



# Article Effects of Canopy Position and Microclimate on Fruit Development and Quality of Camellia oleifera

Yifan Lu<sup>1,2,3</sup>, Yuanyuan Si<sup>1</sup>, Lingyun Zhang<sup>1</sup>, Yongjiang Sun<sup>1</sup> and Shuchai Su<sup>1,2,\*</sup>

- Key Laboratory of Forest Silviculture and Conservation of the Ministry of Education, The College of Forestry, Beijing Forestry University, Beijing 100083, China
- <sup>2</sup> National Energy R&D Center for Non-food Biomass (NECB), Beijing Forestry University, Beijing 100083, China
- <sup>3</sup> International Science & Technology Cooperation Base of Forestry Biomass Energy, Beijing Forestry University, Beijing 100083, China
- \* Correspondence: sushuchai@sohu.com; Tel.: +86-10-62336044

Abstract: Camellia oleifera is an economic tree species in southern China and is famous for its oil. The surrounding climate is filtered by the tree itself, resulting in the canopy microclimate, which affects the growth and fruit quality of C. oleifera. This study investigated the effect of canopy positions on microclimate and fruit growth, maturation and qualities by comparing the differences in canopy position. This study also considered the relationship between microclimate and fruit qualities during the oil conversion period. The fruit qualities and microclimate were studied by dividing the canopy into two vertical layers and horizontal layers, creating the following canopy positions: upper outer canopy (UO), upper inner canopy (UI), lower outer canopy (LO) and lower inner canopy (LI). The light intensity increased significantly from inside to outside and from top to bottom in the canopy; however, there were no significant differences in temperature and relative humidity. At maturity, the moisture content of fruits and kernels in UO and LO was approximately <5% of those in UI and LI. The soluble sugar content increased by 10.90%, 8.47% and 6.84% in UO, UI and LO in November, while no significant change was observed in LI. The kernel oil content (KOC) obtained a higher value in UO and UI at maturity. However, KOC decreased by 5.16%, 3.02%, 3.10% and 0.67% in UO, UI, LO and LI in November. Light intensity in September and October was correlated, and temperature and relative humidity in August and September were correlated.

Keywords: Camellia oleifera; canopy position; canopy microclimate; fruit quality; fruit development

# 1. Introduction

*Camellia oleifera* Abel. is one of the major woody oil trees in the world, with a history of over 2000 years. It has been widely grown in the south of the Yangtze River in China, such as in Jiangxi, Hunan, Guangxi and Hainan provinces [1], and it is also distributed in India and Vietnam [2]. Edible tea oil is extracted from mature *C. oleifera* fruit, which is rich in unsaturated fatty acids ( $\leq$ 90%), where the oleic acid content of unsaturated fatty acids is more than 80%. The extracted tea oil is beneficial for the human cardiovascular system and has antioxidant properties, helping to enhance the antioxidant capacity of human skin when used in skin care products [3]. Meanwhile, the composition of tea oil is extremely similar to that of olive oil, which is known as "Oriental olive oil" [4,5].

In 2020, the planting area of *C. oleifera* reached 4.53 million hectares, with an annual output of 0.627 million tons of oil [6]. Due to the promotion of tea oil in market, the yield and quality of *C. oleifera* oil have become the major factors that could improve the competitive advantage of *C. oleifera*. Several factors, such as biological factors, environmental factors and management level, influence the quality of *C. oleifera* fruit. Previous studies on improving *C. oleifera* yield and quality of focused on roots [7], phosphate-solubilizing bacteria [8], soil property [9], fertilizer and organic mulching material [1]. However, research on the



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**Copyright:** © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). relationship between microclimate and *C. oleifera* fruit growth is scarce. Research on improving fruit yield and quality focuses on pruning, a commonly used method, that changes canopy microclimate by changing canopy structure [10].

A microclimate is composed of various factors, such as light intensity, temperature, relative humidity, ultraviolet radiation (UV radiation), infrared radiation (IR radiation), vapor pressure deficit (VPD), precipitations and wind. A canopy microclimate is a special environment important for plant growth while the canopy structure is filtered by different natural climatic factors [11–13]. Changes in canopy factors affect the microenvironment and, consequently, fruit growth [14–16]. In recent years, studies have been conducted on the relationship between microclimate and fruits of several tree species. Zhang et al. found a strong correlation between microclimate and fruit quality and yield; the yield and quality of fruits in the lower canopy was especially limited due to lower light intensity [11]. The canopy position is a highly influential factor in fruit quality before harvesting. Different canopy positions vary the fruit development environment, including light, temperature, water content, etc., which indirectly affects the quality and metabolism of peach fruit [17,18]. According to a study on grapes, the mechanical removal of leaves before blooming led to a change in the microclimatic condition of the fruit by increasing light intensity, temperature, surface area and exposure time to sunlight. There was no negative impact of pre-bloom mechanical leaf removal on fruit physiology or quality. In addition, the improvement of microclimate enhanced flavonoid accumulation [19]. In pears, microclimatic factors had consequential effects on fruit quality; at the highest and lowest light penetration levels, the quality of fruit was the most pronounced [20].

In previous studies, the effect of temperature and relative humidity on fruit quality was ignored [21]. Moreover, a detailed study on the correlation between C. oleifera fruit and microclimate (light intensity, temperature and relative humidity) was conducted by Wen et al.; microclimate, especially light intensity, affected fruit yield and quality significantly from July to October, which improved from bottom to top, from the inner to outer canopy [10]. The research focused on the relationship between mature fruits and year-round microclimate [10]. However, it lacked details on C. oleifera fruit growth under different canopies and the relationship between fruit quality and microclimate during the oil conversion period. The meteorological factors involved in the development of external phenotype and the accumulation of nutrients in fruits were not studied. The growth period of C. oleifera is mainly from May to October; the fruit volume increases rapidly between June and August, accounting for 66% to 75% of the total volume. The fruit oil conversion period starts in July and peaks in October. Most C. oleifera fruits ripen in October. When C. oleifera matures, the fruit cracks, and the seeds fall off naturally. Adequate light and suitable temperature and humidity are conducive to the accumulation of carbohydrates in fruits. On the contrary, insufficient light or high temperature, which indirectly affect changes in relative humidity in the microenvironment, may affect the development of fruits [22–24]. The economic value of C. oleifera is derived from its oil, which is produced during the oil conversion period, so it is important to focus on physiological changes during the oil conversion period. In this study, we measured fruit quality characteristics and compared the differences of these characteristics under canopy position. The correlations in fruit qualities were also determined. Meanwhile, we explored microclimate factors and the relationship between microclimate and fruit quality during the oil conversion period. Our aim was to investigate the effect of canopy positions on the microclimate factors, fruit growth, maturation and qualities. The relationship between microclimate and fruit qualities during the oil conversion period was also investigated. These results are expected to adjust the tree shape and microclimate factors in future studies.

## 2. Materials and Methods

## 2.1. Experimental Area and Material

The trees of *C. oleifera* 'Hua Xin' grown in the ShanPu Seedlings Co., Ltd. nursery (Zhuzhou, China; 27°37′12′′ N, 113°7′48′′ E) were used for the experiment. The plantation density was 120 trees per hectare. The temperature and precipitation data of the

experimental area during the period 2019–2022 are presented in Figure S1. The trees that were neither clipped nor chemically treated during the period 2019–2022 were selected for this study. Approximately 40 7-year-old trees grown adjacent to each other (planting distances =  $2.5 \text{ m} \times 3 \text{ m}$ ) with similar canopy structure and size were selected for sampling in the experiment. The average height of the trees was  $2 \pm 0.5 \text{ m}$ , and the average crown width was  $2 \pm 0.5 \text{ m}$ .

## 2.2. Light Intensity, Temperature and Relative Humidity

Considering the tree trunk as the center, the canopy was divided into an inner canopy (0.5–0.7 m) and an outer canopy (0.7–1.2 m) horizontally, and a lower canopy (1–1.2 m) and an upper canopy (1–1.2 m) vertically. The canopy was divided into four large canopy positions in total: upper outer (UO), upper inner (UI), lower outer (LO) and lower inner (LI) (Figures S2 and S3).

Based on the four large canopy positions, each canopy (centered at the trunk) was further divided into four directions: north, west, south and east. There were 16 small areas in total. The acquisition of microclimate data was performed at an interval of 10 days on sunny days. The values of microclimatic factors were recorded regularly during the daytime in triplicates (at 8:00, 11:00, 14:00 and 17:00). The experiment was carried out from top to bottom, and from the outside to the inside of the tree canopy. Light intensity was measured with a digital illuminometer (UT383, UNI-T, Dongguan, China). Temperature and humidity were measured with a handheld portable temperature/humidity measuring instrument (testo635-1, Testo, Lenzkirch, Germany).

## 2.3. Fruit Quality

Since there were rare differences among fruits from the 4 small areas with each large canopy, we sampled fruits evenly from 4 large canopy positions (picked from east, north, west and south, respectively, and mixed them in each canopy position). The growth period of fruit was from May to October 2021, and the tea fruits were collected from all four large positions at the following dates: 5 (JE1), 15 (JE2) and 26 (JE3) June; 6 (JY1), 16 (JY2), 26 (JY3) July; 7 (A1), 18 (A2), 27 (A3) August; 7 (S1), 17 (S2), 27 (S3) September; 7 (O1), 17 (O2), 27 (O3) October; and 1 (N1) November.

The data for fruit weight (FW), lateral diameter, longitudinal diameter, fruit shape index (FSI), fresh seed content (FSC) and fresh kernel content (FKC) were obtained at the time of sample collection. Pericarp, seed coat and the kernel were separated using a sharp blade, and the data for FSC and FKC were recorded. Fruit moisture content (FMC), seed moisture content (SMC) and kernel moisture content (KMC) were determined after drying up to constant weight at 100–105 °C in an oven for over 72 h. Dry seed content (DSC) and dry kernel content (DKC) were obtained by comparing dry weight to SMC and KMC, respectively. Moreover, dried kernels were used to measure soluble sugar content (SSC) and protein content (PTC). Anthrone colorimetry was used to determine SSC [25,26]. The PTC was determined by the Kjeldahl method [27]. *C. oleifera* seed oil was extracted using a Soxtec 2050 extraction system (Foss Analytical, Hillerød, Denmark).

The following equations were used to analyze fruit quality traits [1,10,11]:

FSI = Longitudinal diameter/Lateral diameter.

FSC = Fresh seed weight/Fresh fruit weight.

FKC = Fresh kernel weight/Fresh fruit weight.

FMC =  $(1 - dry \text{ fruit weight}) \times 100\%$ .

SMC =  $(1 - dry \text{ seed weight/fresh seed weight}) \times 100\%$ .

KMC =  $(1 - dry kernel weight/fresh kernel weight) \times 100\%$ .

DSC = dry seed weight/dry fruit weight  $\times$  100%.

DKC = dry kernel weight/dry fruit weight  $\times$  100%. FOC = oil weight/dry fruit weight  $\times$  100%.

KOC = oil weight/dry kernel weight  $\times$  100%.

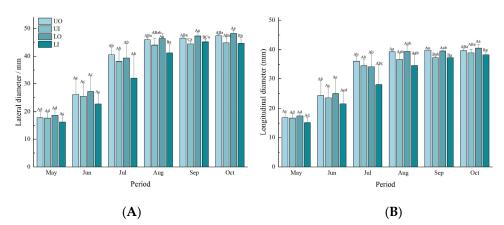
# 2.4. Statistical Analysis

Fruit quality and microclimatic factor data were processed using Microsoft Office Excel 2017. The data were analyzed by one-way analysis of variance, ANOVA, and the means were contrasted by Duncan's multiple comparisons test ( $p \le 0.05$ ) using IBM SPSS Statistics 20 (IBM, Armonk, NY, USA). Continuous dynamic graphs were drawn using Origin 2017. Design-Expert 13 was used to plot 3D graphs of light intensity distribution throughout the horizontal canopy.

## 3. Results

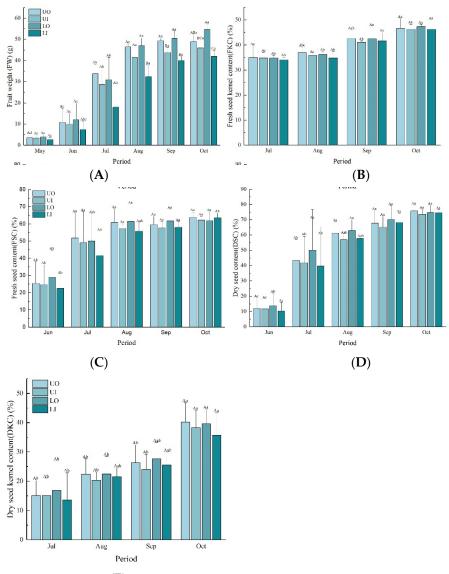
## 3.1. Fruit Development

Based on changes in volume, *C. oleifera* fruit growth and development were observed over six months. In August, the lateral and longitudinal diameters increased by 14.39, 12.68, 12.15 and 9.24 mm and 11.77, 11.05, 9.23 and 6.46 mm in UO, UI, LO and LI, respectively (Figure 1). The analysis of fruit data of different positions in the same month showed that the lateral diameter was not significantly different before July. In October, the maximum values of lateral and longitudinal diameter were 48.23 mm and 40.51 mm, and the minimum values were 44.54 mm and 38.22 mm (Table S2).



**Figure 1.** Variations of lateral diameter (**A**) and longitudinal diameter (**B**) of *C. oleifera* 'Huaxin'. Note: Data are represented as the mean value  $\pm$  standard deviation (SD; *n* = 6). Different uppercase and lowercase letters indicate significant differences ( $p \le 0.05$ ; Duncan's multiple range tests) between locations and periods, respectively.

FW, FSC, FKC, DSC and DKC were also recorded during the growth period. Comparing fruit growth in different months, from May to harvest time, FW increased by approximately 13–16 times (Table S2). *C. oleifera* seeds gained evident weight since June (Figure 2A). The values of FW in July in different canopy positions were over two times larger than those in June, and FW values in July also differed significantly from those in August ( $p \le 0.05$ ). The average values of FSC in June were nearly two times higher than those in July (Figure 2C). DSC increased by over 30% and 10%, respectively, in July and August (Figure 2D). Moreover, FSC and DSC maintained continuous growth after August in LI. With the development of *C. oleifera*, the kernel and seed coat become completely separated in June. Thus, FKC was recorded from July. August and September were critical periods for growth (Figure 2B). FKC showed a nearly 5% increase in both September and October. The values of DKC in October were 13.89%, 14.29%, 11.93% and 10.15% higher than those in September in UO, UI, LO and LI, respectively (Figure 2E).



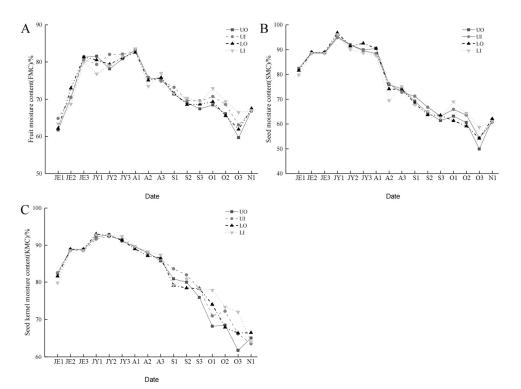
(E)

**Figure 2.** (A) Fruit weight, (B) fresh seed kernel content, (C) fresh seed content, (D) dry seed content, (E) dry seed kernel content of C. *oleifera* 'Huaxin' at different positions of the canopy. Data are represented as mean values  $\pm$  standard deviation (SD; n = 6). Different uppercase and lowercase letters indicate significant differences ( $p \le 0.05$ ; Duncan's multiple range tests) between locations and periods, respectively.

On comparing different positions in the same month (Figure 2A), FW values in LI showed a significant difference with those in UO, UI and LI in August. Additionally, FW in LO ( $12.14 \pm 7.66$  g) was almost twice as high as in LI ( $7.43 \pm 3.97$  g) in June, and FW in July in UO ( $33.71 \pm 4.45$  g) was approximately twice as high as in LI ( $17.93 \pm 10.57$  g). Moreover, FW values in the inner canopy (UI and LI) were always lower than those in the outer canopy (UO and LO). In July, FSC in LI was 9.81% less than that in UO, and DSC in LI was 10.31% less than that in LO (Figure 2C,D, Table S2), whereas no significant difference showed among each position in October. In October, the values of DKC in the outer canopy were higher than those in LI (Table S2). For FSC, DSC and DKC, the values in the outer canopy were higher than those in the inner canopy (LO and LI) between August and September (Figure 2E).

In summary, phenotype-related indexes mainly surged in July–August, during which the growth rate of fruits at different positions in the canopy was different. The growth rate at LI was the slowest among all other positions. Unlike UO, UI and LO, which reached their peak of growth in September, LI reached its peak in October, with a much slower growth rate. Therefore, the phenotypic qualities of fruits at the four canopy positions were similar at the time of harvest.

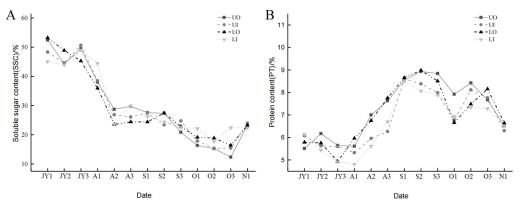
As shown in Figure 3A, peaks of FMC appeared in JE3 and A1 and the values of FMC showed no significant difference among the different canopy positions. In A2, FMC declined by 7.55%, 7.03%, 7.46% and 10.11% in UO, UI, LO and LI, respectively. In October, the values of FMC in the outer canopy ranging from 59.67% to 69.31% were less than those in the inner canopy, ranging from 63.04% to 72.87% (Figure 3A). Compared with FMC in O3, the values of that in N1 increased by 7.37%, 3.75%, 5.61% and 0.57% in UO, UI, LO and LI, respectively. The highest values of SMC appeared in JY1, ranging from 94.86% to 96.11%. The values of SMC in A2 declined sharply, which were 14.62%, 12.16%, 16.34% and 18.19% lower than those in A1 in UO, UI, LO and LI, respectively. The values of SMC in N1 increased by 11.81%, 6.32%, 7.92% and 2.36% in UO, UI, LO and LI, respectively, compared with those in O3 (Figure 3B). As shown in Figure 3C, higher values of KMC appeared in JY1, JY2 and JY3, which ranged from 91.08 to 92.88%, 91.63 to 92.32%, 91.36 to 93.01% and 92.40 to 92.84% in UO, UI, LO and LI, respectively. In O2, the values of KMC in outer canopy were approximately 5% higher than those in inner canopy. Moreover, the value of KMC in UO was 10.24% lower than that in LI in O3. The values of KMC in N1 increased by 3.26% and 0.08%, in UO and LO and decreased by 2.66% and 7.90% in UI and LI, respectively (Figure 3C). The changing trends of KMC in the inner canopy were opposite to those of FSC and SMC in N1.



**Figure 3.** (**A**) Fruit moisture, (**B**) seed moisture, and (**C**) seed kernel moisture content in *C. oleifera* 'Huaxin' from June 2021 to November 2021.

The SSC of fruits in UO and UI peaked in July. For LO and LI, SSC peaked in JY3. However, SSC declined significantly from JY3 to A2 and from S2 to O3 (Figure 4A). The values of SSC in A2 were 20.75%, 23.80%, 21.74%, 25.33% lower than those in JY3 for UO, UI, LO, LI, respectively. The values of O3 were 14.97%, 7.87%, 11.03% and 2.0% lower than those in S2. However, the values of SSC increased by 10.91%, 8.47% and 6.85% in N1 in UO, UI and LO, whereas no significant change appeared in LI (Figure 4A). PTC was stable

during fruit growth. For PTC, the highest values of the outer canopy and the inner canopy appeared in S2 and S1, respectively (Figure 4B). The values of PTC in S2 were 3.33% and 3.01% higher than those in A1 in UO and LO. The values of PTC in S1 were 3.27% and 3.69% higher than those in A1 in LO and LI. Moreover, PTC declined in N1 (Figure 4B), showing an opposite trend to SSC in N1.



**Figure 4.** (**A**) Soluble sugar content and (**B**) protein content in *C. oleifera* 'Huaxin' from July 2021 to November 2021.

*C. oleifera* kernel contained almost no oil in July. Since 18 August, KOC has increased rapidly with approximately 5% oil (Figure 5). Throughout September, the increase in KOC in the outer canopy was significantly higher than that in the inner canopy, resulting in a significantly higher oil content in the outer canopy. KOC peaked at approximately 30% to 40% on October 27. Meanwhile, oil content was the highest in the outer canopy (UO and UI), followed by LO and LI. On November 1, a significant decrease in oil content was observed in all the canopy positions, except for in LI.

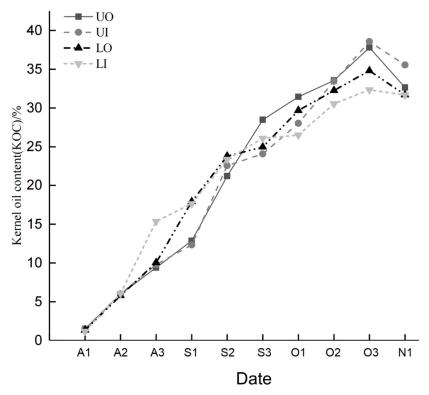
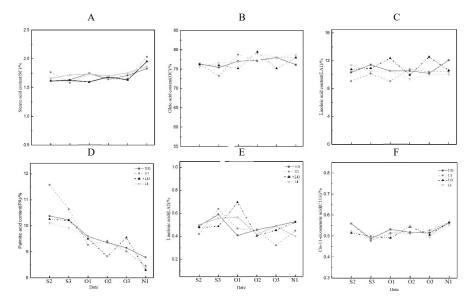


Figure 5. Kernel oil content in C. oleifera 'Huaxin' from August 2021 to November 2021.

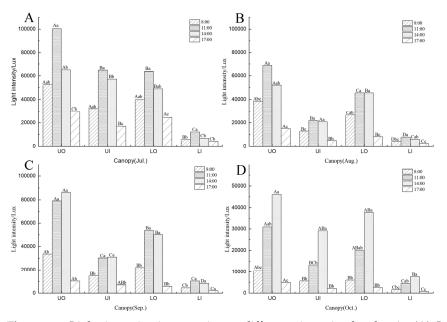
At the end of the oil accumulation phase, the fatty acid composition of C. *oleifera* was found to majorly comprise oleic acid (76–78%), followed by linoleic acid, palmitic acid, stearic acid, linolenic acid and cis-11-dodecenoic acid (Figure 6). Except for the significant decrease in palmitic acid from S2 to N1, no significant change was shown in other acids, and there was no significant difference in fatty acid content among the four canopy positions.



**Figure 6.** (**A**) Stearic acid, (**B**) oleic acid, (**C**) linoleic acid, (**D**) palmitic acid, (**E**) linolenic acid, (**F**) cis-11-dodecenoic acid content in *C. oleifera* 'Huaxin' from September 2021 to November 2021.

# 3.2. Variations in Microclimate within the Canopy between July and October

The variations in temperature and relative humidity in all four positions of the canopy over four months are shown in Tables 1 and 2, respectively. Light intensity variation across the four canopy positions in July–October is shown in Figure 7. Significant differences were observed in microclimate factors in different canopy positions. The lowest and highest light intensity appeared in October and July, respectively, as did the temperature. However, the lowest and the highest relative humidity were recorded in July and August.



**Figure 7.** Light intensity in canopies at different times in the day in (**A**) July, (**B**) August, (**C**) September, (**D**) October. Different uppercase and lowercase letters indicate significant differences ( $p \le 0.05$ ; Duncan's multiple range tests) between locations and times, respectively.

Periods	Location	8:00	11:00	14:00	17:00
July	UO	$32.68\pm1.68\mathrm{b}$	$37.79\pm1.10~\mathrm{a}$	$37.43 \pm 2.19$ a	$36.96 \pm 2.55$ Aa
	UI	$32.84\pm1.62~\mathrm{c}$	$37.71 \pm 1.11$ a	$37.66 \pm 2.37$ a	$34.84 \pm 1.72 \text{ Bb}$
	LO	$32.82\pm1.99~\mathrm{c}$	$38.10\pm0.99~\mathrm{a}$	$37.61\pm2.22$ a	$34.81 \pm 1.80 \text{ Bb}$
	LI	$33.09\pm1.64b$	$37.83\pm0.93~\mathrm{a}$	$37.43\pm2.18~\mathrm{a}$	$34.72\pm1.76~\text{Bb}$
August	UO	$28.84 \pm 3.01 \text{ c}$	$33.56\pm2.43$ ab	$33.82 \pm 2.46$ a	$30.85\pm3.18~\text{Bbc}$
	UI	$28.92\pm3.06\mathrm{b}$	$33.65\pm2.43$ a	$34.07 \pm 2.71$ a	$34.07\pm2.71~\mathrm{Aa}$
	LO	$28.85 \pm 3.09 \text{ c}$	$33.49\pm2.51~\mathrm{ab}$	$34.10\pm2.80$ a	$30.83 \pm 3.21$ Bbc
	LI	$28.88\pm3.20\ c$	$33.38\pm2.44~ab$	$33.82\pm2.58~a$	$30.77\pm3.20~Bbc$
September	UO	$29.93 \pm 0.62 \text{ d}$	$35.24\pm1.96\mathrm{b}$	$36.88 \pm 1.67$ a	$32.48 \pm 1.22 \text{ c}$
	UI	$30.21 \pm 0.53 \text{ d}$	$35.45\pm2.20~\mathrm{a}$	$37.08\pm1.78~\mathrm{a}$	$32.47\pm1.28\mathrm{b}$
	LO	$30.19 \pm 0.59 \text{ d}$	$35.56 \pm 2.32$ a	$37.03\pm1.99$ a	$32.40\pm1.21\mathrm{b}$
	LI	$30.08\pm0.63~d$	$35.45\pm2.16~\text{a}$	$36.95\pm1.74~\mathrm{a}$	$32.34\pm1.21~b$
	UO	$16.95 \pm 3.37 \text{ c}$	$19.71 \pm 2.23  \mathrm{b}$	$22.96 \pm 0.60$ a	$19.21\pm1.50\mathrm{bc}$
October	UI	$16.95\pm3.40~\mathrm{b}$	$19.75\pm2.21\mathrm{b}$	$23.17\pm0.51~\mathrm{a}$	$19.51\pm1.99\mathrm{b}$
	LO	$16.95\pm3.43\mathrm{b}$	$19.73\pm2.16b$	$23.03\pm0.38~\mathrm{a}$	$19.19\pm1.50~\mathrm{b}$
	LI	$16.88\pm3.45~c$	$19.71\pm2.18b$	$23.07\pm0.53~\mathrm{a}$	$19.14\pm1.46~\mathrm{bc}$

**Table 1.** The variations of temperature (°C) at different times of the day in different canopy positions.

Data are represented as the mean values  $\pm$  standard deviation (SD; n = 6). Different uppercase and lowercase letters indicate significant differences ( $p \le 0.05$ ; Duncan's multiple range tests) between locations and times, respectively.

<b>Table 2.</b> The variations of relative humidit	r (%) at different tim	nes of the day in canopies.
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Period	Location	8:00	11:00	14:00	17:00
T 1	UO UI	$66.05 \pm 5.61$ a $65.58 \pm 5.37$ a	$52.46 \pm 1.89 \text{ c}$ $52.27 \pm 2.20 \text{ c}$	$52.24 \pm 4.47 \text{ c}$ $52.11 \pm 4.15 \text{ c}$	$58.92 \pm 6.96 \text{ b} \\ 59.38 \pm 6.50 \text{ b}$
Jul	LO LI	$66.09 \pm 6.14$ a $65.83 \pm 4.84$ a	$51.89 \pm 1.61 \text{ c}$ $52.17 \pm 2.08 \text{ c}$	$\begin{array}{c} 52.16 \pm 4.72 \text{ c} \\ 52.92 \pm 4.32 \text{ c} \end{array}$	$\begin{array}{c} 59.18 \pm 6.64 \text{ b} \\ 59.75 \pm 6.80 \text{ b} \end{array}$
Aug	UO UI LO LI	$84.62 \pm 9.32$ a $84.14 \pm 9.46$ a $84.06 \pm 9.37$ a $84.35 \pm 9.38$ a	$\begin{array}{c} 69.02\pm 6.23 \text{ b} \\ 68.82\pm 6.04 \text{ b} \\ 68.78\pm 6.35 \text{ b} \\ 68.99\pm 6.24 \text{ b} \end{array}$	$\begin{array}{c} 66.61 \pm 4.86 \text{ b} \\ 66.58 \pm 5.16 \text{ b} \\ 66.85 \pm 5.28 \text{ b} \\ 67.20 \pm 6.04 \text{ b} \end{array}$	$77.35 \pm 7.30$ a $77.33 \pm 7.60$ a $77.16 \pm 7.48$ a $77.43 \pm 7.65$ a
Sep	UO UI LO LI	$\begin{array}{c} 73.06 \pm 9.26 \text{ a} \\ 72.84 \pm 9.14 \text{ a} \\ 72.72 \pm 9.10 \text{ a} \\ 72.86 \pm 9.21 \text{ a} \end{array}$	$\begin{array}{c} 56.81 \pm 13.68 \text{ b} \\ 56.20 \pm 13.71 \text{ b} \\ 57.01 \pm 13.01 \text{ b} \\ 56.24 \pm 13.81 \text{ b} \end{array}$	$\begin{array}{c} 50.06 \pm 11.85 \text{ b} \\ 49.94 \pm 12.09 \text{ b} \\ 49.61 \pm 12.26 \text{ b} \\ 50.27 \pm 12.39 \text{ b} \end{array}$	$\begin{array}{c} 57.72 \pm 9.12 \text{ b} \\ 58.04 \pm 9.36 \text{ b} \\ 57.97 \pm 8.94 \text{ b} \\ 57.96 \pm 9.03 \text{ b} \end{array}$
Oct	UO UI LO LI	$81.56 \pm 4.96$ a $81.68 \pm 4.79$ a $81.71 \pm 5.01$ a $81.11 \pm 3.96$ a	$\begin{array}{c} 72.09 \pm 2.19 \text{ ab} \\ 71.93 \pm 2.43 \text{ b} \\ 72.09 \pm 2.15 \text{ b} \\ 72.26 \pm 2.01 \text{ ab} \end{array}$	$\begin{array}{c} 61.64 \pm 11.60 \text{ c} \\ 62.20 \pm 11.05 \text{ c} \\ 61.26 \pm 11.18 \text{ c} \\ 61.98 \pm 11.11 \text{ c} \end{array}$	$70.77 \pm 9.52 \text{ bc} 71.43 \pm 9.09 \text{ bc} 71.33 \pm 9.20 \text{ b} 71.65 \pm 8.98 \text{ b} $

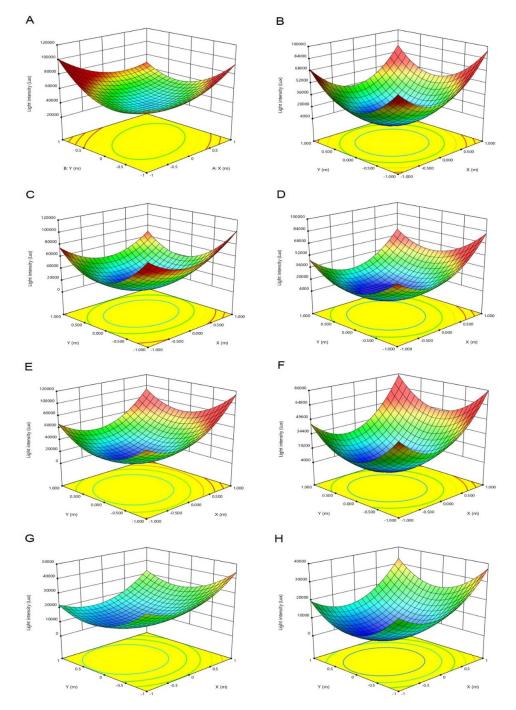
Data are represented as the mean values  $\pm$  standard deviation (SD; *n* = 8). Different uppercase and lowercase letters indicate significant differences (*p*  $\leq$  0.05; Duncan's multiple range tests) between locations and times, respectively.

The values of light intensity between July and October in UO, UI, LO and LI ranged from 4974 to 100,372 Lux, 2139 to 64,723 Lux, 2540 to 63,673 Lux and 758 to 12,119 Lux, respectively. With the development of the canopy, the difference in light intensity evolved differently between the outer and inner canopy. Light intensity in the outer canopy was directly affected by solar radiation, while light intensity in the inner canopy was affected by the canopy. According to Figure 7, light intensity in the outer canopy was higher than that in the inner canopy. During the growth of *C. oleifera*, temperature and relative humidity in different positions at the same time did not show a significant difference. The highest values of temperature, ranging from 32.82 to 38.10 °C, were recorded in UO in July, and the lowest values of relative humidity in positions of UO, UI, LO and LI ranged from 50.06 to 84.62%, 49.94 to 84.14%, 49.61 to 84.06% and 50.27 to 84.35% (Table 2).

# 3.3. Spatial–Temporal Microclimatic Distribution of Canopy

Significant differences were observed in the microclimatic factors in canopies. These factors greatly influenced the fruit growth between July and October. The distribution of light intensity highly varied in canopies. As shown in Figure 8, the light intensity increased from

inner to outer and from lower to upper canopy. The difference in light intensity in the lower canopy was more than that in the upper canopy. The difference in light intensity between the inner and outer canopy, including the upper canopy and lower canopy, showed an inverted "V" trend from July to October. Temperatures in canopies showed a similar changing trend; however, the difference between inner the canopy and the outer canopy was small.

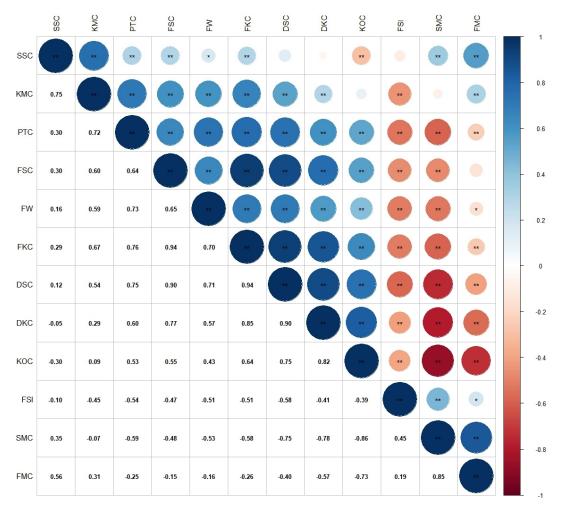


**Figure 8.** Spatial–temporal distribution of light intensity in canopies from July to October. The *Y*-axis is the distance in the east (–) and west (+) directions from the trunk (m); the *X*-axis is the distance in the south (+) and north (–) directions from the trunk (m); the *Z*-axis shows the light intensity. (**A**,**B**) show the light intensity distributions in the lower and upper layers of the canopy in July; (**C**,**D**) show the light intensity distributions in the lower and upper layers of the canopy in August; (**E**,**F**) show the light intensity distributions in the lower and upper layers of the canopy in September; and (**G**,**H**) show the light intensity distributions in the lower and upper layers of the canopy in October.

The trend of relative humidity was opposite to that of light intensity and temperature, which showed a decreasing trend from inner to outer and from lower to upper canopy positions.

## 3.4. Relationships between Fruit Qualities

As shown in Figure 9, a significant correlation was observed in most fruit qualities. FW had the highest positive correlation with FKC (0.94) and DSC (0.90). FSC, FKC, DSC, SSC and PTC had a higher positive correlation with KMC (0.60, 0.67, 0.54, 0.75 and 0.72, respectively). However, SMC and FMC were opposite to KMC. SMC had a significantly negative correlation with PTC, DSC, DKC and KOC (-0.59, -0.75, -0.78 and -0.86). SMC had a negative correlation with DSC, DKC and KOC (-0.40, -0.57 and -0.73). KOC had large correlation coefficients, with values of 0.82 and 0.75, with DKC and DSC (Figure 9).



**Figure 9.** The correlation heatmap between fruit qualities during the whole growing period in *C. oleifera* 'Huaxin'. \* < 0.05, \*\*  $\leq$  0.01.

## 3.5. Relationships between Microclimate and Fruit Qualities

The correlations between microclimatic factors and fruit quality from July to October are given in Table 3.

	Microclimate		Light I	ntensity		Relative Humidity			Temperature				
	Month	Jul	Aug	Sep	Oct	Jul	Aug	Sep	Oct	Jul	Aug	Sep	Oct
	Jul	0.016				0.538 **				-0.395 **			
	Aug	0.094	0.13			0.217	-0.421 **			-0.032	0.259		
Fruit moisture content (FMC)	Sep	0.041	0.033	-0.403 **		0.091	-0.278	0.543 **		0.079	0.138	-0.402 **	
	Oct	0.038	0.139	-0.431 **	-0.527**	-0.086	-0.492 **	0.363 *	0.335*	0.068	0.408 **	-0.370 **	0.226
			0.105	0.101	0.02		0.172	0.000	0.000		0.100	0.070	0.220
	Jul	0.101				0.003				0.162			
Seed moisture content (SMC)	Aug	0.091	0.167			0.135	-0.518 **			0.042	0.348 *		
occu moisture coment (ome)	Sep	-0.016	0.114	-0.318 *		0.007	-0.409 **	0.462 **		0.149	0.313 *	-0.307 *	
	Oct	-0.077	0.197	-0.297 *	-0.398 **	-0.285 *	-0.580 **	0.192	0.276	0.243	0.514 **	-0.189	0.176
	Jul	-0.096				-0.153				0.278			
	Aug	0.011	0.091			-0.089	-0.550 **			0.172	0.426 **		
Kernel moisture content (KMC)	Sep	-0.024	0.145	-0.259		-0.019	-0.406 **	0.404 **		0.178	0.319 *	-0.254	
	Oct	-0.085	0.137	-0.354 *	-0.430 **	-0.11	-0.397 **	0.313 *	0.279	0.116	0.321 *	-0.322 *	0.176
			0.107	0.001	0.100		0.057	0.010	0.27 )		0.021	0.011	0.170
	Jul	-0.116				-0.064				-0.112			
Fresh seed content (FSC)	Aug	0.076	0.164			0.168	-0.418 **			0.04	0.261		
Fresh seed content (FSC)	Sep	0.019	-0.139	0.248		0.179	0.544 **	-0.158		-0.241	-0.460 **	0.151	
	Oct	-0.048	0.245	0.477 **	0.432 **	-0.213	0.158	-0.495 **	-0.271	0.141	-0.024	0.478 **	-0.191
	Jul	-0.112				-0.069				-0.11			
	Áug	0.063	0.121			0.199	-0.215			0.001	0.094		
Fresh kernel content (FKC)	Sep	-0.024	-0.166	0.295 *		0.147	0.580 **	-0.278		-0.213	-0.475 **	0.265	
	Oct	-0.065	0.227	0.496 **	0.440 **	-0.28	0.078	-0.509 **	-0.25	0.167	0.045	0.486 **	-0.177
		0.05				0.447				0.051			
	Jul	-0.05				0.116				-0.251			
Dry seed content (DSC)	Aug	0.052	0.097			0.239	-0.290 *			-0.023	0.145		
Dry seed content (DSC)	Sep	-0.083	-0.185	0.136		0.244	0.682 **	-0.259		-0.223	-0.587 **	0.196	
	Oct	-0.021	0.113	0.520 **	0.520 **	-0.086	0.363 *	-0.545 **	-0.360 *	0.037	-0.214	0.533 **	-0.239
	Jul	-0.125				-0.171				-0.024			
	Åug	0.092	0.123			0.281	-0.171			-0.08	0.047		
Dry kernel content (DKC)	Sep	-0.066	-0.196	0.196		0.128	0.652 **	-0.393 **		-0.187	-0.542 **	0.289 *	
	Oct	-0.001	0.097	0.591 **	0.574 **	-0.255	0.169	-0.639 **	-0.352 *	0.134	-0.032	0.633 **	-0.23
	Jul	-0.041				-0.083				0.109			
Soluble sugar content (SSC)	Aug	0.154	0.422 **			-0.076	-0.106			0.052	0.06		
Soluble sugar collelli (SSC)		0.048	0.152	-0.059		-0.324 *	-0.436 **	-0.117		0.321 *	0.422 **	0.139	
	Sep	0.048	0.152	-0.059		-0.324 *	-0.436 ***	-0.11/		0.521 "	0.422 ***	0.139	

**Table 3.** The correlations between microclimatic factors and fruit qualities during the period July–October.

\*\* indicates a significant difference at the  $p \le 0.01$  level. \* indicates a significant difference at the  $p \le 0.05$  level.

Fruit quality was impacted greatly by light intensity in September and October. Light intensity showed a significant negative correlation with FMC (-0.403) and SMC (-0.318) in September, and with FMC (-0.527), SMC (-0.398) and KMC (-0.430) in October. Moreover, light intensity showed a significant positive correlation with FKC and KOC (0.295 and 0.289, respectively) in September, and with FSC, FKC, DSC, DKC and KOC (0.432, 0.440, 0.520, 0.574 and 0.298) in October.

FMC, SMC, KMC, FSC, FKC, DSC and DKC were affected by relative humidity in August and September (Table 3). FMC, SMC and KMC were significantly negatively correlated with relative humidity in August (-0.421, -0.518 and -0.550), which was the opposite of that in September (0.543, 0.462 and 0.404). Moreover, relative humidity had a positive significant correlation with FSC, PTC and KOC (0.418, 0.576 and 0.620) in August.

FMC, SMC, KMC, PTC and KOC were significantly affected by temperature in August and September (Table 3). In August, temperature showed a significant positive correlation with SMC (0.348) and KMC (0.426), while it showed a significant negative correlation with PTC (-0.464) and KOC (-0.492). Temperature showed a significant negative correlation with FMC (-0.402) and SMC (-0.307) in September, while it showed a significant positive correlation with KOC (0.340).

## 4. Discussion

#### 4.1. The Development of Fruit Qualities and Correlations between Fruit Qualities

The growing season of *C. oleifera* lasted for more than six months, and the direct method to assess fruit growth status is by measuring the fruit volume and weight. The fruit volume was determined by measuring the lateral and longitudinal diameters. Previous studies reported significant fruit weight changes in May, the first rapid growth period appeared between mid–June and mid–July, and the next significant growth period began in late September [28,29], which was consistent with the results of our study. *C. oleifera* seed was mainly composed of water and organic matter [27]. As the *C. oleifera* become mature, moisture content and SSC decreased continuously, whereas PTC and KOC increased. Our results on the fatty acid composition are similar to those of previous studies, where the concentration of oleic acid was the highest [2,27].

The economic value of C. oleifera mainly comes from tea oil; KOC is the most important indicator for judging the maturity and quality of C. oleifera. Our results revealed that KOC peaked in O3; therefore, maturity was most likely to occur by the end of October [28]. Since DSC and DKC showed a higher significant correlation with KOC in our study, the peaks of DSC and DKC were also important to judge fruit maturity and qualities [30,31]. Moreover, during the growth period, SMC and FMC were negatively correlated with most qualities, which implies that the dry matter content increased as the moisture content decreased [32,33]. In our study, FMC and SMC decreased continuously during the growth period and declined rapidly between July and September, possibly because fruit development and oil synthesis demand a large amount of carbohydrates, and the climate and environment in this period were most advantageous for photosynthesis [3]. According to the data from the local weather station, precipitation was the lowest during this year, and the temperature and light intensity were high between July and September. Therefore, continuous high temperature and high-intensity light may be the reason for decreased moisture content [14]. In general, the decrease in moisture content can be explained by the followings: (1) continuous heat and less rainfall led to the evaporation of water in fruit; (2) this period is the nutritional growth period of C. oleifera, which requires a lot of carbohydrates.

Previous studies have revealed that SSC gradually decreases with the increase in KOC [34,35], and SSC and KOC have a significant negative correlation, whereas PTC is positively correlated with KOC [36]. These results are similar to those of our study. This may be because SSC gradually decomposes into protein and tea oil during the maturation of *C. oleifera*. In addition, during the period from O3 to N1, the changing trends of SSC, KOC and PTC were opposite to those of the previous periods, which proved that there was

a mutual transformation between them. Furthermore, the fruit quality between O3 and N1 is rarely discussed in previous studies.

#### 4.2. Variations of Microclimate within the Canopy between July and October

By filtering the surrounding environment and climate, the fruit tree itself prepares its canopy microclimate. The external environment not only directly affects the physiological growth of the tree but also indirectly affects the development and quality of the fruit. According to previous studies, the microclimatic factors are different at different canopy positions, where the high difference in light intensities and low differences in temperature and relative humidity are obvious. This is because *C. oleifera* is a shrub with a small crown [10]. However, all microclimatic factors registered changes every month, reflecting the microclimatic responses to changes in the surrounding environment.

The effect of microclimatic factors on fruit has been extensively studied, especially for light intensity [15,23,37–39]. The spatial continuous distribution of light intensity during the essential months can be reflected using 3D surface plots. The distribution of light intensity in the upper layer was slightly different from that in the lower layer because the upper layer was closer to the external environment, which is consistent with previous studies. Previous studies inferred that the variations in light intensity in canopies were "funnel—shaped". The variation of temperature was similar to light intensity, and that of relative humidity was contrary to that of light intensity. The interaction between microclimate within the canopy and the tree shape is reciprocal. By adjusting the tree shape, growing points with favorable microclimatic conditions could be formed based on studies on monthly fluctuation for the uniform development of tree and fruit [24,40].

## 4.3. The Relationship between Microclimate and Fruit Qualities

Previous studies have reported that fruit yield and quality are mostly affected by the microclimate during July–October. To improve fruit quality and yield, more attention should be paid to the environment and climatic changes during this period. Therefore, our study was primarily focused on the microclimate during this period and on the relationship between microclimate and fruit growth. We found that the light intensity in September and October had a significant positive correlation with fruit qualities, except for moisture content, consistent with the results of previous studies [10,11,41]; the correlations between these factors were not constant and varied between positive and negative randomly. Sometimes, the effect of the current month's microclimate on the next month's fruit quality was more obvious when compared with the current month's fruit quality. Particularly, light intensity and relative humidity in September had a strong correlation with dry matter (DSC and DKC) in October. One probable reason could be that dry matter in C. oleifera accumulated slowly during this period, and the response of fruit quality to the microclimate cannot be reflected immediately. For *C. oleifera*, there may be a process in the response and accumulation. However, in maturity, the dry matter content increased, thereby increasing the oil content.

Microclimatic factors from August to October had significant correlations with fruit qualities. Hence, August–October is considered a critical period. The light intensity had a greater impact on fruit quality in September and October, and temperature and relative humidity had a greater correlation with fruit quality in August and September. Compared with previous results [3,10,21,40], our study focused more on the correlation between microclimate and fruit growth period, which was rare in *C. oleifera* research.

## 4.4. The Impact of Canopy Position and Light Intensity on Fruit Quality

Studies have shown that the quality of fruit majorly varies with canopy positions [23,42,43]. In peaches, it was reported that the upper canopy fruit were more mature compared to the lower canopy fruit during the same period owing to the variation in availability and distribution of light intensity across canopy positions [17,44]. Similarly, in olives, fruits in the upper canopy had a higher maturity index, more fat content and less moisture compared with the fruit in

the lower canopy [45,46]. A similar growth trend was observed in *C. oleifera* maturity. KOC in the upper canopy was higher than that in the lower canopy, and the lowest value of KOC appeared in LI. This may be due to the larger and longer sun exposure on the fruit of the upper canopy than that of the lower canopy [20,47]. Furthermore, this result was also supported by the lower moisture content and SSC at the same period, indicating that more soluble matter was converted into dry matter. In particular, the most significant difference was observed in UO and LI, where the difference in light intensity and that of light absorption between two canopy positions were the largest [48]. In olives, the content of oleic acid varied with different canopy positions at the end of the oil accumulation period [49]. However, no significant difference in fatty acid content was observed in different canopy positions, which may be due to the tree shape and species.

Non–uniform canopies will lead to inconsistencies in fruit growth rate [17], which will cause a lack of uniformity in fruit maturity in different canopy positions at harvesting time. During the rapid growth period, fruit weight and fruit size showed a decreasing trend from outer to inner and from upper to lower canopies. However, fruit weight showed no significant difference in all canopy positions. This result that fruit weight and fruit size are irrelevant with canopies at maturity was also reported in olives and pears [20,46,50]. The distribution of light intensity and assimilation was uneven in canopies, resulting in the inconsistent growth rate of *C. oleifera* fruit in different canopy positions, where LI showed the slowest growth rate. Moreover, non–uniform canopies can also result in the inconsistent maturity of the fruit tree. KOC between O3 and N1 decreased significantly in canopy positions, except for LI. This decrease in KOC may be due to the decomposition of oil caused by the over–maturation of fruit, because fruit in LI was growing slower than that in other canopy positions, and therefore, it was still synthesizing oil. Meanwhile, SSC, FMC, SMC and KMC in canopies increased from O3 to N1, except for LI, which further verified that fruit maturity in different canopies was inconsistent during the same period.

# 5. Conclusions

Our results suggest that there are differences in fruit qualities in the same period among canopy positions, and the maturity in different canopy position is inconsistent. Moreover, there was a significant relationship between fruit qualities and microclimate. Canopy position itself does not affect the development of fruit quality but affects the specific environment of fruit development. Light intensity varies greatly, and temperatures and relative humidity vary slightly in different canopies (July to October). Fruit qualities are affected significantly by microclimate factors during the oil conversion period. Therefore, proper canopies should be prepared by some measurements to regulate the distribution of microclimate factors, especially light intensity in the next study. Moreover, fruit qualities and maturity in different canopy positions will also be regulated with proper canopies and uniform distribution of microclimate.

**Supplementary Materials:** The following supporting information can be downloaded at: https: //www.mdpi.com/article/10.3390/agronomy12092158/s1, Figure S1. (A) Precipitation and temperature variation (B) from 2019 to 2022 in Changsha/Zhuzhou district. Figure S2. Vertical view of canopy partitioning (N, north; W, west; S, south; E, east;). Figure S3. Side view of canopy partitioning (U, upper layer; L, lower layer). Table S1. Changes of lateral diameter and longitudinal diameter in canopies from June to October in 2021. Supplementary Table S2. Fruit development phenotypic traits of *C. oleifera* 'Huaxin' in different positions of the canopy.

**Author Contributions:** Y.L. performed most of the experiment, conducted the data analysis and drafted the manuscript; Y.L. and Y.S. (Yuanyuan Si) designed the experiment and contributed to writing the manuscript; Y.S. (Yongjiang Sun) and L.Z. reviewed and edited the manuscript; S.S. revised the manuscript and supervise the experiments. All authors have read and agreed to the published version of the manuscript.

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