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Analysis of walnut fruit quality based on source-sink relationships

Shiwei Wang, Cuifang Zhang, Cunde Pan, Shanchao Zhao*

Education Department of Xinjiang, Collage of Forestry and Landscape Architecture, Xinjiang Agricultural University/Key Laboratory of Forestry Ecology and Industry Technology in Arid Region, Urumqi, Xinjiang, P.R. China

**Corresponding author: zcf851022@163.com*

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Abstract: In the paper, we investigate the relationship between the walnut fruit quality and the leaf-to-fruit ratio (LFR). The LFR had a significant effect on the physical quality of walnuts ($P < 0.05$), and the fruit volume, fruit fresh weight, fruit dry weight, nut dry weight and kernel dry weight increased with an increasing LFR. However, the LFR had no significant effect on the crude protein and crude fat kernel contents ($P > 0.05$). The number of cells per unit area and the cross-sectional area of the green husk cells with 5L : 1F were significantly higher than those with 1L : 3F and 2L : 3F ($P < 0.05$). The number of cells per unit area of the kernel with 1L : 3F and 2L : 3F was significantly higher than that with the other LFRs ($P < 0.05$). There was no significant difference in the NDW between the natural and girdled fruit-bearing shoots with 2L : 1F ($P > 0.05$). We concluded that the high carbohydrate availability with the high LFR augmented the fruit size by increasing the number of cells in the green husk and kernels. There was no change in the crude protein and crude fat contents in the kernels, possibly due to the proportion of the sugar to the fat distribution not affected by the LFR. Two leaves with good light were necessary to ensure the normal growth and development of one walnut fruit on girdled fruit-bearing shoots.

Keywords: *Juglans regia*; leaf-to-fruit ratio; nut; crude fat; physical quality

The source-sink relationship is an important factor that affects patterns of the light energy capture and photosynthate distribution (Smith et al. 2018). In the process of fruit growth and development, the accumulation and mutual transformation of organic nutrients in a fruit are based on the continuous input of leaf-derived carbohydrates (Hernandez-Santana et al. 2021), which are unloaded into the fruit cells to further combine to form the organic parts in the fruit. Therefore, the fruit quality and economic yield of fruit trees depend on the source-sink interaction. Most researchers regard the leaf-to-fruit ratio (LFR) as the most important indicator for measuring the coordination of the plant source-sink relationships, and define the LFR as the ratio of the number of leaves to the number of fruits (Peng et al. 2012).

Researchers believe that changes in the source-sink relationship have a greater influence on the fruit size than on the fruit quality or any other characteristic. Previous studies have confirmed a positive correlation between the single fruit weight and the LFR (Almanza-Merchán et al. 2011; Choi et al. 2011; Fischer 2012; Baïram et al. 2019). Does a high LFR control the fruit size by regulating the cell size or cell number? Studies on the cell structure have shown that the final size of the fruit

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was mainly determined by the size of the cells, while others thought it depended on the number of cells (Higashi et al. 1999). Many studies have reported the early control of carbohydrate competition during cell division. For example, insufficient light at the flowering stage completely arrests the mitotic activity and causes inflorescence abortion. However, under favourable light conditions, only the distal flower buds are underdeveloped or shed (Nasrallah 1989). The fruit size also largely depends on the distribution of carbohydrates after the cell division (Zamski, Schaffer 1996), which is affected by the temperature (Walker, Ho 1997), fruiting branch density, and fruit number (Heuvelink 2015).

However, there are differing opinions regarding the relationship between the fruit chemical quality and the LFR. Some results showed that the source-sink relationship can affect the chemical quality of the fruit. For example, a high fruit load could reduce the total sugar and phenol contents and the sugar : acid ratio in grapes (*Vitis vinifera* Linn.) (Zhang et al. 2016), it could also reduce the soluble sugar content in persimmons (*Diospyros kaki* Thumb.) (Choi et al. 2011). Moderate fruit thinning can increase the soluble solid and sugar contents, and significantly increase the sucrose content in satsuma mandarins (*Citrus unshiu* Mark) (Kubo et al. 2001). An elevated LFR can improve the dry matter accumulation (Léchaudel et al. 2004) and solid : acid ratio in mangoes (*Mangifera indica* L. cv. 'Keitt') (Du et al. 2011), significantly increase the total soluble sugar content, and reduce the starch and titratable acid contents in pears (*Pyrus pyrifolia* 'Xueqing') (Xu 2015). However, there are also studies that report that the source-sink relationship has no significant effect on the characteristic components of the fruit quality, for example, an increased LFR had no significant effect on the oil content of *Camellia oleifera* kernels (Yuan et al. 2015). The contents of the sugar, acid, and mineral elements in kiwifruit is not affected by the LFR (Fang et al. 2001).

Therefore, to deepen the understanding of the relationship between the walnut fruit quality and the LFR, and provide a theoretical basis for improving the fruit yield and quality of walnut nuts, *Juglans regia* 'Xinxin2' was used as an experimental material to monitor the fruit growth and development, quality and its cellular structure on girdled fruit-bearing shoots with different LFRs through artificial regulation.

MATERIAL AND METHODS

Experimental site and plant material. Our study was conducted in an experimental walnut orchard situated in southern Xinjiang, China (41°1106.31'–41°1247.74'N, 79°1212.76'–79°1357.87'E; 1 394 m a.s.l.), in 2019. The uniform 10-year-old walnut trees (*J. regia* 'Xinxin2') used in these experiments grew at a distance of 5 m × 6 m along east–west oriented rows. The observed trees were sown and planted in 2010 and grafted in 2011. The rootstock was *Juglans regia*. The average crown width of the trees was five metres.

Sink-source manipulation. After the fruit set, the defoliation, girdling, and de-fruiting were performed on the sun-exposed shoots with fully expanded leaves and developing fruits on the south side of 45 trees. Fifteen fruit-bearing shoots per tree were modelled using one of the following 15 LFR value treatments (1L : 1F, 1L : 2F, 1L : 3F, 2L : 1F, 2L : 2F, 2L : 3F, 3L : 1F, 3L : 2F, 3L : 3F, 4L : 1F, 4L : 2F, 4L : 3F, 5L : 1F, 5L : 2F, and 5L : 3F) (Figure 1).

Determination of fruit quality. Fruit samples were collected 45, 70, 90, 110 and 130 days after the full bloom of female flowers (DAF). In each LFR, eight sample plants were randomly selected as repeats and three fruits were randomly selected from each plant. The fruit samples were collected from the girdled fruit-bearing shoots in the south of the canopy about 1.5 m from the ground. The fruit fresh weights (FFWs) were measured using an electronic balance and then the fruit volume

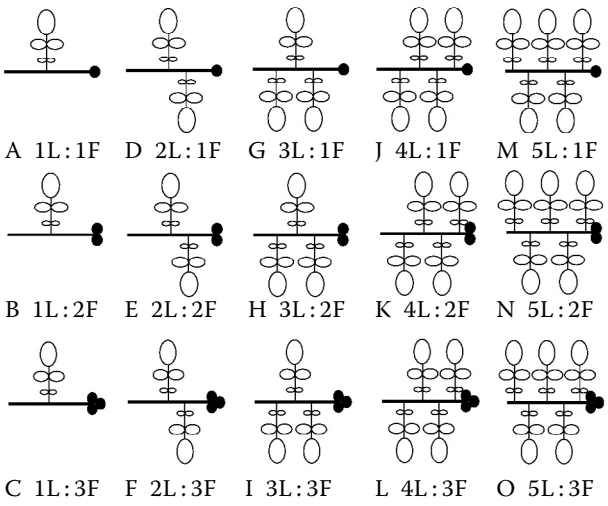

Figure 1. Different leaf-to-fruit ratio manipulations on the girdled shoots of walnut trees

1L : 1F was the girdled shoots with one fruit and one leaf

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(FV) was measured using the drainage method. Finally, the fruit was placed in a constant temperature electric blast drying oven (BK-UV1800PC) to kill the active enzymes in the fruit samples at 105 °C for 30 minutes, then dried at 70 °C to a constant weight to obtain fruit dry weight (FDW). The ripe fruit samples collected on 130 DAF were divided into three parts. One part was used to determine the physical quality, and another part was cut and put into a fixative solution (formalin-alcohol-acetic acid, FAA) to produce paraffin sections. The final part was dried after removing the green husk and the nut dry weight (NDW) was determined. The kernel was then excised and the kernel dry weight (KDW) was measured. Finally, the kernel powder was crushed and passed through a 100-mesh nylon sieve to determine the crude fat and crude protein contents.

Preparation and observation of paraffin section. The green husk and kernel samples were embedded in paraffin and sliced following the protocol of Willey (1971) using a rotary microtome Leica RM2265 (Leica Microsystems Wetzlar, Germany). Cell images were acquired using a DFC495 digital camera (Leica Microsystems GmbH, Germany) and analysed using AutoCAD 2014 to observe the cell number and area. Three procedures were performed for each LFR treatment, and five visual fields were repeatedly observed.

Determination of chemical quality of nuts. The crude fat content in the kernels was determined using the Soxhlet extraction method. The crude protein content in the kernels was determined using the Kjeldahl method.

Data analysis. A variance analysis and an LSD were performed with the SPSS 22.0 statistical analysis software. Microsoft Excel 2007 software was used for sorting the data and the calculations, and SigmaPlot 10.0 software (SigmaPlot 10.0, SPSS Inc. USA) was used for drawing.

RESULTS AND DISCUSSION

Fruit physical quality and LFR. The results revealed that the LFR had a significant effect on the FV, FFW, FDW, NDW and KDW ($P < 0.05$) (Table 1), and these physical quality indicators increased with an increase in the LFR (Figures 2, 3, 4). The quadratic regression curve and linear relationship, respectively, represented the relationship between the LFR and fruit physical quality on the girdled and natural fruit-bearing shoots well (Figure 5). Moreover, there were significant differences in the FFW, FDW, and NDW under different LFRs ($P < 0.05$). However, no significant difference was observed in the FFW with 3L : 1F ($P > 0.05$) and in the NDW with 2L : 1F ($P > 0.05$) between the natural and the girdled fruit-bearing shoots.

The results indicate that the growth of the fruit on the girdled fruit-bearing shoots was strictly dependent on the LFR, and two leaves receiving good light were necessary to ensure normal growth and development of one walnut fruit, which was consistent with the results observed for natural fruit-bearing shoots. The quadratic curve relationship reflecting the relationship between the fruit weight and the LFR (Figure 5) revealed that the increased FFW, FDW and

Table 1. Analysis of variance with multiple response variables testing the effect of the leaf-fruit-ratio on the fruit quality and cells

Response variables	df	SS	MS	*F*-value	*P*-value
Fruit volume	14	15 162.715	1 083.051	52.820	0.012
Fresh weight of fruit	14	7 976.386	569.742	69.269	0.022
Dry weight of fruit	14	414.270	29.591	133.756	0.026
Nut dry weight	14	312.083	22.292	60.579	0.000
Kernel dry weight	14	59.277	4.231	79.625	0.000
Crude protein content	14	49.633	3.545	0.566	0.469
Crude fat content	14	1.959	0.140	0.286	0.223
Cell number of green husk	14	2 940.986	210.070	3.521	0.016
Cell area of green husk	14	2.334	0.160	4.559	0.009
Cell number of kernel	14	6 958.735	497.052	10.010	0.031
Cell area of kernel	14	2.534	0.181	2.732	0.011

df – degree of freedom; SS – sum of squartes of the sample data; MS – mean square sum of the sample data

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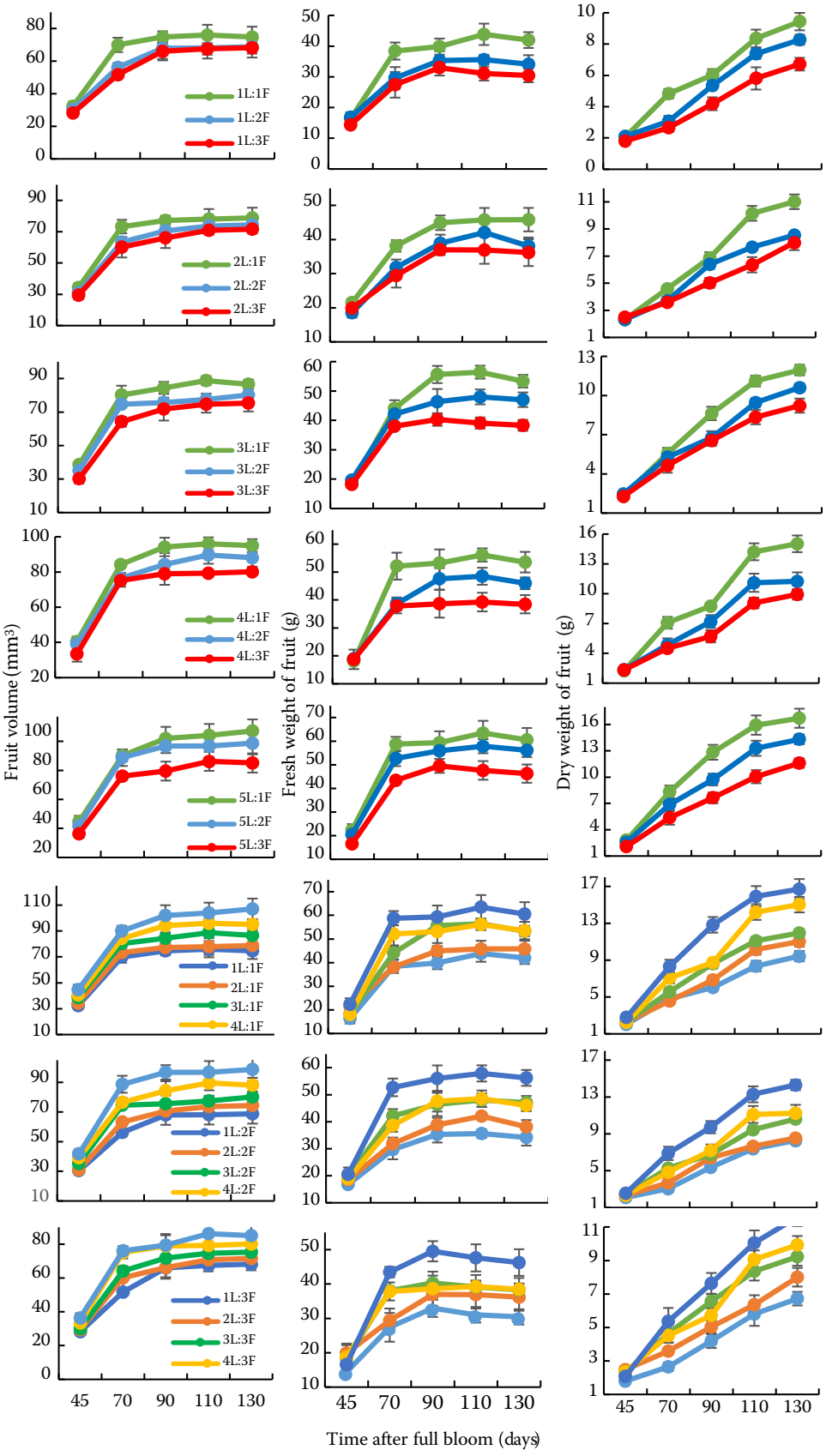

Figure 2. Seasonal variation of the FV, FFW, and FDW of the girdled shoots with different leaf-to-fruit ratio (means ± SD, n = 3)

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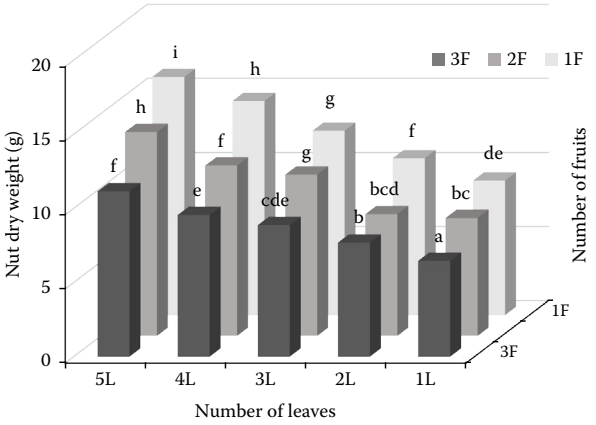

Figure 3. Distribution map of the NDW of the different leaf-to-fruit ratio (means ± *SD*, *n* = 8)
Different lower-case letters indicate significant differences at a 0.05 level

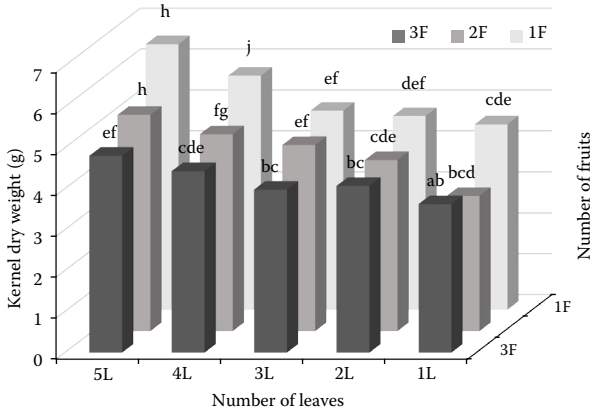

Figure 4. Distribution map of the KDW of the different leaf-to-fruit ratio (means ± SD, *n* = 8)
Different lower-case letters indicate significant differences at a 0.05 level

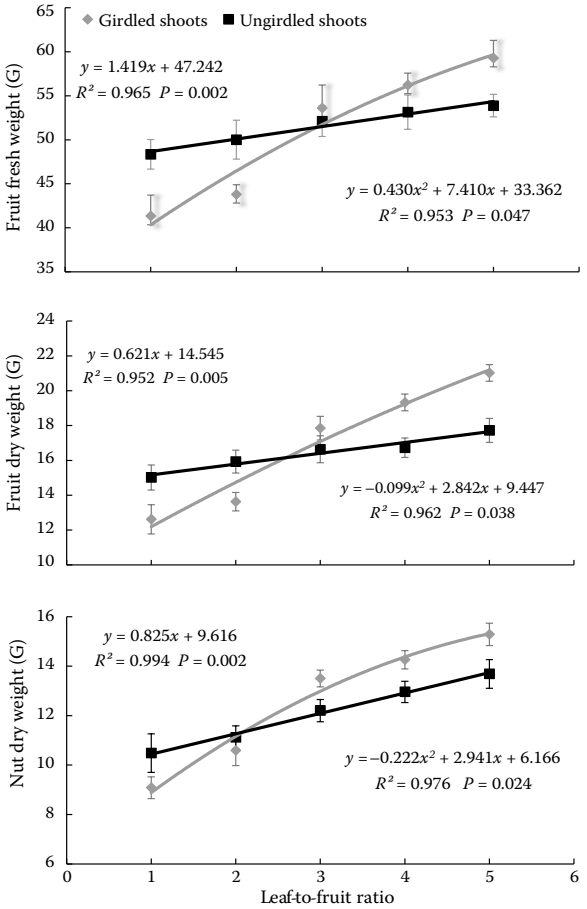

Figure 5. The regression relationship between the leaf-to-fruit ratio and fruit physical quality of the girdled shoots and ungirdled shoots (means ± SD, *n* = 8)

NDW range on the girdled fruit-bearing shoots decreased with an increasing LFR. For example, when the LFR increased from 2L:1F to 3L:1F, the FFW, FDW, and NDW increased by 5.26 g, 2.35 g and 1.83 g, respectively; and when LFR increased from 4L:1F to 5L:1F, the FFW, FDW, and NDW increased by 3.54 g, 1.95 g and 0.94 g, respectively. Research results involving kiwifruit (Famiani et al. 1997) and hickory nuts (Marquard 1987) also showed that the yield of a low LFR was high. In our study, the low yield of the leaves on the girdled fruit-bearing shoots with a high LFR (5L:1F) was consistent with its low net photosynthetic rate (our previous research results).

Fruit cells and LFR. The number of cells per unit area and the cross-sectional area of the green husk and kernel cells with 5L:1F were significantly higher than those with 1L:3F and 2L:3F ($P < 0.05$) (Table 2), which inferred that the large volume of fruit on the girdled fruit-bearing shoots with 5L:1F was caused by a large number of cells comprised of the fruit, and the lower carbohydrate availability on the girdled fruit-bearing shoots with low LFR (1L:3F and 2L:3F) led to a slowdown or even premature cessation of the fruit cell mitosis, which was consistent with the early control of the carbohydrate competition on the cell division reported in previous studies (Nasrallah 1989). The number of cells per unit area in the green husk and kernels on the girdled fruit-bearing shoots with 1L:3F and 2L:3F was significantly higher than those with other LFRs ($P < 0.05$), and the cell size decreased by 30%. We speculated that the availability of carbohydrates affected the cell size, because some studies have shown that the fruit size also depends largely on the distribution of the carbohydrates after the cell division. After the cell division, the cell volume be-

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Table 2. Multiple comparison of the number and size of the cells in the fruits of different leaf-to-fruit ratios (means ± SE, n = 10)

Leaf-to-fruit ratio	Cell number (× 10^{-5}μm^{-2})		Cell area (10^3μm^2)	
	green husk	kernel	green husk	kernel
1L : 1F	62.00 ± 4.57^{ab}	60.00 ± 2.13^{c}	1.53 ± 0.22^{ab}	1.50 ± 0.15^{abc}
1L : 2F	66.33 ± 11.59^{ab}	79.67 ± 4.73^{ab}	1.27 ± 0.09^{abc}	1.13 ± 0.07^{abc}
1L : 3F	78.67 ± 3.06^{a}	93.33 ± 7.57^{a}	0.91 ± 0.37^{c}	0.96 ± 0.08^{c}
2L : 1F	56.33 ± 7.37^{ab}	61.37 ± 3.06^{bc}	1.33 ± 0.09^{abc}	1.47 ± 0.11^{abc}
2L : 2F	57.48 ± 8.50^{ab}	58.02 ± 2.47^{c}	1.46 ± 0.11^{abc}	1.55 ± 0.05^{abc}
2L : 3F	79.00 ± 3.61^{a}	91.67 ± 4.73^{a}	0.96 ± 0.10^{c}	0.98 ± 0.05^{c}
3L : 1F	56.78 ± 16.62^{ab}	60.32 ± 3.61^{c}	1.51 ± 0.25^{abc}	1.50 ± 0.09^{abc}
3L : 2F	55.67 ± 7.09^{ab}	62.00 ± 3.04b^{c}	1.39 ± 0.11^{abc}	1.45 ± 0.09^{abc}
3L : 3F	54.12 ± 10.44^{ab}	59.67 ± 5.51^{c}	1.38 ± 0.09^{abc}	1.52 ± 0.13^{abc}
4L : 1F	56.08 ± 10.54^{ab}	62.03 ± 4.58^{bc}	1.52 ± 0.09^{abc}	1.46 ± 0.11^{abc}
4L : 2F	61.23 ± 13.02^{ab}	53.64 ± 15.24^{c}	1.46 ± 0.36^{abc}	1.65 ± 0.26^{ab}
4L : 3F	55.74 ± 9.29^{ab}	56.13 ± 10.58^{c}	1.55 ± 0.13^{ab}	1.64 ± 0.29^{ab}
5L : 1F	50.67 ± 3.21^{b}	55.67 ± 9.27^{c}	1.78 ± 0.17^{a}	1.81 ± 0.57^{a}
5L : 2F	57.03 ± 2.08^{ab}	56.17 ± 10.54^{c}	1.64 ± 0.08^{ab}	1.64 ± 0.31^{ab}
5L : 3F	56.38 ± 3.78^{ab}	56.78 ± 2.82^{c}	1.53 ± 0.17^{ab}	1.59 ± 0.57^{ab}

gins to increase. The lower carbohydrate availability on the girdled fruit-bearing shoots with the low LFR affects the process of cell enlargement and eventually leads to small cell volumes.

Fruit chemical quality of nuts and LFR. The LFR had no significant effect on the crude protein and crude fat contents in the nut kernels (Table 1) ($P > 0.05$), which was similar to the results of *C. oleifera* (Yuan et al. 2015) and maize (Sala et al. 2007). Research results concerning grapes (Almanza-Merchán et al. 2011), persimmons (Choi et al. 2011), and Xueqing pears (Xu 2015) showed that the percentage of soluble sugars in the fruit on girdled fruit-bearing shoots with a high LFR was higher than those with a low LFR. This was possibly because, in fruit with sugar as the main quality characteristic component, the quality of the fruit depended on the accumulation of sugar, while, in nuts with fat as the main quality characteristic component, the quality of the nut depended on the sugar to fat distribution ratio. A change in the LFR affected the sugar accumulation in the fruit, but did not influence the sugar to fat distribution ratio in the nuts. This distribution ratio is likely to be controlled by genes.

CONCLUSION

The LFR had a significant effect on the physical quality of the fruit on the girdled fruit-bearing shoots ($P < 0.05$), and a high carbohydrate availability with a high LFR increased the FV, FFW, FDW, and KDW by increasing the number of cells in the fruit green husk and kernels; and a low carbohydrate availability with a low LFR led to a lower cell size and diminished cell numbers in the fruit green husk and kernels, resulting in a smaller FV, FFW, FDW, and KDW. However, the LFR had no significant effects on the crude protein and crude fat contents in the kernels ($P > 0.05$), possibly because the changes in the LFR did not alter the sugar to fat distribution ratio in the nuts. Two leaves receiving good light were necessary to ensure normal growth and development of one walnut fruit on the girdled fruit-bearing shoots.

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