Fruit load and canopy shading affect leaf characteristics and net gas exchange of ‘Spring’ navel orange trees

J. P. SYVERTSEN1,2 C. GOÑÍ3 and A. OTERO3

Summary Five-year-old ‘Spring’ navel (Citrus sinensis (L.) Osbeck) orange trees were completely defruited, 50% defruited or left fully laden to study effects of fruit load on concentrations of nitrogen (N) and carbohydrate, net assimilation of CO₂ (Aₗ) and stomatal conductance (gₛ) of mature leaves on clear winter days just before fruit harvest. Leaves on defruited trees were larger, had higher starch concentrations and greater leaf dry mass per area (LDMₐ) than leaves on non-fruited leaves on trees with fruit. Leaves immediately adjacent to fruit were smaller, had lower leaf nitrogen and carbohydrate concentrations, lower LDMₐ, and lower Aₗ than leaves on non-fruited branches of the same trees. Removing half the crop increased individual fruit mass, but reduced fruit color development. Half the trees were shaded with 50% shade cloth for 4 months before harvest to determine the effects of lower leaf temperature (Tₑ) and leaf-to-air vapor pressure difference on leaf responses. On relatively warm days when sunlit Tₑ > 25 °C, shade increased Aₗ and gₛ, but had no effect on the ratio of internal to ambient CO₂ (Cᵢ/Cₑ) concentration in leaves, implying that high mesophyll temperatures in sunlit leaves were more important than gₛ in limiting Aₛ. Sunlit leaves were more photohindered than shaded leaves on cooler days when Tₑ < 25 °C. Shade decreased total soluble sugar concentrations in leaves, but had no effect on leaf starch concentrations. Shading had no effects on canopy volume, yield or fruit size, but shaded fruit developed better external color than sun-exposed fruit. Overall, the presence of a normal fruit crop resulted in lower foliar carbohydrate concentrations and higher Aₛ compared with defruited trees, except on warm days when Aₛ was reduced by high leaf temperatures.

Keywords: carbohydrates, chlorophyll fluorescence, leaf CO₂ assimilation, LVPD, photoinhibition, soluble sugars, starch, stomatal conductance.

Introduction Carbohydrate source–sink relationships between photosynthetic source leaves and vegetative/reproductive growth sinks determine tree growth and fruit yield (Flore and Lakso 1989, Goldschmidt 1999). At least during maximum seasonal demands from fruit sinks, rates of net CO₂ assimilation (Aₗ) can be enhanced in peach leaves (DeJong 1986), in apple leaves (Palmer et al. 1997) and in whole-tree canopies (Giuliani et al. 1997) by high fruit loads. Both Aₛ and photochemical yield from chlorophyll a fluorescence measurements increase with crop load and the requirement for photosynthates in apple trees (Wunsche et al. 2000). Seasonal source–sink relationships are more complex in evergreen citrus trees (Goldschmidt and Koch 1996), because fruit can exist for more than a year and leaves can last for more than 2 years. Competition between young citrus leaves and flowers for limited supplies of photosynthates and mineral nutrients (Guardiola et al. 1984) determine growth, flowering and yield (Sanz et al. 1987). Potted citrus trees with fruit had less vegetative growth (Lenz and Doring 1975) and lower foliar starch concentrations (Lenz and Kuntzel 1974) than deflowered trees. Leaves on fruiting trees had higher Aₛ than leaves on non-fruited trees, especially at low root temperatures of 12 °C (Lenz 1978). In large field-grown citrus trees, however, where there are many alternative carbohydrate sinks, there was no measurable effect of fruit load on photosynthesis (Monselise et al. 1986). In branches of phloem-girdled and defruited trees, carbohydrates and N accumulate (Goldschmidt and Golomb 1982, Sanz et al. 1987), resulting in increased flowering and yield especially in citrus trees that are alternate bearing (Agusti et al. 1992). An accumulation of carbohydrates can reduce Aₛ (Iglesias et al. 2002) by end-product inhibition (Goldschmidt and Koch 1996), but partial defoliation can decrease leaf starch (Iglesias et al. 2002) and increase Aₛ (Syvertsen 1994). On the other hand, competition between fruit and adjacent leaves can lead to lower concentrations of N and other elements (Sanz et al. 1987) in leaves near fruit than in leaves on non-fruiting branches (Smith 1966).

Photosynthesis in sun-acclimated citrus leaves is light-saturated at about one third of full sunlight (Sinclair and Allen 1982, Syvertsen 1984). In warm high-light environments, excess radiation can raise leaf temperature (Tₑ) as much as 9 °C above air temperature (Syvertsen and Albrigo 1980). High leaf
temperatures increase the leaf-to-air vapor pressure difference (LVPD), resulting in lower stomatal conductances \( (g_s; \text{Syvertsen and Salyani 1991}) \) and lower \( A_c \) (Jifon and Syvertsen 2001). Khairi and Hall (1976) attributed high-temperature-induced reduction of \( A_c \) to non-stomatal factors in the mesophyll, whereas LVPD-induced reductions in \( A_c \) were associated with decreased \( g_s \). Reducing solar radiation by about 50% can reduce \( A_c \) in cool low-light climates (Mataa and Tominaga 1998). However, short-term shading with 50% shade cloth in high-light environments can reduce \( T_l \) and LVPD, thereby increasing \( g_s \) and \( A_c \), because there is still adequate light to saturate photosynthetic processes (Jifon and Syvertsen 2001). In sun-exposed grapefruit and orange leaves, reduced photochemical efficiency and supra-optimal leaf mesophyll temperatures were more important than stomatal factors in limiting \( A_c \) (Jifon and Syvertsen 2003). Heat-stress-induced limitations on photoassimilate utilization can also lead to carbohydrate accumulation in leaves (Azcáón-Bieto 1983) and lower \( A_c \), by damaging grana in the chloroplasts and other membrane structures (Nafziger and Koller 1976). Imposing moderate shade in high-light climates can also have practical implications, because properly timed shading during the growing season can increase fruit yield and concentrations of sugars in juice (Jifon and Syvertsen 2001).

Because both crop load and shading can affect tree growth and physiological responses, we determined their combined effects on leaf N, leaf photosynthetic characteristics and carbohydrate concentrations in navel orange trees just before harvest. We also determined shade effects on fruit yield and crop load effects on fruit quality. We tested three hypotheses: (1) the crop requirement for carbohydrates will decrease leaf carbohydrate accumulation and enhance \( A_c \); (2) low N concentrations in leaves adjacent to fruit will limit \( A_c \) and decrease carbohydrate concentrations below those of leaves on non-fruiting branches; and (3) decreased \( T_l \) and LVPD under shade cloth will increase midday \( g_s \) and \( A_c \) of sun-acclimated leaves.

**Materials and methods**

**Experimental trees and treatments**

Experiments were carried out at the INIA Salto Grande Experiment Station located in northwest Uruguay (32° S, 58° W) with uniform 5-year-old ‘Spring’ navel orange (Citrus sinensis (L.) Osbeck) trees on ‘Rubidoux’ (Poncirus trifoliata (L.) Raf.) rootstock. Trees were planted at 7 × 3.5 m spacing on a sandy clay soil, irrigated and fertilized with N at about 100 kg ha\(^{-1}\) year\(^{-1}\).

Trees reached full bloom around September 27, 2001. After the springtime fruit drop period when fruit were about 5 cm in diameter (January 3, 2001), three contrasting fruit load treatments were established on 18 uniform trees. All fruit was removed from six randomly selected trees (no crop), 50% of the average fruit load was removed from throughout the canopy of six trees (half crop) and six trees were left with a full fruit load (full crop). Two months later on March 2, a shade treatment was established by erecting a single horizontal layer of black plastic 50% shade cloth (4 m wide) just above the canopies of nine trees, three trees per crop load class (Shade). The remaining nine trees remained in full sun (Open) as controls. On April 1, we replaced the black shade cloth with aluminized polypropylene 50% shade cloth (Aluminet-50; Polysack Plastic Industries, Nir Yitzhak, Israel) of known transmission characteristics (Cohen et al. 1997). We wanted to duplicate our previous experiments with this shade cloth over citrus trees (Jifon and Syvertsen 2003). The shade treatment remained in place for about 4 months until fruit harvest at the end of June 2001. By mid-May, there was sufficient rainfall to eliminate the need for irrigation until well after harvest.

**Leaf gas exchange and chlorophyll a fluorescence**

Photosynthetic photon flux (PPF), air temperature and relative humidity (RH) above and below the shade screens were recorded continuously with small data loggers (HOBO, Onset, Pocasset, MA). In May and June, just before harvest, \( A_c, g_s \), and transpiration rate \( (E) \) of single leaves were measured with a portable photosynthesis system (CIRAS-1, PP Systems, Haverhill, MA). All gas exchange measurements were made at ambient \( CO_2 \) partial pressure \( (C_a) \) on selected cloudless days between 1100 and 1530 h when photosynthetically active radiation in the open was between 1180 and 1570 \( \mu \text{mol m}^{-2} \text{s}^{-1} \). Gas exchange measurements were made on at least three leaves on each of three trees in the high and low crop load treatments in both the sun and shade treatments. No gas exchange measurements were made on trees with 50% fruit load. We also compared gas exchange characteristics of leaves immediately adjacent to fruit with gas exchange of leaves on non-fruiting branches on unshaded trees with a full crop. All measurements were made on mature 7- to 8-month-old spring flush leaves in fully exposed canopy positions. For the crop load \( \times \) shade measurements, leaves adjacent to fruit were avoided. All leaves were oriented perpendicular to solar radiation during measurements. Leaf temperature, which was determined during the cuvette measurements, was usually 1 to 5 °C above ambient air temperature (Jifon and Syvertsen 2003). When gas exchange measurements were made under sufficiently similar environmental conditions, data from replicate leaves were pooled such that treatment means were from nine to 18 leaves. Leaf-to-air vapor pressure difference was calculated from cuvette air temperature, relative humidity and cuvette \( T_l \), assuming that leaf water was saturated at \( T_l \). We calculated \( C_a/C_t \) from leaf internal \( CO_2 \) partial pressure \( (C_t) \). Photosynthetic water-use efficiency (WUE) was calculated as \( A_c/E \).

Chlorophyll a fluorescence characteristics were measured on the same days as leaf gas exchange measurements in similar leaves. Sampled leaves were dark-acclimated for at least 0.5 h with leaf clips (FL-DC, Opti-Sciences, Tyngsboro, MA) before making fluorescence measurements with a pulse amplitude modulation fluorometer (OS5-FL, Opti-Sciences). The chlorophyll fluorescence parameter \( F_v/F_m \), which represents the maximum efficiency of photosystem II (PSII) photochemistry, was determined following the procedures of van Kooten and Snell (1990) and Maxwell and Johnson (2000),
where $F_m$ is maximal fluorescence intensity with all PSII reaction centers closed, $F_o$ is minimal (ground) fluorescence intensity when all PSII reaction centers are open, and $F_v$ is variable fluorescence ($F_v = F_m - F_o$).

**Leaf size, N and carbohydrates**

On June 24, following the gas exchange and chlorophyll fluorescence measurements, 10 leaves were removed from each tree in each treatment and transported to the laboratory in plastic bags. Leaf areas were measured (Li-Cor 3100, Li-Cor, Lincoln, NE) and the leaf samples were then dried in an oven at 100 °C for 1 h to halt respiration. Drying was continued at 60 °C for at least 72 h. Dried leaves were weighed, ground to a powder and analyzed for total leaf N by Kjeldahl analysis. Total soluble carbohydrates in leaf tissue were analyzed following the methods of Mehuichi et al. (1995). Soluble sugars (fructose + sucrose + glucose) and starch were extracted in 95% ethanol. The soluble fraction was treated with sulfuric acid, whereas the solid fraction was digested with amyloglucosidase after resuspension in aqueous buffer. Both samples were analyzed for total hexoses by HPLC (1100 Series, Agilent Technologies, Palo Alto, CA). Total nonstructural carbohydrates (TNC) were expressed as the sum of soluble sugars and starch expressed in units of glucose on a leaf area basis.

In a separate experiment, seasonal changes in N concentrations in leaves immediately adjacent to fruit (fruit leaves) relative to leaves on non-fruiting branches were evaluated in two nearby, well-fertilized navel orange orchards throughout the previous 2 years. Both classes of leaves were sampled every 2–3 weeks over two seasons from 50 to 225 days after full bloom (in September) until fruit was harvested in June. Only non-fruit leaves were sampled after harvest until 330 days after full bloom. Four leaves in each class were sampled from 50 trees in both orchards and averaged over the two seasons. Total leaf N was determined by Kjeldahl analysis.

**Tree growth and yield**

Canopy volumes were determined by dimensional analysis of canopy width and height (Albrigo et al. 1975) in July 2000 and 2001. Fruit was harvested on June 24, 2001, and tree yield was expressed as total fruit mass per tree. Fruit rind color was measured with a colorimeter on 120 fruits per treatment and expressed as a relative a/b color index ratio. Juice quality was measured by standard laboratory methods for total Brix (refractometer) and acid by titration with 0.1 M NaOH (Wardowski et al. 1995). Shade material was removed at harvest and previous treatment effects on flowering intensity were evaluated in the same trees in September 2002 during maximum bloom. Flowers were counted on randomly selected branches throughout the entire canopy of each experimental tree until 800 to 1000 nodes per tree were evaluated. Return flowering intensity the following year was expressed as number of flowers (100 nodes)$^{-1}$ (Agusti et al. 1992).

**Experimental design and data analysis**

Data were subjected to analysis of variance either as a 3×2 (fruit load × shade) factorial design or as a completely random design comparing fruiting versus non-fruiting leaves. Means were separated by Duncan’s multiple range test (DMRT) at $P < 0.05$. Analyses were carried out with SAS software (SAS Institute, Cary, NC).

**Results**

**Leaf size, N and carbohydrates**

Mean leaf area was significantly increased by the fruit removal treatment, but the shade treatment, which was applied later in the season, did not affect leaf size or leaf dry mass per area (LDM$_a$; Table 1). Leaf N concentration, expressed on a % dry mass basis (N$_m$), decreased after fruit removal, whereas LDM$_a$ increased such that crop removal had no effect on leaf N concentration expressed on a leaf area basis (N$_a$). Shaded leaves had higher leaf N$_m$ than unshaded leaves, but shading had no effect on leaf N$_a$. Leaves adjacent to fruit were smaller, had less LDM$_a$ and lower N$_m$ than leaves on non-fruiting branches. There was no effect of adjacent fruit on leaf N$_m$ relative to leaf N$_m$ of non-fruiting branches. To determine fertilizer require-

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Leaf area (cm$^2$)</th>
<th>N$_m$(%)</th>
<th>LDM$_a$(g m$^{-2}$)</th>
<th>N$_a$(mmol m$^{-2}$)</th>
<th>Soluble sugar (mg g$_{DM}$)</th>
<th>Starch (mg g$_{DM}$)</th>
<th>TNC (g m$^{-2}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full crop (6)</td>
<td>20.5 b$^{1,2}$</td>
<td>2.35 a</td>
<td>156 b</td>
<td>261 ns</td>
<td>51.9 ns</td>
<td>164 b</td>
<td>24.6 b</td>
</tr>
<tr>
<td>Half crop (6)</td>
<td>21.0 b</td>
<td>2.25 ab</td>
<td>157 b</td>
<td>253</td>
<td>54.8</td>
<td>179 ab</td>
<td>26.0 b</td>
</tr>
<tr>
<td>No crop (6)</td>
<td>33.0 a</td>
<td>2.15 b</td>
<td>171 a</td>
<td>261</td>
<td>52.5</td>
<td>217 a</td>
<td>35.6 a</td>
</tr>
<tr>
<td>Open (9)</td>
<td>23.6 ns</td>
<td>2.18 b</td>
<td>162 ns</td>
<td>253 ns</td>
<td>57.3 b</td>
<td>186 ns</td>
<td>28.3 ns</td>
</tr>
<tr>
<td>Shade (9)</td>
<td>22.8</td>
<td>2.35 a</td>
<td>157</td>
<td>263</td>
<td>49.1 a</td>
<td>173</td>
<td>25.8</td>
</tr>
<tr>
<td>Fruit leaves$^2$ (3)</td>
<td>13.3 b</td>
<td>2.27 ns</td>
<td>154 b</td>
<td>248 b</td>
<td>48.0 b</td>
<td>168 ns</td>
<td>24.3 b</td>
</tr>
<tr>
<td>Non-fruit leaves (3)</td>
<td>29.8 a</td>
<td>2.27</td>
<td>164 a</td>
<td>264 a</td>
<td>56.7 a</td>
<td>188</td>
<td>29.0 a</td>
</tr>
</tbody>
</table>

1 Column means within each group followed by different letters differed significantly at $P < 0.05$; ns = not significantly different.

2 There were no significant interaction effects between treatments.
ments, leaves are typically sampled 120–150 days after bloom (Tucker et al. 1995). We note that this was when leaves on non-fruiting shoots had much higher $N_m$ than leaves adjacent to fruit (Figure 1). During the period of our study, about 190 to 225 days after full bloom, leaf $N_m$ on non-fruiting shoots decreased to values comparable with those of leaves adjacent to fruit.

There was no effect of crop load on foliar soluble sugar concentrations, but fruit removal increased leaf TNC and starch concentrations regardless of unit of expression (Table 1). Foliar soluble sugar concentrations were reduced by the shade treatment compared with the open treatment, but shade did not significantly decrease leaf starch or TNC concentration. Leaves on non-fruiting branches had higher concentrations of sugars, starch and TNC than leaves adjacent to fruit.

**Leaf gas exchange and chlorophyll a fluorescence**

On two similar, relatively warm, measurement days, May 29 and June 11, maximum midday air temperatures varied only from 25 to 25.5 °C, and RH varied between 63 and 68% regardless of shade treatment. Shade treatment decreased mean PPF from 1450 to 507 $\mu$mol m$^{-2}$ s$^{-1}$. Shade screens reduced mean midday $T_l$ by 2.4 °C and reduced mean LVPD by 0.5 kPa compared with the open treatment (Table 2). Stomatal conductance and $A_g$ increased in response to shading, but there was no effect of shade on $E$ or $C_i/C_a$. Shading increased leaf WUE, but had no effect on chlorophyll a fluorescence parameters.

Fruit removal resulted in a more than 40% reduction in $A_c$ (Table 2) that was paralleled by decreases in $g_s$ and $C_i/C_a$. Leaf transpiration also declined in defruited trees such that there was no effect of fruit removal on leaf WUE. The decrease in transpiration resulted in an increase in LVPD within the measurement cuvette and a more than 1 °C increase in $T_l$ that was apparently attributable to fruit removal. Although leaves on defruited trees had an increase in midday $F_m$, there was no effect of crop load on $F_v/F_m$.

On June 6, a cool clear day, maximum midday air temperature was about 19 °C and RH was 60–61%. Mean maximum PPF was 1570 and 555 $\mu$mol m$^{-2}$ s$^{-1}$ in the open and under the shade cloth, respectively. Midday sunlit leaf temperatures were less than 25 °C (Table 3). The shade cloth decreased $T_l$ and LVPD but had no effect on the relatively low midday $g_s$ and $A_g$ values or on $C_i/C_a$. Shade effects on $E$ were also not significant, but shade increased WUE. Although all $F_v/F_m$ values were relatively high, shade increased $F_v/F_m$ above that of full-crop trees in the open. Fruit removal reduced $A_g$, $g_s$ and $E$ about 45% below that of leaves on trees with a full crop load, but fruit removal had no effect on $C_i/C_a$ or WUE. Chlorophyll a fluorescence parameters of leaves were unaffected by fruit removal.

On June 13, a warm bright but hazy day, we compared gas exchange characteristics of leaves adjacent to fruit with leaves on non-fruiting branches on fully cropped trees in the open. Midday air temperature was 26 °C, RH was 72% and mean PPF was 1185 $\mu$mol m$^{-2}$ s$^{-1}$. Leaves on non-fruiting branches had higher $A_g$, $g_s$ and $E$ than leaves adjacent to fruit, but there was no effect of adjacent fruit on $C_i/C_a$ or leaf WUE (Table 4). Leaf temperatures in the cuvette were unaffected by the relative positions of leaves to fruit, but higher $E$ lowered LVPD of leaves on non-fruitting branches. Leaves on non-fruiting branches had lower $F_v$ than leaves adjacent to fruit, but there was no effect of adjacent fruit on leaf $F_v/F_m$.

**Tree growth, yield and fruit quality**

There were no significant effects of crop load or shade on tree canopy volume or growth (Table 5). Neither fruit yield per tree nor fruit size was affected by shade treatment, but fruits from the 50% crop treatment were larger than fruits from trees with a full crop. Fruit from full-crop trees had more orange coloration than fruit from half-crop trees and shaded fruit had a more orange coloration than fruit from trees in the open. Fruit from the full-crop treatment also had significantly higher ($P < 0.05$) Brix than fruit from the half-crop treatment (10.1 versus 9.6° Brix), but there were no other significant effects of crop load or shade treatment on peel thickness, Brix, acid or Brix/acid ratio at harvest (data not shown). Fruit removal increased flowering 2.6-fold in the following year, but the 4-month shade treatment during the previous season did not affect return bloom.

**Discussion**

Based on foliar carbohydrate concentrations just before fruit harvest, carbon was limiting in fruiting trees because there was competition between leaves and fruit. Early removal of fruit resulted in increases in leaf size, LDM, and TNC (Table 1).
Because starch is the major storage carbohydrate in citrus leaves (Goldschmidt and Golomb 1982), starch may have contributed to the changes in leaf mass (Bondada and Syvertsen 2003). Wintertime leaf $N_a$ was unaffected by crop load, and leaf $N$ concentrations were apparently adequate to support high $A_c$, except in leaves immediately adjacent to fruit. Based

---

Table 2. Effects of crop load and shade treatments on midday leaf temperature ($T_l$), net gas exchange and chlorophyll fluorescence characteristics of well-irrigated trees on relatively warm, clear days (May 29 and June 11, 2001). Values are means of 18 leaves per treatment. Abbreviations: LVPD = leaf-to-air vapor pressure difference; $E$ = transpiration rate; $g_s$ = stomatal conductance; $A_c$ = CO$_2$ assimilation; $C_i/C_a$ = total internal CO$_2$ partial pressure/ambient pCO$_2$; WUE = water-use efficiency; $F_o$ = minimal (ground) fluorescence intensity; and $F_v/F_m$ = maximum efficiency of photosystem II.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cover</th>
<th>$T_l$ ($°C$)</th>
<th>LVPD (kPa)</th>
<th>$E$ (mmol m$^{-2}$)</th>
<th>$g_s$ (mmol m$^{-2}$)</th>
<th>$A_c$ (µmol m$^{-2}$ s$^{-1}$)</th>
<th>$C_i/C_a$</th>
<th>WUE ($A_i/E$)</th>
<th>$F_o$</th>
<th>$F_v/F_m$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full crop</td>
<td>Open</td>
<td>27.2</td>
<td>2.1</td>
<td>2.84</td>
<td>147</td>
<td>9.77</td>
<td>0.63</td>
<td>3.44</td>
<td>191</td>
<td>0.83</td>
</tr>
<tr>
<td>Full crop</td>
<td>Shade</td>
<td>24.8</td>
<td>1.6</td>
<td>2.85</td>
<td>196</td>
<td>11.15</td>
<td>0.68</td>
<td>3.93</td>
<td>186</td>
<td>0.84</td>
</tr>
<tr>
<td>No crop</td>
<td>Open</td>
<td>28.5</td>
<td>2.6</td>
<td>1.51</td>
<td>64</td>
<td>4.77</td>
<td>0.61</td>
<td>3.06</td>
<td>239</td>
<td>0.8</td>
</tr>
<tr>
<td>No crop</td>
<td>Shade</td>
<td>26</td>
<td>2</td>
<td>1.61</td>
<td>87</td>
<td>7.09</td>
<td>0.57</td>
<td>4.32</td>
<td>213</td>
<td>0.83</td>
</tr>
</tbody>
</table>

Treatment means

| Full crop Open | 26.1 b$^1$ | 1.8 b       | 2.84 a       | 168 a               | 10.37 a                  | 0.65 a                         | 3.65 ns    | 189 b         | 0.84 ns |     |
| Full crop Shade| 27.3 a     | 2.3 a       | 1.56 b       | 75 b                | 5.86 b                   | 0.59 b                         | 3.65       | 226 a         | 0.82    |     |
| No crop Open   | 27.8 a     | 2.3 a       | 2.19 ns      | 107 b               | 7.34 b                   | 0.62 ns                         | 3.25 b     | 215 ns        | 0.82 ns |     |
| No crop Shade  | 25.4 b     | 1.8 b       | 2.21         | 140 a               | 9.05 a                   | 0.62                           | 4.13 b     | 199           | 0.84    |     |

Table 3. Effects of crop load and shade treatments on midday leaf temperature ($T_l$), net gas exchange and chlorophyll fluorescence characteristics of well-irrigated trees on a relatively cool, clear day (June 6, 2001). Values are means of nine leaves per treatment. See abbreviations in Table 2.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cover</th>
<th>$T_l$ ($°C$)</th>
<th>LVPD (kPa)</th>
<th>$E$ (mmol m$^{-2}$)</th>
<th>$g_s$ (mmol m$^{-2}$)</th>
<th>$A_c$ (µmol m$^{-2}$ s$^{-1}$)</th>
<th>$C_i/C_a$</th>
<th>WUE ($A_i/E$)</th>
<th>$F_o$</th>
<th>$F_v/F_m$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full crop</td>
<td>Open</td>
<td>24.4</td>
<td>2.1</td>
<td>1.83</td>
<td>94</td>
<td>6.78</td>
<td>0.6</td>
<td>3.7</td>
<td>212</td>
<td>0.79 b</td>
</tr>
<tr>
<td>Full crop</td>
<td>Shade</td>
<td>22</td>
<td>1.7</td>
<td>1.72</td>
<td>105</td>
<td>7.43</td>
<td>0.61</td>
<td>4.41</td>
<td>183</td>
<td>0.85 a</td>
</tr>
<tr>
<td>No crop</td>
<td>Open</td>
<td>24.2</td>
<td>2.2</td>
<td>0.99</td>
<td>49</td>
<td>3.71</td>
<td>0.63</td>
<td>3.45</td>
<td>222</td>
<td>0.84</td>
</tr>
<tr>
<td>No crop</td>
<td>Shade</td>
<td>21.3</td>
<td>1.8</td>
<td>0.99</td>
<td>58</td>
<td>3.92</td>
<td>0.64</td>
<td>3.92</td>
<td>208</td>
<td>0.83</td>
</tr>
</tbody>
</table>

Treatment means

| Full crop Open | 23.2 ns$^1$ | 1.9 ns      | 1.77 a       | 100 a               | 7.11 a                   | 0.61 ns                         | 4.06 ns    | 198 ns         | 0.82 ns |     |
| Full crop Shade| 22.8       | 2           | 0.99 b       | 54 b                | 3.82 b                   | 0.64                           | 3.69       | 215            | 0.84    |     |
| No crop Open   | 24.3 a     | 2.1 a       | 1.41 ns      | 72 ns               | 5.25 ns                  | 0.62 ns                         | 3.58 b     | 217 ns         | 0.82 b  |     |
| No crop Shade  | 21.7 b     | 1.8 b       | 1.35         | 82                   | 5.68                     | 0.63                           | 4.16 a     | 196           | 0.84 a  |     |

Crop × shade$^2$ ns ns ns ns ns ns ns ns ns ns 0.001

Table 4. Effects of being adjacent to fruit (fruit leaves) versus non-fruit leaves on midday leaf temperature ($T_l$), net gas exchange and chlorophyll fluorescence characteristics of leaves on unshaded, fully cropped trees on a relatively warm, clear day (June 13, 2001). Values are means of 15 leaves per treatment. See abbreviations in Table 2.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$T_l$ ($°C$)</th>
<th>LVPD (kPa)</th>
<th>$E$ (mmol m$^{-2}$)</th>
<th>$g_s$ (mmol m$^{-2}$)</th>
<th>$A_c$ (µmol m$^{-2}$ s$^{-1}$)</th>
<th>$C_i/C_a$</th>
<th>WUE ($A_i/E$)</th>
<th>$F_o$</th>
<th>$F_v/F_m$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruit leaves</td>
<td>28.0 ns$^1$</td>
<td>2.3 a</td>
<td>1.71 b</td>
<td>82 b</td>
<td>4.34 b</td>
<td>0.69 ns</td>
<td>2.60 ns</td>
<td>146 b</td>
<td>0.83 ns</td>
</tr>
<tr>
<td>Non-fruit leaves</td>
<td>27.3</td>
<td>2.0 b</td>
<td>2.47 a</td>
<td>131 a</td>
<td>8.07 a</td>
<td>0.65</td>
<td>3.35</td>
<td>158 a</td>
<td>0.83</td>
</tr>
</tbody>
</table>

$^1$ Paired column means followed by different letters differed significantly at $P < 0.05$; ns = not significantly different.
Table 5. Effects of crop load and shade treatments on tree canopy volume (CV), annual CV growth (in July), yield characteristics, fruit color and return bloom (in September 2002). Values are means from six or nine trees (n) per treatment except fruit color index values, which are means of 120 fruits per treatment.

<table>
<thead>
<tr>
<th>Treatment means</th>
<th>CV (m$^3$)</th>
<th>CV Growth (2001–2000) (m$^3$)</th>
<th>Yield tree$^{-1}$ (kg)</th>
<th>Fruits tree$^{-1}$</th>
<th>Fruit mass (g)</th>
<th>Fruit color index (a/b)</th>
<th>Flowers (100 nodes)$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full crop (6)</td>
<td>6.9 ns$^1$</td>
<td>3.2 ns</td>
<td>42.5 a</td>
<td>187 a</td>
<td>232 b</td>
<td>0.34 a</td>
<td>61 c</td>
</tr>
<tr>
<td>Half crop (6)</td>
<td>7.2</td>
<td>3.6</td>
<td>24.9 b</td>
<td>96 b</td>
<td>262 a</td>
<td>0.30 b</td>
<td>112 b</td>
</tr>
<tr>
<td>No crop (6)</td>
<td>7.1</td>
<td>3.9</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>163 a</td>
</tr>
<tr>
<td>Open$^2$ (9)</td>
<td>7.4 ns</td>
<td>3.9 ns</td>
<td>33.7 ns</td>
<td>136 ns</td>
<td>256 ns</td>
<td>0.29 b</td>
<td>101 ns</td>
</tr>
<tr>
<td>Shade (9)</td>
<td>6.7</td>
<td>3.3</td>
<td>33.8</td>
<td>147</td>
<td>238</td>
<td>0.35 a</td>
<td>124</td>
</tr>
</tbody>
</table>

$^1$ Column means within each group followed by different letters differed significantly at P < 0.05; ns = not significantly different.

$^2$ There was no significant interaction effects between crop load × shade treatments.

on calculations from Turrell (1961), there were about 30,000 leaves on these 5-year-old orange trees and a mean of 187 fruits per tree (Table 5). There were typically two leaves immediately adjacent to each fruit, so non-fruiting leaves outnumbered the estimated 374 fruiting leaves by about 80:1. Thus, leaves adjacent to fruit represented a relatively small percentage of the total leaves in a canopy. On relatively warm measurement days, fruit sink demand for carbon apparently maintained higher $A_c$ than leaves on non-fruiting trees (Table 2). Seasonal changes in citrus leaf carbohydrates have been taken to support the idea that fruit sink strength stimulates leaf photosynthesis (Lenz 1978, Borras et al. 1984), and direct effects of early fruit removal on reduced $A_c$ in the field have recently been reported (Iglesias et al. 2002). The parallel increase in $C/C_s$ with $A_c$, however, implied that $g_s$ was dominant over non-stomatal factors in the mesophyll in maintaining higher $A_c$ in response to fruit sink strength stimulation (Farquhar and Sharkey 1982). The larger transpirational water loss from leaves on fruiting trees lowered the LVPD sufficiently that the resulting higher $g_s$ became a more important determinant of $A_c$ than the carbohydrate sink demand. In contrast, on a relatively cool day when overall gas exchange rates were lower, leaves on fruiting trees again had higher $A_c$ and $g_s$ than leaves on non-fruiting trees, but in this case, there was no effect of crop load on $C/C_s$ (Table 3). Thus, internal mesophyll factors such as sink strength were dominant over stomatal limitations (Jifon and Syvertsen 2003) in determining the crop load maintenance of high $A_c$ on cool days.

Crop load had no effect on leaf concentrations of soluble sugars, but leaves on non-fruiting trees had higher concentrations of starch than leaves on fruiting trees. The lower gas exchange rates in leaves on non-fruiting trees were related to their higher starch and TNC concentrations compared with leaves on normally cropping trees. This implies that, in the absence of fruit sink demand for carbohydrates, the accumulation of starch in leaves may have inhibited photosynthesis (Lenz 1978, Iglesias et al. 2002). On relatively warm days, leaves on non-fruiting trees also had higher $F_o$ than leaves on fruiting trees, which may implicate changes in chloroplast membrane function, perhaps related to the higher concentrations of carbohydrates in chloroplasts (Bondada and Syvertsen 2003). However, there were only small effects of crop load on photoinhibition as measured by $F_v/F_m$ at midday, and all $F_v/F_m$ values were relatively high (> 0.79). Such small changes in $F_v/F_m$ were probably transient (Ort 2001) and of little physiological consequence.

Although there were relatively few leaves adjacent to fruit compared with the total number of leaves present in the canopy, there was competition between leaves and adjacent fruit. Thus, leaves adjacent to fruit were smaller with lower $N_e$ (Sanz et al. 1987), lower soluble sugars, starch and LDMa, compared with leaves on non-fruiting branches (Table 1). The lower $N_e$ could have been responsible for the lower $A_c$ and carbohydrate concentrations in leaves adjacent to fruit compared with leaves on non-fruiting branches. We did not evaluate the effects of distance from fruit on photosynthesis of leaves along the same branch, but the effects of fruit on adjacent leaves were opposite to the $A_c$ responses to crop load in most leaves in the canopy. Such contradictions may have contributed to the confusion in the literature about the influences of fruit sink demand on citrus photosynthesis in the field (Goldschmidt and Koch 1996).

There are several possibilities that could explain why fruit from the full-crop treatment had better exterior color than fruit from the half-crop treatment (Table 5). High crop load can reduce leaf $N$ concentrations and high $N$ in leaves can delay color development (Reese and Koo 1975). It is possible that the half-crop treatment had higher $N$ concentration in the peel and, hence, poorer fruit color development compared with fruit in the full-crop treatment. This possibility is unlikely, however, because there was no effect of crop load on leaf $N_e$, and leaves in the half-crop treatment generally had lower leaf $N$ concentration than leaves in the full-crop treatment (Table 1). Second, there could have been fruit-load effects on shading within canopies. The half-crop trees grew slightly, but not significantly, more than the full-crop trees, which could have increased shade within the canopy of half-crop trees. However, fruit from the shaded treatment had better external...
color development than fruit in the open treatment. Third, fruit from full-crop trees may have accumulated sugars earlier and developed better fruit color than the half-crop trees. Although we did not measure $A_i$ in half-crop trees, fruit from full-crop trees had higher Brix than fruit from half-crop trees. Thus, the full-crop load was a stronger carbohydrate sink and may have maintained a higher $A_i$ than the half-crop load. This idea was supported by the slightly higher starch concentrations in leaves on the half-crop trees than in leaves on the full-crop trees. The increase in return bloom was probably related to the higher carbohydrate reserves in the previously defruited trees than in full-crop trees, because such responses mimic the alternating annual carbohydrate concentrations and yields that occur in alternate-bearing citrus trees (Goldschmidt and Golomb 1982).

On relatively warm days, the increases in $g_s$ and $A_i$ that were attributable to the shade treatment were not reflected in changes in $E$ or $C_i/C_a$. Although shade increased $g_s$, it also decreased LVPD (the driving force for transpiration) thereby compensating for the higher $g_s$ so that $E$ was unchanged. The shade-induced increase in $A_i$ without a change in $C_i/C_a$ demonstrates the relative importance of non-stomatal factors such as high temperature in the mesophyll limiting photosynthesis (Khairi and Hall 1976) or reduced photochemical efficiency in sun-exposed leaves (Jifon and Syvertsen 2003). When overall leaf temperatures were lower on relatively cool days, shaded trees had lower $T_l$ and LVPD than sunlit leaves, but there was no shade effect on the already low leaf gas exchange rates even though sunlit leaves had slightly lower $F_i/F_m$. The shade treatment decreased the short-term accumulation of soluble sugars (Table 1), but shade had no effect on accumulation of starch and TNC. Thus, effects of shade on increased midday net gas exchange and the accumulation of sugars did not translate into more starch accumulation or tree growth.

The horizontal shade screens increased $A_i$ only on relatively warm days and probably only during midday hours because the shaded tree canopies received direct sunlight in the morning and late afternoon (Jifon and Syvertsen 2003). This study focused only on fully exposed leaves on the outside of the canopy so the results cannot be extrapolated to other canopy positions or to whole trees. Although interior leaves were mutually shaded in our experimental trees because leaf area indices were more than 9 (Syvertsen and Lloyd 1994), long-lived citrus leaves can acclimate to changing light environments even after they mature (Syvertsen 1984, Syvertsen and Smith 1984). Shade screens not only reduce direct radiation on outer canopy leaves, but can also increase the fraction of diffuse radiation on interior shaded leaves (Cohen et al. 1997), which may explain why shade had little effect on canopy growth, fruit yield or return bloom the following year. Such a redistribution of light within the canopy has been shown to increase dry matter production in tomato plants (Aikman 1989).

In summary, total fruit removal increased leaf area, leaf starch concentration and LDMa, but decreased $A_i$ relative to leaves on full-cropping trees. The sink demand from mature fruit maintained high midday $A_i$ on clear winter days. Removal of half the crop increased individual fruit mass, but reduced fruit color development. Crop load also reduced return bloom the following year. In leaves immediately adjacent to fruit, however, fruit competed with leaves for resources because these leaves were smaller, had lower leaf N concentration, lower carbohydrate concentrations and LDMa along with lower $A_i$ than leaves on non-fruiting branches on the same trees. The 50% shade treatment decreased midday $T_l$ and evaporative demand, while increasing $A_i$ and $g_s$. Super-optimal temperatures in the mesophyll of sunlit leaves were more important than stomatal limitations on photosynthesis. Shade decreased total soluble sugars in leaves but had no effect on leaf starch or return bloom the following year. Over the study period, there were no effects of shade on canopy growth, yield or fruit size, but shaded fruit developed better external color than sun-exposed fruit. In warm climates, there may be a potential for properly timed shade treatments to increase midday assimilation of CO2 and to improve color of fruit in exposed canopy positions.

Acknowledgments

This research was supported by a Uruguay INIA/UF cooperative research grant and the Florida Agricultural Experiment Station. Approved for publication as Journal Series No. R-09230.

References


TREE PHYSIOLOGY ONLINE at http://heronpublishing.com

Downloaded from https://academic.oup.com/treephys/article-abstract/23/13/899/1650690 by guest on 29 March 2019


