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EFFECT OF THE MIST MICRO-SPRAY TIME ON PHOTOSYNTHETIC SPATIAL HETEROGENEITY OF GRAPE CANOPY

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KEYWORDS

air temperature,
chlorophyll,
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transpiration rate

ABSTRACT

In grape, high temperatures and low humidity prolong midday depression of foliar photosynthesis and dramatically reduce fruit yield. In this study, we explored the effects of various mist micro-spray treatments on grape photosynthesis at a test site in Shanshan County (N, 42.91°; E, 90.30°), Turpan, Xinjiang, China. We tested four different mist micro-spray durations including 1 h (WP1), 2 h (WP2), 3 h (WP3), and 0 h (CK). WP1, WP2, and WP3 affected canopy air temperature and humidity for 5 h, 7 h, and 9 h, respectively. At 12:30, WP1, WP2, and WP3 had the strongest cooling effect and altered temperature by -5.12 °C, -5.09 °C, and -5.17 °C respectively. The relative chlorophyll content was higher in the upper than the lower canopy leaves. There were no differences in the same leaf layers across treatments. The net photosynthesis and transpiration rates and stomatal conductance were higher for the upper than the lower canopy leaves. Compared with CK, the mist micro-spray treatments mitigated "midday depression" in the upper leaves and eliminated it altogether in the lower leaves. Mist micro-spray for 1 h d-1 most effectively improved grape leaf photosynthesis. The findings of the present study lay an empirical foundation for improving grape leaf photosynthesis and fruit yield.

INTRODUCTION

Photosynthesis provides energy for crop growth and determines harvest yield and quality (Rubio et al., 2003; Garnier et al., 2004). Photosynthetic characteristics are greatly affected by environmental factors (Ueda et al., 2000; Midgley et al., 2004; John et al., 2007). Undesirable noontime environmental conditions such as excessive photosynthetically active radiation (Wu et al., 2019; Zhang et al., 2018), high or low air temperature (Zuo et al., 2017), and low relative humidity (Yuan et al., 2020) may induce "midday depression" in photosynthesis that can result in negative photosynthetic rate, lower photosynthetic efficiency, and significantly reduce crop yield. In earlier studies, various measures were proposed to increase photosynthesis including shading (Tang et al., 2019), air temperature control (Yang et al., 2018), air humidity (Wei et al., 2019) in order to prevent "midday depression". However, most of the aforementioned experiments were conducted exclusively on greenhouse crops. Hence, the effects of these modalities on field crops are unknown.

Grape (*Vitis vinifera* L.) is a perennial deciduous vine whose fruit has high commercial value. It has an extensive planting area in Turpan, Xinjiang, China. It is the primary revenue source for the farmers there and a pillar of the local tourism industry (He, 1999). However, the arid climate and high summertime temperatures in the region may hinder grape leaf photosynthesis and significantly reduce vineyard production (Zhang et al., 2015). Therefore, it is necessary to assess the influences of decreasing the air temperature and increasing the air humidity in the vineyard canopy.

Micro-sprinkler irrigation has been widely utilized in many areas. Compared with surface irrigation, it conserves water and cools and humidifies the canopy atmosphere. Thus, it is used extensively in urban green areas, pastures, and other locations (Wang et al., 2007). Micro-sprinkler irrigation produces a larger wet area and more soil evaporation than drip irrigation. For these reasons, it is unsuitable for vineyards. Consequently, a combination of canopy mist micro-spray and drip irrigation for vineyard humidification, cooling, and irrigation was proposed here.

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Ripe grape clusters are susceptible to yellow rot and cracking under high local humidity (Zou & Qi, 2010). Hence, mist micro-spray was performed only during young plant growth in the present study.

MATERIAL AND METHODS

Material

The field trial was conducted during April–September 2017 at the experimental base of the Xinjiang Grape and Fruit Development Research Center (Shanshan County Horticulture Field, Turpan, Xinjiang, China, N, 42.91°; E, 90.30°, 419 masl). The annual average rainfall and evaporation were 25.3 mm and 2,751 mm, respectively, the annual sunshine duration was 2,900–3,100 h, and the frost-free period was > 192 d. The soil type was gravel sandy loam. The test plants were 37-years old Thompson seedless grapes (*Vitis vinifera* L. var Wuhebai). They were planted in large ditches in an east-west orientation. Each ditch was 1.0–1.2 m wide and 0.5 m deep. Small sheds 1.5 m high in the front and 0.8 m high in the rear were used for cultivation. The plant spacing was 1.2–1.5 m and the row spacing was 3.5 m.

Experimental design

One ditch was laid with three drip irrigation tubes. Each of these comprised two drip irrigation belts placed at 30 cm on both sides of the main root. One drip irrigation belt was also placed at the main root itself. The flow rate was 3.2 L h⁻¹, the distance between drippers was 30 cm, and the irrigation quota was 9,150 m³ ha⁻². For this reason, one control and three mist micro-spray treatments were used in this field trial. The mist micro-spray treatments were applied for 1 h (WP1), 2 h (WP2), and 3 h (WP3) while the control (CK) lacked mist micro-spray. Each treatment was performed in triplicate. The mist micro-spray device (cross-type atomizing micro-sprinkler head, Ruichen, Hebei, China) had a spray diameter of 200 cm, a flow rate of 40 L h⁻¹, an emitter distance of 2 m, and an emitter position of 50 cm below the middle of the scaffold. The micro-sprinkler started at 12:00 daily and was operated from May 30 to July 5 during young plant growth.

Here, real-time canopy air temperature and humidity were monitored and canopy upper and lower leaf photosynthetic characteristics and chlorophyll content were measured during young plant growth. The light saturation characteristics of the upper and lower canopy leaves were investigated and high-temperature photosynthesis was evaluated. Data from these assays provided an empirical reference for improving grape leaf photosynthesis in high-temperature regions.

Index measurement

Determination of canopy air temperature and humidity

Automatic air temperature and humidity recorders (EasyLog-USB-2; Lascar Electronics, Whiteparish, UK) were placed in a louver box 50 cm below the canopy. Three automatic canopy air temperature and humidity recorders were placed per treatment group and data were logged every 30 min.

Chlorophyll determination

Relative leaf chlorophyll content was measured with a handheld chlorophyll meter (SPAD-502 Plus; Konica Minolta, Tokyo, Japan). Three normally growing and moderately colored leaves in the upper and lower canopy layers were selected per treatment group and measured every 5 d (Liu et al., 2018). The leaves were measured 6× and the upper and lower layers in each treatment were measured 18× in total. The averages of each set of 18 measurements were used to calculate the chlorophyll content in the leaves of the upper and lower canopy layers.

Determination of photosynthetic data

Normally growing and moderately colored leaves in the upper and lower canopy layers were selected per treatment group on seven sunny days during young plant growth and measured every 5 d. The leaves selected were the same as those used in the relative chlorophyll content measurements. Photosynthesis data were measured for the selected leaves every 2 h from 8:00 to 20:00 using a portable photosynthesis measurement system (CIRAS-3; PP Systems, Amesbury, MA, USA).

Determination of photosynthetically active radiation

Photosynthetically active radiation was measured with a small automatic monitoring weather station (HOBO, Onset Computer Corp, Bourne, MA, USA). The erection height was 4.5 m and recordings were taken every 30 min.

Statistical analysis

Data processing, calculations, and plot design were performed in Excel 2010 (Microsoft Corp., Redmond, WA, USA). The Duncan method was used in SPSS v. 20.0 (IBM Corp., Armonk, NY, USA) to test the significance of the difference between treatment means. $P = 0.05$ and $P = 0.01$ ($0.01 < P \leq 0.05$) between dataset pairs were considered statistically significant, $P \leq 0.01$ was considered highly statistically significant, and $P > 0.05$ was considered statistically nonsignificant.

RESULTS AND DISCUSSION

Diurnal grape canopy air temperature and humidity characteristics under different treatments

We measured and recorded canopy air temperature (Fig. 1a) and humidity (Fig. 1b) once every 30 min during young plant growth. For CK, the diurnal variation in canopy air temperature reached a minimum of 21.45 °C at 6:30 and a maximum of 36.71 °C at 14:30. The difference between the minimum and maximum air temperatures was 15.26 °C. Between 13:00 and 18:00, the canopy air temperature was always > 35 °C. After the mist micro-spray started at 12:00, the air temperatures for WP1, WP2, and WP3 were always lower than that of CK. The temperature reduction began at 12:30. The observed temperature decreases in WP1, WP2, and WP3 lasted ~5 h, ~7 h, and ~9 h, respectively. The maximum difference in canopy air temperature between WP1 and CK, between WP2 and CK, and between WP3 and CK were -5.12 °C, -5.09 °C, and -5.17 °C, respectively. WP1, WP2, and WP3 were ≥ 5 °C cooler than CK for 0.5 h, 1.5 h, and 2.5 h, respectively. The canopy air humidity and air temperature changed in the opposite direction for all

treatments. The canopy air humidity (57.19%) reached a peak at 7:00. The minimum canopy air humidity (33.45%) was measured at 15:30. The difference between the maximum and minimum canopy air humidity was 23.74%. The CK canopy air humidity was < 36% between 13:00 and

18:00. The durations of canopy cooling and humidification were about the same for WP1, WP2, and WP3. Compared with CK, the air humidity measurements in WP1, WP2, and WP3 were at their maxima (59.54%, 59.83%, and 59.34%, respectively) at 12:30.

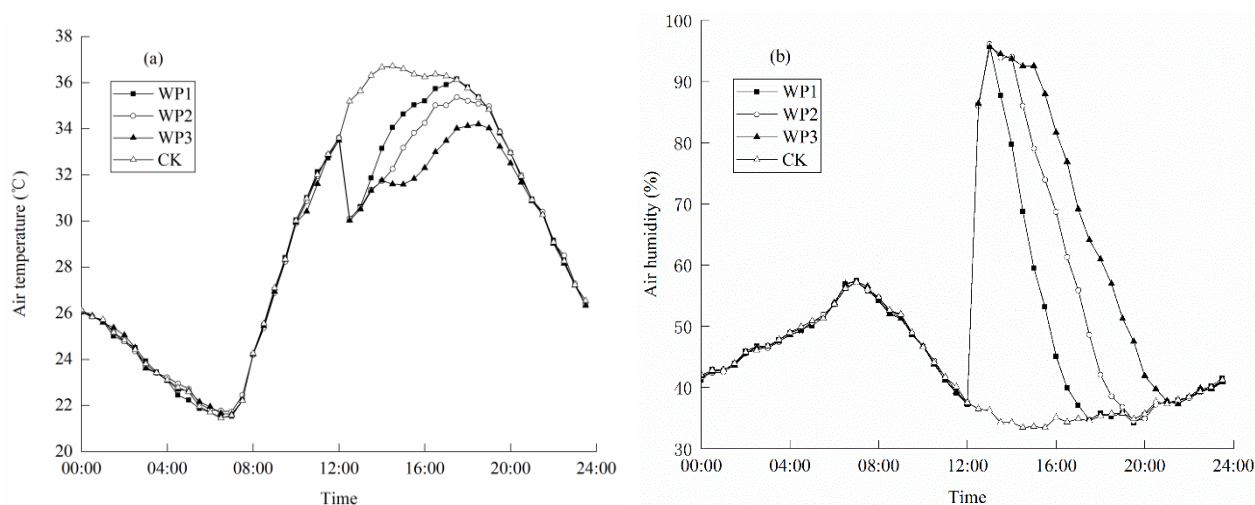


FIGURE 1. Diurnal changes in canopy air temperature (a) and humidity (b) under various treatments.

Changes in relative chlorophyll content in the upper and lower grape leaves under different treatments

Chlorophyll drives photosynthesis which, in turn, is vital to crop growth and development. Here, we observed differences in the relative chlorophyll content in the upper (Fig. 2a) and lower (Fig. 2b) grape leaves under the various treatments. The relative chlorophyll content of the upper leaves of WP1, WP2, WP3, and CK increased with time and

ranged from 41–46, no significant differences were observed among treatments ($P > 0.05$). The lower leaves of all treatments had similar chlorophyll content ($P > 0.05$). The average relative chlorophyll content was significantly higher in the upper than the lower leaves ($P < 0.01$). Hence, mist micro-spray did not alter the relative chlorophyll content of the grape leaves.

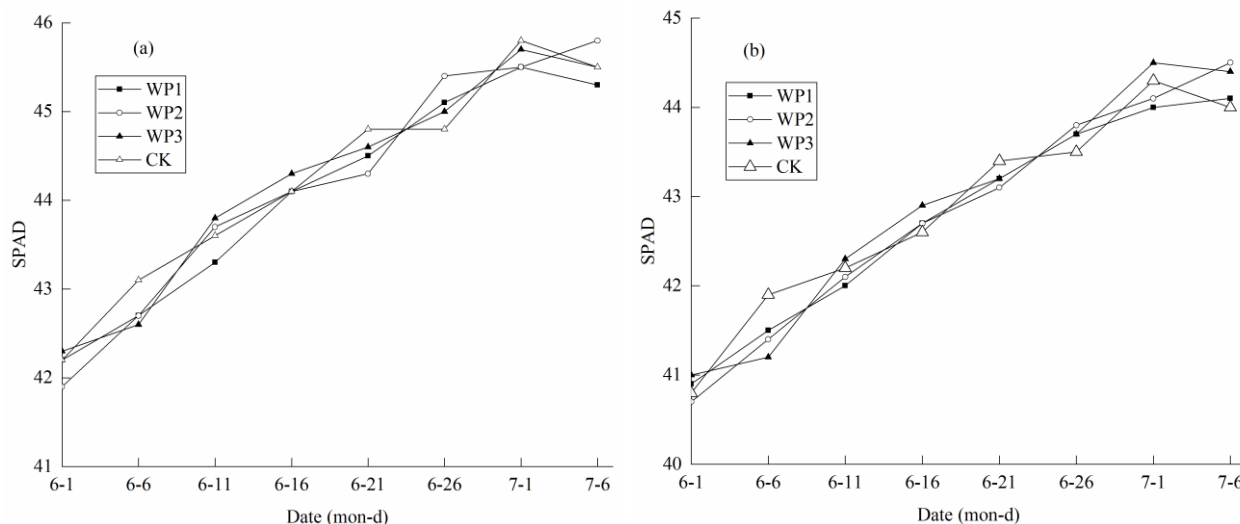


FIGURE 2. Changes in relative chlorophyll content of the upper (a) and lower (b) leaves during young plant growth.

Light response curves for upper and lower grape leaves and daily changes in photosynthetically active radiation in the experimental area

The relative chlorophyll content of the upper and lower canopy leaves significantly differed among the various treatments. However, there was no difference in relative chlorophyll content for the same leaf layers under the various treatments. Thus, we only plotted the

light response curves for the upper and lower leaves under CK (Fig. 3). Pn1 and Pn2 are the net photosynthetic rates of the upper and lower leaves, respectively. The net photosynthetic rates of the upper and lower leaves gradually increased and then leveled off with increasing light intensity. When the photosynthetically active radiation was zero, the net photosynthetic rate was negative. The net photosynthetic rate gradually increased with photosynthetically active radiation. The net photosynthetic rate went from negative to

positive. At a photosynthetically active radiation level of 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$, the increase in net photosynthetic rate slowed down and the rate eventually stabilized. The curve delineating the change in net photosynthetic rate in the lower leaves was generally lower than that for the upper leaves. The light saturation points for the upper and lower leaves were 1,437 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 1,397 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively. Their maximum net photosynthetic rates were 19.29 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 15.72 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively. However, the net photosynthetic rates of the upper leaves were lower than

those for the lower leaves and ranged from 0–200 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Thus, the lower leaves had higher light utilization efficiency at light intensity < 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ while the upper leaves had higher light utilization efficiency at light intensity > 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

The photosynthetically active radiation increased over time, reached a peak, and declined thereafter (Fig. 4). Between 11:45 and 17:45, the grape leaves were under light saturation and the photosynthetically active radiation was > 1,437 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

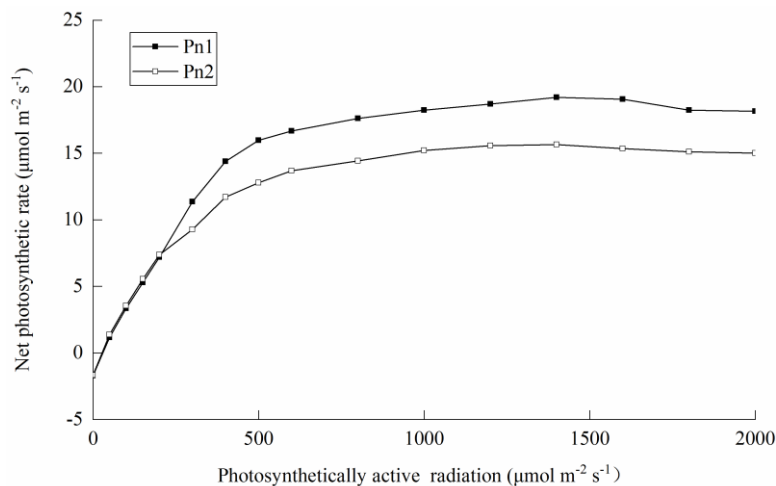


FIGURE 3. Changes with light intensity in the net photosynthetic rates of upper and lower leaves during young plant growth.

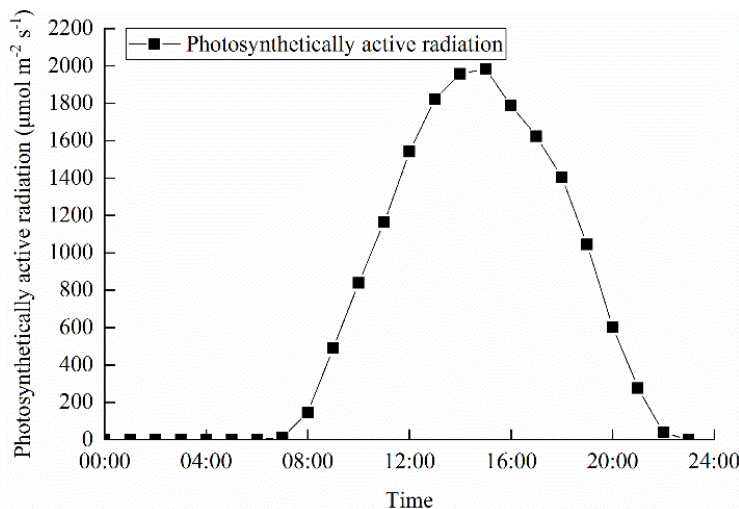


FIGURE 4. Diurnal changes in photosynthetically active radiation.

Diurnal variation in the photosynthetic characteristics of the upper and lower leaves under the various treatments

Diurnal trends in the net photosynthetic rate of the upper and lower leaves under the various treatments

We measured the net photosynthetic rates of the upper (Fig. 5a) and lower (Fig. 5b) leaves under the various treatments. The chlorophyll content, light intensity, and net photosynthetic rate of the upper leaves were higher than those of the lower leaves.

The net photosynthetic rates of the upper leaves in all treatments increased between 10:00 and 12:00 but the differences between treatments were small ($P > 0.05$). The net photosynthetic rates for WP1, WP2, and WP3 were all > 0.8 $\mu\text{mol m}^{-2} \text{s}^{-1}$ higher than that of CK. At 14:00, the

“midday depressions” in net photosynthetic rate were 16.32 $\mu\text{mol m}^{-2} \text{s}^{-1}$, 16.26 $\mu\text{mol m}^{-2} \text{s}^{-1}$, 16.34 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and 15.44 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for WP1, WP2, WP3, and CK, respectively. At 16:00, all net photosynthetic rates were recovered and reached 17.19 $\mu\text{mol m}^{-2} \text{s}^{-1}$, 17.20 $\mu\text{mol m}^{-2} \text{s}^{-1}$, 17.29 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and 16.29 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for WP1, WP2, WP3, and CK, respectively. CK showed the smallest increase in net photosynthetic rate (0.85 $\mu\text{mol m}^{-2} \text{s}^{-1}$). The canopy air temperature was relatively low between 18:00 and 20:00. Hence, the canopy leaves no longer required cooling. However, the water spraying times of WP2 and WP3 (2 h and 3 h, respectively) resulted in continued cooling and the WP3 cooling maintenance time was 9 h. Therefore, the net photosynthetic rate of WP1 was slightly higher than those of WP2, WP3, and CK.

Diurnal variation in the net photosynthetic rates were similar for both the lower and upper leaves under all treatments. At 14:00, only the lower leaves under CK presented with "midday depression". The differences between WP1 and CK, between WP2 and CK, and between WP3 and CK were $1.01 \mu\text{mol m}^{-2} \text{s}^{-1}$, $1.15 \mu\text{mol m}^{-2} \text{s}^{-1}$, and $1.20 \mu\text{mol m}^{-2} \text{s}^{-1}$, respectively. At 16:00, the differences between WP1 and CK, between WP2 and CK, and between

WP3 and CK were all $0.9 \mu\text{mol m}^{-2} \text{s}^{-1}$. The mist micro-spray treatments partially alleviated "midday depression" in the upper leaves and totally eliminated "midday depression" in the lower leaves. For these reasons, there was greater net improvement in the photosynthetic rates of the lower leaves than in those of the upper leaves. Overall, WP1 most effectively conserved water and improved the midday photosynthetic rate.

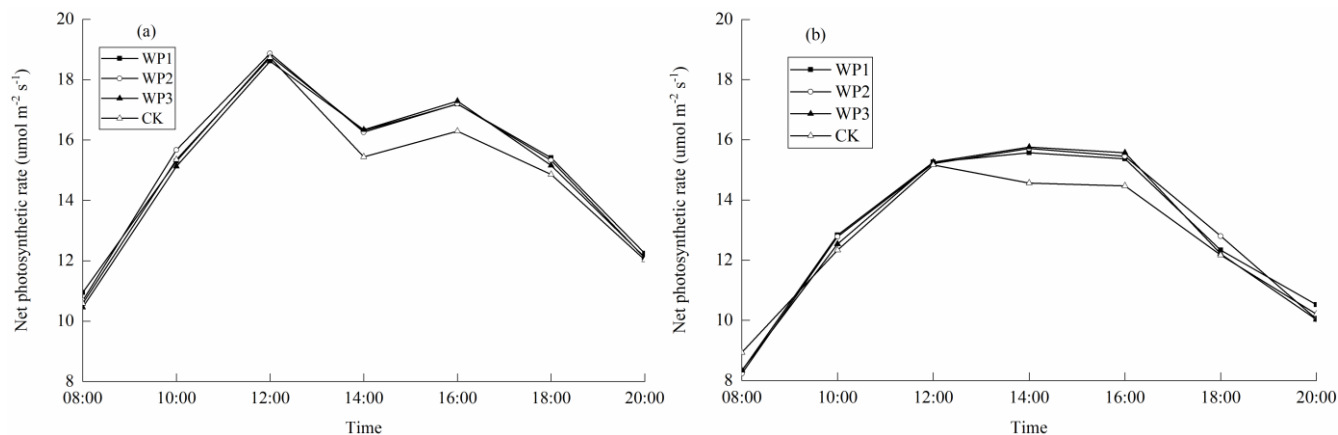


FIGURE 5. Diurnal variation in the net photosynthetic rates of the upper (a) and lower (b) leaves under the various treatments.

Diurnal variation in the transpiration rates of the upper and lower leaves under different treatments

We measured and compared the transpiration rates of the upper (Fig. 6a) and lower (Fig. 6b) leaves under the various treatments. For all treatments, the upper leaves showed a "midday depression" in transpiration rate. In all cases, the transpiration rates peaked at 12:00 and at 16:00. However, the differences among treatments in terms of upper leaf transpiration rate were small between 8:00 and 12:00. By 14:00, the solar radiation intensity and the temperature were high. Consequently, stomatal conductance and transpiration rate decreased in order to prevent excessive water loss. The transpiration rates for WP1, WP2, and WP3 were lower than that of CK between 14:00 and 20:00.

However, the transpiration rate of WP3 was the lowest possibly because the noontime water spray increased the canopy air humidity and inhibited foliar transpiration.

For all treatments, the transpiration rates were generally higher for the upper than the lower leaves. The diurnal variation in the transpiration rate of the lower leaves in WP1, WP2, and WP3 showed a single peak at 14:00. The maximum transpiration rates for WP1, WP2, and WP3 were $8.51 \text{ mmol m}^{-2} \text{ s}^{-1}$, $8.63 \text{ mmol m}^{-2} \text{ s}^{-1}$, and $8.45 \text{ mmol m}^{-2} \text{ s}^{-1}$, respectively. However, the transpiration rates of the lower leaves under CK presented with a double peak and the lowest value was recorded at 14:00. The transpiration rates of the leaves in WP1, WP2, and WP3 were still lower than those for the leaves under CK between 18:00 and 20:00.

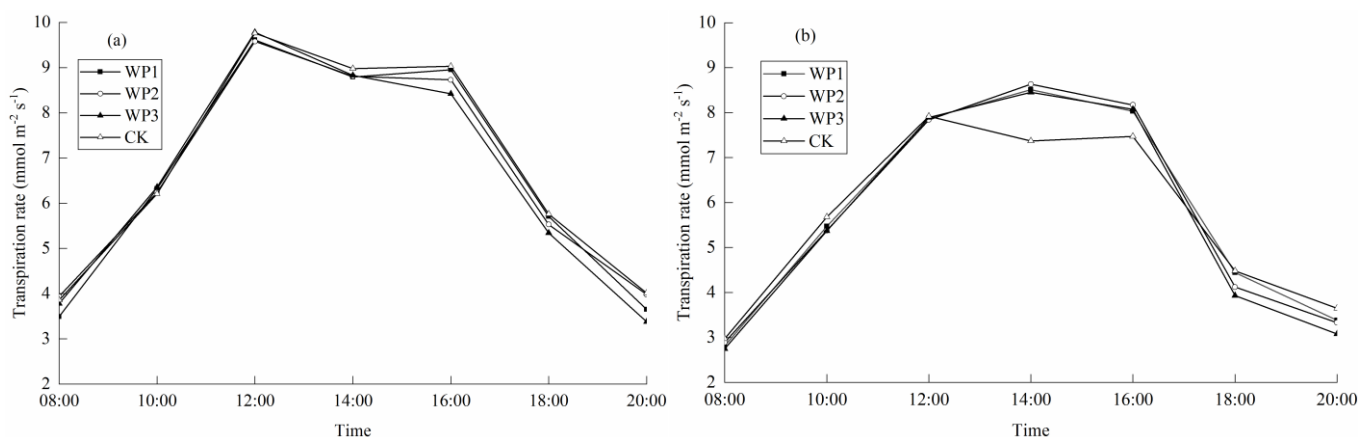


FIGURE 6. Diurnal variation in the transpiration rate of the upper (a) and lower (b) leaves under various treatments.

Diurnal characteristics of stomatal conductance of the upper and lower leaves under different treatments

We measured the diurnal changes in stomatal conductance of the upper (Fig. 7a) and lower (Fig. 7b) leaves under different treatments. For the upper leaves under

all treatments, the diurnal variations in stomatal conductance were generally consistent with those of the transpiration rate. However, the stomatal conductances under WP1, WP2, and WP3 were higher than that under CK between 14:00 and 20:00. After 12:00, the canopy air humidity increases which, in turn, increases the stomatal conductance relative to CK.

The upper leaves had greater stomatal conductance than the lower leaves. The lower leaves under WP1, WP2, and WP3 displayed a single peak at 14:00. Stomatal conductance decreased with time for all treatments but remained higher for WP1, WP2, and WP3 than CK. After 16:00, the

differences between treatments in terms of stomatal conductance were comparatively smaller. An explanation is that the micro-spraying had stopped several hours earlier and the absence of mist lowered the relative humidity and opened the stomata.

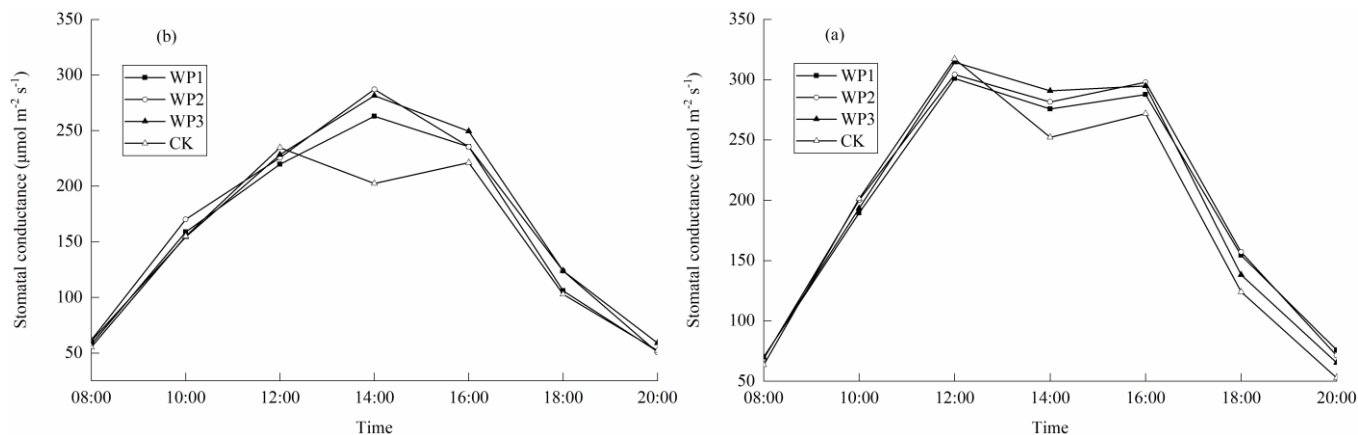


FIGURE 7. Diurnal variations in the stomatal conductance of the upper (a) and lower (b) leaves under different treatments.

Mechanism of "midday depression" in the upper and lower leaves of the canopy

"Midday depression" in crops is caused by a combination of factors, including stomatal limitation and non-stomatal limitation (Gao et al., 2018). The former comprises a decrease in stomatal conductance and inhibition of foliar CO₂ uptake that reduces photosynthesis. The latter consists of external factors that damage chloroplast structure, lower photosynthetic pigment content, decrease photosynthetic enzyme activity, and suppress reactive oxygen metabolism (Murata & Nishiyama, 2017; Nakamura & Izumi, 2018). Stomatal limitation is usually caused by dehydration and low humidity (Farquhar & Sharkey, 1982). In contrast, non-stomatal limitation is often the result of intense light radiation and high or low air temperature. Under high air temperature and low air humidity, stomatal- and non-stomatal limitation combine and severely limit photosynthesis. Here, "midday depression" was relatively stronger in the upper leaves under CK than it was in those under the other treatments. Nevertheless, the mist micro-spray did not entirely eliminate "midday depression". Overall, the mist micro-spray only prevented stomatal limitation in the upper leaves. We propose that it was excessively high leaf temperature caused by non-stomatal-restricted active light radiation and not excessively high air temperature that caused "midday depression" in the upper leaves. The mist micro-spray could cool and humidify both the inside and outside of the canopy. However, the upper grape leaves served as the underlying surface in the vineyard. When a leaf layer is exposed to solar radiation, its internal temperature is always higher than the air temperature. Though the air temperature decreased, the "midday depression" persisted. Hence, non-stomatal limitation was strong because of the light radiation and the leaf temperature. Although the lower leaves of CK exhibited "midday depression", those of WP1, WP2, and WP3 did not. For this reason, the factors affecting "midday depression" in the lower leaves were primarily stomatal limitation and secondarily non-stomatal limitation. The former was caused by high air temperature and low air humidity.

Under conditions of sufficient soil moisture and vapor pressure, stomatal conductance is affected mainly by light intensity and leaf temperature (Liu et al., 2017; Zhang et al., 2018). The most critical factors affecting transpiration are saturated water vapor pressure deficit and short-term light intensity (Granier et al., 1996; Zhang et al., 2015). A previous study suggested that stomatal conductance determines crop transpiration (Duan et al., 2019). The findings of the present study corroborate this conclusion. However, the transpiration rates were lower and the stomatal conductances were higher for the mist micro-spray treatments than CK. This mist micro-spray treatments may have increased relative canopy humidity which, in turn, reduced the vapor pressure deficit and allowed more CO₂ to enter the leaves. Moreover, the mist micro-spray cooled the leaves which, in turn, required no excessive transpiration to reduce their internal temperature. Consequently, air resistance to transpiration increased. These changes increased the stomatal conductance, decreased the transpiration rate, and increased the net photosynthetic rate. Overall, the mist micro-spray cooled and humidified the canopy, reduced "midday depression" in the upper leaves, eliminated "midday depression" in the lower leaves, and increased photosynthesis.

Differences between the upper and lower grape canopy leaves

The leaves were in a state of light saturation between 11:45 and 17:45 (Figs. 3 and 4). However, another study reported that the optimum temperature range for grape leaf photosynthesis was 25–30 °C during light saturation (Sheng et al., 2004). Mist micro-spraying at 12:00 lowered the air temperature from 34–36 °C to 30–31 °C. After cessation of the mist micro-spray, both temperature reduction and humidification gradually decreased until ambient temperature and humidity were reached. According to the optimal photosynthetic temperature of grape leaves, cooling was not required between 18:00 and 20:00. Nevertheless, WP3 continued to reduce canopy temperature, increase

humidity, and inhibit photosynthesis. Here, the chlorophyll content was higher in the upper leaves than it was in the lower leaves. As the lower leaves are shaded, they lack adequate light radiation and cannot effectively biosynthesize chlorophyll (De et al., 2013; Xia et al., 2018). Both the upper and lower leaves have similar light saturation points. However, the net photosynthetic rate is higher in the upper than the lower leaves. This finding is consistent with those reported in earlier studies (Zhang et al., 2000; Alemán F, 2001). The lower leaves are poorly adapted to strong light possibly because of their relatively lower long-term light exposure (Meng et al., 2007; Zheng et al., 2010).

The present study was based on the optimum photosynthetic temperature of grape leaves. Mist micro-spray treatments were applied to the canopy to enhance its photosynthesis. Nevertheless, these treatments did not sustain the temperature at the optimal level for photosynthesis. Moreover, the temperature and mist micro-spray were inconsistent. In future research, then, various emitter flow rates and distance settings combined with different light intensities and temperatures should be tested in order to optimize mist micro-spray under a wide range of conditions.

CONCLUSIONS

Here, mist micro-spray treatments effectively reduced air temperature and increased humidity in grape leaf canopy. After cessation of the mist micro-spray, the canopy air temperature and humidity gradually declined to the same levels as those of the ambient atmosphere. There were no differences in the same leaf layer across all treatments in terms of chlorophyll content. Nevertheless, the chlorophyll content and light saturation point were higher in the upper than the lower leaves. Furthermore, the net photosynthetic and transpiration rates and stomatal conductance were generally higher for the upper than the lower leaves. The “mist micro-spray + drip irrigation” method can increase stomatal conductance in both the upper and lower leaves, reduce the transpiration rate, and increase the net photosynthetic rate. This irrigation method may also eliminate “midday depression” in the lower leaves and reduce “midday depression” in the upper leaves. The net photosynthetic rate in the leaves that were mist micro-sprayed for 1 h d⁻¹ was slightly higher than that in the leaves that were mist micro-sprayed for 2 h d⁻¹ or 3 h d⁻¹. Mist micro-spraying for 1 h d⁻¹ conserves water and is optimal for grape leaf photosynthesis.

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