



Article

Metabolic Response of *Malus domestica* Borkh cv. Rubin Apple to Canopy Training Treatments in Intensive Orchards

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Abstract: In this study, we used apple tree (*Malus domestica* Borkh.) cv. Rubin grafts on dwarfing P60 rootstock. Our planting scheme was single rows with 1.25 m between trees and 3.5 m between rows. The aim of this study was to determine the impact of canopy training treatments, as a stress factor, on metabolic response to obtain key information on how to improve physiological behavior and the management of the growth and development of apple trees. The results indicated that all applied canopy training treatments significantly increased the total phenol and total starch contents in apple tree leaves. The total starch increased from 1.5- to almost 3-fold in all treatments, especially during the 2017 harvesting season, compared to the control. The fructose, sorbitol, and ratio of chlorophyll *a* to *b* in leaves also significantly increased. Higher precipitation levels induced changes in the accumulation of secondary metabolites in apple tree leaves and fruits during the 2017 harvesting season. The total phenol content significantly increased in apple tree leaves in all treatments, but the fructose content decreased. We observed the same tendencies in total phenolic content and glucose concentration in apple fruits. Therefore, the defense reaction might be a preferred option for apple tree cultivation and the optimization of its growth and development.

Keywords: apple tree; carbohydrates; photosynthetic pigments; secondary metabolites; stress response



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1. Introduction

Plant species respond to biotic and abiotic stresses through molecular changes in their tissues; biochemical, physiological, and morphological modifications; or/and adaptations [1–3]. Perennial plants have developed three major strategies to adapt to stress conditions: tolerance, avoidance, and escape [4]. Stress-caused growth strategies are produced through physiological changes, which enable the management of fruit tree growth and yield. In contrast to the widely analyzed drought stress, which has been examined for relatively short durations, disturbing the nutrient supply using technological tools can successfully produce incipient physiological and biochemical long-term responses, suppress growth, and induce developmental processes [5,6]. Over long periods, trees can develop stress avoidance modifications, but what physiological and biochemical responses change during this process remains unclear. Plant defense mechanisms are related to a group of interconnected processes; therefore, plant resistance and/or susceptibility cannot be explained by a single mechanism.

The biochemical compounds involved in plant defense mechanisms indirectly affect plant growth and development through plant metabolism [7,8]. Plants use phenolic compounds for different functions such as resistance to pathogens, pigmentation, growth, and reproduction, among many others [9–11]. Phenolics are also responsible for antioxidant capacity in fruits and vegetables [12]. A number of factors such as climate conditions, soil composition, and canopy training treatments; harvest methods; fruit maturity; and storage

conditions have a substantial impact on the chemical composition and quality of agricultural products [13]. According to Xu et al. [14], the inverse correlation between phenolic concentrations and air temperature can produce differences in the phenolic concentration in plants during different growing seasons, which most likely depends on air temperature and precipitation rate. Cold climatic conditions during plant growth increase the antioxidant properties in plants, which is related to the strategies to counter oxidative stress. The dependence between the location of origin, harvest year, and content of antioxidants in fruits and vegetables might be related to variability in light levels and ambient temperature [15].

During apple maturity, the amount of phenolic compound changes, and a new compound is formed. Phenolic compounds in apple are very stable [16,17]; however, the composition of phenolic compounds in different plant tissues is strongly influenced by environmental conditions, such as UV light, temperature, and nutrition [18]. Some phenolics are difficult to identify because they quickly change to the insoluble pool of phenolics. As such, the total content of the phenolic profile can be used instead [19,20]. A few hydroxycinnamic acids, flavanols (epicatechin, procyanidin B1, and catechin), and dihydrochalcones may be involved in the defense mechanism in apple leaves [11,21]. Carbohydrates are the major source of energy driving prebiotic metabolism; however, they may be diverted from their main metabolic path to plant defense mechanisms, producing phenolic compounds in their leaves [22]. Thus, metabolic changes that occur under stress conditions affect the bioavailability of essential nutrients. Benyas et al. [23] noted that variations in chlorophyll synthesis strongly depend on a plant's reaction to stressors. Plant chlorophylls, as the major pigments in photosynthesis, are related to growth potential and productivity [24]. Moreover, the main role of photosynthetic pigments such as chlorophylls and carotenoids is to prevent stress-induced oxidative damage to genetic material and lipid peroxidation [25,26].

The domestic apple (*Malus domestica* Borkh.) can grow in a wide range of ecological sites worldwide. In addition, apple is one of the most consumed fruits. Its popularity is determined by its long shelf life and its nutritional properties. Apples occupy an important place in the human diet due to the favorable ratio of sugars, acids, and phenolic compounds, which strengthen the body and protect against diseases [27,28]. Although since 2015, the total area of harvested apples has started to decrease, the apple yield has remained at 83–86 million tons per year [29]. In this study, we highlight the metabolic response of apple tree and fruit to canopy training treatments during the vegetation season, as stress factors. We provide key information on how to improve physiological behavior and the management of the growth and development of apple trees. Metabolic reaction might be a preferred option for apple tree cultivation and the optimization of growth, development, and resistance, providing new strategies that can be applied to apple orchards and introduced to commercial gardens in Lithuania as well as in regions with a similar climate in northeastern Europe.

The main objectives of this study were to evaluate the most appropriate canopy training treatments to optimize the physiological processes of apples trees and the quality of their fruits.

2. Materials and Methods

We conducted a field experiment at the Lithuanian Research Center for Agriculture and Forestry (LAMMC), Institute of Horticulture, in an intensive orchard (Babtai, Lithuania, 55°60' N, 23°48' E). We grafted apple tree (*Malus domestica* Borkh.) cv. Rubin on dwarfing P60 rootstocks. Apple P60 rootstock is the low vigorous rootstock (growth of about 21–40% compared to seedling rootstock) used in Poland. Its properties are suitable for cultivation in Lithuanian climatic conditions. We planted apple trees in 2010 in single rows spaced 1.25 m apart with 3.5 m between rows. Pest and disease management was carried out according to integrated plant protection practices, and the orchard was not irrigated. The soil conditions of the experimental orchard were as follows: clay loam, pH 7.3, humus 2.8%, P₂O₅ 255 mg kg⁻¹, and K₂O 230 mg kg⁻¹. We fully randomized three single trees per replicate of a treatment. We used six canopy training treatments: 1. hand pruning each year forming

a slender spindle (control); 2. mechanical pruning (each year); 3. trunk incision using chain saw + mechanical pruning (each year); 4. mechanical pruning (each year) + spraying with prohexadione-calcium; 5. mechanical pruning + hand pruning + summer pruning (performed in the middle of August, removing the most vigorous and vertical shoots); 6. mechanically pruning one side, changing sides annually. We applied canopy training treatments every year from 2016 to the same orchard area.

2.1. Meteorological Conditions

We collected the meteorological data from iMetos meteorological station in an intensive orchard. The air temperature and precipitation in the last three years were widely variable, as presented in Figure 1A,B. Air temperature during every vegetation period was close to the multiannual average, except at harvest time in 2019. The precipitation during the vegetation period (May to September) was much higher than the multiannual average (average of 100 years), especially during harvest time in 2017 and 2019. During the bloom period, the precipitation was low in all three years.

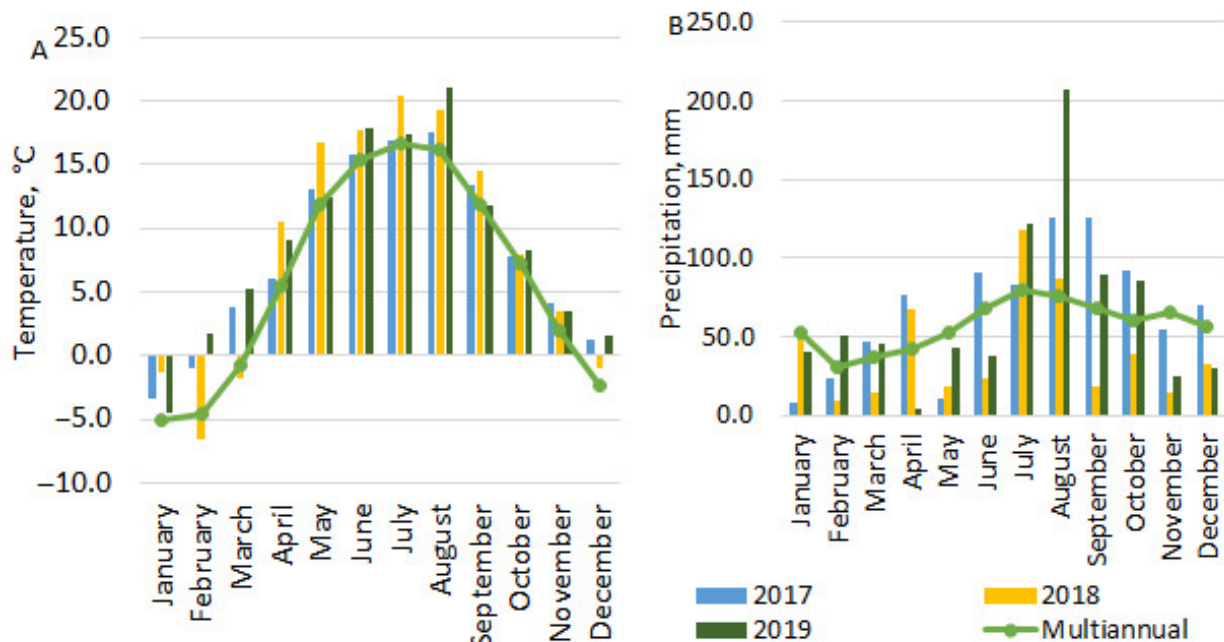


Figure 1. Meteorological conditions ((A), temperature, °C; (B), precipitation, mm) during three vegetation periods (2017, 2018, and 2019).

2.2. Sample Preparation

We collected leaf samples to determine the physiological response and fruit samples to assess the internal fruit quality in the different canopy training treatments. We randomly selected 20 intact, fully developed leaves from the middle part of the branch, from the whole canopy from 3 trees in each replicate for biochemical analyses. We removed the central vein and stalk, chopped the remaining parts of the apple leaf, and immediately froze the samples in liquid nitrogen after weighing for future biochemical analysis.

For fruit analysis, we collected samples from the whole canopy using full randomization. We took five apples from three trees in each replicate at harvest time at commercial ripening (BBCH 87–88). We divided each apple into four parts, removed the seed box, and took a sample from each part of the pulp. We weighed the composite sample of five apples for further analysis. The remaining apples were crushed and dried to air-dry weight to determine the elemental composition.

2.3. Biochemical Analyses

2.3.1. Assessment of Photosynthetic Pigments Using High-Performance Liquid Chromatography (HPLC)

Chlorophylls and carotenes were evaluated using HPLC with diode array detection (DAD) on a Shimadzu 10A (Japan). The leaf sample (0.5 g) was ground and mixed with 80% glacial acetone. The prepared extract was left at 4 °C for 24 h and centrifuged at 10,000 rpm for 15 min. Then, the solution was filtered using a 0.22 µm PTPE syringe filter (VWR International, Delaware County, PA, USA). The sample was separated on a Chromagabond C30 3 µm 120 Å, 15 cm × 2.1 mm column (ES Industries). A 10 µm sample was injected; the column oven temperature was set to 20 °C. The pigments were eluted for 30 min with gradient solvent system A (methanol:water, 4:1) and B (ethyl acetate) at a flow rate of 0.2 mL min⁻¹. The initial conditions were 20% B for 2.5 min, followed by a linear gradient to 30% B at 5 min, holding at 30% B for 5 min, then increasing until 80% B in 2.5 min, 87% B in 7.5 min, and 100% in 5 min, and again to 20% B until the end of the run. We used the calibration method to quantify chlorophylls and carotenes (mg g⁻¹ FW).

2.3.2. Assessment of Soluble Sugars Using Ultra-Performance Liquid Chromatography (UPLC)

The soluble sugar (fructose, glucose, and sorbitol) content was evaluated using HPLC with evaporative scattering detection (ELSD) [10]. A leaf sample (0.5 g) or a fruit sample (1 g) was ground and mixed with deionized water. The prepared extract was left at room temperature for 4 h and centrifuged at 14,000 rpm for 15 min. Based on Brons and Olieman [30], we performed a cleanup step prior to the chromatographic analysis: we mixed 1 mL of the supernatant with 1 mL of 0.01% (*w/v*) ammonium acetate in acetonitrile, which we incubated for 30 min at 4 °C. After incubation, we centrifuged samples at 14,000 rpm for 15 min and filtered the sample through a 0.22 µm PTPE syringe filter (VWR International, Radnor, PA, USA). Analysis was performed on a Shimadzu Nexera UPLC (Kyoto, Japan) system. Separation was performed on a Supelcosil 250 × 4 mm NH₂ column (Supelco, Delaware County, PA, USA) using 77% acetonitrile as the mobile phase at a 1 mL min⁻¹ flow rate. We used the calibration method for sugar quantification (mg g⁻¹ in FW).

2.3.3. Bioassay of Total Starch by Colorimetric Method

The total starch content was determined using 0.5 g of plant sample (leaves and fruit) using a total starch Megazyme assay kit, which is based on the use of thermostable α-amylase and amyloglucosidase (Megazyme International Ireland Limited, Wicklow, Ireland) [31] for the determination of starch in samples, which also contains D-glucose and/or maltodextrins.

2.3.4. Bioassay of Total Phenolic Compounds Using Colorimetric Method

The total phenolic content was determined using 1 g of sample (leaves or fruit) homogenized with liquid nitrogen and diluted with 10 mL of 80% MeOH. The mixed extracts were left for 24 h in the fridge (4 °C) and subsequently centrifuged at 5000 rpm for 5 min. We mixed 0.1 mL of extract with 0.2 mL of 10% Folin–Ciocalteu reagent (Folin reagent diluted with bidistilled water 1:10) and with 0.8 mL of 7.5% (*w/v*) sodium carbonate. After 20 min, the absorbance was measured at 765 nm using a Genesys 6 spectrophotometer (Thermospectronic, Waltham, MA, USA) against distilled water as the blank. Gallic acid was used as the standard; the total phenolics were evaluated using a calibration curve.

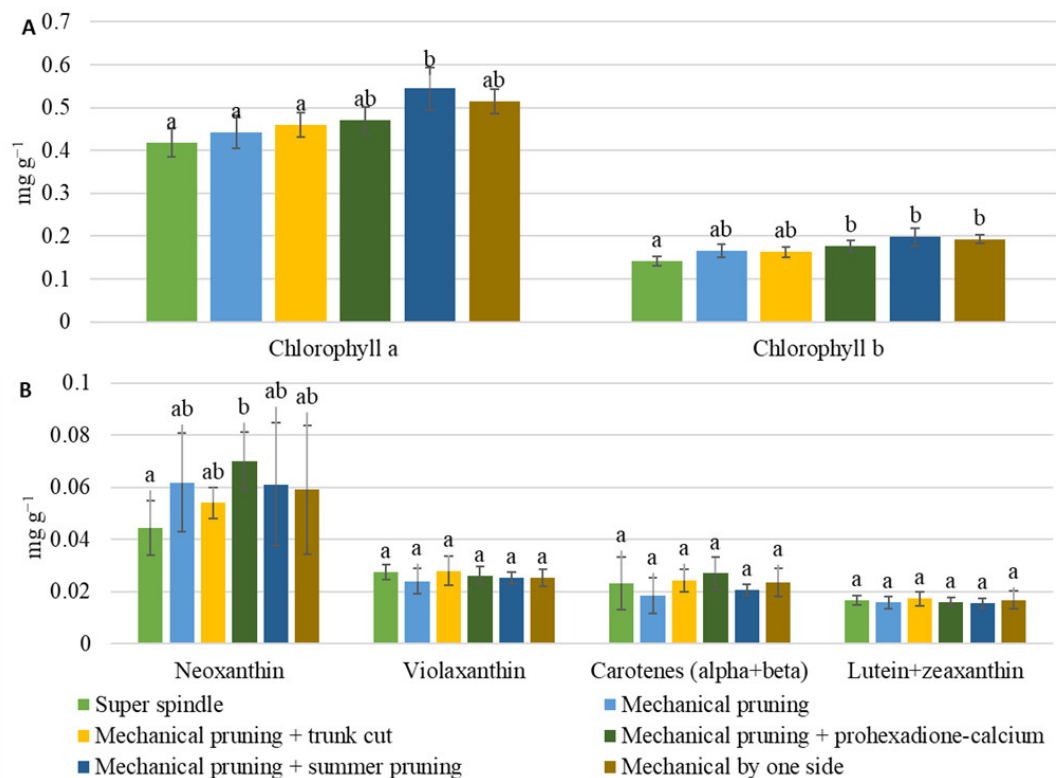
2.4. Statistical Analysis

MS Excel Version 2010 and XLStat 2017 Data Analysis and Statistical Solution for Microsoft Excel (Addinsoft, Paris, France) statistical software were used for data processing. The data are presented as the mean of three years ± standard deviation (*n* = 3 replications). One- and two-way analysis of variance (ANOVA) was carried out along with Tukey's

multiple comparisons test for statistical analyses, $p \leq 0.05$. Differences were considered to be significant at $p < 0.05$.

3. Results

Canopy training treatments had a significant effect on chlorophyll a and b, neoxanthin, and lutein + zeaxanthin content in cv. Rubin leaves at harvest time. Summer pruning, as additional pruning, activated chlorophyll a activity, the content of which significantly increased at harvest time in leaves up to 24% (from 0.417 to 0.544 mg g⁻¹) compared to trees subjected to hand pruning (Figure 2A). Mechanical pruning together with prohexadione-calcium, summer pruning, and pruning on one side significantly increased chlorophyll b concentration by 16–20% compared to hand pruning. Significant differences were produced by different canopy training treatments through an accumulation of neoxanthin and lutein + zeaxanthin. The interaction between years and canopy training treatments was only significant for neoxanthin and violaxanthin (Figure 2B). According to our results, neoxanthin concentration increased up to 37% (from 0.044 to 0.070 mg g⁻¹) through spraying with prohexadione-calcium compared to hand pruning.



F actual						
	Chlorophyll a	Chlorophyll b	Neoxanthin	Violaxanthin	Carotenes (alpha+beta)	Lutein+zeaxanthin
Factor A (canopy training treatment)	*	*	*	ns	ns	*
Factor B (year)	*	*	*	*	ns	*
Interaction AB	ns	ns	*	*	ns	ns

Figure 2. The effect of canopy training treatments on chlorophylls (A) and carotenoids (B) in apple cv. Rubin leaves at BBCH 87–88. The mean value of three years ($n = 3 \times 3 = 9$) $\pm$ standard deviation is presented. The data were processed using two-way analysis of variance (ANOVA), Tukey's (HSD) test at $p = 0.05$. Different letters in blocks indicate significant differences in treatment, LSD, Fisher's protected least: * $p < 0.05$; ns—not significant.

The total amount of phenolic compounds of apple leaves varied from 73.46 mg g⁻¹ to 101.07 mg g⁻¹ and from 3.56 mg g⁻¹ to 4.95 mg g⁻¹ in fresh weight in the effect of different canopy training treatments. The factorial analyses showed significant dependence of the accumulation of total phenols in fruits on years and different canopy training treatments. Interaction between combined factors (years and canopy training treatments) also significantly changed the concentration of total phenols in apple fruits. The total amount of phenols in apple fruits were almost 15-fold lower compared with the amount of total phenolic compounds in leaves. The higher positive effect of almost all treatments on the total amount of phenolic compounds in apple fruits were determined during harvest time (Figure 3). Mechanical pruning significantly increased the total phenols up to 13% (from 69.87 to 73.37 mg g⁻¹) in leaves and up to 28% (from 3.57 to 4.95 mg g⁻¹) in fruits. The factorial analyses showed significant dependence of the accumulation of total phenols in leaves on different canopy training treatments. The higher significant positive effect on the total amount of phenolic compounds in leaves was determined after mechanical pruning with additional summer pruning. It increased from 69.87 to 77.22 mg g⁻¹.

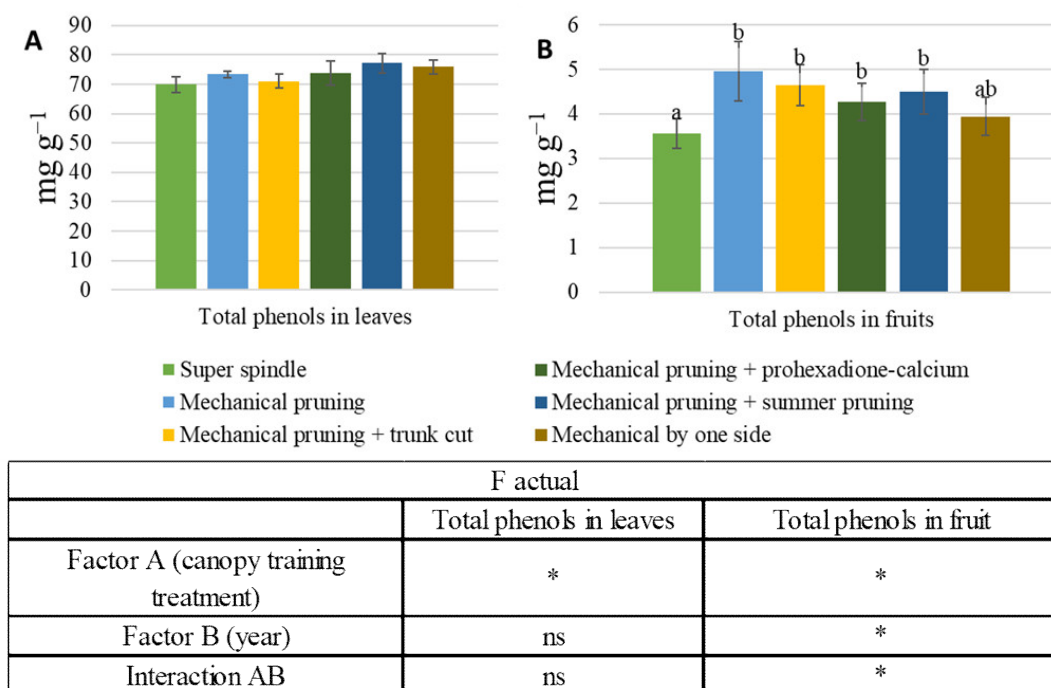


Figure 3. The effect of canopy training treatments on total phenols in cv. Rubin leaves (A) and fruits (B) at BBCH 87–88. The mean value of three years ($n = 3 \times 3 = 9$) $\pm$ standard deviation is presented. The data were processed using a two-way analysis of variance (ANOVA), the Tukey (HSD) test at the confidence level $p = 0.05$. The different letters in blocks indicate significant differences in treatment, LSD–Fisher’s protected least: * $p < 0.05$; shows significant differences, ns—no significant differences.

According to analysis of variances, canopy training treatments had a significant effect on sucrose in fruit and fructose and total starch in both leaves and fruit (Figure 4). The content of carbohydrates also depended on years; meanwhile, regarding the fruit, year only had a significant influence on glucose content. Mechanical pruning with additional treatments increased the content of fructose in leaves up to 16–23% compared to the control—a super spindle. Meanwhile, mechanical pruning without additional treatments increased the content of total starch in leaves by almost two times (Figure 4A). Meanwhile, mechanical pruning without additional treatments significantly decreased glucose content in fruits by up to 42% (from 22.68 to 13.3 mg g⁻¹) compared with pruning with a super spindle. However, mechanical pruning with additional treatments significantly decreased

total starch content in fruits from 28 to 68% compared to mechanical pruning without additional treatments and pruning with a super spindle.

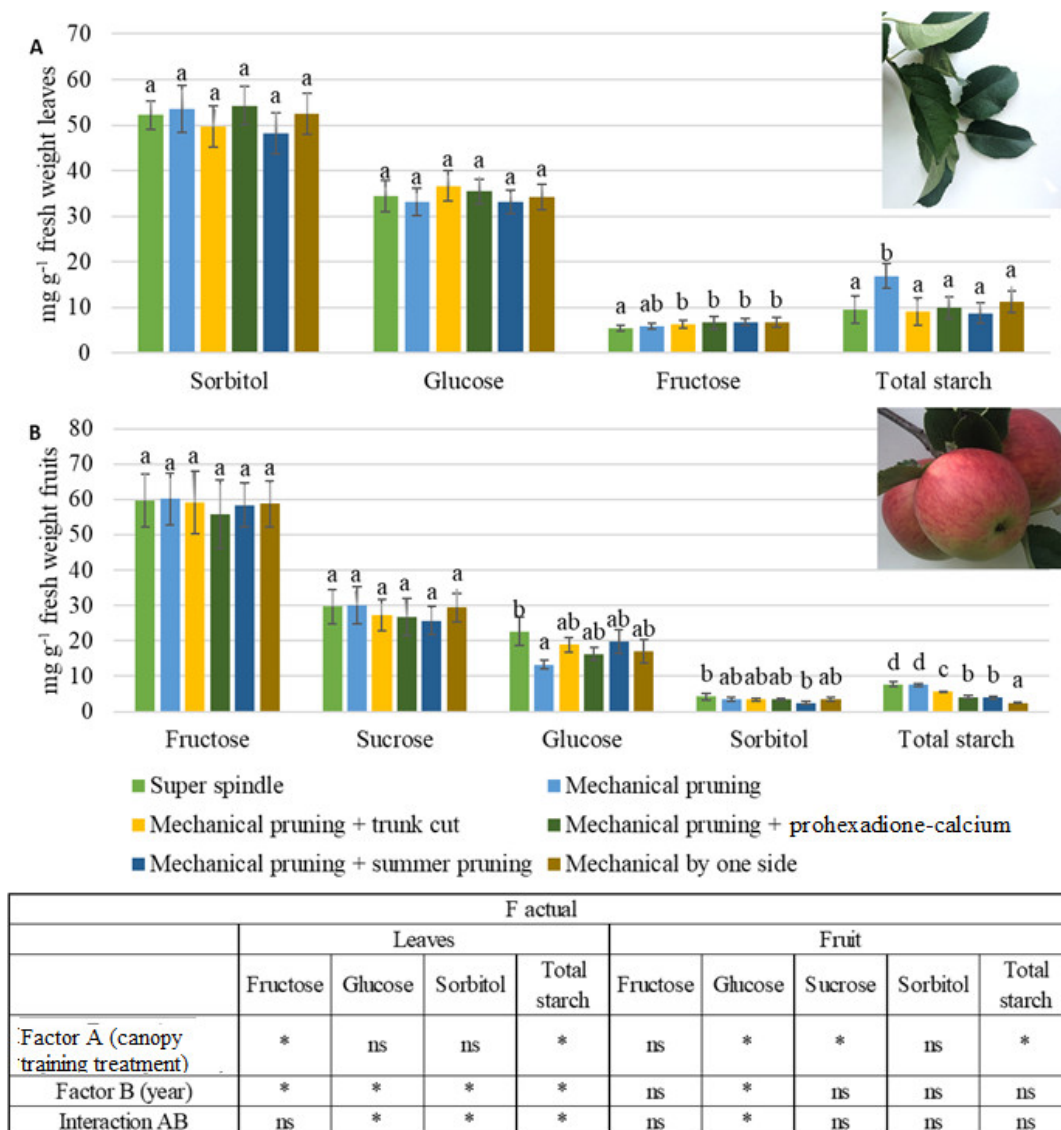


Figure 4. The effect of canopy training treatments on carbohydrates in cv. Rubin leaves (A) and fruits (B) at BBCH 87–88. The mean value of three years ($n = 3 \times 3 = 9$) $\pm$ standard deviation is presented. The data were processed using a two-way analysis of variance (ANOVA), the Tukey (HSD) test at the confidence level $p = 0.05$. The different letters in blocks indicate significant differences in treatment, LSD–Fisher’s protected least: * $p < 0.05$ shows significant differences, ns—no significant differences.

All treatments have been in use since 2016, and since then, the yield has been recorded (Figure 5). In all years, except 2018, mechanical pruning significantly increased cv. Rubin yields compared to manual pruning, whereas in 2018, manual pruning showed no significant differences from mechanical pruning and The contents of this figure are not legible. In order to convert a clear PDF document, whilst retaining its high quality, we kindly request the provision of figures and schemes at a sufficiently high resolution (min. 1000 pixels width/height, or a resolution of 300 dpi or higher). mechanical pruning with trunk cut. Mechanical pruning increased yield by up to 40–150% compared to manual pruning; depending on the year, it was about 12 tons per hectare per year.

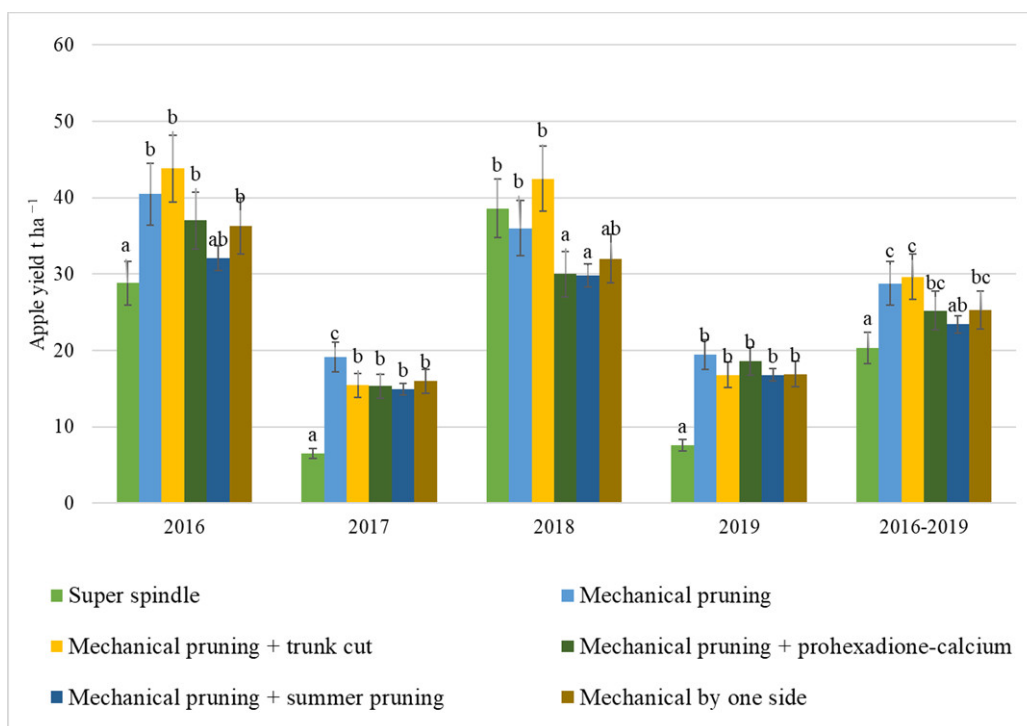


Figure 5. The effect of canopy training treatments on cv. Rubin yield 2016–2019. The mean value ($n = 3$) $\pm$ standard deviation is presented. The data were processed using a two-way analysis of variance (ANOVA), the Tukey (HSD) test at the confidence level $p = 0.05$. The different letters in year indicate significant difference in treatment.

4. Discussion

The physiological processes and metabolism of the fruit tree are highly dependent on chlorophyll and carotenoids, which are receptors for light energy. The chlorophyll content is primarily associated with plant vitality [32], and secondarily, chlorophylls can also act as antioxidants [33]. Significant effects were determined from different canopy training treatments on the accumulation of photosynthetic pigments, except violaxanthin and lutein + zeaxanthin. Meanwhile, factorial analyses showed significant dependence of the accumulation of all photosynthetic pigments, except lutein + zeaxanthin, on vegetation season (Figure 2). The interaction between vegetation season and canopy training treatments was only significant on neoxanthin and violaxanthin. Some authors indicated that carotenoids are the precursors of two plant phytohormones, abscisic acids (ABA) and strigolactones, which are the main regulators for plant stress response and development. [34,35].

Under abiotic stress conditions, plants activate a number of genes, therefore increasing the levels of several metabolites and proteins. Some of these metabolites may be responsible for protection against these stresses. Secondary metabolites, mostly phenolic compounds, have been identified in apple leaves [36,37]. However, some phenolics are difficult to identify due to their quick change in the content of insoluble phenolics, e.g., proanthocyanidins or hydroxycinnamic acids, which participate in the stiffening of cell walls. For this reason, some authors suggested using the total content of phenolic profile [19,20]. The increase in total phenolic content in leaves can be caused by stress in apples after pruning [38]. Because of the mechanical pruning, all branches of the tree that fall within the cutting edge are removed, causing higher stress, compared to manual pruning, where only certain branches are cut without damaging others. The factorial analyses showed that total phenols were significantly affected by different canopy training treatments. Other authors indicated that the accumulation of a higher amount of total phenols in leaves increases plant susceptibility and resistance to diseases or creates a chemical barrier inhibiting the spread of some pathogens [17,20,39,40]. Our results showed the total amount of phenols

in apple fruits were almost 15-fold lower compared with the amount of total phenolic compounds in leaves (Figure 3). The factorial analyses confirmed significant dependence of the accumulation of total phenols in fruits on years and canopy training treatments.

The natural durability of a trunk is related to carbohydrates, which are responsible for the sustained formation of phenolic components during trunk formation. Ontogenetic and environmental impacts, such as stage of organ maturation, mineral nutrition, toxic gases, climate, pathogenic, and symbiotic interactions can affect the allocation and partitioning of carbohydrates between and within the individual organs in the tree [41]. Crop-load can also affect the biochemical parameters of the apple tree. Studies show that less vigorous rootstock has a greater effect on the crop-load, not only on blooming and yield but also on leaf biochemical parameters [42,43]. Some studies have shown that super-dwarfing rootstocks with intensive crop-load conditioned nitrogen deficiency in leaves. Meanwhile, more vigorous rootstocks are less responsive to crop-load [43]. High crop-load can lead to low nitrogen content in leaves, which also leads to lower sugar content. Cv. Rubin was grafted into a dwarfing P 60 rootstock in our experiment, which according to the other authors is moderately sensitive to crop-load; however, from our results, it is seen that the differences in yield did not affect the sugar content in the leaves (Figure 4). Comparing the yield results, the lowest yields were obtained using hand pruning (super spindle), and it was these apple trees that accumulated the least fructose in the leaves. These results indicate that the yield did not reduce nutrient uptake and biochemical processes in the apple leaves.

Significant effects were determined for different canopy training treatments on leaf fructose and total starch concentrations. Summarizing the results, the amount of total starch increased from 1.5-fold to almost 3-fold in all treatments compared to the control (Figure 4). Interaction between canopy training treatments and years significantly affected the concentration of glucose, sorbitol, and total starch in apple tree leaves (Figure 4). Sorbitol is one of the most important transport carbohydrates in apple trees [44–46]. According to our results, sorbitol accumulates in leaves at harvest time (Figure 4), due to lower sink activities [47]. Kelc et al. [48] reported that the lowest concentration of sorbitol at the end of the plant vegetation season is associated with lower activity of photosynthesis. Some authors [49,50] noted that it could be affected by the removal of fruits. Sorbitol is implicated in drought-stress tolerance as well [46,51]. According to our results, the highest concentration of sorbitol in apple tree leaves was related to higher temperature and lower precipitation during the 2018 vegetation season (Figure 1). Factorial analyses also confirmed the significant dependence of accumulation of carbohydrates in leaves on years (Figure 4). In deciduous plants, such as apple trees, the carbohydrate reserve levels vary during the growth cycle; they are low during the blossom period and the beginning of fructification and high at the end of the growing season [44,52]. During the harvest, sugars are no longer transported to apple fruits; thus, more of them are retained in the leaves and intensively transported in storage locations for the winter [53–56]. In fruits, soluble sugars such as sucrose, fructose, and glucose are relevant for fruit growth, development, and fruit quality. The taste and flavor of the fruit is dependent on the composition and concentration of sugars as well as their balance with the acids [57,58]. Other authors indicated that the concentration and distribution of sugars in parenchyma cells in fruits are affected by developmental processes [59–63] and environmental factors [64]. A significant effect was determined of different canopy training treatments on glucose and sucrose concentration (Figure 4). Sorbitol, as a key compound of carbohydrate metabolism, acts as a signal regulating stamen development and for next-year developmental bud processes, such as pollen tube growth and resistance in apple [65,66]. Meanwhile, factorial analyses showed significant dependence of accumulation only of glucose in apple fruits on vegetation season. The same tendency was observed for the combined impact of the years and canopy training treatments on glucose concentration in apple fruits.

5. Conclusions

In summary, all canopy training treatments resulted in the significant increase in the total phenol and total starch content in both apple tree leaves and fruits. This suggests that both stress and defense response processes not only stimulate metabolic changes in apple trees and fruits but also may result the biosynthesis of bioactive compounds for the pharmaceutical or nutritional value. Summarizing all results, we recommend the application of mechanical pruning, prohexadione-calcium, and trunk cutting for apple cv. Rubin. This not only reduces the need for handwork but also successfully obtains high-quality yields and activates the physiological processes of the fruit tree.

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References

- Masouleh, S.S.S.; Sassine, Y.N. Molecular and biochemical responses of horticultural plants and crops to heat stress. *Ornam. Hortic.* **2020**, *26*, 148–158. [\[CrossRef\]](#)
- Muchate, N.S.; Nikalje, G.C.; Rajurkar, N.S.; Suprasanna, P.; Nikam, T.D. Plant Salt Stress: Adaptive Responses, Tolerance Mechanism and Bioengineering for Salt Tolerance. *Bot. Rev.* **2016**, *82*, 371–406. [\[CrossRef\]](#)
- Hasanuzzaman, M.; Kamrun Nahar, K.; Alam Md, M.; Roychowdhury, R.; Fujita, M. Physiological, Biochemical, and Molecular Mechanisms of Heat Stress Tolerance in Plants. *Int. J. Mol. Sci.* **2013**, *14*, 9643–9684. [\[CrossRef\]](#) [\[PubMed\]](#)
- Kooyers, N.J.; Greenlee, A.B.; Colicchio, J.M.; Oh, M.; Blackman, B.K. Replicate altitudinal clines reveal that evolutionary flexibility underlies adaptation to drought stress in annual *Mimulus guttatus*. *New Phytol.* **2015**, *206*, 152–165. [\[CrossRef\]](#) [\[PubMed\]](#)
- Kumar, R.; Bohra, A.; Pandey, A.K.; Pandey, M.K.; Kumar, A. Metabolomics for plant improvement: Status and prospects. *Front. Plant Sci.* **2017**, *8*, 1302. [\[CrossRef\]](#) [\[PubMed\]](#)
- Saud, S.; Fahad, S.; Yajun, C.; Ihsan, M.Z.; Hammad, H.M.; Nasim, W.; Amanullah, Jr.; Arif, M.; Alharby, H. Effects of Nitrogen Supply on Water Stress and Recovery Mechanisms in Kentucky Bluegrass Plants. *Front. Plant Sci.* **2017**, *8*, 983. [\[CrossRef\]](#)
- Isah, T. Stress and defense responses in plant secondary metabolites production. *Biol. Res.* **2019**, *52*, 39. [\[CrossRef\]](#)
- Mazid, M.; Khan, T.A.; Mohammad, F. Role of secondary metabolites in defense mechanisms of plants. *Exp. Biol. Med.* **2011**, *3*, 232–249.
- Popa, V.I. Wood bark as valuable raw material for compounds with biological activity. *Celul. Hârtie* **2015**, *64*, 5–17.
- Ma, D.; Li, Y.; Zhang, J.; Wang, C.; Qin, H.; Ding, H.; Xie, Y.; Guo, T. Accumulation of Phenolic Compounds and Expression Profiles of Phenolic Acid Biosynthesis-Related Genes in Developing Grains of White, Purple, and Red Wheat. *Front. Plant Sci.* **2016**, *7*, 528. [\[CrossRef\]](#)
- Slatnar, A.; Mikulič-Petkovešek, M.; Veberič, R.; Štampar, F. Research on the involment of phenoloics in the defence of horticultural plants. *Acta Agric. Slov.* **2017**, *107*, 183. [\[CrossRef\]](#)
- Arıcı, S.E.; Kafkas, E.; Kaymak, S. Phenolic compounds of apple cultivars resistant or susceptible to *Venturia inaequalis*. *Pharm. Biol.* **2014**, *52*, 904–908. [\[CrossRef\]](#) [\[PubMed\]](#)
- Tolić, M.T.; Krbavčić, I.P.; Vujević, P.; Milinović, B.; Jurčević, I.L.; Vahčić, N. Effects of Weather Conditions on Phenolic Content and Antioxidant Capacity in Juice of Chokeberries (*Aronia melanocarpa* L.). *Polish J. Food. Nutr. Sci.* **2017**, *67*, 67–74. [\[CrossRef\]](#)
- Xu, C.; Zhang, Y.; Zhu, L.; Huang, Y.; Lu, J. Influence of growing season on phenolic compounds and antioxidant properties of grape berries from vines grown in subtropical climate. *J. Agric. Food Chem.* **2011**, *59*, 1078–1086. [\[CrossRef\]](#) [\[PubMed\]](#)
- Kalt, W. Effects of production and processing factors on major fruit and vegetable antioxidants. *J. Food Sci.* **2005**, *70*, 11–19. [\[CrossRef\]](#)
- Renard, C.M.G.C.; Dupont, N.; Guillermin, P. Concentrations and characteristics of procyanidins and other phenolics in apples during fruit growth. *Phytochemistry* **2007**, *68*, 1128–1138. [\[CrossRef\]](#) [\[PubMed\]](#)
- Liaudanskas, M.; Viškelis, P.; Kviklys, D.; Raudonis, R.; Janulis, V. A comparative study of phenolic content in apple fruits. *Int. J. Food Prop.* **2015**, *18*, 945–953. [\[CrossRef\]](#)

18. Lattanzio, V.; Cardinali, A.; Ruta, C.; Fortunato, I.M.; Lattanzio, V.M.T.; Linsalata, V.; Cicco, N. Relationship of secondary metabolism to growth in oregano (*Origanum vulgare* L.) shoot cultures under nutritional stress. *Environ. Exp. Bot.* **2009**, *65*, 54–62. [\[CrossRef\]](#)
19. Treutter, D. Biosynthesis of phenolic compounds and its regulation in apple. *Plant Growth Regul.* **2001**, *34*, 71–89. [\[CrossRef\]](#)
20. Skłodowska, M.; Mikiciński, A.; Wielanek, M.; Kuźniak, E.; Sobiczewski, P. Phenolic profiles in apple leaves and the efficacy of selected phenols against fire blight (*Erwinia amylovora*). *Eur. J. Plant Pathol.* **2017**, *151*, 213–228. [\[CrossRef\]](#)
21. Mikulic-Petkovsek, M.; Slatnar, A.; Stampar, F.; Veberic, R. Phenolic compounds in apple leaves after infection with apple scab. *Biol. Plant* **2011**, *55*, 725–732. [\[CrossRef\]](#)
22. Rosa, M.; Prado, C.; Podazza, G.; Interdonato, R.; González, J.A.; Hilal, M.; Prado, F.E. Soluble sugars–metabolism, sensing and abiotic stress: A complex network in the life of plants. *Plant Signal. Behav.* **2009**, *4*, 388–393. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Benyas, E.; Hassanpouraghdam, M.B.; Zehbtsalmasi, S.; Khatamian Oskoei, O.S. Allelopathic effects of *Xanthium strumarium* L. shoots aqueous extracts on germination, seedlings growth and chlorophyll content of lentil (*Lens culinaris* Medic.). *Rom. Biotechnol. Lett.* **2010**, *15*, 5223–5228.
24. Zepka, L.Q.; Jacob-Lopes, E.; Roca, M. Catabolism and bioactive properties of chlorophylls. *Curr. Opin. Food Sci.* **2019**, *26*, 94–100. [\[CrossRef\]](#)
25. Alexieva, V.; Ivanov, S.; Sergiev, I.; Karanov, E. Interaction between stresses. *Bulg. J. Plant Physiol.* **2003**, *29*, 1–17.
26. Khanal, N.; Bray, G.E.; Grisnich, A.; Moffatt, B.A.; Gray, G.R. Differential Mechanisms of Photosynthetic Acclimation to Light and Low Temperature in *Arabidopsis* and the Extremophile *Eutrema. Salsugineum*. *Plants* **2017**, *6*, 32. [\[CrossRef\]](#)
27. Boyer, J.; Liu, R.H. Apple phytochemicals and their health benefits. *J. Nutr.* **2004**, *3*, 5. [\[CrossRef\]](#)
28. Gharras, H. Polythenols: Food sources, properties and applications. *Int. J. Food Sci. Technol.* **2009**, *44*, 2512–2518. [\[CrossRef\]](#)
29. FAOSTAT. 2020. Available online: <http://www.fao.org/faostat/en/#data/QC/visualize> (accessed on 1 January 2022).
30. Brons, C.; Olieman, C. Study of the high-performance liquid chromatographic separation of reducing sugars, applied to the determination of lactose in milk. *J. Chromatogr. A* **1983**, *259*, 79–86. [\[CrossRef\]](#)
31. McCleary, B.V.; Gibson, T.S.; Mugford, D.C. Measurement of Total Starch in Cereal Products by Amyloglucosidase- α -Amylase Method: Collaborative Study. *J. AOAC Int.* **1997**, *80*, 571–579. [\[CrossRef\]](#)
32. Li, Y.; He, N.; Hou, J.; Xu, L.; Liu, C.; Zhang, J.; Wang, Q.; Zhang, X.; Wu, X. Factors Influencing Leaf Chlorophyll Content in Natural Forests at the Biome Scale. *Front. Ecol. Evol.* **2018**, *6*, 64. [\[CrossRef\]](#)
33. Roca, M.; Chen, K.; Pérez-Gálvez, A. Chlorophylls. In *Handbook on Natural Pigments in Food and Beverages: Industrial Applications for Improving Food Color*; Elsevier Ltd.: Amsterdam, The Netherlands, 2016; pp. 125–158.
34. Walter, M.H.; Strack, D. Carotenoids and their cleavage products: Biosynthesis and functions. *Nat. Prod. Rep.* **2011**, *28*, 663–692. [\[CrossRef\]](#) [\[PubMed\]](#)
35. Yuan, H.; Zhang, J.; Nageswaran, D.; Li, L. Carotenoid metabolism and regulation in horticultural crops. *Hort. Res.* **2015**, *2*, 15036. [\[CrossRef\]](#) [\[PubMed\]](#)
36. Picinelli, A.; Dapena, E.; Mangas, J.J. Polyphenolic pattern in apple tree leaves in relation to scab resistance: A preliminary study. *J. Agric. Food Chem.* **1995**, *43*, 2273–2278. [\[CrossRef\]](#)
37. Petkovsek, M.M.; Slatnar, A.; Stampar, F.; Veberic, R. The influence of organic/integrated production on the content of phenolic compounds in apple leaves and fruits in four different varieties over a 2-year period. *J. Sci. Food Agric.* **2010**, *90*, 2366–2378. [\[CrossRef\]](#) [\[PubMed\]](#)
38. Viškelis, J.; Liaudanskas, M.; Uselis, N.; Kviklys, D. Internal fruit quality depends on the regulation of apple-tree vegetative growth. In Proceedings of the XXX International Horticultural Congress IHC2018: International Symposium on Cultivars, Rootstocks and Management Systems of 1281, Istanbul, Turkey, 12–16 August 2018; pp. 643–648. [\[CrossRef\]](#)
39. Hua, Y.; Guo, H.; Zhou, X.G.; Li, X.; Yang, S.; Song, Y.; Ma, N.; Chai, C.; Qiao, X.; Li, L. Correlations between soluble sugar and phenol contents in leaves and pear scab resistance. *J. Plant Pathol. Microbiol.* **2014**, *5*, 1–4. [\[CrossRef\]](#)
40. Iqbal, M.; Sharma, M.; Ali, R.F.; Yousuf, M.; Hussain, A. In vitro antioxidant activity and spectrophotometric quantification of total phenolic and flavonoid contents of *Malus domestica*. *Trop J. Pharm. Res.* **2013**, *3*, 452–471.
41. Magel, E.; Einig, W.; Hampp, R. Carbohydrates in trees. *Dev. Crop. Sci.* **2000**, *26*, 317–336. [\[CrossRef\]](#)
42. Kviklys, D.; Samuolienė, G. Relationships among the rootstock, crop load, and sugar hormone signaling of apple tree, and their effects on biennial bearing. *Front. Plant Sci.* **2020**, *11*, 1213. [\[CrossRef\]](#)
43. Samuolienė, G.; Viškelienė, A.; Sirtautas, R.; Kviklys, D. Relationships between apple tree rootstock, crop-load, plant nutritional status and yield. *Sci. Hortic.* **2016**, *211*, 167–173. [\[CrossRef\]](#)
44. Loescher, W.H.; Mccamant, T.; Keller, J.D. Carbohydrate reserves, translocation, and storage in woody plant roots. *Hortscience* **1990**, *25*, 274–281. [\[CrossRef\]](#)
45. Cheng, L.; Zhou, R.; Reider, E.J.; Sharkey, T.D.; Dandekar, A.M. Antisense inhibition of sorbitol synthesis leads to up-regulation of starch synthesis without altering CO₂ assimilation in apple leaves. *Planta* **2005**, *220*, 767–776. [\[CrossRef\]](#)
46. Li, M.; Li, P.; Ma, F.; Dandekar, A.M.; Cheng, L. Sugar metabolism and accumulation in the fruit of transgenic apple trees with decreased sorbitol synthesis. *Hort. Res.* **2018**, *5*, 60. [\[CrossRef\]](#) [\[PubMed\]](#)
47. Nii, N. Changes of Starch and Sorbitol in Leaves Before and After Removal of Fruits from Peach Trees. *Ann. Bot.* **1997**, *79*, 139–144. [\[CrossRef\]](#)

48. Kelc, D.; Lakota, M.; Vindis, P. Measurements of sugars in leaves on apple trees. In *DAAAM International Scientific Book*; Katalinic, B., Ed.; DAAAM International: Vienna, Austria, 2016; pp. 207–216. [\[CrossRef\]](#)
49. Wibbe, M.L.; Blanke, M.M. Effects of defruiting on source–sink relationship, carbon budget, leaf carbohydrate content and water use efficiency of apple trees. *Plant Physiol.* **1995**, *94*, 529–533. [\[CrossRef\]](#)
50. Hudina, M.; Colaric, M.; Stampar, F. Primary metabolites in the leaves and fruits of three pear cultivars during the growing season. *Can. J. Plant Sci.* **2006**, *87*, 327–332. [\[CrossRef\]](#)
51. Wang, Z.; Stutte, G.W. The role of carbohydrates in active osmotic adjustment in apple under water stress. *J. Am. Soc. Hortic. Sci.* **1992**, *117*, 816–823. [\[CrossRef\]](#)
52. De Almeida, G.K.; Fioravanco, J.C. Yield of ‘Royal Gala’ apple trees in response to pruning before or after leaf drop. *Pesqui. Agropecu. Bras.* **2018**, *53*, 427–434. [\[CrossRef\]](#)
53. Greer, D.H.; Wünsche, J.N.; Halligan, E.A. Influence of postharvest temperatures on leaf gas exchange, carbohydrate reserves and allocations, subsequent budbreak, and fruit yield of ‘Braeburn’ apple (*Malus domestica*) trees. *N. Z. J. Crop Hortic. Sci.* **2002**, *30*, 175–185. [\[CrossRef\]](#)
54. Greer, D.H. Limitations to photosynthesis of leaves of apple (*Malus domestica*) trees across the growing season prior to and after harvest. *Photosynthetica* **2019**, *57*, 483–490. [\[CrossRef\]](#)
55. Fanwoua, J.; Bairam, E.; Delaire, M.; Buck-Sorlin, G. The role of branch architecture in assimilate production and partitioning: The example of apple (*Malus domestica*). *Front. Plant Sci.* **2014**, *5*, 338. [\[CrossRef\]](#)
56. Melke, A. The physiology of chilling temperature requirements for dormancy release and bud-break in temperate fruit trees grown at mild winter tropical climate. *J. Plant Res.* **2015**, *4*, 110–156. [\[CrossRef\]](#)
57. Yamaki, S. Isolation of vacuoles from immature apple fruit flesh and compartmentation of sugars, organic acids, phenolic compounds and amino acids. *Plant Cell Physiol.* **1984**, *25*, 151–166. [\[CrossRef\]](#)
58. Colaric, M.; Veberic, R.; Stampar, F.; Hudina, M. Evaluation of peach and nectarine fruit quality and correlations between sensory and chemical attributes. *J. Sci. Food Agric.* **2005**, *85*, 2611–2616. [\[CrossRef\]](#)
59. Li, M.; Feng, F.; Cheng, L. Expression patterns of genes involved in sugar metabolism and accumulation during apple fruit development. *PLoS ONE* **2012**, *7*, e33055. [\[CrossRef\]](#) [\[PubMed\]](#)
60. Katz, E.; Boo, K.H.; Kim, H.Y.; Eigenheer, R.A.; Phinney, B.S.; Shulaev, V.; Negre-Zakharov, F.; Sadka, A.W. Label-free shotgun proteomics and metabolite analysis reveal a significant metabolic shift during citrus fruit development. *J. Exp. Bot.* **2011**, *62*, 5367–5384. [\[CrossRef\]](#) [\[PubMed\]](#)
61. Nardozza, S.; Boldingh, H.L.; Osorio, S.; Höhne, M.; Wohlers, M.; Gleave, A.P.; MacRae, E.A.; Richardson, A.C.; Atkinson, R.G.; Sulpice, R.; et al. Metabolic analysis of kiwifruit (*Actinidia deliciosa*) berries from extreme genotypes reveals hallmarks for fruit starch metabolism. *J. Exp. Bot.* **2013**, *64*, 5049–5063. [\[CrossRef\]](#) [\[PubMed\]](#)
62. Desnoues, E.; Gibon, Y.; Baldazzi, V.; Signoret, V.; Génard, M.; Quilot-Turion, B. Profiling sugar metabolism during fruit development in a peach progeny with different fructose-to-glucose ratios. *BMC Plant Biol.* **2014**, *14*, 336. [\[CrossRef\]](#) [\[PubMed\]](#)
63. Li, M.; Li, D.; Feng, F.; Zhang, S.; Ma, F.; Cheng, L. Proteomic analysis reveals dynamic regulation of fruit development and sugar and acid accumulation in apple. *J. Exp. Bot.* **2016**, *67*, 5145–5157. [\[CrossRef\]](#) [\[PubMed\]](#)
64. Ruan, Y.L. Sucrose metabolism: Gateway to diverse carbon use and sugar signaling. *Annu. Rev. Plant Biol.* **2014**, *65*, 33–67. [\[CrossRef\]](#) [\[PubMed\]](#)
65. Meng, D.; He, M.; Bai, Y.; Xu, H.; Dandekar, A.M.; Fei, Z.; Cheng, L. Decreased sorbitol synthesis leads to abnormal stamen development and reduced pollen tube growth via a MYB transcription factor, MdMYB39L, in apple (*Malus domestica*). *New Phytol.* **2018**, *217*, 641–656. [\[CrossRef\]](#) [\[PubMed\]](#)
66. Meng, D.; Li, C.; Park, H.J.; González, J.; Wang, J.; Dandekar, A.M.; Turgeon, B.G.; Cheng, L. Sorbitol modulates resistance to *Alternaria alternata* by regulating the expression of an NLR resistance gene in apple. *Plant Cell* **2018**, *30*, 1562–1581. [\[CrossRef\]](#) [\[PubMed\]](#)