh, 1960), macadamia (*Macadamia* sp.) (Sakai and Nagao, 1985) and lychee (*Litchi chinensis* Sonn.) (Stern *et al.*, 1995), are characterized by the abscission of thousands of flowers and immature fruit, with only a small percentage of these fruit persisting to harvest.

One of the first attempts to characterize flower and fruit abscission of the avocado was a branch study conducted in Trinidad utilizing numerous cultivars (Papademetriou, 1975). More than 95% of observed flowers abscised within 1 week of the end of bloom and fruit abscission was complete 4–12 weeks after the end of bloom, depending on the cultivar. Final fruit set estimates ranged from 0 to 0.66% per branch. In studies of fruit subsamples monitored on 'Fuerte' trees in Mexico (Pérez *et al.*, 1988) and 'Hass' trees in Israel (Adato and Gazit, 1977b), it was found that most fruit abscised within a few months of the end of the bloom, followed by a gradually declining rate of fruit abscission, with fruit abscission ceasing in October. During observations of bloom in Florida, Davenport (1982) found that many commercially important cultivars, including 'Hass' and 'Fuerte', were characterized by high rates of fruit abscission, whereas others had low initial fruit set rates and limited abscission. In one of the few studies designed to characterize and quantify whole-tree flower and fruit abscission in avocado, researchers in Israel found that individual 'Fuerte' avocado trees can lose hundreds of thousands of flowers and

thousands of developing fruit in a single season with a final fruit set of < 0.02% (Lahav and Zamet, 1975). Fruit abscission was also monitored on 'Hass' and 'Ettinger' trees during the study, and for all three cultivars, fruit loss was intense during May and June, but was negligible by September (Northern Hemisphere). In a study of two 'Fuerte' trees in South Africa, more than 400,000 flowers and 10,000 developing fruit abscised per tree within a 10-week period (Slabbert, 1981). In Japan, 'Fuerte', 'Bacon' and 'Zutano' abscised tens of thousands of flowers and hundreds of fruit per tree (Inoue and Takahashi, 1990). Cultivar differences and year effects were pronounced. The highest fruit set value in this study was < 0.04%. In a small study (two trees observed for 2 years), 'Fuerte' trees in California lost hundreds of thousands of flowers per season, with a mean final fruit set of 0.07% (Cameron *et al.*, 1952). In a study of ten alternate bearing 'Hass' trees in California, an average of 75,000 and 339,000 flowers abscised in 'off' and 'on' crop years, respectively (Garner and Lovatt, 2008) and the majority of these flowers were not pollinated (Garner, 2004). Abscission of immature fruit peaked approximately 1 month after peak flower abscission with an average of 50 and 280 fruit per day in 'off' and 'on' crop years, respectively (Garner and Lovatt, 2008). Researchers using both open and hand pollination have noted that many fruit abscising during this time period, typically referred to as June drop, are unfertilized enlarged ovaries (Tomer and Gazit, 1979; Sedgley, 1980). Taken together, these studies of avocado flower and fruit abscission indicate that excessive abscission occurs in the spring and summer, the early and June fruit drop periods, respectively, after which the fruit abscission rate declines, typically reaching zero fruit per week in autumn, though the intensity, timing and longevity of the abscission periods varies with both cultivar and location. When abscission of immature fruit decreases, the abscission of mature fruit from the preceding year's crop begins, suggesting that the two abscission processes are regulated independently (Garner and Lovatt, 2008). The 'off' or 'on' crop status of the tree does not affect the timing of flower and fruit abscission, percentage fruit set, or the average diameter or biomass of individual fruit abscising at similar

phenological stages, despite differences in the magnitude of the number of reproductive structures abscising (Garner and Lovatt, 2008). This suggests that yield is not solely resource-limited and that alternate bearing is neither the cause of nor the result of flower or fruit abscission.

Although the loss of large numbers of fruit is a natural thinning process for most forest species and fruit tree crops, '... from a horticultural point of view, this self-regulatory mechanism may be too strong for fruit species, such as... avocado...' (Bangerth, 2000). Wolstenholme (1990) listed the avocado among subtropical evergreen crops domesticated relatively recently and described the need for new cultural practices to maximize their productivity. Avocado trees are physiologically capable of bearing much larger crops. By determining the energy required to produce 1000 kg of apple, 'Valencia' orange (*Citrus sinensis* L.) or avocado fruit, and then comparing yields of high-intensity apple, orange and avocado production, it was estimated that a well-managed avocado orchard at an equivalent level of technology is theoretically capable of averaging 32.5 t ha<sup>-1</sup> of fruit with 17% oil content (Wolstenholme, 1986). Worldwide, yields over the last decade have averaged significantly less than this (Avocadosource.com, 2003).

### Alternate Bearing

Avocado trees, especially the 'Hass' cultivar, are prone to alternate bearing (also called biennial, irregular or uneven bearing), repeating cycles of a light, low yield 'off' crop ('off' tree, 'off' year) followed by a heavy, high yield 'on' crop ('on' tree, 'on' year). Although, in general, the 'on'/'off' cycles are biennial, in some cases, an 'on' year can be followed by two or more consecutive 'off' years, and vice versa. In addition, yield reductions from the 'on' to 'off' year can be 100%, with no fruit the year following the 'on' crop (Whiley *et al.*, 1992). The phenomenon is widespread, occurring in both deciduous and evergreen trees under commercial production and in nature, where it is called 'masting' (Monselise and Goldschmidt, 1982). Monselise and Goldschmidt (1982) concluded that alternate bearing appears to be inherent in

polycarpic perennial plants and observed that it can occur over a large geographical area, an entire orchard, an individual tree, part of a tree or even for one branch.

The negative economic impact of alternate bearing on commercial avocado industries locally as well as internationally is substantial. The 'on' crop is characterized by a large number of small-sized fruit of reduced commercial value at harvest (Lovatt, 2011a). For the 'off' crop, fruit size is large but there are too few fruit to provide the grower with a good income. In addition, the reduced amount of fruit in the 'off' year can result in a loss of fresh fruit market share that jeopardizes the sustainability of an industry. Years with low fruit volume can also compromise the development and sustainability of value-added product industries. Furthermore, uneven crop loads, especially within an orchard, complicate orchard management, including the use of plant growth regulators to increase yield and fruit size (Garner *et al.*, 2011). For a grower-orientated crop management discussion of alternate bearing, see Whiley *et al.*, Chapter 12, this volume.

### Triggers of alternate bearing

Alternate bearing in avocado, as reported for apple and pear, is typically initiated by climatic events, e.g. freeze, low or high temperatures, or lack of rain during bloom or fruit set, which result in low flower number or cause excessive flower and/or fruit abscission (Jonkers, 1979). This results in an 'off' crop, which is typically followed by an 'on' crop the next year, depending on how long it takes the trees to recover. Conversely, optimal conditions during bloom and fruit set that preclude natural flower and fruit thinning result in an 'on' crop that is followed by an 'off' crop. Cultural practices can initiate alternate bearing. Inadequate irrigation or fertilization that result in excessive flower and fruit abscission, create an 'off' crop that, with timely correction of these cultural conditions, can be followed by an 'on' crop. Excessive pruning, resulting in low flower or fruit number and vigorous vegetative shoot growth will result in an 'off' crop followed by heavy flowering on the new vegetative shoots

and an 'on' crop. Delayed harvest has been proposed as a factor that exacerbates alternate bearing in avocado (Whiley *et al.*, 1996a,b; Paz-Vega, 1997). Once initiated, alternating 'on'/'off' yield cycles are perpetuated by the effect of fruit number (crop load) on factors within the tree that ultimately impact floral intensity the following year (van der Walt *et al.*, 1993).

### Perpetuation of 'on'/'off' yield cycles

The physiological processes by which the 'on'/'off' production cycles become entrained constitute a key determinant of annual avocado tree productivity that are not well defined. An 'on' year is initiated with an intense bloom that sets and matures the 'on' crop that in turn causes a low floral intensity 'off' bloom and an 'off' crop the following year, whereas the 'off' bloom sets and matures an 'off' crop that results in an intense 'on' bloom the next spring (Garner and Lovatt, 2008). The bloom that sets the 'on' crop is earlier, consistent with bud break being less inhibited in the presence of the 'off' crop set the previous year, and occurs over a longer period than the 'off' crop bloom (Garner and Lovatt, 2008). Results of research designed to determine whether yield one year influenced the number of reproductive structures that abscised from 'Hass' avocado trees the following year, demonstrated that the 'off' or 'on' crop status of the tree did not influence the percentage fruit set or average fruit diameter or biomass of individual fruit that abscised at similar developmental stages. Furthermore, the number of mature fruit in the previous year's crop did not influence flower or fruit abscission of the current crop. In both years of the research, as abscission of the immature fruit decreased, abscission of the preceding year's crop (mature fruit) increased, indicating the processes were controlled independently and not driven by crop load. During the study, neither weather conditions nor tree nutrient status was associated with key abscission events. Taken together, the results of this research provide strong evidence that the previous year's yield does not influence current year flower or fruit abscission of the 'Hass' avocado. Thus, the normal seasonal abscission of reproductive structures, in

the absence of a triggering climatic or cultural event, appears to be an independent process that does not contribute to the entrainment of 'on'/'off' yield cycles of the 'Hass' avocado during alternate bearing. Further, the results of this research provided additional evidence that final yield of the 'Hass' avocado is strongly related to the number of floral shoots (inflorescences) at bloom (Garner and Lovatt, 2008).

Typically, avocado trees in the humid subtropics produce two major vegetative shoot flushes in spring and summer of each year, respectively (Scholefield *et al.*, 1985; Whiley *et al.*, 1988; Whiley and Schaffer, 1994), with avocado-growing areas like California having a third, autumn vegetative shoot flush (Salazar-García *et al.*, 1998), and some avocado-growing areas of Mexico having an additional (fourth) winter vegetative shoot flush (Salazar-García *et al.*, 2006). The vegetative shoots in each of these flushes have the potential to produce floral shoots the following spring. The heavy 'on' crop suppresses both the number and length of vegetative shoots that develop (Lahav and Kalmar, 1977; Wolstenholme *et al.*, 1990). Schaffer *et al.* (1991) reported that individual fruits influenced shoot growth. Non-fruiting shoots produced multiple vegetative shoot flushes, whereas fruiting shoots flushed only once in spring. In addition, the vegetative shoot at the apex of indeterminate floral shoots that set fruit do not produce summer or autumn vegetative shoots (Davenport, 1982; Salazar-García *et al.*, 1998). In contrast, avocado trees that set an 'off' crop in spring produce a significant number of summer and autumn vegetative shoots that contribute floral shoots to bloom the next spring (Year 2).

In California, for 'Hass' avocado trees in an 'off' year (Year 1), 70–80% of the floral shoots at bloom (Year 2) were produced by the Year 1 summer vegetative shoots, with spring and autumn vegetative shoots each contributing between 10–15% of the Year 2 floral shoots (Tables 6.4 and 6.5) (Lovatt, 2011a). Summer vegetative shoots produce a greater number of floral shoots than spring and autumn vegetative shoots because floral shoots are borne in the axil of the leaves along the length of a summer vegetative shoot (Thorp *et al.*, 1993; Salazar-García and Lovatt, 1998). In California, Year 1 summer shoots that develop during 'off' crop

**Table 6.4.** Contribution of Year 1 spring and summer plus autumn shoots, with and without fruit on 'on' and 'off' crop 'Hass' avocado trees, to return bloom in Year 2. (Lovatt, 2011a.)

	Total	Summer +	
		Spring	Autumn
No. floral shoots/4 shoots			
<b>Fruiting shoots</b>			
'On' crop tree	0.3 c <sup>a</sup>	0.2 c	0.1 b
'Off' crop tree	1.7 bc	0.7 bc	1.0 b
<b>Non-fruiting shoots</b>			
'On' crop tree	4.2 b	2.3 a	1.9 b
'Off' crop tree	10.2 a	1.9 ab	8.3 a
<i>P</i> -value	<0.0001	0.0076	<0.0001

<sup>a</sup>Values in a vertical column followed by different letters are significantly different at the *P*-value specified by Fisher's Protected LSD Test.

years produce 80% of the Year 2 determinate floral shoots (Lovatt, 2011a). Determinate floral shoots, which are more productive than indeterminate floral shoots (Buchholz, 1986; Lovatt *et al.*, 1994; Thorp *et al.*, 1994; Salazar-García and Lovatt, 1998), are produced by both apical and axillary buds of summer flush vegetative shoots (Salazar-García and Lovatt, 1998). Autumn shoots that flower produce only a single terminal indeterminate floral shoot (Thorp *et al.*, 1993; Salazar-García and Lovatt, 1998). Late-developing autumn shoots remain vegetative. The 'on' crop of young developing fruit inhibits summer and autumn vegetative shoot growth, thereby reducing the contribution of these shoots to bloom the next spring (Tables 6.4 and 6.5). This results in an 'off' bloom produced predominantly by the Year 1 spring flush of vegetative shoots and characterized by a significantly greater proportion of indeterminate to determinate floral shoots.

The importance of the summer and autumn shoots to the intensity of the return bloom was confirmed by removing all summer and autumn vegetative shoots or just the autumn vegetative shoots from 'off' crop 'Hass' avocado trees. The floral intensity at return bloom and the return yield of these trees were compared to non-treated 'off' and 'on' crop trees. At return bloom the next year, non-treated 'off' crop control trees and 'off' crop trees with only the autumn flush vegetative shoots removed flowered profusely, whereas 'off' trees with the summer

plus autumn shoots removed had a weak bloom and low return yield (2 kg per tree); 'on' crop control trees yielded 10 kg per tree (Lopez-Jimenez and Lovatt, summarized in Lovatt, 2011a).

### Whole tree and localized effects of fruit

The effect of the setting crop on return bloom is both localized to shoots that set fruit and a function of the total number of fruit per tree (crop load). The interaction between the localized and whole tree effect of fruit on return bloom was determined by comparing the number of floral shoots produced by Year 1 spring shoots that set fruit (fruiting shoots) and shoots that did not set fruit (non-fruiting shoots) for 'on' and 'off' crop avocado trees (Table 6.4) (Lovatt, 2011a). Maximum return bloom was attained on non-fruiting shoots of 'off' crop trees. These data also confirm the greater contribution of summer plus autumn shoots versus spring shoots of 'off' crop trees to return bloom the following year. The combined localized and whole tree effect of fruit was strongest for buds on Year 1 spring shoots that set fruit on 'on' crop trees, where the full effect of fruit in

alternate bearing was expressed. Comparison of fruiting and non-fruiting shoots on 'off' crop trees provided evidence that 'Hass' avocado fruit exert a strong effect on return bloom, localized to shoots that set fruit. Similarly, a whole tree effect of fruit was evident for non-fruiting shoots of 'on' crop trees. These data, and similar data not presented herein, document that for trees setting an 'on' crop, return bloom and yield was highly dependent on the number of shoots that did not set fruit. This fact reinforces using cultural practices that reduce crop load at the proper time through fruit thinning, by hand, chemical fruit removal or pruning, to mitigate alternate bearing.

### The cumulative effect of fruit from set to harvest

Classic fruit removal experiments designed to identify when the 'on' crop was exerting its effect on the contribution of spring, summer and autumn flush vegetative shoots to return bloom of the 'Hass' avocado in California, demonstrated that the effect was cumulative over time and the sum of the effects on the three flushes of shoots (Table 6.5). For this experiment, only the current setting fruit were present on the trees, the previous year's mature crop having

**Table 6.5.** Effect of the 'off' and 'on' crop and fruit removal from 'on' crop 'Hass' avocado trees on the contribution of Year 1 spring, summer and autumn vegetative shoots to return bloom (Year 2).

	Spring shoots (no./branch)			Summer (S) and autumn (A) shoots (no./branch)			
	Floral shoots	Vegetative shoots	Inactive buds	Total S + A shoots	Floral shoots	Vegetative shoots	Inactive buds
'Off' crop trees	10.6cde <sup>a</sup>	0.7c	0.8a	30.7c	66.8bc	4.3bcd	0.5a
'On' crop trees with fruit removed							
June	14.4abc	2.9bc	2.3a	42.8ab	80.5ab	3.3bcd	1.1a
July	21.8ab	2.0bc	1.6a	50.1a	119.0a	2.4bcd	1.3a
August	21.8ab	2.1bc	0.8a	35.6bc	92.5ab	2.1bcd	0.4a
September	23.8a	1.4c	0.6a	24.6cde	62.4bc	1.4cd	0.1a
October	13.9bcd	0.8c	1.9a	18.0e	33.0cd	0.8d	0.4a
November	13.8bcd	2.1bc	1.5a	21.1de	28.6cd	9.5abcd	1.9a
December	5.0cde	5.9a	2.4a	17.5e	7.6d	12.6abc	2.6a
January	2.9e	2.6bc	2.8a	27.4cde	19.4d	17.5a	2.1a
'On' crop trees	4.5de	4.1ab	3.1a	22.6de	19.1d	13.3ab	1.7a
<i>P</i> -value	0.0010	0.0036	0.1388	0.0003	0.0002	0.0533	0.4319

<sup>a</sup>Values in a vertical column followed by different letters are significantly different at specified *P*-values by Fisher's Protected LSD Test.



been harvested before June. Young developing fruit were removed from different sets of 'on' crop trees monthly from June through January. The number of floral and vegetative shoots and inactive buds at bloom the following spring were quantified.

For Year 1 spring flush shoots, fruit removal from June through September significantly increased the percentage bud break and number of floral shoots produced at return bloom (Table 6.5). Progressively later fruit removal from October through December reduced both the percentage bud break and the floral intensity of Year 1 spring shoots, to a number equal to and not significantly different from the contribution of Year 1 spring shoots of either 'off' or 'on' crop trees, respectively. This suggests that buds on Year 1 spring flush shoots remained both floral and viable through December.

Removal of the 'on' crop in June and July increased the number of summer vegetative shoots that developed and the number of floral shoots produced by these shoots at return bloom. Progressively later fruit removal reduced the growth of summer shoots and commensurately, their contribution to bloom. However, the average number of floral shoots produced per summer shoot was 2.4–2.6 from July through September. Later fruit removal resulted in growth of autumn flush vegetative shoots. A significant proportion of the apical buds of the autumn flush shoots in this study remained vegetative. At bloom, many dormant buds abscised. Whether these buds were floral and still viable remains to be determined.

Leaving the mature 'on' crop of fruit on the trees through the spring and summer to October of Year 2 (the young setting Year 2 crop was removed in June from these trees) reduced return bloom in Year 3 to a similar degree as young developing fruit of an 'on' crop (Table 6.6). The reduction in floral shoot number was due to a decrease in the number of vegetative shoots that developed during the summer of Year 2 in both cases. The mature crop was harvested in mid-October; there was no effect on autumn vegetative shoot growth and the contribution of the autumn flush shoots to return bloom. Thus, the practice of delaying the harvest of a heavy 'on' crop through summer to attain greater fruit size or using 'on-tree fruit storage' to wait for better market prices indeed exacerbates alternate bearing, as was also found by Whiley *et al.* (1996a, b). The data reinforce the benefit of an early harvest prior to this critical period of avocado tree phenology and explain the relatively high yields obtained in semi-warm highland tropical and humid subtropical climates, since in these regions harvest is usually done 1–3 months before bloom (Salazar-García *et al.*, 2007c).

#### Relationship between the effect of the 'on' crop and floral development

The 'on' crop clearly inhibits bud break of buds from which summer vegetative shoots develop, thereby reducing the floral intensity of the

**Table 6.6.** Effect of delayed harvest of the mature 'on' crop until October of Year 2 on the contribution of the Year 2 summer shoots to return bloom in Year 3 compared to the effects of the setting 'on' and 'off' crops of the 'Hass' avocado in California.

	Floral shoots			Vegetative	Inactive buds
	Total	Indeterminate	Determinate	shoots	
	Average no./8 shoots per tree				
'Off' crop control	18.0a <sup>a</sup>	5.0a	13.0a	0.2c	24.4b
Mature 'on' crop only	5.2b	3.0a	2.2bc	3.2b	26.8ab
'On' crop control	8.8ab	4.0a	4.8abc	5.0ab	36.2a
<i>P</i> -value	0.0248	0.3344	0.0409	0.0016	0.0494

<sup>a</sup>Values in a vertical column followed by different letters are significantly different at specified *P*-values by Fisher's Protected LSD test.

return bloom. The further decrease in floral intensity with progressively later fruit removal evident in the spring following the 'on' crop (Table 6.5) might be due to inhibition of bud break of viable floral buds in spring. Alternatively, the 'on' crop might inhibit floral development creating non-viable buds that abscise during spring bloom. Increased 'exposure' to the 'on' crop could logically lead to greater paradormancy and reduced bud break. From an evolutionary viewpoint, inhibition of bud break of viable floral buds, as opposed to inhibition of floral development, ensures the reproductive success of the tree at any point in time that the current crop is lost.

In the absence of data on floral development collected concurrently with fruit removal treatments, the timing of key stages of floral development of 'Hass' avocado reported by Salazar-García *et al.* (1998) and described earlier in this chapter was contrasted with the timing of the cumulative effect of the 'on' crop on floral intensity. The goal was to identify possible effects of the 'on' crop on floral development.

Flowering proceeds in three stages: competent (able to respond to a floral signal), determined (committed to floral development, reversion to vegetative growth cannot occur) and expressed (morphological changes associated with inflorescence or flower development are evident) (Taiz and Zeiger, 2006). Since commercial avocado orchards are comprised of trees with scions derived from buds from adult trees, all buds are competent. Avocado is an autonomous flowering species (Venning and

Lincoln, 1958; Salazar-García *et al.*, 1998), which means that individual vegetative shoots elongate to produce a genetically determined number of nodes at which time the shoot apical meristem (SAM) undergoes an epigenetic change, associated with phase transition from vegetative growth (i.e. leaf production) to reproductive growth (inflorescence development). It must be noted that at the level of genetic regulation of flowering, there is significant 'cross-talk' between the autonomous and vernalization (i.e. low temperature) flowering pathways; thus, flowering in autonomous species can be induced by low temperature (Taiz and Zeiger, 2006).

Salazar-García *et al.* (1998) established that the primary chemical signal for annual phase transition of avocado occurred in the summer, at the end of July–beginning of August (Northern Hemisphere), with four secondary axis inflorescence meristems evident by 30 August, consistent with the commitment of the primary meristem to floral development (Salazar-García *et al.*, 1999) (Figs. 6.6 and 6.11). From August through October, secondary axis meristems of the inflorescence developed, with ten secondary axis meristems present by 30 October. Despite high temperatures (27–42°C) that occurred from August to October in the year of this research, reversion of apical buds to vegetative growth was not observed (Salazar-García *et al.*, 1998), consistent with apical buds of summer flush shoots being irreversibly committed to flowering. In Table 6.5, it can be seen that progressively later removal of the 'on' crop through September resulted in fewer summer vegetative shoots.

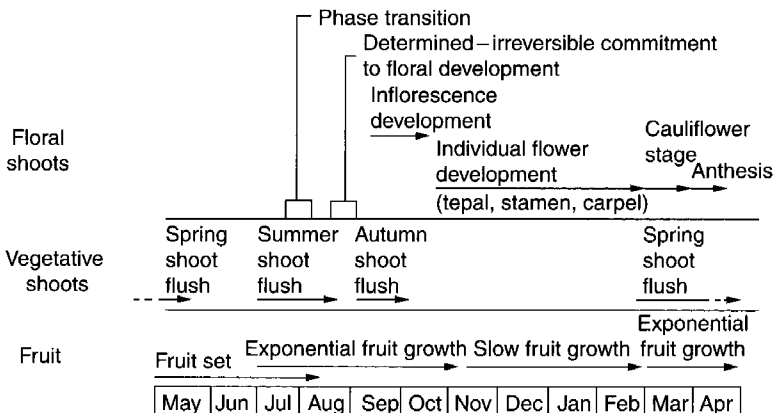


Fig. 6.11. 'Hass' avocado tree phenology in California (after Salazar-García *et al.*, 1998).

However, these shoots consistently contributed two to three floral shoots per summer shoot at return bloom. In addition, there was a significant increase in the number of floral shoots contributed by spring shoots to return bloom over this period compared to both 'on' and 'off' crop trees. These results suggest that fruit do not inhibit phase transition on spring or summer shoots. In addition, inhibition of phase transition would have resulted in a concomitant increase in vegetative shoot number at return bloom. This did not occur (Table 6.5). The low number of floral shoots produced per summer shoot might suggest the possibility that the 'on' crop inhibits phase transition of some axillary buds, which become determined at a later time than the apical bud. Consistent with this interpretation, the number of determinate floral shoots is reduced by 50% when fruit removal is delayed to September.

By 30 November, development of terminal flowers on secondary and tertiary inflorescence axes was initiated, but by 30 December, only the oldest (basal) secondary axis had cymes with fully formed flowers. By 30 January, the reproductive structures (gynoecium and stamen) were developing in the oldest flowers (Salazar-García *et al.*, 1998). The data in Table 6.5 document the dramatic reduction in floral shoot number that occurred when the 'on' crop was not removed until December. This comparison suggests the possibility that the 'on' crop might inhibit subsequent development of the more apical secondary axes and individual flowers, which could result in unviable buds on Year 1 spring and summer shoots at bloom.

For autumn flush vegetative shoots, delaying fruit removal from October through January reduced floral shoot number and increased vegetative shoot number at spring bloom (Table 6.5), suggesting the likelihood that phase transition was inhibited for late-developing autumn flush vegetative shoots. Whether this was due to the presence of the 'on' crop or climate was not resolved in this study, but 'on' crop trees produced more vegetative shoots at return bloom than 'off' crop trees.

The canopy of the avocado tree is comprised of vegetative shoots of varied developmental ages that render floral development asynchronous, a condition that typifies the interactions between climate and the presence of fruiting and non-fruiting shoots (Schaffer

*et al.*, 1991). Despite this, results of fruit removal experiments are useful for determining when the 'on' crop affects return bloom and thereby provide insight into a possible mechanism.

### **Relationship between carbohydrate availability and the effect of the 'on' crop on floral intensity at return bloom**

The starch concentration of a tissue is an excellent barometer of carbohydrate availability. Starch concentrations of vegetative tissues have repeatedly been demonstrated to be greater at the end of the 'off' crop year and lower at the end of the 'on' crop year for deciduous and evergreen tree crop species (reviewed by Paz-Vega, 1997). It is noteworthy that in alternate bearing pistachio (*Pistacia vera*), the differences in starch concentration are accompanied by similar differences in the concentrations of nitrogen, phosphorus and potassium between 'off' and 'on' crop years (Rosecrance *et al.*, 1998). The high oil content of the avocado fruit provides a logical explanation for the depletion of carbohydrate reserves during the production of an 'on' crop. What is of interest is whether differences in starch concentration between 'on' and 'off' crop trees exist at the time when differences in crop load are known to impact tree phenology. For example, are there differences in the amount of carbohydrate available to support summer vegetative shoot growth and phase transition, during floral organogenesis or at spring bud break related to crop load?

Paz-Vega (1997) reviewed the literature reporting seasonal changes in starch concentrations in relationship to vegetative and reproductive growth of alternate bearing avocado trees. Differences in starch concentration resulting from differences in crop load occurred predominantly in the winter and spring, at which time carbohydrate availability could logically influence late stages of floral development and/or spring bud break. In light of the results of on-going research, it is tempting to speculate that sugar signalling might be an important aspect of the hormonal control of bud break (reviewed in Anderson *et al.*, 2010).

For both 'on' and 'off' crop trees, starch concentrations declined during flowering and fruit set; no differences in starch concentration were reported to occur in summer. Whiley and

inflorescence number and increased vegetative shoot number of apical buds primarily on autumn shoots (Salazar-García and Lovatt, 1998). Stimulation of vegetative shoot growth at the apex of indeterminate floral shoots by GA<sub>3</sub> inhibited bud break of axillary buds. Progressively later applications of GA<sub>3</sub> in December, January or February, when the proportion of fully formed inflorescences was greater, had no effect on the number of floral shoots produced by summer or autumn flush shoots.

In apple, GA<sub>1</sub> and GA<sub>4</sub> accumulate in buds of 'on' crop trees at the time of floral bud development with a concomitant reduction in return bloom (Kittikorn *et al.*, 2010). However, for avocado it must be determined whether GA<sub>1</sub> or GA<sub>4</sub> (or other avocado GAs) have the same effect and whether the GA is exported from the fruit at the proper time to influence phase transition or subsequent floral development. Thus, the putative role of GA in inhibiting floral development during the 'on' crop year of avocado remains to be demonstrated.

#### *Auxin, cytokinin and abscisic acid*

The question of whether the inhibition of summer vegetative shoot growth that occurs during the 'on' crop year is due to hormones was investigated. The effects of 'off' and 'on' crops and fruit removal from 'on' crop 'Hass' avocado trees on endogenous hormone concentrations of buds were quantified (Eyheraguibel and Lovatt, unpublished). As in previous experiments, June fruit removal from 'on' crop trees increased the number of summer vegetative shoots to a value above that of both 'on' and 'off' crop control trees with a concomitant increase in floral shoot number at return bloom that was greater than that of 'on' crop trees and equal to that of 'off' crop trees. In response to removal of the 'on' crop in June, by August there was a significant increase in the concentration of the cytokinin IPA to a value greater than that of 'on' and 'off' crop trees. In addition, the concentration of IAA was significantly reduced. Bud concentrations of ABA were not influenced by crop load or June fruit removal. The ratio of auxin to cytokinin was significantly greater in buds from 'on' crop trees than 'off' crop trees. Trees with the 'on' crop removed in June had the lowest auxin to

cytokinin ratio. Taken together, these results suggest that bud break of the buds producing the summer vegetative shoot flush are inhibited on 'on' crop trees by a high auxin to cytokinin ratio typical of apical dominance (endodormancy), but more accurately described as paradormancy due to the putative role of fruit. Concurrent analysis of roots indicated that crop load and fruit removal had no effect on root hormone homeostasis during the summer months.

The results suggest that IAA is exported from the fruit and accumulating in the buds apical to the fruit. Although a controversial idea, it is consistent with the conclusion of Cowan *et al.* (2005) that IAA export from avocado fruit is essential to maintain fruit growth. In contrast, IAA accumulation in apical buds could be due to reduced polar transport from the shoot apex (Bangerth, 2009) and thus, be an indirect effect of the fruit. Studies with the *Arabidopsis* auxin transport mutant *pin1* provided evidence that normal polar transport of IAA was not critical to transition from vegetative to reproductive growth; *pin1* mutants produced inflorescences. Normal polar transport of IAA was, however, essential for formation of flower primordia within the inflorescence (Cheng and Zhao, 2007). IAA also regulated the number and identity of floral organs that developed.

The buds from 'on' crop trees with all fruit removed each month from June through October, respectively, had high IPA concentrations and low IAA and ABA concentrations by February relative to 'on' crop trees that did not have their fruit removed until November, December or January ( $P < 0.0001$ ) (Eyheraguibel and Lovatt, unpublished). The low IPA and high IAA and ABA concentrations of buds from 'on' crop trees with fruit removed after October corresponded with the reduction in floral shoot number for these trees ( $P = 0.0002$ ) compared to 'off' crop trees and 'on' crop trees with fruit removed prior to October. Concurrent analysis of roots indicated that crop load and fruit removal had no effect on root hormone homeostasis.

Whereas changes in bud hormone concentrations obtained in response to fruit removal were clear, the results obtained for hormone concentrations in buds from 'off' and 'on' crop

trees were frequently not significantly different, because in our early studies we did not keep close track of whether buds were collected from fruiting or non-fruiting shoots (Lovatt, 2006). However, taken together, the results provide evidence that bud concentrations of IPA decreased and IAA and ABA increased, and hence the ratios of IAA and ABA to cytokinin increased in apical and axillary buds in November through February of the 'on' crop year in parallel with the decrease in the number of buds that underwent bud break in spring.

To test the possibility that these changes in bud hormone homeostasis were related to inhibition of spring bud break, 'on' crop 'Hass' avocado trees were trunk injected in January (Year 2) with the auxin-transport inhibitor tri-iodobenzoic acid (TIBA) combined with a cytokinin. The treatment increased the total number of floral shoots that developed at return bloom, with the greatest contribution due to an increase in determinate floral shoots borne on summer shoots, most notably on fruiting shoots ( $P < 0.10$ ) (Lovatt, 2010). In subsequent experiments, trunk injecting 'on' crop 'Hass' avocado trees in July and again in January increased the number of determinate floral shoots borne on summer flush shoots, and total floral shoot number at return bloom to a value intermediate to 'off' and 'on' crop trees (Lovatt, 2011b).

In recent reviews of the role of hormones in floral induction, evidence pointed to cytokinins among all the hormones as having a promotive role in floral induction in woody perennials, with IAA and GA having inhibitory effects on floral induction (Hanke *et al.*, 2007; Bangerth, 2009). Apical dominance is the classic example of the interaction between IAA and cytokinin in regulating development. In apical dominance, IAA negatively regulates cytokinin biosynthesis at the node of the shoot (point of attachment and vascular connection of the leaf and axillary bud to the shoot) by controlling the expression level of the gene adenosinephosphate isopentenyltransferase (IPT), which encodes the key regulatory enzyme in cytokinin biosynthesis (Tanaka *et al.*, 2006). This interaction might similarly play a regulatory role in flowering.

Whether IAA and cytokinins have a direct effect on phase transition or subsequent stages of floral development in avocado remains to be

determined. However, at the present time, the data presented above suggest that changes in bud hormone homeostasis, resulting in a high auxin to cytokinin ratio, inhibit only bud break in summer, since return bloom is increased on both spring and summer flush shoots even when fruit are removed from 'on' crop trees as late as September (Table 6.5). The potential inhibition of phase transition of axillary buds on summer flush shoots, which are delayed in their development relative to apical buds, and of the apical buds of autumn flush shoots must be considered.

The relationship between the amount of summer vegetative shoot growth and the floral intensity of the return bloom in alternate bearing is not unique to avocado. It has been demonstrated for citrus (*Citrus reticulata*) (Verreyne and Lovatt, 2009) and olive (*Olea europaea*) (Sibbett, 2000). Moreover, inhibition of bud break of summer shoots by a high ratio of IAA to cytokinin and spring bud break by high ratios of IAA and ABA to cytokinin during the 'on' crop year has previously been demonstrated for *C. reticulata* (Verreyne, 2005).

#### Other hormones

Kittikorn *et al.* (2010) identified an interesting relationship among floral bud initiation, crop load, GAs, 9,10-ketol-octadecadienoic acid (KODA) and jasmonic acid (JA) in apple. Concentrations of GA<sub>1</sub> and GA<sub>4</sub> were greater in buds of 'on' crop trees, which produced 17% floral buds, relative to trees with fruit removed that produced 67% floral buds. Fruit removal greatly increased bud concentrations of KODA and JA prior to initiation of floral bud development. In contrast, GAs accumulated at the start of floral bud initiation of 'on' crop trees. There is no known role for either KODA or JA in floral development in perennial tree crops, though promotion of flowering has been documented for herbaceous annual plants (Krajncič *et al.*, 2006).

#### Future research

The next significant advances in understanding how fruit number (crop load) in the current year regulates floral intensity the following year will likely come from studies on the regulation of flowering at the level of the gene. Most of our

knowledge regarding the genetic regulation of floral development has been derived from studies with the model plant *Arabidopsis thaliana*. Comparative analyses between *A. thaliana* and other plants, such as rice (*Oryza sativa*), poplar (*Populus trichocarpa*) and apple (*Malus domestica*) have demonstrated the extensive conservation of pathways regulating flowering, but have also identified features distinct from *A. thaliana* (Hayama and Coupland, 2004; Hanke *et al.*, 2007; Jansson and Douglas, 2007). Homologues of several floral identity genes have already been identified in avocado (*Persea americana* and *P. borbonia*) (Chanderbali *et al.*, 2006, 2009).

Analysis of floral gene expression will make it possible to distinguish between inhibition of bud break and inhibition of floral development in alternate bearing avocado trees. In addition, new, powerful genetic approaches will make it possible to better decipher the roles of the various plant hormones in regulating avocado flowering. For example, in a recent study using a combination of quantitative genetics, quantitative trait loci (QTL) detection and candidate gene mapping, genetic determinants of alternate bearing were identified in a segregating population of apple, produced from a cross between cultivars exhibiting differences in tree architecture and alternate bearing (Guitton *et al.*, 2012). The pattern of alternate bearing was based on yield per tree over six consecutive years. Flowering genes, including floral integrator and floral meristem identity genes, did not co-locate with QTLs for alternate bearing. In contrast, the genes regulating the synthesis, degradation and transport of IAA and GA co-located with the alternate bearing QTLs. The authors concluded that the flowering genes analyzed do not directly control alternate bearing, but suggested that their regulation by hormones was the process leading to alternate bearing. Thus, genes regulating the amount of IAA or GA in the shoot apical meristem were identified as the determinants of alternate bearing in apple. Tree architecture was also identified as an important factor in alternate bearing (Guitton *et al.*, 2012). QTLs for vegetative shoot growth and branching intensity co-located with QTLs for flower and fruit production. There was also a positive relationship between shoot length and return bloom over consecutive years. These results confirm the relationship among vegetative shoot growth,

canopy complexity and yield that can presently be managed through common horticultural practices. The research results of Guitton *et al.* (2012) not only provide insight into potential determinates of alternate bearing in avocado, but also reinforce the use of genetic approaches in current and future investigations of alternate bearing in avocado.

## Conclusions

It is anticipated that rapid progress towards unravelling the regulation of avocado flowering and fruitfulness will be made in the near future by employing the tools of molecular biology and molecular genetics. However, this progress will be possible only because of significant advances in our understanding of avocado tree phenology, floral development, pollination biology, fruit retention and fruit growth made over the past decade using classic research approaches. The results of these investigations established when key developmental events occur in the phenology of the avocado tree and the factors influencing them. This information is fundamental to the direction of future research and development of cultural practices.

More than a decade ago, the foundational avocado phenology model of Whitley *et al.* (1988) stimulated similar research in other avocado-producing countries. Subsequently, avocado tree architecture was deciphered revealing the importance of sylleptic versus proleptic shoots to flowering and productivity (Thorp and Sedgley, 1993a,b). Additionally, the contribution made by each of the annual vegetative shoot flushes to floral intensity the following spring is now known for several avocado-growing regions. The seminal research of Salazar-García *et al.* (1998) established that July–August (Northern Hemisphere) is the time of phase transition for summer flush vegetative shoots, rather than winter. This information coupled with the subsequent identification of when floral buds are determined (committed to floral development), including the macro- and microscopic anatomical characteristics associated with irreversible commitment to flowering (Salazar-García *et al.*, 1999), will prove invaluable for

elucidating the genetic and hormonal control of this process. Collectively, information developed over the past decade provides not only researchers with an invaluable set of tools, but also growers. Further, adaptation of this information to different climatic zones will continue to prove instructive in how and when to manipulate vegetative shoot growth for maximum bloom, or to shift the time of bloom to increase fruit set or change fruit maturity date. At a minimum, this information will help growers avoid the use of cultural practices that will reduce flowering and yield.

Continued research on the importance of pollination, pollinizers and pollinators and recent research clarifying the contribution of outcrossing, close pollination and self-pollination to fruit set have led to practical solutions for increasing yield. During this past decade, increased integration of the factors that regulate fruit set and fruit growth has been achieved, including the unique functions of avocado's C<sub>7</sub> sugars, the role of hormones and, most recently, aspects of genetic regulation of avocado fruit cell division in relation to the small-fruit variant of 'Hass' (Dahan *et al.*, 2010).

Hormonal regulation of flowering, fruit set and fruit development is an area of avocado reproductive biology where significant advances could also be made through the use of molecular genetic tools and new, more sensitive methods for quantifying plant hormones. Development of new plant growth regulator strategies to increase yield, fruit size and quality are anticipated.

Knowledge gained from research with *A. thaliana*, *P. trichocarpa* and *M. domestica* will provide guidance for fruitful avenues of research in avocado. It appears that understanding the hormonal control of flowering and fruit development in avocado will be integral to identifying physiological and genetic mechanisms by which fruit number (crop load) one year regulates flowering the next. If, as Guittou *et al.* (2012) concluded, hormones are the key determinant of alternate bearing through regulation of shoot growth and floral development, it is possible that, in the future, the negative effects of the 'on' crop could be mitigated with strategies that use exogenously applied plant growth regulators and do not require fruit thinning.

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