Acta Sci. Pol. Hortorum Cultus, 19(1) 2020, 53–59

https://czasopisma.up.lublin.pl/index.php/asphc ISSN 1644-0692 e-ISSN 2545-1405 DOI: 10.24326/asphc.2020.1.5

ORIGINAL PAPER

Accepted: 17.04.2019

INFLUENCE OF PARTIAL SPUR LEAVES REMOVAL ON FRUITLET SHEDDING, FRUIT QUALITY AND SHOOT GROWTH IN APPLE TREES AS A BASIS FOR MECHANICAL THINNING

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ABSTRACT

Knowledge of the physiology of natural fruitlet shedding is especially important to insure thinning efficiency in apple production. The effect of partial spur leaf removal on shedding, as well as on fruit quality and bourse shoot growth, was investigated. Removing spur leaves increased shedding, while there was no effect of partial leaf removal on mature fruit quality in terms of weight, firmness and sum of sugars, detected by HPLC-RI and resulted in lower contents of citric, fumaric and shikimic acids, detected by HPLC-UV. Growth of bourse shoots was poorer in treatments with leaf removal. To summarize, a decreased number of spur leaves caused more intensive fruitlet abscission and poorer bourse shoot growth but had no negative influence on fruit size. These results show the potential use of decreasing spur leaf area to stimulate more intensive natural shedding to support fruitlet thinning, which could be used in combination with prospective mechanical thinning.

Key words: *Malus domestica*, shedding, fruit quality, bourse shoots, sugars, organic acids

INTRODUCTION

Apple trees (*Malus domestica* Borkh.) have three periods of natural shedding (flowers, fruitlets and fruits) during the growing season. The most intensive natural fruitlet shedding occurs a few weeks (4–6) after full bloom in apples, which is also known as physiological drop or June drop and lasts from six to ten weeks, with one, two or three peaks [Goffinet et al. 1996, Jakopic et al. 2015, Jakopic et al. 2016]. Physiological fruit drop is not able to guarantee high fruit quality and a suitable return bloom in the following season [Radivojevic et al. 2014, Eccher et al. 2015]. To overcome these shortcomings, flower- and/or fruitlet thinning is an efficient and necessary measure in apple fruit production [Dennis 2000, Jakopic et al. 2013, Lakso and Goffinet 2013]. In conventional or integrated systems, this measure is often done with chemical thinners but there are not any good organically certified thinning materials for apples. An alternative could be new technologies of mechanical thinning which are currently being commercially used or tested [Damerow and Blanke 2009, Blanke 2011]. The machines, which have rotating spindles with ropes or strings attached, can also remove spurs and damage leaf tissue [Ngugi and Schupp 2009]. Regardless of thinning method used, good understanding of the process of fruitlet shedding is a basis for implementing thinning, which is an important cultural practice in apple production.

The intensity of natural shedding depends on various factors. Fruit abscission may occur as a response to both endogenous and/or exogenous cues [Eccher et al. 2015]. To integrate the many environmental and physiological factors affecting the carbohydrate supply and demand balance and, consequently, fruit abscission in

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an apple tree, Lakso et al. [2001] developed a carbohydrate balance model. The model demonstrates that carbon metabolism may be a key aspect and possibly an integrator of fruit abscission. At about 10 to 20 days after bloom, the demand for carbohydrates and other nutrients from various sinks exceeds what the sources can supply [Lakso et al. 1999]. The carbon balance may integrate the negative effects of shading, application of photosynthetic inhibitors, elevated temperatures, chemical thinning or a combination of various factors [McArtney et al. 2004, Zhou et al. 2008].

In the first few weeks after bloom, the carbohydrate support for fruit growth comes from the spur leaves [Lakso and Goffinet 2013]. A clear reduction in fruit growth rate was found 72 hours after shading the trees [Zhou et al. 2008]. The abscission of young apple fruitlets is caused mainly by a correlative dominance effect of adjacent fruit and/or nearby shoots [Bangerth 2000]. The limitation of light decreases photosynthesis intensity and, consequently, carbohydrate synthesis. Heavy shading to 12% of full sun led to complete de-fruiting of the trees, while having no effect on shoot growth [Lakso and Goffinet 2013].

Mature and well-managed apple trees develop approximately similar numbers of spur and extension leaves in each season. Yield was not affected by partial defoliation (30, 50 or 70%) of whole trees soon after June drop, after defoliation, net photosynthesis of individual leaves was much higher than before defoliation on all trees with a low number of fruits [Stampar et al. 1999].

Because of the leaf damage which can occur during mechanical thinning the influence of spur leaves was investigated. The specific aim of the study was to discover the significance of spur leaves on natural shedding, fruitlet development, and quality of mature fruits, as well as the influence on one-year shoot growth. To evaluate the importance of spur leaves at bloom, one or two thirds of leaves were removed or all leaves were left on branches.

MATERIAL AND METHODS

The study was carried out at the orchard of the Horticultural Center of the Biotechnical Faculty in Orehovlje near Nova Gorica (latitude 45.87 N, longitude 13.61 E, altitude 48 m). The orchard is protected by a hail net and equipped with an irrigation system, rows are NE-SW oriented, planting distance is 3.3 × 1.2 m, and trees are trained in the solaxe training system. In the 2016 growing season, natural June shedding of ‘Golden Delicious’ apple trees grafted on M9 rootstock was monitored. At full bloom, 21 uniform spur-bearing branches were chosen on 21 trees, on east side in the middle of tree canopy, their circumferences were measured at base and thinning was carried out on crop load of seven flower clusters (each with five flowers) per cm^2 of branch. Branches were girdled at branch collar and divided into three leaf removal treatments: 2/3 (in which 2/3 of spur leaves were removed), 1/3 (in which 1/3 of spur leaves were removed) and 0/3 (in which no spur leaves were removed). Before removing the spur leaves, the leaves were divided into three size classes and 1/3 or 2/3 were removed according to size class at flowering (15th April). Each treatment was replicated on seven branches.

After the June drop was finished (14th July), clusters were examined and attached fruitlets were counted. Technologically mature fruits were harvested on 12th September, when it was exactly determined for each fruit on which branch it had grown, how many fruits were in the cluster, and the position of each fruit in the cluster. All fruits were weighed and their firmness and total soluble solids content measured. For chemical analysis, 18 fruits were randomly chosen from each of the three treatments and 3 together used for one sample for analysis of sugar and organic acids content, so each analyze was done in 6 replicates per treatment. Analyses were done by the high liquid performance content (HPLC) system using the method previously described by Jakopic et al. [Jakopic et al. 2013, 2016].

At the end of the growing season (10th October), annual shoot growth was measured on each of the seven shoots per treatment, whereby the length of bourse shoots was measured on each branch. There were no long vegetative shoots without generative buds.

Data were statistically analyzed with the Statgraphics Plus program for Windows 4.0 (Statgraphics Technologies, Virginia, USA), using one-way analysis of variance (ANOVA). Differences were estimated with Duncan’s test. *P*-values of less than 0.05 were considered statistically significant. Multivariate statistical analysis (hierarchical cluster analysis, discriminate analysis, and classification) was conducted to interpret differences in all analyzed variables among treatments. Ward's method based on Euclidean square distance was used to interpret differences among the parameters of all three treatments.

RESULTS AND DISCUSSION

Effect on fruitlet shedding. Natural shedding of apple fruitlets appears a few weeks after bloom. At this time, only spur leaves around flower clusters are developed on the apple tree. These leaves have to produce assimilates for all sinks at that time, limited assimilates production is at least one reason for fruitlet shedding. Removing a proportion of spur leaves affected apple fruitlet shedding. If 1/3 or 2/3 of the leaves were removed at bloom, fruitlet drop was at least 10% more intensive, although differences were not statistically significant (Fig. 1). The number of retained fruitlets in a cluster was lower (Fig. 2). Iwanami et al. [2012] also found out that the abscission of fruitlets is influenced by the number of leaves in the cluster. In some apple cultivars, the fewer leaves there were in a cluster, the larger the number of fruitlets undergoing abscission was [Iwanami et al. 2012].

Effect on fruit quality. In addition to the number of remaining fruits depending on the treatment, the quality of mature fruits was estimated. Fruit weight at harvest was not statistically significantly different among treatments in which spur leaves were removed (Fig. 3). No differences among the treatments indicate that a decrease in the area of spur leaves is not solely responsible for fruit development. In mandarins, Antoine et al. [2016] reported that fresh weight and fruit diameter were significantly reduced, by about 30% and 20%, respectively, for early 80% defoliation and by about 35% and 15%, respectively, for late defoliation, when compared to the control, while it modified the plant's source : sink ratio.

In terms of fruit quality, fruit firmness and total soluble solids were also investigated. There were no differences among treatments in fruit firmness (Fig. 3) but there were differences among treatments in the content of total soluble solids (TSS).

TSS content was higher in fruits from branches with 2/3 removed leaves than from others. This may seem a bit unexpected but it could be at least partly explained on the basis of other results. In the same treatment, fruitlet shedding was the most intensive and, consequently, fewer fruits (sinks) were retained on the branch (Fig. 1). In addition, poor bourse shoot growth was observed in the same treatment. In contrast, Stampar et al. [1999] reported that partial defoliation (30, 50 or 70%) of whole trees soon after June drop affected soluble solids, the lowest content of soluble solids was found on trees with 70% leaf loss.

Among sugars, fructose, glucose and sucrose, as well as the alcohol sugar sorbitol, were analyzed in mature fruits. The main analyzed sugar was fructose, followed by glucose and sucrose (Tab. 1).

Removing spur leaves resulted in an increase of fructose content in mature fruits (Tab. 1). Campbell et al. [1991], who analyzed the concentration of sucrose (as the main sugar) as well as glucose and fructose in nectar from 'Delicious' apples from girdled and/or defoliated individual spurs, concluded that the sugar concentration of apple nectar does not depend on the presence of spur leaves but rather on translocated materials from other sources.

Although removing a share of spur leaves at bloom had no effect on sorbitol content, Archbold [1999] reported that removing all leaves between girdle and fruit nearing 70 days after bloom in 'Gala' apple induced a reduction of sorbitol content and resulted in a reduction in fruit growth in a few days after the measure. Antoine et al. [2016] established in mandarin production that an 80% decrease in the fruit to leaf ratio leads to decreased fruit growth and modification of the primary metabolism, especially by decreasing sucrose and increasing glucose, fructose and citric acid. This indicates that the timing and duration of carbohydrate limitation have different effects on changes of acidity, sugar accumulation and fruit development.

More than sugars, organic acids showed a dependence on removal of spur leaves. Removing 1/3 of spur leaves resulted in statistically lower contents of all analyzed organic acids (citric, shikimic and fumaric), with the exception of malic acid (Tab. 1). Antoine et al. [2016] also reported that the quantity of citric acid decreased soon after early and late defoliation but concentrations at the end of the experiment were higher in both defoliation treatments than in the control. On the other hand, partial defoliation affected the malic acid quantity, which dropped sharply and significantly 48 h after defoliation and, at harvest, fruits

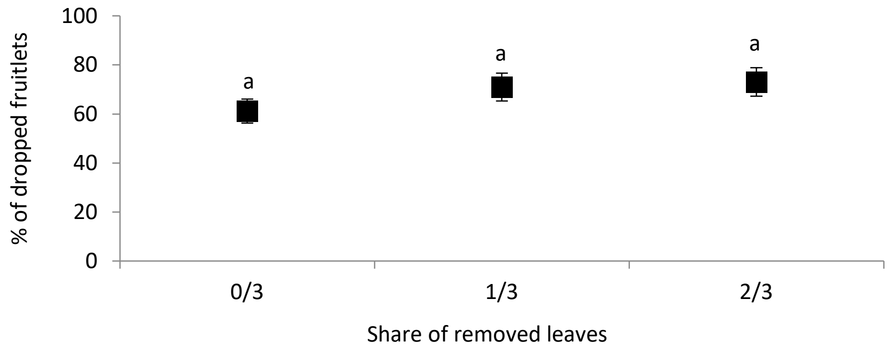

Fig. 1. Share of dropped fruitlets after June drop on branches on which 0, 1/3 or 2/3 spur leaves were removed at blooming. Average values and standard errors are presented. Different letters indicate statistically significant differences among treatments at $p < 0.05$

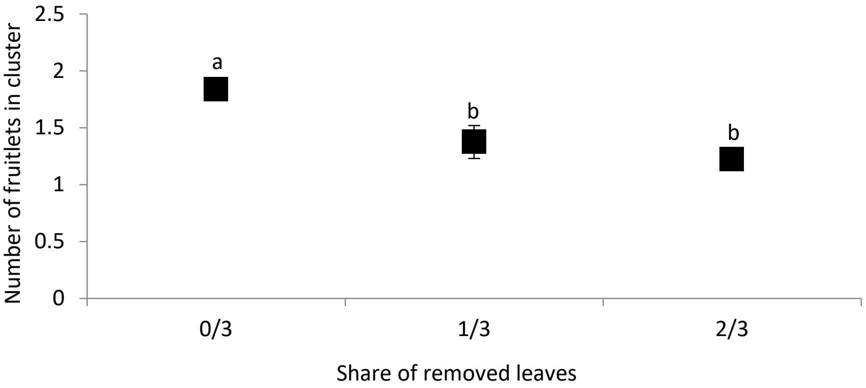

Fig. 2. Number of fruitlets in a cluster after June drop on branches on which 0, 1/3 or 2/3 spur leaves were removed at blooming. Average values and standard errors are presented. Different letters indicate statistically significant differences among the treatments at $p < 0.05$

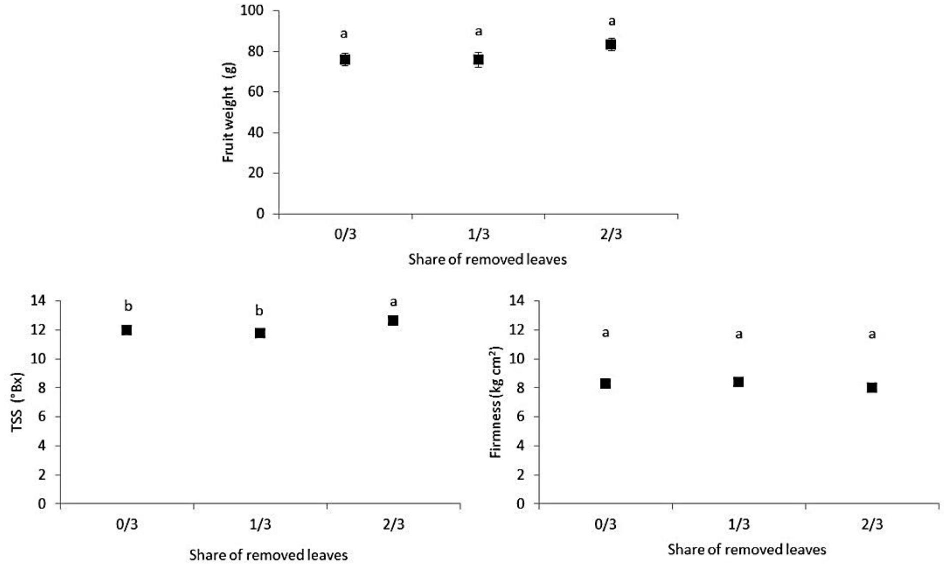

Fig. 3. Weight, total soluble solids (TSS) and firmness of mature apples from branches on which 0, 1/3 or 2/3 spur leaves were removed at blooming. Average values and standard errors are presented. Different letters indicate statistically significant differences among the treatments at $p < 0.05$

Table 1. Content levels of individual sugars (mg g^{-1} FW) and organic acids (µg g^{-1} FW) in apple fruits depending on treatment. Different letters indicate statistically significant differences among the treatments at $p < 0.05$

	Share of removed leaves			*p*-value
	0/3	1/3	2/3	
Malic acid	3724.3 ±256.6	3246.2 ±211.2	3714.1 ±133.1	n.s.
Citric acid	1137.6 ±97.7 a	792.6 ±41.2 b	1168.4 ±101.8 a	$p = 0.0132$
Shikimic acid	38.9 ±1.7 a	29.4 ±2.1 b	29.9 ±2.5 b	$p = 0.0009$
Fumaric acid	4.8 ±0.3 a	3.5 ±0.3 b	4.1 ±0.3 b	$p = 0.0113$
Sum of acids	4905.5 ±277.2 a	4071.7 ±200.5 b	4916.5 ±1283.2 a	$p = 0.0394$
Sucrose	7.3 ±0.5	9.2 ±1.1	9.9 ±0.6	n.s.
Glucose	26.3 ±0.5	24.2 ±1.0	23.7 ±1.0	n.s.
Fructose	62.0 ±0.5 b	63.7 ±0.6 ab	65.4 ±0.6 a	$p = 0.0024$
Sorbitol	1.8 ±0.1	1.9 ±0.1	2.1 ±0.1	n.s.
Sum of sugars	97.5 ±0.7	99.0 ±1.8	101.1 ±1.1	n.s.

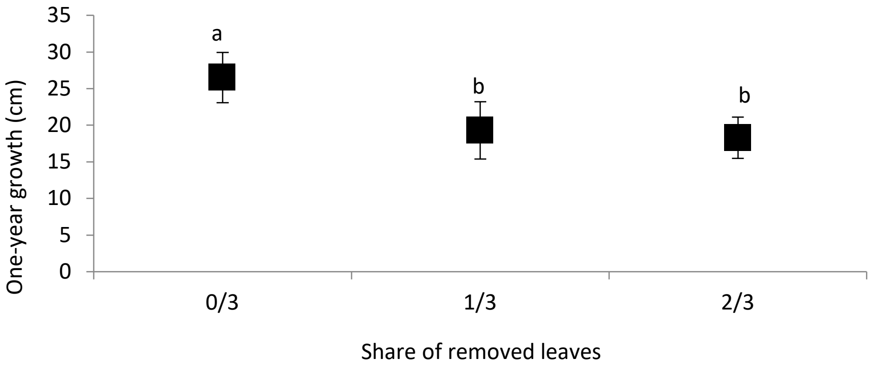

Fig. 4. One-year growth of brunches from branches on which 0, 1/3 or 2/3 spur leaves were removed at blooming. Average values and standard errors are presented. Different letters indicate statistically significant differences among the treatments at $p < 0.05$

Fig. 5. Dendrogram for analysed parameters of branches on which 0, 1/3 or 2/3 spur leaves were removed, using Ward's method based on square Euclidian distance

had accumulated less malic acid in comparison to the control [Antoine et al. 2016].

Changes in the source : sink ratio results in an altered sugar supply and fruit growth, as well as in fruit acidity [Etienne et al. 2013]. In peach and mango, a decrease in malate has been observed early after an increase in the source : sink ratio, followed by an increase near maturity. The opposite effect was observed for citrate content, with an increase after an increase in the source : sink ratio and a decrease near maturity [Wu et al. 2002, Lechaudel et al. 2005]. The accumulation of organic acids in fruits without removing leaves may therefore indicate less intensive respiration. In our research, this was not confirmed for apple fruit development, which may be because the processes involved in metabolism and accumulation of malic and citric acid in mesocarp cells are under both genetic and environmental control [Etienne et al. 2013].

Effect on annual shoot growth. In apple trees, spurs leaves first develop from a generative bud, then the flower cluster and later also bourse shoots might grow. In our study, annual growth of bourse shoots was measured and the results suggest that their final length also depends on the amount of partial leaf removal. On branches on which 1/3 or 2/3 of leaves were removed at bloom, the growth of one-year-old shoots was less than on branches on which no leaves were removed (Fig. 4).

On apple trees in which the source : sink ratio was increased by removing fruits, net photosynthesis was reduced [Veberic et al. 2003]. In our study, there was more intensive shoot growth in non-defoliated treatments, which indicates that growth was a consequence of the changed carbohydrate accumulation because of the changed source : sink ratio.

Leaves on lateral (bourse) shoots develop after bloom [Lakso and Goffinet 2013]. Initially, growing vegetative shoots do not send any assimilates, and they even get assimilates, and in this way, they can compete with fruitlets for carbohydrates. Additionally, extension shoot leaves first support the growth of shoots with carbohydrates and, when they have more than 12 leaves, begin to support fruit growth. For the last two thirds of the season, both bourse and shoot leaves support the fruit [Lakso and Goffinet 2013]. Because the growth of bourse shoots was less intensive and finished earlier, supporting the fruits was also earlier and did not influenced on lower fruit quality parameters in leaf removing treatments at harvest.

Similarities and discrepancies among treatments with removal of spur leaves were determined using hierarchical cluster analysis (Fig. 5). All investigated parameters (fruitlet shedding, sugar and organic acids content and shoot growth) were included in a combined cluster analysis as a similar dendrogram. As expected, the highest similarity was observed among treatments from branches in which 1/3 or 2/3 spur leaves were removed. The similarity among them may be connected to the interrupted flow of assimilates to other sinks than fruits on the branch.

CONCLUSIONS

A decreased number of spur leaves caused more intensive fruitlet abscission and poorer bourse shoot growth but had no negative influence on fruit size. These results show the potential use of decreasing spur leaf area to stimulate more intensive natural shedding to support fruitlet thinning of apple fruitlets.

ACKNOWLEDGMENTS

This work is part of the program Horticulture P4-0013-0481 supported by the Slovenian Research Agency (ARRS).

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