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Modulation of tolerance of "Hamlin" sweet orange grown on three rootstocks to on-tree oleocellosis by summer plant water balance supply

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ABSTRACT

Citrus fruit is prone to on-tree oleocellosis (OTO), which decreases its fresh market value due to deterioration in the fruit peel. This study aimed to evaluate the effects of Lichi16-6 trifoliata (*Poncircus trifoliate*, LC), Goutoucheng sour orange (*Citrus aurantium*, GT), and Rangpur lime (*C. limonia* Osbeck, RL) rootstocks on the occurrence of OTO, plant water status, reactive oxygen species (ROS) scavenging capacity of 12-year-old "Hamlin" sweet orange (*C. sinensis* Osbeck) trees in both 2014 and 2015 cropping seasons. The results showed that "Hamlin" trees grown on RL maintained higher water potential and relative water content (RWC) in leaves and fruit peels as well as higher transpiration in the morning and afternoon compared with scions grown on the other two rootstocks. This improved water status in the morning resulted in a lower diurnal range of rind oil release pressure (ΔRORP) and the incidence of OTO per tree (IOPT). However, although "Hamlin" trees grown on GT had a significantly higher transpiration in the morning, leaves of "Hamlin" trees grown on GT had the lowest transpiration in the afternoon compared with those grown on the other two rootstocks. This exacerbated water status in the afternoon resulted in higher malondialdehyde and ROS in leaves and severity of OTO per tree (SOPT). In conclusion, the tolerance of "Hamlin" sweet orange grown on three rootstocks to OTO was modulated by plant water balance supply during OTO-sensitive stage. Also, the ΔRORP and ROS scavenging capacity could be selected as indicators to assess IOPT and SOPT, respectively.

1. Introduction

Oleocellosis (or oil spotting) is a physiological disorder that occurs after the rupture of the peel oil gland, causing obviously visible pitting due to the released oil, which is phytotoxic to pericarp cells (Shomer and Erner, 1989; Chikaizumi, 2000; Montero et al., 2012). This has become a significant economic problem worldwide in citrus crops because of the high losses in exportable fresh fruit (McDonald et al., 2000). Citrus fruit is highly sensitive to oleocellosis during both the postharvest storage (Alferez and Burns, 2004; Fischer et al., 2009) and on-tree ripening (Zheng et al., 2011), termed as postharvest oleocellosis (PHO) and on-tree oleocellosis (OTO), respectively.

Hitherto, some reports proved that PHO resulted from the rupture of oil glands due to various mechanical injuries during harvesting, handling, and marketing and that PHO lesions are often > 10 mm in diameter (Fischer et al., 2009; Montero et al., 2009). As a result, PHO can often be prevented by picking the fruit of susceptible cultivars and in sensitive orchards in the late afternoon and placing it into picking sacks and bins with great care (Eckert and Eaks, 1989; Fischer et al., 2009).

Compared with PHO, OTO can also result in lesions of approximately 5–8 mm in diameter (Chikaizumi, 2000; Zheng et al., 2016), and up to 80% of fruit can be affected in sensitive orchards (Almela et al., 1990; Melgar et al., 2011). Some reports concluded that OTO could also arise from various types of damages, including insect attack, hail damage, or wind rub (Kotzé, 1988). Moreover, the occurrence of this disorder varied every year, among groves, cultivar, and fruit of a given tree (Oliveira et al., 2000; Zheng et al., 2010c). However, an effective method is yet lacking to avoid the OTO since the mechanism underlying its development has not been understood clearly, especially with respect to the causes of rupture of oil glands.

Previous studies have shown clear differences in albedo thickness among fruit of the major citrus cultivars, and fruit of Ortanique tangor with a thin albedo was more tolerant to PHO compared with that of Navelate Navel orange tree with a thick albedo. This spongy tissue may

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Table 1
IOPT in fruit in the rapid-expanding stage (August) to the near-harvest stage (December) and SOPT in fruit of Hamlin sweet orange trees grown on three rootstocks LC, GT, and RL in both 2014 and 2015 cropping seasons.

Season	Rootstock	IOPT (%)				SOPT
		August	September	October	December	December
2014	LC	8.2 ± 2.2 aA	56.0 ± 3.1 bB	57.0 ± 3.9 bB	58.9 ± 2.9 bB	0.6 ± 0.0 b
	GT	6.1 ± 1.4 aA	43.3 ± 2.1 cB	42.0 ± 2.9 cB	45.0 ± 2.8 cB	0.7 ± 0.1 b
	RL	7.5 ± 0.5 aA	32.6 ± 3.8 dB	34.0 ± 1.3 dB	32.1 ± 1.6 dB	0.4 ± 0.1 c
2015	LC	10.2 ± 2.4 aA	76.0 ± 2.9 aB	77.0 ± 2.9 aB	78.9 ± 1.6 aB	0.7 ± 0.1 b
	GT	8.1 ± 1.2 aA	53.3 ± 2.3 bB	52.0 ± 2.6 bB	55.0 ± 2.4 bB	1.3 ± 0.2 a
	RL	9.5 ± 0.7 aA	33.6 ± 3.2 dB	34.0 ± 3.1 dB	32.1 ± 3.7 dB	0.5 ± 0.0 c

IOPT, incidence of on-tree oleocellosis per tree; GT, Goutoucheng sour orange; LC, Lichi16-6 trifoliata; RL, Rangpur lime; SOPT, severity of on-tree oleocellosis per tree.
Note: Data (means ± SD, $n = 3$) followed by different uppercase letters in the same row denote significantly different changes in IOPT in fruit in the rapid-expanding stage (August) to the near-harvest stage (December), and data (means ± SD, $n = 3$) followed by different lowercase letters in the same column denote significantly different pretreatments ($P \leq 0.05$).

exert a buffering effect on sharp variations in water status, modulating the response of peel to stress (Alferez et al., 2010; Cronjé et al., 2017). Previous studies have shown that exogenous $Ca(NO_3)_2$ improved water potential, catalase (CAT), and superoxide dismutase (SOD) activity in both leaves and fruit peel tissues and reduced the occurrence of OTO in fruit of Newhall Navel orange tree in water-deficit stress during summer (Zheng et al., 2016).

On the basis of the aforementioned information and response of OTO occurrence in "Hamlin" (*Citrus sinensis* L. Osbeck; Zheng et al., 2010a) and Trovita sweet orange (Zheng et al., 2010b) trees to rootstocks, the present study hypothesized that tolerance to oleocellosis depended on the ability of peel tissues to cope with the water-deficit stress by maintaining the balance of water supply and reactive oxygen species (ROS) metabolism in citrus trees. Therefore, the plant water status [water potential, relative water content (RWC), transpiration, and stomatal conductance] and ROS scavenging capacity (ROS generation rate and antioxidant enzyme activities) were measured in "Hamlin" sweet orange trees grown on different rootstocks under local weather conditions.

2. Material and methods

2.1. Plant material

The present study was conducted for 2 years consecutively (2014 and 2015) in an experimental orchard of the Citrus Research Institute, Chongqing, China (CRIC, latitude, 29°45′51″ N; longitude, 106°22′21″ E; altitude, 240 m above sea level). The study used 12-year-old "Hamlin" sweet orange trees grafted on the rootstocks of Lichi16-6 trifoliata (*Poncircus trifoliate*, LC), Goutoucheng sour orange (*C. aurantium*, GT), and Rangpur lime (*C. limonia* Osbeck, RL), spaced $3 \times 4\ m^2$ apart. All experiments were conducted in a randomized complete block design with three trees on each rootstock as one replication. The experiment was replicated three times.

All trees without irrigation underwent the same conventional cultural care, and seedcakes at 40.0 kg/tree in March and compound fertilizer at 10.0 kg/tree in July were applied during the experiment. The main characteristics of the seedcakes were total nitrogen (N) 53.5 g/kg, total phosphorus (P) 6.2 g/kg, total potassium (K) 10.2 g/kg, and total magnesium (Mg) 3.5 g/kg, and the percentages of N, P_2O_5, and K_2O in the compound fertilizer were 13%, 10%, and 21%, respectively.

2.2. OTO parameters

A total of 20 fruit per tree, yielding 60 fruit per replicate and 180 fruit in total from 9 trees, grown on each rootstock were used to evaluate the incidence of OTO per tree (IOPT) and severity of OTO per tree (SOPT) according to the protocol described in the study by Zheng et al. (2010c, d). The dynamic changes in IOPT were measured every month at the site from fruit in the rapid-expanding stage (July) to the near-harvest stage (December). SOPT was evaluated at harvest in both 2014 and 2015 seasons. Moreover, the total number of fruit with OTO occurrence was recorded as$x$, and the total number of OTO spots with diameter > 0.25 cm and ≤0.25 cm was recorded as $x1$ and $x2$, respectively. The IOPT and SOPT were calculated by the equations IOPT = $x/60$ and SOPT = $\Sigma(x1 \times 0.5 + x2 \times 0.25)/60$.

2.3. Rind oil release pressure

A total of 5 fruit per tree, yielding 15 fruit per replicate and 45 fruit in total from 9 trees, grown on each rootstock were used to analyze the rind oil release pressure (RORP). A digital penetrometer (Magness Taylor Pressure Tester, Canada; Oberbacher, 1965) was used for the analysis at two equational regions at 8:00, 12:00, and 18:00 on three selected sunny days from August to September in both 2014 and 2015 cropping seasons. Moreover, the difference between the maximum and the minimum values of RORP on the selected days was recorded as the diurnal range of RORP (ΔRORP; Zheng et al., 2016a).

2.4. Plant water status

On the aforementioned three selected days, stomatal conductance and transpiration of 12 leaves, randomly marked on 6 out of 9 trees grown on each rootstock, were measured using a portable CO_2/H_2O gas exchange device (Li-6400, Li-Cor, NE, USA; large 6.0-cm^2 leaf chamber) between 8:00 am and 18:00 pm during the OTO-sensitive stage (July to September) in both cropping seasons.

In view of high humidity of the air in the morning and the highest air temperature around 16:00 pm in the afternoon under the conditions investigated in this study, three leaves and 4 fruit peel samples per tree, yielding 9 leaves and 12 fruit peels per replicate and 27 leaves and 36 fruit peels in total from 9 trees, grown on each rootstock were collected to measure the water potential using a PSYPRO Water Potential System (Wescor Inc., UT, USA) with 8 C-52 chambers at 16:00 pm.

Immediately after the water potential measurements, leaves and fruit peel samples were collected to determine the RWC based on the study by Claussen (2005). RWC was calculated using the equation RWC (%) = (FW–DW)/(TW–DW) × 100,

where FW and DW are the fresh weight and dry weight, respectively, and TW is the weight of tissue after being soaked in water for 4 h at room temperature.

2.5. Air temperature, wind speed, and soil humidity

The air temperature, wind speed, and soil humidity (soil water