



**ARTICLE**

## Effects of PBO and PP<sub>333</sub> on Shoot Growth, Nutrient Accumulation, and Fruit Quality in *Carya illinoensis* cv. 'Shaoxing'

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### ABSTRACT

To enhance the productivity of *Carya illinoensis* cv. 'Shaoxing' and mitigate the loss of flowers and fruits, the effects of different concentrations of Piperonyl Butoxide (PBO) wettable powder (2, 5, and 10 g·L<sup>-1</sup>) and Paclobutrazol (PP<sub>333</sub>) (150, 300, and 450 mg·L<sup>-1</sup>, based on active ingredients) on 6-year-old 'Shaoxing' plants were investigated with water sprayed as the control. The results showed that: (1) Treatment with 10 g·L<sup>-1</sup> PBO and 450 mg·L<sup>-1</sup> PP<sub>333</sub> significantly inhibited the excessive growth of 'Shaoxing' branches. Also, 10 g·L<sup>-1</sup> PBO exhibited the best diameter increment effect on fruiting branches, and 150 mg·L<sup>-1</sup> PP<sub>333</sub> exhibited the best diameter increment effect on vegetative branches. (2) The content of soluble sugar and soluble protein in leaves treated reached the highest level after treatment with 450 mg·L<sup>-1</sup> PP<sub>333</sub>, while the content of starch sugar in leaves reached the highest level after treatment with 300 mg·L<sup>-1</sup> PP<sub>333</sub>. The application of PBO and PP<sub>333</sub> mitigated the decline in N, P, K, and other nutrient levels observed in the leaves of 'Shaoxing'. As the PBO and PP<sub>333</sub> concentrations increased, the nutrient elements in the leaves first increased and then decreased. Among them, 300 mg·L<sup>-1</sup> PP<sub>333</sub> treatment exhibited the best effect on increasing the content of N, P, and K in the leaves at the late stage of fruit development. (3) In terms of fruit setting rate and nutritional quality of 'Shaoxing' fruit, 5 g·L<sup>-1</sup> PBO treatment showed the most promising effect on improving fruit setting rate, 150 mg·L<sup>-1</sup> PP<sub>333</sub> exhibited the best effect on improving reducing sugar and decreasing tannin content in the kernel, 10 g·L<sup>-1</sup> PBO had the best effect on improving the crude fat content, and 2 g·L<sup>-1</sup> PBO had the best effect on improving the cellulose content in the kernel. (4) Principal component analysis showed that 450 mg·L<sup>-1</sup> PP<sub>333</sub> treatment had the most comprehensive regulatory effect on the growth and development of current-year branches, leaves, and fruits of 'Shaoxing'. This study provided a theoretical basis and data reference for the growth and development of *C. illinoensis* cv. 'Shaoxing' fruits from the perspective of the application of plant growth regulators.

### KEYWORDS

*Carya illinoensis* cv. 'Shaoxing'; branch control; fruit quality; PBO; PP<sub>333</sub>



## Nomenclature

<i>C.illinoensis</i>	<i>Carya illinoensis</i>
PBO	Piperonyl butoxide
PP <sub>333</sub>	Paclobutrazol

## 1 Introduction

*Carya illinoensis* K. Koch is a member of the walnut family (Juglandaceae), which is native to the United States and Mexico. It was introduced into China during the late 19th and early 20th centuries [1]. The flowering period of *C. illinoensis* is from April to May, while the fruiting period extends from May to October. Following planting, the tree begins to bear fruits in its fourth to sixth years and enters the full fruiting period in its seventh to eighth years [2]. Among the numerous varieties introduced into China, ‘Shaoxing’, ‘Pawnee’, ‘Mahan’, and ‘Xibu’ occupy a dominant position. ‘Shaoxing’ is particularly important for its extensive promotion in Huanjiang Maonan Autonomous County, Hechi City, Guangxi Zhuang Autonomous Region [3]. ‘Shaoxing’ exhibits excellent adaptability and high-quality fruits. A 6-year-old plant can enter the initial production phase. The oil content of the fruit kernel can reach 68.1%, with the linoleic acid reaching about 12.56% [1]. However, the introduction of *C. illinoensis* has resulted in several undesirable phenomena, including excessive growth of branches and leaves, a low yield of fruits, severe flower and fruit drop, and poor fruit quality. Additionally, reproductive obstacles such as a poor flowering period and poor pollination and fertilization during reproduction contribute to a further reduction in fruit yield [4]. These phenomena have significantly affected the yield and productivity of *C. illinoensis* cv. ‘Shaoxing’, consequently affecting the income of local growers. To summarize, inhibiting excessive branch growth, promoting flowering and fruiting, and increasing fruit quality during the introduction and cultivation of ‘Shaoxing’ to improve economic benefits and facilitate the sustainable development of the *C. illinoensis* industry in the region is extremely important.

The rational application of plant growth inhibitors and retardants represents a crucial strategy for regulating the vegetative and reproductive growth of plants. It can also be applied to mitigate flower and fruit drop and enhance fruit quality [5,6]. Paclobutrazol (PP<sub>333</sub>, C<sub>15</sub>H<sub>2</sub>N<sub>3</sub>OCl), also known as chlorobutrazol, is a triazole compound. It can reduce cell division and elongation by inhibiting the synthesis of endogenous gibberellin in plants, thus inhibiting plant growth or the growth of branches. It is a commonly used chemical in agricultural and forestry production because it can efficiently regulate shoot growth, promote fruit development, and inhibit the excessive proliferation of branches and leaves [7]. However, the prolonged application of PP<sub>333</sub> results in environmental contamination and the emergence of drug-resistant plants, which can lead to branch death, diseases, and yield reduction. To overcome these limitations, Piperonyl Butoxide (PBO) has emerged as a promising alternative in recent years for treating fruit trees. PBO is a compound preparation comprising a range of growth regulators (e.g., uniconazole, cytokinins, and auxin derivatives), swelling agents, colorants, trace elements, and other components. It has several functions, including the inhibition of excessive shoot growth, an increase in chlorophyll content, the shortening of internodes, the promotion of flowering, an improvement in fruit quality, and an increase in plant resistance [8]. In recent years, PP<sub>333</sub> and PBO have been applied extensively in the cultivation of various fruit trees. The application of PP<sub>333</sub> can increase the yield and quality of fruit produced by different tree species, including *C. illinoensis* cv. ‘Pawnee’ [9], *Anacardium occidentale* [10], *Citrus reticulata* [11], and others. Additionally, PP<sub>333</sub> can inhibit the longitudinal growth of branches. Treatment with 1500 mg·L<sup>-1</sup> PP<sub>333</sub> considerably increased the starch content and C/N ratio in *Taxus chinensis* leaves [12]. The combination of 100 mg·L<sup>-1</sup> NAA and 200 mg·L<sup>-1</sup> PP<sub>333</sub> can inhibit the growth of *C. illinoensis* seedlings, stimulate root development, and increase the root-shoot ratio [13]. Compared to treatment with auxin, ABA, and salicylic acid, treatment with PP<sub>333</sub>

strongly inhibited the vegetative growth of *Olea europaea* and increased the fruit-setting rate. Foliar spraying is a more effective method than soil treatment [14]. PBO was found to be effective when applied to a range of fruit trees, such as *Pyrus Bret Schneider* Rehd. [15] and *Vitis labrusca* × *Vitis vinifera* ‘Kyoho’ [16], which can control the growth of new shoots and promote the development and maturation of fruits. The application of PBO significantly promoted the growth of *Malus Pumila* Mill. Shoots, inhibit the growth of new shoots, and increase the chlorophyll content of leaves, fruit weight, and fruit shape index [17]. To summarize, PP<sub>333</sub> and PBO can inhibit the growth of branches and internode elongation in various plant species. Moreover, they exert regulatory effects on fruit quality, yield, and plant growth. Additionally, PBO can increase the proportion of carbohydrates in leaves, stimulate the accumulation of photosynthetic products, and promote early fruit maturation. However, further studies are needed to gain a deeper understanding of the effects of PP<sub>333</sub> and PBO on *C. illinoensis* cv. ‘Shaoxing’ and determine the optimal treatment concentration.

*Carya illinoensis* is planted in a large part of Hechi City, Guangxi Zhuang Autonomous Region, China. Some studies have shown that the appropriate application of PP<sub>333</sub> and PBO can facilitate the growth and yield of *C. illinoensis*. The application of PP<sub>333</sub> and PBO during the growth and development period may address the problems of flower and fruit drop, a low fruit setting rate, and reduced yield during the introduction and cultivation of *C. illinoensis* cv. ‘Shaoxing’. In this study, 6-year-old *C. illinoensis* cv. ‘Shaoxing’ plants were used to conduct experiments. During the flowering and fruiting periods, different concentrations of PBO and PP<sub>333</sub> solutions were applied to the leaves by spraying. The effects of treatment with different growth regulators on the regulation of shoots and fruits, nutrient accumulation, and fruit quality of *C. illinoensis* cv. ‘Shaoxing’ plants were analyzed. From the perspective of plant growth regulator application, this study provided a theoretical basis for regulating the growth of introduced *C. illinoensis* in Huanjiang County.

## 2 Materials and Methods

### 2.1 Test Sites

The experimental site was located in Huanjiang Maonan Autonomous County, Hechi City, Guangxi Zhuang Autonomous Region (108.56°E, 24.51°N, altitude 470.3 m). The area has a subtropical monsoon type of climate. The annual average number of solar irradiation hours is 1396, the annual average temperature is 19.9°C, the frost-free period is 290 days, and the annual average precipitation is 1389.1 mm. Most of the precipitation occurs during the spring and summer months, accounting for about 70% of the annual total. The study was conducted from May to September 2019.

### 2.2 Experimental Design

Based on the scheme proposed by Zhang et al., a two-factor randomized block design was used to apply different concentrations of PBO and PP<sub>333</sub> plant growth regulators to the leaves of 6-year-old *C. illinoensis* cv. ‘Shaoxing’ plants (Fig. A1), with clear water serving as the control (CK) [18] (Table A1). The experiment comprised seven treatments, with 21 plants allocated to each treatment, seven plants allocated to each replicate, and three replicates. During the experiment, the plants that displayed consistent growth and no pests or diseases were selected for further study. Three branches of the ‘Shaoxing’ sample tree were selected from the east, south, west, and north of the middle of the crown for marking and numbering. Foliar sprays of the growth regulators were applied twice: once on May 2 and again on July 15 (an interval of 75 days). Each tree was sprayed with about 700–800 mL of solution until the leaf surfaces were saturated and then dripped (Fig. A1). PBO wettable powder, sourced from Huaye Agricultural Technology Co., Ltd. (Jiangyin, China), was prepared at concentrations of 2, 5, and 10 g·L<sup>-1</sup>, referring to the total formulation concentration according to the manufacturer’s specifications; PP<sub>333</sub> wettable powder (containing 15% active ingredients), obtained from Guoguang Agricultural Chemical Co., Ltd. (Chengdu,

China), was prepared at concentrations of 150, 300, and 450 mg·L<sup>-1</sup> based on the actual active ingredient content (Fig. A2). Throughout the experiment, maintenance and management conditions, such as irrigation and fertilization, were maintained consistently, and weeds were removed 1–2 times per month.

## 2.3 Determination of Indices

### 2.3.1 Shoot Growth Indices

The growth of the branch internodes was measured on days 19 and 147 after the flowering (an interval of 128 days). Three fruiting branches and three vegetative branches with comparable growth states were randomly selected from the east, south, west, and north of the middle part of the crown of the sample plant for numbering. The diameter of the base of the branch and the length of the third internode were measured using a digital vernier caliper, with an accuracy of 0.01 mm. Each treatment was repeated three times.

### 2.3.2 Leaf Physiological Indices

As described by Jia et al., the fruit development period of *C. illinoensis* can be divided into four distinct phases: the fruit slow growth period, the fruit rapid expansion period, the fruit hardcore period, and the fruit kernel maturity period. The contents of soluble sugar, soluble protein, and starch in the leaves of the ‘Shaoxing’ were determined at 19, 83, 117, and 147 days after flowering [19]. The healthy leaves situated at the periphery of the sample branches in the east, west, south, and north of the plant were mixed and bagged, representing a single plant unit. The samples were quickly transferred to a biological sample refrigerator, transported to the laboratory, and placed in an ultralow temperature refrigerator at –80°C for subsequent analysis. The content of soluble sugar and starch in the leaves was determined by anthrone colorimetry [20], whereas the soluble protein content was determined by the Coomassie brilliant blue G-250 method [21].

### 2.3.3 Leaf Nutrient Indices

The leaves of ‘Shaoxing’ sample branches were collected at 19, 83, 117, and 147 days after flowering, de-enzyme at 105°C for 0.5 h in an oven, and then adjusted to 80°C until a constant weight was reached. The dried materials were pulverized, filtered, and stored in a sealed container at room temperature in a cool environment to determine the total N, P, and K contents in the leaves. The total N content in the leaves was determined by H<sub>2</sub>SO<sub>4</sub>-H<sub>2</sub>O<sub>2</sub> digestion using the Kjeldahl method. The total phosphorus content of the leaves was determined using the molybdenum antimony colorimetric method, whereas the total potassium content was determined by flame photometry [22].

### 2.3.4 Fruit Setting Rate

The number of female flowers on each branch of each plant was investigated at the late flowering stage (May 2), and the number of fruit set on each branch of each plant was investigated at the fruit maturity stage. The fruit setting rate, defined as the percentage of fruit set to the number of female flowers was calculated.

### 2.3.5 Nutrient Indices of the Fruit Kernel

At 147 days after the flowering of ‘Shaoxing’, 4–6 fruits were collected from the east, south, west, and north of the branches of each sample plant in each treatment, and 10 fruits were randomly selected after classification and treatment of the harvested fruits. After removing the peel and kernels, the samples were placed in an ultralow temperature refrigerator set at –80°C to determine the nutrients. The tannin content in the kernels was determined by spectrophotometry [23]. The crude fat content of the fruit was determined by the Soxhlet extraction method [24]. The soluble protein content of the kernels was determined by the Coomassie brilliant blue G-250 method [21]. The reducing sugar content in the kernels was determined by the 3,5-dinitrosalicylic acid method [25]. The cellulose content in the kernels was determined by the nitric acid-ethanol method [26].

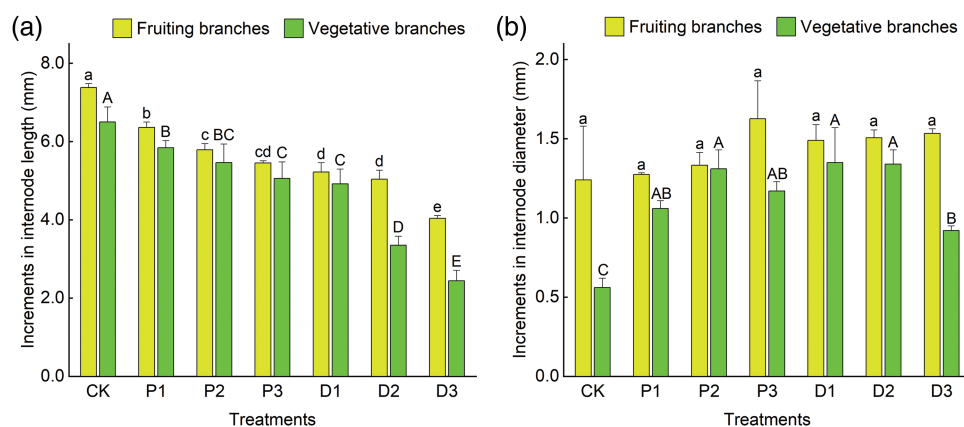
## 2.4 Data Analysis

The data were analyzed using Microsoft Excel 2016 (USA). IBM SPSS 20.0 (USA) was used to conduct multivariate analysis of variance and principal component analysis; the Duncan method ( $\alpha = 0.05$ ) was used for multiple comparisons. The principal component analysis included a correlation matrix, in which 16 indicators closely related to the shoot control and fruit promotion in ‘Shaoxing’ were selected. The number of principal components was determined according to an eigenvalue greater than 1.0. The function expression of the principal component was listed based on the eigenvector of the correlation matrix of ‘Shaoxing’. Finally, the comprehensive scores of the effects of PBO and PP<sub>333</sub> on ‘Shaoxing’ were calculated and compared based on the principal component value.

## 3 Results

### 3.1 Effects of PBO and PP<sub>333</sub> on the Growth of Fruiting Branches and Vegetative Branches of ‘Shaoxing’

Different concentrations of PBO and PP<sub>333</sub> significantly affected the increase in fruit branch length and vegetative branch length of ‘Shaoxing’ ( $p < 0.05$ ) (Fig. 1a, Table A2). Among all treatments, the 450 mg·L<sup>-1</sup> PP<sub>333</sub> (D3) treatment had the most significant inhibitory effect on the growth of fruit branches and vegetative branch length. The increase in the internode lengths of the fruiting branches and vegetative branches were 4.04 and 2.44 mm, respectively, which were 45.26% and 62.46% lower than those of the control group (7.38 and 6.50 mm, respectively). Regarding the application of the two growth regulators, the increase in the internode length of fruiting branches and vegetative branches decreased with an increase in the concentration of the growth regulators. The optimal inhibitory concentrations of the two growth regulators on fruiting branches and vegetative branches were 10 g·L<sup>-1</sup> PBO (P3) and 450 mg·L<sup>-1</sup> PP<sub>333</sub> (D3), respectively.



**Figure 1:** Effects of different treatments on the increase in internode length and diameter of fruiting branches and vegetative branches of ‘Shaoxing’. (a) Effects of different treatments on the increase in the internode length of fruiting branches and vegetative branches. (b) Effects of different treatments on the increase in the internode diameter of fruiting branches and vegetative branches. Different lowercase letters indicate significant differences in the growth of fruiting branches among the different treatments ( $p < 0.05$ ), and different uppercase letters indicate significant differences in the growth of vegetative branches among the different treatments ( $p < 0.05$ ); the error line represents the standard error

Different concentrations of PBO and PP<sub>333</sub> significantly affected the increase in vegetative branch diameters in the ‘Shaoxing’ ( $p < 0.05$ ), whereas their impact on fruiting branch diameter growth was not statistically significant ( $p > 0.05$ , Table A2) (Fig. 1b). In all treatments, the increase in branch diameter

reached a maximum value of 1.63 mm after treatment with 10 g·L<sup>-1</sup> PBO (P3), which was 31.45% greater than that of the control group (1.24 mm). The increase in the internode diameter of vegetative branches reached a maximum value of 1.35 mm after treatment with 150 mg·L<sup>-1</sup> PP<sub>333</sub> (D1), which was 141.07% greater than that of the control group (0.56 mm). As the PBO and PP<sub>333</sub> concentrations increased, the increase in the internode diameter of fruiting branches increased, and the increase in the internode diameter of vegetative branches first increased and then decreased. The optimum concentrations of the two growth regulators to promote an increase in the diameter of fruiting branches were 10 g·L<sup>-1</sup> PBO (P3) and 450 mg·L<sup>-1</sup> PP<sub>333</sub> (D3), and the optimum concentrations to promote an increase in the diameter of vegetative branches were 5 g·L<sup>-1</sup> PBO (P2) and 150 mg·L<sup>-1</sup> PP<sub>333</sub> (D1).

To summarize, both PBO and PP<sub>333</sub> significantly inhibited the excessive growth of vegetative branches and fruiting branches. The inhibitory effect of PP<sub>333</sub> was stronger than that of PBO, and the inhibitory effect of PP<sub>333</sub> on vegetative branches was stronger than that on fruiting branches. Treatment with 450 mg·L<sup>-1</sup> PP<sub>333</sub> (D3) had the greatest inhibitory effect on the growth of the internode of fruiting branches and vegetative branches. The PBO and PP<sub>333</sub> treatments significantly increased the diameter of the vegetative branches of ‘Shaoxing’. Treatment with 10 g·L<sup>-1</sup> PBO (P3) had the strongest effect on increasing the diameter of fruiting branches, whereas treatment with 150 mg·L<sup>-1</sup> PP<sub>333</sub> (D1) had the greatest effect on effect on vegetative branches.

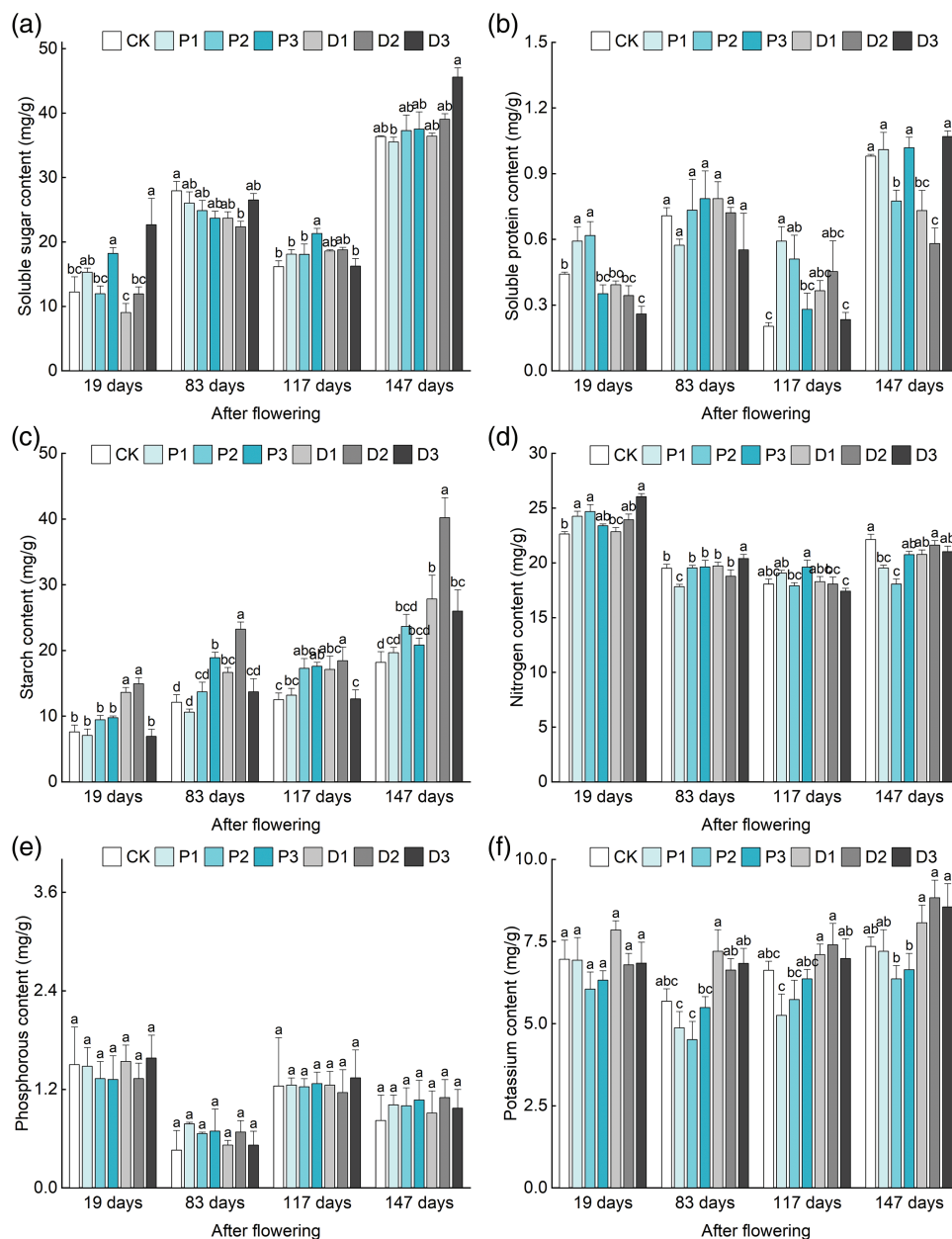
### **3.2 Effects of PBO and PP<sub>333</sub> on the Physiological Indices and Nutritional Status Indices of ‘Shaoxing’ Leaves**

#### **3.2.1 Effects of PBO and PP<sub>333</sub> on the Physiological Indices of ‘Shaoxing’ Leaves**

Different concentrations of PBO and PP<sub>333</sub> significantly affected the soluble sugar content in the leaves of ‘Shaoxing’ plants ( $p < 0.05$ ) (Fig. 2a, Table A3). Following the transition to different stages after anthesis (days 19 to 147), the soluble sugar content of the leaves in each treatment group increased, decreased, and finally increased. The changes in soluble sugar content in the leaves of the ‘Shaoxing’ at different stages of fruit development were as follows. At 19 days after flowering, the soluble sugar content of the leaves treated with 450 mg·L<sup>-1</sup> PP<sub>333</sub> (D3) reached a maximum value of 22.65 mg·g<sup>-1</sup>, which was 85.35% greater than that recorded in the control group. At 83 days after flowering, the soluble sugar content of the leaves in all treatment groups was lower than that in the control group. At 117 days after flowering, the soluble sugar content of the leaves treated with 10 g·L<sup>-1</sup> PBO (P3) reached a maximum value of 21.31 mg·g<sup>-1</sup>, which was 31.87% greater than that of the control group. At 147 days after flowering, the soluble sugar content of leaves treated with 450 mg·L<sup>-1</sup> PP<sub>333</sub> (D3) reached a maximum value of 45.59 mg·g<sup>-1</sup>, which was 25.45% greater than that of the control group. These results indicated that 10 g·L<sup>-1</sup> PBO (P3) and 450 mg·L<sup>-1</sup> PP<sub>333</sub> (D3) had the strongest effects on the soluble sugar content in the leaves of ‘Shaoxing’ fruits during the fruit development period (19–147 days after flowering) across all treatment groups.

Different concentrations of PBO and PP<sub>333</sub> significantly affected the soluble protein content in the leaves of ‘Shaoxing’ plants ( $p < 0.05$ ) (Fig. 2b, Table A3). Following the transition to different stages after flowering (19–147 days after flowering), the soluble protein content of the leaves in each treatment group increased, decreased, and finally increased. The changes in the soluble protein content in the leaves of the ‘Shaoxing’ at different stages of fruit development were as follows. At 19 days after flowering, the soluble protein content of the leaves treated with 5 g·L<sup>-1</sup> PBO (P2) reached a maximum value of 0.62 mg·g<sup>-1</sup>, which was 40.91% greater than that of the control group. At 83 days after flowering, the soluble protein content of the leaves treated with 10 g·L<sup>-1</sup> PBO (P3) reached a maximum value of 0.79 mg·g<sup>-1</sup>, which was 11.26% greater than that of the control group. At 117 days after flowering, the soluble protein content in the plants in the 2 g·L<sup>-1</sup> PBO (P1) treatment group reached a maximum value of 0.59 mg·g<sup>-1</sup>, which was significantly greater than that in the control group, indicating an increase of 195.00%. At 147 days after flowering, the soluble protein content in the plants in the 450 mg·L<sup>-1</sup> PP<sub>333</sub>

(D3) treatment group reached a maximum value of  $1.07 \text{ mg}\cdot\text{g}^{-1}$ , but the difference was not significant compared to that in the control group. These results indicated that treatment with PBO and  $\text{PP}_{333}$  did not significantly affect the soluble protein content in the leaves of ‘Shaoxing’. However, it partly mitigated the decrease in soluble protein content in leaves from days 83 to 117 after flowering.



**Figure 2:** Effects of different treatments on the physiological indices of ‘Shaoxing’ leaves. (a) Effects of different treatments on the soluble sugar content. (b) Effects of different treatments on the starch content. (c) Effects of different treatments on the soluble protein content. (d) Effects of different treatments on the N content in leaves. (e) Effects of different treatments on the P content in leaves. (f) Effects of different treatments on the K content in leaves. Different lowercase letters indicate significant differences ( $p < 0.05$ ), and the error line represents the standard error

Different concentrations of PBO and PP<sub>333</sub> significantly affected the starch content in the leaves of 'Shaoxing' plants ( $p < 0.05$ ) (Fig. 2c, Table A3). Following the transition to different stages after flowering (19–147 days after flowering), the starch content of the leaves in each treatment gradually increased. The changes in starch content in the leaves of 'Shaoxing' plants at different stages of fruit development were as follows. At 19 days after flowering, the starch content of the leaves treated with 300 mg·L<sup>-1</sup> PP<sub>333</sub> (D2) reached a maximum value of 14.92 mg·g<sup>-1</sup>, which was 96.32% greater than that of the control group. At 83 days after flowering, the starch content of the leaves treated with 300 mg·L<sup>-1</sup> PP<sub>333</sub> (D2) reached a maximum value of 23.22 mg·g<sup>-1</sup>, which was 91.90% greater than that of the control group. At 117 days after flowering, the starch content of the leaves treated with 300 mg·L<sup>-1</sup> PP<sub>333</sub> (D2) reached a maximum value of 18.42 mg·g<sup>-1</sup>, which was 47.48% greater than that of the control group. At 147 days after flowering, the leaf starch content of the leaves treated with 300 mg·L<sup>-1</sup> PP<sub>333</sub> (D2) reached a maximum value of 40.19 mg·g<sup>-1</sup>, which was 121.07% greater than that of the control group. To summarize, among the PBO treatments, 5 g·L<sup>-1</sup> PBO (P2) had the strongest effect on promoting the starch content of 'Shaoxing' leaves during the fruit development period, whereas 300 mg·L<sup>-1</sup> PP<sub>333</sub> (D2) had the greatest effect among the PP<sub>333</sub> treatments.

### 3.2.2 Effects of PBO and PP<sub>333</sub> on the Nutritional Status Indices (Total N, P, and K Content) of 'Shaoxing' Leaves

Different concentrations of PBO and PP<sub>333</sub> significantly affected the N content in the leaves of 'Shaoxing' plants ( $p < 0.05$ ) (Fig. 2d, Table A3). With the changes in the different stages after flowering (19–147 days after flowering), the N content of the leaves in each treatment gradually decreased. The changes in the N content in the leaves of 'Shaoxing' at different stages of fruit development were as follows. At 19 days after flowering, the N content in the leaves treated with 450 mg·L<sup>-1</sup> PP<sub>333</sub> (D3) reached a maximum value of 26.03 mg·g<sup>-1</sup>, which was 15.02% greater than that of the control group. At 83 days after flowering, the N content of the leaves treated with 450 mg·L<sup>-1</sup> PP<sub>333</sub> (D3) reached a maximum value of 20.39 mg·g<sup>-1</sup>, which was 4.56% greater than that of the control group. At 117 days after flowering, the N content of the leaves treated with 10 g·L<sup>-1</sup> PBO (P3) reached a maximum value of 19.61 mg·g<sup>-1</sup>, which was 8.58% greater than that of the control group. At 147 days after flowering, the N content in the leaves of the control group reached a maximum value of 22.13 mg·g<sup>-1</sup>, while the N content in the group treated with 300 mg·L<sup>-1</sup> PP<sub>333</sub> (D2) was the highest, which was 6.28% lower than that of the control group, and this difference was not statistically significant. These findings indicated that the nitrogen content of the leaves decreased during the fruit development period. The PP<sub>333</sub> treatment partially increased the nitrogen content of leaves in the late stage of 'Shaoxing' fruit development. This resulted in a delay in the leaf yellowing period and an increase in the cycle of photosynthesis.

Different concentrations of PBO and PP<sub>333</sub> did not significantly affect the P content in the leaves of 'Shaoxing' plants ( $p < 0.05$ ) (Fig. 2e, Table A3). With the changes in the different stages after flowering (19–147 days after flowering), the P content of the leaves in each treatment decreased, increased, and finally decreased. The changes in leaf P content at different stages of 'Shaoxing' fruit development were as follows. At 19 days after flowering, the leaf P content of the leaves treated with 450 mg·L<sup>-1</sup> PP<sub>333</sub> (D3) reached a maximum value of 1.58 mg·g<sup>-1</sup>, which was 5.33% greater than that of the control group. At 83 days after flowering, the P content of the leaves treated with 2 g·L<sup>-1</sup> PBO (P1) reached a maximum value of 0.78 mg·g<sup>-1</sup>, which was 69.57% greater than that of the control group. At 117 days after flowering, the P content of the leaves treated with 450 mg·L<sup>-1</sup> PP<sub>333</sub> (D3) reached a maximum value of 1.34 mg·g<sup>-1</sup>, which was 8.06% greater than that of the control group. At 147 days after flowering, the P content of the leaves treated with 300 mg·L<sup>-1</sup> PP<sub>333</sub> (D2) reached a maximum value of 1.10 mg·g<sup>-1</sup>, which was 34.15% greater than that of the control group. These results indicated that the application of PP<sub>333</sub> and PBO at different concentrations did not significantly change the P content of leaves. However,



the P content of the leaves treated with PBO and PP<sub>333</sub> was greater than that of the control group during the middle and late stages of fruit development (83–147 days after flowering). These findings suggested that spraying PP<sub>333</sub> may retard the decline in leaf P content during the late stages of ‘Shaoxing’ fruit development.

Different concentrations of PBO and PP<sub>333</sub> significantly affected the K content in the leaves of ‘Shaoxing’ plants ( $p < 0.05$ ) (Fig. 2f, Table A3). With the changes in the different stages after flowering (19–147 days after flowering), the K content of the leaves in each treatment first decreased and then increased. The changes in the K content in the leaves at different stages of ‘Shaoxing’ fruit development were as follows. At 19 days after flowering, the K content in the leaves treated with 150 mg·L<sup>-1</sup> PP<sub>333</sub> (D1) reached a maximum value of 7.85 mg·g<sup>-1</sup>, which was 12.79% greater than that of the control group. At 83 days after flowering, the total K content of the leaves treated with 150 mg·L<sup>-1</sup> PP<sub>333</sub> (D1) reached a maximum value of 7.20 mg·g<sup>-1</sup>, which was 26.76% greater than that of the control group. At 117 days after flowering, the K content of the leaves treated with 300 mg·L<sup>-1</sup> PP<sub>333</sub> (D2) reached a maximum value of 7.40 mg·g<sup>-1</sup>, which was 11.78% greater than that of the control group. At 147 days after flowering, the K content of the leaves treated with 300 mg·L<sup>-1</sup> PP<sub>333</sub> (D2) reached a maximum value of 8.83 mg·g<sup>-1</sup>, which was 20.14% greater than that of the control group. Our results indicated that while the application of PBO and PP<sub>333</sub> did not lead to a notable alteration in the K content in leaves, the K content of leaves treated with different concentrations of PP<sub>333</sub> during the fruit development period (19–147 days after flowering) was greater than that of the control group. These findings suggested that spraying PP<sub>333</sub> can increase the K content of leaves during the fruit development of ‘Shaoxing’ fruits to a certain extent.

### **3.3 Effects of PBO and PP<sub>333</sub> on the Fruit Setting Rate and Nutritional Quality of ‘Shaoxing’ Fruits**

#### **3.3.1 Effects of PBO and PP<sub>333</sub> on the Fruit Setting Rate of ‘Shaoxing’**

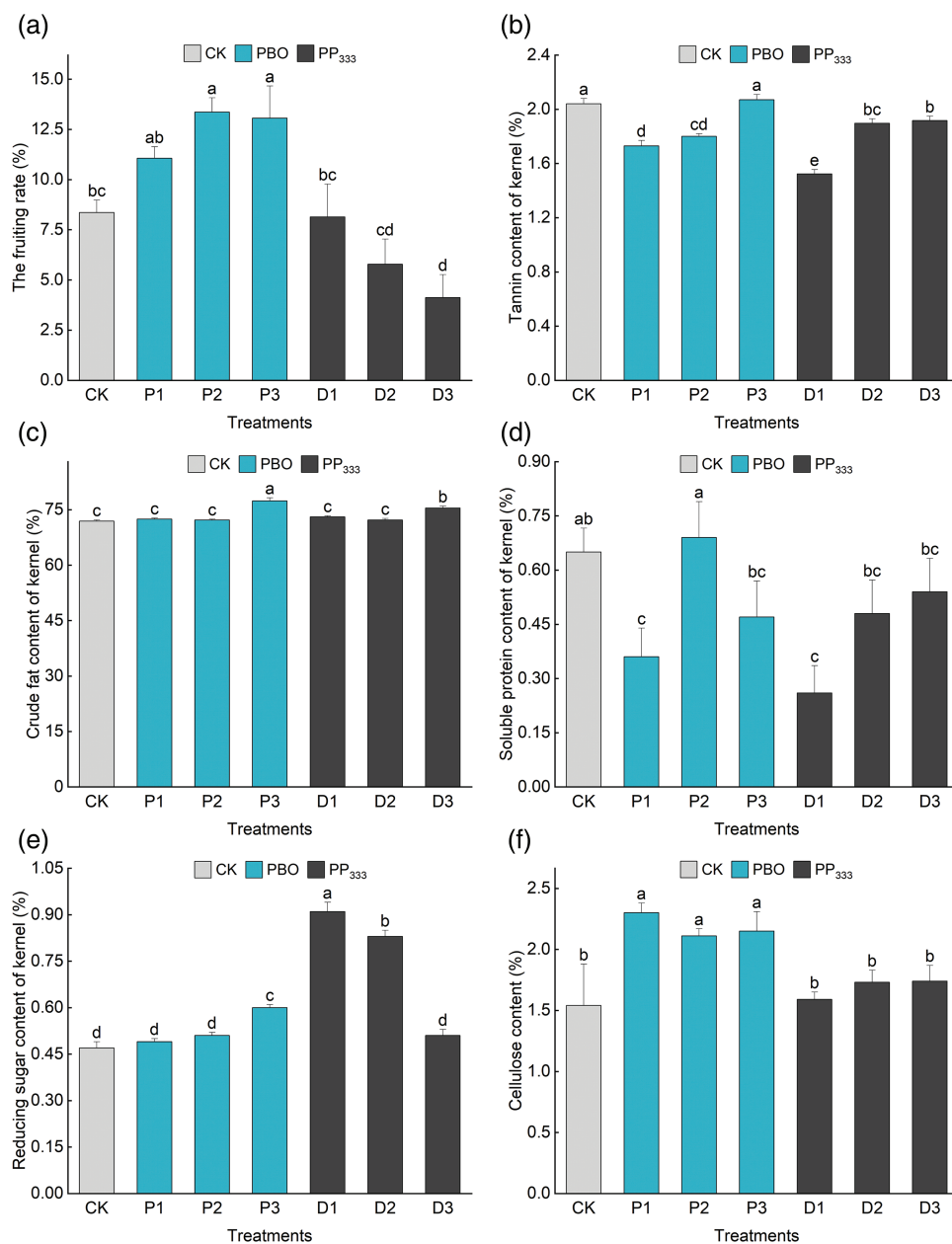
Different concentrations of PBO and PP<sub>333</sub> had significant effects on the fruit setting rate of ‘Shaoxing’ ( $p < 0.05$ ) (Fig. 3a, Table A4). In all the treatments, the fruit setting rate reached a maximum of 13.36% under the 5 g·L<sup>-1</sup> PBO (P2) treatment, which was 59.81% greater than that of the control group (8.36%). After treatment with the two growth regulators, the fruit setting rate gradually decreased as the PP<sub>333</sub> concentration increased but gradually increased as the PBO concentration increased. The fruit-setting rate of ‘Shaoxing’ was significantly different between the P2, P3, and D3 treatment groups and the control group (CK), and the fruit-setting rate of ‘Shaoxing’ branches under the P2 and P3 treatments was significantly greater than that of the CK, indicating that 2–10 g·L<sup>-1</sup> PBO treatment can improve the fruit setting rate of ‘Shaoxing’.

#### **3.3.2 Effects of PBO and PP<sub>333</sub> on the Nutritional Quality of ‘Shaoxing’ Fruits**

Different concentrations of PBO and PP<sub>333</sub> significantly affected the tannin content of ‘Shaoxing’ fruits ( $p < 0.05$ ) (Fig. 3b, Table A4). Among all treatments, the tannin content in the 150 mg·L<sup>-1</sup> PP<sub>333</sub> (D1) treatment group was the lowest (1.52%), which was 25.49% lower than that in the control group. After applying the two growth regulators, the tannin content gradually increased with an increase in PBO and PP<sub>333</sub> concentrations. Except for treatment with 10 g·L<sup>-1</sup> PBO (P3) and 450 mg·L<sup>-1</sup> PP<sub>333</sub> (D3), the tannin content of the remaining treatments was considerably lower than that of the control group. These findings suggested that low concentrations of PBO (2–5 g·L<sup>-1</sup>) and PP<sub>333</sub> (150–300 mg·L<sup>-1</sup>) effectively decreased the tannin content in ‘Shaoxing’ fruit.

Different concentrations of PBO and PP<sub>333</sub> significantly affected the content of crude fat in ‘Shaoxing’ fruit ( $p < 0.05$ ) (Fig. 3c, Table A4). Among all treatments, the crude fat content of the 10 g·L<sup>-1</sup> PBO (P3) treatment group reached a maximum value of 77.38%, which was 7.62% greater than that of the control group. After treatment with the two growth regulators, the content of crude fat in fruits increased gradually as the PBO and PP<sub>333</sub> concentrations increased. The crude fat content in the 10 g·L<sup>-1</sup> PBO (P3)

and 450 mg·L<sup>-1</sup> PP<sub>333</sub> (D3) treatment groups was significantly greater than that in the control group, indicating that high concentrations of PBO (10 g·L<sup>-1</sup>) and PP<sub>333</sub> (450 mg·L<sup>-1</sup>) increased the crude fat content in ‘Shaoxing’ fruit and improved fruit quality.



**Figure 3:** Effects of different treatments on the fruit setting rate and fruit quality of ‘Shaoxing’. (a) Effects of different treatments on the fruit setting rate. (b) Effects of different treatments on the tannin content in fruit kernels. (c) Effects of different treatments on the crude fat content of fruit kernels. (d) Effects of different treatments on the soluble protein content of fruit kernels. (e) Effects of different treatments on the reducing sugar content of fruit kernels. (f) Effects of different treatments on the cellulose content of fruit kernels. Different lowercase letters indicate significant differences ( $p < 0.05$ ), and the error line represents the standard error

Different concentrations of PBO and PP<sub>333</sub> significantly affected the soluble protein content in ‘Shaoxing’ fruit ( $p < 0.05$ ) (Fig. 3d, Table A4). Among all treatments, the soluble protein content of the fruit treated with 5 g·L<sup>-1</sup> PBO (P2) reached a maximum value of 0.69%, which was 6.15% greater than that of the control group. For the two growth regulators, the soluble protein content first increased and then decreased as the PBO concentration increased but gradually increased as the PP<sub>333</sub> concentration increased. Compared to the control group, the 10 g·L<sup>-1</sup> PBO (P1) and 150 mg·L<sup>-1</sup> PP<sub>333</sub> (D1) treatment groups presented significantly lower soluble protein contents; however, no significant difference in the soluble protein content was recorded between the other treatment groups and the control group. Additionally, 5 g·L<sup>-1</sup> PBO (P2) significantly increased the soluble protein content in fruit compared to the other treatments.

Different concentrations of PBO and PP<sub>333</sub> significantly affected the reduced sugar content in ‘Shaoxing’ fruit ( $p < 0.05$ ) (Fig. 3e, Table A4). Among all treatments, the reduced sugar content of fruits treated with 150 mg·L<sup>-1</sup> PP<sub>333</sub> (D1) reached a maximum value of 0.91%, which was 102.22% greater than that of the control group. After applying the two growth regulators, the content of reducing sugar gradually increased as the PBO concentration increased but gradually decreased as the PP<sub>333</sub> concentration increased. The reduced sugar content of all treatment groups was greater than that of the control group. Additionally, the reducing sugar content in the 10 g·L<sup>-1</sup> PBO (P3), 150 mg·L<sup>-1</sup> PP<sub>333</sub> (D1) and 300 mg·L<sup>-1</sup> PP<sub>333</sub> (D2) treatment groups was significantly greater than that in the control group, indicating that high concentrations of PBO (10 g·L<sup>-1</sup>) and low concentrations of PP<sub>333</sub> (150–300 mg·L<sup>-1</sup>) could significantly increase the reducing sugar content in ‘Shaoxing’ fruit.

Different concentrations of PBO and PP<sub>333</sub> significantly affected the cellulose content of ‘Shaoxing’ fruit ( $p < 0.05$ ) (Fig. 3f, Table A4). Among all treatments, the cellulose content of the fruits treated with 10 g·L<sup>-1</sup> PBO (P1) reached a maximum value of 2.30%, which was 49.35% greater than that of the control group. After applying the two growth regulators, the cellulose content of the fruit first increased then decreased as the PBO concentration increased but gradually increased as the PP<sub>333</sub> concentration increased. The cellulose content of all PBO treatment groups was significantly greater than that of the control group. Additionally, the cellulose content of the fruit increased slightly in the PP<sub>333</sub> treatment group, suggesting that the application of PBO may increase the cellulose content of the fruit.

To summarize, the application of PBO and PP<sub>333</sub> significantly affected the nutritional quality of *C. illinoensis* cv. ‘Shaoxing’ fruits. The 2 g·L<sup>-1</sup> PBO (P1) treatment had the strongest effect on increasing the fruit cellulose content. The 5 g·L<sup>-1</sup> PBO (P2) treatment had the greatest effect on increasing the soluble protein content of the fruits. The 10 g·L<sup>-1</sup> PBO (P3) treatment had the greatest effect on increasing the sugar content of the fruits. The 150 mg·L<sup>-1</sup> PP<sub>333</sub> (D1) treatment had the greatest effect on reducing the fruit tannin content and increasing the fruit reducing sugar content.

### 3.4 Principal Component Analysis

The effects of different concentrations of PBO and PP<sub>333</sub> on the growth index, leaf index, and fruit quality of ‘Shaoxing’ were determined by analyzing the variance of these indices. The results showed significant differences between the different treatments. A comprehensive evaluation and analysis of the effects of growth regulators on ‘Shaoxing’ were conducted via principal component analysis, and the comprehensive treatment effects of the two growth regulators at different concentrations were evaluated holistically.

#### 3.4.1 Principal Component Extraction

The characteristic value and contribution percentage of principal components serve as the basis for screening principal components. In total, 16 related indices are closely associated with shoot control and fruit promotion in *C. illinoensis* cv. ‘Shaoxing’, was selected for analysis. Principal components were extracted using the IBM SPSS software. Four principal components with eigenvalues exceeding 1.0 were

selected as comprehensive indices to assess the overall effect of PBO and PP<sub>333</sub> on shoot control and fruit promotion in *C. illinoensis* cv. 'Shaoxing' (Table 1).

**Table 1:** Complete variable interpretation

Principal component	Eigenvalue	Percentage (%)	Cumulative percentage (%)
1	6.326	39.540	39.540
2	3.529	22.055	61.596
3	3.006	18.789	80.385
4	1.562	9.760	90.145

### 3.4.2 Principal Component Score

Among the comprehensive scores of the four principal components with eigenvalues greater than 1.0 (Table 2), the highest score was for the 450 mg·L<sup>-1</sup> PP<sub>333</sub> (D3) treatment group. The comprehensive scores for the PP<sub>333</sub> and PBO treatment groups were higher than those of the CK. Except for the 150 mg·L<sup>-1</sup> PP<sub>333</sub> treatment, the scores of the PP<sub>333</sub> treatments were greater than those of the PBO treatment. These results indicated that treatment with PBO and PP<sub>333</sub> positively affected the growth and development of 'Shaoxing' fruits. The overall effect of the PP<sub>333</sub> treatment was more favorable than that of the PBO treatment group.

**Table 2:** Ranking of principal components in different treatments

Treatment	Principal component 1	Principal component 2	Principal component 3	Principal component 4	Comprehensive score	Raking
P1	-2.102	-0.781	0.504	-0.739	-0.472	6
P2	-2.182	-1.302	0.923	1.719	-0.290	5
P3	-0.319	0.956	2.910	-0.798	0.350	3
D1	1.659	-2.145	-1.097	-1.781	-0.276	4
D2	3.252	-1.300	-0.520	1.430	0.459	2
D3	2.611	3.066	-0.078	0.133	0.857	1
CK	-2.920	1.506	-2.642	0.035	-0.628	7

## 4 Discussion

### 4.1 Effects of PBO and PP<sub>333</sub> on the Branch Growth of 'Shaoxing'

The growth of plant branches can be altered by plant growth regulators. PBO and PP<sub>333</sub> can regulate the growth of plant branches by inhibiting various processes, including the oxidation of kaurene to kaurene acid [27–29]. The results of this study showed that medium and high concentrations of PBO (5–10 g·L<sup>-1</sup>) and PP<sub>333</sub> (300–450 mg·L<sup>-1</sup>) inhibited the excessive growth of fruiting branches and vegetative branches of *C. illinoensis* cv. 'Shaoxing' to a certain extent promoted the periclinal division of branch cells, which was similar to the results of *C. illinoensis* cv. 'Mahan' [18], *Zamioculcasz zamiifolia* [30], *Ficus carica* [31], and others. PP<sub>333</sub> inhibited plant cell division and the synthesis of gibberellic acid 3, thereby inhibiting the growth of the plant stem's subapical meristem. However, PP<sub>333</sub> did not inhibit the growth of the apical meristem but decelerated cell elongation, thus inhibiting the longitudinal growth of branches. The regulatory effect was closely related to the concentration and the species of the plant [32–34]. The results

of this study indicated that an increase in the concentrations of PBO and PP<sub>333</sub> was associated with an increase in the inhibitory effect on the elongation of the fruiting and vegetative branches of 'Shaoxing'. Additionally, a notable increase in the diameter increment of fruiting branches was observed. Similarly, the application of PP<sub>333</sub> resulted in a notable inhibition of the growth of *Betula alnoides*, with a strong correlation between the concentration and degree of inhibition [35]. Compared to the 100 mg·L<sup>-1</sup> PP<sub>333</sub> treatment, the 200 mg·L<sup>-1</sup> PP<sub>333</sub> treatment could significantly increase the branch diameter of *C. illinoensis* cv. 'Pawnee' [9]. To summarize, the optimal concentrations of PBO and PP<sub>333</sub> can facilitate the lateral growth of the branches of *C. illinoensis* cv. 'Shaoxing' while simultaneously inhibiting the elongation of the internodes, thus promoting the formation of short, thick branches.

#### 4.2 Effects of PBO and PP<sub>333</sub> on the Physiological Indices and Nutritional Status Indices of 'Shaoxing' Leaves

The leaf is essential for photosynthesis, respiration, and transpiration in plants. The physiological indices and elemental composition of leaves strongly influence plant growth and reproduction [36]. The results of this study indicated that foliar application of PBO and PP<sub>333</sub> may facilitate the accumulation of carbohydrates in the leaves of 'Shaoxing' plants, leading to an increase in the contents of soluble sugar, soluble protein, and starch in leaves. The contents of soluble sugar and soluble protein in leaves treated with 450 mg·L<sup>-1</sup> PP<sub>333</sub> (D3) were the highest, while the content of starch in leaves treated with 300 mg·L<sup>-1</sup> PP<sub>333</sub> (D2) was the highest. Similarly, PP<sub>333</sub> treatment was found to significantly increase the soluble sugar content in *Ligustrum lucidum* leaves [37]. Compared to that in the control group, the starch content in the leaves of *C. illinoensis* cv. 'Mahan' significantly increased in response to 250 mg·L<sup>-1</sup> PBO treatment [18]. A high concentration of PP<sub>333</sub> was found to significantly increase the soluble sugar content in the leaves of *Mangifera indica* L. [38]. Plant growth regulators such as PBO and PP<sub>333</sub> may facilitate the transformation and transfer of organic substances, including carbohydrates, to the leaves, thus increasing the adaptability of plants to environmental stress. Administering plant growth regulators may increase the concentration of nitrogen in the leaves, thereby postponing the onset of leaf yellowing and increasing the duration of the leaf photosynthetic cycle [39–41]. Nitrogen (N), phosphorus (P), and potassium (K) are essential for plant growth and development. N plays a key role in plant biology, serving as a vital component of plant proteins, nucleic acids, and chlorophyll. It is involved in several essential processes, including plant growth, photosynthesis, and metabolic activity. P is a component of ATP, and K can regulate the activity of various enzymes within plant cells, thus increasing the ability of plants to withstand drought [42,43]. Treatment with PBO and PP<sub>333</sub> did not significantly alter the contents of N, P, and K in the leaves. However, the PP<sub>333</sub> treatment, along with the select PBO treatment groups, delayed the decrease in N, P, and K contents in leaves at the later stage of fruit development. This result was similar to the finding that PP<sub>333</sub> treatment can increase the contents of N, P, and K in leaves during the growth and development of *Passiflora coerulea*. These changes may be attributed to the indirect influence of growth regulators on the accumulation of N, P, and K through effects on plant growth, photosynthesis, and hormone metabolism [44,45]. In this study, PBO treatment promoted the accumulation of N and P in the leaves of 'Shaoxing', which did not favor the accumulation of K. PBO treatment may have increased the content of soluble protein in the leaves of 'Shaoxing', thus increasing the content of N. This finding was similar to the results reported for *Amorpha fruticose* and *Mytilaria laosensis* [46,47]. Moreover, no significant effects were detected on the P content between treatments, which may be attributed to natural biological variation in P uptake and partitioning between plant samples. Factors such as environmental conditions and plant physiological status may also contribute to this variability [48,49]. In conclusion, foliar application of PBO and PP<sub>333</sub> can increase carbon and nitrogen nutrient levels in the leaves of *C. illinoensis* cv. 'Shaoxing' during the middle and late stages of fruit development, and delay the decrease in N, P, and K contents in the leaves.

### 4.3 Effects of PBO and PP<sub>333</sub> on the Fruit Setting Rate and Nutrients in ‘Shaoxing’ Fruit

We found that PBO, at both 200 and 10 g·L<sup>-1</sup>, significantly improved the fruit-setting rate of *C. illinoensis* cv. ‘Shaoxing’. Similarly, another study showed that applying PP<sub>333</sub> or PBO can significantly increase the fruit setting rate and fruit yield of *Corylus heterophylla* × *Corylus avellan* hybrid hazels [50], *Litchi chinensis* [51], *Dimocarpus longan* [52], *Pyrus brestschneideri* Rehd. [53], and other plants. This may be associated with the high concentration of PBO, which contains relatively high levels of uniconazole, trace elements, and other components that facilitate the accumulation of nutrients in the fruit [54]. The contents of crude fat, protein, reducing sugar, and cellulose are important nutrients in the kernels of *C. illinoensis* cv. ‘Shaoxing’ fruits and the tannin content is proportional to the bitterness and astringency of the fruit. A reduction in tannin content results in a corresponding decrease in the perceived bitterness and astringency of the fruit [55–57]. In this study, treatment with PP<sub>333</sub> (150–450 mg·L<sup>-1</sup>) and PBO (2–5 g·L<sup>-1</sup>) significantly reduced the tannin content of ‘Shaoxing’ kernels, and all treatments increased the crude fat, cellulose, and reducing sugar contents of the kernels. Similarly, spraying 300 mg·L<sup>-1</sup> PP<sub>333</sub> on leaves during the fruit growth period can significantly reduce the tannin content in the kernels of the fruits of *Juglans regia* L., thus increasing the overall palatability of the fruit [58]. The foliar application of PBO at the full-bloom stage can substantially increase the soluble sugar content in the fruits of *Pyrus spp.* [59]. The promotion of plant growth by plant growth regulators may occur due to alterations in nutrient utilization efficiency and the optimization of plant nutrient performance [60,61].

## 5 Conclusion

In this study, we investigated the effects of different concentrations of PBO and PP<sub>333</sub> on branch growth, leaf physiological indices, and fruit nutritional quality in *C. illinoensis* cv. ‘Shaoxing’. The results showed that treatment with 450 mg·L<sup>-1</sup> PP<sub>333</sub> had the most significant inhibitory effect on the growth of fruiting branches and vegetative branches. Treatment with 10 g·L<sup>-1</sup> PBO or 150 mg·L<sup>-1</sup> PP<sub>333</sub> increased the diameter of branches, and treatment with 5 g·L<sup>-1</sup> PBO significantly increased the fruit setting rate. The PBO and PP<sub>333</sub> treatments significantly increased the content of carbon and nitrogen nutrients in leaves during the later stage of fruit development, while also delaying the decrease in the levels of N, P, and K. Additionally, PBO and PP<sub>333</sub> increased the crude fat and cellulose contents while reducing tannin levels, thereby improving the taste of fruits. Principal component analysis showed that the overall control effect of the PP<sub>333</sub> treatment was greater than that of the PBO treatment; applying 450 mg·L<sup>-1</sup> PP<sub>333</sub> resulted in the highest comprehensive score. To summarize, foliar application of 450 mg·L<sup>-1</sup> PP<sub>333</sub> to 6-year-old *C. illinoensis* cv. ‘Shaoxing’ plants in May (the late flowering stage) and July (the rapid expansion period of the fruit) are recommended for production purposes. Additionally, irrigation and fertilization management should be optimized to facilitate greater accumulation of nutrients by plants, promote the development of short and thick branches, and improve fruit quality. This study focused on the 6-year-old *C. illinoensis* cv. ‘Shaoxing’ in Hechi City, Guangxi; however, the different *Carya illinoensis* varieties used in agroforestry production need to be further investigated.

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**Availability of Data and Materials:** All of the data developed or examined during this investigation are available in this article and its supplementary materials, or they can be provided at reasonable request.

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## Appendix A

**Table A1:** Concentrations of PBO and PP<sub>333</sub>

Treatment	Reagent	Concentration
CK	Clear water	–
P1	PBO	2 g·L <sup>-1</sup> (wetable powder)
P2	PBO	5 g·L <sup>-1</sup> (wetable powder)
P3	PBO	10 g·L <sup>-1</sup> (wetable powder)
D1	PP <sub>333</sub>	150 mg·L <sup>-1</sup> (active ingredients)
D2	PP <sub>333</sub>	300 mg·L <sup>-1</sup> (active ingredients)
D3	PP <sub>333</sub>	450 mg·L <sup>-1</sup> (active ingredients)

Note: To prepare the solutions, for PBO treatments (P1, P2, and P3), weigh out the required amount of PBO wettable powder based on the desired concentration: e.g., for P1 (2 g·L<sup>-1</sup>), weigh 2 g of PBO wettable powder and add it to 998 g of water in a 1 L volumetric flask. Stir until fully dissolved to prepare a 1 L solution. For PP<sub>333</sub> treatments (D1, D2, and D3), calculate the amount of PP<sub>333</sub> wettable powder based on the active ingredient content (15%): e.g., for D1 (150 mg·L<sup>-1</sup>), weigh 1 g of PP<sub>333</sub> wettable powder and add it to 999 g of water in a 1 L volumetric flask. Stir until fully dissolved to prepare a 1 L solution. The same procedure is followed for other concentrations.

**Table A2:** F-value and *p*-value of Shoot Growth Indices

Index	Fruiting branches length	Vegetative branches length	Fruiting branches diameter	Vegetative branches diameter
F-value	43.943	49.629	0.771	7.356
<i>p</i> -value	0.000	0.000	0.605	0.001

**Table A3:** F-value and *p*-value of leaf nutrient accumulation indices

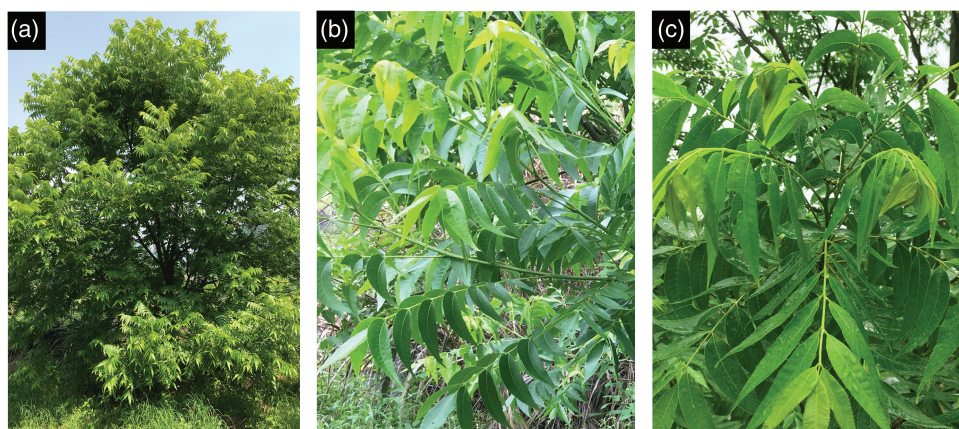
Index	Soluble sugar (19 days after flowering)	Soluble sugar (83 days after flowering)	Soluble sugar (117 days after flowering)	Soluble sugar (147 days after flowering)	Soluble protein (19 days after flowering)	Soluble protein (83 days after flowering)
F-value	5.903	2.015	3.385	4.929	9.248	1.123
<i>p</i> -value	0.003	0.013	0.028	0.007	0.000	0.398
Index	Soluble protein (117 days after flowering)	Soluble protein (147 days after flowering)	Starch content (19 days after flowering)	Starch content (83 days after flowering)	Starch content (117 days after flowering)	Starch content (147 days after flowering)
F-value	3.359	9.275	13.477	12.882	3.143	10.921
<i>p</i> -value	0.029	0.000	0.000	0.000	0.036	0.000
Index	Nitrogen content	Nitrogen content	Nitrogen content (117 days	Nitrogen content (147 days	Phosphorous content	Phosphorous content

(Continued)

<b>Table A3 (continued)</b>						
Index	Soluble sugar (19 days after flowering)	Soluble sugar (83 days after flowering)	Soluble sugar (117 days after flowering)	Soluble sugar (147 days after flowering)	Soluble protein (19 days after flowering)	Soluble protein (83 days after flowering)
	(19 days after flowering)	(83 days after flowering)	after flowering)	after flowering)	(19 days after flowering)	(83 days after flowering)
F-value	7.962	3.673	4.832	24.780	0.130	0.515
<i>p</i> -value	0.001	0.021	0.032	0.000	0.990	0.760
Index	Phosphorous protein (117 days after flowering)	Phosphorous protein (147 days after flowering)	Potassium content (19 days after flowering)	Potassium content (83 days after flowering)	Potassium content (117 days after flowering)	Potassium content (147 days after flowering)
F-value	0.332	0.162	1.221	12.557	7.800	22.343
<i>p</i> -value	1.000	0.983	0.352	0.002	0.004	0.000

**Table A4:** F-value and *p*-value of fruit quality indices

Index	The fruiting rate	Tannin content	Crude fat content	Soluble protein	Reducing sugar	Cellulose content
F-value	9.314	30.091	20.113	2.671	124.114	10.482
<i>p</i> -value	0.000	0.000	0.000	0.006	0.000	0.000

**Figure A1:** *Carya illinoensis* cv. 'Shaoxing' plant and foliar spray treatment

Note: (a) 6-year-old *Carya illinoensis* cv. 'Shaoxing' plant. (b) The leaves of 'Shaoxing' before foliar spray treatment. (c) The leaves of 'Shaoxing' after foliar spray treatment.



**Figure A2:** PBO and PP<sub>333</sub> wettable powder

Note: (a) PBO wettable powder was sourced from Huaye Agricultural Technology Co., Ltd., China (Technical indicators: medium element content (Ca + Mg)  $\geq$  10.0%). (b) Pacllobutrazol (PP<sub>333</sub>) wettable powder was sourced from Guoguang Agricultural Chemical Co., Ltd., China (containing 15% active ingredients).