

Phytopigments, Proline, Chlorophyll Index, Yield and Leaf Nitrogen as Impacted by Rootstock, Training System, and Girdling in “Aztec Fuji” Apple

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Abstract

World overpopulation, scarcity of water and finite arable land mandate a more efficient use of these natural resources. To remain sustainable, modern-day apple (*Malus domestica* Borkh.) fruit production utilizes high-density orchards on semi-dwarfing, precocious rootstocks. Nonetheless, little attention has been paid to the use of size-controlling practices such as trunk girdling in these modern orchard systems. The impact of two rootstocks, two tree architecture (training) systems, and three levels of girdling (in spring) on chlorophyll index (CI), proline, chlorophyll content (Chl; a, b, a + b), carotenoids, anthocyanin, yield, fruit weight and leaf nitrogen (N) in “Aztec Fuji” were studied in 2015 and 2016. Trees on “Bud 9” had up to 5% higher chlorophyll indices in leaves on side branches than those on “Nic 29” at each sampling time in 2015 and 2016. However, trees on “Nic 29” had up to 5.5% chlorophyll index in terminal branches, 14% higher Chl b, 54% higher leaf proline in 2015, up to 5% higher leaf nitrogen (N) in 2015 and 2016, 82% higher yields in 2015, and up to 7% higher fruit weight in 2015 and 2016 than those on “Bud 9”. Trees trained into a central leader (CL) architecture had up to 6% higher chlorophyll index and 4% higher leaf N in terminal branches 2015 and 2016, 43% more terminal leaf proline content in 2015, but 13% less yield in 2016 than those with a tall spindle training (TS). Terminal leaves from trees receiving a bark girdling in 2015 (BG15) had up to 6.5% lower leaf N, 7% less chlorophyll index, and between 27% to 56% lower proline content than those from the ungirdled check and score girdled treatment in 2015 (SG15). Also, trees with BG15 and a bark girdling in both 2015 and 2016 (BG1516) treatments had significantly less leaf N concentration than all other treatments or the ungirdled check. Trees that were

score girdled in 2015 and repeat score girdled in 2016 (SG1516) had 21% higher yield and 12% higher fruit weight than those that were ungirdled.

Keywords

Canopy Architecture, Cambium Ringing, Growth Control, High Density Orchards, *Malus x domestica*

1. Introduction

World overpopulation mandates the efficient use of natural resources, including water and land. Establishment of high-density apple (*Malus domestica* Borkh) orchards, using new designs on dwarfing rootstocks have become popular in many progressive apple-producing regions around the globe. In apples, these new systems typically result in improved fruit quality and yield and rootstocks can influence several postharvest attributes, such as ripening, color, shape and fruit size [1] [2] [3] [4] [5]. It has been reported in the NC-140 (1984) cooperative planting that apple fruit ripening is negatively correlated with tree vigor, as the most dwarfing rootstocks resulted in the earliest ripening [1]. Rootstocks can also influence scion leaf and fruit mineral concentrations and thus, indirectly affect yield and fruit quality [6] [7] [8].

Selection of suitable tree architecture in a high-density orchard is mainly determined by rootstock vigor and soil type. Clements reported that performance of “McIntosh” and “Honeycrisp” apples on “Bud 9”, “M.26” and “MM.106” rootstocks, with a central leader (CL), vertical axis (VA), or tall spindle (TS) tree architectures. In both cultivars, trees on “Bud 9” had the highest cumulative yield over 2008-2010 (during 3rd through 5th leaf), followed by those on “M.26” and “MM.106” rootstocks. In addition, their studies found that trees trained to TS had the highest production per hectare, followed by those with VA and CL [9].

Girdling is one of the most practical methods for inducing artificial stress [10]. This practice has been used in several deciduous and evergreen tree species [10]-[16]. Girdling is the removal of a circumferential strip of bark down to the vascular cambium, and if practiced during the early stages of growing season it temporally halts the flow of photosynthates from top of the tree to the root system. Girdling trunks or large branches enhanced fruit set and some quality attributes, particularly fruit color, when performed at specific growth stages on various fruit crops in some studies [14] [17] [18] [19] [20]. It has been suggested that girdling in fruit trees can increase carbohydrate supply during fruit maturation, leading to the enhancement of red coloration by increasing anthocyanin synthesis [21]. Girdling apple trees 10 days after petal fall only slightly improved red color in two of three cultivars but increased yellow background color development of fruit skins [22]. It has been reported that mid-summer trunk girdling increased red coloration and intensity both years and improved market-grade pack out in “Jonagold” apple [23]. This effect was not caused by advanced ma-

turity. In that study, girdling reduced fruit size only on trees of low N status [23]. Post-bloom trunk girdling increased fruit set and flower bud formation and reduced vegetative growth on apple trees [24] [25] [26] [27]. It has been reported that fruit on girdled limbs of apples had higher fruit dry weight and dry weight concentration than on non-girdled limbs [28]. Leaf area on non-girdled limbs was unaffected by crop load but increased dramatically on girdled limbs with a crop load of less than one fruit per square centimeter limb cross-sectional area. These leaves also had a low photosynthetic rate, high stomatal resistance, and high internal CO₂ [28].

The impact of girdling on yield and fruit quality attributes have also been studied in other fruit crops. In Argentina, girdling pear (*Pyrus communis*) trees increased yield in two seasons [29]. Color on stone-fruits (*Prunus* spp.) was improved after trunk girdling at the pit-hardening stage [17] [18]. It has been reported that branch girdling, at the stage of rapid fruit growth, on “Hass” avocado (*Persea americana*) increased the fruit mass by 35% [30]. Vine girdling in table grapes (*Vitis vinifera*) increased berry and cluster weight [12] [31] but decreased soluble solids concentration and titratable acidity [31].

Scoring is cutting through cambium layer without removing bark. In this method, flow of solutes through phloem will be stopped or slowed down for a shorter period than by girdling [12]. Other scientists observed that scoring and girdling increase yield without decreasing return bloom in “Triumph” persimmon (*Diospyros virginiana*) [32]. Scoring and girdling were equally effective in increasing fruit size in peach and nectarine (*Prunus persica*) [17] [33]; and loquat (*Eriobotrya japonica*) [34]. However, bark girdling may impair trees and vine health if callusing is slow or inadequate [12] [33]. In a study with orange (*Citrus reticulata*) trees, vascular connectivity of the phloem was re-established much faster after scoring than after girdling [35].

Chen *et al.* [36] reported that proline is one of four amino acids that rises during drought stress as you approach wilting [36] and that finding was later confirmed [37] [38] [39]. Yelenosky [39] found that proline is a major source of soluble nitrogen in citrus leaves. They also suggested that as a metal chelator and an antioxidant defense molecule, proline plays an important role in stress. Okumoto *et al.* [40] focused on understanding biochemical mechanisms of proline accumulation under stress conditions. They reported that proline is a major component of cell wall proteins and is regulated by developmental and environmental signals [40]. Other scientists observed a rise of proline in a wide range of plant species as a result of transcriptional and post-transcriptional regulation [38].

Measuring the amount of proline concentration appears to be a great way of monitoring artificial or natural stresses in several plants. When considering girdling as artificial stress for plants, considering amount of proline as a stress indicator factor in most plant species may be beneficial in evaluating girdling effects. Ibrahim *et al.* [41] reported that girdling branches or limbs decreased the chlorophyll a, b and total chlorophyll (Chl) concentrations and promoted caro-

tenoids concentrations, fruit set and fruit yield of Washington navel orange leaves compared to the Control. Also, other scientists reported that the concentrations of Chl. a, Chl. b, and total chlorophyll a + b decreased with girdling pistachio shoots [15]. Davie *et al.* [30] reported that leaves on girdled and scored branches of avocado without fruit showed a decrease in photosynthesis rate after treatment and stopped photosynthesizing 14 days after treatment. They also reported branches with fruit took longer to show a reduction in photosynthesis. Nevertheless, Rivas *et al.* [42] reported that girdling trunks of mandarin trees had no significant effect in total chlorophyll concentrations between control and girdled trees. However, other scientists reported that girdling had positive effects on total chlorophyll and chlorophyll (a) content in “Balady” mandarin leaves, but reduced chlorophyll (b) content [43].

Tang *et al.* [44] evaluated the effects of girdling on carbohydrate-induced senescence. They reported that girdling reduced chl. a, b, carotenoids, ratio of a:b and chlorophyll:carotenoids. In addition, Proietti *et al.* [45] reported that fruit dry mass increased, but chlorophyll concentration decreased in parallel with reduction of photosynthetic rate (P_N) in olive trees in response to girdling.

Despite the use of dwarfing rootstocks and modern training systems in modern apple orchards, there is no report on the influence of tree girdling and scoring on the performance of apple trees under these conditions. Therefore, the objective of this experiment was to study the effect of two rootstocks, two training systems, and two levels of girdling on growth, yield, and fruit quality attributes at harvest-time and leaf nitrogen (N) status in “Aztec Fuji” apple trees over 2015 and 2016 seasons compared to non-girdled controls.

2. Materials and Methods

2.1. Orchard Establishment and General Cultural Practices

The experimental orchard was established in the spring and early summer of 2010, on a north/south orientation, at the Parma Research and Extension Center, University of Idaho (43.7853°N, 116.9422°W) on a sandy loam soil with an approximate pH of 7.3. The experimental site is a semi-arid climate with an annual precipitation of approx. 297 mm. Climate conditions of this experimental site were almost similar to those in the Pacific Northwest [46]. General orchard cultural practices such as irrigation and thinning were reported in a different aspect of this comprehensive study elsewhere [5] [47] [48].

Total nitrogen of 60 g/tree as UAN 32 (urea and ammonium nitrate, 32% N) was applied through the driplines. Each year, two fertigation of 30 g/tree N were made: one in late-May and second one two weeks later. Potassium was applied as 30 g K/tree as potassium oxide, via fertigation, once a year in late-May. Phosphorous, as monoammonium phosphate (61% P_2O_5), was applied at the rate of 150 g of formulation to each tree-planting hole, only once at the time of planting. Micronutrients, particularly, iron and zinc, were sprayed twice in spring and once in early summer each year. Calcium (Ca) was sprayed three times

with cover sprays during spring every year.

2.2. Rootstock and Tree Training Treatments

“Aztec Fuji” trees on “M.9”, RN 29 (“Nic 29”) and *Budagovsky* 9 (“Bud 9”) rootstocks (C & O Nursery, Wenatchee, WA) were planted at 0.9 × 3.90 m spacing with a north-south row orientation. “Snow Drift” crab apple (*Malus* × “Snow-drift”) on “M.26” EMLA rootstock (C & O Nursery, Wenatchee, WA) was inter-planted in each row as a pollinizer after every 10th “Aztec Fuji” tree. Trees were trained to either central leader (CL) or tall spindle (TS) during the dormant season in early-March each year. In trees with a CL training system, four main scaffolds were kept permanently and the other side branches above these scaffolds were kept shorter at about 45° angle to form a “Christmas-tree shape”. Trees with a TS system were trained to have between 14 to 18 side branches, which were trained to 110° from the vertical axis with tree ties. After the first year, only side branches with thicker than 1/2 to 2/3 diameter of the main leader were removed, leaving a 12cm-stub for regrowth. Tree leaders in both CL and TS were maintained at approx. 3.75 m in height.

2.3. Girdling Treatments

Each rootstock block contained 12 trees, where 6 of the adjacent trees in a row received TS training while 6 trees on the adjacent row received CL training. In 2015, trees were in full bloom on April 11, and girdling treatments were applied on April 27th. In 2015, paired trees of these 6 tree-plots received one of the three girdling treatments as follows: 1) Control or No Girdling (NOGD), where no girdling was applied; 2) Bark Girdling (BG15) at 30 cm above the graft union, where a complete 3 - 4 mm ring of bark with cambium was removed by cutting with a sharp knife so that xylem wood tissue was exposed; 3) Score Girdling (SG), where the bark of tree trunk was scored in a spiral pattern, going one and half turns around the tree trunk, from 30 cm above the bud union with a sharp linoleum utility knife. In 2016, trees were in full bloom on April 15 and girdling treatments were applied on May 16th. In 2016, the experimental arrangement was similar 2015, except for the fact that the second of each paired tree of the girdled treatments was not girdled or scored respectively. Where trees were girdled in 2016, this took place at approximately 6 cm above the 2015 bark girdling (BG15) site. This treatment was designated BG1516. Where trees were scored in 2016, this also took place approximately 6 cm above the 2015 score girdling site (SG15). Trees scored in both years were designated SG1516.

2.4. Terminal and Side Branch Chlorophyll Index

To determine chlorophyll index (CIs), four random terminal and side branches in each experimental tree were tagged and their final growth was measured in August, September and October 2015 and also June and October 2016, using a SPAD meter. CIs between 10 am and 2 pm were determined using equipment

based on a new technology (Field Scout Chlorophyll Meter, Model CM 1000, Spectrum Technologies, Aurora, IL, USA) and determined according to the method described by Mahdavi *et al.* [49] [50]. The equipment uses the ambient and reflected 700 and 840 nm lights to calculate the relative CI. It measures conical viewing areas between 30 and 180 cm from lens. The equipment measures index of relative chlorophyll content ranges from 0 to 999.

2.5. Leaf Nitrogen Measurements

Thirty leaves were sampled from each tree at random from the middle of the current-season shoots in mid-August each year. Leaves were placed in a cooler and taken to the University of Idaho Pomology and Viticulture laboratories each year for analysis. Leaves were washed in a mild solution, containing 1% Liqui-Nox anionic detergent (AlcoNox Inc., White Plains, NY, USA), rinsed in four different 25-L containers of distilled water, and dried in a forced-air oven at 65°C. Dried leaves were ground to pass through a 40-mesh screen using a Cyclotec Sample Mill (Model 1093; Tecator, Hoganas, Sweden). Leaf N concentration was determined by combusting the dry leaf tissues using a LECO Protein/Nitrogen Analyser (Model FP-528; LECO Corp., St. Joseph, MI, USA).

2.6. Yield and Fruit Weight Attributes

Twenty fruits per tree were randomly sampled for quality evaluation and yield was recorded from each tree on October 17-20 during 2015-2016.

2.7. Proline, Chlorophyll a, b, a + b, Carotenoids, Anthocyanin Measurements

Free proline concentrations were determined according to the method described by Bates *et al.* [51]. Also, chlorophyll a, b and a + b concentrations were measured according to the method described by Arnon [52]. Total leaf anthocyanin and carotenoid concentrations were measured determined according to the method described by Sims & Gamon [53].

3. Experimental Designs and Statistics

The experimental design was a randomized complete block split-split plot (in both years) with two rootstocks as the main effects, two methods of tree training system as sub-plot, girdling treatments as sub-sub plots, and 4 blocks (replicates). Field data were collected throughout 2015 and 2016. At harvest leaf N concentration and individual tree yields were recorded. Analyses of variance was conducted using SAS (SAS Institute, Cary, NC, USA), with GLM and means were compared by least significant difference (LSD) at $P \leq 0.05$.

4. Results and Discussion

4.1. Interactions

Other than a case between rootstock and tree architecture for yield per tree in

2016, no significant interaction was observed between rootstock-training or rootstock-training-girdling treatments for any measurements in this study. Thus, only direct effects of rootstock, tree architectures, and type of girdling are reported in **Tables 1-3**.

Table 1. The influence of rootstock, canopy architectures, and cambium disconnection on terminal and side branch chlorophyll index, measured by SPAD meter in “Aztec Fuji” apple in 2015 and 2016.

Treatment	2015						2016			
	August 15		September 9		October 16		June 30		October 20	
	Terminal	Side	Terminal	Side	Terminal	Side	Terminal	Side	Terminal	Side
Rootstock										
Nic 29	61.72 a ^x	54.30 b	59.07 a	51.52 b	59.46 a	53.00 b	58.42 a	53.75 b	58.95 a	50.49 b
Bud 9	60.47 a	56.86 a	57.42 b	52.78 a	56.36 b	54.08 a	58.21 a	55.65 a	57.90 b	51.31 a
Tree Training										
Central Leader	62.80 a	56.00 a	59.97 a	52.47 a	58.59 a	54.45 a	58.73 a	54.32 a	57.97 a	49.65 a
Tall Spindle	59.32 b	55.16 a	56.48 b	51.85 a	57.09 b	52.60 a	57.98 b	55.09 a	56.89 b	51.94 a
Girdling^z										
NOGD	62.35 a	55.51 a	58.43 a	52.83 a	59.26 a	54.28 a	59.11 ab	55.03 ab	57.85 ab	51.99 ab
BG15	58.62 b	53.42 b	56.88 b	50.48 b	56.48 b	51.66 b	58.52 ab	55.85 a	58.09 ab	52.70 a
SG15	62.49 a	57.89 a	59.42 a	53.22 a	57.79 a	54.59 a	59.30 a	55.42 a	60.09 a	50.79 ab
BG1516	57.35 b	52.50 b	56.32 b	48.65 b
SG1516	56.78 b	54.99 ab	57.65 ab	49.81 ab

^xAbbreviations: NOGD = no girdling (control); BG15 = bark girdling in 2015; SG15 = score girdling in 2015; BG1516 = bark girdling in 2015 and 2016; SG1516 = score girdling in 2015 and 2016. ^zMean separation within columns in each group of treatments by LSD at 5% level.

Table 2. The influence of rootstock, canopy architectures, and cambium disconnection on leaf nitrogen (N) proline, chlorophyll a, b, and a + b, carotenoids, and anthocyanins in “Aztec Fuji” apple.

Treatment	Proline (mg/g dwt)	Chlorophyll (mg/g dwt)			Carotenoids (mg/gr dwt)	Anthocyanins (mg/gr dwt)
		a	b	Total (a + b)		
Rootstock						
Nic 29	0.7779 a ^x	29.53 a	26.26 a	55.78 a	0.0177a	0.0542 a
Bud 9	0.5047 b	30.74 a	23.05 b	53.79 b	0.0178 a	0.0631 a
Tree Training						
Central Leader	0.7491 a	30.67 a	24.63 a	55.31 a	0.0176 a	0.0603 a
Tall Spindle	0.5228 b	29.54 b	24.67 a	54.21 b	0.0179 a	0.0568 a
Girdling^z						
NOGD	0.7965 a	30.71 a	26.66 a	57.37 a	0.0174 a	0.0599 a
BG15	0.5100 b	28.81 a	22.29 b	51.10 b	0.0175 a	0.0627 a
SG15	0.6478 ab	30.95 a	24.69 b	55.64 a	0.0184 a	0.0521 a

^xAbbreviations: NOGD = no girdling (control); BG15 = bark girdling in 2015; SG15 = score girdling in 2015; BG1516 = bark girdling in 2015 and 2016; SG1516 = score girdling in 2015 and 2016. ^zMean separation within columns in each group of treatments by LSD at 5% level. No interaction existed among any treatments.

Table 3. The influence of rootstock, canopy architectures, and cambium disconnection on leaf nitrogen, yield, and fruit weight in “Aztec Fuji” apple.

Treatment	Leaf N (% dwt)		Yield (kg/tree)		Fruit weight (g)	
	2015	2016	2015	2016	2015	2016
Rootstock						
Nic 29	2.30 a ^x	2.33 a	46.1 a	47.3 a	238.4 a	246.6 a
Bud 9	2.19 b	2.26 b	25.3 b	45.8 a	229.1b	230.5 b
Tree Training						
Central Leader	2.29 a	2.27 a	38.8 a	43.4 b	235.7 a	242.7 a
Tall Spindle	2.20 b	2.32 a	31.3 a	49.1 a	231.4 a	234.0 a
Girdling ^z						
NOGD	2.30 a	2.30 b	36.2 a	41.6 b	213.5 b	223.3 b
BG15	2.16 b	2.41 a	36.5 a	51.9 a	243.8 a	241.6 ab
SG15	2.28 a	2.32 b	32.1 a	46.6 ab	240.0 a	236.0 ab
BG1516	.	2.17 c	.	45.0 ab	.	242.0 ab
SG1516	.	2.27 b	.	50.3 a	.	249.3 a
Significance ^y						
Rootstock	**	**	**	NS	**	**
Tree training	**	NS	NS	*	NS	NS
Rootstock*tree training	NS	NS	NS	*	NS	NS
Rootstock*tree training* girdling	NS	NS	NS	NS	NS	NS

^zAbbreviations: NOGD = no girdling (control); BG15 = bark girdling in 2015; SG15 = score girdling in 2015; BG1516 = bark girdling in 2015 and 2016; SG1516 = score girdling in 2015 and 2016. ^ySignificance: Significant at 1% level if shown by **, at 5% level if shown by *, and no significant if shown by NS.

^xMean separation within columns in each group of treatments by LSD at 5% level or less.

4.2. Rootstock Effect

Leaves from terminal and side branches of trees on both “Bud 9” and “Nic 29” rootstocks had lower CI during early growing season (August 15, 2015 and June 30, 2016) than those in late season (October 16, 2015 and October 20, 2016) (Table 1), due to the declining trend of seasonal leaf nitrogen [8] and perhaps chlorophyll content. Side branches of trees on “Bud 9” had significantly higher CI (up to 5%) than those on “Nic 29” rootstock during all sampling times in 2015 and 2016 (Table 1). In contrast, terminal branches of trees on “Nic 29” rootstock tended to have up to 6% higher CI than those on “Bud 9” in both 2015 and 2016. Terminal buds of trees on dwarf rootstocks, such as “Bud 9”, grows slower and cease before those on more vigorous rootstocks such as “Nic 29” [3], perhaps leading to a higher distribution of nitrogen and chlorophyll in the side branches in trees on “Bud 9”. Leaf samples were taken from terminal shoots, and leaves from trees on “Nic 29” had significantly (up to 5%) higher concentration of N than those on “Bud 9” (Table 3).

Leaves from terminal shoots of trees on “Nic 29” rootstock had 54% higher proline, 14% higher chlorophyll b, and about 4% higher total chlorophyll (a + b) than those on “Bud 9” rootstock, perhaps due to their higher N concentrations (**Table 2**). The impact of rootstock on the actual total chlorophyll content of terminal shoots (**Table 2**) parallels the results found in their non-destructive chlorophyll index measurements (**Table 1**). However, there was no significant difference between two rootstocks for the levels of carotenoids and anthocyanins.

Trees on “Nic 29” also had up to 7% larger average (heavier) fruit than those on “Bud 9” in both 2015 and 2016, despite their 83% higher yield in 2015 (**Table 3**). Usually heavier yield is associated with smaller fruit size. However, trees in all treatments were thinned at about 15 cm spacing at the time of hand thinning. Therefore, higher yield and larger fruit size in trees on “Nic 29” compared to those on “Bud 9” is due to their larger canopy, leading to a higher leaf/fruit ratio in trees on “Nic 29”. It is extremely difficult to determine true effects of rootstock on the scion cultivar fruit quality and other characteristics. This is mainly because confounding effects such as rate of light penetration, crop load, and tendency of biennial bearing can confuse the results. Similar to our assessment, Autio [54] experienced the same challenge in evaluating the impact of six rootstocks on fruit quality attributes of “Delicious” apple. However, he concluded that fruit size consistently was largest from trees on “M.9” EMLA and smallest from trees on “OAR 1” rootstocks.

4.3. Tree Architecture Effect

Trees with CL architecture had up to 6% higher terminal shoot chlorophyll index than did those with a TS system in both 2015 and 2016 (**Table 1**). However, side branch chlorophyll indices in these two training systems were statistically similar (**Table 1**). Also, Trees with a CL system had 43% higher proline content and 4% higher chlorophyll a than did those with a TS training system in 2015 (**Table 2**). While, there were no significant differences between two training systems, nor between carotenoids and anthocyanin concentrations. The impact of training system on the actual total chlorophyll content of terminal shoots (**Table 2**) parallels the results found in their non-destructive chlorophyll index measurements (**Table 1**). In addition, Trees with a CL system in 2015 measurement had 4% higher leaf N as compared to trees with TS system. Nevertheless, trees with a TS training had 13% higher yield than those with a CL training (49.1 vs. 43.3 kg/tree), while fruit weight was similar in both tree training systems (**Table 3**). There was a significant interaction between rootstock and tree architecture for yield per tree in 2016 (**Table 3**). In this interaction, trees on “Nic 29” with a CL training had significantly lower yield (39.9 kg/tree) than those on “Nic 29” with a TS tree training that had 53.9 kg/tree in 2016. Trees on “Nic 29” with a CL training had denser and darker canopies (personal observation), resulting in excessive shading and lower fruit bud formation, and thus lower yield than those on “Nic 29” with a TS training.

Trees on “Nic 29”, with either a CL or TS training system, needed more time for training and pruning, and this issue should be considered when choosing this rootstock. “Aztec Fuji” trees on “Nic 29” rootstock can be suitable for planting at farther than 0.9 m spacing between trees in a row and in the lighter soil in the region. “Bud 9” is also a suitable rootstock for “Aztec Fuji” apple if extra-large fruit size is not the main objective of apple production.

4.4. Girdling Effect

Bark girdling in 2015 (BG15) significantly reduced CI in terminal branches than NOGD (up to 7%). Also, bark girdling in 2015, repeated in 2016 (BG1516) treatments reduced leaf N concentrations than all other treatments (up to 11%) (**Table 2**). The total leaf chlorophyll content and chlorophyll b in bark-girdled trees was also lower than those of non-girdled (up to 12% and 20% in Chl a + b and Chl b, respectively) (**Table 2**). Reduction of leaf chlorophyll as a result of bark girdling is in general agreement with previous reports [20] [30] [41] [42] [44] [45]. Girdling reduced leaf chlorophyll content in citrus trees, perhaps because girdling increases carbohydrates, resulting in changes to PSII system [42]. In addition, our results revealed that bark girdling (BG15) decreased proline content of leaves as compared to those on non-girdled (56%) and score-girdled (27%) trees (**Table 2**). Trees receiving BG15 or BG1516 had significantly lower leaf N than other treatments (up to 6.5% and 11% for BG15 and BG1516, respectively) (**Table 2**) which resulted in improved fruit color in these trees (data not shown). The results are in agreement with Schechter *et al.* [28] who reported that nutrient concentrations; including N in leaves on girdled non-fruiting limbs were generally lower than those in the other treatments. The impact of girdling in their non-fruiting “Sturdeespur Delicious” agreed with our results in fruiting “Aztec Fuji” apples. Impacts of girdling on nutrient partitioning in apple fruit tissue on different rootstocks warrant further investigation. Also, better color in the fruit from apple trees with lower leaf N was previously reported by Fallahi *et al.* [19], which can be due to lower chlorophyll content in the skin of these fruit. Girdling can reduce resistance to cold stress in grape vines [12] and tree fruit (Fallahi, data not published). Thus, lower proline content of trees receiving a bark girdling treatment in 2015—girdled trees may make these girdled trees susceptible to extreme sub-freezing temperatures, and the authors have repeatedly observed this situation (data not shown).

Girdling treatments in 2015 did not affect yield per tree in 2015. This result was expected because flowers of 2015 crop were initiated, and fruit of this season were already set before we applied our girdling treatments in May 2015. However, trees with BG15 or SG1516 treatments had significantly (21% to 25%) higher yield in 2016 compared to those with NOGD treatment (**Table 3**), because girdling or scoring in 2015 resulted in more fruit initiation and fruit set, leading to higher crop in 2016. Enhancing yield/tree by girdling is in general agreement with the result of Ibrahim *et al.* [41] on Washington navel orange trees.

Fruit weight in 2015 and 2016 was affected by girdling treatments. In 2015,

both bark and score girdled trees (BG15 and SG15) had 14% and 12.5% (respectively) higher fruit weight than non-girdled trees (**Table 3**). However, in 2016 only trees receiving repeated of score girdling on the same tree (SG1516) had about 12% higher fruit weight than NOGD (**Table 3**). These results suggest that carbohydrates accumulation is increased in the girdled trees. Similar to our results, Nguyen & Chung [55] reported that a simple S-shape scoring increased fruit weight and size in “Wax” apple. Based on our two-year data, we conclude that score and bark girdling in one year is sufficient to increase fruit weight of the current year and the yield in the following year. However, if both fruit weight and higher yield are the critical objectives of fruit production, annual bark scoring should be practiced in each year. Similarly, Mostafa & Saleh [36] found that girdling increased fruit weight in “Balady” mandarin.

Trees, which are bark girdled, needed about 6 weeks to heal. Trees, which are score girdled need about 3 weeks to heal. Many apple growers, particularly in the humid regions, are concerned that girdling or scoring during early growing season might introduce fireblight bacteria through the wounded areas. Although we did not observe any sign of fireblight incident in these experimental trees, one can postpone the girdling or scoring to late June or early July when fire blight inoculum is reduced in the orchard. This practice must be tested in each apple growing region before it is widely recommended to growers. It is extremely important to allow enough time to the girdling or scoring wounds to heal to avoid possible freeze damage. In general, reduced leaf N concentrations of girdled trees may reduce photosynthesis in the long-term. The positive effects of girdling on yield and fruit weight may encourage some orchardists to use this method in fruit crop trees; however they should be aware of possible cold damage if temperatures plunge down to temperatures below which cells are acclimated.

5. Conclusion

From our 2-year study, we concluded that leaves from terminal and side branches of trees on both “Bud 9” and “Nic 29” rootstocks had lower CI during early growing season than those in late season. Side branches of trees on “Bud 9” had higher CI than those on “Nic 29” rootstock. In contrast, terminal branches of trees on “Nic 29” rootstock tended to have higher CI than those on “Bud 9” in both 2015 and 2016. Trees with CL architecture had higher terminal shoot chlorophyll index than did those with a TS system. Also, leaves from trees with a CL system had higher proline and chlorophyll a than those with a TS training system. Frequency of girdling and scoring practices in combination with different rootstocks and training systems had impacts on growth, yield, fruit size, chlorophyll index and proline. However, there may be some major long-term effects of girdling on tree performance, cold tolerance, and biennial bearing and thus, a longer-term investigation is warranted.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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