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# Photoperiodic phytochrome-mediated vegetative growth responses of container-grown citrus nursery trees



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#### ARTICLE INFO

#### ABSTRACT

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Keywords: Day length Citrus sinensis Poncirus trifoliata Carbohydrates Night interrupt long noted that these rootstocks exhibit much slower growth during the winter than their non-trifoliate counterparts (e.g., 'Volkamer' lemon, 'Sour Orange'). Since 2007 citrus nursery trees in Florida must be grown in greenhouses to protect them from the Asian citrus psyllid, the vector of huanglongbing (citrus greening). Greenhouse production of citrus nursery trees has greatly increased production costs and, thus, the desire to determine why trifoliate-type rootstocks grow poorly during the winter. We hypothesized that trifoliate-type rootstocks, because of their deciduous habit, respond to photoperiod and exhibit slow growth under short days (photoperiods <12 h). Our objective was to determine the effect of photoperiod on the growth of container-grown trees of the two most common trifoliate-type rootstocks, 'Carrizo' citrange and 'Swingle' citrumelo with and without 'Hamlin' sweet orange scions. Three weeks after budding, all trees were placed in growth chambers under three different photoperiods: short days (SD -10 h photoperiod), long days (LD - 14 h) and short days + night interrupt (SD-NI - 10 h photoperiod + 1 h night interrupt) for 14 weeks, and maintained at 28/21 °C day/night temperature. All trees, regardless of being budded or not, had reduced growth under SD conditions, whereas the trees under SD-NI grew similar to those under LD. Average tree growth during the 14-weeks was 19, 52 and 55 cm, across all rootstock-scion combinations for SD, LD and SD-NI treatments, respectively. The difference in growth between budded and non-budded trees in the SD treatment was not significant, but was highly significant in LD and SD-NI. Across rootstock-scion combinations, the average numbers of new nodes produced per tree were 13, 30 and 32 nodes in the SD, LD and SD-NI treatments, respectively, indicating that the increased growth under LD and SD-NI conditions was not simply a result of internode elongation. Net CO<sub>2</sub> assimilation was higher under the SD and SD-NI treatments compared with LD, but there were no significant differences in whole-plant total nonstructural carbohydrate concentrations as a result. The ability of a 1-h night interrupt to overcome the SD response indicates that the photoperiod effect observed is a phytochrome-mediated response.

In Florida, most citrus trees are grown on rootstocks with trifoliate orange parentage. Nurserymen have

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#### 1. Introduction

Florida citrus nurseries produce more than three million trees annually [Florida Department of Agriculture and Consumer Services (FDACS), 2010]. Traditionally, citrus nursery trees were produced in field nurseries, and greenhouse-grown containerized trees accounted for only 35% of total production in the state (Davies and Zalman, 2008). However, as of January 2007, all citrus nursery trees in Florida must be grown in containers in greenhouses that meet specific governmental regulations for pest and disease exclusion (Florida Department of State, 2010). This shift from traditional field-grown to greenhouse container-grown systems has dramatically increased production costs and limited propagation space. In addition, production problems that were previously viewed as minor are now seen as major, chief among them being erratic, uneven scion growth particularly during the winter on trifoliate orange-type (*Poncirus trifoliata* (L.) Raf. and its hybrids) rootstocks. Grower observations indicate that budded trees on all rootstocks grow more slowly during the winter months, but that this problem

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is exacerbated on trifoliate-type rootstocks, to the point that many nurseries cannot propagate trees on these rootstocks for several months each year. Understanding why trifoliate-type rootstocks in particular have slow and uneven growth during winter is critically important since these rootstocks account for >80% of all citrus tree propagations in Florida (FDACS, 2010).

Photoperiod is one of the major abiotic factors affecting the growth of trees during winter months (Callaham, 1962). Low temperatures coupled with short photoperiods are known to enhance dormancy and reduce vegetative growth in many tree species, while high temperatures and long days promote vegetative growth (Kozlowski and Pallardy, 2002; Nelson and Dickson, 1981). Effects of low temperature on assimilate partitioning, vegetative growth and photosynthesis have been well documented in woody species (Greer, 1983; Greer and Warrington, 1982; Howell and Weiser, 1970; Sirtautas et al., 2011; Ushio et al., 2008). However, for photoperiod most research has focused on flowering responses, with relatively few studies on vegetative growth responses. In citrus, fall-budded 'Washington' navel orange trees are reported to exhibit significantly greater growth under long day conditions provided by supplemental light from dusk to 2200 h compared with natural photoperiod (Nauer et al., 1979). Vegetative growth of satsuma mandarin (Citrus reticulate Blanco) grown under 16 h photoperiod (Inoue, 1989) and some rootstocks of trifoliate parentage such as 'Carrizo' citrange (Citrus sinensis  $L \times P$ . trifoliata) (Warner et al., 1979) have also been documented to respond positively to long davs.

Trifoliate orange is unique among the citruses in that it is deciduous and enters deep dormancy allowing it to survive as far north as 42° N latitude (Davies and Albrigo, 1994). Although there is limited research to determine the environmental signal responsible for growth cessation and dormancy induction in trifoliate orange, photoperiod is likely to play a role. The hypothesis that trifoliate orange is responsive to short photoperiods is supported by the observation that in subtropical regions such as Florida the species becomes quiescent later in the season than in more northern climates (Young, 1977). This may be due to the fact that day length changes occur more slowly and are not as great at low compared with high latitudes. Piringer et al. (1961) found that growth of trifoliate orange slowed markedly under short day conditions (8 h photoperiod), further suggesting sensitivity to photoperiod. Although temperature is also likely involved in the slowing of growth and onset of dormancy in trifoliate orange as it is in many other citrus genotypes (Cooper, 1960; Young, 1961, 1969; Young and Peynado, 1962), it is unlikely that this is a major factor in temperature-controlled greenhouse nurseries.

The literature provides evidence that day length influences growth in citrus trees, specifically trifoliate orange, but none of these studies indicate whether these growth responses are photosynthetic (i.e., carbohydrate-related) or phytochrome-mediated photoperiodic effects. To improve nursery management recommendations it would be beneficial to know whether trifoliate orange rootstocks are truly responsive to photoperiod via phytochrome and whether this response is imparted to non-trifoliate scions budded onto these common rootstocks.

Photoperiod has also been reported to influence carbohydrate partitioning in many species, which may be related to vegetative growth changes. Higher soluble sugar content was observed in the shoot apices of wheat (Mohapatra et al., 1983) and barley (Cottrell and Dale, 1986) under short photoperiods (8 h). *Arabidopsis* plants under very short photoperiods (2, 3, 4 and 8 h) showed an increase in their rate of starch synthesis and a decrease in starch degradation (Gibon et al., 2009). In citrus, soluble sugar levels generally increase and starch levels decrease in winter (Dugger and Palmer, 1969); although, this relationship has not been shown to be in response to photoperiod and may be related to fruit maturation during winter. We hypothesized that the vegetative growth of trifoliate orangetype rootstocks is responsive to photoperiod and this response is mediated by phytochrome, but that this response is reduced when sweet orange (*C. sinensis*) scion varieties are grafted on trifoliatetype rootstocks. To test this hypothesis and study the effects of photoperiod on the growth and carbohydrate partitioning of trifoliate orange, we conducted experiments using the two most common trifoliate-type rootstocks in Florida ['Carrizo' citrange and 'Swingle' citrumelo (*C. × paradisi* Macfad. × *P. trifoliata*)] with and without 'Hamlin' sweet orange scions.

#### 2. Materials and methods

#### 2.1. Plant material

A total of 144 trees were used in the experiment, 72 of 'Carrizo' citrange and 72 of 'Swingle' citrumelo. Half of the trees on each rootstock (36) were budded with 'Hamlin' sweet orange scions using the inverted-T method. This resulted in four rootstock-scion combinations: 'Carrizo' non-budded (Car), 'Swingle' non-budded (Sw), 'Hamlin' on 'Carrizo' (Ham/Car) and 'Hamlin' on 'Swingle' (Ham/Sw). All rootstock trees were grown from seed germinated at the same time and, thus, were of a uniform age. The trees were obtained from a commercial citrus nursery approximately 1 month after budding when the success of the bud take could be assured, but when scion growth was still <3 cm. All trees were grown in 0.95-L pots (MT38; Stuewe and Sons, Tangent, OR) using a proprietary soilless potting media consisting of peat moss, composted pine bark and perlite (SOG10-2-NOF; Florida Potting Soils, Atlanta, GA). All rootstock sprouts were removed, when present, on the budded trees. In an effort to produce trees of similar initial size and growth habit, the non-budded trees were pruned to approximately 15 cm (the height of the inserted bud on the budded trees) at the time they were obtained from the nursery and a single lateral bud was allowed to grow. No attempts were made to remove any lateral branches from the new growth on either the budded or non-budded trees during the experimental period.

#### 2.2. Experimental conditions

The plants of each treatment were placed in growth chambers (Conviron model E15; Controlled Environments, Ltd., Winnipeg, Manitoba, Canada) set to maintain the experimental conditions. Twelve trees of each rootstock-scion combination were grown under each of the following three photoperiod treatments: short days (SD) - 10 h photoperiod, long days (LD) - 14 h photoperiod, and short days + night interrupt (SD-NI) - 10 h photoperiod + 1 h night interrupt in the middle of the dark period. These photoperiods were chosen to approximate the longest and shortest natural day lengths in Polk County, FL (27.96° N, 81.70° W) where >40% of nursery propagations occur (FDACS, 2010). The use of a night interrupt treatment is the classic method to determine if a photoperiod response is photosynthetic or phytochrome-mediated by affecting the phytochrome red to phytochrome far-red ratio without significantly affecting the daily light integral (DLI). A 1 h night interrupt in the middle of dark period has been shown to be effective in breaking the dark period while having little to no effect on net photosynthesis (Thomas and Vince-Prue, 1997). All growth chambers were set to maintain 28/21 °C day/night temperature (night temperature was not adjusted during the 1 h night interrupt). Photosynthetic photon flux (PPF) at plant height was maintained at 450  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> with a red to far-red ratio of 4:1 during the day, and 100  $\mu mol\,m^{-2}\,s^{-1}$ with a red to far-red ratio of 3:1 during the night interrupt using a combination of fluorescent and incandescent lamps. This provided a DLI of 16.2 mol m<sup>-2</sup> d<sup>-1</sup> under SD, 22.68 mol m<sup>-2</sup> d<sup>-1</sup> under LD

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