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Research Article

Effect of Leaf-to-Husk Ratios on Carbohydrate, Yield and Quality in Chinese Chestnut (*Castanea mollissima* Bl)

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Abstract: To study the influence of different leaf-to-husk ratios to the yield, fruit quality (starch, soluble sugars, proteins and fats.) and carbohydrate in different organs (foliages, stems, cupules and ovaries) of Chinese chestnut (*Castanea mollissima* Bl.) trees, 3 levels were set up with different leaf-to-husk ratios, which was 5:1, 10:1 and 15:1. The results indicated that the contents of fructose, glucose, inositol, sucrose, xylose, arabinose and sum of individual sugars, differed significantly among different organs of the different treatments. The leaf-to-husk ratios of 15:1 had the higher yield, higher total soluble sugar, starch and protein and lower fat concentrations. Low leaf-to-husk ratio influenced the accumulation of starch and the development of the ovary of Chinese chestnut.

Keywords: Carbohydrates, *Castanea mollissima* Bl, leaf-to-husk ratio, quality, source-sink relationships, yield

INTRODUCTION

Carbohydrate plays an important role in yield and quality. It supplies the required energy for the growth of fruit (Myers and Kitajima 2007). With the development of scientific research, application of sink-source theory to guide practice receiving widespread attention (Jones *et al*., 1996). Several reviews have covered different aspects of sink-source relations, such as the control of leaf-to-fruit ratio or the regulation of photosynthetic machinery and sink system (Khan *et al*., 2007; Wu *et al*., 2008; Yasumura, 2009; Kang *et al*., 2010). The sink-source theory believed that output of plants not only depends on the efficiency of photosynthesis, translocation of assimilates and formation of active sinks and depends on the intensity and size. It is important to understand the response of trees with appropriate sink-source relationship. The small source is difficult to achieve a high yield and the sink-source uncoordinated, as a result of the library feedback regulation of the source, similarly is hard to obtain the extraordinary production. Leaves have a significant effect on yield responses (Lawlor, 2001), fruit growth is regulated by a critical leaf number for the greatest photosynthetic capacity and most efficient metabolism (Lone *et al*., 2008). Fruit is one of the most important sinks that require a continuous supply of building materials (Proietti, 2000; Hoch, 2007). Fruit yield increased proportionally to the number of fruits, but the fruit weight was not affected, as long as the fruit load was below the threshold level (Heuvelink, 1997; Marcelis, 1992). In other fruit trees, which with high fruit loads were partitioned primarily to fruits, whereas those with low fruit loads were to permanent tree parts (Lenz, 2009; Proietti *et al*., 2006; Claudia *et al*., 2011). Adjusting leaf-to-fruit ratio is one of the significant elements to produce the highest fruit quality fruits. Hence, sink-source may be manipulated by different levels of leaf-to-fruit ratios on branches (Famiani *et al*., 2000).

The Chinese chestnut (*Castanea mollissima* Bl.) is a major commercial nut-bearing tree (Donis-Gonzalez, 2008). The interval between flowering and harvest can last more than four months. In chestnut growing the main problem is the high percentage of empty cupules, resulting in not satisfactory yields. About the causes of this phenomenon not much is known. Many studies have been performed on the cultivation of Chinese chestnut in order to enhance economic output. Shi and Stosser (2004, 2005) studied the reproductive biology, the flower bud differentiation and pollen tube growth in vim of Chinese chestnut.

The most common reserve carbohydrate in Chinese chestnut fruits is starch, although other carbohydrates, such as glucose, inositol, sucrose have also been shown to be mobilized within the plant and utilized as sources of energy. In particular, very few information has been conducted on the effects of leaf-to-husk ratio on carbohydrates, yield and quality characteristics in Chinese chestnut, which would be very useful for a better understanding of the biological and physiological processes for optimising cultural practices, particularly pruning. For these reasons, a study of the effects of different leaf-to-husk ratios of shoots on Chinese

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chestnut fruit growth were investigated, in order to obtain more information on relationships between leaf area and fruit growth and related processes such as carbohydrates of assimilates within the tree and regulation of the yield and quality.

MATERIALS AND METHODS

Plant material and growing conditions: The experiments were conducted in 2011 and 2012 at Qianxi, Hebei Province, China. Several 12-year-old Chinese chestnut trees, spaced 3×3 m apart and trained to a perpendicular V system were selected for this experiment. All trees received conventional care and had a good balance between vegetative and reproductive activities. At the both years, twenty days after full bloom (last-June), defoliation or fruit thinning were performed to give leaf-to-husk ratios of 5:1, 10:1 and 15:1 was performed on the shoots. Un-thinned Chinese chestnut trees in the same rows served as control.

Carbohydrate analyses: The carbohydrate types and the content in different organs were examined during the development of fruit stage. The carbohydrate was determined according to the method of Tan *et al*. (2010) on an GC-MS system (Trace-GC Ultra) connected to a Trace-DSQ mass selective detector with EI ionization of full scan and selected ion monitoring (Thermo-Finnigan, USA) at the College of Biological Sciences and Biotechnology, Beijing Forestry University.

Measurement of yields and qualities: At harvest, the fruit rate, single cupule weight, single kernel weight and yield were determined. Moreover, soluble carbohydrate, proteins, fats and starch contents were also evaluated. Twenty fruits per branch were collected randomly for the evaluation of parameters of yield and ten fruit per branch were collected randomly for internal quality.

RESULTS AND DISCUSSION

Effect of leaf-to-husk ratio on carbohydrate: Different leaf-to-husk ratio resulted in significantly decreased the contents of fructose and inositol in the leaves of the fruit-bearing shoot of Chinese chestnut. The decreased amplitude of fructose is up to about 30%. No significant effect was observed among different treatments. At the leaf-to-husk ratio of 5:1 and 15:1, the inositol was not statistically significant. Content of glucose in leaves was highest in the ratio of 5:1, compared with control increased 32.94% and the lowest in the ratio of 15:1. There were significant differences between treatments in concentrations of sucrose in the leaves of Chinese chestnut. Content of sucrose is highest at the ratio of 10:1, 11 times as big as control. The sum of sugars has the highest level at the ratio of 10:1(Table 1). It can be seen, there was a significant effect of the leaf-to-husk ratio on leaf carbohydrate concentration.

The content of fructose tended to be higher in stem on fruit-bearing shoots with low leaf-to-husk ratios. Glucose concentrations in stem at the leaf-to-husk ratio of 5:1 and 10:1 were significantly higher than that of control. Compared to the control, glucose in stem was significantly reduced only by treatment 15:1 (Table 2). The content of inositol in stem was highest at the leaf-to-husk ratio of 10:1, compared with control increased 41.55%. Different levels of leaf-to-husk ratio reduced the contents of sucrose and total sugar in stems (Table 2).

The content of fructose and glucose tended to be lower in cupules with low leaf-to-husk ratios. Inositol

Table 1: Effect of leaf-to-husk ratio on the carbohydrate in leaves of Chinese chestnut

leaf-to-husk ratio	Fructose (ng/mg)	Glucose (ng/mg)	Inositol (ng/mg)	Sucrose (ng/mg)
5:1	55.68b	204.59a	254.79c	120.49b
10:1	58.35b	170.23b	322.05b	220.16a
15:1	60.55b	122.38c	242.37c	98.790c
CK	82.15a	153.79b	387.53a	20.950d

Different letters indicate significantly difference values ($p<0.05$)

Table 2: Effect of leaf-to-husk ratio on the carbohydrate in stems of Chinese chestnut

leaf-to-husk ratio	Fructose (ng/mg)	Glucose (ng/mg)	Inositol (ng/mg)	Sucrose (ng/mg)
5:1	116.55a	255.25a	516.31b	578.620c
10:1	94.440b	244.87a	684.43a	701.790b
15:1	102.36b	25.250c	544.05b	586.140c
CK	30.07 0c	106.17b	483.74c	1949.03a

Different letters indicate significantly difference values ($p<0.05$)

Table 3: Effect of leaf-to-husk ratio on the carbohydrate in husks of Chinese chestnut

leaf-to-husk ratio	Fructose (ng/mg)	Glucose (ng/mg)	Inositol (ng/mg)	Sucrose (ng/mg)	Xylose (ng/mg)	Arabinose (ng/mg)
5:1	14.78c	58.19b	162.74a	91.12b		
10:1	15.28c	63.78b	157.74a	155.19a		
15:1	24.74b	99.42a	133.25b	70.020c		
CK	36.99a	92.52a	114.01c	49.470d	1.89	0.65

Different letters indicate significantly difference values ($p<0.05$)

Table 4: Effect of leaf-to-husk ratio on the carbohydrate in ovaries of Chinese chestnut

leaf-to-husk ratio	Fructose (ng/mg)	Glucose (ng/mg)	Inositol (ng/mg)	Sucrose (ng/mg)
5:1	69.84 0d	880.22c	1057.12b	3223.66a
10:1	600.24a	787.06d	959.510c	2339.25b
15:1	547.25b	935.27a	1086.00a	233.42 0c
CK	534.82c	898.57b	964.780c	120.970d

Different letters indicate significantly difference values (p<0.05)

Table 5: Effect of leaf-to-husk ratio on yields

leaf-to-husk ratio	Fruit rate (%)	Single cupule weight (g)	Single kernel weight (g)	Yield (kg/hm^2)
5:1	25.60c	27.58c	5.75d	2529d
10:1	29.18b	34.72b	6.08c	2659c
15:1	34.71a	39.03a	8.12a	2888a
CK	33.30a	39.92a	7.73b	2798ab

The values reported are the average of 10 observations; Values with different letters are significantly different at p = 0.05

Table 6: Effect of leaf-to-husk ratio on starch

leaf-to-husk ratio	Amylose content (mg)	Amylopectin content (mg)	Starch content (mg)
5:1	14.94c	20.04c	34.98c
10:1	16.82b	26.16b	42.98b
15:1	18.64a	31.50a	50.13a
CK	18.52a	31.67a	50.19a

The values reported are the average of 10 observations; Values with different letters are significantly different at p = 0.05

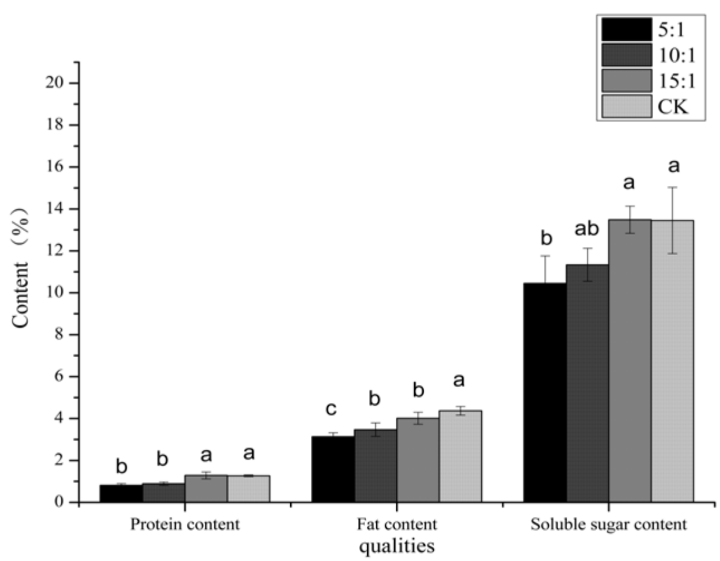

Fig. 1: Effect of leaf-to-husk ratio on fats, proteins and soluble sugars

concentrations in cupules were not significantly different, in the low and medium leaf-to-husk ratio treatments, but 42.74% and 38.36% higher than that of control. Inositol concentrations in cupules was highest at the leaf-to-husk ratio of 10:1. Xylose and arabinose only appear in the control (Table 3). Leaf-to-husk ratio at 10:1 resulted in the highest fruit increment in cupules of Chinese chestnut.

Compared to the control, fructose concentration in ovary was significantly reduced almost 90% of the control under the leaf-to-husk ratio of 5:1 (Table 4). Contents of glucose and inositol were highest in ovary at the leaf-to-husk ratio of 15:1 and lowest at the leaf-to-husk ratio of 10:1. There was a significant effect of the leaf-to-husk ratio on the concentrations of sucrose. The sucrose concentrations of ovary were 27 times than control at the leaf-to-husk ratio of 5:1. The result indicates that low leaf-to-husk-ratio was beneficial to the accumulation of soluble sugar in chestnut ovary.

Effect of leaf-to-husk ratio on yields: The fruit rate and single cupule weight was not significantly different compared to the control. At the leaf-to-husk ratios of 15:1, the Fruit rate and single cupule weight at the leaf-to-husk of 10:1 were significantly lower than that of control. The fruit rate and single cupule weight, reduced 23.12 and 30.94%, respectively at the rate of 5:1 (Table 5). The highest Leaf-to-husk ratio (15:1) resulted in a significantly higher single kernel weight and yield, compared to other treatments. The result showed that the nut size and yield were affected by the ripening stages, which were influenced by lower leaf number.

Effect of leaf-to-husk ratio on qualities: There was not a significantly effect at the leaf-to-husk ratio of 15:1 on the concentrations of amylose, amylopectin and total starch. At the leaf-to-husk ratio of 5:1, the concentrations of amylose, amylopectin and total starch in fruits were significantly lower than that of control and total starch reduced by 30.30% (Table 6). It is showed that low leaf number affects the accumulation of starch.

There was a significant effect of the leaf-to-husk ratio on the concentrations of soluble sugars, proteins and fats (Fig. 1). Soluble sugar and protein concentrations were not significantly different, but fat concentrations were lower, in the high leaf-to-husk ratio treatments, when compared to the control. The leaf-to-husk ratio of 5:1 resulted in the lowest increment of soluble sugars, proteins and fats. Thus, low leaf fruit number influences the index of fruit quality, seriously.

CONCLUSION

In conclusion, assimilates can be easily translocated and it is necessary to have about 10 leaves per husk to ensure normal fruit development, but the 15:1 mode is the most reasonable one. At the leaf-to-husk ratios of 10:1 indicate an easy accumulate more sucrose and soluble sugar in reserve tissues for fruiting in the following year. More soluble sugar accumulation in stems and against the sugar transport to cupules and ovaries at a high leaf-to-husk ratios. Contents of inositol were highest in leaves and cupules. The sucrose concentration was highest in stem. Sucrose concentrations were significantly higher in leaves, cupules and ovaries at the leaf-to-husk ratio of 15:1 and control, when compared to the leaf-to-husk ratio of 5:1 and 10:1. The results showed that the increased assimilate supply to fruit resulting from an increased leaf-to-husk ratio, low leaf number against the accumulation of starch and influence the development of ovary. Quality of less developed fruit from trees with low leaf-to-fruit ratios was significantly lower than that of more mature fruit grown on trees with high leaf-to-fruit ratios (Usenik *et al*., 2010; Choi *et al*., 2011). It seems that higher leaf-to-fruit ratio contributes to better assimilate supply of fruit, faster fruit development and better fruit quality. Various populations of leaves play an important roles in supplying carbohydrates to developing fruits (Lang, 2001; Vaast *et al*., 2005). Therefore, when the tree is able to guarantee a large amount of assimilates it is possible to reach a good fruit size need 15 leaves per husk. Setting leaf-to-husk ratio reasonably the fruits of Chinese chestnut should be absorb more nutrients, raised the rate of fruit set availably, which is in agreement with previous studies (Poiroux-Gonord *et al*., 2012; Choi *et al*., 2012; Iwanami *et al*., 2012). Unilaterally magnify the effects of sink or source is not true. The high yield and quality of Chinese chestnut must take care of the harmony with sink-source characteristics.

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