Introduction

Citrus trees are perennial evergreens, which sustain a complex tree structure with one to three distinct annual vegetative growth flushes. After a sufficient induction period, with the onset of growth-promoting conditions during spring (increase in temperatures and sufficient water and mineral nutrient supply), flowers develop from buds on vegetative shoots that originated from vegetative shoots in the different growth flushes of the previous 12-month season. This article is the second in a series of three articles on the physiology of citrus flowering, and will focus on the important chronological events pertaining to the development of a citrus flower, as influenced by endogenous factors, environmental conditions and cultural practices.

Inleiding

Sitrus is ‘n immergroen boom wat h komplekse boomstruktuur onderhou deur een tot drie jaarlikse vegetatiewe groei-fases. Na induksie van nuutgevormde vegetatiewe lote deur genoegsame geakkumuleerde lae temperatuur en/of water stress gedurende die herfs en winter, ontwikkel blomme in die lente vanuit knoppe van lote wat gedurende die vorige 12-jaar seisoen gevorm het.

Hierdie artikel is die tweede in h reeks van drie artikels wat handel oor die fisiologie van blom-ontwikkeling in sitrus en die belangrike stappe van blom-ontwikkeling, soos beinvloed deur interne plant-kondisies, omgewingsfaktore en produksie-praktyke.
ing that of water stress (Moss, 1969) and low temperatures in cooler, winter-rainfall areas (Valiente and Albrigo, 2004). Both flower-promoting stimuli result in the cessation of root growth, which strongly resembles a common mechanism of flower induction initiation. For many years, the hypothesis surrounding this common mechanism was that of an obligatory/required tree “rest” period, similar to that of winter dormancy in pome and stone fruit species.

However, a significant breakthrough in understanding flower induction has recently come about with the successful identification of a key flowering gene, the FLOWER-ING LOCUS T (“FT-gene”) in the model plant, Arabidopsis, and with the expression of its homologues and the direct relationship to flowering also being confirmed in perennial fruit tree species such as citrus (Nishikawa et al., 2007). The intensity of FT-gene expression in reaction to species-specific environmental stimuli strongly correlates with time of flowering and flower intensity, and currently provides a viable model of flower induction in both annual/biennial and perennial flowering plants. The accepted model states that FT-gene expression in plant leaves in response to an environmental cue results in FT-protein synthesis and transport via the phloem to a susceptible meristem. Bud transition from a vegetative to reproductive state only initiates under growth-promoting environmental conditions such as warm temperatures and increased water supply, once the accumulation of FT-proteins in the bud have reached a certain species-specific threshold level (Nishikawa et al., 2007).

In citrus, a sufficient period of cool temperature (15-20°C) and/or a prolonged period of water stress, are the main inductive stimuli that lead to expression of the citrus FT-gene (CiFT) in leaves, stems and buds (Nishikawa et al., 2007) (Fig. 1). After sufficient induction, the CiFT-mRNA is translated to the CiFT-protein, which is transported via the phloem to susceptible buds, and results in reproductive development under growth-promoting conditions during spring (Fig. 1). Loss of function in the CiFT-gene delays flowering, whereas over-expression leads to very early and/or intense flowering (Nishikawa et al., 2010).

**Flower initiation and differentiation**

Flower initiation in citrus involves the transition of bud meristematic tissue from a vegetative to reproductive state in response to levels of sufficiently accumulated CiFT-proteins in the bud (Davenport, 1990). Flower initiation is the process within the bud, during which the flower parts start to develop at a molecular level into a state significantly distinguishable from vegetative, non-flower- ing buds (Lord and Eckard, 1985). The differentiation of a bud occurs with the onset of growth-promoting conditions during spring and results in bud transformation into either
a vegetative or reproductive state. Non-differentiating buds remain dormant as a result of sprouting inhibition incurred by presence of fruit (Verreyenne and Lovatt, 2009), insufficient growth-promoting conditions (Moss, 1969) or age of the bud/shoot (Schneider, 1968).

Flowering buds differentiate first - approximately 3 to 4 weeks earlier than those on vegetative shoots, with the terminal flower (generally leafy) developing before the flowers in the lateral positions (generally leafless) (Abbott, 1935). Flowers differentiating from the terminal position of a shoot are more advanced (Lord and Eckard, 1985), and with the exception of lemon, lime, grapefruit and satsuma mandarin, fruit set is higher and eventual fruit size larger compared to lateral positions (Krajewski and Rabe, 1995).

Except for an increased photo-assimilate supply to ovaries obtained from the additional leaves on leafy inflorescence, these flowers are also a stronger sink than those of leafless inflorescences due to better developed xylem and a higher number of vascular bundles (Erner and Shomer, 1996). Delayed bud-break and sprouting usually produces leafy inflorescence (Lenz, 1966), which could be explained by increased temperatures at the time of bud-break, because warm temperatures prior to and during flower differentiation are associated with the sprouting of predominantly leafy inflorescence, and low temperatures with the development of leafless inflorescence (Moss, 1969).

**The regulation of flower development events**

The lack of flowering response on receptive shoots after exposure to favorable environmental conditions is proof that additional factors influence bud-fate and flowering potential. The presence of fruit at different phenological stages (García-Luis et al., 1995; Koshita et al., 1999; Valiente and Albrigo, 2004), cultivar differences in response to climate and water supply (Davenport, 1990), and time of harvest (early vs. late) (Verreyenne and Lovatt, 2009) all point to the presence of an endogenous, self-regulating system that pertains to the intensity of flowering response. Concomitantly, the majority of research findings strongly support a self-regulatory model that involves an endogenous signal transmitted within the plant (hormones), with the intensity of transmittance resulting in inhibition or up-regulating of flowering response in the presence of growth-promoting conditions. In addition, the molecular response to the hormonal signal is to a large degree dependent on the measure of available plant metabolic energy (carbohydrates) and the prevalence and intensity of factors influencing the availability thereof.

**The role of endogenous hormones**

**Auxins (IAA):** Citrus trees sustain a complex tree structure with the development of new shoots during vegetative growth flushes in spring, summer and autumn. After a sufficient induction period and with an increase in temperature and water and mineral-nutrient supply, flowers develop from buds on these vegetative shoots. The development of new flowering sites/bearing units during the various vegetative growth flushes is therefore of critical importance to ensure a sufficient return bloom. Fruit is a major source of natural, endogenous auxins (most importantly indole acetic acid (IAA)), and their presence during the summer is directly correlated to the inhibition of new flower-bearing units (shoots) (Fig. 2) and resultant poor return bloom - a mechanism proposed to be similar to apical dominance (Verreyenne, 2005).

Early removal of fruit high in IAA, allows for the sprouting of new potential flower-bearing units, increase in available flowering sites and intensified flowering during return bloom (Verreyenne and Lovatt, 2009). In addition, summer fruit removal (hand thinning) results in a significant increase in the size of remaining fruit (Stander, 2014) and results in the decrease in an important group of endogenous hormones, gibberellins (GA), which directly influence flower-bud development at the molecular level.

**Gibberellins (GA):** The inhibiting effect of gibberellins (GA’s) on flowering of perennial fruit trees is well documented (Bangert, 2009). The effect on flower induction in citrus is furthermore supported by the lack of reproductive inflorescence obtained after exogenous applications of gibberellic acid (GA3) under prevailing flower inductive conditions (low temperature and/or water stress) (Goldschmidt et al., 1985; Guardiola et al., 1982; Monselise and Halevy, 1964). Only recently has light been shed on the metabolic method of inhibition of exogenously applied GA during flower induction, with Muñoz-Fambuena et al., (2012) demonstrating a reduction in the expression of the key flower-promoting gene, the citrus flowering locus T (CiFT) in the leaves and buds of sweet orange (Citrus sinensis), in response to GA3. A similar response was obtained in mandarin (Citrus reticulata) by Goldberg-Moeller et al. (2013).

Fruit rinds and developing seeds are a major source of endogenous GA (Ben- Cheikh et al., 1997) and the suggestion of the inhibiting effect of endogenous GA on flowering is substantiated by Koshita et al. (1999) who related the lack of flower bud formation to a three-fold increase of GA-content in the leaves of fruit-bearing shoots of satsuma mandarin (Citrus unshiu) during the time of flower induction (Fig. 2). The regulatory role of GA conforms to the evolutionary adaptation of perennial plants that restricts saturation of buds by flowers and allows for sprouting of vegetative shoots to maintain plant longevity beyond two growing seasons.
However, from a commercial perspective, even a slight imbalance of a particular factor such as GA, can lead to detrimental consequences to successful citrus production over the short- and long-term. In years of heavy fruit loads, especially in late-maturing cultivars that are harvested after winter, such as ‘Nadorcott’ and ‘Or’ mandarins (Citrus reticulata) as well as ‘Valencia’ sweet orange (Citrus sinensis), the inhibition of CiFT-expression by excessive number of fruit (the major source of endogenous GA), is one of the main factors resulting in the manifestation of alternate bearing.

The role of carbohydrates

Although suggested by Koch (1996), the direct involvement of total carbohydrates or its different components, such as the non-reducing sugar, sucrose, in the transcriptional control of flower-gene expression in perennial fruit trees has thus far not been conclusively proven. Nevertheless, the indirect implementation of carbohydrates’ effect on flowering is very well documented and adheres to the “nutritional hypothesis” as related to citrus flower development.

Strong and convincing evidence suggests that the lack of flowers developing after a severe “on”-year in alternate bearing cultivars as a consequence of extreme depletion of carbohydrates in tree storage organs (Verreyne, 2005; Van der Merwe, 2012) (Fig. 3). Concomitantly, an increase in flower numbers is obtained from de-fruiting treatments of trees during an “on”-year (Goldschmidt and Golomb, 1982; Stander, 2014). By early removal of a large number of fruit, a strong sink for photo-assimilates is removed and therefore results in the restoration of carbohydrate levels in leaves and shoots to a level sufficient for development and maintenance of an adequate number of flowers during the return bloom. However, the sole effect of carbohydrates on flowering following fruit removal is inconclusive, as removal of fruit also involves the elimination of a major natural source of endogenous hormones such as GA and IAA that may have influenced bud-fate (Moss, 1971).

Girdling during flower induction provides a useful experimental tool to manipulate tree carbohydrate levels without having to remove fruit. The increase in flower intensity resulting from autumn girdling (Fig. 4) is strongly correlated to a spike in carbohydrate availability for energy utilization during flower induction (Goldschmidt, 1999). However, once again the important interplay of hormone/carbohydrate balance comes into play. The breaking of vascular connectivity between potential flowering buds and other organs resulting from the girdling treatment could possibly restrict the transportation of other, more specific signals (such as hormones) that may control or have a significant influence on flower formation.

Conclusions

Taking all of the above-mentioned aspects into consideration, flowering response in citrus is a factor of three important variables that can only be optimized if fully understood and carefully considered by a citrus grower, as follows:

1. Flowering is a factor of the amount of available flowering sites/bearing units. Are the production practices embracing nutritional, irrigation and crop management practices that will ensure the development of new vegetative flush during August/September, November/December and February/March?

2. Flowering is a factor of the level of self-regulation within a bearing unit. Is the particular cultivar under consideration an early or late cultivar (fruit harvested after flower induction) and in the case of the latter, how can fruit load be managed to a commercially acceptable level, without fruit inhibiting CiFT-gene expression?

3. Flowering is a factor of the intensity level of flower induction conditions. Will enough low temperatures accumulate during May-July in the particular production area, and if not, can sufficient water stress be applied during this period to stimulate necessary CiFT-gene expression, without neglecting fruit quality and tree health?

The worst-case scenario for citrus flower development will be where inadequate new bearing shoots are formed in the summer or autumn immediately prior to winter induc-
tion conditions and crop load cannot be regulated to i) support the development of new bearing shoots and ii) avoid the depletion of carbohydrate reserves. Cultivar choice, such as the planting of a seedy (seed = high in GA), late-maturing cultivar (harvested after winter) in an area with historically moderate average autumn and winter temperatures (>15°C) (little or no CiFT expression) will place an additional burden on flowering potential.

References cited