Final Report

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Effect of Different Photoperiod and LED Lighting Regimes on The Growth and Physiology of Containerized Citrus Nursery Trees

SUBMITTED TO
CALIFORNIA CITRUS NURSERY BOARD

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Introduction

Citrus industry in California Faces one of the largest challenges to date, the HLB (*Huanglongbing*), a deadly disease spread by the Asian citrus psyllid, *Diaphorina citri*. Due to devastation by this disease in Florida, propagation of citrus in California requires insect exclusion facilities. Faster year-round propagation is critical for containerized nurseries to offset the investment in new exclusion facilities. Citrus nurseries under these facilities currently face problems of poor bud push, and slow scion growth in fall-budded container-grown trees. Rootstocks with the deciduous Trifoliate orange (*Poncirus trifoliata*) parentage commonly used in California respond to photoperiod changes in fall, resulting in dormancy induction due to colder temperatures and shorter days. Since winter temperatures in California are much lower than those in Florida, research done in California conditions with California specific rootstock/scion combination, will open a new set of avenues for our industry. The hypothesis is that the use of supplemental lighting in smart ways- like using night-interrupt and extension of daylength (EoD) would trigger growth responses in nursery trees overcoming slow growth in citrus nursery trees during Fall.

Currently, very few nurseries use light to extend the photoperiod with conventional lighting that burns energy for long hours, costing thousands of dollars. There is an interest in research using LED lights with smart lighting regimes to enhance plant growth by tapping classic physiological responses of plants.

During Fall, when day length shortens, plants cease their growth to save carbohydrates for their survival during the longer dark periods as plants detect the duration of darkness to control their growth. It takes only a short exposure to red/far-red light to alter the interconvertible forms of phytochrome in leaves. Therefore, only a short interruption of the dark period by red light is enough to induce long-day effects in plants. Maintaining high light intensities increases nursery costs of production. Therefore, in this study, phytochrome signaling effects were explored by using low intensity (10 µmol. m^{-2}. s^{-1}) lights during NI

and EoD periods to overcome the short-day effect by changing the red to far-red light ratio of the spectrum. Low light intensity below the light compensation point also ensures the minimal photosynthetic activity during that period and it results in maintaining the same Daily Light Integral received by the trees across all treatments. EoD with Far-red light also needs to be studied, because exposure to far-red light alters the forms of phytochrome as well. Not much work has been done in woody trees, and the effects of far-red light need to be explored in citrus, as it may push restricted shoot growth during winter. EoD and NI treatments at low light intensity and supplemental far-red light were applied to see if they may provide a commercially feasible way to increase propagation speed during winter.

MATERIALS AND METHODS

Plant Material

Seventy-two trees were used in each experimental run. These trees came from commercial citrus nursery (Tree source nursery, Woodlake, CA). Thirty-six were unbudded seedlings of 'Carrizo' citrange (*Citrus sinensis* L. × *Poncirus trifoliata*) and another half were 'Carrizo' budded with 'Clementines' (*C. deliciosa* x *C. sinensis*). All of the rootstocks were of uniform age and budded at the same time. Trees were allowed to stay in the commercial citrus nursery for approximately one month until budding success was assured. Trees were then transferred to a greenhouse at California State University, Fresno, where they were potted and acclimatized for two weeks. Trees were potted into 1-gallon pots (30 cm × 10 cm) using soilless coconut coir medium. The daily water use of trees was determined by a gravimetric method. Trees were well irrigated and excess water was allowed to drain to reach field capacity. After field capacity was reached, the weight of the pots was recorded every 24 hours for 3 consecutive days to quantify the amount of daily water use by trees. All rootstock growth below the budding position was removed in the budded trees. Initial growth data was taken from every tree before start of the experiment.

Tree girth, shoot growth, and number of leaves and shoots were recorded. To enable a growth comparison, all unbudded trees were topped to a 35 cm height, comparable to the initial height of the budded trees. No further pruning or removal of growth was done after the trees were put into the growth chambers and treatments were imposed. Trees were irrigated every three days with 200ml of water per tree as determined by the gravimetric method explained earlier. Macronutrients N, P, K (21-21-21) were applied through fertigation (30gm of fertilizer per 30 liters of water) every two weeks. Micronutrients Mg, S, Mn and Zn (Hye-Green Micro-Mix, Gar by Tootelian, Inc., Reedley, Ca) were applied as a foliar spray (6g/L) every two weeks.

Experimental Conditions

Each run of the experiment was performed under controlled environmental conditions imposed by growth chambers (A1000, Conviron, Inc, Winnipeg, Canada). Growth chambers were equipped with LED lights (PRO 650e, Lumigrow) controlled by the cloud-based Lumigrow smart-PAR system. Nine budded and nine unbudded trees were placed in each of the 4 different growth chambers. Four different photoperiodic treatments were assigned randomly to the growth chambers (see Table 1).

Table 1: Different photoperiodic treatments used in the experiment

Treatment	Photoperiod
EoD*	10h LED (500 µmol $m^{-2}s^{-1}$) + 4h Extension of Daylength (10 µmol $m^{-2}s^{-1}$)
NI	10h LED (500 µmol $m^{-2}s^{-1}$ + 1h Night Interrupt (10 µmol $m^{-2}s^{-1}$)
Far-red	10h LED (500 µmol $m^{-2}s^{-1}$) + 4h Far-red light in first run
	10h LED (500 µmol $m^{-2}s^{-1}$) + 10h Far-red light in second run
Control	10h LED (500 µmol $m^{-2}s^{-1}$) as a short-day

*EoD= Extension of Daylength, NI= Night Interrupt, Far-red= far-red light supplementation, Control= Short day control.

Here light intensity in every treatment for 10 Here light intensity in every treatment for 10 LED periods was set to 500 µmol m-2s-1, which was determined by running light response curves with a LI-6800 (LI-COR Biosciences, Nebraska, USA) before start of the experiment. The light intensity for both NI and EoD was set to 10 µmol m-2s-1, which was below the light compensation point in light response curves. This procedure minimizes photosynthetic activity during the NI and EoD periods. Low light intensities were used just as a signal to create a phytochrome-mediated photoperiodic response by altering the Pr/Pfr ratio without changing the Daily Light Integral (DLI) across the treatments. DLI was calculated by following formula:

DLI (moles/day) = [Light intensity µmol m-2s-1 x Total number of hours x 36000 seconds] ÷ 1000000

DLI of 21.6 moles/day was maintained for the lighting regimes to create uniform total light input for the plants. DLI is the total photosynthetically active radiation (PAR) received by the plant on a daily basis, which is a function of intensity and duration of light. 1h Night Interrupt was given from 12:30 am to 1:30 am to interrupt the dark period, and EoD extended the day length from 10h to 14h. NI and EoD were used to overcome the short-day effect that was maintained in the 10h LED control. Light intensity during 1h NI and 4h EoD was 10 µmol $m^{-2}s^{-1}$. Far-red light was used as an EoD treatment for 4 hours after 10h LED in the first run. In the second run, the far-red light was kept on throughout the 10h LED as a supplementary light to enhance its effect. Temperature in all of the growth chambers was set to 25/15 °C for day/night in the first run which was reduced to 21/13 °C for day/night in the second run. Humidity was set to 80%. Environmental conditions in growth chambers were chosen to mimic the actual citrus nursery conditions during winter. Photosynthetic photon flux was maintained at 500 µmol $m^{-2}s^{-1}$ with a red to blue light ratio of 9:1 during the 10h LED period in all treatments. Trees were shuffled every three days within the growth

chambers. Watchdog Micro Station 1000 series data loggers (Spectrum Technologies, Inc.) equipped with quantum light sensors were installed in all growth chambers to monitor and record the temperature, RH and light every 30 minutes. The light spectrum was monitored periodically using a spectroradiometer (Spectrum Technologies, Inc.). Plants were kept for 12 weeks under experimental conditions.

Data Collection

Shoot Growth and Number of Nodes

Growth data were collected before the start of the experiment and bi-weekly throughout the experiment from each tree. Total shoot length was recorded which was cumulative of the length of all branches and the stem of the tree. Total number of nodes (leaf number) and branches were also recorded every two weeks for each of the trees.

Stem Diameter

Stem diameter was recorded every two weeks from a marked position on the stem 2 inches above the soil line, for each tree using a digital Vernier caliper.

Leaf Area

Leaf area was measured every two weeks, non-destructively, from 5 representative leaves from each tree using a portable leaf area meter (LI-3000C, LI-COR Biosciences, Nebraska, USA).

Total Chlorophyll Content

Total chlorophyll content was determined using a colorimetric method (Inskeep and Bloom, 1985), before the start and at the end of the experiment in both runs. Three trees were randomly selected from both budded and unbudded trees from each treatment for chlorophyll estimation. Two leaf discs (1/4-inch diameter) were excised from each selected

tree by a hole punch. Leaf discs were then placed in clean test tubes and 2 ml of N, N-dimethylformamide was added to each test tube containing the leaf discs. Test tubes were then placed in the dark for 72 hours. After 72 hours, the tubes were vortexed, and 1.5 ml of the solution was transferred to a cuvette to read its absorbance at 647 and 664 nm in a spectrophotometer. Chlorophyll concentration in mg/l was then calculated from absorbance readings by using the following formula:

Chl a = 12.70 (A_{664}) – 2.79 (A_{647})

Chl b = 20.70 (A_{647}) – 4.62 (A_{664})

Total Chlorophyll content = Chl a + Chl b)

(Here A_{647} = absorbance at 647 nm and A_{664} = absorbance at 664 nm)

Instantaneous Net Photosynthetic Rate

CO_2 assimilation rate was measured every week from three budded and three unbudded trees in each photoperiodic treatment with a portable photosynthesis system (LI-COR 6800, LI-COR Biosciences, Nebraska, USA). A fluorometer chamber with a 6 cm^2 round aperture was used by matching the light intensity (500 µmol $m^{-2}s^{-1}$) and spectrum (9 Red: 1 Blue) in the growth chambers. CO_2 assimilation rate was measured from fully expanded mature leaves after the lights were turned on for at least two hours to assure that maximum photosynthetic activity was being measured. Total daily photoassimilation was estimated by multiplying the total leaf area of the tree with average instant photosynthetic rate of the treatment.

Total Fresh and Dry Weight

Trees were harvested destructively at the end of the experiment and separated into roots, shoots/stem and leaves. The fresh weight of each fraction was recorded, and then each fraction was oven-dried at 65°C for 24 hours so that its dry weight could be recorded. Root: shoot ratio was calculated both on fresh and dry weight basis by dividing the fresh/dry

weight of below ground parts (roots) by fresh/dry weights of aboveground parts (stem + leaves).

Data Analysis

The experiment was carried out in a completely randomized design. Treatments were randomly assigned to the growth chambers in each run. Data were analyzed separately for budding types and for the two different runs of the experiment. Analysis and graphical presentation of data was done by Graphpad Prism software 8.0 (La Jolla, CA, USA) using analysis of variance. Mean differences between the treatments were tested for significance with Tukey's test (P=0.05).

RESULTS AND DISCUSSION

Vegetative Growth

Photoperiodic treatments were able to push shoot growth and the number of leaves by overcoming the short-day effect, as in previous studies (Brar and Spann, 2014; Inoue, 1989; Warner et al., 1979). Vegetative growth increased in response to increasing day length in woody plants (Kozlowski and Pallardy, 2002; Garner and Allard, 1920). In this both runs of this study, shoot growth was significantly higher in NI than in the control for both budded (see Table2) and unbudded (see Table3) trees. NI in unbudded trees had a significantly higher number of shoots than all other treatments in the second run (see Table 3). There were no differences among treatments for the number of shoots in budded trees (see Table 2). Shoot growth in EoD was also significantly higher than the control in unbudded trees for both runs (see Table 3), but for budded trees the effect was seen only in the second run (see Table 2). Far-red treatment was able to push the shoot growth significantly higher than the control in budded trees in both runs (see Table 2), but this was not the case for unbudded trees (see Table 3). Moreover, there was no effect of Far-red treatment on leaf numbers in either budded or unbudded trees in either run. Previous studies on different crops have

reported effects of far-red light on shoot elongation (Miyashita et al., 1994; Davis and Simmons, 1994; Ito et al., 2014) but there were no differences for number of leaves. Far-red light is known to increase shoot growth by internodal elongation without any increase in node count (Casal, 2013). Under Far-red treatment, the number of shoots in both budded and unbudded trees were significantly less than all other treatments in both runs, though differences were found to be significant only in unbudded trees (see Fig. 1), which corroborates previous studies in various crops that have stated the role of far-red light in inhibiting side shoot growth (Tucker, 1976; Reddy et al., 2013).

Figure 1: Representative unbudded trees (Carrizo' citrange (Citrus sinensis L. × Poncirus trifoliata) under different photoperiodic treatments as listed under from left to right. *NI= Night Interrupt, EoD= Extension of Daylength, Far-red= far-red-light supplementation, Control= Short day control.

Number of leaves was recorded to were representative of the number of nodes and NI had a significantly higher number of leaves than the Control and Far-red treatments in unbudded trees in both runs (see Table 3); whereas in budded trees, this was observed only in the second run (see Table 2). EoD had a significantly higher number of leaves than the Control and Far-red treatments in unbudded trees in both runs, and likewise, in budded trees this was only observed in the second run.

Budded trees did not respond positively to EoD in the first run. This may be due to the budding effect of the evergreen scion (*C. deliciosa x C. sinensis*) as compared to the deciduous trifoliate rootstock 'Carrizo', which has been well documented to be responsive to photoperiod (Brar and Spann, 2014; Inoue, 1989; Warner et al., 1979). Unbudded rootstock in this experiment responded positively to photoperiodic treatments in both runs. Yet shoot growth and the number of leaves were higher than the control only in second run under EoD and in both runs under NI in budded trees (see Table 2). These results suggest that photoperiod-responsive trifoliate rootstocks may restrict scion vegetative growth in short-day conditions.

There were no significant differences between the treatments for stem girth in both budded and unbudded trees in either run. There were no differences among the treatments for the leaf area in both budding types in either run. Budded trees had higher leaf area than unbudded trees in both runs regardless of the treatments. Total growth was greater in the first run than in the second run as the temperature was reduced to 13°C and 21°C (night and day) from 15 C° and 25 C° (night and day) to see the effect of photoperiodic treatments at low temperatures typical of fall. Trees still responded to the photoperiodic treatments after the temperature changes. This suggested that the photoperiodic response of citrus trees is independent of the temperature changes. These results corroborate with the previous studies done by Brar and Spann (2013) in which they reported that citrus responds to photoperiodic

changes until the temperature falls below a critical range (10-15 °C). Sharples and Burkhart (1954) reported 13 °C as the critical temperature for citrus growth.

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Table 2: Effect of different photoperiodic treatments on increments in growth parameters of budded citrus trees in two experimental runs.

	Budded- 1st run				Budded- 2nd run			
Parameter	Treatment[x]				Treatment[x]			
	EoD	NI	Far-red	Control	EoD	NI	Far-red	Control
Leaf number	3.66a*	13.33a	6.66a	12.20a	5.22a*	5.00a	3.44b	3.55b
Shoot length (cm)	7.88b	15.43a	14.57a	7.62b	6.33a	5.55a	6.22a	3.33b
Shoot number	1.22a	1.22a	1.22a	2.00a	0.88a	0.55a	0.44a	0.77a
Stem diameter (mm)	1.14a	1.16a	1.12a	1.04a	0.81a	0.77a	0.98a	1.13a
Leaf Area (mm^2)	11.41d	16.39ab	12.31cd	14.27abc	12.21a	11.16a	12.81a	12.78a
Root: Shoot	0.88ab	0.70b	0.87ab	0.98a	0.47b	0.51bc	0.59ac	0.63a

*Mean separation by Tukey's test, $P < 0.05$ with experimental runs analyzed separately.

[x] EoD= Extension of Daylength, NI= Night Interrupt, Far-red= far-red light supplementation, Control= Short day control.

Table 3: Effect of different photoperiodic treatments on increments in growth parameters of unbudded [13] citrus trees in two experimental runs.

Parameter	Unbudded- 1st run				Unbudded- 2nd run			
	Treatment[x]				Treatment[x]			
	EoD	NI	Far-red	Control	EoD	NI	Far-red	Control
Leaf number	16.75a*	22.17a	7.55b	4.60b	11.22b*	14.89a	3.88d	7.88c
Shoot length (cm)	25.33a	29.00a	11.44b	8.33b	19.11b	24.22a	7.33d	13.00c
Shoot number	2.11ab	2.33ab	0.667b	2.77a	2.11b	3.33a	1.00c	2.00b
Stem diameter (mm)	1.38a	1.31a	1.38a	1.21a	1.86ab	1.94a	1.51bc	1.61bc
Leaf Area (mm^2)	8.35a	7.51a	8.45a	7.33a	9.09a	8.55a	8.67a	8.68a
Root: Shoot	0.81b	0.82b	0.96a	0.98a	0.46b	0.49b	0.63a	0.64a

*Mean separation by Tukey's test, $P < 0.05$ with experimental runs analyzed separately.

[x] EoD= Extension of Daylength, NI= Night Interrupt, Far-red= far-red light supplementation, Control= Short day control.

Root: Shoot (Dry Weight Partitioning)

Trees were harvested after 90 days and separated into leaves, stems and roots to record their fresh and dry weights. Root-to-shoot ratio on a dry weight basis was computed to assess the assimilate partitioning between above- (leaves and stems) and below-ground portions. NI had significantly lower root: shoot than the control in both budded and unbudded trees for both the runs (see Fig. 2). Shoot growth in citrus is known to be inversely correlated with root growth (Bevington and Castle, 1985). EoD had significantly lower root: shoot ratio than the control in unbudded trees in both runs (see Fig. 2), but for budded trees this ratio was significantly lower only in the second run (see Fig 2), and no differences were found in the first run (see Fig. 2). No differences in root: shoot ratio were seen between Far-red and Control treatments, suggesting no translocation of reserves from roots to stems occurring in response to short-day length (see Fig. 2). Instead, stem elongation may be due to an increase in auxin synthesis at the apex and young leaves which results in production of abscisic acid in axillary buds and restricts bud outgrowth, as found in tomato (Tucker, 1976). This hypothesis would explain why the Far-red treatment failed to elongate stems in unbudded trees, as those were topped to uniform height before putting them in the growth chambers, which entailed removal of the apical region where auxin synthesis would have taken place.

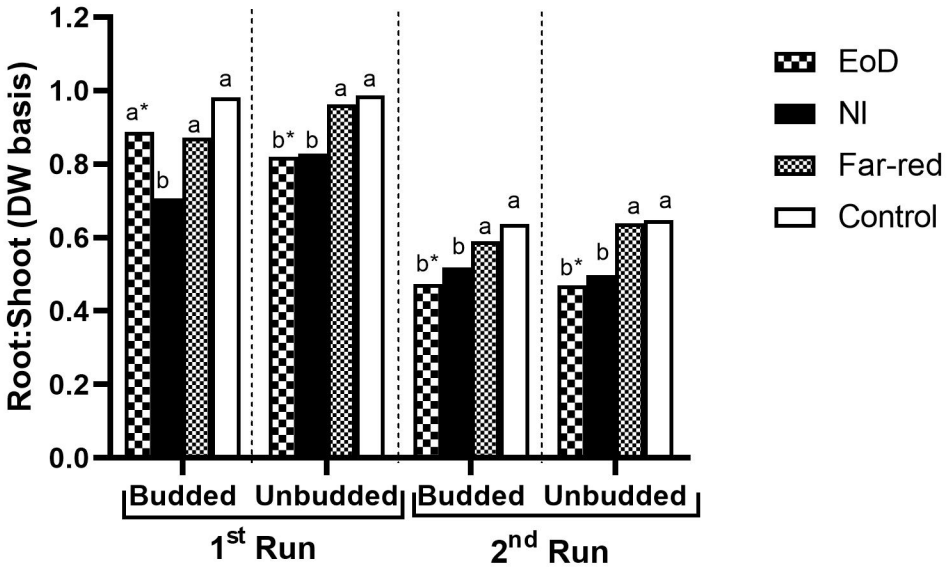

Figure 2. Root-to-shoot ratio (Dry weight basis) of budded and unbudded containerized citrus trees under four different photoperiods treatments in two experimental runs. EoD= Extension of Daylength, NI= Night Interrupt, Far-red= far-red light supplementation, Control= Short day control.

*Mean separation within budding types and experimental runs by Tukey's test, P <0.05.

As discussed earlier, supplemental light intensities used in the NI and EoD treatments were below the light compensation point, and far-red light is not photosynthetically active. Therefore, minimal added photosynthetic activity was possible during these treatments, but plants in the NI and EoD treatment nevertheless grew significantly more than control plants. This pattern is explained by root: shoot ratio on a dry weight basis, as the trees which had higher vegetative growth had less dry weight in roots, as opposed to control trees, which had saved the same photoassimilates in their roots and increased their root mass in response to 10-hour day length (Goldschmidt, 1982). This result suggests that low intensity (10 μmol. m-2.sec-1) light during these treatments was able to trigger the classic phytochrome effect by altering the phytochrome forms by changing the red-light absorbing phytochrome form to far-red-light.

Different Tissue Dry Weight Partitioning

There was a significant effect of photoperiodic treatments on root, shoot and leaf dry weights. In the first run, root dry weights of budded trees under NI, EoD and Far-red treatment were significantly less than the control, and there were no differences among treatments for leaves and shoot dry weights (see Table 4). In the second run, in budded trees NI and EoD had less root dry weight than Control and Far-red, though the differences were not significant (see Table 4). There were no differences for shoot and leaf dry weights among the treatments in budded trees (see Table 4). Unbudded trees under NI and EoD treatments in both runs had significantly higher dry weight in shoots than control (see Table 5). Far-red also had higher dry weight in shoots than control in both runs, but the difference was not significant in the first run in unbudded trees (see Table 5). There were no differences among treatments for leaf or root dry weights in unbudded trees in either run (see Table 5). Dry weight partitioning between roots, shoots and leaves among the treatments in budded and unbudded trees suggested that budded trees under 10-hour day length stored reserves in roots whereas unbudded trees under 10-hour day length stored reserves in shoots. This result further suggests that trifoliate rootstocks impart photoperiodic sensitivity to budded scions.

Table 4. Tissue dry weights of budded trees under four different photoperiodic treatments for two separate runs of experiment.

	Budded- 1st run				Budded- 2nd run			
Parameter	Treatment[x]				Treatment[x]			
	EoD	NI	Far-red	Control	EoD	NI	Far-red	Control
Roots (mg)	5084ab*	4239b	4976b	6360a	1629a*	1515a	2030a	1904a
Shoots (mg)	4438a	3672a	3860a	4252a	2244ab	1743b	2293a	1975ab
Leaves (mg)	1876a	2454a	1607a	2206a	1218a	1168a	1152a	1047a

*Mean separation by Tukey's test, P <0.05 with experimental runs analyzed separately.
[x] EoD= Extension of Daylength, NI= Night Interrupt, Far-red= far-red light supplementation, Control= Short day control.

Table 5. Tissue dry weights of unbudded trees under four different photoperiodic treatments for two separate runs of experiment.

	Unbudded- 1st run				Unbudded- 2nd run			
Parameter	Treatment[x]				Treatment[x]			
	EoD	NI	Far-red	Control	EoD	NI	Far-red	Control
Roots (mg)	5825a*	5793a	6152a	5614a	2618a*	2981a	2915a	3130a
Shoots (mg)	5646a	5155ab	4942ab	4170b	4088a	4294a	3158b	3302b
Leaves (mg)	1609a	1554a	1518a	1661a	1589a	1727a	1423a	1550a

*Means separation by Tukey's test, P<0.05 with experimental runs analyzed separately.
[x] EoD= Extension of Daylength, NI= Night Interrupt, Far-red= far-red light supplementation, Control= Short day contro

Introduction- Total non-structural carbohydrate partitioning in citrus

In the first part of this study the effect of different photoperiods with smart LED lighting on growth of citrus nursery trees was studied. It was found that night interruption and extension of day length treatments were able to push the restricted growth as compared to the 10-hour short-day control. Therefore, the purpose of this study was to test if the effect of different photoperiods was phytochrome-mediated or photosynthetic. Light has two functions in plants: it is a source of energy for photosynthesis and a signal for various developmental processes. In this study the second function of light, as a signal or phytochrome effect of light was explored. Night interrupt, extension of daylength and far-red-light treatments were used as a source of signal to alter the forms of phytochrome to induce long day effects in plants. Low intensity light (10 µmol. m-2. s-1) which is below the light compensation point was used in the treatments to alter the forms of phytochrome (Pr/Pfr). This low intensity light could not have a photosynthetic effect, thus in this study, the effect of LED light treatments on photosynthesis and total non-structural carbohydrates partitioning was studied to confirm if vegetative growth differences seen in the first part of the study were mediated by phytochrome effect of light.

Plants synthesize carbon through photosynthesis and utilize it for structural biomass and respiration. A small fraction of synthesized carbon is kept in the form of nonstructural carbohydrates (starch and soluble sugar). Sucrose, a soluble carbohydrate is a major product of photosynthesis which can be stored and translocated to actively growing regions (Lewis, 1984), whereas starch is a major storage carbohydrate of woody species (Roper et al., 1988).

Shorter day length resulted in increased starch accumulation, decreased translocation and concentration of foliar sucrose in soybean (Chatterton and Silvius,

1979) and tobacco (Huber et al., 1984). Iglesias et al. (2002) documented that the accumulation of sugar in the leaves results in an inhibition of photosynthesis though feedback mechanisms. Overaccumulation of starch could damage chloroplast functioning and an excess of soluble sugars may cause osmotic stress (Krapp et al., 1991; Webber et al., 1994). Shorter photoperiod increased total nonstructural carbohydrates in leaves of clover (Boller and Nösberger, 1983) and blueberry (Darnell, 1991). Photoperiodic changes have been documented to alter the carbohydrate partitioning in Dioscorea spp. (Chu and Ribeiro, 2002), *Lolium temulentum* (Périlleux and Bernier, 1997) and Aloe vera (Paez et al., 2000).

Citrus is known to store carbohydrates in winter and translocate them in the following spring for early season growth flush (Zamski and Schaffer, 1996) which is a characteristic of deciduous fruit trees (Schaffer et al., 1986). Citrus continues to accumule reserves even when demands of developing fruits are not met (Fishier et al., 1983), just as a general survival strategy (Zamski and Schaffer, 1996). Therefore, translocating reserves for vegetative growth might increase the vegetative growth in winter and also lead to higher assimilation rates. Girdling, a very common practice in citrus, is performed to avoid the translocation of assimilates from leaves to roots when photosynthetic activity is high, in order to serve the needs of above ground parts for growth and development (Salisbury and Ross, 1992). However, girdling cannot be done in young citrus nursery trees. Therefore, we need to more fully understand the role of the accumulation and distribution of reserves on vegetative growth and photosynthesis in young containerized citrus nursery trees by studying in detail the partitioning of carbohydrates into different plant parts.

MATERIALS AND METHODS

Data Collection

Total Fresh and Dry Weight

Trees were harvested destructively at the end of the experiment and separated into roots, shoots/stem and leaves. The fresh weight of each fraction was recorded, and then each fraction was oven-dried at 65°C for 24 hours so that its dry weight could be recorded.

Total Non-Structural Carbohydrates Quantification

After harvest and dry weight determination, every fraction from every tree was then ground with a Thomas Wiley mill (Wiley® Mill 4 1/2 HorsePower) and allowed to pass through a 2 mm sieve, then further ground with a CT 293 Cyclotec™ (FOSS Analytical, Denmark) and allowed to pass through a 0.5 mm sieve. Non-structural carbohydrates were quantified in the ground material from each fraction of each tree. Soluble sugar and starch content were quantified using the protocol described by Bailey (1958) with a few modifications. Soluble sugars were extracted by mixing 25 mg of dried ground tissue in 1 ml of ultrapure water and incubating it at 70°C for 15 minutes. Extracts were then centrifuged (10 minutes at 13000 rpm), and the supernatant was decanted, diluted, vortexed and loaded into a 96-well tray along with the glucose standards. Anthrone reagent (0.1% by vol. of H_2SO_4) was mixed vigorously with the samples that had been loaded onto the tray. The plate was then incubated at 85 C for 20 minutes, allowed to stay at room temperature for 10 minutes and was read with a plate reader by measuring absorbance at 620 nm.

Starch was analyzed from the same sample by removing the supernatant, washing the pellet with ethanol followed by ultrapure water to remove any remaining soluble

sugars. Starch degradation was done by boiling (100°C for 10 minutes) the pellet saved from the soluble sugars analysis, adding 100 µl of 70 units/ml amyloglucosidase from *Aspergillus niger* (Sigma-Aldrich, Co.) and 100 µl 7 units/ml of alpha amylase from *Aspergillus oryzae* (Sigma-Aldrich, Co.). The pH was controlled by adding 500 µl of 0.2 M acetate buffer, pH 5.5. Digests were then incubated at 37°C for 4 hours. After incubation, digests were centrifuged (5 minutes, 13000 rpm), 50 µl was decanted, diluted, vortexed and loaded onto a 96-well tray along with Anthrone reagent (0.1% by vol. of H_2SO_4) which was mixed vigorously into each sample by pipetting up and down several times. The plate was incubated (85°C for 20 minutes), left at room temperature for 10 minutes and then read with a plate reader measuring absorbance at 620nm.

Total starch content and soluble sugars were calculated by multiplying the concentrations by the total dry weights of leaves, shoots and roots.

RESULTS AND DISCUSSION

Budded Trees

Total starch content in roots was less in the NI, EoD and Far-red treatments as compared to the control in both experimental runs (see Table 6). These differences were significant only between NI and Control treatments, but we could still see the short-day control on assimilate storage in roots when comparing the photoperiodic treatments. This agrees with the results of previous studies in citrus which documented the storage of assimilates in roots in response to dormancy or short days (Salisbury and Ross, 1992; Zamski and Schaffer, 1996). NI had the least starch in shoots as compared to the control in both runs (Table 6) and lowest level of starch amongst all other treatments in the second run (see Table 6). NI and EoD also had a lower starch content in the leaves of budded trees as compared to the control in the second run (see Table 6). This might be due to significantly higher vegetative growth (leaves + shoot growth) in the NI and EoD

treatments than in the control which resulted in the use of total non-structural carbohydrates for vegetative growth. Previous studies done by Jones et al. (1974) and Jones and Steinacker (1951) in citrus also found that carbohydrate levels drop during the period of growth flushes and increase during the period of dormancy. Citrus leaves serve the early season growth needs as they remain evergreen and photosynthetic throughout the season (Goldschmidt, 1999).

Far-red trees had lower starch content in roots as compared to the control for budded trees in both runs (see Table 6) and the highest starch and soluble sugars in shoots in the second run (see Table 6), although differences were not significant, but still it suggests the translocation of non-structural carbohydrates from roots to shoots which may have resulted in shoot elongation in budded trees as compared to the control trees.

Total soluble sugars were less in roots of NI and EoD as compared to control in budded trees in both runs but differences were not significant. There were also no significant differences for soluble sugars in leaves and shoots of budded trees in both runs (see Table 6).

Table 6: Effect of different photoperiodic treatments on total non-structural carbohydrates (TNC) in leaves, shoots and roots of budded citrus trees in two experimental runs.

	Budded- 1st run				Budded- 2nd run			
Parameter	Treatment[x]				Treatment[x]			
	EoD	NI	Far-red	Control	EoD	NI	Far-red	Control
Starch (mg)								
Leaves	28.79a*	81.55a	35.46a	39.66a	103.3a*	104.2a	139.4a	129.7a
Shoots	337.1a	241.4a	219.1a	301.4a	195.3ab	121.4b	217.8a	161.5ab
Roots	645.5ab	492.3b	595.4ab	737.4a	285.6ab	245.2b	341.5a	367.5a
Soluble sugars (mg)								
Leaves	175.0a	227.6a	137.1a	190.0a	76.02a	66.39a	72.75a	61.79a
Shoots	144.6a	134.0a	133.7a	129.0a	112.8a	78.57a	121.7a	111.5a
Roots	225.7a	208.5a	180.8a	247.9a	97.95a	86.62a	110.8a	122.1a
Root: shoot (TNC)	1.16a	1.07a	1.52a	1.48a	0.77b	0.81b	0.76b	1.06a

* Mean separation by Tukey's test, P <0.05 with experimental runs analyzed separately.

[x] EoD= Extension of Daylength, NI= Night Interrupt, Far-red= far-red light supplementation, Control= Short day control.

Unbudded Trees

In the first run only, trees under NI, EoD and Far-red treatments had more total starch content in shoots than did the control, but these differences were not statistically significant. For starch content in roots, the NI, EoD and Far-red trees had less total starch content the control, but these differences were not statistically significant. Kramer and Kozlowski (1979) have documented that any perennial part of a tree can serve as a storage organ for reserves which allows them to deposit and use it at any part of the season. There were no differences among treatments for leaf starch content in both the runs. Trees under NI and EoD had numerically higher soluble sugars in shoots and leaves than the control in both runs, but statistically, these differences were not found to be significant (see Table 7). More soluble sugars in above ground parts of trees under NI and EoD treatments would explain their significantly higher vegetative growth as compared to the control trees. There were no differences amongst photoperiodic treatments for leaf soluble sugars in roots in both the runs (see Table 7).

Far-red trees had numerically less starch content in shoots, roots and leaves than the control in the second run only, but these differences were also not statistically significant. Furthermore, in unbudded trees Far-red was not able to elongate the shoots as compared to budded trees where we also saw translocation of reserves. Far-red light was not able to induce shoot elongation in unbudded trees, probably due to the topping of trees to achieve uniform height before the experiment began which removed the actively growing apex region where auxin production occurs.

Table 7: Effect of different photoperiodic treatments on total non-structural carbohydrates in leaves, shoots and roots of unbudded citrus trees in two experimental runs.

	Unbudded- 1st run				Unbudded- 2nd run			
Parameter	Treatment[x]				Treatment[x]			
	EoD	NI	Far-red	Control	EoD	NI	Far-red	Control
Starch (mg)								
Leaves	90.77a*	118.9a	110.8a	128.4a	268.3a*	245.4a	208.6a	241.7a
Shoots	385.6a	386.8a	422.8a	262.6a	447.0a	414.8a	331.1a	441.1a
Roots	895.7a	803.0a	859.2a	803.3a	447.4b	461.3b	494.5ab	605.9a
Soluble sugars (mg)								
Leaves	189.9a	184.2a	151.7a	172.4a	136.4a	158.8a	115.2a	120.4a
Shoots	258.2a	210.8a	204.6ab	135.8b	218.5ab	238.0a	170.9b	206.0ab
Roots	250.1a	246.6a	254.3a	240.4a	157.0a	165.4a	183.3a	173.6a
Root: shoot (TNC)	1.24a	1.20a	1.22a	1.61a	0.54b	0.57ab	0.79a	0.71ab

* Mean separation by Tukey's test, P <0.05 with experimental runs analyzed separately.
[x] EoD= Extension of Daylength, NI= Night Interrupt, Far-red= far-red light supplementation, Control= Short day control.

Root: Shoot Ratio

Root: shoot ratio was calculated to see if the above and below ground partitioning of total non-structural carbohydrates (starch + soluble sugars) in the trees differed among the treatments. NI and EoD had lower root: shoot ratio for total non-structural carbohydrates (starch + soluble sugars) as compared to the control in both budding types and runs (see Fig. 3). Differences were not found to be significant which was expected as all photoperiodic treatments received the same daily light integral but still, this suggested possible translocation of photoassimilates between above and below ground parts of citrus trees in response to the photoperiodic treatments. Zamski and Schaffer (1996) had documented storage of carbohydrates in roots by citrus during dormancy and translocating them in spring for early growth flush. This had also been well documented by labelling studies in Apple (Quinlan, 1969), Pecan (Lockwood, 1978) and other deciduous fruit trees (Schaffer et al., 1986). Storage of total non-structural carbohydrates in roots probably compromised the vegetative growth in the control trees as citrus is known to accumulate reserves even when the demands of developing fruits are not met (Fishier et al., 1983), which is a general survival strategy (Zamski and Schaffer, 1996). Any kind of stress which inhibits vegetative growth cessation, results in translocation of assimilates to storage organs (Bradford and Hsiao, 1982). Short day length in the Control treatment restricted the vegetative growth due to storage of non-structural carbohydrates in roots whereas in NI and EoD treatments, translocation of reserves from roots resulted in increased vegetative growth. McCamant (1988) describes a similar response, that decline in root starch at low temperatures was correlated with above ground growth which ceased when trees were de-budded or topped.

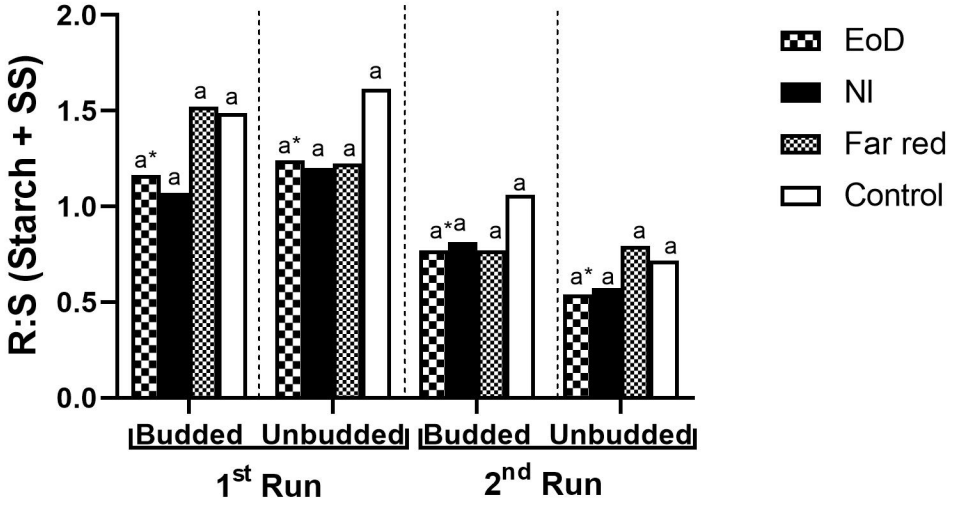

Figure 3: Root: shoot ratio of total non-structural carbohydrates (starch + soluble sugars) under different photoperiodic treatments in budded and unbudded containerized citrus trees in two experimental runs.
EoD= Extension of Daylength, NI= Night Interrupt, Far-red= far-red light supplementation, Control= Short day control.

*Mean separation within budding types and experimental runs by Tukey's test, P <0.05.

There were no significant differences for instant photosynthesis measurements among different photoperiodic treatments in both budded and unbudded trees in either runs (see Fig. 4). This was expected as all treatments received the same amount of light. Total photoassimilates were calculated by multiplying instant photosynthesis with total leaf number and leaf area for each day in each photoperiodic treatment. In budded trees, total photoassimilates were significantly higher in NI and control as compared to EoD and Far-red light treatments in the first run (see Table 8), whereas NI had significantly lesser photoassimilates than the other treatments in the second run (see Table 8). In unbudded trees total photoassimilates were significantly higher in NI, EoD and Control than for the Far-red light treatment in the first run (see Table 9) whereas, NI and EoD had significantly higher total photoassimilates than Control and Far-red light treatments in the second run (see Table 9). Consequently, photoassimilates were not the limitation that restricted vegetative growth in the control trees as they had the same amount of total

photoassimilates as did the NI trees. This can be explained by the root: shoot ratio data which showed the storage of photoassimilates in roots as compared to its investment in the above ground vegetative growth in Control trees. Similarly, in the second run where unbudded trees under Control and Far-red-light treatment had significantly less photoassimilates than did NI and EoD trees, were possibly due to the significantly higher leaf number in NI and EoD, which ultimately resulted in higher photoassimilates produced and invested in aboveground growth by the trees. Unbudded trees responded more positively to photoperiodic treatments in terms of leaf number and shoot growth than budded trees, which ultimately, may have resulted in more photoassimilates in the unbudded trees under NI and EoD treatments.

Far-red had lower photoassimilates in both budded and unbudded trees in both runs probably due to lower leaf numbers as compared to the other treatments. Control trees had the same amount of photoassimilates as the NI and EoD trees in the first run for both budding types, but these photoassimilates did not end up in vegetative growth in the control as compared to NI and EoD trees. Indeed, Control and Far-red-light trees had higher root biomass than did the NI and EoD trees for both budding types and in both runs.

Total chlorophyll content was recorded to see the effect of different photoperiods on it. In both experimental runs, there was a numerically higher total chlorophyll content in trees under NI and EoD photoperiods as compared to the Control for both budded and unbudded trees (see Tables 7 & 8). Differences were not significant in either case.

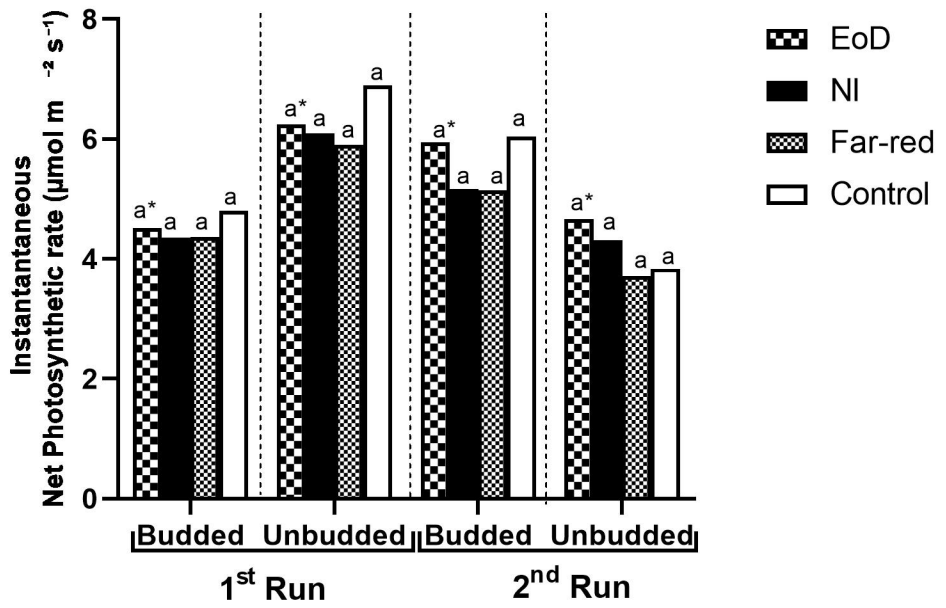

Figure 4: Instantaneous Net photosynthetic rate of budded and unbudded containerized citrus trees under different photoperiodic treatments in two experimental runs. EoD= Extension of Daylength, NI= Night Interrupt, Far-red= far-red light supplementation, Control= Short day control.

*Mean separation within budding types and experimental runs by Tukey's test, P <0.05.

Table 8: Effect of different photoperiodic treatments on physiological parameters of budded citrus trees in two experimental runs.

	Budded- 1st run				Budded- 2nd run			
Parameter	Treatment[x]				Treatment[x]			
	EoD	NI	Far-red	Control	EoD	NI	Far-red	Control
Total Photoassimilates per day (µmol)	4666b*	7080a	4713b	6965a	4166a*	2494b	2997a	3157a
Instant Photosynthesis ($\mu molm^{-2}s^{-1}$)	4.51a	4.36a	4.36a	4.80a	5.94a	5.17a	5.15a	6.03a
Total chlorophyll content (mg/l)	7.13a	5.04a	4.93a	4.93a	7.41a	7.75a	6.78a	6.04a

*Mean separation by Tukey's test, $P < 0.05$ with experimental runs analyzed separately.

[x] EoD= Extension of Daylength, NI= Night Interrupt, Far-red= far-red light supplementation, Control= Short day control.

Table 9: Effect of different photoperiodic treatments on physiological parameters of unbudded citrus trees in two experimental runs.

	Unbudded- 1st run				Unbudded- 2nd run			
Parameter	Treatment[x]				Treatment[x]			
	EoD	NI	Far-red	Control	EoD	NI	Far-red	Control
Total Photoassimilates per day (µmol)	6825a*	6274a	5130b	6870a	4060ab*	4593a	2511c	3202bc
Instant Photosynthesis ($\mu molm^{-2}s^{-1}$)	6.24a	6.09a	5.90a	6.89a	4.66a	4.31a	3.71a	3.83a
Total chlorophyll content (mg/l)	8.40a	7.69a	6.93a	6.30a	3.81a	5.10a	5.53a	3.08a

*Mean separation by Tukey's test, P <0.05 with experimental runs analyzed separately.

[x] EoD= Extension of Daylength, NI= Night Interrupt, Far-red= far-red light supplementation, Control= Short day control.

Starch Partitioning

Regardless of the photoperiodic treatment, roots were found to have higher total starch content than the shoots and leaves for both budding types and in both runs (see Fig. 5). Leaves had the least storage of starch as compared to shoots and roots for both budding types and runs (see Fig. 5). There were no differences in the distribution of soluble sugars among different plant parts in both budded and unbudded trees in either runs. This suggests the role of roots as a major storage organ for starch in containerized citrus trees in winter, in response to low temperatures and shorter photoperiods. Given that the NI and EoD trees were able to stimulate translocation of these starch reserves to above ground parts which might be the reason for increased vegetative growth as compared to control in first part of this study. It appears that the roots are storing enough carbohydrates to potentially allow manipulation in temperature and photoperiod in the greenhouse to circumvent the restricted vegetative growth of containerized citrus trees in the winters.

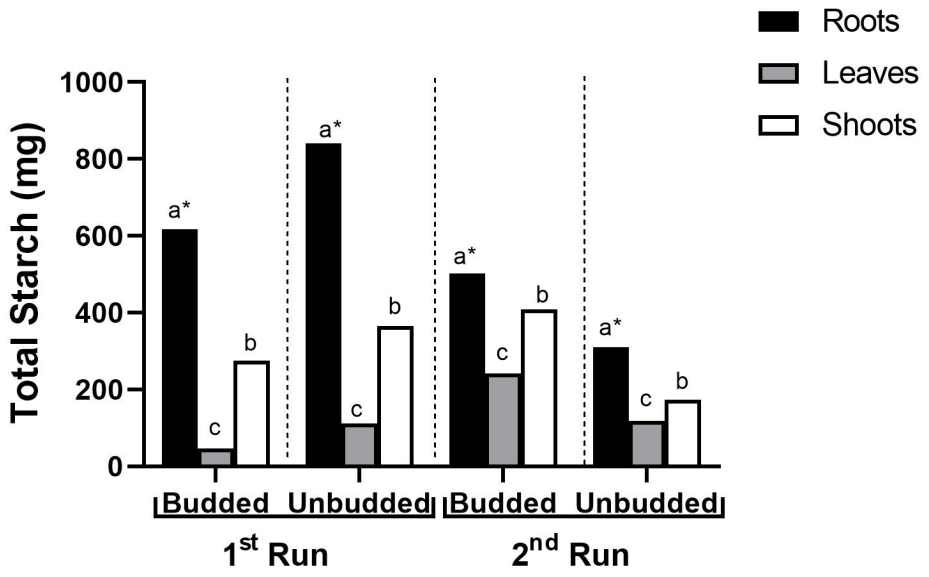

Figure 5: Total starch content partitioning between roots, leaves and shoots of containerized citrus nursery trees with all treatments combined in two experimental runs.
EoD= Extension of Daylength, NI= Night Interrupt, Far-red= far-red light supplementation, Control= Short day control.

*Mean separation within budding types and experimental runs by Tukey's test, P <0.05.

CONCLUSIONS

Night interrupt at low light intensity were able to increase vegetative growth of the budded and unbudded containerized citrus nursery trees at winter temperatures. The observation that photoassimilate concentration in the NI trees was similar to the short-day Control and root: shoot ratio of NI trees was lower as compared to the control trees, strengthened the argument for a phytochrome-mediated control of vegetative growth by low light intensity treatments. It suggests that the short-day control trees were saving the photoassimilates in their roots instead of using them for their above ground vegetative growth. This short-day effect was overcome with low intensity light supplementation in smart ways such as night interruption and extension of day length, which may have triggered the phytochrome to translocate the reserves to above ground parts for vegetative growth. Starch was found to be stored in roots in both budded and unbudded trees regardless of the treatments which means that the rootstock plays an important role in growth cessation for budded citrus trees in nurseries during the fall. Therefore, this is an important information which can be exploited to understand the dormancy induction during winters in citrus or any other woody trees.

Temperature was dropped down to 21/13 for day/night temperature in the second run but still there were significant differences in growth parameters, which is an important information for nurseries that photoperiodic effect of low intensity LED lights is still valid at lower temperatures. Therefore, LED lights can be used to create these photoperiods which can push the restricted growth of citrus trees during the winters. Vegetative growth can be in terms of bud push of newly budded seedlings, shoot growth and leaves. Trifoliate rootstock 'Carrizo' used in this experiment was also found to be responsive to photoperiodic treatments and further responsible for the cessation of scion growth during winters. NI and EoD can also be used to push the growth of rootstock seedlings which will ultimately fasten the cycle of citrus

propagation. Far-red light was not able to increase the number of nodes, but it still could be used for stem elongation in budded trees as stem elongation did not compromised the stem diameter growth which is critical for citrus nurseries. Far-red effect was seen in budded trees as they had intact actively growing apex region and therefore could be used to push growth of newly budded trees in the nursery during winters. NI treatment was more consistent than EoD treatment in terms of vegetative growth, indeed had less energy consumption, as the lights were turned on at very low intensity for only one hour. Therefore, NI may be economically applicable to push the growth of both budded and unbudded containerized citrus nursery trees during winter months. Nursery growers have several incentives to adopt such technologies, as the loss of production efficiency in winter months causes a considerable economic loss. Nurseries stop budding the trees in October and start in February due to the poor growth of citrus trees during these months. In addition to losing growth time and uniformity, resources like water, nutrients, electricity are wasted along the way. They put the shade cloth over the green house to conserve more heat in the greenhouse which further reduces the amount of light penetration into the greenhouse and leading to the growth restriction. Therefore, LED lights are potential solution to the problem of poor growth during winter months in citrus nurseries if used in smart ways such as night interruption, extension of day length and using far-red spectrum at low light intensities.

FUTURE WORK

Citrus

In this study we were able to increase the vegetative growth of budded and unbudded containerized citrus nursery trees. This experiment was performed in the growth chambers by mimicking the actual environmental conditions of citrus nursery greenhouse during fall. Results were consistent over the two experimental runs for vegetative growth. Therefore, this study can now be further tested in the commercial citrus nursery settings during the winters. LED lights can be installed in the commercial greenhouse to see the effect of night interruption, extension of

day length and far-red light supplementation on growth of budded and unbudded citrus nursery trees during fall. In this study LED lights were used to mimic the spectrum, intensity and duration of natural day light during winter months which was a disadvantage as it was difficult to mimic the changing day length and temperature over the season. Roots were found to be storing the significant starch content regardless of the treatments, which can be exploited for speeding up the vegetative growth by manipulating the temperature or photoperiod throughout the season.

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