Athens Institute for Education and Research
ATINER

ATINER's Conference Paper Series
AGR2015-1792

Physiological Aspects of Biennial Bearing by Manipulating Crop Load in Apple Trees

Giedre Samuoliene
Senior Researcher
Lithuanian Research Centre for Agriculture and Forestry
Lithuania

Alina Ceidaite
PhD Student
Lithuanian Research Centre for Agriculture and Forestry
Lithuania

Ramunas Sirtautas
Researcher
Lithuanian Research Centre for Agriculture and Forestry
Lithuania

Darius Kviklys
Senior Researcher
Lithuanian Research Centre for Agriculture and Forestry
Lithuania

ATINER CONFERENCE PAPER SERIES No: AGR2015-1792

An Introduction to ATINER's Conference Paper Series

ATINER started to publish this conference papers series in 2012. It includes only the papers submitted for publication after they were presented at one of the conferences organized by our Institute every year. This paper has been peer reviewed by at least two academic members of ATINER.

Dr. Gregory T. Papanikos
President
Athens Institute for Education and Research

This paper should be cited as follows:

Samuoliene, G., Ceidaite, A., Sirtautas, R. and Kviklys, D. (2016). **"Physiological Aspects of Biennial Bearing by Manipulating Crop Load in Apple Trees",** Athens: ATINER'S Conference Paper Series, No: **AGR2015-1792.**

Athens Institute for Education and Research
8 Valaoritou Street, Kolonaki, 10671 Athens, Greece
Tel: + 30 210 3634210 Fax: + 30 210 3634209 Email: info@atiner.gr URL: www.atiner.gr
URL Conference Papers Series: www.atiner.gr/papers.htm

ISSN: 2241-2891
14/01/2016

Physiological Aspects of Biennial Bearing by Manipulating Crop Load in Apple Trees

Giedre Samuoliene

Alina Ceidaite

Ramunas Sirtautas

Darius Kviklys

Abstract

Effect of crop load distribution of apple trees on bearing behaviour was tested with the 'Ligol' apple tree on P60 rootstock. The crop load was adjusted to 6 inflorescences per cm^2 of the trunk cross-sectional area. The flower buds were thinned in May at the pink bud stage as follows: even distribution on the tree – (I) control; from one side of tree – (II) bare side and (III) fruiting side; from individual branches throughout all the tree – (IV) bare branch and (V) fruiting branch; and from inside and outside of the tree – (VI) bare inside and (VII) fruiting outside. JA is associated with flower induction and gibberellins (GA_1, GA_3, GA_7) - with flower inhibition in apple trees, as higher amounts of JA and lower GA levels were found in buds of these treatments where inflorescences were removed. Significantly lower ABA contents were detected in all treatments in comparison to even distribution of inflorescences. A significantly higher total amount of non-structural carbohydrates, due to a significant increase of glucose, was detected in buds where inflorescences were not removed. A strong negative correlation between ABA/sucrose and IAA/glucose shows that increased contents of floral inhibitors lead to the decrease of sugar signalling molecules where flowers were not removed. Thus, localized flowering inhibition depends on the critical ratio of the inhibitor and promoter signalling molecules that are exported to the buds. The lowest yield was found when inflorescences were removed from individual branches throughout the tree. Due to hormonal and sugar signalling flowering stimulus in buds of treatments where inflorescences were removed, thus better return bloom in next spring was achieved when buds were removed from one side of the tree.

Keywords: Carbohydrates, phytohormones, return bloom, yield

Acknowledgments: This research was funded by a grant (No. MIP-036/2014) from the Research Council of Lithuania.

Introduction

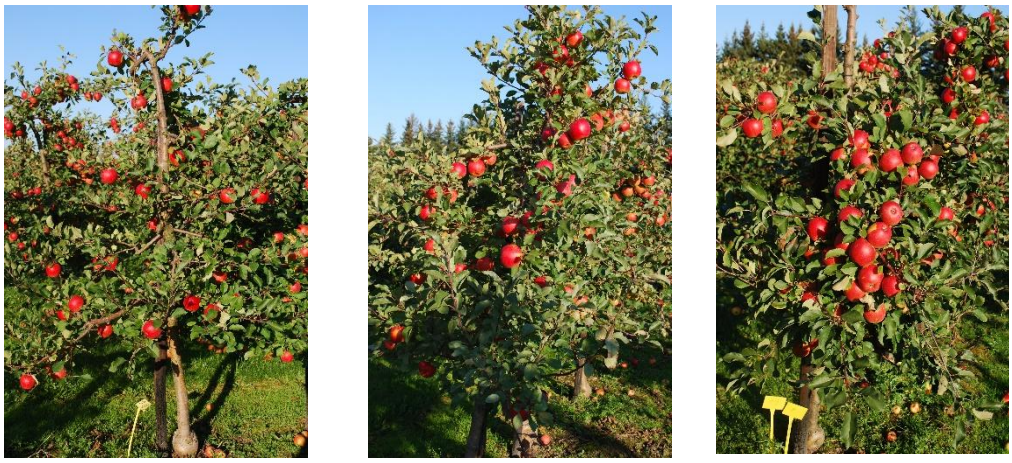
The biennial bearing of apple trees is an actual problem worldwide and in Lithuania as well (Kviklys et al. 2013), as a number of commercially important cultivars are biennial. Such cultivars produce extensive numbers of flower clusters in one year, which leads to flower inhibition for the next year (Dennis 2000; Nichols et al. 2011). Heavy fruit crops evoke physiological changes, related with flowering control, in trees. Floral initiation stimulated by endogenous factors, such as hormonal inhibition (Pellerin et al. 2012), carbon limitation (Dennis 2000), carbohydrate metabolism and related photosynthetic indices (Zhou and Quebedeaux 2003), results in biennial bearing. Some studies demonstrated that the ratio of inhibitor (gibberellins, abscisic acid, indole-3 acetic acid) to promoter (jasmonic acid, zeatin) phytohormones is critical in flowering processes of trees (Kittikorn et al. 2010; Ramírez et al. 2004; Turktas et al. 2013). Moreover, the ratio of inhibitor to promoter hormones coming from seeds and leaves respectively, is estimated at each shoot apical meristem. Thus, biennial bearing is caused by a local effect of inhibiting flower development for the next year (Pellerin et al. 2012). Another internal factor regulating flowering is sugar signalling. It has been reported that the sucrose levels increased after flowering inductive stimuli in apical buds (Moghaddam and Ende 2013). Sugar balance, glucose, fructose and sucrose, in the apex strongly regulates flowering in many species. It has been reported that high sucrose levels are associated with high T6P levels, but this correlation depends on the activity of sucrose splitting enzymes, such as invertases, and have a strong impact on sucrose to hexose ratios (Wingler et al. 2012). Thus, the relation between sugar metabolism, signalling and floral transition is in great importance. On the other hand, endogenous non-structural carbohydrate levels are associated with photosynthetic carbon assimilation. It was reported that photosynthesis is generally inhibited accumulation of assimilates in leaves (Smith et al. 2005). Heavy crop load, defined as fruit units per trunk cross-sectional area (TCSA), affects carbohydrate accumulation, especially during fruit development, when fruits become major sink organs (Wünsche et al. 2005). Generally, an increase in crop load stimulates photosynthesis, chlorophyll content, but there is no difference in the ratio of chlorophyll and between fruiting and non-fruiting trees, and decreases leaf area and dry mass per unit leaf area (Vemmos 1994; Wünsche et al. 2005) and yield in the subsequent year (Krishnamurthy et al. 2013; Turktas et al. 2013). Physiological aspects of apple tree biennial bearing are complex. Floral initiation depends on interaction between environmental factors as well as on the harmonious action of hormonal and sugar status and signalling pathways. Thus, the effect of crop load distribution within the apple tree on bearing behaviour was tested with the 'Ligol' apple tree on P60 rootstock. The role, amount and ratio of phytohormones and non-structural carbohydrates, as well as some characteristics of leaves were evaluated.

Materials and Methods

A field experiment was carried out in an intensive orchard of Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry, using the apple cultivar 'Ligol' grafted on P 60 rootstock. The crop load was adjusted to 6 inflorescences per cm^2 of trunk cross-sectional area (TCSA). The flower buds were thinned in May at the pink bud stage as follows: even distribution on the tree – (I) control; from one side of tree – (II) bare side and (III) fruiting side; from individual branches throughout all tree – (IV) bare branch and (V) fruiting branch; and from inside and outside of the tree – (VI) bare inside and (VII) fruiting outside (Figure 1).

Figure 1. *The Distribution of Fruits on the Tree. (A) Even Distribution on the Tree – (I) Control; (B) From One Side of the Tree – (II) Bare Side and (III) Fruiting Side; (C) From Individual Branches Throughout the Tree – (IV) Bare Branch and (V) Fruiting Branch*

(A) (B) (C)



Yield (kg per tree and t per ha) and fruit weight (g) was evaluated in October. Return bloom was observed in May.

Phytohormones were analysed in axillary buds in the middle of September and in the middle of March according to Vitti et al. (2013) on Shimadzu HPLC (Japan) chromatography with ultra high-speed LC/MS. Separation of phytohormones (gibberellins – GA_1, GA_3 and GA_7; indole-3-acetic acid – IAA; jasmonic acid – JA; abscisic acid – ABA; zeatin) was performed on YMC-UltraHT Pro C18 (3.0x75mm) (Japan). Mobile phase – double distilled water (0.5% formic acid) – methanol (90:10, v/v) in positive mode and double distilled water (0.5% formic acid) – methanol (80:20, v/v) in negative mode, flow rate- 0.2ml/min.

Fructose, glucose and sucrose were analysed in axillary buds in the middle of September. About 1g of fresh plant tissue was grounded and diluted with +70º C 4 ml double distilled water. The extraction was carried out for 24 h. The samples were filtered using cellulose acetate (pore diameter 0.22 µm) syringe filters. The analyses were performed on Shimadzu HPLC (Japan)

chromatography with a low temperature evaporative light scattering detector (ELSD-LTII), oven temperature was maintained at +40º C. Separation of carbohydrates was performed on EC 250/4 NUCLEOSIL Carbohydrate column (250 x 4mm) (Germany), mobile phase – acetonitrile – water (79:21, v/v), flow rate- 2ml/min.

The sensitivity of the HPLC methods was established using a method validation protocol (ICH, 2005).

Photosynthetic pigments (chlorophylls (Chl) a and b and carotenoids (Car)) were measured by spectrophotometric (Genesys 6, ThermoSpectronic, USA) method of Wetshtein (Gavrilenko and Zigalova, 2003) in 100% extract of acetone in the middle of July. 0.2 g of fresh leaves were grounded with $CaCO_3$ with small volumes of acetone, filtered and diluted to a final volume of 50 ml using 100 % acetone. The absorbance of the samples were measured at 440.5 and 662 nm for Chl a and b and at 644 nm for Car.

The leaf area (cm^2) was measured with a leaf area meter (AT Delta-T Device, UK) in the middle of July.

The data analysis was processed using a one-way analysis of variance Anova, the Fisher's LSD Test at the confidence level $p = 0.05$. Data was processed using Statistica 10 software.

Results and Discussion

The biennial bearing of apple trees is mainly associated with an extensive number of flower clusters (Nichols et al. 2011; Pellerin et al. 2012). Physiological explanation of such tree behaviour is based on hormonal, sugar signalling pathways, as well as interaction with tree organs (leaves, apical meristems, fruits) (Guitton et al. 2012). The inhibitory effect of gibberellins on flowering initiation in apple trees is reported by many authors (; Guitton et al. 2012; Mutasa-Göttgens and Hedden 2009). Ramírez et al. (2004) showed that floral initiation of 'Golden Delicious' was inhibited by endogenous gibberellins, such as GA_1, GA_4 and iso-GA_7. Moreover, ABA, IAA and GA may act together or independently to inhibit flower initiation in apple trees. Whereas zeatin and jasmonic acid promotes flowering initiation (Bangerth 2006; Kittikorn et al. 2010). These data are in agreement with the obtained results as generally, higher amounts of promoter phytohormones (zeatin and JA) and lower levels of inhibitory phytohormones (ABA, IAA and GAs) were found in buds of these treatments where inflorescences were removed (Table 1). Higher contents of JA and lower contents of GAs were found in bare side in September, but the opposite accumulation of IAA and GAs was in March, moreover the biggest recovery of return bloom was in this treatment, because of more intensive flowering in bared side (Table 5). Higher contents of all tested phytohormones were detected in the bare inside comparing to fruiting outside in September and March. In comparison to fruiting branch, bigger contents of zeatin, IAA and GA_7 were in the bare branch in September. However, contents of all phytohormones, except of ABA, increased in March (Table 1). The basics of phytohormonal floral induction pathway and the action

of phytohormones were expressed in the treatment where inflorescences were thinned from one side of the apple tree. Thus, the results confirm that JA is associated with flower induction and gibberellins (GA_1, GA_3, GA_7) - with flower inhibition in apple trees. Turktas et al. (2013) also states that molecular messengers (e.g. gibberellins) are effective inhibitors of the floral induction in olives. Thus, the relative balances between GAs-like compounds and ABA concentrations may act as a key regulator of floral development and as alternate bearing in many fruit trees (Krishunamurthy et al. 2013).

Table 1. *Effect of Thinning on Content of Phytohormones ($\mu g\ g^{-1}$ FM) in Axillary Buds*

Treatment	Zeatin	JA	ABA	IAA	GA_1	GA_3	GA_7	Total GAs	Promoter : inhibitor
					September				
Even distribution	19.8	494.2	60.7	292.7	54.4	168.6	671.8	894.8	1 : 2.4
Bare side	9.0a	333.9	19.0a	174.5a	29.3a	36.2a	241.2a	306.8a	1 : 1.5
Fruiting side	10.9a	319.6a	21.0a	216.9a	37.6a	49.9a	279.0a	366.4a	1 : 1.8
Bare branch	13.4a	351.7a	20.2a	238.4a	31.0a	69.0a	291.5a	391.5a	1 : 1.8
Fruiting branch	9.9a	452.4	22.1a	198.5a	32.5a	78.7a	284.4a	395.6a	1 : 0.96
Bare inside	14.9a	450.3a	25.9a	94.6a	57.1b	116.6a	577.3a	751.1a	1 :1.9
Fruiting outside	9.4a	280.8a	19.1a	147.7a	30.2a	36.4a	161.4a	228.0a	1 : 1.4
LSD05	2.66	50.79	8.02	47.86	2.64	9.26	29.22	27.17	
					March				
Even distribution	96.7	507.4	173.1	378.3	66.5	78.0	549.4	694.0	1 : 2.1
Bare side	31.8a	320.4	27.7a	2207.7b	42.9a	40.1a	133.2a	216.2a	1: 7.0
Fruiting side	39.3a	579.5	15.0a	2845.7b	25.3a	22.8a	97.6a	145.7a	1 : 4.9
Bare branch	242.2b	1589.1b	431.2b	795.8	256.1b	202.6b	1490.6b	1949.3b	1 : 1.7
Fruiting branch	142.6b	1313.7b	451.0b	527.8	121.4b	148.2b	538.2	807.8	1 : 1.2
Bare inside	37.8a	231.6a	39.2a	1208.5b	24.1a	24.1a	83.3a	131.5a	1 : 5.1
Fruiting outside	13.0a	79.0a	3.9a	476.0	9.3a	7.5a	28.4a	45.3a	1 : 5.7
LSD05	38.67	250.75	53.13	457.86	20.55	37.35	181.44	178.60	

Means ± SE, n = 3 trees, 25 buds from 1 tree. Means followed by letters are significantly different ($P \leq 0.05$) from control (even distribution).

Significantly higher amounts of fructose, glucose and sucrose, except of sucrose in bare branch and inside, were detected in all treatments compared to even distribution of inflorescences (Table 2). Higher contents of the total carbohydrates, mainly due to higher contents of glucose, were in the buds, where inflorescences were not removed in spring. Higher contents of fructose and sucrose were found in the bare side comparing to the fruiting side. However, an increase of fructose, glucose and sucrose in the fruiting branch and fruiting outside, in comparison to bare branch and bare inside respectively was found. The ratio of hexose to sucrose was significantly higher in different thinning treatments as compared with even thinning (control), nevertheless, there were no common tendencies between treatments. It seems that hexoses, especially glucose, play an important role in the long-distance signal transduction pathway of flowering induction in apple trees. This is in agreement with Koch (2004), as the author stated that hexoses tend to have greater signaling potential in flowering induction, while sucrose promotes cell differentiation. Moreover, relative ratios of hexoses to sucrose are regulated by

invertase, which cleaves sucrose into glucose and fructose, stimulates changes in shoot apical meristems and results in flowering (Heyer et al. 2004). Thus, sucrose can act as a flowering signal directly or a signal can arise via glucose or UDP-glucose and fructose (Li et al. 2011).

Table 2. *Effect of Thinning on Distribution of Non-Structural Carbohydrates (mg g^{-1} FM) in Axillary Buds*

Treatment	Fructose	Glucose	Sucrose	Hexose to Sucrose ratio	Hexose plus Sucrose
			September		
Even distribution	12.6	47.3	2.90	20.6	62.8
Bare side	24.8b	110.2b	4.77b	28.3b	139.8b
Fruiting side	22.2b	111.8b	3.78b	36.7b	141.2b
Bare branch	27.1b	92.0b	2.61	45.6b	121.7b
Fruiting branch	27.7b	118.3b	5.27b	27.8b	151.3b
Bare inside	22.2b	63.3b	2.94	29.1b	88.4b
Fruiting outside	25.7b	108.8b	4.57b	29.5b	139.4b
LSD05	1.17	3.65	0.47	3.79	4.80

Means ± SE, n = 3 trees, 25 buds from 1 tree. Means followed by letters are significantly different ($P \leq 0.05$) from control (even distribution).

As the flowering signaling pathway is a complex and multicomponent phenomenon, so its regulation depends not only from one signaling molecule. Critical aspects of plant growth and development are regulated by hormone-based signaling pathways cross-talk with sugars (Eveland and Jackson 2011). Whereas Krishnamurthy et al. (2013) found a slight decreasing trend of both carbohydrates and IAA, while zeatin ribose increased, and stated that this could be due to rapid utilization by the developing fruits in the on year compared to off year. A strong correlation between ABA to sucrose and IAA to glucose was found in our treatment. A strong negative correlation between ABA to sucrose and IAA to glucose shows increased contents of floral inhibitors that lead to the decrease of sugar signalling molecules in the treatments where flowers were not removed (Table 3). Such data suggests that localized flowering inhibition depends on the critical ratio of inhibitor and promoter signalling molecules that are exported to buds. Sugars and ABA tend to act synergistically during rapid cell division, for example ABA and sucrose act synergistically (Eveland and Jackson 2011). According to Mishra et al. (2009), increasing concentrations of glucose induced genes for auxin biosynthesis and transport had a different effect on individual auxin receptors, thus glucose and auxin may act either antagonistically or synergistically. There is evidence, that auxin regulates GA biosynthesis during various plant development stages. Besides, IAA and GA may act together or independently to inhibit flowering in perennial fruit trees (Bangerth 2006). Positive strong correlation between IAA to GAs (Table 3) shows that an increase in the IAA content leads to an increase of gibberellins.

Table 3. *Phytohormones and Non-Structural Carbohydrates Correlation (r) Depending on Flower Removal*

Treatment	ABA to Sucrose	IAA to Glucose	IAA to total GAs
	September		
Control	0.78	0.98	0.99
Bare side	1.00	-0.84	1.0
Fruiting side	-0.87	0.87	1.0
Bare branch	0.90	0.99	1.0
Fruiting branch	0.87	1.00	1.0
Bare inside	0.96	0.95	1.0
Fruiting outside	-0.83	-0.82	1.0

Fruits are the major sink organs influencing transport of carbohydrates from leaves to fruits. These effects occur during fruit development and become more intensive during the fruit maturation stage. Thus, the thinning of inflorescences influences the partitioning of carbohydrates between plant organs and affects leaf characteristics and physiology (Nii 1997). Significantly bigger amounts of chlorophyll a and a significant decrease of chlorophyll b and the leaf area was found in treatments where inflorescences where thinned or left in different sides of the tree in comparison to even thinning. A decrease of chlorophyll b and the leaf area was found in fruiting branch and fruiting outside treatments. Generally, lower amounts of chlorophylls a and b and smaller leaf area were found where inflorescences were not thinned in spring. A significantly higher ratio of chlorophyll a to b was detected in bare or fruiting side or bare or fruiting branch treatments in comparison to even thinning. There were no significant changes in the dry mass per unit leaf area and carotenoid contents, except of carotenoid decrease where inflorescences were left on separate branches (Table 4). Nii (1997) also showed that the leaf area and dry mass per unit leaf area decreased with increasing number of fruits, at the fruit-maturation stage (middle of July). Even thinning, thinning inflorescences from separate branch or from inside of the tree lead to low sugar accumulation, especially of glucose and sucrose (Table 2), and an increase in the leaf area was noticed (Table 4). This is in agreement with Teo et al. (2006), as leaf area increased with a decrease of sucrose in different apple tree lines.

Table 4. *The Effect on Thinning on Photosynthetic Pigments Content and Some Leaf Characteristics*

Treatment	Chl a	Chl b	Chl a / b	Car	Leaf area	SLW
			July			
Even distribution	0.68	0.40	1.67	0.35	45.85	0.045
Bare side	0.85b	0.35a	2.44b	0.33	41.31a	0.049
Fruiting side	0.84b	0.34a	2.51b	0.34	38.09a	0.048
Bare branch	0.89b	0.37	2.42b	0.37	44.47	0.051
Fruiting branch	0.70	0.34a	2.10b	0.30a	41.16a	0.046
Bare inside	0.73	0.43	1.71	0.35	44.90	0.042
Fruiting outside	0.63	0.35a	1.81	0.33	42.90	0.048
LSD05	0.058	0.047	0.298	0.047	3.798	0.007

Means ± SE, n = 3 trees, 15 leaves from 1 tree. Means followed by letters are significantly different ($P \leq 0.05$) from control (even distribution). SLW – dry mass per unit leaf area; Chl – chlorophyll; Car – carotenoids.

The thinning of inflorescences from one side of the tree or from a separate branch resulted in a significant decrease of the total yield and yield per tree. The thinning of inflorescences from the inside of the tree conditioned the biggest fruit weight (Table 5). It could be explained that all fruits were exhibited to sun and there were no shadowed fruits inside the tree, what usually results to lower quality and smaller fruits. Pellerin et al. (2012) demonstrated that the bigger percentage of inflorescence thinning resulted in a higher ratio of return bloom. The highest ratio of promoter (zeatin and JA) to inhibitor (ABA, IAA and GAs) hormones was in buds after even thinning 1:2.4 in autumn, but it almost did not changed in spring (1:2.1) (Table 1), this resulted in lowest return bloom rate (Table 5). The significantly highest rate of the return bloom (Table 5) and an increase of promoter to inhibitory hormones ratio in spring (Table 1) was in treatments where inflorescences were thinned from one side of the tree or from inside of the tree. Pellerin et al. (2012) proposed a model where the critical inhibitor to promote hormone ratios of fruiting shoot apical meristems is 1:1 up to 1:16 and the amount of required thinning was 0% and 78% respectively. Thus, the bigger contents of promoter hormones result in a greater thinning rate. On the other hand, floral bud inhibition may also occur when carbohydrate reserves are scarce, which is typical after a high yield fruit production (Turktas et al. 2013).

Table 5. *Effect of Thinning on the Fruit Set and Quality*

Treatment	Yield, t per ha	Yield, kg per tree	Fruit weight, g	Return bloom, No. flower clusters cm^{-2} TCSA
		October		May
Even distribution	58.2	25.5	0.244	4.9
Fruiting side	54.0	23.6	0.259	6.4b
Fruiting branch	49.1a	21.5a	0.255	5.5
Fruiting outside	57.3	25.1	0.265b	5.8b

Means ± SE, n = 5. Means followed by letters are significantly different ($P \leq 0.05$) from control (even distribution).

Conclusion

JA is associated with flower induction and gibberellins (GA_1, GA_3, GA_7) - with flower inhibition in apple tree, as higher amounts of JA and lower GAs levels were found in buds of these treatments where inflorescences were removed. Strong negative correlation between ABA to sucrose and IAA to glucose shows increased contents of floral inhibitors that lead to a decrease of sugar signalling molecules in treatments where flowers were not removed. Thus, localized flowering inhibition depends on the critical ratio of the inhibitor and promoter signalling molecules that are exported to buds. Even thinning resulted the biggest yield, but fruits were the smallest. Moreover, thinning of inflorescences from one tree side resulted in a better return bloom the next spring, especially due to intensive bloom of the bared side.

References

Bangerth F (2006) Flower induction in perennial fruit trees: still an enigma? Acta Hort 727:177-195. doi: http://bit.ly/1Zma6XH.

Dennis FG (2000) The history of fruit thinning. Plant Growth Regul 31:1-16. doi: http://bit.ly/201NqOR.

Eveland AL, Jackson DP (2011) Sugars, signalling, and plant development. JExp Bot 63:3367-3377. doi: http://1.usa.gov/1P2l84Q.

Gavrilenko VF, Zigalova TV (2003) Big practice of growing physiology. Moscow. [In Russian].

Guitton B, Kelner J-J, Velasco R, Gardiner AE, Chagné D, Costes E (2012) Genetic control of biennial bearing in apple. J Exp Bot 63(1): 131-149. doi: http://1.usa.gov/1PcNWBy.

Heyer AG, Raap M, Schroeer B, Marty B, Willmitzer L (2004) Cell wall invertase expression at the apical meristem alters floral, architectural, and reproductive traits in Arabidopsis thaliana. Plant J 39:161-169. doi: http://1.usa.gov/201NtdA.

ICH (2005) Harmonised Tripartite Guideline. Validation of Analytical Procedures: Text and Methodology Q2(R1). http://www.ich.org/LOB/media/MEDIA417.pdf

Kittikorn M, Shiraishi N, Okawa K, Ohara H, Yokoyama M, Ifuku O, Yoshida S, Kondo S (2010) Effect of fruit load on 9,10-ketol-oktadecadienoic acid (KODA), GA and jasmonic acid concentrations in apple buds. Sci Hortic 124:225-230. doi: http://1.usa.gov/1SPG5Qg.

Koch K (2004) Sucrose metabolism: regulatory machanisms and pivotal roles in sugar sensing and plant development. Curr Opin Plant Biol 7:235-246. doi: http://bit.ly/1PZGjBJ.

Krishnamurthy KS, Ankegowda SJ, Srinivasan V, Hamza S (2013) Influence of carbohydrates, mineral nutrients and plant hormones in alternate bearing of black pepper (*Piper nigrum* L.). Am J Plant Sci4:1960-1967. http://bit.ly/1Q7rorg.

Kviklys D, Kviklienė N, Bielicki P, Bite A, Lepsis J, Univer T, Univer N, Uselis N, Lanauskas J (2013) Baltic fruit rootstock studies: evaluation of apple (*Malus domestica* Borkh.) new rootstocks. Zemdirbyste 100(4): 441-446. doi: http://bit.ly/1RGanWO.

Li P, Wind JJ, Shi X, Zhang H, Hanson J, Smeekens SC, Teng S (2011) Fructose sensitivity is suppressed in Arabidopsis by the transcription factor ANAC089 lacking the membrane-bound domain. Proc Natl AcadSci USA 108:3436-3441. doi: http://bit.ly/1ZwGtbF.

Mishra B, Singh M, Aggrawal P, Laxmi A (2009) Glucose and auxin signalling interaction in controlling Arabidopsis thaliana seedlings root growth and development. PLoS One 4502. doi: http://bit.ly/1OQLHIn.

Moghaddam MRB, Van den Ende W (2013) Sugars, the clock and transition to flowering. Plant Sci 4:1-6. doi: 10.3389/fpls.2013.00022.

Mutasa-Göttgens E, Hedden P (2009) Gibberellin as a factor in floral regulatory networks. J Exp Bot 60:1979-1989. doi: http://bit.ly/1OjO7xy.

Nichols D, Embree CG, Fillmore SAE (2011) Dormant spur-wood pruning severity impacts on vegetative growth, blossom intensity and fruit weight of 'Honeycrisp' apple trees. Acta Hortic 903:681-687. doi: http://bit.ly/23158UW.

Nii N (1997) Changes of starch and sorbitol in leaves before and after removal of fruits from peach trees. Ann Bot 79:139-144. doi: http://bit.ly/1n1nxRf.

Pellerin BP, Buszard D, Georgallas A, Nowakowski RJ (2012) A novel framework to consider endogenous hormonal control of apple tree flowering. HortSci 47(5):589-592. doi: http://bit.ly/1N91JI5.

Ramírez H, Benavides A, Robledo V, Alonso R, Gómez J (2004) Gibberellins and cytokinins related to fruit bud initiation an apple. Acta Hortic 636:409-413. doi: http://www.actahort.org/books/636/636_50.htm.

Smith AM, Zeeman SC, Smith SM (2005) Starch degradation. Ann Rev Plant Biol 56:73-98. doi: http://bit.ly/1JM5mcf.

Teo G, Suzuki Y, Uratsu SL, Lampinen B, Ormonde N, Hu WK, DeJong TM, Dandekar AM (2006) Silencing leaf sorbitol synthesis alters long-distance partitioning and apple fruit quality. http://bit.ly/1OQLPaV. [14.05.2015].

Turktas M, Inal B, Okay S, Erkilic EG, Dundar E, Hernandez P, Dorado G, Unver T (2013) Nutrition metabolism plays an important role in the alternate bearing of the olive tree (*Olea europaea* L.). Plos One 8(3):1-15. doi: 10.1371/journal.pone.0059876.

Vemmos SN (1994) Net photosynthesis, stomatal conductance, chlorophyll content and specific leaf weight of pistachio trees (cv. Aegenes) as influenced by fruiting. J Hortic Sci 69:775-782. Doi: 10.1080/14620316.1994.11516512.

Vitti A, Nuzzaci M, Scopa A, Tataranni G, Remans T, Vangronsveld J, Sofo A (2013) Auxin and cytokinin metabolism and root morphological modifications in Arabidopsis thaliana seedlings infected with cucumber mosaic virus (CMV) or exposed to cadmium. Int J Mol Sci 14:6889-6902. doi: file:///C:/Users/Vartotojas/Downloads/ijms-14-06889.pdf.

Wingler A, Delatte TL, O'Hara LE, Primavesi LF, Jhurreea D, Paul MJ, Schluepmann H (2012) Trehalose 6-phosphate is required for the onset of leaf senescence

associated with high carbon availability. Plant Physiol 158:1241-1251. doi: http://1.usa.gov/1KeWAye.

Wünsche JN, Greer DH, Laing WA, Palmer JW (2005) Physiological and biochemical leaf and tree responses to crop level in apple. Three Physiol 25:1253-1263. doi: http://bit.ly/1KeWBlI.

Zhou R, Quebedeaux B (2003) Changes in photosynthesis and carbohydrate metabolism in mature apple leaves in response to whole plant source-sink manipulation. J Am Soc Hortic Sci 128(1):113-119. doi: http://bit.ly/1J4F6tz.