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Effect of crop load on phytohormones, sugars, and biennial bearing in apple trees

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Abstract

The amount and composition of phytohormones, sugars, and some other leaf characteristics depending on a crop load were evaluated in apple (*Malus domestica* Borkh. cv. Ligol grafted on P 60 rootstock) trees in order to prevent biennial bearing. The crop load was adjusted to 12 (control, unthinned), 8, 4, and 0 (non-fruiting) inflorescences (or fruits) per cm^2 of trunk cross-sectional area (TCSA). Inflorescences were removed in May before flowering. Phytohormones were analyzed in axillary buds and leaves in September. Results show that, in contrast to the unthinned trees, thinning to 4 fruits cm^{-2}(TCSA) resulted in a significant decrease of yield per tree, but a significant increase of fruit mass, return bloom, and leaf area. The heavy crop load resulted in suppressed bloom in the following year. Composition and content of phytohormones was changed considerably. Moreover, thinning resulted in an increased hexose accumulation. Such data suggest that flowering inhibition depended on the phytohormones that were exported to buds and on sugar-hormone signalling cross-talk.

Additional key words: abscisic acid, auxins, chlorophylls, gibberellins, hexoses, jasmonic acid, leaf area, sucrose, zeatin.

Introduction

The apple tree (*Malus domestica* Borkh.) is among the most important commercial fruit crops grown worldwide. The problem with many cultivars is alternate bearing as result of an excessive number of flower clusters and fruits (Guiton *et al*. 2012, Pellerin *et al.* 2012). The timing of transition from vegetative growth to flowering is of paramount importance (Bernier *et al.* 1993). Floral induction in perennial fruit trees is distinct from that of annual or biennial plants because it is a quantitative process with a significant proportion of above ground meristems remaining vegetative, whereas all meristems terminating plant life are induced at once in annual and biennial plants (Samuoliene and Duchovskis 2006, Bangerth 2009). In several species, cold is the major factor stimulating flower initiation. The required vernalization length is in connection with development and size of the plants, more developed ones demanding a shorter period. Besides temperature, the day length may also play a basic role in flower initiation. Those inductive factors stay in a tight correlation with each other (Zeevaart 2006). Perennial plants have a long juvenile period but in the adult phase, their meristems can initiate floral organs annually. The different flowering-promoting factors are perceived by different parts of the plant. Temperature is perceived by all plant parts, vernalization mainly by the shoot apex, and photoperiod by mature leaves. Therefore, this implies that these parts interact and that the fate of the apical meristem – remaining vegetative or becoming reproductive – is controlled by an array of long-distance signals from the entire plant. Transport of sucrose from leaves to apical meristem (Bernier *et al.* 1993) and an increase in content of gibberellins (GAs) are observed during flowering induction (Gibson 2004). Generally, four major flowering pathways, such as induction through the photoperiod, temperature/autonomous, energy (sucrose), and regulation by GAs, have been characterized in herbaceous species and at the molecular level especially in *Arabidopsis* (Corbesier and Coupland 2005). However, genetic analyses in *Arabidopsis* showed that several independent flowering inducing pathways exist. Numerous experiments have shown that misexpression of *FLOWERING LOCUS T FT*-like genes perturbs the time of flowering in range of plants, and FT-like proteins act as universal promoters of flowering (Pin and Nilsson 2012, Lifschitz *et al.* 2014). Although the mechanisms by

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Abbreviations: ABA - abscisic acid; Cars - carotenoids; Chl - chlorophyll; GAs - gibberellins; IAA - indole-3-acetic acid; JA - jasmonic acid; TCSA - trunk cross-sectional area.
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which FT homologs induce flowering in different plant species remains unclear, there is evidence that FT-like proteins act as mobile flowering signals interacting with analogous proteins at shoot apical meristem (Zeevaart 2006). Moreover, florigen and antiflorigen are equally important constituents of floral induction. Expression of *FT/FTL1*-like (*FTL*) genes of gymnosperms in *Arabidopsis* results in delayed flowering (Klintenäs *et al.* 2012). Kotoda *et al.* (2010) showed that in apple two FT homologs exhibit distinct expression patterns. A *MdFT1* is essentially expressed in apical meristem during floral transition, whereas expression of *MdFT2* peaks at a later stage in reproductive tissue and developing fruits. Tränker *et al.* (2010) also demonstrated that *MdFT1* is responsible for inducing flowering, and that the function of an apple *FT1* gene is conserved in annual herbaceous species as well as in perennial woody species. The apple tree is autonomous flowering plant whose floral initiation depends on vegetative development in the growing season before anthesis (Wilkie *et al.* 2008). Flower induction, flower initiation, flower differentiation, and blooming are the main stages of flowering (Samuolienė *et al.* 2009). Flower initiation is the key developmental stage for apple trees (Guiton *et al.* 2012), and it is inhibited by environmental conditions such as high crop load (Nichols *et al.* 2011), irradiance (Kittikorn *et al.* 2011), temperature (Kviklys and Robinson 2010), and drought or GAs produced by seeds (Kittikorn *et al.* 2010).

There is a practice of regulating the number of flowers as the distance between flower clusters in one year influences the development of floral buds for the next year (Meland 2009). Lowering a crop load from untreated trees down to 9 fruits cm^{-2} of trunk cross-sectional area (TCSA) decreases fruit yield but improves fruit mass and quality. Moreover, consistent annual production was achieved by thinning to 6 fruits cm^{-2}(TCSA) (Embree *et al.* 2007). Nichols *et al.* (2011) also suggested that a reduced number of flower clusters to between 40 and 60 m^{-3}(canopy) is necessary to control biennial bearing in cv. Honeycrisp.

Consistent annual yields may be achieved by reducing the number of fruits per tree, which leads to an increased relative amount of leaf area per fruit thereby enhancing the availability of photoassimilates for the remaining fruits (Meland 2009). However, Wünsche *et al.* (2005) showed that an increased crop load stimulates accumulation of chlorophylls (Chls) and photochemical efficiency of photosystem II in leaf chloroplasts.

Endogenous factors, such as non-structural saccharides or phytohormones, affect plant development (Bangerth 2009). The main sugars synthesized in apple leaves are sorbitol, which accounts for 60 to 70 % of photosynthates produced in leaves, and sucrose (Wei *et al.* 2014). Sucrose is the main sugar transported through the plant and, among other functions, acts as signalling molecule for flowering induction. Li *et al.* (2012) indicated that, compared with model plants, metabolism and accumulation of sugars in apple are unique, as almost all sorbitol and a half of sucrose are converted to fructose. Moreover, the inhibitory role of GAs in flowering perennials, and especially apple trees, is distinguished (Wilkie *et al.* 2008). Ramírez *et al.* (2004) noted that endogenous GAs (possibly GA_1, GA_4, and GA_7) produced by seeds inhibit floral initiation in cv. Golden Delicious. A higher content of GA_1 and GA_4 was found in apical buds at the time of floral initiation on blossom-thinned heavily cropping cv. Fuji (Kittikorn *et al.* 2010).

Cellejas and Bangerth (1997) posited a hormone balance hypothesis that suggests that seeds export hormones which inhibit flowering, and leaves export hormones which promote flowering, and their ratio is critical for flowering. In more recent years, Pellerin *et al.* (2012) proposed a model to explain the effect of hormones on canopy structure of a mature tree and on flowering. Bertelsen *et al.* (2002) suggested that GAs might suppress floral initiation by enhancing polar transport of indole-3-acetic acid (IAA) from seeds. Moreover, if GAs are indeed floral inhibitors, IAA may stimulate their synthesis in the bud. Other endogenous hormones jasmonic acid (JA) and cytokinins are associated with flower induction and may promote floral initiation in apple (Bangerth 2009, Kittikorn *et al.* 2010). Thus, the ratio of different hormones coming from seeds and leaves may be a cause of biennial bearing. In agreement, Dennis (2000) suggested that biennial bearing may be result of signalling molecules such as sucrose and hormonal inhibition of floral initiation by nearby seeds.

The purpose of this study was to investigate the involvement of phytohormones and sugars in flowering initiation of the apple cv. Ligol and some yield and leaf characteristics to determine the most appropriate crop load in order to prevent biennial bearing.

Materials and methods

A field experiment was carried out in an intensive orchard at the Institute of Horticulture, the Lithuanian Research Centre for Agriculture and Forestry, using the apple (*Malus domestica* Borkh.) cultivar Ligol grafted on the P 60 rootstock. The trees were planted at 3.5 × 1.25 m and trained as slender spindle. Pest and disease management was carried out according to the rules of integrated plant protection. Eight single trees were fully randomized. The TCSA was measured 20 cm above the grafting union before vegetation. The crop load was adjusted to 8, 4 and 0 (not fruiting) inflorescences (or fruits) per cm^2 of TCSA. Unthinned trees served as control and an average crop load was 12 inflorescences (or fruits) per cm^2 of TCSA. Flowers were removed in early May before flowering at the pink bud stage. The final number of fruits per cm^2 of TCSA, yield (kg per

tree) and fruit mass (g) were evaluated in October. Return bloom was observed in May.

Phytohormones were analyzed in axillary buds and leaves in the middle of September and in the middle of March according to Vitti *et al.* (2013) using high pressure liquid chromatography (HPLC; *Shimadzu,* Kyoto, Japan) combined with ultra-high-speed mass spectrometry (LC/MS). Separation of phytohormones (GAs – GA_1, GA_3, and GA_7; IAA; JA; abscisic acid, ABA; and zeatin) was performed on a *YMC-UltraHT Pro C18* column (3.0 × 75 mm) (*Shimadzu*). A mobile phase – double distilled water [with 0.5 % (v/v) formic acid] + methanol (90:10, v/v) in a positive mode, and double distilled water [with 0.5 % (v/v) formic acid] + methanol (80:20, v/v) in a negative mode; a flow rate of 0.2 cm^3 min^{-1}.

Sucrose was analyzed in axillary buds in the middle of September. About 1 g of fresh tissue was ground in 4 cm^3 of double distilled water and the extraction was carried out at 70 ºC for 24 h. The samples were filtered using cellulose acetate (pore diameter 0.22 μm) syringe filters. The analyses were performed using a *Shimadzu* HPLC with a low temperature evaporative light scattering detector (*ELSD-LTII*), and oven temperature was maintained at 40 ºC. Separation of saccharides was performed on an *EC 250/4 NUCLEOSIL* (4 × 250 mm) column, a mobile phase – acetonitrile + water (79:21, v/v), a flow rate of 2 cm^3 min^{-1}. The sensitivity of the HPLC methods was established using a method validation protocol (*ICH 2005*; http://www.ich.org/LOB/media/MEDIA417.pdf).

Some leaf characteristics were measured during fruit maturation in the middle of July. Content of Chl *a*, Chl *b*, and carotenoids (Cars) was measured with a spectrophotometer *Genesys 6* (*Spectro Analytical Instrumenst*, Kleve, Germany) in an acetone extract according to Gavrilenko and Zigalova (2003). Fresh leaves (0.2 g) were ground with powdered $CaCO_3$ and small volumes of 100 % acetone, filtered, and diluted to a final volume of 50 cm^3 with acetone. The absorbance of the samples was measured at 440.5 and 662 nm for Chls *a* and *b* and at 644 nm for Cars. Leaf area was measured with a leaf area meter (*AT Delta-T Device*, Cambridge, UK).

Data were processed using one-way analysis of variance (*ANOVA*) and Duncan's multiple range test at $\alpha = 0.05$. The *Statistica 10* software was used for data processing.

Results

A significantly higher content of zeatin (50.6 %) and JA (33.5 %), was found in axillary buds of the unthinned trees compared with those thinned to 8 fruits cm^{-2}(TCSA) in September (Table 1). On the other hand, thinning had no significant effect on zeatin content in leaves, but significant differences in JA content between not fruiting trees and those thinned to 4 fruits cm^{-2}(TCSA) were found. However, the accumulation of zeatin and JA was much lower in axillary buds in spring.

Thinning to 8 fruits cm^{-2}(TCSA) caused about a 60 % lower accumulation of ABA and IAA in axillary buds in autumn. However, the lowest amounts of ABA and IAA were found in leaves of the unthinned trees. In axillary buds in spring, a significant decrease in ABA accumulation was in 4 fruits cm^{-2}(TCSA), and increase in 12 fruits cm^{-2}(TCSA). Thinning to 4 fruits cm^{-2}(TCSA) resulted in a significant increase of IAA, GA_1 and GA_3 content in spring. A low amount of total GAs was found in axillary buds of the not-fruiting trees in autumn. However in spring, relatively high amounts of GA_1, GA_3,

Table 1. The effect of crop load [0, 4, 8, and 12 inflorescences cm^{-2}(trunk cross-sectional area)] on content of phytohormones [μg g^{-1}(FM)] determined in 3 trees, 5 leaves, or 25 buds per each tree. Means followed by the same letter are not significantly different ($P > 0.05$) according to Duncan's multiple range test. Promoters of flowering are zeaxanthin and JA, others are inhibitors.

Organs	Crop load	Zeatin	JA	ABA	IAA	GA_1	GA_3	GA_7	Total GAs	Promoter: inhibitor
Leaves	12	12.1ab	1273.7abc	61.2a	347.3a	89.3abc	57.4a	643.1a	789.8a	1:0.9
(September)	8	14.6b	1584.9bc	99.8c	649.9c	95.7bc	115.4c	1032.6c	1243.7c	1:1.2
	4	13.8ab	1702.0c	107.3c	669.8c	99.7c	103.1c	960.9b	1162.7b	1:1.1
	0	12.2ab	790.1a	80.7b	513.6b	74.2a	79.0b	1153.8d	1307.1c	1:2.4
Axillary buds	12	34.8d	694.3c	132.2d	417.0b	119.5c	253.8c	1150.2d	1523.4d	1:2.8
(September)	8	17.2a	461.4a	53.0a	316.5a	54.4a	168.6b	650.5b	873.6b	1:2.6
	4	25.8c	623.4b	91.7c	377.1b	65.8b	150.6b	862.5c	1078.9c	1:2.4
	0	21.5b	463.3a	76.8b	793.8c	68.3b	86.3a	482.5a	637.2a	1:3.1
Axillary buds	12	1.18b	4.76a	12.27d	47.34a	0.53a	0.32ab	66.42b	67.26c	1:21.4
(March)	8	2.05c	6.87a	5.02b	31.35a	2.23b	0.11ab	0.76a	3.10a	1:4.4
	4	8.75d	160.91c	1.39a	775.6b	8.42c	3.53b	4.00a	15.95b	1:4.7
	0	0.52a	19.33b	9.57c	74.67a	0.99ab	0.32ab	2.54a	3.85a	1:4.4

Table 2. The effect of crop load [0, 4, 8, and 12 inflorescences cm^{-2}(trunkcross-sectional area)] on content of non-structural saccharides [mg g^{-1}(FM)] in axillary buds in September determined in 3 trees and 25 buds per each tree. Means followed by the same letter are not significantly different ($P > 0.05$) according to Duncan's multiple range test.

Crop load	Fructose	Glucose	Sucrose	Hexose/sucrose	Hexose+sucrose
12	11.46b	32.28b	4.25bcd	10.34b	47.98b
8	12.32bcd	51.63c	3.04a	21.04c	67.00c
4	14.90d	56.70c	3.78b	18.94c	75.37c
0	6.02a	21.54a	4.95d	5.57a	32.51a

Table 3. The correlation between phytohormones and non-structural saccharides in axillary buds in September as influenced by a crop load [0, 4, 8, and 12 inflorescences cm^{-2}(trunk cross-sectional area)]. It was determined in 3 trees and 25 buds per each tree.

Crop load	ABA to sucrose	IAA to glucose	IAA to GA_1	IAA to GA_3	IAA to GA_7	IAA to total GAs
12	0.98	0.98	-1.0	1.0	-1.0	-1.0
8	1.00	0.93	-1.0	-1.0	1.0	-1.0
4	0.99	1.00	1.0	-1.0	-1.0	-1.0
0	0.87	0.92	1.0	1.0	1.0	0.5

and GA_7 were established in buds of the unthinned trees. An opposite effect of thinning was observed in leaves where the lowest amount of total GAs in leaves of the unthinned trees was found. The promoter/inhibitor ratio was highest in axillary buds in spring and especially in the unthinned trees.

The lowest content of hexoses and the highest content of sucrose were detected in axillary buds of the non-fruiting trees (Table 2). Increases of fructose (about 55 %) and glucose (about 60 %) content and a decrease of sucrose (about 31 %) was at 4 and 8 fruits cm^{-2}(TCSA) compared with the non-fruiting trees. Thus, the lowest and highest ratios of hexoses to sucrose and the total amount of saccharides were in axillary buds at the non-fruiting trees and at 4 and 8 fruits cm^{-2}(TCSA), respectively.

Table 4. The effect of crop load [0, 4, 8, and 12 inflorescences cm^{-2}(trunk cross-sectional area)] on fruit set, yield, fruit mass, and return bloom determined in 5 trees. Means followed by the same letter are not significantly different ($P > 0.05$) according to Duncan's multiple range test.

Crop load	Final number of fruits [cm^{-2}(TCSA)]	Yield [kg $tree^{-1}$]	Fruit mass [g]	Return bloom, [number of. flower clusters cm^{-2}(TCSA)]
12	11.7c	33.5c	236.4a	3.7d
8	7.4b	25.6abc	240.7abc	5.0c
4	4.9a	18.5a	272.3c	8.0b
0	0	0	0	13.2a

The correlation analysis shows that the increased content of ABA and IAA led to the increased content of sucrose and glucose, respectively, as a strong positive correlation was found (Table 3). No common trend for accumulation of GAs influenced by IAA was detected. A strong negative correlation shows that the increased IAA content decreased GA_1 content at 12 and 8 fruits cm^{-2}(TCSA), GA_3 at 8 and 4 fruits cm^{-2}(TCSA), and GA_7 at 12 and 4 fruits cm^{-2}(TCSA), whereas a strong negative correlation between IAA and total GAs was evident in all the thinning treatments except for the non-fruiting trees.

Table 5. The effect of crop load [0, 4, 8, and 12 inflorescences cm^{-2}(trunk cross-sectional area)] on content of photosynthetic pigments [mg g^{-1}(FM)] and leaf area [cm^2] determined in July in 3 trees and 25 leaves per each tree. Means followed by the same letter are not significantly different ($P > 0.05$) according to Duncan's multiple range test.

Crop load	Chl *a*	Chl *b*	Total Chl	Car	Leaf area
12	0.71b	0.38ab	1.10b	0.35b	40.05a
8	0.68ab	0.40b	1.08ab	0.34ab	45.85abc
4	0.58ab	0.37ab	0.98ab	0.30ab	43.62bc
0	0.65ab	0.38ab	1.03ab	0.33ab	45.93c

The non-fruiting apple trees formed a significantly larger (12.8 %) leaf area in comparison with the unthinned trees (Table 5). The content of Chl *a*, total Chl, and Cars was higher in leaves of the unthinned trees than in other treatments by 9.9, 7.2, and 5.7 %, respectively (Table 5). The final fruit number decreased up to 0.3 and 0.6 fruits in the crop load of 12 and 8 fruits cm^{-2}(TCSA) but increased up to 0.9 fruits in the crop load of 4 fruits cm^{-2}(TCSA) at the end of the experiment (Table 4). The yield per tree was highest in the unthinned trees and decreased by 1.3 times per each thinning step. On the other hand, the biggest fruit mass was in the crop load of

4 fruits cm^{-2}(TCSA) and decreased by 11.6 and 13.2 % in 8 and 12 fruits cm^{-2}(TCSA). The biggest return bloom was at the crop load of 4 fruits cm^{-2}(TCSA), and the lowest one in the unthinned trees.

Discussion

Most commercially grown apple cultivars have an over-abundant flower density, which results in biennial bearing in apple trees. The hormonal pathway of floral initiation in apple differs from the model plant *Arabidopsis*, as alternate bearing induced by heavy fruit load is associated with GAs which act as floral inhibitors (Ramírez 2004, Wilkie *et al.* 2008) for the following year (Stephan *et al.* 2001). According to the results, the ratio of promoter to inhibitor hormones was about 3.0 (not thinned), 2.0 [8 and 4 fruits cm^{-2}[TCSA)], and 1.3 (not fruiting) times higher in axillary buds than in leaves in autumn (Table 1). Bangerth (2006) showed that GAs and auxin inhibit floral initiation and cytokinins act as floral promoters in perennial fruit trees. This is in agreement with Ramírez *et al.* (2004), as the application of exogenous cytokinins to spurs at the time of flower initiation increases the number of flowers, and GA_1, GA_4, and iso-GA_7 were reported as inhibitors. Jasmonic acid is at high concentrations in thinned apple trees when GAs are low and *vice versa* in heavily bearing trees (Kittikorn *et al.* 2010). Generally, unlike zeatin, a lower content of JA was found in axillary buds than in leaves in autumn. However, a decrease of both promoter and inhibitor phytohormones was observed in spring in all the thinning treatments (Table 1). On the other hand, compared with autumn, the ratio of promoter to inhibitor phytohormones increased 1.4 times in the non-fruiting trees, about 1.8 times in the 4 and 8 fruits cm^{-2}(TCSA), and 7.6 times in the unthinned trees in spring. Moreover, the yield per tree was highest and return bloom was lowest in the unthinned trees (Table 4). Thus, the heavy crop load resulted in an increase of inhibitor phytohormones and return bloom was suppressed the following year. Pellerin *et al.* (2012) found that critical inhibitor to promoter hormone ratios of 1:1, 1:4, or 1:16 require 0, 60, and 78 % of thinning, respectively.

Physiological aspects of plant development are complex and the response is associated with multi-component flower initiation pathways. Developmental changes in shoot apical meristems are sensitive to long-distance sugar signalling. Thus, saccharides may control transition to flowering (Teo *et al.* 2006, Moghaddam and Van den Ende 2013). A significant decrease of the hexose to sucrose ratio and total sugar amount because of the low content of hexoses was found in the non-fruiting trees (Table 2). The crop load adjusted to 8 and 4 inflorescences cm^{-2}(TCSA) resulted in a significant increase of the hexose to sucrose ratio and total sugar content mainly due to a significant increase of glucose. Li *et al.* (2012) indicated that apple is unique in terms of sugar metabolism and accumulation, as almost all sorbitol and a half of sucrose are converted to hexoses by invertase.

Flowering is induced by both sugars and phytohormones. There is strong evidence of cross-talk between sugar and phytohormone signalling which determines transition from vegetative growth to generative development. Sugars and ABA act synergistically during transition from rapid cell division to cell enlargement and accumulation of storage reserves (Eveland and Jackson 2012). On the other hand, ABA and glucose act antagonistically during seed germination (Leon and Sheen 2003). Strong positive correlations between ABA and sucrose and between IAA and glucose shows a synergistic action (Table 3). Thus, the importance of sucrose and glucose for flowering is emphasized by the strong positive correlation between inhibitor hormones and sugar signalling molecules. Mishra *et al.* (2009) suggested that glucose and auxin may act both antagonistically or synergistically. Due to this fact more than half of the genes affected by auxin were regulated by glucose in *Arabidopsis*. Complex relationships also exist between inhibitor hormone levels and flowering. Removal of apical buds resulted in lower IAA and total GAs content (Bangerth 2006). Negative correlation between IAA and total GAs shows that when content of IAA increased, the amount of GAs decreased (Table 3). Strong negative correlations between IAA and total GAs content in axillary buds of fruiting apple trees suggested that content of GAs is more important for floral inhibition than content of IAA, as a significantly lower rate of return bloom was observed compared with non-fruiting trees. Moreover, strong positive correlations between inhibitor hormones and sugars, between IAA and GA_1, and between GA_3 and GA_7 were found (Table 3) and resulted in the highest rate of return bloom following year in non-fruiting trees (Table 4).

It was reported that a low number of fruits leads to enhanced flowering in the following year and promotes biennial bearing (Dennis 2000, Nichols *et al.* 2011). Consistent annual production is achieved by thinning to 6 fruits cm^{-2}(TCSA) in cv. Honeycrisp (Embree *et al.* 2007). These reports are in agreement with the obtained data, as more intensive thinning resulted in a greater return bloom the following year (Table 4). Based on return bloom, expected yields would be twice and three times higher at thinning levels 4 and 8, respectively, compared to the control. The apple yields achieved with different thinning treatments or based on return bloom are higher than average yields under Lithuanian climatic conditions (Kviklys *et al*. 2012).

In contrast to thinning to 4 fruits cm^{-2}(TCSA), thinning to 12 fruits cm^{-2}(TCSA) resulted in a significantly higher yield per tree and in a significant decrease in fruit mass. Meland (2009) also reported that

fruit masses are highest with the lowest crop load and decrease with increasing crop loads. Wünsche *et al.* (2005) showed that an increase in crop load increases Chl content. However, different thinning did not lead to significant changes in Chl *a*, Chl *b*, and Cars content (Table 5). On the other hand, Vemmos (1994) observed an increase of Chl content without any differences in Chl *a*/*b* ratio and a decrease in leaf area at an increasing crop load. We also observed that the increased crop load resulted in a decrease of leaf area (Table 5). This could be associated with carbon transport during fruit maturation, as non-structural saccharides are the primary end-products of leaf photosynthesis, and the increase of hexose content was observed in the fruiting trees (Table 2).

Conclusions

In contrast to the unthinned trees bearing 12 fruits cm^{-2}(TCSA), thinning to 4 fruits cm^{-2}(TCSA) resulted in the significant decrease in yield per tree, the significant increase in fruit mass, return bloom, and leaf area. The heavy crop load (the unthinned trees) resulted in an increase of inhibitor phytohormones and return bloom was suppressed the following year. Such data suggest that flowering inhibition depended on a critical ratio of inhibitor to promoter hormones that were exported to buds. Moreover, thinning resulted in the increased hexose accumulation. Thus, flowering may be determined by the phytohormones transported from leaves or buds and by sugar-hormone interactions. The data obtained in autumn are not as informative as those obtained in early spring, according to which flowering and yield may be predicted.

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