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EFFECT OF TWO GIRDLING DATES ON CARBOHYDRATE ACCUMULATION IN PLANT TISSUES AND FRUIT QUALITY OF BARBERRY

Mina MOQHADDAM*, Zeinab AREFNEZHAD, Mehdi KHAYYAT
University of Birjand, Iran

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ABSTRACT

The present study was designed to investigate the effects of two girdling dates (late April and early September) on fruit quality and carbohydrate accumulation in barberry shrubs (*Berberis vulgaris* L.) during the years 2016–2017. One year old shoots were ringed and fruit characteristics were monitored for two continues year. Data showed that the girdling caused a significant increase in the ascorbic acid content in fruit. Similarly, anthocyanin and total acidity were affected by girdling compared to control in 2017. September-girdled shrubs had the highest amount of fresh mass but the dry mass was not changed. It was shown that chlorophyll b increased in the girdled shrubs compared to control. September girdling reduced the carbohydrate content in leaves. In contrast, it increased the carbohydrate content in shoots. In addition, there was a significant difference between starch in the shoot and starch in leaf. Owing to the obtained results, early September girdling caused a broad range on fruit quality.

Keywords: starch, chlorophyll, anthocyanin, ringing, seedless barberry

INTRODUCTION

Seedless barberry (*Berberis vulgaris* L.), belongs to the Berberidaceae family that grows in the mountainous north-east of Iran, Khorasan (Fatehi-Hassanabad et al. 2005). Barberry is one of the plants that contain berberine and has had a long history in eastern and western medical tradition (Chevallier 2001), and it is still used in northern Europe (Fatehi-Hassanabad et al. 2005). Bark, root, rhizomes, stem, leaf, and fruit are used in medicine (Arayne et al. 2007).

The removal of a ring of phloem (girdling, ringing) is a horticultural practice that results in an accumulation of carbohydrates and used to influence tree growth and development and fruit growth, especially in citrus, grape, peach, and other fruit tree crops (Roper & Williams 1989; Schaper & Chacko Csiro 1993; Goren et al. 2004). According to the result of some preliminary experiments, the timing of treatment is important (Noel 1970; Priestley 1976).

Ringing interrupts the phloem pathway and consequently change the pattern of distribution (Mason & Maskell 1928). By girdling, the flow of sap stops, carbohydrates and starch accumulate above the girdle, and also in production and translocation of certain plant hormones are arrested (Davie et al. 1995). Moreover, it influences the metabolic activity of shrubs, primarily by increasing carbohydrate accumulation above the girdle and reducing the sink strength for photosynthate below the girdle (Martin et al. 1994; Nordgren et al. 2003). In addition, reduction in stomatal conductance (gs) and increase in leaf water potential have been observed in girdled shrubs (Williams et al. 2000). Trunk girdling is one of the many options to control excessive shoot growth and to accelerate fruit maturation.

Girdling has been widely used in many fruit tree crops, mainly to increase flowering, fruit set and fruit size (Goren et al. 2004). Horticultural effects of girdling are a lot. As example, all stages of reproductive organs, development, flowering, fruit

*Corresponding author:
e-mail: moghaddam.mina222@gmail.com; yas.aref91@gmail.com; mhdkhayyat@gmail.com

set and in many instances fruit maturity and quality are influenced by girdling. However, the mechanism(s) through which girdling operates are not yet fully understood (Janick 2003). Khandaker et al. (2011) reported that ringing could improve yields and physical and chemical properties of fruit. Zhao et al. (2013) reported that girdling in mid-May increased ascorbic acid and decreased titratable acid in apple. It has been reported that ringing increased total phenolic content in peach fruit (Kubota et al. 1993) and increased the level of anthocyanin in the berry skin of crimson seedless grapes (Brar et al. 2008). In citrus, girdling a few weeks before flowering increased leaf chlorophyll content and carbohydrate concentration in various flowering and vegetative shoots (Rivas et al. 2008). Girdling date did not significantly affect fresh mass in persimmon (Choi et al. 2010). Delayed maturation of the fruit manifesting in the late change of color to red late fruit-color change in barberry and also low fruit quality are the most important problems related to barberry. So, the aim of this study was to investigate the effect of two girdling dates on the accumulation of anthocyanin and the quality of fruit in barberry.

MATERIAL AND METHODS

This study was conducted in the research orchard of the Faculty of Agriculture, University of Birjand (Iran) in 2016–2017. Similar 50 year-old shrubs were spaced 3 m in rows and 3 m between rows were selected as plant materials. The 45 one-year-old shoots with the same length (45 ± 5 cm), diameter, and productivity were tagged for future treatments. On each shrub, three shoots were chosen. The 2-mm width ringing was performed in late April and early September at the base of each shoot. Generally, ringing was performed on same shrubs in 2016 and 2017. Fruits were harvested on mid-October and separately juiced for chemical measurements by hand.

Ascorbic acid content was determined by indophenol method, and the value was expressed as milligram of ascorbic acid per 100 ml of juice (Ranganna 1977).

The total anthocyanin content of juice was determined by the pH-differential method using two buffer systems consisted of potassium chloride (pH 1, 0.025 M) and sodium acetate (pH 4.5, 0.4 M). One milliliter of juice sample was mixed with 10 mL of buffer, and the absorbance (A) was measured at 510 and 700 nm using a Unico 2100 spectrophotometer (Wagner 1979).

Total acidity (TA) was determined by titration of 1 ml of juice with 0.2 M of NaOH, and the results were calculated as a percentage of citric acid (Cochran et al. 1986).

The total phenolics of juice was determined by Folin-Ciocalteu method at a wavelength of 725 nm and expressed as a percentage of gallic acid (Chuah et al. 2008).

Chlorophylls in leaves were determined in May, June, and October. About 0.025 g of leaves was homogenized in 5 ml of 80% acetone. The samples were stored for 24 h in a fridge, and then the absorbance was read at 470, 645, and 663 nm by Unico 2100 spectrophotometer (Lichtenthaler 1987).

Fresh mass of 100 berries was measured. To determine the dry mass, 100 berries were oven dried at 70 °C for 72 h and dry mass was recorded.

Shoots and leaves samples were taken from the shrubs at late-May and late-October. Shoots and leaves were separately oven dried at 50 °C for 70 h to determine total carbohydrate. Total carbohydrate was determined according to the anthrone method by Unico 2100 spectrophotometer (Mocready et al. 1950) and the starch amount was determined using colorimetric method (Magel 1991).

The trial was conducted as completely randomized block design with three treatments and five replications. In each replication, 3 shoots were chosen for sampling. The treatments included were (1) control shrubs (the non-girdled shoots), (2) girdled shoots in late April, and (3) girdled shoots in early September. Obtained data were analyzed by Genstat Ninth Edition, and mean values were compared at the level of 5% according to LSD test. Obtained results in 2016 and 2017 were analyzed separately.

RESULTS AND DISCUSSION

Fresh mass was not significantly affected by girdling (2016) (Table 1). September girdling had the highest fresh mass (38.2 g) in 2017 and the lowest (16.1 g) in 2016 by control treatment.

Fruit mass is an important quality parameter of fruit production. Bark ringing or girdling significantly increased the fruit mass as well as yield (Sharif et al. 2007). Girdling can improve carbohydrate availability to fruits and as a consequent lead to an increase in fruit-set and yield as well as a number of fruits (Goren et al. 2004; Rivas et al. 2004). Our results are in agreement with the findings of Mostafa & Saleh. (2006), who reported that girdling increased the fruit mass in balady mandarin orange. Dry mass was not significantly affected by girdling in 2017.

Generally, girdling significantly increased the ascorbic acid content in fruits (Table 1). The highest ascorbic acid content was obtained in the second girdling in September 2016. In the following year (2017), both girdling dates (April and September) significantly increased the ascorbic acid content in fruits. The effect of girdling time on ascorbic acid was significant in 2017. Cultural practices such as girdling determine the crop load and fruit size, which can influence the nutritional composition of fruits (especially nitrogen) and may indirectly affect the vitamin C content (Lee & Kader 2000). Zhao et al. (2013) stated that vitamin C content in apple increased with girdling.

In the first year, anthocyanin was not affected by girdling, but both April and September girdling significantly increased anthocyanin content in the second year. The lowest anthocyanin content (69 mg·dm^{-3}) was observed in September 2016 (Table 1). Anthocyanin pigments are responsible for the red, purple, and blue colors of many fruits and also they have possible health benefits as dietary antioxidants (Ronald & Wrolstad 2001). Girdling accelerated ripening and also had positive effects on anthocyanin accumulation in the fruits (Khandaker et al. 2011). Accumulation of anthocyanin was also reported in girdled grape by El-Hammady and Abd-El-Hamid (1995). Girdling increased significantly TA compared with control in 2017 (Table 1).

It was reported that girdling of Italia grape at the beginning of ripening significantly reduced titratable acidity (Carreño et al. 1998). However, the opposite response was found with girdling in apple (Arakawa et al. 1998).

Content of total phenolics in fruits with average value of 64.5 was not influenced by girdling (Table 1) that was disagreement with the result obtained in peach (Kubota et al. 1993).

There were no significant differences in chlorophyll a content among different sampling time. The lowest amount of chlorophyll b (4.59) and the ratio of chlorophyll b to chlorophyll a (0.306) were observed in control.

The effect of girdling was significant on chlorophyll a content, chlorophyll b content, and the ratio of chlorophyll b to chlorophyll a contents. Girdling in April did not increase the chlorophyll a and chlorophyll b. The amount of chlorophyll a content, chlorophyll b content, and the ratio of chlorophyll b to chlorophyll a ratio were highest in September girdling when compared to those observed for the first time in April (Table 2).

Table 1. The effect of girdling on fruit characteristics of barberry separately for each year of investigation

Girdling date	F.m. of 100 berries (g)	D.m. of 100 berries (g)	Ascorbic acid (mg·100 ml^{-1})	Anthocyanin (mg·dm^{-3})	Total acidity (g·100 ml^{-1})	Total phenolics (mg gallic acid·g^{-1} DM)
2016						
Control	16.1a ± 3.4	-	1500b ± 623	145a ± 36.5	55.4a ± 11.9	-
April girdling	16.8a ± 3.1	-	1500b ± 661	142a ± 31.2	39.3a ± 15.4	-
September girdling	19.1a ± 3.2	-	2167a ± 597	169a ± 29.8	46.5a ± 15.1	-
2017						
Control	24.5c ± 4.2	3.6a ± 0.7	840c ± 353.5	81b ± 134.0	32.0c ± 8.1	64.7a ± 0.007
April girdling	33.8b ± 1.2	4.1a ± 0.3	1119a ± 353.5	122a ± 31.1	41.5a ± 11.1	64.5a ± 0.003
September girdling	38.2a ± 0.8	4.5a ± 1.0	1095b ± 204.1	124a ± 26.6	39.1b ± 12.1	64.4a ± 0.0006

In each column, separately for year of investigation, means with the same letter are not significantly different at 5% level of probability using LSD

Table 2. The effect of girdling and sampling time on leaves chlorophyll of barberry (2017)

Effect		Chl a ($\mu g \cdot cm^{-3}$)	Chl b ($\mu g \cdot cm^{-3}$)	Chl b/Chl a
Sampling Time				
May		14.0a ± 0.6	4.6b ± 0.8	0.31c ± 0.05
June		13.1a ± 1.02	5.0a ± 0.5	0.38b ± 0.02
October		13.8a ± 0.9	6.0a ± 0.3	0.44a ± 0.01
Treatment				
Control		14.3a ± 0.7	5.6b ± 0.4	0.39a ± 0.01
April girdling		10.7b ± 0.6	3.0c ± 0.6	0.29b ± 0.05
September girdling		15.8a ± 0.5	7.0a ± 0.2	0.44a ± 0.0007
Treatment	Sampling time			
Control	May	14.24a ± 0.7	6.0a ± 1.0	0.42a ± 0.04
	June	13.75a ± 1.7	4.5b ± 0.1	0.34b ± 0.03
	October	14.95a ± 1.8	6.3a ± 0.6	0.42a ± 0.01
April girdling	May	11.86a ± 0.5	0.8c ± 0.8	0.06c ± 0.06
	June	9.70a ± 1.6	3.7b ± 1.1	0.35a ± 0.06
	October	10.63a ± 0.9	4.7b ± 0.5	0.45a ± 0.03
September girdling	May	15.79a ± 1.1	7.0a ± 0.4	0.44a ± 0.001
	June	15.79a ± 1.1	7.0a ± 0.4	0.44a ± 0.001
	October	15.79a ± 1.1	7.0a ± 0.4	0.44a ± 0.001

In each column means with the same letter are not significantly different at 5% level of probability using LSD

Accordingly, in our study, girdling increased the leaf chlorophyll b content in both the years. Several authors have proposed that total leaf carbohydrate content and starch increased as a result of girdling (Rivas et al. 2008). It may be due to the accumulation of chlorophyll content and increased photosynthesis in the girdled branch (Khandaker et al. 2011).

The highest amount of chlorophyll b was recorded in second girdling treatment on three sampling time, whereas the lowest amount was obtained during the first girdling. The ratio of chlorophyll a to chlorophyll b was not significantly different from other treatments during the sampling, but the lowest value was observed in the control and second girdling on second and third sampling time (Table 2).

Total carbohydrate content in leaves was significantly affected by ringing. The highest significant carbohydrate content of leaves was obtained in girdled shrubs at April (Table 3). Also, the carbohydrate content in shoots at harvest time showed highly significant differences because of treatments. Removal of a strip of phloem from the main trunk by girdling actually blocks the transport of sugars to the roots; large amounts of carbohydrates produced by photosynthesis will accumulate in vegetative organs above the girdle or be used for fruit development. Girdling has been shown to increase the carbohydrate concentration above the girdle in *Vitis vinifera* L. (Roper & Williams 1989).

Table 3. The effect of girdling on total carbohydrate content of barberry leaves and shoots (2017)

Girdling date	Tissue type	
	leaves ($mg \cdot g^{-1}$ DM)	shoots ($mg \cdot g^{-1}$ DM)
Control	25.6a ± 0.9	3.3b ± 0.6
April girdling	43.9a ± 1.2	0.1b ± 0.6
September girdling	14.9b ± 0.8	49.4a ± 2.1

Note see Table 2

There were significant differences between starch in the shoots (0.212) and starch in leaves (0.226) (Table 4), but the interaction of treatment and tissue type on starch did not differ significantly compared to control (Table 5). Girdling affected the activity of key enzymes involved in carbohydrate metabolism of the growing apple and decreased the starch level (Berüter & Feusi 1997). Li et al. (2003) reported that concentration of starch in girdled tree leaves was thrice that of control tree leaves and in girdled tree bark, it was twice that of control tree bark.

Table 4. The effect of girdling on starch of barberry (2017)

	Starch (DM %)
Girdling date	
Control	0.217a ± 0.003
April girdling	0.224a ± 0.008
September girdling	0.217a ± 0.003
Tissue type	
Leaves	0.226a ± 0.005
Shoots	0.212b ± 0.0008

In each column means with the same letter are not significantly different at 5% level of probability using LSD.

Table 5. The effect of treatment and tissue type on starch in barberry (2017).

Girdling date	Tissue type	Starch (DM in %)
Control	Leaves	0.223a ± 0.006
	Shoots	0.211a ± 0.0007
April girdling	Leaves	0.234a ± 0.017
	Shoots	0.215a ± 0.002
September girdling	Leaves	0.222a ±0.005
	Shoots	0.212a ±0.001

Note see Table 1

CONCLUSION

However, the effect of girdling on plant is not clear but it has been proved that simple action such as ringing causes changes in fruit characteristic. So, it must be considered as an option for better management of orchards. According to our study, girdling in September resulted in a stronger effect on fruit quality of barberry plant. Hence, late girdling would be more beneficial for improving fruit quality.

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