Study on Photosynthetic Characteristics of Hybrid Mulberry in Autumn under Herbaceous Cultivation

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Abstract [**Objectives**] This study was conducted to clarify the physiological mechanism of growth of hybrid mulberry after autumn cutting in herbaceous cultivation. [**Methods**] The net photosynthetic rate (Pn), stomatal conductance (Gs), intercellular CO_2 concentration (Ci) and transpiration rate (Tr) of leaves were measured under different treatment conditions using a Li-6400XT portable photosynthetic measurement system. [**Results**] After harvest of mulberry shoots in autumn, leaf Pn decreased with the extension of branch and leaf growth time, while Gs, Ci and Tr showed a trend of first increasing and then decreasing. The Pn was affected by factors such as leaf positions, mulberry varieties, cutting, and fertilization, which was manifested by the 6th to 10th mature leaves > the 2nd to 4th tender leaves, 'Nongsang 14' > hybrid mulberry, intermediate cut > uncut, and normal fertilization > no fertilization, all showing significant differences ($P < 0.05$). Combined with the results of Gs, Ci and Tr measurements, it was found that the changes in leaf Pn were mainly related to non-stomatal factors. Timely cutting and harvesting during summer and autumn could significantly improve the photosynthetic rate of mulberry leaf, which was beneficial for extending the late autumn growth period of hybrid mulberry under herbaceous cultivation. [**Conclusions**] This study provides a theoretical reference for mulberry shoot harvesting techniques in summer and autumn.

Key words Hybrid mulberry; Herbaceous cultivation; Harvest of mulberry shoots; Photosynthetic rate; Leaf age

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Photosynthesis is the foundation of plant growth and development, and over 90% of the dry matter of mulberry comes from the photosynthesis of mulberry leaf[1]. Studying the photosynthetic characteristics of mulberry is of great significance for enhancing their photosynthetic function, increasing organic matter accumulation, and improving the yield and quality of mulberry leaf, and can also provide a theoretical basis for improving mulberry cultivation techniques. For many years, scholars at home and abroad have conducted a series of studies on the photosynthetic characteristics and influencing factors, gene expression regulation, and other aspects of mulberry[2-5], but there are still few research reports on the annual growth and photosynthetic characteristics of hybrid mulberry in herbaceous cultivation.

Herbaceous cultivation of mulberry is a new technology developed at the beginning of this century[6]. It mainly utilizes the characteristics of hybrid mulberry, such as fast growth, shearing resistance, and slender branches, to cultivate dense tree shapes without trunks, and implement annualmulberry shoot harvesting and shoot rearing. The biggest difference from traditional mulberry leaf harvesting is the implementation of mulberry shoot harvesting during the summer and autumn growth seasons, resulting in multiple growth cycles and periodic changes in mulberry physiological functions[7-9]. Therefore, in this study, with four-year-old hybrid mulberry as the research object, the photosynthetic characteristics in the middle and late autumn periods were explored under different conditions after the third harvest of mulberry shoots, aiming to provide theoretical reference for mulberry shoot harvesting techniques in the summer and autumn periods of hybrid mulberry.

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Fangying XU (1997-), female, P. R. China, master, devoted to research about landscape plant resources and applications.

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Materials and Methods

Plant materials

The test mulberry plantation is located in the modern sericulture demonstration park of Jiangsu Huajia Silk Co., Ltd. in Miaotou Village, Pingwang Town, Suzhou City. In the spring of 2018, the plantation was built using small seedlings, with an average row spacing of about 80 cm, plant spacing of 20-25 cm, and planting density of about 60 000 plants/hm^2. The tested hybrid mulberry variety was 'Guisang 12', with a tree age of 4 years. The mulberry shoots were harvested in a herbaceous cultivation mode, with 4 harvests per year. Before spring germination and after each harvest, compound fertilizer (N-P-K ratio 15-15-15) and urea were applied in ditches, at a rate equivalent to 600 kg/hm^2 of pure nitrogen throughout the year. After the third harvest of mulberry shoots on August 30, 2021, the main photosynthetic parameters of mulberry leaf under different stages and treatment conditions were measured three times from early October to early November. Grafted mulberry 'Nongsang 14' (harvested on September 10) was used as the control.

Experimental design

(1) The growth period was divided into three periods: photosynthetic parameters were measured on day 40 (G_1), day 60 (G_2), and day 70 (G_3) after the third harvest of mulberry shoots. (2) Leaf position: Leaves were selected from different parts of new shoots (from top to bottom, the 2nd to 10th leaf positions) to measure photosynthetic parameters. (3) Two different treatments were set for the cutting stages: after the third harvest of

mulberry shoots (H_1) and after intermediate cutting (H_2). The photosynthetic parameters of the same leaf position were measured in late autumn. (5) The fertilization amount was divided into four levels: the fertilization amounts applied per hectare in various harvests were converted to the amounts of pure nitrogen, that is, 150 kg (1 times the amount, F_1), 225 kg (1.5 times the amount, F_2), and 375 kg (2.5 times the amount, F_3), and no fertilization (F_0) as the control.

Determination of photosynthetic parameters

The Li-6400XT portable photosynthetic measurement system was used to measure the net photosynthetic rate (Pn), stomatal conductance (Gs), intercellular CO_2 concentration (Ci) and transpiration rate (Tr) of leaves. During the measurement, a built-in LED light source was used with a light intensity of 1 500 pmol/(m^2 · s), and the flow rateof sample chamber was set to 500 μmol/s, and the atmospheric CO_2 concentration was about 400 μl/L. The measurement time was from 9:00 a. m. to 11:00 a. m. on a clear and windless day. From each group, mature leaves with similar growth from the 6^{th} to 7^{th} positions of new shoots were selected for measurement. The grafted mulberry 'Nongsang 14' was used as the control, and six leaves were measured in each group to obtain an average value as the measurement value.

Data analysis

Data processing and graph drawing were conducted using EXCEL2013, and significant differences were analyzed using the LSD method of SPSS software.

Results and Analysis

Analysis on photosynthetic characteristics of hybrid mulberry in different autumn periods under herbaceous cultivation

Compared with traditional cultivation of mulberry harvested in summer or spring, hybrid mulberry under herbaceous cultivation can also be harvested multiple times during the summer and autumn growth periods. After cutting, new shoots can sprout again, and the branches and leaves enter a new growth period. From the results of photosynthesis measurements at different periods (Table 1), it could be seen that during the period from day 40 (G1) to day 70 (G3) after the third harvest of mulberry shoots (October 10 to November 10), the net photosynthetic rate (Pn) and transpiration rate (Tr) showed a continuous downward trend with the growth period, and the differences between different periods reached a significant level ($P < 0.05$). Unlike this, stomatal conductance (Gs) and intercellular CO_2 concentration (Ci) showed a trend of first increasing and then decreasing. Compared with day 40 (G1) after harvesting of mulberry shoots, the Gs and Ci significantly increased on day 60 (G2), indicating that the reason for the significant decrease in Pn was mainly caused by non-stomatal factors. On day 70 (G3), the Gs significantly decreased, while the Ci significantly increased, indicating that the main reason for the significant decrease in Pn in hybrid mulberry during this period was influenced by stomatal factors, which might be related to the low-temperature environmental conditions in late autumn.

Table 1 Measurement results of photosynthetic parameters in hybrid mulberry at different growth periods in autumn

Growth period	Pn//μmol/(m^2 · s)	Gs//μmol/(m^2 · s)	Ci//μmol/mol	Tr//mmol/(m^2 · s)
G1	14.74 ± 0.29 a	0.12 ± 0.003 b	169.89 ± 16.55 c	3.6 ± 0.07 a
G2	8.78 ± 0.36 b	0.16 ± 0.010 a	303.49 ± 9.82 a	2.11 ± 0.11 b
G3	4.51 ± 0.45 c	0.04 ± 0.005 c	232.13 ± 38.17 b	0.81 ± 0.09 c

Different letters in the table indicate the significance of differences between different experimental plots ($P < 0.05$), and the measured leaf positions are the 6^{th} to 7^{th} leaves. The same below.

Analysis of photosynthetic characteristics at different leaf positions of hybrid mulberry in autumn under herbaceous cultivation

After the third harvest of mulberry shoots on August 30, the branches and leaves of hybrid mulberry grew more vigorously in the mid autumn period, but the growth rate gradually slowed down after entering the late autumn period. From the results of photosynthesis measurements at different leaf positions (Table 2), the photosynthetic rate also significantly decreased. The net photosynthetic rate presented middle mature leaves (6 – 10 positions) > upper tender leaves (2 – 4 positions), and grafted mulberry Nongsang 14 (Pn-NS) > hybrid mulberry (Pn-1). Secondly, during the mid autumn period (late September), after an additional intermediate cutting (harvesting of mulberry shoots), hybrid mulberry could quickly sprout and grow again, entering the vigorous growth stage again. And the net photosynthetic rate (Pn-2) of leaves also significantly increased, approaching the level of Nongsang 14 (Pn-NS) in the control area.

Table 2 Measurement results of photosynthetic parameters at different leaf positions of hybrid mulberry in autumn μmol/(m^2 · s)

Leaf position	Pn-1	Pn-2	Pn-NS
2	1.43 ± 0.53 a	3.22 ± 0.56 a	2.24 ± 0.53 a
4	3.19 ± 0.79 a	6.33 ± 1.42 ab	4.96 ± 0.87 a
6	4.51 ± 0.45 b	8.91 ± 0.71 b	9.04 ± 1.30 b
8	4.68 ± 0.77 b	8.32 ± 0.87 b	7.82 ± 1.08 b
10	4.70 ± 0.30 b	–	8.96 ± 1.46 b

When measured on November 10, the new shoots in the middle cutting area (Pn-2) had less than 10unfolded leaves, so they were not measured.

Effects of autumn cutting stages on photosynthetic characteristics of hybrid mulberry under herbaceous cultivation

After the third harvest of mulberry shoots on August 30, the growth of branches and leaves began to enter a slow growth period in late September. At this time, adding another harvest of mulberry shoots can not only promote the germination and growth of hybrid mulberry again, but also restore the photosynthetic capacity of new branches and leaves. From the measurement results of several photosynthetic parameters (Table 3), it could be seen that the

net photosynthetic rate (Pn) of the cut area (H_2) increased by 97.56% compared with the uncut area (H_1), but it was still slightly lower than the control area Nongsang 14 (Hck). Meanwhile, its stomatal conductance (Gs) also increased by 50.00%, while the intercellular CO_2 concentration (Ci) decreased by 43.98%, indicating that the vigorous growth of new branches and leaves after intermediate cutting was mainly due to the improvement of photosynthetic efficiency of mesophyll cells.

Table 3 Measurement results of photosynthetic parameters in hybrid mulberry at different cutting stages in autumn

Cutting stage	Pn//μmol/(m² · s)	Gs//μmol/(m² · s)	Ci//μmol/mol	Tr//mmol/(m² · s)
H_1	4.51 ±0.45 b	0.04 ±0.004 b	232.13 ±38.17 a	0.81 ±0.09 b
H_2	8.91 ±0.71 a	0.06 ±0.005 a	161.32 ±32.45 b	1.29 ±0.11 a
Hck	9.64 ±0.74 a	0.07 ±0.004 a	186.50 ±31.77 ab	1.55 ±0.15 a

Effects of fertilization rate on photosynthetic characteristics of hybrid mulberry in autumn

Hybrid mulberry usually sprout early, and have a long growth cycle, during which the branches and leaves grow vigorously, and the leaves fall off late. They require "large amounts of water and fertilizer" to meet their growth and development needs. Under the conditions of herbaceous cultivation, branches and leaves are cut for multiple times throughout the entire growth season to harvest mulberry shoots. It not only affects the photosynthetic function of leaves, weakens the growth and development ability of branches and leaves, but also takes a large amount of mineral elements from the soil. Therefore, soil fertilization status has a significant impact on the growth and development of hybrid mulberry under herbaceous cultivation. From the results of photosynthesis measurements at different fertilization rates (Table 4), compared with the control area without fertilization (F_0), several photosynthetic indicators at fertilization rate F_1 significantly increased. However, there were no significant differences between different fertilization treatments, and even a slight downward trend was observed. Therefore, in terms of photosynthetic efficiency, it indicated that the fertilization amount rate of 150 kg/hm² nitrogen every season could meet the nutritional requirements of hybrid mulberry under herbaceous cultivation.

Table 4 Effects of different fertilization rates on photosynthetic parameters of hybrid mulberry during the mid autumn period

Treatment of fertilization rate	Pn//μmol/(m² · s)	Gs//μmol/(m² · s)	Ci//μmol/mol	Tr//mmol/(m² · s)
F_0	12.81 ±0.3 b	0.09 ±0.002 c	132.15 ±7.13 b	2.71 ±0.07 d
F_1	14.74 ±0.29 a	0.12 ±0.003 a	169.89 ±5.85 a	3.60 ±0.07 b
F_2	14.11 ±0.51 a	0.11 ±0.003 b	159.73 ±10.03 a	3.13 ±0.09 c
F_3	14.35 ±0.28 a	0.12 ±0.002 a	158.33 ±4.39 a	3.66 ±0.05 b

Conclusions and Discussion

The herbaceous cultivation of hybrid mulberry mainly utilizes its characteristics of fast branch and leaf growth, strong shearing resistance, long growth period, and slender branches to cultivate dense tree shapes without trunks. During the growth period, new shoots are harvested multiple times, achieving multiple batches of mulberry shoot harvesting and shoot rearing throughout the year, greatly improving the labor productivity of mulberry cultivation and silkworm rearing[1]. The biggest difference between it and traditional harvesting techniques is that multiple mulberry harvests are also carried out during the summer and autumn growth periods, resulting in different characteristics of branch and leaf growth and photosynthesis during various growth periods.

From the measurement results of comprehensive photosynthetic parameters, it could be seen that when the branches and leaves of hybrid mulberry entered a slow growth period in late autumn, their photosynthetic rate significantly decreased (Table 1, G_2, G_3), which was because at this time, hybrid mulberry had already accumulated a certain amount of branches and leaves, and the photosynthetic function of leaves gradually weakened. In this regard, before hybrid mulberry enter the slow growth stage, if a timely harvest of mulberry shoots can be carried out, it can not only promote the germination and growth of the branches and leaves again, but also prolong the autumn growth period relatively. Meanwhile, in this study, it could significantly enhance the photosynthetic function of new branches and leaves (Table 3, H_1/H_2), which is similar to the result in the harvest of mulberry shoots of *Morus alba* var. *multicaulis* (Perrott.) Loud. in late autumn[10]. It can be seen that timely cutting and harvesting have a positive effect in improving the leaf yield of hybrid mulberry in autumn under herbaceous cultivation and regulating the layout of autumn silkworm rearing production.

The photosynthesis of mulberry is influenced by various factors. The relationship between leaf age and photosynthesis is also very close. The photosynthetic capacity of newly unfolded young leaves is relatively low, and as the leaves grow and develop, the photosynthetic rate of mulberry leaf gradually increases until the mature leaves reach their maximum value. And after maintaining the peak for a period of time, the photosynthetic rate will gradually decrease. The results of this study indicated that the net photosynthetic rate of hybrid mulberry leaf was closely related to leaf position. The net photosynthetic rate of tender leaves at positions 2 – 4 was lower, while mature leaves at positions 6 – 10 were higher. The difference between tender and mature leaves was significant, while there was no significant difference between mature leaves (Table 2). It is different from the result of the peak at the 7th leaf position reported by Wu[11], which may be related to the actual

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team, understory economic team, resource utilization team, and forest health and willness team, as well as the traditional Chinese medicine processing team of Chongqing Academy of Chinese Medicine Materia Medica. The understory breeding team of Chongqing Academy of Animal Sciences has also cooperated deeply with enterprises to improve the construction of the industrial chain.

Increasing investment and leading the upgrading of industrial foundation

We should take full advantage of the opportunity for rural revitalization and rely on the characteristic resources of *Taxus* in Pengshui County to develop industries such as underforest planting and aquaculture according to local conditions, and continue to explore a three-dimensional economic model of "forest agriculture", such as forest grain, forest vegetables, forest fungi, forest herbs and forest poultry. The government provides advice and guidance for farmers who transfer land, and enterprises provide technical guidance for business management and underforest economy. Farmers will receive real benefits and utilization, which will increase their enthusiasm for protecting the resources of *Taxus*, which further provide basic guarantees for the sustainable development and utilization of *Taxus* resources in the future. Meanwhile, we will promote the deep integration of *Taxus* with industries such as tourism, health, elderly care and education, develop healthy tourism for *Taxus* in Pengshui County, and create a unique ecological tourism brand for *Taxus*.

Conducting science popularization and education and increasing resource protection efforts

Led by the Pengshui Autonomous County Government and promoted by relevant government departments and local township governments, we should organize standardized management systems and protection measures and strengthen science popularization and publicity to protect existing *Taxus* resources. Meanwhile, enterprises vigorously promote the *Taxus* industry through ecotourism, expand the brand awareness of *Taxus* health care, and promote *Taxus* forest health and willness tourism.

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physiological age of leaves, and this relationship varies depending on mulberry varieties and cutting stages. That is, the net photosynthetic rate of Nongsang 14 was higher than that of hybrid mulberry, which was related to the physiological age of leaves at the same leaf position. Moreover, under the condition of intermediate cutting after harvest of mulberry shoots, the photosynthetic capacity could be significantly improved (Table 2, Pn-NS/Pn-1). Therefore, appropriately increasing the number of harvesting times during the autumn growth period of hybrid mulberry can promote the duration of vigorous growth of hybrid mulberry branches and leaves, which is equivalent to extending the autumn growth period of herbaceous cultivation of hybrid mulberry, which is beneficial for improving mulberry leaf yield.

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