Research Article

Vegetative shoot flush dynamics of 'Pera' sweet orange on three rootstock cultivars

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Abstract

The dynamics and intensity of new shoot flushes of 'Pera' sweet orange scions [*Citrus* × *sinensis* (L.) Osbeck] grafted onto 'Rough' lemon (*Citrus* × *limonia* var. *jambhiri* Lush.), 'Swingle' citrumelo [*Citrus* × *aurantium* var. *paradisi* x *Poncirus trifoliata* (L.) Raf.] and 'Sunki' mandarin (*Citrus reticulata* 'Sunki') rootstocks were evaluated in the field at a citrus farm located in a northern region of the state of Sao Paulo, Brazil. Every 20 days for 16 months, new shoots were counted within a square frame of 0.25 m^2 set on the central portion of the canopy and classified based on their phenological stages. Trees on 'Swingle' rootstock produced a lower area under the flush shoot dynamics curve (AUFSD) and mean number of new shoots than trees on 'Rough' lemon or 'Sunki' mandarin. For trees on all three rootstocks, new shoot intensities varied significantly over time with the greatest number of new shoots developing during late spring and early summer. Increases in minimum air temperature and available soil water were important indicators of overall emergence of new shoots.

Keywords: *Citrus* spp., scion-rootstock combination, phenological stages, plant growth

Introduction

Vegetative growth of citrus trees occurs in flushing cycles throughout the year, with the periods of growth and vegetative dormancy regulated mainly by variation in climatic conditions. The genetic nature of the scion and the rootstock species, which is used to control tree vigor and increase tolerance to biotic and abiotic factors, also plays an important role. For instance, new shoot development tends to be more frequent on lemons and acid limes than on sweet oranges (Spiegel-Roy and Goldschmidt 1996; Primo-Millo and Agustí 2020).

Knowledge of the impact of the rootstock on vegetative growth of citrus trees is important to determine planting densities of new orchards and other related cultural practices, such as pruning and harvesting (Forner-Giner et al. 2014), and also to improve pest management, especially of Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama, vector of *Candidatus* Liberibacter asiaticus (Las). Las is the bacterium associated with huanglongbing (HLB), presently the most important citrus disease worldwide (Bové 2006). Since the population dynamics of ACP is consistently associated with presence of new shoots on citrus trees (Yamamoto et al. 2001; Tsai et al. 2002; Kistner et al. 2016), understanding flushing dynamics helps to improve not only ACP management, but also HLB management. In this study, we investigated the dynamics of vegetative shoot flushes of 2.5-year-old 'Pera' sweet orange scions grafted onto three rootstock cultivars, 'Rough' lemon, 'Swingle' citrumelo and 'Sunki' mandarin. The period of the year during which new vegetative shoot flushes developed, the number of new shoots produced and the flushing intensity as AUSFD were determined every 20 days for 16 months.

Materials and Methods

Experimental area and plant material

The experiment was carried out between August 2017 and December 2018 in blocks at the Citrus Experimental Station located in the Bebedouro municipality (20°53'16'' S, 48°28'11'' W; 600 m above sea level) in northern São Paulo State (SPS), Brazil. The predominant climate condition of the region is Cwa, characterized as subtropical with a dry winter (Köppen climate classification system) (Rolim et al. 2007). Meteorological variables were monitored daily using a CR10 Measurement and Control System (Campbell Scientific Inc) installed at ca. 600 m distance from the experimental blocks. The sequential water balance [water deficit (WD, mm) and actual evapotranspiration (AET, mm)] were calculated between assessment dates as proposed by Thornthwaite and Mather (1955) using an available water capacity of 100 mm. The potential evapotranspiration (PET, mm) was estimated using the FAO-56 Penman–Monteith (FAO PM) method (Allen et al. 1998).

The experimental area consisted of a non-irrigated 0.8 ha block of 'Pera' sweet orange grafted onto 25 rootstocks cultivars, randomly distributed within the block, with three of the 25 rootstocks cultivars chosen for the assessments: 'Rough' lemon, 'Swingle' citrumelo and 'Sunki' mandarin.

The trees were 2.5 years of age and spaced 2.0 m within-row and 5.0 m between-rows (1,000 trees ha^{-1}), with the planting rows positioned at 51° of azimuthal orientation. During the evaluation period, the trees were not pruned and received the standard management practices recommended to grow citrus in SPS (Carvalho et al. 2005). In an attempt to avoid Liberibacter infection, the trees were treated with systemic and contact insecticides during the first three years, and after that, with contact insecticides, which were sprayed every 10 to 15 days.

Evaluation of vegetative flushing and mean number of new shoots

Every 20 days, a squared frame of 50 cm x 50 cm was positioned in the outer surface of the canopy, from 1.5 m to 2.0 m above ground level, and all new shoots present within the frame projection were counted and classified. Eight trees per scion-rootstock combination randomly selected in the field were assessed, with each tree taken as a replication (Carvalho et al. 2020). Only trees with a healthy overall appearance were used. The 20-day assessment interval was chosen because it is shorter than the time required for a given new shoot to develop from stage V1 (bud swelling) to V6 (complete tissue maturation), which varies from 35 to 45 days at air temperatures of 24 to 26ºC (Cifuentes-Arenas et al. 2018).

Area under the flush shoot dynamics curve (AUFSD)

During the whole assessment period, flushing intensity for each tree was determined by calculating the area under the flush shoot dynamics curve (AUFSD) per m^2 of canopy, which is given by the formula

$$AUFSD = \sum_{i=1}^{n-1} \left(\frac{NS_{i+1} + NS_i}{2} \right) * (T_{i+1} - T_i)$$

where NS_i is the number of new shoots at stages v1 to v6 in the i^{th} observation, and $T_{i+1} - T_i$ the number of days between observations (Campbell and Madden 1990).

Data analysis

A split-plot analysis of variance for repeated measures taken overtime was applied to the data for the mean number of new shoots, in a fully randomized design. The rootstock was the main plot and the assessment date was the subplot. In analysis, the total 24 assessment dates were grouped in every three consecutive dates, which corresponded to eight periods of distinct seasonal climatic conditions. AUFSD was calculated for each sampled tree, with the same trees continuing to be evaluated up to the last evaluation date. Significant differences were detected by comparison of the means with the Tukey test ($P < 0.05$).

Pearson correlation analysis were performed to assess the relationship between the mean number of new shoots and the daily average values of maximum (T_{max}), minimum (T_{min}) and average (T_{avg}) temperature (°C) and accumulated rainfall (mm), taken during the 20 days prior to the evaluation date, in order to evaluate the influence of the environment on flushing intensity.

All analyses were performed using the statistical software R, version 3.6.1 (R Development Core Team 2015).

Results

Over the entire 16-month evaluation period, the highest and lowest values, respectively, for environmental parameters evaluated were (i) solar radiation 28.0 and 15.5 MJ m^{-2} day^{-1}, (ii) evapotranspiration 4.6 and 0.7mm day^{-1}, (iii) maximum absolute daily temperatures 37.3 °C and 15.7 °C, and (iv) and minimum absolute temperatures 22.2 °C and 6.2 °C. The accumulated rainfall was 1,690 mm, distributed mainly during the spring and summer months (Figure 1).

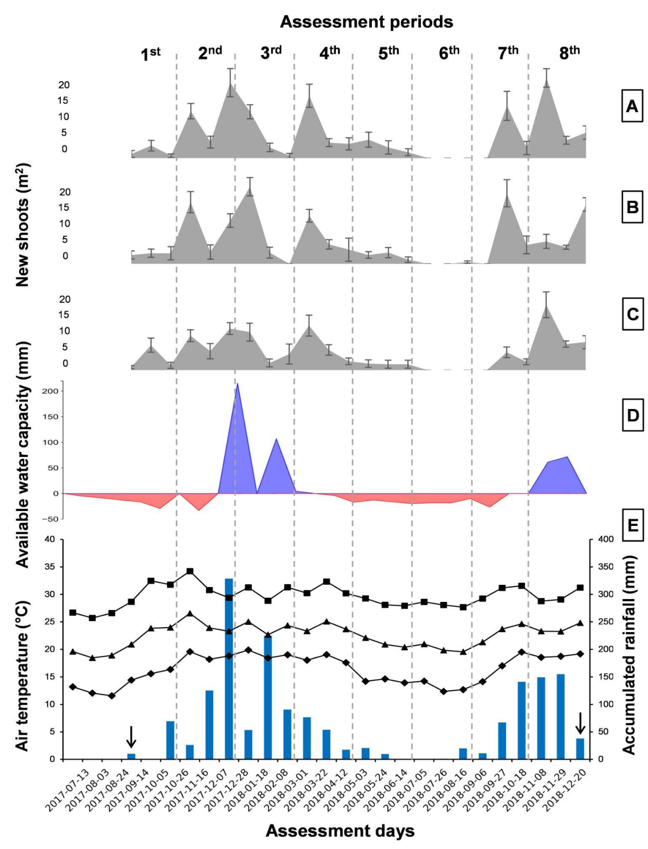

Fig. 1. New shoots per m^2 inside a 0.25 m^2 frame positioned at 1.5-2.0 m high in the canopy of 'Pera' sweet orange trees grafted onto: **A)** 'Rough' lemon, **B)** 'Sunki' mandarin, and **C)** 'Swingle' citrumelo. **D)** Water balance [water deficit (red area) and excess (blue area), in millimeters] during the assessment period. **E)** Accumulated rainfall (blue bars) and maximum (■), average (▲) and minimum (♦) daily air temperature registered during the periods between sampling dates (20 days), from

August 2017 to December 2018 in Bebedouro, SP. The arrows indicate the first and last assessment dates.

Rootstock and assessment date interaction was not significant ($F_{7,14}$ = 1.20; P = 0.2718). However, the mean number of new shoots differed among rootstocks ($F_{2,14}$ = 3.26; P < 0.05), with 'Swingle' citrumelo inducing significantly less new shoots (3.7 new shoots per m² of canopy) than 'Sunki' mandarin or 'Rough' lemon (4.9 and 4.6 new shoots per m² of canopy, respectively). 'Sunki' and 'Rough' lemon did not differ from each other.

The intensity of the flushing events was analyzed by the AUFSD. This allowed simplifying the analysis, since it combines the intensity and frequency of the new shoots in a single value. The AUFSD for 'Swingle' citrumelo (1,730 NS-m^2) was significantly lower than that for 'Sunki' mandarin (2,234 NS-m^2) or 'Rough' lemon (2,168 NS-m^2) ($F_{2,21}$ = 6.06; P < 0.01) (Figure 2).

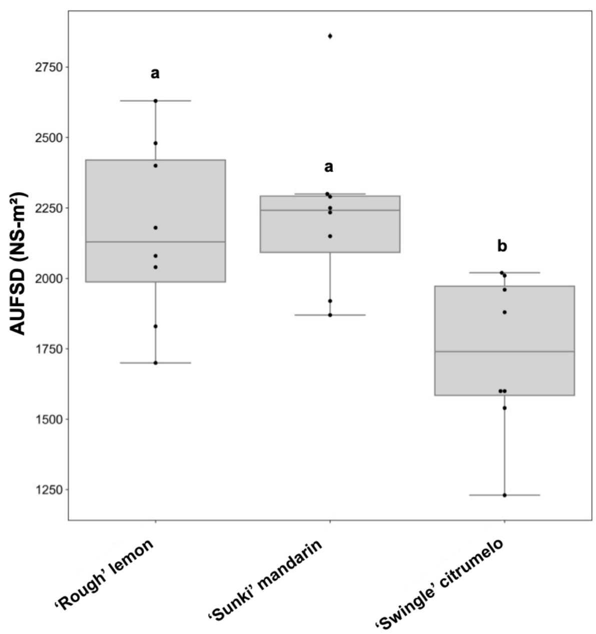

Fig. 2. Boxplots of the area under the flush shoot dynamics curve (AUFSD) of 'Pera' sweet orange scions grafted onto 'Rough' lemon, 'Sunki' mandarin and 'Swingle' citrumelo rootstocks, 2.5 year-old, evaluated from August 2017 to December 2018 in Bebedouro, SP, Brazil.

Also, there were significant differences between assessment periods ($F_{7,14}$ = 24.12; P < 0.0001). Higher number of new shoots were detected during the 2nd (October to December 2017) and 8th (November to December 2018) assessment periods, with 8.4 and 8.1 flushes per m² of canopy, respectively, followed by the 4th (March to April 2018), 3rd (December 2017 to February 2018) and 7th (September to October 2018) periods (5.8, 5.0, 4.4 flushes per m² of canopy, respectively). During the 1st (August to October 2017), 5th (May to June 2018) and 6th (July to August 2018) assessment periods, 2.0, 1.9 and 0.03 flushes per m² of canopy were observed (Figure 1).

Correlation analysis indicated positive association between the number of new shoots and daily average minimum temperature for 'Sunki' (r = 0.65; P < 0.01), 'Rough' lemon (r = 0.59; P < 0.01) and 'Swingle 'citrumelo (r = 0.53; P < 0.01). The accumulated rainfall was positively correlated with the number of new shoots for 'Sunki' (r = 0.50; P < 0.01), 'Rough' lemon' (r = 0.51; P < 0.01) and 'Swingle 'citrumelo (r = 0.44; P < 0.01).

The number of new shoots were also correlated with the accumulated rainfall during periods with and without water deficit. During periods of excess water, the number of new shoots were positively correlated and significant with the rainfall for 'Sunki' (r = 0.77; P < 0.01), 'Rough' lemon' (r = 0.80; P < 0.01) and 'Swingle 'citrumelo (r = 0.71; P < 0.01). In drought periods, the association was positive and significant for 'Sunki' (r = 0.84; P < 0.01) and 'Rough' lemon' (r = 0.49; P < 0.01), but not significant for 'Swingle 'citrumelo (r = 0.10; P > 0.05).

Discussion

We evaluated the abundance of new shoots on canopies of 2.5-year-old 'Pera' sweet orange grafted onto three rootstock varieties in the northern region of SPS, Brazil, from August 2017 to December 2018. Flushing dynamics were similar for the three rootstocks, but new shoot intensity was lower on trees growing on 'Swingle' than on 'Rough' lemon or 'Sunki' mandarin rootstocks.

Oliveira (2017) also found a similar flushing pattern on young trees of six sweet orange scion varieties, including 'Pera', grafted onto 'Sunki', 'Swingle' and 'Rangpur' lime (*C.* × *limonia* Osbeck var. *limonia*) in irrigated and non-irrigated orchards in the central region of SPS, Brazil. But, contrary to our results, there were no differences for the mean number of new shoots counted in a square frame of the same size used in the study reported herein, regardless of the rootstock and the irrigation conditions.

In the present study, an increase in minimum air temperature in combination with an increase in available soil water resulted in new shoot growth. The lower number of new shoots produced by 'Pera' sweet orange on 'Swingle' citrumelo rootstock may be related to its lower drought tolerance compared with 'Rough' lemon and 'Sunki'. Drought stress is known to restrict vegetative growth in citrus plants, especially on young trees (Rodríguez-Gamir et al. 2010; Bowman and Joubert 2020). In drought periods, the emergence of new shoots was lower for 'Pera' sweet orange on 'Swingle' citrumelo than on the two other rootstocks. In addition, 'Pera' sweet orange on 'Swingle' citrumelo seems to present slower responses in new shoot growth, as observed by the minor peaks of flushes after water deficit periods (Figure 1).

New shoot intensities also varied significantly over time with the greatest number of new shoots developing during late spring and early summer, when there is an increase in temperature and water availability to the trees, just after a period of bud dormancy (Spiegel-Roy and Goldschmidt 1996). Major periods of new shoot emergence in late spring and early summer seasons for young and mature citrus trees were also observed by Oliveira (2017) and Carvalho et al. (2020) in SPS, Brazil, Catling (1969) in South Africa, and Hall and Albrigo (2007) in Florida.

During flushing periods, citrus pests are attracted to the new shoots for feeding and reproduction, which is the case for the Asian citrus psyllid (ACP) (Yamamoto et al. 2001; Kistner et al. 2016). Therefore, spray applications should be more frequent during these periods. Increase in frequency of spray applications is also warranted by a rapid expansion of the new shoots during these periods, which leads to a reduction in the protection period provided by insecticide sprays (De Carli et al. 2018).

Despite the reduced intensity of new shoots observed for 'Pera' sweet orange on 'Swingle' citrumelo, the most prevalent citrus rootstock in use in SPS, Brazil (Carvalho et al. 2019), flushes were detected for 'Pera' sweet orange grafted onto this rootstock throughout the year. Previous field studies have not shown any difference in HLB incidence attributed to 'Swingle' citrumelo and other rootstocks (Oliveira 2017). So, the lower shoot intensity of 'Pera' scion on 'Swingle' citrumelo does not support any change in the recommended frequency of insecticide sprays to control ACP. Studies on rootstocks with more contrasting vigor than those used here in different climate regions are in progress.

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