Project title: Study of whole-canopy and leaf gas exchange, water relations, chlorophyll fluorescence to understand the effects of cultural practices on growth and productive capacity of apple trees

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Objectives
During the past four years, a semi-automated system for whole-canopy gas exchange measurements was successfully designed, developed and used for several experiments in central WA. The overall objectives of the proposed research aimed at improving knowledge of effects of interaction between cultural practices and environment on tree physiology, with particular emphasis on carbohydrate production, tree productivity, fruit quality and sustained cropping. The main experimental research objectives of the project were:

1. Study the physiological consequences of the use of chemical thinners.
2. Study the repercussions of reduced irrigation techniques on gas exchange, water relations and productivity of apple trees.
3. Evaluate the consequences of the application of kaolin-based particle film on net photosynthetic rate, transpiration rate and fruit quality of apple trees.
4. Determine if application of a new product containing harpin (Messenger™) results in enhanced apple photosynthetic activity as suggested by preliminary data.
5. Establish cooperation with scientists at other institutions.

Significant findings
The whole-canopy net photosynthesis approach was successfully utilized in eastern Washington as a tool to study the response of carbon assimilation to experimental or common horticultural practices.

1. Effects of chemical thinners on carbon assimilation
Objective of the present study was to investigate the possibility of the use of alternative strategies for fruit thinning by studying chemicals which have shown promising results on experimental trials. Trials were conducted in 2001 and 2002, both in greenhouse and field conditions. Crabapple trees were used for greenhouse trials, whereas field trials were conducted in commercial apple orchards. In 2001, two field experiments were performed on apple trees. The treatments were 1) unsprayed control; 2) vegetable oil emulsion (VOE, 8% v/v); 3) NC99 (5% v/v); or 4) combination of Crocker’s fish oil (CFO, 2% v/v) and lime sulfur (LS, 2% v/v). In both cases it appeared that the combination of CFO+LS induced a depression in photosynthetic activity in the days immediately following application (Fig. 1). However such depression was not recorded in crabapple (data not shown). The photosynthesis depression appeared to last for 3-7 days. In trees treated with CFO+LS, leaves and flowers exhibited evident necrosis. Chlorophyll fluorescence was the same in all treatments, indicating that the biochemistry of the photosynthetic machinery was not affected by the chemical thinners.
In 2002, the experiment was repeated in the same orchard using the same chemical thinners but no major differences were observed among treatments (Fig. 2). Only on May 3, assimilation rate in treated trees was lower than in control. However, this seemed to be a rather isolated phenomenon, rather than a consequence related to treatment application. The leaf necrosis reported in year 2001 for the CFO+LS treatment was not recorded in 2002. It is possible that the physical damage observed in 2001 was caused by a combination of the chemical (most likely LS) action and the pressure used to apply the solution with the handgun. This hypothesis is validated by the fact that, in 2002, treatments were applied with the PropTec tower sprayer, which requires a much smaller pressure to deliver the product.
2. **Deficit irrigation trial**

The effects of partial rootzone drying (PRD) and season-long deficit irrigation (DI) on carbon assimilation and water relations of apple trees were investigated in 2001 and 2002. In 2001, differences in soil water content among treatments became significant in mid summer. By the end of the season, differences between the control and the deficits were close to 0.10 m$^3$ m$^{-3}$. Differences among treatments in predawn $\psi$ were statistically significant only 104 days after full bloom (DAFB) (Fig. 3), but no significant differences were recorded on the other dates. Significant differences in mean midday $\psi$ data began to show 90 DAFB and continued throughout the season. Midday $\psi$ in PRD trees was always 0.28-0.39 MPa lower than in control trees. Leaf net gas exchange in control trees was higher than in PRD trees 78, 109, and 120 DAFB (Fig. 4). Whole-canopy net carbon exchange (NCER) was monitored three times throughout the season. In general, NCER data suggested that carbon assimilation was higher in well-irrigated trees (Table 1). Fruit diameter measurements throughout the season showed no negative effect of either PRD or DI on fruit growth (data not shown). Fruit quality characteristics were not affected by the treatments, but fruit from trees grown under PRD irrigation were smaller than control and DI trees (data not shown).

![Fig. 3. Predawn (A) and midday (B) water potential measured on leaves of ‘Fuji’ apple trees subjected to normal irrigation, partial rootzone deficit (PRD), or regulated deficit irrigation (DI). Treatment means indicated with the same letter are not significantly different ($P \leq 0.05$). Data collected in 2001.](image-url)
Fig. 4. Effects of irrigation techniques on leaf gas exchange of ‘Fuji’ apple trees. PRD = partial rootzone deficit; DI = regulated deficit irrigation. [A] A, leaf net assimilation rate, [B] E, leaf transpiration rate, [C] gs, stomatal conductance. Treatment means indicated with the same letter are not significantly different (P ≤ 0.05).

Table 1. Whole-canopy net assimilation rate (A_{WC}) and transpiration rate (E_{WC}) of ‘Fuji’ apple trees subjected to different irrigation techniques. PRD, partial rootzone deficit; DI, regulated deficit irrigation. Data represent the mean of the values calculated between 1000 and 1500 HR. Numbers with different letters within rows indicate significant differences at P ≤ 0.05.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Time</th>
<th>Control</th>
<th>PRD</th>
<th>DI</th>
</tr>
</thead>
<tbody>
<tr>
<td>A_{WC} (µmol m^{-2} s^{-1})</td>
<td>July (74-76 DAFB)</td>
<td>6.1 a</td>
<td>4.7 b</td>
<td>4.9 b</td>
</tr>
<tr>
<td></td>
<td>Aug. (116-118 DAFB)</td>
<td>4.0 a</td>
<td>2.8 c</td>
<td>3.3 b</td>
</tr>
<tr>
<td></td>
<td>Sept. (144-146 DAFB)</td>
<td>3.1 a</td>
<td>2.3 b</td>
<td>1.8 c</td>
</tr>
<tr>
<td>E_{WC} (mmol m^{-2} s^{-1})</td>
<td>July (74-76 DAFB)</td>
<td>2.2 a</td>
<td>2.1 a</td>
<td>2.1 a</td>
</tr>
<tr>
<td></td>
<td>Aug. (116-118 DAFB)</td>
<td>2.3 a</td>
<td>1.8 b</td>
<td>2.2 a</td>
</tr>
<tr>
<td></td>
<td>Sept. (144-146 DAFB)</td>
<td>2.2 a</td>
<td>1.6 b</td>
<td>1.8 b</td>
</tr>
</tbody>
</table>

In 2002, neither water potential nor gas exchange data (Fig. 5 and Fig. 6, respectively) showed any differences among the irrigation treatments.
Fig. 5. Predawn [A] and midday [B] water potential measured on leaves of ‘Fuji’ apple trees subjected to normal irrigation, partial rootzone deficit (PRD), or regulated deficit irrigation (DI). PRDS indicates PRD trees treated with Surround WP. Treatment means indicated with the same letter are not significantly different ($P \leq 0.05$). Data were collected in Quincy, WA in 2002.

Fig. 6. Effects of irrigation techniques on leaf gas exchange of ‘Fuji’ apple trees. PRD = partial rootzone deficit; DI = regulated deficit irrigation. [A] A, leaf net assimilation rate, [B] E, leaf transpiration rate, [C] $g_s$, stomatal conductance. Treatment means indicated with the same letter are not significantly different ($P \leq 0.05$).

3. **Particle film effects on gas exchange and water relations in apple trees**

Effects of two different particle films, Surround WP and Raynox, on photosynthesis and fruit quality of 4-year-old ‘Fuji’/M.26 were examined during the 2000 growing season. Despite minimal differences detected among treatments on a daily basis, neither particle film seemed to
induce any sensitive modification to average seasonal photosynthetic activity (Fig. 7). Particle films did not alter fruit growth at any time during the season (data not presented). The two particle films did not induce any effect on fruit load, fruit size, and fruit maturity characteristics (background color, firmness, soluble solids, titratable acids, starch/iodine score) (Table 2).

Fig. 7. Mean whole-canopy net assimilation rate in trees treated with Surround WP and Raynox. Data represent integrated values of measurements collected between 11:00 a.m. and 2:00 p.m. PST.

Table 2. Fruit maturity characteristics in trees treated with Surround WP and Raynox during summer 2000.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Average fruit weight (g)</th>
<th>Firmness (lbs)</th>
<th>Soluble solids (brix)</th>
<th>Titratable acids (%)</th>
<th>Starch Content (score)</th>
<th>Hue (º)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>337.8 a</td>
<td>14.6 a</td>
<td>16.0 a</td>
<td>0.36 a</td>
<td>4.2 a</td>
<td>33.7 a</td>
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<tr>
<td>Surround WP</td>
<td>286.0 a</td>
<td>15.5 a</td>
<td>15.8 a</td>
<td>0.41 a</td>
<td>4.1 a</td>
<td>40.4 a</td>
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<tr>
<td>Raynox</td>
<td>324.5 a</td>
<td>14.9 a</td>
<td>16.4 a</td>
<td>0.38 a</td>
<td>4.2 a</td>
<td>30.6 a</td>
</tr>
</tbody>
</table>

4. **Messenger™ application to apple trees**

In general, Messenger™ did not affect significantly leaf net photosynthesis (Fig. 8). Net photosynthesis data collected at whole-canopy level at 113-114 DAFB confirmed the results obtained from leaf measurements (Table 3, Fig. 9A) and significant differences between the two treatments were not detected. Carbon assimilation rate was higher in trees treated with Messenger at most times during the day at 134-135 DAFB (Fig. 9B); however, differences between the daily averages of the two treatments were not significantly different (Table 3). Although average transpiration rate was lower in Messenger-treated trees at 113-114 DAFB (Table 3), this result was not observed at leaf level (Fig. 8) or at different times of the day (Fig. 9C). Overall, our results do not indicate that Messenger caused any increase in carbon assimilation rate, as indicated by data collected on other crops or by preliminary results in apple.

Table 3. Whole-canopy net assimilation rate (A\text{WC}) and transpiration rate (E\text{WC}) of apple trees treated with Messenger. Data represent the mean of the values calculated between 800 and 1300 HR. Numbers with different letters within rows indicate significant differences at $P \leq 0.05$. 
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Date</th>
<th>Control</th>
<th>Messenger</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A_{WC}$ (µmol m$^{-2}$ s$^{-1}$)</td>
<td>113-114 DAFB</td>
<td>7.65 a</td>
<td>6.03 a</td>
</tr>
<tr>
<td></td>
<td>134-135 DAFB</td>
<td>5.54 a</td>
<td>4.35 a</td>
</tr>
<tr>
<td>$E_{WC}$ (mmol m$^{-2}$ s$^{-1}$)</td>
<td>113-114 DAFB</td>
<td>4.70 a</td>
<td>3.59 b</td>
</tr>
<tr>
<td></td>
<td>134-135 DAFB</td>
<td>2.50 a</td>
<td>2.48 a</td>
</tr>
</tbody>
</table>

Fig. 8. Effects of Messenger application on net carbon assimilation rate of apple leaves. *, ** indicate means statistically different at $P \leq 0.05$ and $P \leq 0.01$, respectively.
Fig. 9. Effects of Messenger application on whole-canopy net photosynthesis rate [A, B] and transpiration rate of apple trees treated with Messenger. Application was performed twice during the growing season, at 113-114 DAFB [A, C] and 134-135 DAFB [B, D].

5. **Cooperation with other institutions**

In 2001, I spent six weeks spent at the HortResearch Centre located in Nelson, New Zealand, to calibrate the inflatable cuvettes for whole-canopy gas exchange studies. A successful experiment was conducted to study the consequences of the application of particle film (‘Surround WP’) on gas exchange. ‘Surround WP’ appeared to cause a reduction in carbon assimilation rate at the leaf level. However, at whole-canopy level, no differences in gas exchange rates were observed. Inflatable cuvettes were compared and calibrated against the rigid and more sophisticated ones available at the HortResearch, Nelson Research Centre. There was a positive correlation ($r^2 = 0.81$, $P \leq 0.0001$) between data collected with the two types of cuvettes, therefore indicating that data collected with the inflatable cuvettes are accurate and reliable. In the ‘Surround WP’ experiment, leaf net photosynthesis appeared to be 12% lower in ‘Surround WP’-coated leaves than in untreated leaves. Measurements of the light properties of leaves indicated a higher proportion of reflected light treated leaves, which can explain the reduced net photosynthesis observed. Interestingly, such reduction was not detected at whole-canopy level.

Collaboration with Dr. Lakso led to an experiment conducted to study the effects of changing light levels in apple. Data were collected in 2001 at the WSU Columbia View Research Orchard. Results of that particular experiment are indicated in Dr. Lakso final report.

**Dissemination of results**

Oral and poster presentations delivered at industry and scientific meetings during the three years I worked at WSU-TFREC with the support of WTFRC:

1. Lombardini, L. *Whole plant photosynthesis: a sensitive technique to study the effects of cultural practices on growth and productive capacity of apple trees.* (Poster) 95th Meeting


Manuscripts containing results from the above mentioned experiments submitted for publication:


Two additional manuscripts, which include results from the deficit irrigation and chemical thinner trials, are in preparation.

**Acknowledgments**
I would like to thank Jim McFerson, the Commissioners, and the WTFRC staff for their support in the research I conducted while working at WSU-TFREC. The three years spent conducting exciting and cutting-edge research in central Washington have been extremely important and formative for my professional career.