

## Long-term effect of branch bending on fruiting capacity, biomass and xylem vessel anatomical structure of walnut (*Juglans regia*) c.v. Xiangling and Qingxiang

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### ABSTRACT

The experiment was conducted to find out the effect of branch bending with ten years on fruiting capacity, biomass and xylem vessel anatomical structure in walnut (*Juglans regia* L.) c.v. Xiangling and Qingxiang trees. Both the percentage of fertile fruits and nut yield were investigated in ten successive years from the trees being planted during 2008. And the biomass of trees and xylem vessel anatomical structure of annual semi-woody branches were measured during 2017. Although the response of both varieties was not similar, branch bending could increase the percentage of fertile fruits and nut yield only in early 3-6 years. In both varieties during ten years, branch bending reduced the trunk diameter above 20 cm ground, the width of vessel, vessel area and vessel total area/xylem transect area, increased the crown area and dry weight of above-ground significantly, but had no effect on above-ground and under-ground fresh weight. There were no more positive effects of branch bending in long-term cultivation of Xiangling and Qingxiang walnuts. It seems that more physiological responses need to be concerned to evaluate the long-term effects of branch bending.

**KEY WORDS:** Branch bending, Percentage of fertile fruits, Yield, Biomass, Xylem vessel, Vessel area, Crown area.

Walnut (*Juglans regia* L.) trees are most ancient and worldwide commercial forestry with large scale more than 5 600 thousands ha now in China, where it has been cultivated for more than 2 000 years (Chen *et al.*, 2014). And branch-bending practice in cultivation for walnut trees had been referenced from apple after 2000 (Wei *et al.*, 2013). The branch bending could increase walnut tree budding rate, net photo-synthetic rate of leaves, spur formation, yield and root activity in the same year or the next year (Wei *et al.*, 2013; Li *et al.*, 2014a; Li *et al.*, 2014b). Dai (1996) reported the promotional effects of branch bending on young pair trees with timelessness. Branch bending is a widely used cultural practice in orchards (Colaric *et al.*, 2007), which is proved to reduce vegetative growth and increase flower buds and spur formation in apple through redistributing carbon-to-nitrogen (C/N) and hydraulic conductance of sap (Lauri and Lespinasse 2001; Zhang, 2017; Han *et al.*, 2007).

An increase in vegetative growth of plants can lead to a higher growth performance in plant biomass, which is positively correlated with yield (Egli 1997; Rigsby and Board 2003; Board and Modali 2005). The number, diameter, and length of vessels directly determine the xylem hydraulic efficiency and transportation function, thus determining redistribution of material and energy in plants and growth characteristic of plants (West and Brown 1999). It was found that branch bending has an effect on hydraulic conductance of sap (Han *et al.*, 2007). The information regarding shoot architecture of walnut trees was well documented (Wei *et al.*, 2013; Li *et al.*, 2014a, 2014b). However, effect of branch bending on plant vessel system and biomass is not known. Therefore, an experiment was conducted to evaluate the effect of branch bending on percentage of fertile fruits, yield, xylem vessel anatomical structure and biomass and, to analyze the timelessness of branch bending in absence of confounding factors such as rootstock and fertilizer, and water management.

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## MATERIALS AND METHODS

The trees of walnut cv. Xiangling and Qingxiang grafted on *Juglans regia* L. were planted in a commercial orchard (31°31'N and 110°50'E) in Baokang country in Hubei province, China, at a spacing of 6 m × 7 m during 2007. The orchard is at an altitude of 810 m with an annual precipitation of 800-1 000 mm and an annual mean temperature of 12-15°C. The soil of orchard had 49.68 g/kg available N, 11.9 g/kg available P, 68.4 g/kg available K, 4.73 mg/g available Fe, 0.29 mg/g g/kg available B, and 1.07 mg/g available Zn. Drip irrigation with fertilizers and other orchard practices including pest control were applied every year.

Bending all one-year-old branches at an angle of 90° in every March from 2008 to 2016 were selected. Four treatments were applied:

- Treatment I: Xiangling walnut trees with branch bending.
- Treatment II: Xiangling walnut trees without branch bending.
- Treatment III: Qingxiang walnut trees with branch bending.
- Treatment IV: Qingxiang walnut trees without branch bending.

Each treatment had ten trees with random arrangement in orchards. Percentage of fertile fruits with every tree was calculated in every April and July during 2007 - 2016. Percentage of fertile fruits = number of fruits/number of flowers × 100%. And nut yield of every tree was weighted at the end of September. Trunk diameter above 20 cm ground and crown area were measured in December 2016.

Biomass was measured during January 2017. Three randomly selected trees in every treatment were fully excavated. The roots were rinsed in tap water to clean the soil and then dried with bare cloth. Fresh weight of above-ground and under-ground parts were measured separately. Then they were dismembered to be dried at 60°C for 72 h in a forced-air oven to a constant weight.

Annual semi-lignified branches were randomly selected from the canopy periphery of every tested tree at the end of June 2016. They were fixed for paraffin and xylem vessel extraction. Paraffin and xylem vessel extraction were determined according to Liu *et al.* (2008). The data were subjected to SAS 8.1 with one-way ANOVA. And the means were compared using the least significant difference (LSD) test at  $P < 0.05$ .

## RESULTS AND DISCUSSION

Compared with non-branch-bending trees, branch bending could significantly improve percentage of fertile fruits in Xiangling and Qingxiang trees 3-6 years after planting (Fig. 1). The peak of percentage of fertile fruits of Xiangling walnut trees with branch-bending appeared during sixth year. It declined rapidly after eighth year. The percentage of fertile fruits from other three treatments was relatively stable after eighth years. And the successive sequence was treatment IV > treatment II > Treatment III.

The branch bending could make Qingxiang walnut trees to have production one year ahead, but had no effect on Xiangling walnut trees (Fig. 2). Although yield of branch-bending trees were higher than non-branch bending trees of Xiangling walnut 5 years after planting and Qingxiang walnut 7 years after planting, yield with branch-bending treatment of Xiangling walnut trees declined rapidly after 8 years planting. On the contrary, yield of non-branch-bending Qingxiang walnut trees were rising all the time 10 years after planting.

As branch-bending treatments allowing greater percentage of fertile fruits and higher yield in early years based on their more leaf area per unit (Wertheim *et al.*, 2001; Jackson, 1989; Zhang *et al.*, 2017), the response of both varieties was not similar. It seems that branch bending has to be more concerned for long-term effect on walnut trees.

Compared with the biomass in corresponding parts of both varieties, trunk diameter above 20 cm ground,

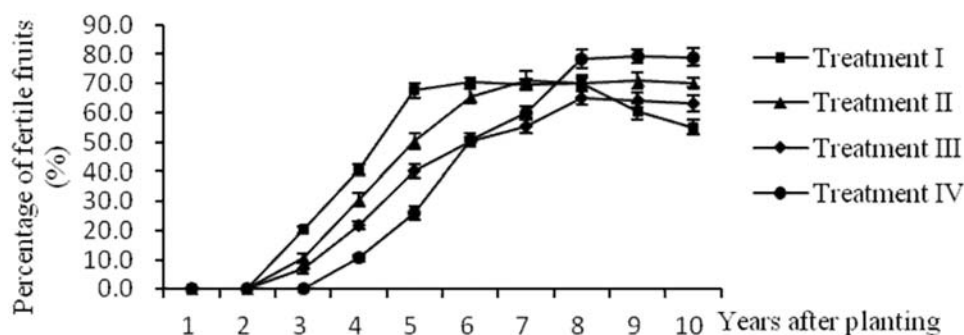


Fig. 1: Percentage of fertile fruits of all treatments during different planting years

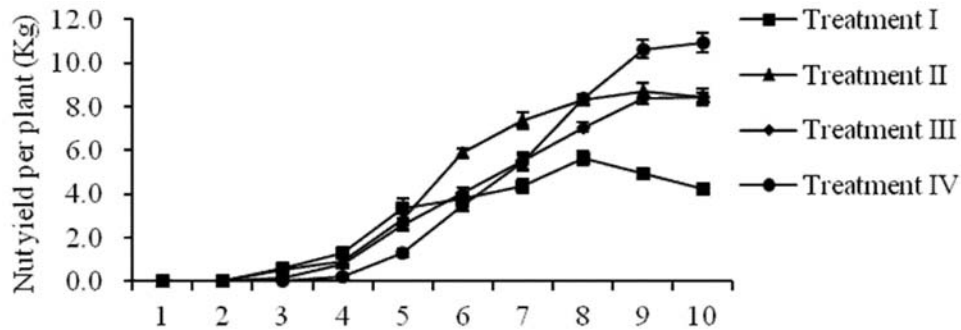


Fig. 2: Nut yield per plant in all treatments during different planting years.

crown area, and DW of above-ground biomass of Qingxiang walnut were higher than Xiangling walnut significantly (Table 1). It means that there are differences in biomass accumulation in different varieties. Compared with non-branch-bending treatment, branch bending treatments of both varieties reduced the trunk diameter above 20 cm ground, but increased the crown area and DW of above-ground biomass significantly. For all that, it had no effect on FW of above-ground biomass and under-ground biomass accumulation. The dry matter accumulated from photosynthesis of branch-bending treatment is more used for crown extension, but less for thickening growth of trunk and root growth comparatively.

The branch-bending treatment significantly reduced the width of vessel, vessel area and vessel total area/xylem transect area of both varieties branches, especially more than 50% decline of vessel total area/xylem transect area of Qingxiang walnut trees (Table 2).

Compared with Xiangling trees, branch-bending treatment reduced more significantly length of vessel but increased the vessel density. Still, branch-bending treatment had no effect on xylem area/transect area.

With the prolonging of planting time, smaller increase or even decrease of percentage of fertile fruits and yield with branch-bending treatments in both varieties may be related to nutrient transport of vessel. Adler *et al.* (2008) reported that plants can adapt to environment by adjusting their morphology, physiology, biomass allocation patterns and characteristics of water diversion system, so as to achieve optimal allocation of resources and water balance. This adaptation cause differences in physiological characteristics, internal anatomical structure and external morphological characteristics of plants, which can change the water balance process and water transmission characteristics of plants (Jansen *et al.*, 2011; Markesteijn *et al.*, 2011). The branch-bending treatments

**Table 1.** Biomass of different treatments after planting over ten years

Treatment	Trunk diameter (cm)	Crown area (m <sup>2</sup> )	Above-ground biomass (kg)		Under-ground biomass (kg)	
			Fresh weight	Dry weight	Fresh weight	Dry weight
Treatment I	13.50 ± 0.68d	20.05 ± 0.41c	10.99 ± 0.46b	3.89 ± 0.17d	9.77 ± 0.37b	3.91 ± 0.19b
Treatment II	14.57 ± 0.48c	17.73 ± 0.45d	11.66 ± 0.40ab	4.16 ± 0.15c	10.64 ± 0.49ab	4.34 ± 0.31a
Treatment III	16.23 ± 0.53b	24.28 ± 0.72a	13.00 ± 1.03ab	5.02 ± 0.36b	11.52 ± 0.78a	4.32 ± 0.11a
Treatment IV	18.72 ± 0.72a	22.84 ± 0.30b	14.60 ± 0.47a	5.73 ± 0.13a	11.64 ± 0.62a	4.40 ± 0.09a

**Table 2.** Comparison of vessel anatomical structure of different treatments after planting over ten years

Treatment	Length of vessel/μm	Width of vessel/μm	Vessel density (number/mm <sup>2</sup> )	Vessel area/mm <sup>2</sup>	Vessel total area/xylem transect area	Xylem area /transect area
Treatment I	284 ± 62 bc	86.4 ± 21.3 b	23.6 ± 10.0 ab	0.313 ± 0.155 b	0.146 ± 0.072 b	0.33 ± 0.08 a
Treatment II	339 ± 128 ab	119.5 ± 33.1 a	19.2 ± 8.2 bc	0.565 ± 0.286 a	0.231 ± 0.117 a	0.32 ± 0.03 a
Treatment III	238 ± 89 c	52.1 ± 18.6 c	27.6 ± 14.7 a	0.112 ± 0.274 c	0.066 ± 0.044 c	0.29 ± 0.02 ab
Treatment IV	342 ± 76 a	104.3 ± 33.0 a	15.5 ± 9.1 c	0.435 ± 0.237 ab	0.145 ± 0.079 b	0.26 ± 0.03 b

significantly reduced the width of vessel, vessel area and vessel total area/xylem transect area in both varieties, then reduced the water and nutrient transport efficiency, which may be one of the important causes for serious abscission and yield reduction. As the vessel is an important channel for nutrient delivery from the root system (Wang, 2000), the results means that branch-bending treatment significantly reduced the efficiency of root nutrient dredging.

## CONCLUSION

Although the response of both varieties was not similar, branch bending could increase the percentage of fertile fruits and nut yield only during early 3-6 years. For both varieties in ten years, branch bending reduced the trunk diameter above 20 cm ground, width of vessel, vessel area and vessel total area/xylem transect area, increased the crown area and dry weight of above-ground significantly, but had no effect on above-ground and under-ground fresh weight. Thus, there were no more positive effects of branch bending in long-term cultivation of Xiangling and Qingxiang walnuts. It seems that more physiological responses need to be concerned to evaluate the long-term effects of branch bending.

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