

REVIEW ARTICLE

Regulation of floral initiation in horticultural trees

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Abstract

The intention of this review is to discuss floral initiation of horticultural trees. Floral initiation is best understood for herbaceous species, especially at the molecular level, so a brief overview of the control of floral initiation of *Arabidopsis* (*Arabidopsis thaliana* (L.) Heynh.) precedes the discussion of trees. Four major pathways to flowering have been characterized in *Arabidopsis*, including environmental induction through photoperiod and temperature, autonomous floral initiation, and regulation by gibberellins. Tropical trees are generally induced to flower through environmental cues, whereas floral initiation of temperate deciduous trees is often autonomous. In the tropical evergreen tree mango, *Mangifera indica* L., cool temperature is the only factor known to induce flowering, but does not ensure floral initiation will occur because there are important interactions with vegetative growth. The temperate deciduous tree apple, *Malus domestica* Borkh., flowers autonomously, with floral initiation dependent on aspects of vegetative development in the growing season before anthesis, although with respect to the floral initiation of trees in general: the effect of the environment, interactions with vegetative growth, the roles of plant growth regulators and carbohydrates, and recent advances in molecular biology, are discussed.

Key words: Apple, *Arabidopsis*, floral induction, floral initiation, mango, trees.

Introduction

Floral initiation includes all of the developments necessary for the irreversible commitment by the meristem to

produce an inflorescence (Kinet, 1993). Control of floral initiation is not restricted to the developing meristem, but may involve signals from other areas of the plant.

Autonomous flowering is where internal developmental cues lead to floral initiation. 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.

Some aspects of flowering in trees make them especially challenging for physiologists, breeders, and growers; first, the juvenile phase, which lasts for several years during which time no flowering or fruiting occurs; second, interactions between vegetative growth, flowers, and fruit of the previous year on floral initiation in the current year, affect growers through phenomena such as biennial bearing, and make interpretation of research data difficult for scientists.

The purpose of this review is to discuss floral initiation in horticultural trees. However, floral initiation is best understood for herbaceous species, so the herbaceous literature will be reviewed briefly first, to serve as a framework for the discussion of trees. Several extensive reviews of the genetic or physiological control of floral initiation of herbaceous species have been undertaken, including Bernier *et al.* (1993), Kinet (1993), Boss *et al.* (2004), Corbesier and Coupland (2005), and Bernier and Perilleux (2005). Case studies on floral initiation in apple (*Malus domestica* Borkh.) and mango (*Mangifera indica* L.) are then presented, because considerable research has been done on these species and they exemplify the differences between temperate deciduous and tropical evergreen tree flowering systems. Finally, floral initiation in horticultural trees as a whole is discussed.

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Floral initiation in *Arabidopsis thaliana* (L.) Heynh, and other herbs and grasses

Arabidopsis is a model plant used extensively for studying the molecular biology and genetics of flowering. *Arabidopsis* is particularly useful because the entire genome has been sequenced, and it is an easily transformable plant with a short life cycle that produces many seeds. The genetic diversity available through different ecotypes, the winter annual and summer annual, and through a large range of mutants, is also useful to researchers.

Four major pathways have been shown to lead to flowering in *Arabidopsis*: photoperiodic, autonomous, gibberellins (GA), and vernalization, and their actions at the genetic level have been revealed to a large degree (Fig. 1). Flowering in *Arabidopsis* can be enabled by regulation of the expression of repressors, or actively promoted by endogenous or environmental signals (Boss *et al.*, 2004). These pathways eventually converge by regulating the floral meristem identity genes (Pineiro and Coupland, 1998).

Photoperiodic induction

Photoperiod is sensed in the leaves, with long-day (LD) and short-day (SD) plants flowering in response to the change in the dark period, requiring short and long dark periods, respectively. In *Arabidopsis*, *CONSTANS* (*CO*) mRNA expression is regulated by the circadian clock and peaks toward the end of the day (Suarez-Lopez *et al.*, 2001). Under LD conditions the photoreceptors cryptochrome 1 and 2 and phytochrome A act antagonistically to phytochrome B to stabilize the *CO* protein, allowing it then to up-regulate *FT* (Valverde *et al.*, 2004). Both *CO* and *FT* proteins are expressed specifically in the vascular tissues of the leaf (Takada and Goto, 2003). Once expressed, the *FT* protein is then transported via the phloem to the meristem (Corbesier *et al.*, 2007), so that in *Arabidopsis* at least, the *FT* protein acts as a 'florigen'. *FT* is a bZIP transcription factor that is preferentially expressed at the shoot apex. *FT* forms a protein complex with *FD* in order to interact with floral meristem identity genes such as *APETALA 1* (*API*) (Abe *et al.*, 2005; Wigge *et al.*, 2005). Together or individually the floral meristem identity genes convert shoot meristems into flowers (Pineiro and Coupland, 1998).

Genes homologous to *FT* have been found in several other species and, as for *Arabidopsis*, their proteins appear to fulfil the role of 'florigen'. For example, in rice, *Oryza sativa* L., a SD plant, *Hdl* is a *CO* orthologue and an output of the circadian clock, which promotes or suppresses *FT* orthologues under SD and LD conditions, respectively (Izawa *et al.*, 2008). Under inductive conditions *Hd3a* mRNA, an *FT* orthologue, accumulates in the leaf blade and the protein is transported to the shoot apical meristem (SAM) where it promotes flowering

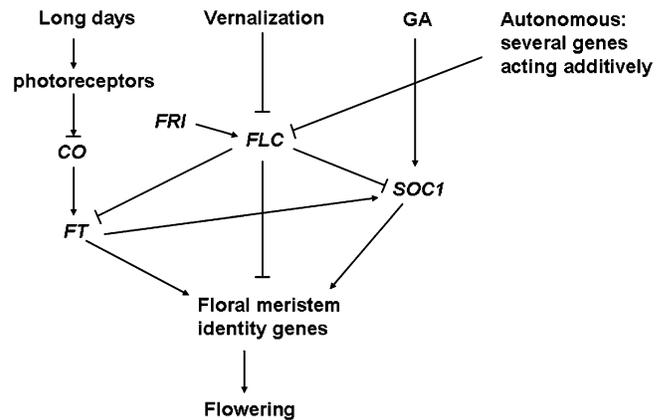


Fig. 1. Floral initiation in *Arabidopsis* occurs through the photoperiodic, vernalization, GA or the autonomous pathways. Pointed arrows represent positive regulation, 'T' arrows represent negative regulation, and both pointed and 'T' arrows represent positive and negative regulation. Under LD, photoreceptors stabilize *CO* allowing up-regulation of *FT*, the *FT* protein is transported from the leaf to the meristem where it interacts with floral meristem identity genes, leading to flowering. Vernalization suppresses the floral repressor *FLC* both in the meristem and the leaf, the autonomous pathway also suppresses *FLC* through several genes that act additively. GA promotes flowering by up-regulating *SOC1*, and also appears to speed flowering through interactions with the other pathways.

(Tamaki *et al.*, 2007). *FT* orthologue proteins have also been implicated as 'florigens' in cucurbits (Lin *et al.*, 2007) and tomato (Lifschitz and Eshed, 2006).

GA floral induction

The GA pathway also actively promotes flowering in *Arabidopsis*. Under SD conditions, GA_4 , which is most likely produced in the leaves and transported to the meristem, up-regulates one or both of the genes *LEAFY* (*LFY*), a floral meristem identity gene (Blazquez *et al.*, 1998; Eriksson *et al.*, 2006), and *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*), a 'floral integrator' (Bernier and Perilleux, 2005), leading to flowering. GA-deficient mutants have delayed flowering under short days but flower on time under long days (Wilson *et al.*, 1992). However, double mutants of the photoperiodic and GA pathways flowered later under long days than double mutants of the photoperiodic and autonomous pathways (Reeves and Coupland, 2001), suggesting some interaction between the photoperiodic and GA pathways.

The interactions between photoperiodic induction and GA are well understood in *Lolium temulentum* L., a LD flowering grass. Long days cause up-regulation of GA biosynthesis genes in the leaves, an increase in GA concentrations in the leaves, and an increase in GA export to the SAM (King *et al.*, 2006). The *L. temulentum* homologues of *Arabidopsis*' *CO* (*LtCO*) and *FT* (*LtFT*) also respond to long days with increased transcription levels in the leaves. Importantly, exogenous applications

of GA to the leaves in SD conditions resulted in flowering without increasing *LtFT* levels. King *et al.* (2006) concluded that GA was acting as a 'florigen' and that *LtFt* could either act as a 'florigen' or enable the production and transport of other signals. While GA plays an important role in promotion of flowering in both *Arabidopsis* and *L. temulentum*, only in *L. temulentum* has it been shown to act as a 'florigen' in response to photoperiodic induction.

Vernalization

Flowering in response to exposure to cold temperatures, -1 °C to 10 °C, for extended periods is termed vernalization (Simpson and Dean, 2002). Variation at the *FRIGIDA* (*FRI*) locus appears to be the main determinant of flowering time in accessions of *Arabidopsis* with the winter annual habit, that is, those that require vernalization; dominant alleles of *FRI* require vernalization while many early flowering types carry the non-dominant *fri* allele (Gazzani *et al.*, 2003). *FRI* acts to suppress flowering by increasing the levels of *FLOWERING LOCUS C* (*FLC*) mRNA, but may also act to suppress flowering through pathways independent of *FLC* (Michaels and Amasino, 1999). *FLC* is a MADS domain gene (Michaels and Amasino, 1999). MADS domain genes are a large group and have a range of plant developmental functions, although those associated with flowering have been most commonly investigated. Variation in *FLC* also confers variation in the vernalization phenotype; in late flowering *FLC* mutants (*FLC* is over expressed), vernalization speeds up flowering and reduces the expression of *FLC* to undetectable levels. *FLC* represses expression of *SOCI*, which prevents up-regulation of *FD* in the meristem (Searle *et al.*, 2006). *FLC* also inhibits transcription of *FT* in the leaf (Searle *et al.*, 2006); therefore vernalization is sensed in both the leaves and meristem to suppress expression of *FLC*.

Vernalization is also well understood in winter cereals, barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*) in particular. Vernalization hastens flowering by promoting expression of the *VRN1* gene (Trevaskis *et al.*, 2003), a floral integrator related to *APETALA1* in *Arabidopsis* (Yan *et al.*, 2004), which down-regulates the floral repressor *VRN2* (Trevaskis *et al.*, 2006). Flowering is hastened in wheat and barley in long days through *VRN3* expression (an *FT* homologue) (Turner *et al.*, 2005). It has been proposed that in genotypes requiring vernalization, *VRN2* expression prevents flowering in long days until after vernalization (Trevaskis *et al.*, 2007).

Autonomous flowering

The autonomous pathway also acts upon the expression of *FLC* although independently of vernalization. In this case, several genes act additively to suppress the expression of

FLC (Michaels and Amasino, 1999), but it is unclear why they act at particular stages of plant development (Boss *et al.*, 2004). The autonomous pathway may also assist the photoperiodic and GA pathways through its action upon the floral repressors (Reeves and Coupland, 2001).

In addition to these four floral pathways the flowering of *Arabidopsis* appears to be regulated by more general aspects of plant metabolism (Bernier and Perilleux, 2005). For example, Corbesier *et al.* (1998) found that sucrose was important both as a signal and assimilate in the flowering of a starchless mutant; Eriksson *et al.* (2006) found that sucrose could act synergistically with GA to promote flowering in the absence of long days; and Bagnall and King (2001) reported that there were complex interactions between light quality and quantity and the availability of assimilates during LD induction.

Case study 1: floral induction in mango

Mango, *Mangifera indica* L., is an evergreen tropical tree cultivated in the tropics and subtropics. With respect to floral initiation, mango has been more extensively researched than any other tropical tree species. The juvenile period for mango varies with cultivar but can be approximately three years for particular cultivars (Salomon and Reuveni, 1994). Floral initiation occurs during late autumn and early winter; flower panicles emerge from the terminal and sub-terminal buds and grow continuously until anthesis occurs in the spring (Fig. 2).

Most mango cultivars bear irregularly. This has been largely attributed to variation in flowering and, in subtropical areas, fruit retention (Whiley, 1993). Variations in the amount of flowering may be within trees from year to year, between trees in the same year, and between branches on the same tree. Some Indian cultivars are reputedly biennially bearing, having distinct 'on' and 'off' years (Pandey, 1989).



Fig. 2. Floral induction in mango occurs in response to cool temperatures perceived by mature leaves (L), which are necessary for floral initiation. Flower panicles (FP) originate from terminal or subterminal buds of the most recent vegetative flush.

Many of the cultivars grown in Australia, including 'Kensington Pride', flower irregularly, but without predictable 'on' and 'off' years (Blaikie and Kulkarni, 2002).

Under subtropical conditions mango flowers in response to cool temperatures (Whiley *et al.*, 1989; Batten and McConchie, 1995; Shu and Sheen, 1987; Chaikiattiyos *et al.*, 1994; Nunez-Elisea and Davenport, 1994), although the temperatures reported for cool temperature induction in mango are much higher than those reported for vernalization of *Arabidopsis* above. Whiley *et al.* (1989) found that eight out of ten mango cultivars flowered at a day/night temperature regime of 15/10 °C, and only one of the cultivars flowered at 20/15 °C, while the other nine cultivars grew vegetatively; and Shu and Sheen (1987) found that 100% of 'Haden' mangoes flowered at 19/13 °C, 60% at 25/19 °C and 0% at 31/25 °C. Interestingly, four cultivars that flowered at 30/20 °C in the work of Sukhivibul *et al.* (2000) failed to flower at 20/15 °C in the work of Whiley *et al.* (1989). It is possible that the larger diurnal temperature difference was a significant factor, as speculated by Batten and McConchie (1995) for lychee (*Litsea chinensis* Sonn.). Reports on the duration of cold temperature needed for floral initiation vary from 4 d (Reece *et al.*, 1946) to 2 weeks (Shu and Sheen, 1987) in cultivar 'Haden' and up to 35 d in 'Tommy Atkins' and 'Keitt' (Yeshitela *et al.*, 2004).

There is evidence for a phloem mobile floral stimulus (florigen) in mango. Kulkarni (1986, 1988b, 1991) examined mango flowering by cross-grafting cultivars with different inductive requirements. While the rootstock was under inductive conditions it could promote flowering in the defoliated scion under conditions non-inductive for the scion cultivar, so long as the rootstock had leaves. However, when leaves remained on the scion, flowering was inhibited and subsequent growth was vegetative. Similarly, juvenile mango plants have the ability to flower after grafting to a mature plant so long as the juvenile plant is defoliated and the adult plant has leaves (Singh, 1959), indicating signals from the leaves of the adult plant promote flowering and can overcome juvenility, while leaves from juvenile plants inhibit flowering. In separate studies, when branches were girdled and decapitated, the growth from axillary buds was floral if leaves were allowed to remain on the plant for more than 4 d under inductive conditions (Reece *et al.*, 1946, 1949). Floral initiation will occur in the presence of even a fraction of a mature leaf, but the proportion of stems initiating reproductive, as opposed to vegetative, growth decreases with increasing distance from the leaves, and as the number of leaves decreases (Davenport *et al.*, 2006). From these observations, there seems to be a floral stimulus in mango that is transient, graft transmissible, and generated by the leaves.

Vegetative growth in mango is through episodic flushing, which is more frequent as temperature increases

(Whiley *et al.*, 1989). Episodic or recurrent flushing, common in subtropical and tropical trees, is where apical or axillary buds are released and the new shoots expand continuously through several nodes and then mature. After a period of dormancy, the cycle begins again with further bud release. The timing of flush development is important for successful flowering because bud release, for vegetative or reproductive growth, can only occur from mature flush (Nunez-Elisea and Davenport, 1995). In addition, buds appear to be receptive to the floral stimulus for only a small portion of the flush development cycle, because floral induction seems to require inductive temperatures approximately to coincide with bud release. Recently emerged buds on plants of cultivar 'Irwin', growing in warm, non-inductive conditions initiated flower panicles when they were moved to florally inductive ambient winter conditions, so long as the buds were less than approximately 10 mm in length (Batten and McConchie, 1995). Treatments such as pruning that manipulate the timing of flush development and synchronize canopy flushing have been successful in increasing flowering intensity (Yeshitela, 2005).

Under tropical conditions, in which cold inductive temperatures may be brief, erratic or non-existent in some seasons, it is unclear what exactly leads to floral initiation; however, some factors improve the likelihood of flowering. First, some cultivars flower more reliably than others (Pandey, 1989) and will flower at higher temperatures. For example 'Florigon' was the only cultivar to flower at the higher temperature treatment of 20/15 °C of ten cultivars tested by Whiley *et al.* (1989). Second, treatments which reduce vegetative vigour such as paclobutrazol (GA biosynthesis inhibitor), and manipulate the timing of flush development, such as water stress (Davenport, 2003), may help if they serve to focus bud release around the time of inductive temperatures, although this requires testing. Water stress may be used only indirectly to promote flowering, as described below for lychee. Experimentally, water stress *per se* has not been shown to induce flowering or decrease the cold requirement of mango (Chaikiattiyos *et al.*, 1994; Nunez-Elisea and Davenport, 1994). Third, potassium nitrate is thought to induce flowering (Bondad and Apostol, 1979), but seems to be ineffective in many environments (Davenport and Nunez-Elisea, 1997).

There is some correlative evidence for regulation of floral initiation in mango by plant growth regulators (PGRs), GA in particular. Evidence comes from measurements of endogenous GA, the effects of exogenous GA, and the effects of GA biosynthesis inhibitors. The concentration of GA in terminal stems of cultivar 'Khiew Sawoey' decreased during the 16 weeks before panicle emergence in trees that subsequently flowered, and increased over the same period in terminal stems of trees that remained vegetative (Tongumpai *et al.*, 1991). This

raises the possibility of a direct inhibitory role of GA in mango floral initiation. Exogenous applications suggest that GA may indirectly regulate mango flowering by delaying bud release, because applied GA delays bud release and does not inhibit flowering so long as bud release occurs under florally inductive conditions (Nunez-Elisea and Davenport, 1998). GA biosynthesis inhibitors such as paclobutrazol (Rademacher, 1995) both hasten and increase the flowering intensity of mango (Kulkarni, 1988*a*; Blaikie *et al.*, 2004) and also reduce vegetative vigour (Winston, 1992). So paclobutrazol may directly promote flowering, or act indirectly by increasing the likelihood of bud release during floral inductive conditions. Therefore, GA may inhibit floral initiation endogenously or may act indirectly by influencing the timing of bud release.

In summary, the only factor shown experimentally to induce flowering in mango is temperature below 15–20 °C, with florally inductive temperatures varying between cultivars. Floral initiation is affected by the cycle of flush development, and the timing and intensity of flowering can be manipulated by exogenous applications of PGRs. The evidence for the internal regulation of floral initiation by GA is not conclusive.

Case study 2: floral initiation in apple

Apple is a temperate, deciduous tree grown commercially from the tropics to high latitude temperate regions (Lakso, 1994). Apple has a long juvenile phase which can be greater than six years; however, grafting onto dwarfing rootstocks can reduce the juvenile phase (Kotoda *et al.*, 2006). Spring bloom in apple is part of the cycle of reproductive development that begins with floral initiation in the preceding summer (Abbott, 1970). The process is not continuous, but broken by the period of rest associated with the winter months, during which no development occurs. The apple inflorescence is a determinate raceme which often has five flowers (Westwood, 1978). Apple is an autonomous flowering plant, as are many other temperate deciduous tree crops.

Apple flowers are borne on two types of shoots, spurs and long shoots (Fig. 3). The spur is a short shoot in which extension growth is limited to the production of a rosette with few leaves (Abbott, 1970). The axis of the spur is called the ‘bourse’ which can continue to produce short shoots from the axillary buds in the following seasons (Luckwill, 1970). Long shoots are the extension shoots of the current season’s growth, and, particularly in new cultivars, can produce flowers from both terminal and axillary buds (Tromp, 2000).

Meristems of developing vegetative buds include several appendages: bud scales, bracts, transition leaves, and true leaves. The vegetative buds must be fully developed for the transition to floral buds to occur (Buban and Faust,

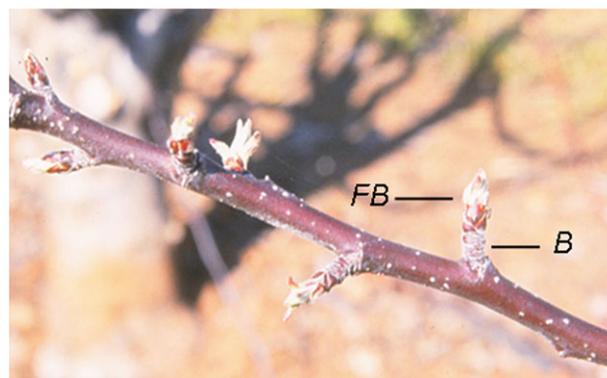


Fig. 3. Apple flower buds (FB) are often initiated on terminal buds of short shoots called spurs, the axis of the spur is called the bourse (B). Flowers are initiated during the growing season before winter dormancy, anthesis occurs in the spring when the chilling requirement of winter dormancy has been satisfied and temperatures are suitable for growth.

1982). The appendages begin developing when growth resumes in spring. A critical appendage (node) number in vegetative buds was suggested by Fulford (1965, 1966*a*) and reported as 16 for ‘Golden Delicious’ (Luckwill and Silva, 1979). Attainment of the critical node number appears to be the prerequisite for a bud to make the transition from vegetative to floral; no compelling reason can be cited but there is experimental evidence supporting this critical node requirement (Luckwill and Silva, 1979; Hirst and Ferree, 1995; McArtney *et al.*, 2001; Bertelsen *et al.*, 2002). The rate of node development needs to be fast enough to ensure the critical node number is reached before the end of the growing season; this is most important at high latitudes with short growing seasons (Faust, 1989). The first sign of transition from vegetative to reproductive growth is doming of the apical meristem which, in spurs, may occur approximately 50 d after full bloom (Kotoda *et al.*, 2000; Foster *et al.*, 2003). Lateral floral meristems and bracts then develop until the terminal and lateral flowers have initiated sepals (Foster *et al.*, 2003), this differentiation may continue throughout the autumn until the onset of winter dormancy (Sung *et al.*, 2000). The flowers are completed after the release of dormancy between bud burst and anthesis (Sung *et al.*, 2000).

Environmental conditions exert some control over floral initiation in apple. Heat unit accumulation could not consistently account for the timing of floral initiation in ‘Royal Gala’ (McArtney *et al.*, 2001), but the temperature during the growing season can affect the intensity of floral initiation and it may be that temperatures which induce high vegetative vigour reduce floral initiation (Tromp, 1976, 1980). Low irradiance has been shown to inhibit floral initiation on spurs. Flowering on spurs was not affected at up to 30% shading but was totally inhibited when shading increased to 70% of the available light in cultivar ‘McIntosh’ (Cain, 1971).

Many cultivars of apple have a tendency to bear alternately. Heavy fruit loads accentuate biennial bearing by reducing flower production (Luckwill, 1970). Early work also demonstrated that reducing fruit load could increase flower production and that the effect was reduced the longer fruit removal was delayed (Harley *et al.*, 1942). On individual spurs, increased bourse shoot length and increased leaf area were found to increase flowering (Nielsen and Dennis, 2000), while defoliation (Ramirez and Hoad, 1981) and the presence of fruit diminished flowering (Fulford, 1966b). There is evidence that the inhibitory effect of fruit load on flowering is due to GA export from the seeds. Luckwill (1970) noted high levels of GA in apple seeds during embryo growth from approximately 5 weeks to a maximum at 9 weeks after full bloom. The inhibitory effect of seeds on flower production was demonstrated by experiments using the cultivar 'Spencer Seedless' that can be manipulated to produce seedless or seeded fruit; seeded fruit inhibited flower production on spurs while seedless fruit did not (Chan and Cain, 1967; Nielsen and Dennis, 2000).

GA inhibit floral initiation in apple (Tromp, 1982; McLaughlin and Greene, 1984; Bertelsen *et al.*, 2002). GA may act by reducing the rate of node development; GA application in 'Pacific Rose' reduced bud appendage production, bud size, and both delayed and reduced the transition of buds from vegetative to floral (Bertelsen *et al.*, 2002). While GA inhibit flowering, cytokinin can promote flowering when applied soon after full bloom (McLaughlin and Greene, 1984). Cytokinin application as zeatin increased flowering on spurs and replaced the need for leaves in floral initiation on defoliated spurs (Ramirez and Hoad, 1981). Other plant growth regulators also affect floral initiation in apple. For example, daminozide increases flower bud formation (Ramirez and Hoad, 1981; McLaughlin and Greene, 1984), and has been reported to both decrease GA tissue concentrations (Hoad and Monselise, 1976) and increase cytokinin tissue content (Ramirez and Hoad, 1981). Ammonia increased floral initiation and stem arginine content when applied as a four mM ammonium sulphate nutrient solution (Rohozinski *et al.*, 1986).

Orthologues of floral meristem identity genes and MADS domain genes of *Arabidopsis* have been identified in apple. Two orthologues of *LEAFY* named *AFL1* and *AFL2* were isolated from cultivar 'Jonathan' and were subsequently shown to hasten flowering in transformed *Arabidopsis* plants (Wada *et al.*, 2002). Further, expression of *AFL1* in the apple meristem began when phenotypic transition of buds from vegetative to floral was observed. Two members of the MADS domain gene family, similar to *AGL2* and *AGL4* from *Arabidopsis*, have been isolated in cultivar 'Fuji'. Both were expressed in the early stages of flower development and one was expressed during the later stages (after dormancy) (Sung

et al., 2000). The apple gene *MdTFL1* delayed flowering in transformed *Arabidopsis* plants (Kotoda and Wada, 2005), and transformed 'Orin' apple plants expressing antisense *MdTFL1* RNA flowered as little as 8 months after transfer to the greenhouse, compared with the non-transformed control plants that did not flower in 6 years, indicating that *MdTFL1* has a role in maintaining the juvenile phase in apple (Kotoda *et al.*, 2006). Some of the early flowering transgenic *MdTFL1* antisense expressing plants had continuous flowering habits and altered branch architecture due to varying flower positions, indicating additional roles of *MdTFL1* in the timing and location of floral initiation. Upstream regulation of these apple flowering genes by the environment or endogenous signals is not understood.

Research investigating floral initiation in apple implicates seasonal developmental processes and hormonal relationships as the major factors driving floral initiation as opposed to specific environmental stimuli.

Other tree crops

Environmental control of floral initiation

A range of subtropical and tropical tree species can be induced to flower by exposure to low temperature: mango, lychee (Menzel and Simpson, 1995), macadamia (*Macadamia integrifolia* Maiden and Betche) (Nakata, 1976), avocado (*Persea americana* Mill.) (Buttrose and Alexander, 1978) and orange (*Citrus sinensis* L.) (Moss, 1976). Olive (*Olea europaea* L.), an evergreen tree generally grown in Mediterranean environments, can also be induced to flower under cool conditions (Hackett and Hartmann, 1964). One major difference between cool temperature induction in subtropical and tropical trees, and vernalization in herbaceous species, is the temperature required: subtropical and tropical trees often require temperatures around 15–20 °C whereas vernalization in herbaceous species requires temperatures between –1 °C and 10 °C.

With respect to temperate deciduous species, high temperatures (up to 30 °C) increased inflorescence production in grapevine, *Vitis vinifera* L., while temperatures of 21 °C and below increased tendril production (Buttrose, 1970), but for southern high bush blueberry (*Vaccinium corymbosum* L.), 28 °C inhibited floral induction compared with 21 °C (Spann *et al.*, 2004).

Therefore cool temperatures induce flowering in several tropical and subtropical horticultural trees. In temperate deciduous horticultural trees, temperature can affect the intensity of floral initiation, but it is not clear if temperature provides an inductive stimulus.

The site of perception of cool florally inductive temperatures has not been extensively investigated. In mango, cool temperatures may be sensed in the leaves because mature leaves appear to be the source of an essential floral

stimulus (described above in the mango case study). Lychee may be similar given that mature leaves also appear necessary for floral initiation (Menzel *et al.*, 2000; Ying and Davenport, 2004). However high root temperatures can inhibit floral initiation in lychee even when shoots are exposed to florally inductive temperatures (O'Hare, 2004), implicating either perception by the roots and long-distance signalling or heat transfer via the transpirational stream. In citrus, in contrast, leaves are not necessary for floral initiation in cool inductive temperatures (Davenport, 2000) or for floral induction via water stress (Southwick and Davenport, 1986), indicating that both the cold temperature and water stress stimuli that induce flowering may be perceived in the stem or buds.

Photoperiodic induction, a common mechanism in herbaceous species, has rarely been demonstrated in trees. Southern high bush blueberry flowers in response to short days of 8 h, but not under long days or short days with a 1 h night interruption (Spann *et al.*, 2003); this is most likely a response to photoperiod. In avocado, both the time to flowering and floral initiation were decreased by short days of 9 h compared with 15 h (Buttrose and Alexander, 1978), but these effects may be related to differences in photosynthetic period and daily carbon assimilation rather than to photoperiod.

Light intensity also influences floral initiation in perennials; as described for apple above. In kiwi fruit, *Actinida chinensis* Planch., shaded shoots produced fewer floral buds and fewer inflorescences per shoot than exposed shoots (Grant and Ryugo, 1984). In olive, both high and low light intensity treatments reduced floral initiation (Stutte and Martin, 1986). Similarly in the ornamental tree, *Metrosideros excelsa* Soland. ex Gaertn., the greatest flowering intensity occurred at intermediate light intensities (Henriod *et al.*, 2003). Although variations in light intensity can affect floral initiation quantitatively, it is most likely not an inductive stimulus in these cases but a secondary factor perhaps related to assimilate production and its effects on growth.

Water stress has been demonstrated to induce flowering experimentally in two citrus horticultural tree species. Tahitian limes, *Citrus latifolia* Tan., flowered on resumption of daily irrigation after both cyclic and constant water stress for as little as 2 weeks (Southwick and Davenport, 1986). When the same Tahitian lime plants had completed a vegetative flush 2 months later, water stress and subsequent resumption of adequate watering resulted in floral initiation again. Rewatering following water stress also caused floral initiation in lemon, *Citrus limon* (L.) Burm. f., but not in lychee, mango or avocado (Chaikiattiyos *et al.*, 1994). It is not clear if the inductive stimulus is provided by the period of water stress or the subsequent watering. Water stress can also act indirectly to promote floral initiation by checking vegetative flushing, as demonstrated for lychee (Stern *et al.*, 1998).

Vegetative growth and floral initiation

Horticultural tree species vary both in the types of shoots that produce flowers and where on these shoots the flowers are borne. The location of the flowers and the timing of floral initiation influence how flowering and vegetative growth interact.

Several subtropical species, including lychee, tend to produce inflorescences from terminal buds; others, such as avocado, from both terminal and axillary buds; and still others, like macadamia, *Macadamia integrifolia* Maiden and Betche, *M. tetraphylla* Johnson, and hybrids, from axillary buds.

Similar to apple, several other temperate deciduous species, such as pear, *Pyrus communis* L. (Faust 1989), and sweet cherry, *Prunus avium* L. (Webster and Shepherd, 1984), initiate flowers on specialized spur structures as well as on current season's growth. On the other hand, peach, *Prunus persica* L. Batsch (Dorsey, 1935), and apricot, *Prunus armeniaca* L., (Jackson, 1969), initiate flowers in lateral buds of the current season's growth.

In the subtropical trees lychee, avocado, and macadamia, flowering is dependent on bud release during cool florally inductive temperatures (Olesen, 2005). This is largely regulated by maturity of the most recent flush (discussed below), but the likelihood of bud release and flowering can also be affected by characteristics of the shoot, as for macadamia (JD Wilkie *et al.*, unpublished data). In temperate deciduous species, however, the release of pre-existing floral or vegetative buds in the spring after winter dormancy is dependent on satisfying the chilling requirements (Rohde and Bhalerao, 2007).

Vegetative growth in lychee is through recurrent flushing, with the interval between successive flushes dependent on the prevailing weather conditions (Olesen *et al.*, 2002). There is only a small part of this cycle when the new shoots are receptive to floral induction, that being around the time of early flush development when the expanding buds are no more than a few millimetres in length (Batten and McConchie, 1995). Therefore, vegetative shoots that are not mature by late autumn often do not flower, because the cyclic nature of flush development means they will initiate new growth only after cool florally inductive winter conditions have passed. Macadamia is similar except that the cycle of flush development generally affects the flowering behaviour of mature shoots at a distance from the most recent apical flush instead of the flowering behaviour of the most recent apical flush itself (Olesen, 2005). Avocado is also similar, and intermediate to lychee and macadamia in its flowering behaviour (Olesen, 2005).

The timing of vegetative growth also affects floral initiation in some temperate deciduous tree crops, where floral initiation occurs in the growing season before

anthesis. In grapevine, the undifferentiated primordia have the potential to produce inflorescences or tendrils depending on their location and climatic conditions; inflorescences tend to be formed in developing latent buds and tendrils in growing shoots (Boss *et al.*, 2003). Floral initiation in peach is also reliant on, but not inhibited by, vegetative growth; floral initiation occurs in buds of the current season's growth and begins when the buds are approximately four nodes back from the growing tip (Dorsey, 1935).

Excessive vegetative growth has often been cited as being antagonistic to flower bud initiation in apple and some other temperate fruit trees (Luckwill, 1974; Faust, 1989; Forshey and Elfving, 1989). Consistent with this, dwarfing rootstocks increase early flowering of apple (Luckwill, 1974); growth retardants such as daminozide can increase flower bud initiation of apple (McLaughlin and Greene, 1984); and for sweet cherry, as regrowth in response to pruning increases, floral initiation decreases (Guimond and Andrews, 1998). However, treatments that increase vigour do not always decrease floral initiation; greater shoot growth due to increasing temperatures (20 °C compared to 13 °C) during the growing season also increased flower bud initiation of apple (Zhu *et al.*, 1997). These inconsistencies may be due to effects of the treatments that are independent of vigour, for example, dwarfing rootstocks may induce early flowering independent of their effect on vegetative growth; or high temperature during the growing season may promote flowering independently of vegetative growth.

The role of plant growth regulators

The literature on the role of GA in the floral initiation of woody perennials is vast and inconsistent. However, there is evidence to suggest that endogenous GA can inhibit floral initiation and that GA can also inhibit floral initiation through effects on shoot growth, as discussed for mango above. For example, applied GA inhibits floral initiation in avocado (Salazar-Garcia and Lovatt, 1998), citrus (Lord and Eckard, 1987), sweet cherry (Lenahan *et al.*, 2006), and peach (Garcia-Pallas and Blanco, 2001); reduced levels of endogenous GA have been correlated with floral initiation in citrus (Koshita *et al.*, 1999), lychee (Chen, 1990); and GA biosynthesis inhibitors have improved flowering in mango (Winston, 1992), and lychee (Menzel and Simpson, 1990), and macadamia (Nagao *et al.*, 1999).

Floral initiation in grapevine occurs in uncommitted primordia of developing latent buds destined for dormancy and subsequent release in the following spring (Srinivasan and Mullins, 1980). The uncommitted primordia have the potential to produce tendrils as well as inflorescences, but tendrils tend to be produced only when uncommitted primordia initiate growth in the same season

in which they were produced, that is, without undergoing winter dormancy (Boss *et al.*, 2003). However, in a dwarf, GA-insensitive mutant of grapevine, only inflorescences and no tendrils were produced from uncommitted primordia of the expanding shoots (Boss and Thomas, 2002). Thus endogenous GA appear to inhibit floral initiation in grapevine.

GA applications in citrus that inhibit flowering reduce the number of buds that are released in spring but not the proportion of buds that produce floral shoots (Garcia-Luis *et al.*, 1986). Thus the effect seems to be on shoot growth rather than floral initiation. Applied GA also affects shoot growth in apple by reducing the rate of node development, lessening the chances of the buds reaching the critical appendage number (Bertelsen *et al.*, 2002).

The presence of fruit inhibits floral initiation in several species including the pome fruits (Weinbaum *et al.*, 2001) and citrus (Garcia-Luis *et al.*, 1986). Large crops can lead to poor floral initiation in the following year and induce a cycle of biennial bearing. GA exported from the seeds of pome fruits (Chan and Cain, 1967) and some part of citrus fruit to the buds (Garcia-Luis *et al.*, 1986) is thought to be involved in the inhibition.

Cytokinins may also be involved in floral initiation. Endogenous cytokinin levels in buds of lychee increase at the onset of florally initiation and differentiation, and exogenous applications increase floral initiation (Chen, 1991), although there is no evidence that cytokinins can replace the florally inductive stimulus. Application of the growth retardant maleic hydrazide to 'Japanese pear', *Pyrus pyrifolia* Nakai, increased both endogenous cytokinin levels and floral initiation (Ito *et al.*, 2001).

Ethylene has long been used to promote flowering commercially in pineapple (*Ananas comosus* (L.) Merr.) (Turnbull *et al.*, 1999); there are also indications that it promotes flowering in apple (Bukovac *et al.*, 2006).

More comprehensive accounts on the role PGRs in floral initiation of several horticultural trees are available, including apple (Buban and Faust, 1982; Dennis and Neilsen, 1999), mango (Davenport and Nunez-Elisea, 1997), and citrus (Davenport, 1990).

The role of carbohydrates

Carbohydrates have two roles in plant development, in the general provision of energy and carbon skeletons for growth and in the regulation of metabolism. The challenge for research into floral initiation is in designing experiments that separate out these roles.

In the LD flowering perennial shrub *Fuchsia hybrida* Hort. ex Sieb. & Voss, floral induction can occur in the presence of long days or high irradiance short days (King and Ben-Tal, 2001). The extent of floral initiation under high irradiance SD conditions is correlated with the sucrose concentration at the apex, and under these conditions

sucrose may be acting as a florigen. This is not so for LD induction because the sucrose concentration did not increase at the apex with the increase in daylength (King and Ben-Tal, 2001). As a perennial shrub, *F. hybrida* is somewhat removed from horticultural trees but does demonstrate a potential direct effect of carbohydrates in floral signalling in a perennial species.

The need for carbohydrates for floral initiation has often been investigated by measuring levels of stored carbohydrates, or imposing treatments such as girdling that modify the levels of stored carbohydrates, and correlating these with flowering intensity. The results have been mixed. Girdling increased flowering intensity in olive (Lavee *et al.*, 1983), lychee (Menzel and Simpson, 1987), and citrus (Goldschmidt *et al.*, 1985) indicating increased stored carbohydrates can increase floral initiation, because girdling has been reported to increase levels of stored carbohydrates in some horticultural trees (Goldschmidt *et al.*, 1985; Menzel *et al.*, 1995). Further, a study of stored carbohydrates in biennial bearing citrus found that high and low levels of stored carbohydrates corresponded with high and low levels of floral initiation, respectively (Goldschmidt and Golomb, 1982). However, other experiments with citrus have revealed complex interactions between cool inductive temperatures, PGRs, fruit load, and girdling treatments on the flowering intensity (Goldschmidt *et al.*, 1985). In olive, both high and low light intensity treatments, in which high and low levels of stored carbohydrate were measured, respectively, resulted in decreased flowering relative to the control, and the effects of carbohydrate were minor compared with the inhibition of flowering due to the presence of fruit (Stutte and Martin, 1986). It is unclear whether increased flowering intensity in treatments that also increase the availability of carbohydrates in horticultural trees is due to the action of carbohydrates as a floral stimulus or an energy source.

Flowering genes in horticultural trees

Orthologues of *Arabidopsis* flowering genes have been identified in several tree species. Studies to determine the function of these genes generally involve correlation of gene expression with floral initiation/development or transgenic studies, where flowering genes from the perennial species are inserted into a related perennial species or *Arabidopsis*.

In general, perennial flowering gene orthologues have been shown to function akin to their *Arabidopsis* namesakes. For example, Satsuma mandarin *FT* orthologue mRNA levels increased with the seasonal onset of cool temperatures during the time of floral induction (Nishikawa *et al.*, 2007); there is evidence that *LEAFY* orthologues isolated from sweet orange (Pillitteri *et al.*, 2004b) and grapevine (Boss *et al.*, 2006) act as floral promoters; and evidence that *TFL1* orthologues isolated from citrus

(Pillitteri *et al.*, 2004a) and grapevine (Boss *et al.*, 2006) act as floral inhibitors.

There is now some understanding of how the expression of flowering genes integrates with the environment and flowering time in horticultural trees. In the temperate deciduous tree poplar, *Populus* sp., *FT* orthologue expression increased in long days and appeared to be at least partly influenced by the timing of *CO* orthologue transcription; additionally, *FT* orthologue expression varied seasonally and corresponded to the timing of floral initiation in long days, while expression of an *FT* orthologue in young transformed poplar plants removed the juvenile phase (Bohlenius *et al.*, 2006). In Satsuma mandarin, *FT* orthologue mRNA levels increased with the time spent under florally inductive conditions (Nishikawa *et al.*, 2007). In sweet orange, *LFY* and *API* orthologue RNA levels increased during and after florally inductive cool temperatures while RNA of the *TFL* orthologue was absent (Pillitteri *et al.*, 2004a). In transgenic hybrid citrus, *Citrus sinensis* L. Osbeck × *Poncirus trifoliata* L. Raf., over-expression of *LFY* and *API* orthologues substantially reduced the juvenile phase, but flowering still appeared to be under both environmental and endogenous control because it occurred only once a year in the spring (Pena *et al.*, 2001).

Conclusions

So far, the literature on floral initiation in herbaceous plants has been reviewed, in particular *Arabidopsis*, then mango, apple, and horticultural trees in general. It is apparent that floral initiation in trees is controlled by a range of factors which may include environmental stimuli, developmental cues, and other interactions with vegetative growth and PGRs. It is also apparent that rarely can one factor be considered in isolation.

Annual plants, such as *Arabidopsis*, may have several pathways to flowering which include induction in response to environmental stimuli or autonomous initiation. Horticultural trees generally initiate flowers in response to either an environmental stimulus or autonomously.

There is some evidence that the mechanisms through which environmental stimuli act are similar between annual plants and horticultural trees. For example, the roles of *CO* and *FT* in *Arabidopsis* appear to be similar to those of orthologues in poplar (Bohlenius *et al.*, 2006) and the role of *TFL1* in *Arabidopsis* appears to be similar to that of the orthologue in grapevine (Boss *et al.*, 2006).

However, there may also be differences. Vernalization acts on the meristem and leaves in *Arabidopsis* to suppress floral repressors, but in mango cool temperatures are sensed in the mature leaves which then generate a signal that is exported to the meristem to promote flowering. Thus the floral response to cool temperatures in

mango appears to be more analogous to photoperiodic induction in *Arabidopsis*, or to the effects of ambient temperature on genes of the autonomous flowering pathway (Blazquez *et al.*, 2003).

Two major differences exist between tropical and temperate deciduous horticultural trees with respect to floral initiation. First, tropical species such as mango initiate flowers in response to an environmental stimulus, while temperate deciduous species, such as apple, initiate flowers autonomously. Second, temperate deciduous horticultural trees undergo a period of dormancy between floral initiation and anthesis, while in tropical species, including mango, floral development is continuous from floral induction to anthesis.

Notwithstanding these differences, the purpose is the same, flowering under environmental conditions suitable for successful reproduction. Tropical species such as mango and lychee often use a predictable environmental stimulus, cool winter temperatures, to induce flowering. Temperate deciduous species initiate flowers during the growing season but initiate growth in the following spring only after the chilling requirements of winter dormancy have been satisfied and temperatures are suitable for growth.

Research in trees is expensive, slow, and has often been focused on limits to production in horticultural species. Recent advances in the understanding of the genetic control of floral initiation in herbaceous plants such as *Arabidopsis* have provided a platform from which trees can be studied.

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