FINAL PROJECT REPORT
WTFRC Project Number: AP-06-604

Project Title: High temperature stress on apple fruit peel: physiology and detection

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Other funding Sources

Agency Name:
Amount awarded:
Notes:

Total Project Funding: $82,967

Budget History:

Item	Year 1:	Year 2:	Year 3:
Salaries	22,000	23,188	
Benefits	8,249	9,070	
Wages			
Benefits			
Equipment	0	0	
Supplies	10,230	10,230	
Travel	0	0	
Miscellaneous	0	0	
Total	40,479	**42,488**	

Objectives

The overall objective is to better understand the underlying physiology of high temperature stress to apple fruit peel with an ultimate goal of detecting and reducing high peel temperature-induced fruit disorders both preharvest and postharvest. The specific objectives are:

1) To determine how high temperature affects the balance of photooxidation and photoprotection of apple fruit peel, leading to sunburn browning;
2) To determine if chlorophyll fluorescence reflects the damage of high temperature on fruit peel and varietal differences during the growing season;

Significant Findings

1) Maximum photosystem II (PSII) quantum efficiency (Fv/Fm) of the sun-exposed peel of well-exposed fruit in the southwest canopy decreased during the day in response to high peel temperatures, and very little recovery was made during overnight dark relaxation, indicating that the high peel temperature has damaged the PSII centers of the peel.

2) After a couple days of high temperature exposure, more fruit in the west side of the canopy had very low Fv/Fm value than those in the east side. This difference corresponds to the different profiles of peel temperatures and sunburn occurrence between the two sides of the canopy. This along with the diurnal Fv/Fm data indicates that Fv/Fm is a very sensitive indicator of high temperature stress in apple peel.

3) Compared with the non-sunburned peel, the sunburned peel had lower chlorophyll content, lower Fv/Fm, lower net oxygen evolution rate, and lower activities of key photosynthetic enzymes, but higher activities of antioxidant enzymes and higher content of antioxidant metabolites and higher xanthophyll cycle activity on a chlorophyll basis, and higher hydrogen peroxide and malondialdehyde content. This indicates that high peel temperature most likely has increased the photooxidation potential, rather than decreased the photoprotective capacity of fruit peel.

4) Controlled temperature treatments of fruit peel samples in the dark showed that high peel temperature led to decreases in Fv/Fm and net O_2 evolution, and appearance of "K" step in chlorophyll a fluorescence transient. This indicates that high temperature has damaged the oxygen evolution complex of the PSII, leading to oxidative stress.

5) Simultaneous high temperature and high light treatment decreased Fv/Fm and O_2 evolution of Gala peel more than high temperature or high light alone. The clear "K" step in chlorophyll fluorescence, which appeared in the high temperature treatment, disappeared under simultaneous high temperature and high light treatment. This indicates that high temperature mainly affects the oxygen evolution complex of the PSII (the donor side) whereas high light mainly affects the acceptor side of the PSII.

6) Apple cultivars differ in their responses to high temperature and high light stress. Of the cultivars tested, Red Delicious is most tolerant of high temperature and high light stress whereas Cameo is the least tolerant. Our data show that chlorophyll fluorescence is an effective tool for testing varietal difference in tolerance to high temperature and high light stress.

Methods

<u>1. Determine diurnal changes of Fv/Fm in relation to peel temperature</u> This experiment was carried out on mature Gala/M.9 trees (spacing:15 X 6.5 feet) at WSU TFREC on July 21. Fifty well-exposed fruit on the southwest part of the canopy were selected the day before and the

temperature of the sun-exposed side of each fruit was monitored with a thermocouple connected to a data logger. In addition, the temperature of the shaded side of 3 fruit was also monitored along with ambient temperature. Every 4 hours starting from pre-dawn (5:00), ten fruit were dark-adapted for 30 min and then measured for Fv/Fm. The pre-dawn values of Fv/Fm were also measured the next day.

2. Determine the distribution of fruit peel Fv/Fm and sunburn occurrence on the east side and west side of the canopy after exposure to high temperatures. Ten well-exposed fruit from each side (east and west) of the canopy were selected and their peel temperatures were monitored as above from July 23 to 25. All the fruit from the east side and west side of the canopy were harvested separately and Fv/Fm of the sun-exposed peel was measured at pre-dawn on July 25. The percentage of fruit with sunburn was counted on separate trees with similar canopy size and structure in the morning on July 26.

3. Compare the sunburned and non-sunburned fruit in terms of photosynthetic capacity, chlorophyll fluorescence, and antioxidant system. The sun-exposed peel of non-sunburned and sunburned fruit (80 fruit each) was taken from the east and west side of the canopy from 9:15 to 10:00AM and from 4:00 to 4:45PM on July 25, respectively. The samples were immediately frozen in liquid nitrogen and stored until analysis.

4. Determine chlorophyll fluorescence and oxygen evolution of the sun-exposed peel of 'Fuji' fruit in response to controlled high temperature treatments. At approximately 100 days after full bloom (mid-August), well-exposed fruit on the west side of the canopy of Fuji/M.9 trees were taken right after sunset and the sun-exposed side of each fruit was marked. All the fruit were dark-adapted overnight at 22C and fruit peel samples (0.5 mm thick, 1 cm^2) were taken from the sun-exposed side. The peel samples were placed between two layers of wet paper towel and the assembly was put onto the bottom of a small aluminum foil vessel with the top covered with aluminum foil. Then, the vessel was directly floated on water in a water bath, the temperature of which was controlled by a refrigerated water bath and the temperature equilibrium between the fruit peel and water was reached within 1 to 2 min. The peel samples were exposed to 25, 35, 40, 42, 44, 46 or 48°C in the dark for 30 min. Chlorophyll a fluorescence transient and photosynthetic O_2 evolution were measured after the peel samples had been kept in the dark at room temperature for 30 min after each temperature treatment.

5. Determine the effect of simultaneous high temperature and high light stress on the sun-exposed peel of Gala fruit. Well-exposed fruit were harvested from the west side of Gala/M.9 trees on August 16, 2007, wrapped in wet paper towel and put into plastic bags immediately. After overnight dark adaptation in the lab, the peel discs with 3 mm thickness were cut from the sun-exposed side and were put onto 4 layers of wet cheesecloth in a stainless steel water jacket, the temperature of which was controlled by a refrigerated water bath. Light was provided by a tungsten lamp. Peel disc were treated with high temperature (45 ºC) in the dark, high light (1600 µmol m^{-2} s^{-1}) at room temperature, or cross stress of high light and high temperature (45 ºC, 1600 µmol m^{-2} s^{-1}) for 0, 15, 30 and 45 min, respectively. After dark-adaptation for one hour, chlorophyll a fluorescence transients and O_2 evolution were measured.

6. Determine varietal difference in response to high temperature and high light stress. In late August, well-exposed fruit of Cameo, Fuji, Gala, Golden Delicious and Red Delicious were harvested from the west side of the canopy, wrapped in wet paper towel and put into plastic bags immediately. After overnight dark adaptation in the lab, the peel discs with 3 mm thickness were cut from the sun-exposed side and were put onto 4 layers of wet cheesecloth on a stainless steel water jacket, the temperature of which was controlled by a refrigerated water bath. The light

intensity was controlled by a tungsten lamp. Discs were treated with different temperatures (30, 35, 40, 42, 44, 46 and 48 ºC) in the dark or in the light (1200 µmol m^{-2} s^{-1}) for 30 min. After dark adaptation for 1 h, chlorophyll a fluorescence transients were measured. For all the experiments, chlorophyll fluorescence was measured with a Handy PEA and oxygen evolution was measured with ChloroLab 2 (Hansatech, UK).

Results and Discussion

1. Diurnal changes of PSII quantum yield (Fv/Fm) of the sun-exposed peel and the shaded peel in relation to peel temperature on a hot day in central WA

On July 21 of 2006, the temperature of the sun-exposed peel of well-exposed fruit in the southwest part of the canopy increased almost linearly from 5:00 to 15:20, reaching 50C at 15:20, and then stayed above 45.9C till 17:30. In contrast, the shaded peel only reached a maximum of 40.7C between 17:00 and 17:30 when the highest ambient temperature was 35.5C.

Fv/Fm of the sun-exposed peel at pre-dawn was around 0.53, and remained essentially unchanged till 9:00. However, the Fv/Fm decreased linearly from 9:00 to 17:00, and then increased slowly from 17:00 to 5:00 the next day. The rapid decrease in Fv/Fm from 9:00 to 17:00 corresponds well with the period of high fruit peel temperature. Even at pre-dawn the next day Fv/Fm has not fully recovered to the previous pre-dawn level, indicating severe oxidative damage has occurred in the peel. In contrast, Fv/Fm of the shaded peel was higher than in the sun-exposed peel and remained unchanged throughout the entire day. The large error bar in the Fv/Fm data for the sun-exposed peel is due to the fact that there were large variations in Fv/Fm value among individual fruits.

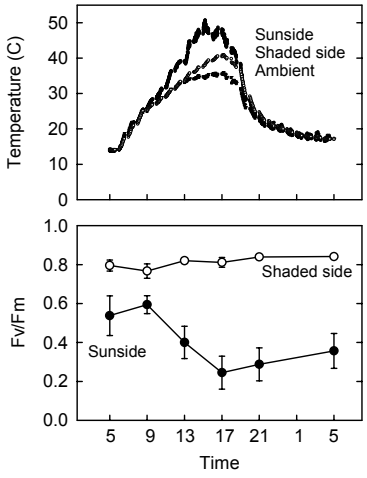

Fig 1

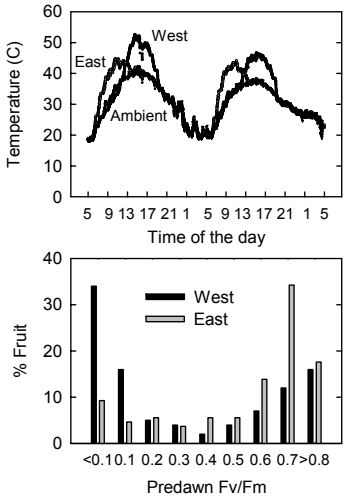

Fig 2

Fig 1. Diurnal changes of peel temperature and Fv/Fm in the sun-exposed peel and the shaded peel of well-exposed fruit in the southwest part of the canopy on July 21, 2006.

Fig 2. Diurnal changes of the temperature of the sun-exposed peel in the east and west sides of the canopy on July 23 and 24 and fruit distribution in terms of Fv/Fm measured at pre-dawn on July 25, 2006.

2. Distribution of fruit peel Fv/Fm and sunburn occurrence in the east side and west side of the canopy after exposure to high temperatures.

We compared the distribution of fruit Fv/Fm between east and west sides of the canopy after a couple days of high temperature exposure (July 23 and 24). As shown in Fig 2, the % fruit with an Fv/Fm value less than 0.1 was much higher in the west side (34%) than in the east side (9%) of the tree canopy whereas the % fruit with an Fv/Fm value between 0.7 and 0.8 was much lower in the west side (13%) than in the east side (34%). These numbers correspond well with the difference in temperature profiles between the two sides. Interestingly, both east and west sides had 16 to 18% of the fruit with an Fv/Fm value higher than 0.8, which indicates that both sides have equal number of shaded fruit. Counting the number of sunburned fruit on each side showed that the west side had 21.9% with sunburn whereas the east side had only 6.1%.

3. Comparison of sunburned and non-sunburned fruit peel

Photosynthetic oxygen evolution: As light level increased, net O_2 evolution rates for both non-sunburned and sunburned peels increased almost linearly first, then reached a saturation point, beyond which O_2 evolution showed little response to increasing light level (Fig 3). At each given light level, photosynthetic O_2 evolution rate was significantly lower in the sunburned peel than in the non-sunburned peel (Fig. 3). The quantum yield for O_2 evolution (the initial slope of each curve) was much lower in the sunburned peel than in the non-sunburned peel, whereas the light saturation point was higher in the sunburned peel than in the non-sunburned peel.

Hydrogen peroxide and malondialdehyde: The sunburned peel had higher concentrations of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA, an indicator of oxidative lipid metabolism) compared with the non-sunburned peel (Fig. 4), which clearly indicates that oxidative damage has occurred.

Activities of key photosynthetic enzymes: Compared with the non-sunburned peel, the sunburned peel had lower activities of key photosynthetic enzymes, including ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), NADP-glyceraldehyde-3-phosphate dehydrogenase, phosphoribulokinase, and stromal fructose-1,6-bisphosphatase (Data not shown). However, the activities of these enzymes decreased to a lesser extent than the net O_2 evolution rate.

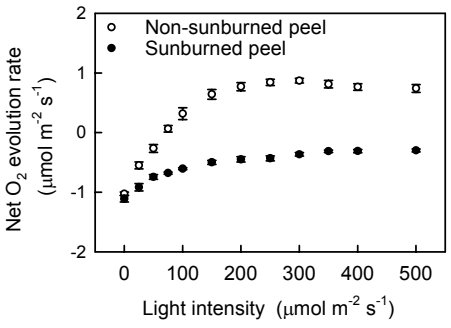

Fig 3

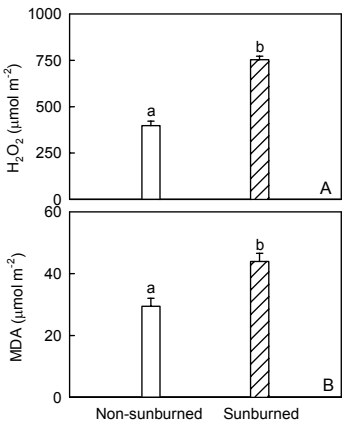

Fig 4

Fig 3. Light response of net oxygen evolution of sunburned and non-sunburned peels.
Fig 4. Hydrogen peroxide and malondialdehyde (MDA) content in sunburned and non-sunburned peels.

Reflectance and pigments: The sunburned peel had higher reflectance averaged over 400 – 700 nm. Reflectance spectra showed that the sunburned peel reflected more light in the range between 420 and 700 nm than the non-sunburned peel (Data not shown).

Chlorophyll, xanthophyll cycle pool size and lutein contents expressed on a peel area basis, and β-carotene and neoxanthin contents expressed on a peel area or Chl basis were lower in the sunburned peel than in the non-sunburned peel, whereas the contents of xanthophyll cycle pool size, zeaxanthin and antheraxanthin, and lutein expressed on a chlorophyll basis were higher in the sunburned peel than in the non-sunburned peel. This indicates that more chlorophylls were degraded relative to xanthophylls and other carotenoids. Almost all the xanthophyll cycle pool was converted to zeaxanthin and antheraxanthin in both sunburned and non-sunburned peels.

Antioxidant enzymes and metabolites: Activities of ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase, and glutathione reductase were higher in the sunburned peel than in the non-burned peel, whereas there was no significant difference in superoxide dismutase or catalase activity between the two peel types (Data not shown).

The content of total ascorbate (reduced + oxidized), total glutathione (reduced + oxidized) and reduced glutathione was higher in the sunburned peel than in non-sunburned peel, but the ratio of both reduced ascorbate to total ascorbate and reduced glutathione to total glutathione were lower in the sunburned peel than in the non-sunburned one. No significant difference was observed in reduced ascorbate content between the sunburned and the non-sunburned peels (Data not shown).

Chlorophyll a fluorescence transients: When overnight dark-adapted fruit was exposed to a saturating pulse of light, chlorophyll a fluorescence showed a characteristic rise from minimal fluorescence (Fo) to maximum fluorescence (Fm) in the non-sunburned peel (Fig 5). However, the fluorescence signal of the sunburned peel was much lower and reached Fm at a much earlier stage followed by little change in fluorescence intensities. Chlorophyll fluorescence turns out to be the most sensitive of all the responses we have measured on the sunburned peel.

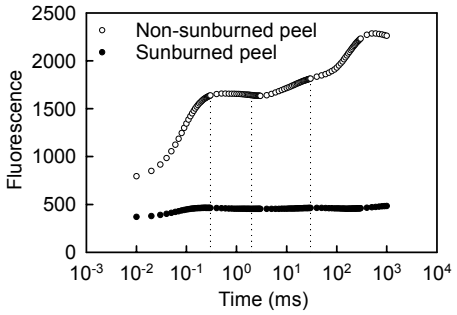

Fig 5. Chlorophyll fluorescence induction curves of sunburned and non-sunburned fruit.

4. Chlorophyll a fluorescence transients, Fv/Fm and photosynthetic oxygen evolution of the sun-exposed peel in response to temperature treatments

When the peel temperature increased from 25 to 35C, neither Fo nor Fm showed any significant change (Fig 6A). However, as peel temperature increased further, Fm decreased whereas Fo increased. In addition, the shape of the chlorophyll fluorescence induction curve changed. When the peel temperature reached 44 - 48°C, a very clear peak (called "K" step) at 300 μs appeared, followed by a pronounced dip. After exposure to 46 and 48°C, maximal fluorescence was already reached at "K" step followed by a rapid decrease to a level close to or

below Fo (Fig. 6A). Fv/Fm changed very little from 25 to 40°C, and then dropped rapidly with further increase in temperature (Fig. 6B).

Net photosynthetic O_2 evolution rates remained unchanged as temperature increased from 25°C to 40°C, then dropped rapidly with any further increase in temperature (Fig. 6C). After exposure to 46 and 48C, the net O_2 evolution rate became negative. However, heat stress showed no effects on dark respiration.

Decreases in Fv/Fm and net O_2 evolution, coupled with appearance of "K" step in chlorophyll a fluorescence transient indicate that high temperature has damaged the oxygen evolution complex of the PSII, leading to oxidative stress. However, the lack of a clear K step in the sunburned peel (Fig 6) suggests that there is interaction between high peel temperature and high light.

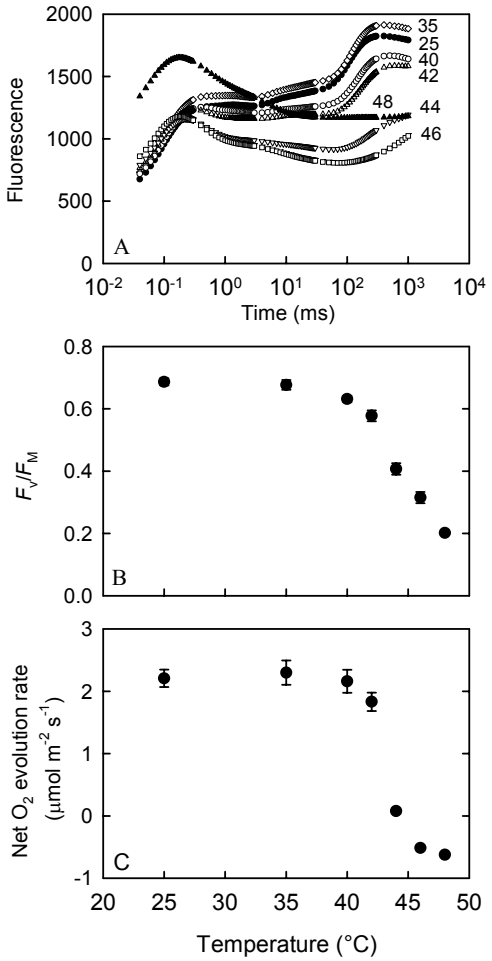

Fig 6. Chlorophyll fluorescence transient (A), maximum PSII efficiency, Fv/Fm (B), and net oxygen evolution of the sun-exposed peel of Fuji fruit in response to temperature treatments.

5. Responses of Fv/Fm, chlorophyll a fluorescence transients and photosynthetic oxygen evolution of the sun-exposed peel in response to high temperature and high light treatments

Simultaneous high temperature and high light treatment decreased peel Fv/Fm more than high temperature or high light treatment alone (Fig 7). Peel oxygen evolution rate was significantly lower in the simultaneous high temperature and high light treatment than in the high

temperature or high light treatment alone (Fig 8A). Dark respiration was not significantly affected (Data not shown). This clearly indicates that high temperature coupled with high light causes more damage to fruit peel than high temperature or high light alone.

Chlorophyll fluorescence transients at the end of the 45 min treatment showed a clear "K" step in the high temperature treatment (in the dark) alone, but the "K"step disappeared in the simultaneous high temperature and high light treatment. This suggests that high temperature mainly affects the oxygen evolution complex of the PSII (the donor side) whereas high light mainly affects the acceptor side of the PSII.

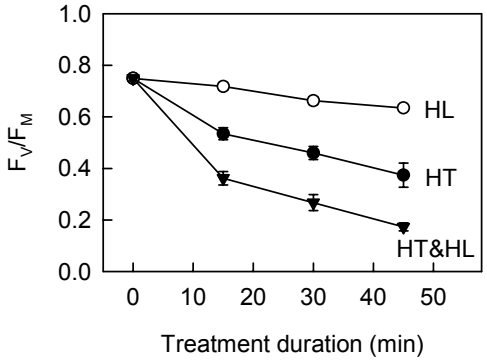

Fig 7. Maximum quantum yield of PSII (Fv/Fm) of the sun-exposed side of Gala apple peel in response to high temperature (45 ºC, HT), high light (1600 μmol m^{-2} s^{-1}, HL), and high temperature with high light (HT&HL) treatments.

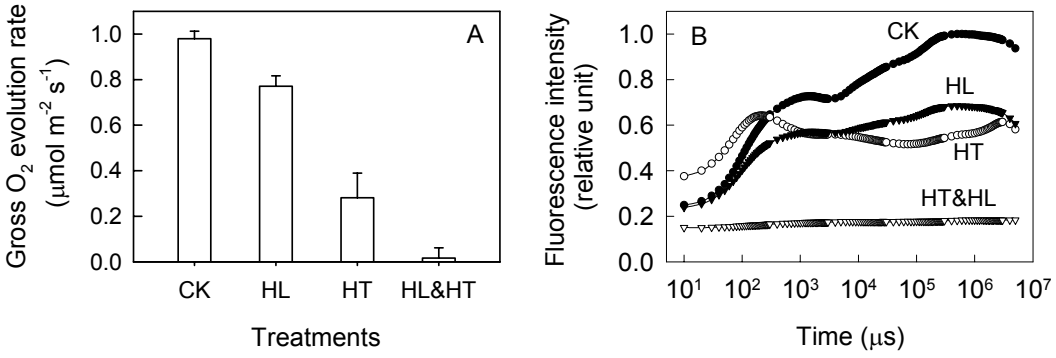

Fig 8. Oxygen evolution rate (A) and chlorophyll fluorescence transients (B) of 'Gala" peel in response to high light (HL), high temperature (HT) and high light with high temperature (HL&HT) treatments for 45 min.

6. Varietal difference in response to high temperature and high light stress

When treated in the dark, the Fv/Fm value of the sun-exposed peel of Cameo, Fuji, Gala, Golden Delicious and Red Delicious remained unchanged as the treatment temperature increased from 30 to 40 °C (Fig 9A). With further increases in treatment temperature, varietal difference showed up. Fv/Fm of Cameo peel started to decrease at 42°C, whereas that of Red delicious

didn't decrease until temperature increased to 46°C. At any given temperature from 42 to 48 °C, Red Delicious had the highest Fv/Fm whereas Cameo had the lowest Fv/Fm.

When treated under high light, Fv/Fm of the sun-exposed peel of all cultivars tested decreased as the treatment temperature increased (Fig 9B). At each given temperature, Red Delicious had the highest Fv/Fm whereas Cameo had the lowest Fv/Fm.

Our data indicate that apple cultivars differ in terms of tolerance to high temperature and high light stress. However, the tolerance mechanism remains to be elucidated.

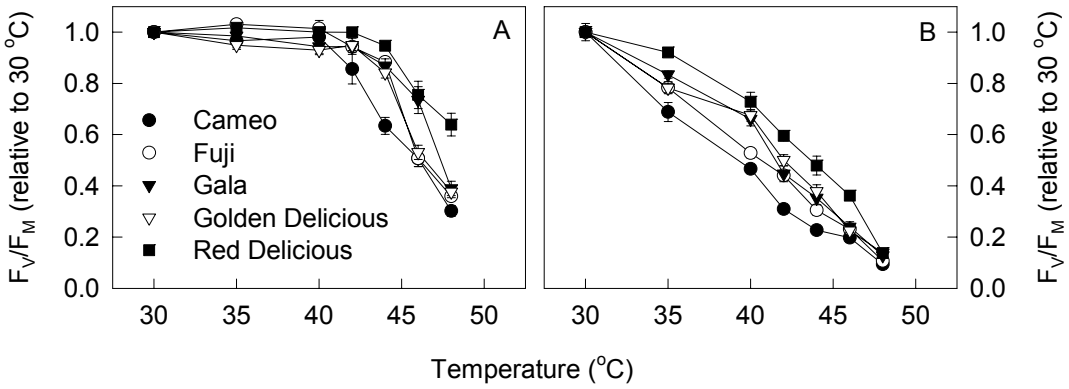

Fig 9. Maximum quantum yield of PSII (Fv/Fm) of the sun-exposed peel of five apple cultivars in response to temperature treatment for 30 min in the dark (A) or under 1200 μmol m^{-2} s^{-1} light (B).