

Comparative effects of kaolin and calcium carbonate on apple fruit surface temperature and leaf net CO₂ assimilation

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Abstract

The use of reflective particles on apple fruits has been suggested as a tool to diminish its thermal charge and thus mitigate stress effects caused by high temperature. The products effectiveness is often expressed in terms of damaged fruit, however it is influenced by the sensitivity of the variety, growing conditions and application method. Therefore, it is necessary to quantify the temperature of the fruits surface (FST) achieved according to the residue deposited to determine the degree of thermal protection for each product. Moreover, the residue deposited in the canopy enhances the albedo on the leaves reduces the availability of incidental light. The goal of this work was to evaluate the efficiency of reflective particles in the reduction of superficial temperature of the fruits and its effect on net CO₂ assimilation rate (A_{CO_2}) in apple trees (*Malus domestica*, Borkh). The fruits were treated with: one, two and four (1X; 2X and 4X) applications of kaolin (treatment K) or calcium carbonate (treatment C) at 2.5% P/V and untreated fruit as control. The residue effect on A_{CO_2} was evaluated in individual leaves at 2X concentration. Both products showed a thermic protective effect as compared with control. The protection degree depended upon the concentration. The highest temperature of the control was 49.8 °C and in these conditions kaolin was significantly more effective than carbonate, the thermic reduction was 1.9 °C vs. 1.3 °C at 2X and 2.5 °C vs 2.1 °C at 4X for kaolin and carbonate, respectively. At 1X there were no statistical differences between products. In turn A_{CO_2} is only negatively affected under low intensities of light (< 700 $\mu\text{moles m}^{-2} \text{s}^{-1}$ of PAR). Higher radiation levels compensate the shading effect over leaves and also the maximum A_{CO_2} (A_{max}) was not affected.

Key words: Heat stress, infrared thermography, leaf photosynthesis, *Malus domestica*, particle film, sunburn protection

Introduction

Stress caused by high temperatures, together with direct solar radiation in apple [*Malus sylvestris* (L.) Mill var. *domestica* (Borkh.) Mansf.] orchards develop a physiopathy called sunburn which significantly affects the quality of the fruits (Schrader *et al.*, 2001; Racskó *et al.*, 2005). Schrader *et al.* (2001) stated two types of sunburn according to fruit surface temperature: “sunburn necrosis” caused by necrosis in superficial tissues when temperature of the fruits surface (FST) reaches 51-52 °C and “sunburn browning”, which is a yellow or bronze stain on the fruit skin caused by lower temperatures (46-49 °C) and the presence of ultraviolet radiation (UV). The damage severity depends mainly of the temperature reached by the fruit surface and also it is influenced by own sensitivity of cultivar, the weather conditions and handling practices (Parchomchuk *et al.*, 1996; Schrader *et al.*, 2003).

The use of reflective particles on fruits has been suggested as a tool to diminish its thermic charge because it reduces the incident radiation that can be absorbed by the fruits (Glenn *et al.*, 2002; 2003, 2009; Wunsche *et al.*, 2004) and thus reduce the incidence of sunburn (Glenn *et al.*, 2002; Gindaba *et al.*, 2005; Wand *et al.*, 2006; Colavita, 2011). The nature of particles generally comprises minerals of high reflectivity, among them kaolin and calcium carbonate are alternatives of relative low cost, safe use, low erosion, reduced particle size and water diffusion ability (Glenn *et al.*, 2003). When the effectiveness of the products

is expressed in terms of damaged fruit, it is influenced by the sensitivity of the variety, growing conditions and application method (Glenn *et al.*, 2002; Erez *et al.*, 2004). Therefore, to determine the degree of thermal protection for each product it is necessary to quantify the FST achieved according to the residue deposited on the fruit surface. Moreover, the residue of the products over the leaf enhances its albedo and reduces the absorption of photosynthetically active radiation. There exists discrepancy over the effect of the use of reflective particles in relation to the rate of net assimilation of CO₂. Some authors have reported positive effects under saturated light conditions on apple trees leaves (Glenn *et al.*, 2001) and pomegranate (*Punica granatum* L.) (Melgarejo *et al.*, 2004). However, Gindaba *et al.* (2007) in apple trees and Rosati *et al.* (2007) in walnuts (*Juglans regia* L.) and almond trees [*Prunus dulcis* (Mill.) DA Webb] did not find a significant effect on the net CO₂ assimilation rate when applying kaolin even under saturated conditions. Contrarily, Schupp *et al.* (2002) reported in Fuji apple trees - treated with three kaolin applications at 3% (m/V) - a smaller productivity and size of fruits, they attributed this to a lesser light availability to develop photosynthesis. In the same line of thought, Wunsche *et al.* (2004b) and Le Grange *et al.* (2004) observed a decreased rate of net CO₂ assimilation in apple trees treated with reflective particles and they associated it with the effect of a lower light incidence.

The aim of the present work was to measure the efficiency of each reflective particle films of kaolin and carbonate calcium in

the reduction of superficial temperature of the fruits and its effect on the net CO₂ assimilation rate in apple trees.

Materials and methods

Degree of thermic protection: The grade of thermic protection was evaluated according to the quantity of residue settled on the surface and to the nature of the protecting product at the moment of highest warming. Two commercial products were used for the development of reflective particle films: one based on kaolin processed up to a diameter lower than 2 μm (K) and another one based on calcium carbonate (97%) and zinc oxide (3%) (C). At harvest time, 70 fruits of similar size were picked from an apple orchard *cv.* Granny Smith, trained as palmette leader in the experimental farm of National University of Comahue (38°50'44"S, 68°04'11"W). They were divided in seven groups each with ten repetitions following a completely random design and then were applied the following treatments: one (1X), two (2X) and four (4X) applications of kaolin or carbonate at 2.5% *m/V* (treatment K or treatment C: 1X; 2X and 4X, respectively) and other fruits with no treatment as (control). The applications were carried out with a hand atomizer and an intermediate drying time was allowed between each one. To allow more homogeneous conditions, all fruits were exposed to the sun simultaneously, in a broad area, far away from the influence of vegetation and the increase of surface temperature was measured through the record of six thermic images from 11:15 h until 15:30 h, moment in which the temperature over them begun to decline. The thermic images were taken using an infrared camera, Handy Thermo TVS-200 Inframetrics thermal IR video imagery system (Nippon Avionics co. LTD, Tokyo, Japan). The focal distance used was 1.0 m, and spatial resolution was 1.68 mm. The emissivity value was adjusted in 0.95 (Hellebrand *et al.*, 2001). In each fruit a circular area of 111 pixels was selected (approximate radius = 1 cm), centered on that pixel of highest temperature identified in the fruit. The average temperature of each area identified (FST) was considered as the highest thermic increase reached by the fruit at the moment of taking measurements. A second grade function, FST *vs.* time (t) was adjusted, and the maximum temperature reached for each treatment (FST_{max}) was determined from the derivative of the function corresponding.

During the test, the air temperature ranged between 21.5 and 29.0 °C, the relative humidity fell from 40 to 25% and the wind did not exceed 0-2 m/s. The highest hourly average radiation was of 624.8 watts m⁻² and was registered at 13:00 PM. For each fruit the residue deposited on the surface (SR) was removed and quantified as μg/cm².

The means differences between treatments were assessed by the least significant difference (LSD) or Fisher test.

Light response: The work was carried on apple trees *cv.* Royal Gala of about 9 years old, homogeneous in height and strength trained as palmette leader, with a plantation frame of 4.0 x 1.5 m, and mechanical irrigation. Five weeks after comercial harvest seven exposed shoots of similar size, strength and orientation distributed in adjacent trees were selected. In middle zone of each bud, three completely expanded adjacent leaves, were randomly treated with: kaolin 5.0 % (m/V) (treatment K); carbonate 5.0% (m/V) (treatment C); and no treatment as control (Control). In

order to avoid accidental contamination during the applications, those marked leaves were protected with aluminum foil. The applications were performed 7 days before taking measurements. The measurement of gas interchange was taken in each leaf, between 09:30 AM (1 h 56 min after sunrise) and 13:30 PM (midday sun), using CIRAS-1 Analyzer; PP System, (Haverhill, MA, USA). The camera settings were programmed with 350 ppm CO₂; 25 °C and 7 levels of photosynthetically active radiation (PAR): 0; 100; 200; 400; 600; 800 and 1000 μmoles m⁻² s⁻¹, respectively. The net CO₂ assimilation (A_{CO_2}) was registered in μmoles m⁻² s⁻¹. Recorded data were fitted to non rectangular hyperbola (Lambers *et al.*, 1998), using a no linear adjustment procedure (Statistica, StatSoft, Inc Tulsa, USA); (Equation 1),

$$A_{CO_2} = \{ \Phi x PAR + A_{max} - [(\Phi x PAR)^2 - 4\Phi x PAR x \Theta x A_{max}]^{1/2} \} / (2\Theta - R_0) \quad \text{Ec. 1}$$

where, A_{CO_2} is the rate of net CO₂ assimilation (μmoles m⁻² s⁻¹); Phi (Φ), is the apparent quantum efficiency and relates CO₂ mass fixed by mole of incidental photon, which graphically is associated to initial slope of the curve; PAR, photosynthetically active incidental radiation (μmoles m⁻² s⁻¹); A_{max} , theoretical rate of net CO₂ assimilation in saturated conditions of PAR; Tita (Θ), is the curvature factor, which varies between 0 and 1. When Θ has values near to 1, the curve changes directly from its initial gradient determined by Φ to a *plateau* (Blackman curve) determined by A_{max} and dark respiration (Ogren, 2003). For those values smaller to 1, the curve becomes a hyperbola. R_0 represents the dark respiration of the leaf under PAR=0 conditions. PAR in $A_{CO_2}=0$ value is the point of light compensation (CP₁) for this level of CO₂.

After gas interchange measurements, leaves were cut and SR was assessed. The leaf area determined with the leaf area meter Li-Cor 3100 (Li-Cor Inc., Lincoln, NE, USA).

Products reflectance: Spectral reflectance of products was assessed in the range of photosynthetically active radiation (PAR) (400-700 nm) over a flat, homogeneous surface of 1.0 cm thickness, using a spectral radiometer FieldSpec™ Analytical Spectral Devices, Inc. (ASD) 5335 Sterling Drive; Suite A Boulder; CO 80301 USA. As a reference, we used the polytetrafluoroethylene standard (PTFE), *Spectralon*™, with a 99% of reflectance (Weidner *et al.*, 1981). All measurements were taken under conditions of natural midday light, over three different surface points, using an average of 5 marks in each point.

Results and discussion

Thermic protection degree: The surface temperature of treated fruit during the warming period was lowered as compared to control for all used concentrations (Table 1). Untreated fruits, exposed to sun and without protective effect of vegetation reached a FST of 49.8 °C, a value of about 20 °C higher than the surrounding air. Measurements of the temperature on exposed fruits surface in each tree have established differences from 10 to 15 °C higher to the air temperature (Thorpe, 1974; Shrader *et al.*, 2003), this issue highlights the importance of thermic influence of the canopy over the fruits surface temperature. Furthermore the thermal distribution in the canopy is not uniform. Stajanko *et al.* (2003) reported FST variations inside the canopy from 24 to 40 °C in Gala apple trees. However, even in fruits isolated

Table 1. Maximum temperature estimated during test (FST_{max}) at the moment of further warming; temperature decrease (TD) of treatments C (calcium carbonate) and K (kaolin) as compared to Control. Amount of residue recovered of fruit surface (SR). Air temperature: 28.9°C

Parameters	Treatment						
	Control	C			K		
		1X	2X	4X	1X	2X	4X
TSF _{max} ^z (°C)	49.8a	48.7b	48.5c	47.7d	48.7bc	47.8d	47.1e
TD (°C)	0	-1.1	-1.3	-2.1	-1.0	-1.9	-2.5
SR ^y (mg/cm ²)	0	250.9a	385.6b	526.2c	204.7a	349.9b	554.3c

^zTSF_{max} is maximum of function $TSF = a.t^2 + b.t + c$ which represents observed values in each treatment.

^yDifferent letters in row indicate significant differences by LSD Fisher test ($P < 0.05$).

from the canopy influence, our results indicate a protective effect achieved by the application of reflective particles. At the moment of highest warming, treatment C significantly lowered surface temperature in relation to control by 1.1; 1.3 and 2.1 °C for 1X, 2X and 4X concentrations, respectively; treatment K: 1.1; 2.1 and 2.5 °C for 1X, 2X and 4X concentrations, respectively (Table 1). Nevertheless, the scale of thermic decrease coincides only partially with the ones of other authors who have worked with the fruits in the plant. Glenn *et al.* (2002), observed a range of thermic decrease with kaolin of 1.4 to 3.4 °C in “Fuji” apple trees and subsequently of 4.0 to 4.3 °C in “Scarlet Delicious” when the average surface residue in these fruits was of 548 and 508 µg cm⁻², respectively. In our working conditions, using comparable amounts of kaolin (554.3 µg cm⁻²), we estimated a maximum decrease up to 2.5 °C on treated fruits in relation to control (Table 1). Gindaba *et al.* (2005) reported temperature decreases of 1.5 to 6.4 °C in “Cripps Pink” and “Royal Gala” apples when two applications of kaolin (6% and 3% *m/V*) were applied at interval of 5 weeks.

SR on the fruits was significantly different between treatments, however, it did not increase in linear proportion with the number of applications at 2.5% *m/V* (Table 1). Application conditions can modify the quantity of settled residue. Glenn *et al.* (2002) also reported important differences in the fruits residue collected with the same number of kaolin applications. This suggests the need to index the effect of reflective particles to the amount of current residue by surface unit, at the moment of taking the measurements.

Light response: The observed values of A_{CO_2} , corresponding to PAR are shown in Fig. 1. Data was represented through a non

rectangular hyperbola (Lambers *et al.*, 1998); with R²: 0.93; 0.96 and 0.92 for K, C and control, respectively. When the PAR level was lower than 700 µmoles m⁻² s⁻¹, A_{CO_2} in Control was higher as compared to treatments. At PAR = 200 µmoles m⁻² s⁻¹ the value of A_{CO_2} reached by the control (4.03 µmoles CO₂ m⁻² s⁻¹) was significantly higher than the treatment K (2.79 µmoles CO₂ m⁻² s⁻¹), while treatment C showed intermediate values, but no significant differences ($P < 0.01$) (Table 2). These results suggest a smaller availability of incident light on treated leaves, which shows itself with detriment to leaf A_{CO_2} to low radiation intensities. The differences between kaolin and carbonate are consistent with the greater reflectance of the first. Reflectance data of products expressed as percentages of the reference pattern, showed a maximum reflectance of 85.5% for kaolin vs. 83.4% for carbonate. The differences of reflectance between products in the PAR range fluctuated between 1.7 and 4.4 % according to the wave length, being kaolin more reflective (Fig. 2). At PAR values higher to 700 µmoles m⁻² s⁻¹ for treatment C and 900 µmoles m⁻² s⁻¹ for treatment K, the shading effect seems to be compensated. A_{CO_2} values on leaves treated with reflective particles film were increased at high levels PAR, though not showing significant statistical differences. This is consistent with non differences observed ($P < 0.05$) in the analysis of parameter A_{max} of equation 1, which represents the maximum assimilation of CO₂ when light is not limiting (Table 2). These results differ with Wunsche *et al.* (2004a), who reported a decrease of 20% of PAR absorption in apple leaves treated with kaolin, with negative effects on net CO₂ assimilation rate. Gindaba *et al.* (2007) reported a negative effect on net CO₂ assimilation rate in apple leaves treated with kaolin at the end of the growing stage. In almond trees, 2000 µmoles m⁻² s⁻¹ of PAR were not sufficient to reach saturation on kaolin treated leaves at 6% (*m/V*), suggesting a strong shading effect on these reflective particles (Rosati *et al.*, 2006). Quantum efficiency, Φ , is related to the ability of electronic transport in the leaf (Iacono *et al.*, 2004). In this work, Φ in control (0.030) was significantly higher as compared to treatments C (0.023) and K (0.020) whereas differences between C and K were not statistically significant ($P < 0.05$) (Table 2). These values reinforce the idea that the reflection of one part of incident radiation negatively affects the photosynthetic rate in individual leaves at low PAR intensities. Similar results in apples were reported by Le Grange *et al.* (2004). The parameter Θ represents the photosynthetic efficiency above the linear sector determined by Φ (Ogren, 1993) and there exists evidences that Θ decreases with the increase of carboxylative ability of Rubisco (Ogren and Evans, 1993). In our working conditions no significant differences were observed in relation

Table 2. Light response of apple leaves cv. Royal Gala, treated with kaolin (K) and calcium carbonate (C), at 5.0% (*m/V*), on apparent quantum efficiency (Φ), theoretical rate of net CO₂ assimilation in saturated conditions of PAR (A_{max}), the curvature factor (Θ); dark respiration (R_o) and luminic compensation point (CP_L)

Treatment	Residue (mg cm ⁻²)	Parameter ^z				
		Φ	A_{max} (µmoles CO ₂ m ⁻² s ⁻¹)	Θ	R_o (µmoles CO ₂ m ⁻² s ⁻¹)	CP_L^y (µmoles RFA m ⁻² s ⁻¹)
K	377.3 a	0.020 a	10.8 a	0.88 a	1.02 a	58.7 a
C	396.4 a	0.023 a	10.5 a	0.83 a	1.24 a	54.7 a
Control	0	0.030 b	11.4 a	0.88 a	1.39 a	46.9 a

^zThe parameters were calculated based on the average of the values generated for each sheet, based on the model:

$$A = \{ \Phi \times RFA + A_{max} - [(\Phi \times RFA)^2 - 4\Phi \times RFA \times \Theta \times A_{max}]^{1/2} \} / (2\Theta - R_o)$$

^y CP_L was calculated at $A = 0$.

Different letters in columns indicate significant differences by LSD Fisher test ($P < 0.05$).

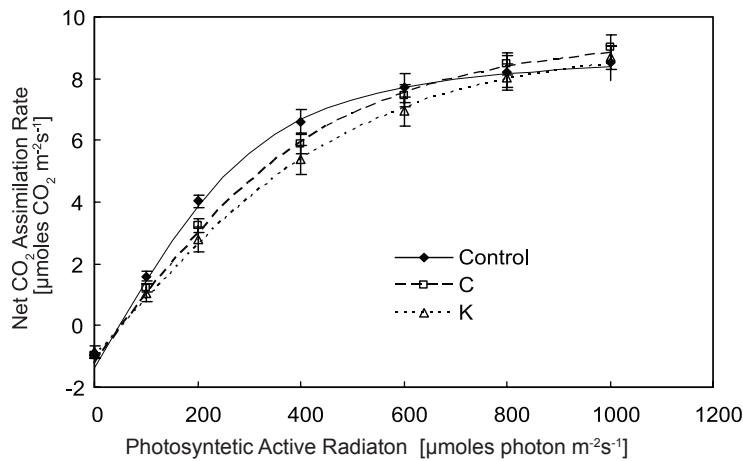


Fig. 1. Net CO₂ assimilation (A_{CO_2}) in response to increasing of photosynthetically active radiation (PAR) in apple leaves cv. Royal Gala, treated with kaolin 5.0% (m/V) (K) and carbonate 5.0% (m/V) (C). The points in each treatment represent the mean \pm standard error.

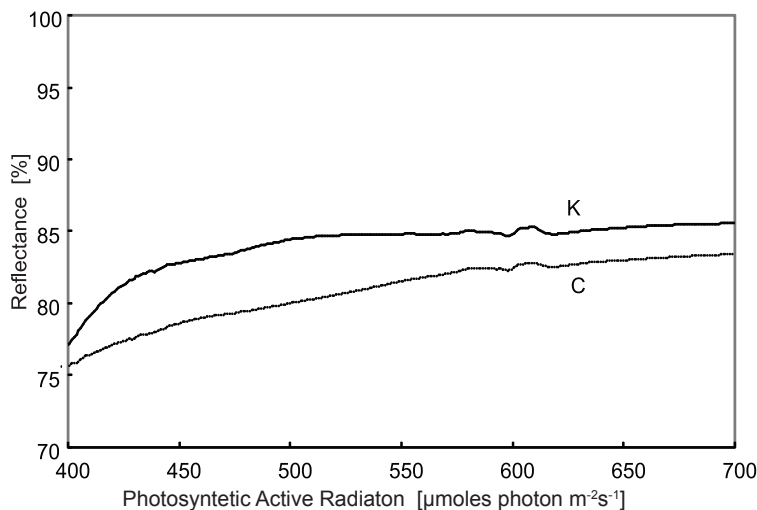


Fig. 2. Percent reflectance by wavelength, of a flat and uniform layer of K and C, within the spectral range of the photosynthetically active radiation.

to this parameter between treatments (Table 2). Lambert *et al.* (1998) demonstrated that both dark respiration (R_o) and light compensation point (CP_L) are higher in plants that are acclimatized to high light intensities compared to plants acclimatized to low light intensities. In this work, the effect of the low light availability caused by the treatments was not enough to establish significant differences over R_o and CP_L during the experiment (Table 2).

Based on our results we can deduce that there exists a thermoprotector effect attributable to treatments. The degree of protection depends on the reflectance and the amount of residue settled on the surface. The amount of the residue settled on the fruit surface is affected by products and application conditions but not directly proportional to the number of applications of one determined concentration. The treatment difference was observed from one residue accumulation threshold. The degree of thermic protection reached in this test suggests the need of combining the use of reflective particle films together with other cultural practices that can contribute to decrease thermic charge on fruits, such as irrigation, training and pruning. In individual leaves A_{CO_2} seems to affect negatively only to low light intensities ($PAR < 700 \mu\text{moles m}^{-2} \text{s}^{-1}$), higher radiation levels compensate the effect of shadowing on the leaves even though not modifying the maximum ability of net CO₂ assimilation.

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