# Effects of Canopy Film Covering on Photosynthesis and Fruit Quality of Kumquat (Postprint)

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## Abstract

Canopy film covering technology has been widely applied in kumquat production. This study used Yangshuo kumquat as experimental material, with canopy film covering as the treatment and no film covering as the control. Changes in environmental factors such as temperature, humidity, and light intensity in the canopy were measured for both treatment and control; leaf area, leaf length, leaf width, and chlorophyll content were observed; photosynthetic indices including net photosynthetic rate, stomatal conductance, transpiration rate, and intercellular CO2 concentration were determined; and fruit quality indicators such as fruit firmness, soluble solids, total sugar, titratable acid, and vitamin C content were analyzed. The results showed that: compared with the control, after canopy film covering treatment, the light intensity in the kumquat canopy decreased, temperature increased, and humidity decreased; leaf area increased and chlorophyll content improved; net photosynthetic rate decreased, with the maximum reduction reaching 21.39% of the control; fruit titratable acid decreased; and fruit quality indicators such as fruit firmness, soluble solids, total sugar, solid-acid ratio, and sugar-acid ratio increased. Kumquat canopy film covering treatment reduced canopy light and decreased net photosynthetic rate, but increased leaf area and chlorophyll content, thereby ensuring the accumulation of photosynthetic assimilates and increasing fruit soluble solids and total sugar; simultaneously, it increased daytime canopy temperature, reduced titratable acid, and increased solid-acid ratio and sugar-acid ratio, thereby overall improving fruit quality.

## Full Text

# Influences of Canopy Film-Covering on Photosynthesis and Fruit Quality in Kumquat

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**Abstract:** Canopy film-covering technology has been widely applied in kumquat production. This study used Yangshuo kumquat as experimental material, with film-covered trees as the treatment and uncovered trees as the control. Environmental parameters including temperature, humidity, and light intensity within the canopy were measured for both treatments. Leaf area, leaf length, leaf width, and chlorophyll content were observed, while photosynthetic indices such as net photosynthetic rate, stomatal conductance, transpiration rate, and intercellular CO concentration were determined. Fruit quality indicators including fruit firmness, soluble solids, total sugar, titratable acid, and vitamin C content were also analyzed. The results showed that compared with the control, film-covering reduced light intensity while increasing temperature and decreasing humidity in the canopy. Leaf area and chlorophyll content increased, while net photosynthetic rate decreased by up to 21.39% compared to the control. Titratable acid content decreased, while fruit firmness, soluble solids, total sugar, solid-acid ratio, and sugar-acid ratio all improved. Although canopy film-covering reduced light intensity and net photosynthetic rate, the increased leaf area and chlorophyll content ensured the accumulation of photosynthates, thereby improving soluble solids and total sugar content. Simultaneously, film-covering elevated daytime canopy temperature, reduced titratable acid, and increased solid-acid and sugar-acid ratios, collectively enhancing overall fruit quality.

**Keywords:** kumquat, canopy film-covering, photosynthetic characteristics, fruit quality, temperature and humidity, light intensity

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Kumquat (*Fortunella crassifolia* Swingle) is one of Guangxi' s characteristic citrus species. By 2017, cultivation area in the region had reached 25,520 hectares with production of 282,800 tons, becoming a pillar fruit industry for farmers in major production areas such as Yangshuo and Rong' an. Canopy film-covering technology has been widely adopted in kumquat cultivation, extending on-tree storage time and market availability, thereby increasing prices and farmer profits. However, few reports have addressed the effects of canopy film-covering on kumquat photosynthesis and fruit quality.

Film-covering technology has been extensively applied to various crops including kumquat and bayberry (Qin et al., 2008; Zhang et al., 2007), particularly in kumquat production where it significantly reduces fruit cracking while maintaining quality (Ou et al., 2010; Mei et al., 2012; Li et al., 2012). Canopy film-covering has been shown to increase soluble solids content in Shatang mandarin and Gonggan mandarin during late-stage on-tree storage (Mai et al., 2014; Zhang et al., 2014). Photosynthesis is a fundamental physiological process affecting fruit sugar and acid accumulation and plays a foundational role in fruit quality formation. Many cultivation practices, including canopy film-covering, influence fruit tree photosynthesis. Studies on rain-shelter cultivated 'Bonanza' navel orange showed that leaf net photosynthetic rate (Pn), stomatal conductance (Gs), intercellular CO concentration (Ci), and transpiration rate (Tr) were all lower than in open-field controls (Tang, 2013). Shading treatments reduced net photosynthetic rate in soybean and strawberry (Li et al., 2018; Peng et al., 2018), while colored film covering (green, red) enhanced photosynthetic rate in ginger leaves (Zhang et al., 2007).

Although domestic reports have addressed the effects of canopy film-covering on kumquat canopy environment and fruit quality, comprehensive studies integrating canopy environment, leaf morphology, photosynthesis, and fruit quality remain limited. This study investigated the effects of canopy film-covering on kumquat photosynthesis and fruit quality to provide theoretical support for production practices aimed at improving photosynthetic efficiency and fruit quality under film-covering conditions.

### 1.1 Experimental Materials

The experiment was conducted from 2017 to 2018 in Baisha Town, Yangshuo County, Guilin City, Guangxi. Healthy, uniformly growing 15-year-old seedling kumquat trees were selected. Intact, healthy leaves were collected from non-fruiting spring shoots, while fruit samples of uniform size without mechanical damage were harvested from the middle, upper, and outer canopy sections in four directions (east, south, west, north).

### 1.2.1 Experimental Design

From December 10, 2017 to March 31, 2018, twenty uniformly growing kumquat trees were randomly selected in the middle section of an orchard with consistent south-facing slope. Ten trees were film-covered (treatment) and ten were left uncovered as controls. On March 20-21, 2018, diurnal changes in photosynthesis of healthy, intact functional leaves on spring shoots were measured for two consecutive days under clear, windless conditions. On March 21, 2018, six fruits per tree (one each from the middle, upper, and four directional positions) were collected, totaling 60 fruits per treatment, which were randomly divided into three groups as replicates. Similarly, six leaves per tree were collected (one from each position), with 60 leaves per treatment. Thirty leaves were used for chlorophyll content determination and 30 for leaf area and morphological measurements. All leaf samples were randomly divided into three groups as replicates. Temperature, humidity, and light intensity recorders were placed in the middle canopy of each treatment to monitor environmental conditions.

#### 1.2.2 Index Determination

Photosynthetic parameters were measured using a Li-6400 portable photosynthesis system under natural light with gas flow set at 500 ml · min [1]. Measurements were taken hourly from 9:00 to 17:00, with three trees measured each time. Three healthy, intact functional leaves (3rd-5th leaf from the top of current-year spring shoots) were randomly selected from the outer upper canopy of each tree, with three observations per leaf averaged as the value for that time point.

Fruit quality analysis employed conventional biochemical methods. Soluble solids were measured with a handheld refractometer. Titratable acid was determined by titration. Total sugar and reducing sugar were measured using the 3,5-dinitrosalicylic acid colorimetric method. Vitamin C was determined by 2,6-dichlorophenol indophenol titration. Fruit longitudinal and transverse diameters were measured with vernier calipers. Single fruit weight was measured with a 0.001 g precision balance. Fruit firmness was measured with a hardness tester (GY-1).

Chlorophyll content was determined by spectrophotometry. Samples were ground into powder in 80% acetone, centrifuged, and supernatant absorbance was read at 663 nm (A ) and 645 nm (A ). Chlorophyll concentration (C) was calculated using the formula C = 20.31×A + 8.05×A , then converted to leaf chlorophyll content based on sample mass.

Leaf morphological indices were measured using a laser leaf area meter (CI-203, CID, USA) for leaf area, and vernier calipers for leaf length, width, and thickness. Canopy environmental parameters were recorded using automatic temperature-humidity-light recorders (L99-LXWS, Luge) fixed in the middle canopy with hourly recording frequency. Data in [Figure 1: see original paper], [Figure 2: see original paper], and [Figure 3: see original paper] represent observations from March 20, 2018.

### 1.3 Statistical Analysis

Experimental data were analyzed using Excel 2010 and SAS 9.0 statistical software.

## 2.1 Effects of Canopy Film-Covering on Canopy Environmental Factors

As shown in , during the film-covering period, monthly average canopy temperature was consistently higher in the film-covered treatment than the control, with the maximum temperature difference (0.9°C) occurring in December 2017. Monthly average humidity was consistently lower in the film-covered treatment, with significant differences from January to March 2018 and a maximum difference of 5.79 percentage points in March 2018. Monthly average light intensity was significantly lower in the film-covered treatment, with the maximum difference of 0.88 klx in February 2018.

**Table 1** Influence of canopy film-covering on temperature, humidity, and light intensity in the canopy

| Month | Average monthly temperature (℃) | Average monthly humidity (%) | Average monthly light intensity (klx) |
|---|---|---|---|
| | Film-covering | Control | Film-covering |
| Dec 2017 | 8.41±0.36 a | 7.51±0.07 a | 89.51±0.26 a |
| Jan 2018 | 8.64±0.51 a | 8.03±0.49 a | 76.43±0.24 a |
| Feb 2018 | 11.92±0.51 a | 11.34±0.49 a | 64.06±1.15 a |
| Mar 2018 | 17.55±0.16 a | 17.35±0.19 a | 80.24±1.03 b |

*Note: Different lowercase letters indicate significant differences between treatment and control at the 0.05 level. The same below.*

Film-covering also affected diurnal changes in canopy temperature, humidity, and light intensity. As shown in [Figure 1: see original paper], film-covering increased canopy air temperature compared to the control, with significant differences at 14:00, but minimal differences from 22:00 to 6:00. Film-covering decreased canopy air humidity, reaching significant differences at 10:00 ([Figure 2: see original paper]). Film-covering significantly reduced canopy light intensity, with less diurnal variation than the control. Light intensity at 18:00 was nearly zero, likely because the recorder was placed in the middle canopy where foliage blocked some light, and because weather conditions became overcast around 18:00 on the observation day, further diminishing already weak light ([Figure 3: see original paper]).

## 2.2 Effects of Canopy Film-Covering on Chlorophyll Content and Leaf Morphology

As shown in , film-covering significantly increased leaf chlorophyll content and leaf area by 27.31% and 33.22%, respectively, compared to the control. Leaf length, width, and thickness also increased but without significant differences.

**Table 2** Influence of canopy film-covering on chlorophyll content and leaf morphology in kumquat

| Treatment | Chlorophyll content (mg · g $^{1}$) | Leaf area ($cm^2$) | Leaf length (cm) | Leaf width (cm) | Leaf thickness (cm) |
|---|---|---|---|---|---|
| Film-covering | 3.058±0.06 a | 19.745±0.02 a | 10.755±1.02 a | 3.698±0.10 a | 0.050±0.001 a |
| Control | 2.402±0.02 b | 14.821±1.20 b | 9.268±0.22 a | 3.528±0.30 a | 0.044±0.001 a |

## 2.3 Effects of Canopy Film-Covering on Photosynthesis

As shown in [Figure 4: see original paper], diurnal variation in kumquat leaf net photosynthetic rate exhibited a "double-peak" pattern (increase-decrease-increase-decrease). Film-covering reduced net photosynthetic rate compared to the control, with significant differences at 10:00, 11:00, and 14:00, decreasing by 21.39%, 19.35%, and 12.57%, respectively.

Stomatal conductance diurnal variation also showed a double-peak pattern ([Figure 5: see original paper]), with the film treatment peaking at 13:00 and the control at 11:00, both reaching minimum values at 16:00. Film-covering treatment showed lower stomatal conductance than the control at 9:00 and 11:00, but slightly higher values at other times, though differences were not significant.

Intercellular CO concentration diurnal variation displayed a "double-inverted-V" pattern (decrease-increase-decrease-increase), reaching minimum values at 12:00 ([Figure 6: see original paper]). Film-covering treatment had higher intercellular CO concentration than the control, with significant differences at 9:00, 11:00, and 15:00, increasing by 10.61%, 34.02%, and 16.96%, respectively.

Transpiration rate diurnal variation showed a pattern of increase-decrease-increase-decrease. Except at 9:00, transpiration rate was higher in film-covered trees than the control, with significant differences at 11:00 and 14:00, increasing by 27.82% and 27.02%, respectively ([Figure 7: see original paper]).

## 2.4 Effects of Canopy Film-Covering on Fruit Quality

As shown in , film-covering increased single fruit weight and significantly increased fruit firmness by 7.35% compared to the control. Longitudinal and transverse diameters also increased significantly by 4.07% and 5.25%, respectively, while fruit shape index decreased slightly.

**Table 3** Effect of canopy film-covering on external fruit quality in kumquat

| Treatment | Single fruit weight (g) | Firmness (×10 Pa) | Longitudinal diameter (cm) | Transverse diameter (cm) | Fruit shape index |
|---|---|---|---|---|---|
| Film-covering | 45.87±1.97 a | 5.55±0.05 a | 3.58±0.24 a | 3.21±0.05 a | 1.12±0.01 a |
| Control | 43.64±1.90 a | 5.17±0.03 b | 3.44±0.20 b | 3.05±0.07 b | 1.13±0.01 a |

As shown in , film-covering significantly increased total soluble solids by 5.96% compared to the control. Total sugar, reducing sugar, and sucrose content also increased. Film-covering decreased titratable acid content, thereby increasing solid-acid and sugar-acid ratios.

**Table 4** Effect of canopy film-covering on internal fruit quality in kumquat

| Treatment | Total soluble solids (%) | Total sugar (%) | Reducing sugar (%) | Sucrose (%) | Titratable acid (%) | Solid-acid ratio | Sugar-acid ratio | Vitamin C (mg·100ml 1) |
|---|---|---|---|---|---|---|---|---|
| Film-covering | 18.30±1.40 a | 16.06±1.76 a | 6.55±0.38 a | 8.08±0.32 a | 0.80±0.09 a | 25.64±1.32 a | 21.78±2.30 a | 19.99±3.79 a |
| Control | 17.27±0.31 b | 14.80±1.04 a | 6.98±0.10 a | 7.43±0.26 a | 0.68±0.06 a | 25.73±2.01 a | 19.70±0.91 a | 16.88±1.30 a |

## 3. Discussion and Conclusion

Photosynthesis is a vital physiological process that significantly influences fruit yield and quality formation in fruit trees. Canopy film-covering cultivation technology has been widely applied in citrus, grape, and other fruit crops with notable economic benefits. Investigating photosynthetic changes under film-covering cultivation provides theoretical guidance for further improving yield and quality. Previous research indicates that light is a crucial factor affecting photosynthesis, with both excessively high and low light intensities impairing photosynthetic efficiency. Zhou et al. (2008) demonstrated that weak light conditions reduced actual quantum efficiency in *Catharanthus roseus*. Li et al. (2018) found that shading decreased net photosynthetic rate, stomatal conductance, and transpiration rate while increasing intercellular CO concentration in soybean. In this study, regardless of film-covering, kumquat leaf net photosynthetic rate showed a double-peak diurnal pattern. However, canopy film-covering significantly reduced canopy light intensity, consequently decreasing net photosynthetic rate—consistent with previous findings.

Temperature also substantially affects photosynthesis, with optimal temperatures varying among fruit tree species, and both high and low temperature stresses reducing photosynthetic capacity. In this study, regardless of treatment, net photosynthetic rate declined to the first diurnal valley at 14:00 when temperature peaked, indicating that high temperature indeed inhibited photosynthesis to some extent. Water is both a substrate for photosynthesis and a medium for physiological and biochemical reactions, primarily affecting photosynthesis by influencing chloroplast hydration and enzyme activities involved in photosynthesis. In this study, canopy air humidity in film-covered trees was lower than the control from 2:00 to 16:00. Under relatively dry conditions, enhanced transpiration caused greater water loss from leaves, reducing both water supply for photosynthesis and enzyme activities involved in photosynthetic reactions, thereby decreasing net photosynthetic rate.

Plant growth is influenced by environmental factors including light, temperature, and water, which affect tree growth and morphological characteristics. Under insufficient light, leaf morphology changes accordingly. Studies have shown that shading reduced stem diameter and root surface area while increasing chlorophyll content in soybean seedlings (Li et al., 2018). Under weak light, chloroplast grana in *Catharanthus roseus* became larger with fewer thylakoid layers, while chlorophyll content increased (Zhou et al., 2008). In this study, canopy film-covering reduced light intensity, prompting kumquat leaves to adapt by growing longer and wider, ultimately developing larger leaf areas to capture more sunlight. Additionally, film-covering increased monthly average canopy temperature, facilitating faster leaf growth and larger leaf area. To cope with reduced light, kumquat leaves accumulated more chlorophyll, similar to findings in soybean (Li et al., 2018). Increased chlorophyll content could partially compensate for reduced light intensity effects on photosynthate formation, maintaining normal sugar-acid metabolism and preserving inherent fruit quality.

The relationships among net photosynthetic rate, stomatal conductance, transpiration rate, and intercellular CO concentration vary among plant species and ecological environments, with inconsistent results in previous studies (Liu et al., 2009). In this study, at 11:00, film-covered trees showed significantly lower stomatal conductance but higher transpiration rate than the control, possibly because higher canopy temperature reduced stomatal aperture, decreasing CO uptake, while lower canopy air humidity increased transpiration rate, reducing leaf water content. This insufficient supply of both CO and water substrates for photosynthesis decreased net photosynthetic rate and caused intercellular CO accumulation, significantly increasing its concentration. Relationships among these four photosynthetic parameters were not consistently patterned at other times, requiring further investigation.

Fruit quality is crucial for economic benefits, with appearance quality being an important component. This study found that canopy film-covering significantly increased fruit firmness, likely by reducing rainwater absorption and slowing cell wall degradation and intercellular space enlargement during ripening—consistent with previous research (Li et al., 2012). Film-covering significantly increased fruit longitudinal and transverse diameters but did not significantly increase single fruit weight, with underlying mechanisms requiring further analysis.

Sugar accumulation significantly influences internal fruit quality formation. Various measures can promote sugar accumulation, including moderate drought (Jiang et al., 2013) and shading treatments (Peng et al., 2018). Previous studies demonstrated that film-covering can improve fruit quality (Ou et al., 2012; Mei et al., 2012; Gao et al., 2013). In this study, film-covering reduced canopy air humidity during the covering period, similar to moderate drought treatment, increasing total sugar, reducing sugar, and sucrose content, resulting in higher soluble solids. Although film-covering reduced net photosynthetic rate, increased leaf chlorophyll content ensured effective photosynthate accumulation. Additionally, film-covering enhanced diurnal temperature variation, favoring sugar accumulation and increasing total sucrose and other photosynthetic products in fruit despite reduced photosynthetic rates.

Acid accumulation also plays an important role in internal fruit quality. Light, temperature, and water all affect acid accumulation. Peng et al. (2018) found that 25% shading reduced titratable acid content in strawberry by 12.84%. Mei et al. (2012) demonstrated that titratable acid in film-covered kumquat fruits was lower than in uncovered fruits during late storage. Previous research also indicates that acid reduction requires certain temperatures (Deng and Peng, 2013). This study found that canopy film-covering reduced titratable acid content in kumquat fruits, possibly related to increased daytime canopy temperature, though underlying mechanisms require further investigation.

In summary, canopy film-covering reduced light intensity and photosynthetic efficiency in kumquat canopies but increased leaf area and chlorophyll content, ensuring photosynthate accumulation and increasing fruit soluble solids and total sugar. Film-covering also elevated daytime canopy temperature, facilitating acid reduction and increasing solid-acid and sugar-acid ratios. Additionally, film-covering improved fruit firmness and storage-transport resistance, collectively enhancing overall fruit quality.

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*Note: Figure translations are in progress. See original paper for figures.*