WTFRC Project # AH-03-306 Final Report

Title: **Photoprotection of apple fruit by xanthophyll cycle**

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Objectives

The overall objective is to better understand the xanthophyll cycle pool size and composition in the peel of apple fruit and the role the xanthophyll cycle plays in protecting fruit from excessive light damage with an ultimate goal to reduce sunburn occurrence on apple fruit. Specific objectives are: 1) To compare xanthophyll cycle pool size and composition between sun-exposed and shaded fruit of selected apple varieties; 2) To characterize the seasonal patterns of xanthophyll cycle pool size and composition under WA conditions; 3) To determine xanthophyll cycle pool size and composition of 'Gala' fruit peel in response to nitrogen supply; 4) To determine the effects of evaporative cooling, partial root-zone drying, and deficit irrigation on xanthophyll cycle pool size and conversion of 'Fuji' apple peel under Washington conditions; 5) To compare xanthophyll cycle pool size and composition between sunburn susceptible and sunburn tolerant apple cultivars; and 6) To determine UV-screening and reflective coating on xanthophyll cycle size and composition.

Significant Findings

- The sun-exposed side of both Gala and Smoothee fruit has a larger xanthophyll cycle pool size and higher activities of antioxidant enzymes than the shaded side, indicating the sun-exposed side has a higher photoprotective capacity than the shaded side.
- Under high light at noon, the xanthophyll cycle in the sun-exposed side operates at its full capacity to dissipate excess absorbed light and the xanthophyll cycle pool size may become limiting.
- Under Washington conditions, xanthophyll cycle pool size decreases as fruit develops while activities of some of the antioxidant enzymes, especially ascorbate peroxidase, show a compensatory increase.
- Xanthophyll cycle pool size in the sun-exposed peel of 'Gala' fruit increased as nitrogen supply increased, indicating that fruit with a good nitrogen status has higher photoprotective capacity than fruit with a low nitrogen status.
- Evaporative cooling increased whereas deficit irrigation and partial root zone drying decreased xanthophyll cycle pool size.
- There were differences between cultivars in xanthophyll cycle pool size and lutein content associated with their susceptibility to sunburn.
- Pre-dawn maximum quantum efficiency (Fv/Fm) appears to reflect the varietal susceptibility to natural event of high fruit peel temperature and high light.
- Coating fruit with Surround didn't affect xanthophyll cycle pool size whereas UV-screening decreased xanthophyll cycle pool size.

Methods

Experiment 1. *Comparison of the sun-exposed side with the shaded side of apple fruit:* Both Gala/M.9 and Smoothee/M.9 trees were grown at a spacing of 1.83 × 4.88m in the field at Cornell Orchards in Ithaca, NY. They were mature trees trained as a central leader system, 3.5 m tall. At about 3 months after bloom, fruit from two canopy positions were selected: those on the exterior part of the canopy that were fully exposed to sunlight and those in the interior part of the canopy close to the central leader that were heavily shaded (receiving only approximately 5 to 8% of the full sunlight at noon). At each canopy position, the sun-exposed side was carefully distinguished from the shaded side for each fruit. Chlorophyll fluorescence measurements were made on both sides of the attached fruit under natural light exposure at noon and at predawn. Fruit peel discs (1 cm^2 each, 1 mm thick) were taken quickly at noon and/or predawn from both the sun-exposed side and the shaded side of the attached fruit, frozen in liquid nitrogen, and stored at -80 °C until analysis of pigments and antioxidant enzymes. For the noon sampling, the incident PFD and surface temperature for the sun-exposed side of exterior fruit, the shaded side of exterior fruit, the sun-exposed side of interior fruit, and the shaded-side of interior fruit was 1800 ±15, 87 ± 5, 121 ± 7, and 40 ± 3 $\mu mol\ m^{-2}\ s^{-1}$, and 34.4 ± 0.4, 27.3 ± 0.2, 27.5 ± 0.3, and 26.9 ± 0.1 °C, respectively.

Experiment 2. Seasonal changes of xanthophyll cycle pool size and composition of Gala fruit was monitored from 59 days after bloom to early August at about 2-week intervals in an 10th leaf Scarlet Gala/M.26 orchard in East Wenatchee, WA for a total of 4 times. At each sampling date, fruit peel discs (1 cm^2 each, 1 mm thick, 2 discs/fruit) of sun-exposed fruits from southwest of the tree canopy were taken at both midday (1:00 to 2:30 PM) and predawn, frozen in liquid nitrogen, and stored at -80 °C until analysis. Chlorophyll fluorescence was measured on the sun-exposed side of fruit after overnight dark adaptation. Fruit size, photon flux density (PFD), air temperature and fruit temperature for each sampling date were listed in the following Table.

Sampling date	*Fruit size (mm)*	*PFD $\mu mol\ m^{-2}\ s^{-1}$*	*Air Temp (°C)*	*Fruit Temp (°C)*
June 19	31.2 ± 0.32	947	22.0	24.6
July 8	41.8 ± 0.89	1125	28.2	35.1
July 22	47.9 ± 0.63	1905	34.7	45.8
August 4	53.8 ± 0.61	1985	27.1	36.7

Experiment 3. *Xanthophyll cycle pool size and composition in apple fruit peel in response to nitrogen supply:* Fourth leaf 'Gala'/M.26 trees grown in sand culture were used in this experiment. The cropload of these trees was adjusted by hand thinning to 5 fruit per cm^2 trunk cross-sectional area at 10 mm king fruit. They were supplied with 2 liters of 2.5, 12.5, or 25 mM nitrogen in a Hoagland's solution twice a week from petal fall to 4 weeks before harvest. Fruit growth was monitored in each N treatment. There were 6 replicates per treatment in a completely randomized design. At 70 days, 100 days, and 120 days after bloom, chlorophyll fluorescence was measured at noon to determine thermal dissipation capacity of the fruit and fruit peel samples (1 cm^2 each, 1 mm thick, 2 discs/fruit) were taken to measure xanthophyll cycle pool size and composition.

Experiment 4. *Effects of evaporative cooling, partial root zone drying, and deficit irrigation on xanthophyll cycle and composition of 'Fuji' fruit peel:* Eleven-year-old 'Fuji'/M.9 trees at Quincy, WA received one of the following 4 treatments: well-watered control, overhead EC on well-watered trees, partial root-zone drying or deficit irrigation. EC treatment started on July 16 and ended about 7 days before the last sampling. The timer settings for the EC treatment were 20 minutes on then 10 minutes off from 11:30 AM to 5:00 PM every day. The EC treatment, although started on the 16th, did not provide adequate cooling until July 22. Trees in partial root-zone drying and deficit irrigation treatments were supposed to receive approximately half of the irrigation of the well-watered control. However, this year the trees received less water, resulting in water stress development. Fruit peel samples were taken on July 15, August 9, and September 8 during the 2004 growing season. At each sampling date, fruit peel discs (1 cm^2 each, 1 mm thick, 2 discs/fruit) of sun-exposed fruits from southwest of the tree canopy were taken at both midday (1:00 to 2:30 PM) and predawn, frozen in liquid nitrogen, and stored at -80 °C until analysis. Chlorophyll fluorescence was measured on the sun-exposed side of fruit after overnight dark adaptation. Fruit size, photon flux density (PFD), air temperature and fruit temperature at each sampling date were also measured.

Experiment 5. Compare xanthophyll cycle pool size and composition between sunburn susceptible and sunburn tolerant apple cultivars. Mature Cameo, Golden Delicious and Red Delicious trees that were in adjacent blocks in an orchard in Wenatchee, WA were used. Fruit peel samples (10 fruit per cultivar) of sun-exposed side were taken from the exterior canopy of the trees from 1PM to 3:00PM on July 19, August 25 and September 13 to determine xanthophyll cycle pool size and conversion. Maximum quantum efficiency (Fv/Fm) of apple peel (10 fruit per cultivar) was measured after overnight dark-adaptation to indicate the damage of high light coupled with high temperature. Fruit size, photon flux density (PFD), air temperature and fruit temperature at each sampling date were also measured.

Experiment 6. Determine UV-screening and reflective coating on xanthophyll cycle size and composition. Mature 'Gala' trees received one of the following three treatments from 2 weeks after petal fall to 2 weeks before harvest: 1) covered with a polycarbonate which transmits 97% of the photosynthetically active radiation but blocks 98% of the entire UV spectrum (2) sprayed with Surround at 25 lbs per acre every 2 weeks; or 3) untreated control. Each treatment was replicated 4 times in a completely randomized design. Sun-exposed fruits were sampled on June 21, August 9, and August 26 to determine xanthophyll cycle pool size and composition.

Chlorophyll and xanthophyll pigments were extracted and analyzed by using an HPLC procedure (Cheng, 2003). Antioxidant enzymes were measured as described by Ma and Cheng (2003).

Results and discussion

1. Comparison of the sun-exposed side with the shaded side of apple fruit

At noon, efficiency of excitation transfer (F_v'/F_m') was lower in the sun-exposed side than the shaded side for both exterior and interior fruit with the sun-exposed peel of the exterior fruit having the lowest value (Fig. 1A). This indicates that the sun-exposed side has a higher thermal dissipation capacity than the shaded side. Maximum quantum efficiency (F_v/F_m) at pre-dawn was only slightly lower in the sun-exposed side than the shaded side for both exterior and interior fruit (Data not shown), which indicates that the sun-exposed side is fairly well protected from high light.

Xanthophyll cycle pool size was larger in the sun-exposed side than the shaded side (Fig. 1B). At noon, the sun-exposed peel had a higher conversion of violaxanthin (V) to zeaxanthin (Z) and antheraxanthin (A) than the shaded peel for both exterior and interior fruit, with A+Z accounting for over 90% of the xanthophyll cycle pool in the sun-exposed side of the exterior fruit (Fig. 1C). Both the xanthophyll cycle pool size and the conversion of violaxanthin to zeaxanthin and antheraxanthin correspond well with the thermal dissipation capacity of peel type as indicated by efficiency of excitation transfer (F_v'/F_m'). This reflects the dependence of thermal dissipation on the operation of the xanthophyll cycle.

Among the antioxidant enzymes, the activity of superoxide dismutase was similar between sun-exposed and shaded peels with slightly lower values in the interior fruit (Fig. 2A). Activities of the enzymes in ascorbate-glutathione cycle: ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase, and glutathione reductase all showed a similar trend, i.e. the sun-exposed peel had a higher activity than the shaded peel for both exterior and interior fruit although the difference between the sun-exposed and the shaded peels of the interior fruit was not as big as that in the exterior fruit (Fig. 2B, C, D, E). Catalase activity was higher in the shaded side than the sun-exposed side for both exterior and interior fruit, with the sun-exposed side of the exterior fruit having the lowest activity (Fig. 2F). Considering that apple fruit peel had 4 to 5 times higher ascorbate peroxidase activity, but much lower catalase activity than leaves (Cheng and Ma, 2003), the reaction catalyzed by ascorbate peroxidase may serve as the main pathway for detoxifying hydrogen peroxide in apple fruit peel.

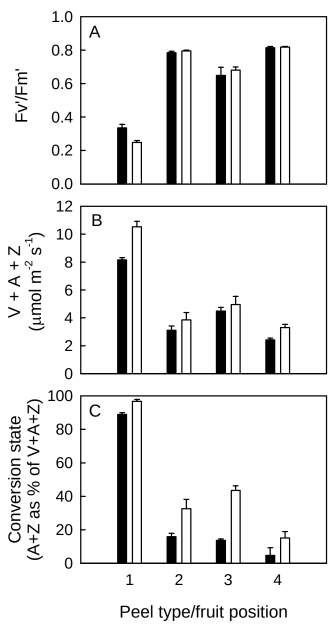


Fig. 1

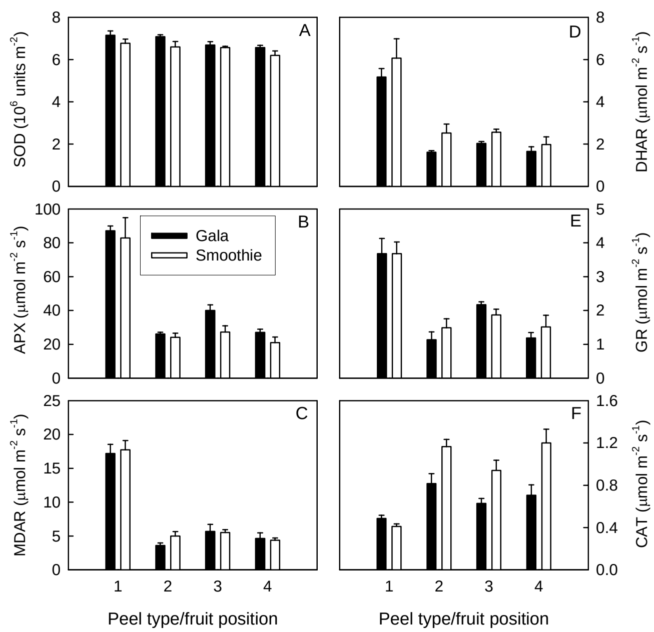


Fig. 2

Fig. 1. Efficiency of excitation transfer, Fv'/Fm' (A), xanthophyll cycle pool size (B), and conversion state (C) in the peel of apple fruit acclimated to light exposure within the tree canopy. Peel type/fruit position: 1: the sun-exposed peel of exterior fruit; 2: the shaded peel of exterior fruit; 3: the sun-exposed side of interior fruit; 4: the shaded side of interior fruit. Solid bar represents Gala whereas empty bar is Smoothee. Each bar is the mean of 4 replicates with standard error. All the measurements were made from samples taken at noon.

Fig. 2. Superoxide dismutase, SOD (A), ascorbate peroxidase, APX (B), monodehydroascorbate reductase, MDAR (C), dehydroascorbate reductase, DHAR (D), and glutathione reductase, GR (E), and catalase, CAT (F) in the peel of apple fruit acclimated to light exposure in the tree canopy. Peel type/fruit position was the same as in Fig. 1. Solid bar represents Gala whereas

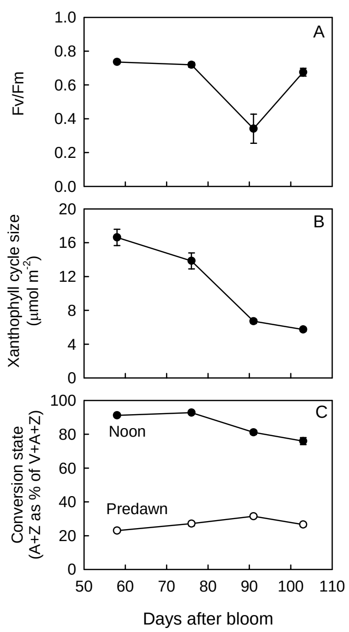
empty bar is Smoothee. Each bar is the mean of 4 replicates with standard error. All the measurements were made from samples taken at noon.

2. Seasonal changes of xanthophyll cycle pool size and activities of antioxidant enzymes

The maximum quantum efficiency (*Fv/Fm*) was significantly lower on the third sampling date than the other three (Fig. 3A). This decrease reflects the photooxidative damage caused by high fruit temperature under high light.

As the season progresses, xanthophyll cycle pool size decreased (Fig 3B). In early August, xanthophyll cycle pool size decreased to about 1/3 of that at 2 months after bloom. Most of the xanthophyll cycle pool was present as violaxanthin and zeaxanthin at midday (Fig. 3C). Interestingly, even after overnight dark adaptation, violaxanthin and zeaxanthin still accounted for about 25 to 30% of the xanthophyll cycle pool size (Fig. 3C). This suggests that apple fruit retain significant amount of zeaxanthin and violaxanthin overnight so that they are ready to dissipate excess absorbed light when sunlight shines on the fruit the next day.

Both superoxide dismutase and dehydroascorbate reductase activities decreased over the sampling period (Fig. 4A, D) whereas ascorbate peroxidase activity increased (Fig. 4B). Monodehydroascorbate reductase activity increased initially, and then leveled off (Fig. 4C). Glutathione reductase activity did not show any significant change (Fig. 4E). Catalase activity remained stable except for a drop on the third sampling date (Fig. 4F). The significant decrease of catalase activity on the third sampling date is very likely caused by the high fruit temperature as catalase is very sensitive to oxidative damage.



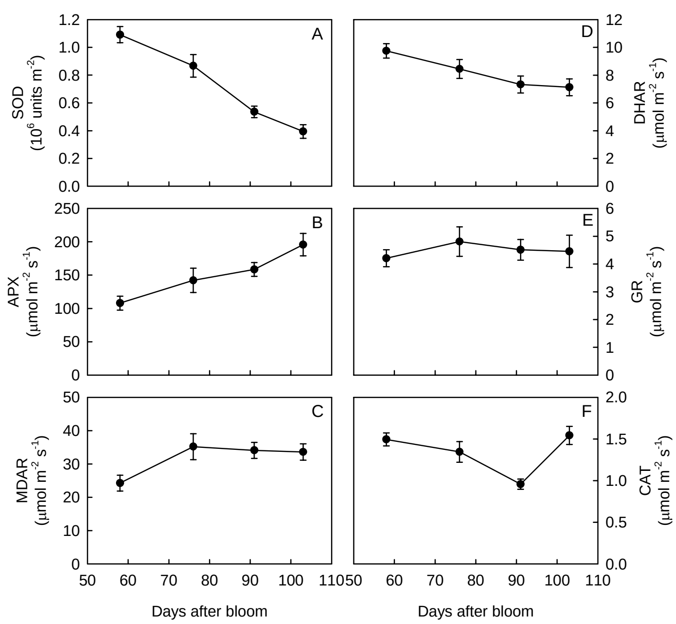


Fig. 3 (Left). Seasonal changes of maximum photosystem II efficiency, Fv/Fm (A), xanthophyll cycle pool size (B), and conversion of violaxanthin (V) to zeaxanthin (Z) and antheraxanthin (A) in the sun-exposed peel of Gala fruit in a Washington orchard. Fv/Fm was measured after overnight dark adaptation. Xanthophyll cycle pool size and conversion were measured at both midday and after overnight dark adaptation. Each point is mean with standard error of 10 fruit for Fv/Fm or 6 fruit for xanthophyll cycle pool size and conversion state.

Fig. 4 (Right). Seasonal changes of superoxide dismutase, SOD (A), ascorbate peroxidase, APX (B), monodehydroascorbate reductase, MDAR (C), dehydroascorbate reductase, DHAR (D), and glutathione reductase, GR (E), and catalase, CAT (F) in the sun-exposed peel of Gala fruit in a

Washington orchard. Samples were taken at midday (1:00 to 2:30PM). Each point is mean with standard error of 6 fruit.

3. Xanthophyll cycle pool size and composition in the peel of 'Gala' fruit peel in response to nitrogen supply

At noon, efficiency of excitation transfer (F_v'/F_m') of the sun-exposed peel was higher in the low N treatment than in the medium or high N treatments (Fig. 5A). This indicates that fruit in the low N treatment has a lower thermal dissipation capacity than the medium or high N fruit. Photochemical quenching coefficient did not differ between fruits in different N treatments (Data not shown). The photosystem II operating efficiency, which represents the proportion of absorbed light used in photochemistry, was higher in the peel of low N fruit compared with medium-N or high N fruit (Fig. 5B). Maximum quantum efficiency (F_v/F_m) of fruit peel after overnight dark adaptation was similar across the N treatments (Data not shown).

On any given sampling date, xanthophyll cycle pool size was larger in the high N fruit than in the low N fruit (Fig. 5C). This corresponds well with the thermal dissipation capacity as indicated by efficiency of excitation transfer (Fig. 5A). As the season progressed, xanthophyll cycle pool size in all N treatments decreased, which is also correlated well with the seasonal change of thermal dissipation capacity of the fruit. At noon, over 95% of the xanthophyll cycle pool in the sun-exposed side was present in the form of zeaxanthin (Z) and antheraxanthin (A) regardless of N treatments (Fig. 5C). This indicates that xanthophyll cycle operates at its full capacity and the xanthophyll cycle pool size may become limiting. Chlorophyll concentration was also higher in the high N treatment than in the medium or low N treatments and decreased as fruit developed (Data not shown).

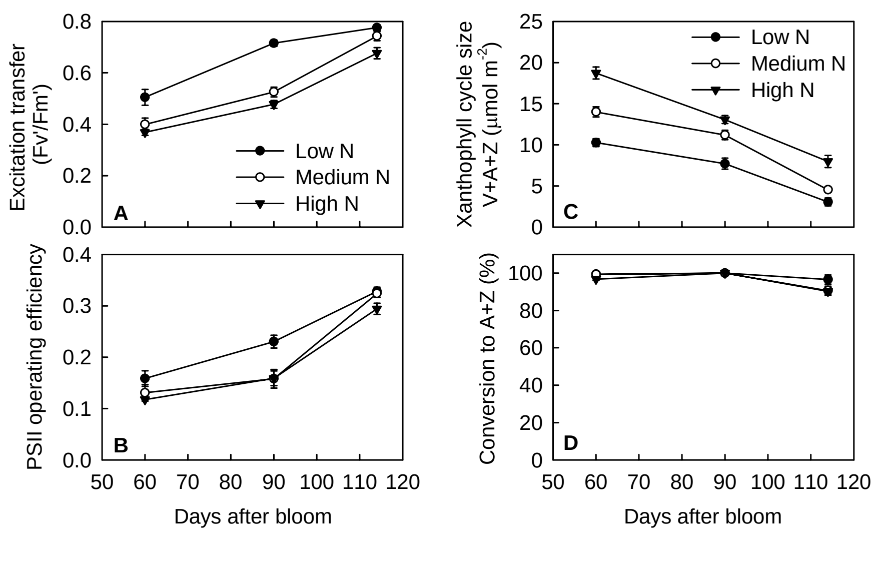


Fig. 5. Efficiency of excitation transfer, Fv'/Fm' (A), photosystem II operating efficiency (B), xanthophyll cycle pool size, Violaxanthin + Antheraxanthin + Zeaxanthin (C), and conversion of xanthophyll cycle to antheraxanthin and zeaxanthin (D) in the peel of 'Gala' fruit in response to nitrogen supply.

We have also measured antioxidant enzymes and metabolites in response to nitrogen treatments. Activities of all the enzymes in the Mehler peroxidase reaction, including superoxide dismutase and enzymes in ascorbate-glutathione cycle were higher in the high N fruit than in low N fruit (Data not shown). This indicates that high N fruit have a high capacity for detoxifying reactive oxygen species generated via direct electron transfer to oxygen under high light conditions.

4. Effects of evaporative cooling, partial root zone drying and deficit irrigation on xanthophyll cycle pool size and composition of 'Fuji' fruit peel.

Across all the treatments, both chlorophyll concentration and xanthophyll cycle pool size decreased as the season progressed (Fig.6), which is similar to what we found on 'Gala' fruit. The difference is that the decrease is less pronounced perhaps because 'Fuji' is a late season variety. Evaporative cooling tended to increase whereas both partial root-zone drying and deficit irrigation tended to decrease both chlorophyll concentration and xanthophyll cycle pool size (Fig. 6). Conversion of xanthophyll cycle to antheraxanthin and zeaxanthin was over 90% at noon with no difference between the treatments. No significant difference was found in maximum quantum efficiency (F_v/F_m) among control, evaporative cooling, partial root-zone drying and deficit irrigation treatments (Data not shown).

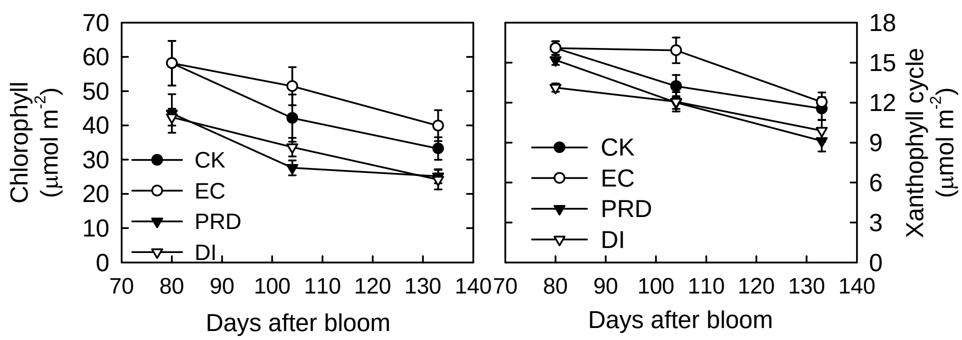


Fig. 6. Effects of evaporative cooling (EC), partial root-zone drying (PRD) and deficit irrigation (DI) on chlorophyll concentration and xanthophyll cycle pool size in the sun-exposed peel of 'Fuji' fruit.

5. Compare xanthophyll cycle pool size and composition between sunburn susceptible and sunburn tolerant apple cultivars.

Red Delicious is less susceptible to sunburn than Cameo and Golden Delicious. The xanthophyll cycle pool size was higher in Red Delicious than in Golden Delicious, but Cameo had a similar size of xanthophyll cycle pool size with Red Delicious (Fig 7C). In contrast, fruit peel lutein content was highest in Red Delicious and lowest in Golden Delicious with Cameo in the middle (Fig 7D). Red Delicious maintained the highest maximum quantum efficiency (Fv/Fm) whereas Golden Delicious had the lowest Fv/Fm with Cameo in between throughout the growing season (Fig 7A), which appears to correlate well with the susceptibility of these three cultivars to sunburn. The lower Fv/Fm measured on July 19 was associated with a fruit peel temperature of 43°C. Chlorophyll content showed variety-specific changes as the season progressed: sharp and linear decrease in Golden Delicious, relatively constant in cameo, and moderate decrease in Red Delicious (Fig 7B).

6. UV-screening and reflective coating on xanthophyll cycle size and composition.

Throughout the growing season, coating 'Gala' fruit with Surround didn't affect the xanthophyll cycle pool size whereas screening UV light with a polycarbonate decreased xanthophyll cycle pool size.

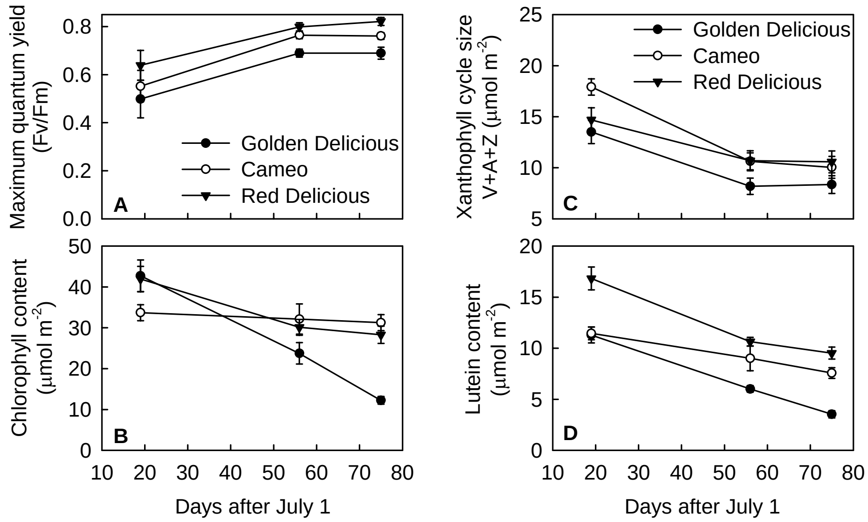


Fig 7. Seasonal changes of maximum quantum efficiency (A), chlorophyll content (B), xanthophyll cycle pool size (C), and lutein content (D) of sun-exposed fruit peel in Golden Delicious, Cameo and Red Delicious in WA.

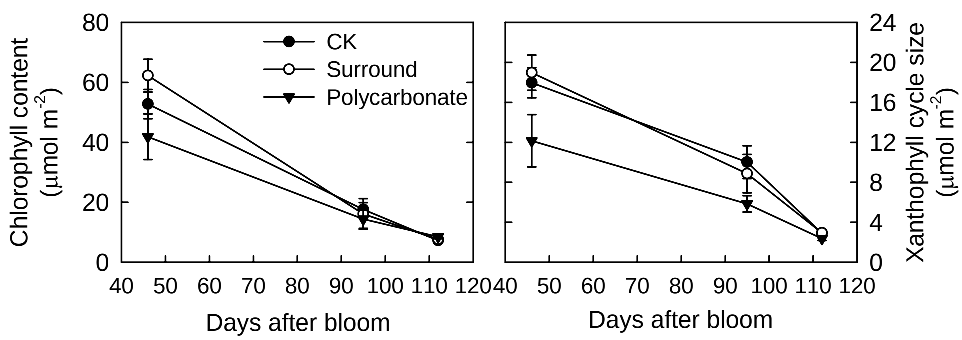


Fig 8. Seasonal changes of chlorophyll content and xanthophyll cycle pool size of sun-exposed peel of 'Gala' fruit in response to Surround treatment and UV-screening treatment via polycarbonate.

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