Foliar S-ABA application in a warm apple production area does not reduce sunburn in 'Granny Smith'

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Highlights:

- Foliar application of S-ABA had no effect on sunburn in apple under South African conditions.
- Repeated applications of S-ABA reduced fruit size and TSS.
- S-ABA had no positive effect on peel antioxidant capacity contrary to previously reported results in Japan.

Abstract

Sunburn is a physiological disorder that affects the visual quality of apple (*Malus Domestica*) fruit. In South Africa, producers estimate yield losses of up to 50% in green cultivars due to sunburn damage. Recent research in Japan has found that foliar application of abscisic acid (S-ABA) reduced sunburn in apples by up to 30%. The aim of this study was to examine the effect of S-ABA application on the occurrence of sunburn and on other fruit quality parameters in ~~‘~~Granny Smith~~’~~, the apple cultivar that suffers most sunburn under South African conditions. Trials were conducted over three growing seasons from 2010-2011 to 2012-2013 in a ‘Granny Smith’ orchard in Grabouw, South Africa. S-ABA was applied at concentrations between 250-1000 ppm and various timings during summer (from November until harvest in March/April). A representative scaffold branch on both sides of the tree was strip picked at commercial harvest resulting in samples of at least 100 fruit per tree for sunburn, fruit color, and red blush assessment. A sub-sample of 20 fruit was randomly selected and used to determine average fruit size, fruit firmness, and internal quality. The application of S-ABA did not decrease sunburn incidence and severity under South African growing conditions. Unlike in the Japanese study, S-ABA application decreased the peel concentration of total antioxidants, total phenolics and reduced ascorbic acid whilst increasing oxidized ascorbic acid. These changes suggest that S-ABA either downregulated synthesis of antioxidants or caused increased oxidative stress. Consistent with S-ABA application possibly causing stress, leaf necrosis was observed when S-ABA was applied just prior to periods of high temperature. Consistent with its physiological role in plants, S-ABA application decreased stomatal conductance and thereby also decreased the net carbon assimilation and transpiration rates, while the stem water potential was increased due to reduced water loss. Concomitant with the decrease in carbon assimilation, there was a significant

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reduction in fruit size,~~ as well as~~ total soluble solids, and titratable acidity with repeated S-ABA applications. S-ABA did not affect fruit maturity. Our results suggest that application of S-ABA to reduce sunburn is ineffective under South African conditions and therefore not recommended. The divergent response to S-ABA in Japan and South Africa may relate to the higher irradiance in South Africa or to the bagging of fruit after S-ABA- application and re-exposure to sunlight before harvest in Japan.

1. Introduction

Sunburn is a physiological disorder of apples that causes discoloration of the fruit surface thereby affecting fruit visual quality (Felicetti and Schrader, 2009). It is a serious problem in many warm apple-growing regions of the world, located in semi-arid climatic zones such as Washington State (USA), South Africa, Israel, Chile and Australia. These production regions experience high air~~mbient~~ temperatures and solar radiation during the growing season. In South Africa, producers estimate yield losses of up to 50% due to sunburn damage (Wand et al., 2006). High ambient temperatures and excessive ir~~solar ~~radia~~tion~~nce cause overheating of the fruit surface, leading to the development of sunburn (Chen et al., 2008; Wünsche et al., 2004a). Three types of sunburn have been identified namely, sunburn necrosis, sunburn browning (Schrader et al., 2001) and photo-oxidative sunburn (Felicetti and Schrader, 2008; Schrader et al., 2003).

Protection against sunburn includes natural defense mechanisms within the fruit peel and cultural practices adopted by growers. Fruit peel sunburn protection is offered by physiochemical properties of the peel such as homogeneity, thickness and composition of the epicuticular wax layer and the amount of hair on the skin surface, (Wünsche et al., 2004a), heat shock proteins (Ferguson et al., 1998; Ritenour et al., 1998), photoprotective pigments (Felicetti and Schrader,

2009) and antioxidant compounds (Yuri et al., 2010). Cultural practices that have been adopted to reduce sunburn include evaporative cooling (Evans, 2004; Gindaba and Wand, 2005), the application of kaolin-based reflective particle films (Gindaba and Wand, 2005; Glenn et al., 2002; Wünsche et al., 2004b) and installation of shade nets above the orchards (Gindaba and Wand, 2005; Iglesias and Alegre, 2006; Smit, 2007). However, the complexity of the underlying physiological mechanisms and environmental processes leading to sunburn has made it difficult to come up with a single solution to the problem (Wünsche et al., 2004a).

Abscisic acid (ABA) is a plant growth regulator that is involved in the signaling and regulation of plant responses to water stress (Kim and van Iersel, 2011). ABA is produced in the roots and transported to the shoots via the xylem where it regulates stomatal closure in leaves and fruit thereby controlling ~~transpirational~~ water loss by transpiration~~from the plant~~ (Zhang and Davies, 1987). Due to the high cost of production and chemical instability, ABA has, until recently, had few applications in horticulture (Cao et al., 2013). However, recent advances in methods of production have made it economically feasible to use ABA in horticultural production (Peppi et al., 2006). ABA has been used in horticulture for the prevention of blossom end rot in tomato plants grown under low calcium conditions by improving calcium transport in xylem, resulting in higher tissue calcium concentrations (De Freitas et al., 2011; De Freitas et al., 2014). The increase in xylar water and calcium transport to the fruit seems to be due to decreased xylar water flow to the leaves in response to ABA application (Ref?). ABA has also been used as a physiological antitranspirant to improve shelf life and quality of pot plants by causing a delayed initiation of wilting (Astacio and Van Iersel 2011; Kim and Van Iersel, 2011; Waterland et al., 2010). Another application has been for the maintenance of postharvest quality and improving the color of red grapes by stimulating anthocyanin production (Cantín et al., 2007; Hiratsuka et al., 2001; Peppi et al., 2006), as a fruit thinner in apples and pears (Greene, 2012; Greene et al., 2011) and to prime young apple trees so as to provide dehydration protection under conditions of water stress (Tworkoski et al., 2011). Recent research in Japan has found that the foliar application of S-ABA (biologically active form of abscisic acid or [5-(1-hydroxy-2,6,6-trimethyl-4-oxo-2-cyclohexen-1-yl)-3-methyl-2,4-pentadienoic acid] reduced sunburn incidence in ‘Tsugaru’, ‘Sensyu’, ‘Yataka’ and ‘Fuji’ apples by up to 30% (Iamsub et al., 2009; Iamsub et al., 2008). The S-ABA application was associated with increased antioxidant levels, thereby purportedly alleviating oxidative damage caused by high ambient temperatures and irradiance (Iamsub et al., 2009).

According to Racsko and Schrader (2012), further research is needed to determine whether S-ABA will also reduce sunburn in climatically harsher regions (such as South Africa) that experience higher summer temperatures and higher levels of irradiance. Therefore, the aim of this research was to examine the effect of S-ABA application on the incidence of sunburn in ‘Granny Smith’ apples under South African conditions. ‘Granny Smith’ is the cultivar that shows the most sunburn in South Africa because of its green peel color???. Considering the various effects of ABA on plant physiology and eco-physiology, which may prove beneficial or detrimental in a commercial apple production setup, other fruit quality parameters were also assessed. Gas exchange and plant water status were assessed to confirm S-ABA responses in ‘Granny Smith’ apple while peel chemical composition was analyzed to determine the previously observed effects of S-ABA on apple pigmentation and antioxidant capacity (Iamsub et al., 2009).

2. Materials and Methods

2.1 Study site and plant material

Trials were conducted over three growing seasons from 2010-2011 to 2012-2013 in a ‘Granny Smith’ orchard at Disseldraai farm in Grabouw, South Africa (Latitude: 34°16’S; Longitude: 19°03’E, Alt 266 m). The region has a Mediterranean-type climate receiving most of its rainfall in winter (June-August). Summers (October-March) tend to be hot and d~~with high d~~aily maximum temperatures~~–~~ in excess of 35 °C are common. M~~and m~~ost summer days are~~ly~~ cloudless~~ days ~~ with high irradiance levels which exceed 30 MJ/m^2/d at times ~~during the growing season ~~(Figure 1, 2 and 3). Tsukuba, Japan, where the original research was done (Iamsub et al., 2009), has a warm temperate climate with significant cloud cover, lower irradiance levels and lower maximum temperatures during the growing season (Figures 2 and 3). The Disseldraai orchard was planted in 1993 on M793 rootstock at a spacing of 4.5 x 2.0 m in an east - west row orientation. The tree rows form~~s~~ a continuous fruiting wall with an average height of 4 m and a canopy width of about 3 m. Normal commercial cultural practices of irrigation, pest management and fertilization were followed. ‘Granny Smith’ is the second most cultivated apple cultivar in South Africa, making up 18.4 % of the total area planted (Hortgro, 2014) and it is highly susceptible to sunburn (Fouché et al., 2010). Therefore, considering the substantial economic losses due to sunburn, it is an important cultivar to conduct sunburn research on.

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2.2 Treatments and experimental design

Four~~The~~ treatments were established in the 2010-2011 growing season~~ consisted~~ each with 10 single tree replicates with buffer trees between treatment plots and buffer rows between treatment rows in a randomized complete block design. The treatments comprised ~~of~~ an untreated control, while 250 ppm S-ABA (Valent BioSciences, Libertyville, IL, USA), 500 ppm S-ABA, and 1000 ppm S-ABA were applied monthly on 13 Jan. 2011 (92 days after full bloom - DAFB, 11 Feb. 2011 (121 DAFB), and 11 Mar. 2011 (149 DAFB). In the 2011-2012 growing season, treatments consisted of an untreated control, and 1000 ppm S-ABA applied at full bloom (FB, 17 Oct. 2011), monthly on 04 Dec. 2011 (48 DAFB), 05 Jan. 2012 (80 DAFB), 03 Feb. 2012 (109 DAFB) and 03 Mar. 2012 (140 DAFB) or whenever ambient temperature was forecasted to exceed 32 °C on 19 Jan. 2012 (94 DAFB), 13 Feb. 2012 (119 DAFB) and 09 March 2012 (144 DAFB) with a minimum gap of two weeks between applications. The arbitrary 32ºC threshold for application was chosen considering the daily temperatures typically experienced during summer in this region and based on fruit peel temperature measurements done ~~by WJ Steyn (pers. comm.)~~ in previous seasons. The aim was to set a threshold that would balance the need for sunburn control with logistic and practical considerations of application and cost. Treatments in 2012-2013 consisted of an untreated control, and 400 ppm S-ABA applied 40 DAFB on 24 Nov. 2012 or 80 DAFB on 03 Jan. 2013. All treatments were applied during early mornings and slow-drying conditions using a motorized knapsack sprayer at a rate of 2 L tree^{-1} (2 222 L ha^{-1}) as a full cover spray. ~~In all three seasons, ten single tree replicates per treatment were used with buffer trees between treatment plots and buffer rows between treatment rows in a randomized complete block design.~~ Different trees were used in each season. Gas exchange

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measurements (Section 2.6.1~~methodology reported below~~) indicated that S-ABA application affected tree physiology and can thus be assumed to have been taken up effectively.

The treatments during the first year (2010-2011) were adapted from Iamsub et al. (2008) and Iamsub et al. (2009), who originally reported the reduction of sunburn incidence after application of S-ABA. For the second season (2011-2012), the early application at full bloom was done to potentially increase xylem water supply to the fruit as reported by De Freitas et al. (2014) in tomato. The treatment before the heat wave was done with the aim to increase the antioxidant capacity in the fruit peel just before photothermal stress – upregulation of antioxidant capacity was the mechanism reported for sunburn reduction by Iamsub et al. (2008) and Iamsub et al. (2009). For the 2012-2013 growing season treatments were imposed after full bloom and at lower concentration to avoid the negative effects observed in the 2011-2012 growing season on fruit size when sprayed at 1 000 ppm at FB.

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2.3 Sunburn and red blush assessment

A representative scaffold branch on both the north and south facing sides of trees was strip picked at commercial harvest resulting in samples of at least 100 fruit per tree. Sunburn incidence and severity was assessed using the Schrader and McFerson scale (Schrader et al., 2003) where 0 represented no sunburn, 1 to 4 refer to increasing levels of sunburn browning, and 5 signifies sunburn necrosis. Samples were assessed for occurrence of red blush using a color chart (Set A 32, Deciduous Fruit Board, South Africa).

2.4 Fruit quality

A sub-sample of 20 fruit was randomly selected and used to determine average fruit size (equatorial diam?). Fruit firmness was measured using a fruit texture analyzer at the equatorial region on opposite sides of the fruit (Güs, GS 20, Strand, South Africa). Percentage starch conversion was measured using the iodine test and starch conversion chart (Unifruco Research Services, Bellville, South Africa). Sub-sample fruit were pooled for juice extraction. A hand-held refractometer (Model N1, Atago, Tokyo, Japan) was used to measure total soluble solids concentration (TSS). Titratable acidity (TA) was determined by titrating 5 g of juice with 0.1 M NaOH with an automated titrator (Model 719 S, Metrohm AG, Hersiau, Switzerland) and expressed as percentage of malic acid.

2.5 Peel chemical composition

Peel chemical composition analysis was done in the 2012-2013 growing season on fruit collected 10 and 20 days after spraying (DAS) following both application dates. Ten fruit representative of those on tagged branches were randomly selected per tree replicate and fruit peel tissue separated from the flesh. The collected peel tissue samples were flash frozen in liquid nitrogen before being finely milled. Milled samples from each tree replicate were pooled together and then stored in a freezer at -80 °C.

2.5.1 Ascorbic acid and glutathione

High-performance liquid chromatography (HPLC) was used for ascorbic acid and glutathione analysis. The analysis was done in three phases according to the method by Jooste (2012) with some minor modifications. Firstly, in the extraction phase, 10 ml of extraction buffer was added to 2.0 g of fresh frozen sample in a centrifuge tube. The extraction buffer was made up of 3% metaphosphoric acid (MPA), 1.0 mM ethylenediaminetetracetic acid (EDTA) and 2% insoluble polyvinylpolypyrrolidone (PVPP). The mixture was vortexed and left to stand for 15 min, after which 1.8 ml was pipetted into a 2.0 ml eppendorf tube and then centrifuged at 20 000 x g for 15 min. Secondly, the analysis phase was done in two steps. The first step involved direct analysis of clean supernatant to identify the reduced forms of ascorbic acid and glutathione. The second step involved reducing ascorbic acid and glutathione from oxidized forms using 20 µl of 400 mM DL-dithiothreitol (DTT) to measure the total ascorbic acid and glutathione in each sample. Oxidized ascorbic acid and glutathione were determined by subtracting reduced values from the total. HPLC analysis was done on Agilent Series 1100 HPLC system (Agilent Technologies, Inc., Waldbronn, Germany) using a photodiode array detector and C_{18} 5-µm stationary phase column protected by a 4.6 mm x 12.5 mm guard cartridge (Zorbax SB-C18, Agilent, USA). A known concentration of ascorbic acid and glutathione was used to identify retention peaks. The final phase involved quantification of ascorbic acid and glutathione and this was done using Chemstation for LC 3D systems software (Rev. B.10.03 (204), Agilent Technologies, Inc., Waldbronn, Germany). Results were expressed as micrograms per gram fresh weight ($\mu g.g^{-1}$ FW).

2.5.2 Total antioxidant capacity

Total antioxidant analysis was done using the 2,2-diphenly-1-picryl hydrazyl (DPPH) radical scavenging assay according to the method by Karioti et al. (2004) with some modifications. The method involved the addition of 40 mL distilled water to 0.5 g fresh frozen sample. The mixture was vortexed at 10 000 rpm for 10 min. In triplicates, 15 µl of clean sample was added into eppendorf tubes together with 735 µl of methanol and 750 µl of 0.1 mM DPPH. The tubes were covered in aluminum foil and incubated at room temperature for 30 min. Absorbance was measured at 517 nm on the spectrophotometer (Cary 50 Bio, Varian, Australia (PTY) Ltd, Melbourne, Australia). The spectrophotometer was blanked with 100% methanol and a standard curve of ascorbic acid from 0 to 2.0 mM was constructed. Total antioxidant activity was expressed as milligrams per gram fresh weight (mg.g^{-1} FW).

2.5.3 Total phenolics

Total phenolics were measured by the Folin Ciocaltreu's (FC) phenol colourimetric method. The method involved the addition of 5 mL of 80% ethanol to 1.0 g fresh frozen sample in a 50 ml centrifuge tube. The mixture was then ground finely with Ultra Turrax. A magnetic rod was added to the mixture and the tubes were placed in a fridge at 4 °C where they were constantly stirred using a magnetic stirrer. In triplicates, 10 µl of the sample, 40 µl of 80% ethanol and 450 µl of 0.1M FC reagent were added to plastic cuvettes. After 5 min 500 µl of 5.6% Na_2CO_3 was added to the cuvette and the mixture vortexed after which it was left to stand for 90 min before readings were taken. The spectrophotometer was blanked with 80% ethanol and a standard curve of Gallic acid (mg/L) from 0 to 2.0 was constructed. Total phenolic concentration was expressed as Gallic acid equivalent (GAE) in milligrams per gram fresh weight (mg.g^{-1} FW).

2.6 Tree eco-physiological status

Tree eco-physiological measurements were taken in the 2010-2011 season to confirm S-ABA uptake by leaves and again in 2012-2013 to assess the duration of the effect of S-ABA. Tree eco-physiological measurements were done on cloud free days, 7 DAS at 123 DAFB for the 2010-2011 growing season to determine whether the S-ABA application had an effect on tree physiology and could therefore be expected to have been taken up by the plant. Since these measurements indicated that S-ABA had physiological effects, no measurements were done in the 2011-2012 growing season. In the 2012-2013 growing season, it was decided to do measurements at 10 and 20 DAS, at 40 and 80 DAFB, to determine the duration of the S-ABA effects.

2.6.1 Gas exchange

Gas exchange measurements (net CO_2 assimilation rate, ~~stomatal conductance~~, transpiration rate) and the stomatal conductance were taken using an infrared gas analyzer~~,~~ (Model LI-6400: ~~(~~Li-Cor, Lincoln, Nebraska, USA). Flow rate was set at 500 µmol s^{-1}, reference carbon dioxide concentration at 380 ppm, leaf temperature at 25 °C and the photosynthetic photon flux density at 1500 µmol m^{-2} s^{-1}. Measurements were taken on sun-exposed leaves on the northern side of the tree. Two fully expanded leaves of the same age, size and health were used per treatment replication. Leaves were sampled from shoulder height and all readings were taken between 08:00 and 11:30.

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2.6.2 Plant water status

The effect of S-ABA on ~~plant water status~~tree water stress was assessed by measuring the midday stem/xylem water potential using a pressure bomb (Model 600:~~,~~ PMS Instrument Co, USA). For each tree replicate, two mature and healthy leaves of the same age, size and proximity to the stem, from inside the canopy were bagged for at least one hour in silver reflective bags to equilibrate the leaf and the stem's xylem water potential before readings were taken. Leaves were sampled from shoulder height and all readings were taken between 12:00 and 13:30.

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2.6.3 Fruit surface temperature

Fruit surface temperature was measured using a hand-held infrared thermometer (Raynger MX4, Raytek Corporation, Santa Cruz, USA) between 14:00 and 14:30 on the sun-exposed part of the fruit. Five fruit per treatment replication were tagged for measurements.

2.8 Statistical analysis

Data were analyzed using the General Linear Models (GLM) procedure of SAS Enterprise Guide 3.0 (SAS Institute Inc., 2004, Cary, NC, USA). Where significant differences occurred ($p \leq 0.05$), means were separated by the Least Significant Difference (LSD). Linear contrasts were fitted where applicable.

3. Results

3.1 Sunburn incidence

The application of S-ABA at different concentrations and timings did not decrease the incidence and severity of the different sunburn types in 'Granny Smith' under South African growing conditions in any of the three growing seasons (1). In fact, in the 2011/12 growing season, the application of 1000 ppm S-ABA at full bloom (FB) and preceding ambient temperature exceeding 32 °C increased sunburn necrosis compared to the control (Table 1). Sunburn incidence was also increased by the application of 1000 ppm S-ABA at FB (Table 1). Only in the 2011-2012 season, was there a significant (P value?) contrast between S-ABA applications and control, with S-ABA application resulting in higher sunburn necrosis compared to the control (Table 1).

3.2 Fruit quality

There were no significant differences (P values?) in fruit weight and diameter between the treatments in the 2010-2011 growing season (Table 2). Repeated applications of S-ABA caused a reduction in fruit size compared to the control in the 2011-2012 growing season (Table 2). Fruit size was not affected by S-ABA applications in the 2012-2013 growing season (Table 2). S-ABA application did not affect the incidence of red blush and fruit firmness in any of the seasons (Table 2).

Repeated applications of S-ABA reduced TSS in the 2010-2011 and 2011-2012 (Table 3). TSS decreased linearly with increasing S-ABA concentration in the 2010-2011 growing season (Table 3). Single applications of S-ABA had no effect on TSS in the 2011-2012 and 2012-2013 growing seasons (Table 3). A similar trend was observed for TA with repeated applications of S-ABA at high concentration (1 000 ppm) causing a significant reduction in TA in the 2010-2011 and 2011-2012 seasons (Table 3). However, repeated applications at lower concentrations (250 and 500 ppm) and single applications of S-ABA had no effect on TA in any of the seasons (Tables 3). There was a linear decrease in TA with increasing S-ABA concentration (Table 3).

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3.3 Peel chemical composition

The application of S-ABA at 40 DAFB did not affect oxidized and total ascorbic acid or oxidized, reduced and total glutathione concentrations in the fruit peel at both 10 and 20 DAS (Table 4). Reduced ascorbic acid was significantly lower in the treated peel compared to the control at 10 DAS, but there was no difference in reduced ascorbic acid concentration between the treatments and control at 20 DAS (Table 4). At 80 DAFB, total ascorbic acid and oxidized ascorbic acid concentrations were significantly higher in the treated peel compared to the control at 10 DAS, but there was no difference at 20 DAS (Table 5). There were no significant differences in oxidized, reduced and total glutathione concentrations, and reduced ascorbic acid concentration between the S-ABA and control in the fruit peel at both 10 and 20 DAS (Table 5). When sprayed at 40 DAFB, S-ABA significantly reduced the concentration of total antioxidants and total phenolics when measured 10 DAS, but not at 20 DAS (Table 6). There were no significant differences in concentration of total antioxidants and phenolics when S-ABA was applied at 80 DAFB, measured at both 10 and 20 DAS (Table 6).

3.4 Tree eco-physiological status

Net carbon assimilation rate, stomatal conductance and leaf transpiration rate were significantly reduced by S-ABA application compared to the control in the 2010-2011 growing season (Table 7). The application of S-ABA at 40 DAFB in 2012-2013 had no effect on net carbon assimilation rate, stomatal conductance or leaf transpiration rate when measured at 10 and 20 DAS (Table 8). Net carbon assimilation rate, stomatal conductance, and leaf transpiration rate were significantly reduced compared to the control by S-ABA application at 80 DAFB when measured at 10 DAS, but not at 20 DAS (Table 8). There were no significant differences in fruit surface temperature between the treatments in any of the seasons (Tables 7, 8). Stem water potential was significantly higher for the 500 ppm S-ABA treatment when measured 7 DAS during the 2010-2011 growing season (Table 7). During the 2012-2013 growing season, stem water potential was significantly higher at 10 DAS for both 40 and 80 DAFB sprays, but not different compared to the control when measured at 20 DAS (Table 8). Leaf necrosis occurred when S-ABA was applied at 1 000 ppm preceding a heat wave (data not shown).

4. Discussion

Exogenous applications of S-ABA have been reported to decrease sunburn in ‘Tsugaru’, ‘Sensyu’, ‘Yataka’ and ‘Fuji’ apples by up to 30% at rates of 100 ppm to 800 ppm in Japan (Iamsub et al., 2008; Iamsub et al., 2009). In contrast to the Japanese studies, the application of S-ABA did not reduce sunburn incidence and severity in ‘Granny Smith’ under the Mediterranean-type growing conditions in South Africa~~n conditions~~. Significant effects of S-ABA application on carbon assimilation, plant water status, and fruit size rule out that the lack of effect on sunburn could be due to inadequate absorption of S-ABA. Although these leaf parameters do not imply that S-ABA was taken up or exported to the fruit, the effects observed on antioxidants in the apple peel (Tables 4-6) ~~do~~ suggest that the fruit~~ did take~~ took up or received S-ABA through the vascular system. Net carbon assimilation rate, stomatal conductance, and leaf transpiration were significantly reduced by S-ABA application when measured 7 and 10 DAS. Similar results have been reported *in vivo* with potato leaves (Baricevic and Stopar, 1994). The reduction in net carbon assimilation rate, stomatal conductance, and leaf transpiration rate can be explained by the stomatal closure induced by S-ABA application. The response did not extend beyond 10 DAS and no significant differences remained at 20 DAS. Baricevic and Stopar (1994) similarly reported that net gas exchange returned to normal 8-16 days after applications. Plant water status represented by the midday stem water potential was significantly improved immediately after application due to reduced tree water loss and the effect diminished over time. Plant water status also returned to normal at 20 DAS~~,~~ suggesting that the effect on plant water status was temporary. Improved plant water status with S-ABA application has also been reported in young apple trees (Tworkoski et al., 2011).

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Iamsub et al. (2009) ascribed the decrease in sunburn to the higher levels of antioxidants observed in the peel of treated apples. S-ABA did not increase the levels of antioxidants in ‘Granny Smith’ peel but rather reduced total antioxidant capacity, total phenolics, and reduced ascorbic acid whilst increasing oxidized ascorbic acid. Sunburn incidence in the Japanese study was very high at around 70% but no information was provided on how sunburn was graded. Sunburn incidence in the control of our study was ca. 45%, 31% and 39% in the 2010-2011, 2011-2012 and 2012-2013 growing seasons, respectively, measured on the Schrader and McFerson scale (Schrader et al., 2003). Careful reading of the Materials and Methods section of Iamsub et al. (2009) indicate that fruit of all treatments were enclosed in white bags after S-ABA treatment. Bagging of fruit is used in Japan to provide protection against pests but also to obtain improved fruit finish (Sharma et al., 2014). The fruit become etiolated within the bags and upon re-exposure to light shortly before harvest, show a considerable spike in anthocyanin accumulation (Ju, 1998). The authors did not indicate whether the bags were removed shortly before harvest for the fruit to develop red color, but it seems likely judging from the good red color of the fruit (Iamsub et al., 2009). This re-exposure of shaded fruit may explain the high incidence of sunburn despite the considerably lower irradiance levels typically measured at Tsukuba, Japan compared to Grabouw, South Africa (Figure 3).

Free radical formation in response to ABA application seems to serve as triggers for plants to develop tolerance of various stresses (Jiang and Zhang, 2001). In work on maize seedlings, Jiang and Zhang (2001) found that treatment with low ABA concentrations (10 to 100 μM) upregulated the antioxidative defense response, but at 1 mM, excessive free radical generation led to oxidative damage. Oxidative stress and the excessive production of free radicals have been implicated in the development of sunburn in apple (Racsko and Schrader, 2012). Under less stressful conditions (lower irradiance and lower vapor pressure deficits - VPD), such as those experienced at Tsukuba, Japan (Figures 2 and 3), increased concentrations of antioxidants after application of S-ABA may have led to the reduction in sunburn as reported. The bagging of apple fruit after S-ABA application in the experiment of Iamsub et al. (2008) may have prevented excessive free radical formation by lowering light incident on the fruit peel. Conversely, application of S-ABA on a day preceding a heat wave increased the incidence of sunburn at harvest. Evidently, a spike in free radical production in response to S-ABA application, may upregulate antioxidant capacity and thereby provide protection against oxidative damage in the fruit peel. However, under more stressful conditions or at higher S-ABA concentrations, excessive free radical formation can exacerbate oxidative damage.

The increase in sunburn when applied at FB may be due to increased sensitivity of young fruit to S-ABA sprays. S-ABA application at full bloom as a thinning agent caused quite severe leaf yellowing in ‘McIntosh’ apple (Greene et al., 2011). The yellowing increased in severity from 50 to 1000 ppm S-ABA. Leaf necrosis was observed when 1 000 ppm S-ABA was applied during periods of high temperatures. Under stress conditions caused by high temperatures, high concentrations of S-ABA can cause phytotoxicity leading to leaf damage (Ibrahim and Jaafar, 2013; Waterland et al., 2010). Also, S-ABA application has been shown to result in increased leaf temperatures due to the reduction in transpiration, which may lead to leaf necrosis (O’Donoughue et al., 2011). No defoliation (although not assessed) was observed in response to S-ABA application. Greene et al. (2011) also did not mention the occurrence of leaf drop despite the severe yellowing of leaves. Therefore, increased exposure of fruitlets to sola radiation~~unlight~~ was probably not the cause of the increase in sunburn after S-ABA application at FB. Also, at this point in the growing season, ambient temperatures we~~a~~re still low and fruit surface

Commented [S12]: Consistency!

temperature rarely reached~~s~~ the threshold for sunburn development. The sunburn sensitivity of apples increases during fruit development (Racsko and Schrader, 2012). This may relate to the finding that the sensitivity of ‘Rosemarie’ pear peel to photothermal stress increased during fruit development (Steyn et al., 2009).

Repeated applications of S-ABA caused a reduction in fruit size. Fruit size also decreased with increasing concentration of S-ABA applied. The reduction in fruit size in our work can be explained by the fact that the application of S-ABA causes stomatal closure resulting in a reduction of CO_2 assimilation rate, consistent with the observations by ~~(~~Greene~~,~~ (2012) and~~;~~ Waterland et al~~.,~~ (2010). During the 2012-2013 growing season, no effect on stomatal closure was observed at 40 DAFB. At 80 DAFB, the application of S-ABA caused stomatal closure until 10 DAS, but not observed at 20 DAS. Therefore, the effect on stomatal conductance was not lasting and inconsistent in our study. An increase in fruit size has been reported from single applications of S-ABA when used as a fruit thinner at petal drop (Greene, 2012). However, this fruit size increase ~~in fruit size~~ is due to the thinning effect of S-ABA at petal drop which leaves fewer fruit to compete for photoassimilates.

The application of S-ABA did not affect fruit maturity judging from fruit firmness and starch conversion data. This is in agreement with previous research (Greene et al., 2011), although an improvement in firmness when S-ABA was applied at petal drop has been observed (Greene, 2012). While single applications of S-ABA had no effect on TSS and TA in this study and as reported by Greene et al., (2011), we found that repeated applications of S-ABA reduced TSS and TA. The S-ABA-induced closure of stomata may decrease photosynthesis and this may result in lower levels of photoassimilates in the fruit, hence lower TSS and TA (Waterland et al., 2010). In contrast, the application of S-ABA at petal drop, which had a thinning effect has been found to increase TSS (Greene, 2012). Fewer fruit were left on the tree after thinning, therefore more photoassimilates are available to the remaining fruit.

5. Conclusion

This research, done over three seasons, showed that foliar S-ABA application at different timings and application rates did not decrease sunburn incidence and severity in 'Granny Smith' apple under South African conditions. In fact, the application of high concentrations of S-ABA at FB and before hot days increased sunburn incidence and severity, possibly by inducing excessive free radical formation with resultant oxidative damage. The positive effect on antioxidant levels observed in Japan was not obtained in our study. However, in Japan, apple fruit were enclosed in white bags immediately after S-ABA application, which may have decreased the extent of free radical formation and facilitated the upregulation of antioxidant defense systems. Further studies on the effect of S-ABA and water stress on the formation of free radicals, oxidative damage and expression of antioxidative enzymes at different irradiance levels and a range of temperatures may provide more insight into how these factors relate to sunburn in apple peel.

The effects of S-ABA at high concentration on stomatal aperture caused a transient decrease in gas exchange that led to a reduction in fruit size and negative effects on internal fruit quality. However, there was a positive effect on tree water status in terms of stem water potential due to stomatal closure and the reduction in transpiration. Based on our results, the application of S-ABA to reduce sunburn is not recommended in regions similar to South Africa such as Washington State USA, Israel, Chile and Australia that experience high temperature and high irradiance.

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Table 1. The effect of S-ABA application on sunburn incidence (SI) , percentage of fruit showing sunburn browning (SB) or necrosis (SN), and sunburn severity of sunburnt 'Granny Smith' apples (SVB) at Disseldraai, Grabouw, during the 2010-2011, 2011-2012 and 2012-2013 seasons.

Treatment	SI (%)	SB (%)[y]	SN (%)[x]	SVB[w]
2010-2011				
1. Control	45.2 ns	28.5 ns	16.7 ns	2.99 ns
2. 250 ppm S-ABA	44.3	29.4	14.9	2.86
3. 500 ppm S-ABA	43.7	28.9	14.8	2.97
4. 1000 ppm S-ABA	35.9	23.4	12.5	2.96
Pr>F	0.1519	0.2165	0.5020	0.8852
S-ABA vs Control	0.2888	0.6316	0.2410	0.6813
S-ABA linear	0.0661	0.0664	0.3865	0.6345
2011-2012				
1. Control	30.7 b	29.4 ab[z]	1.31 b	1.87 ns
2. 1000 ppm S-ABA at FB	38.4 a	35.4 a	3.11 a	1.88
3. 1000 ppm S-ABA monthly	29.9 b	27.3 b	2.60 ab	2.00
4. 1000 ppm S-ABA (>32 ℃)	31.0 b	27.5 b	3.54 a	2.11
Pr>F	0.0384	0.0481	0.0373	0.4701
S-ABA vs Control	0.3466	0.7850	0.0080	0.3695
2012-2013				
1. Control	39.0 ns	37.5 ns	1.50 ns	1.68 ns
2. 400 ppm S-ABA, 40 DAFB	43.7	42.2	1.51	1.70
3. 400 ppm S-ABA, 80 DAFB	39.5	37.0	2.55	1.71
Pr>F	0.2090	0.1999	0.4548	0.9581
S-ABA vs Control	0.2930	0.4423	0.5228	0.7835

[z]Means with a different letter differ significantly at the 5% level (LSD), ns - Not significant, [y]1-4 score, (Schrader and McFerson sunburn chart), [x]5 score, (Schrader and McFerson sunburn chart), [w]1-5 score, with 1 having least sunburn and 5 the most severe (Schrader and McFerson sunburn chart).

Table 2. The effect of S-ABA application on fruit size, flesh firmness and red blush of 'Granny Smith' apples at Disseldraai, Grabouw, during the 2010-2011, 2011-2012 and 2012-2013 seasons.

Treatment	Fruit weight (g)	Fruit diameter (mm)	Fruit firmness (kg)	Red blush[y]
2010-2011				
1. Control	137.8 ns	67.5 ns	8.31 ns	15.2 ns
2. 250 ppm S-ABA	138.8	68.0	8.29	17.6
3. 500 ppm S-ABA	135.4	67.2	8.28	15.5
4. 1000 ppm S-ABA	129.0	66.0	8.27	13.6
Pr>F	0.0857	0.1628	0.9770	0.5360
S-ABA vs Control	0.3052	0.5523	0.6981	0.8690
S-ABA linear	0.0189	0.0315	0.8372	0.1592
2011-2012				
1. Control	164.8 a[z]	71.1 a	8.66 ns	0.93 ns
2. 1000 ppm S-ABA at FB	163.0 a	71.6 a	8.77	0.62
3. 1000 ppm S-ABA monthly	143.6 b	67.2 b	8.63	0.51
4. 1000 ppm S-ABA (>32 °C)	152.5 b	70.1 a	8.60	0.67
Pr>F	0.0008	0.0001	0.4930	0.2583
S-ABA vs Control	0.0082	0.0299	0.8973	0.0699
2012-2013				
1. Control	139.8 ns	68.5 ns	8.01 ns	0.88 ns
2. 400 ppm S-ABA ≈ 40 DAFB	146.2	69.5	8.05	0.70
3. 400 ppm S-ABA ≈ 80 DAFB	143.9	69.4	7.90	0.92
Pr>F	0.7549	0.7237	0.4659	0.2687
S-ABA vs Control	0.4885	0.4287	0.7517	0.5896

[z] Means with a different letter differ significantly at the 5% level (LSD).

ns-Not significant.

[y] 1-12 score, with 1 having no blush and 12 the most blush (A32 chart).

Table 3. The effect of S-ABA application on internal quality of 'Granny Smith' apples at Disseldraai, Grabouw, during the 2010-2011, 2011-2012 and 2012-2013 seasons.

Treatment	TSS (°Brix)	TA (%)	Starch conversion (%)
2010-2011			
1. Control	11.1 a^z	0.66 ab	22.8 ns
2. 250 ppm S-ABA	11.0 b	0.67 a	19.9
3. 500 ppm S-ABA	10.7 bc	0.63 bc	22.5
4. 1000 ppm S-ABA	10.6 c	0.62 c	23.7
Pr>F	0.0060	0.0390	0.3223
S-ABA vs Control	0.0085	0.1515	0.6570
S-ABA linear	0.0150	0.0180	0.0985
2011-2012			
1. Control	11.8 a^z	0.96 a	19.2 ns
2. 1000 ppm S-ABA at FB	11.8 a	0.93 a	19.7
3. 1000 ppm S-ABA monthly	10.8 b	0.80 b	20.1
4. 1000 ppm S-ABA (>32 ℃)	11.0 b	0.84 b	21.6
Pr>F	0.0001	0.0003	0.2097
S-ABA vs Control	0.0002	0.0015	0.1782
2012-2013			
1. Control	11.8 ns	0.79ns	17.0 ns
2. 400 ppm S-ABA ≈ 40 DAFB	11.7	0.84	19.1
3. 400 ppm S-ABA ≈ 80 DAFB	11.6	0.85	18.6
Pr>F	0.4119	0.4065	0.5252
S-ABA vs Control	0.2555	0.1891	0.2746

^{z}Means with a different letter differ significantly at the 5% level (LSD).

ns - Not significant.

Table 4. The effect of S-ABA application at 40 DAFB on the concentration of total ascorbic acid, total glutathione, oxidized ascorbic acid, oxidized glutathione, reduced ascorbic acid and reduced glutathione in the peel of 'Granny Smith' apples at Disseldraai, Grabouw, during the 2012-2013 season.

Treatment	Total ascorbic acid ($\mu g.g^{-1}$ FW)	Total glutathione ($\mu g.g^{-1}$ FW)	Oxidized ascorbic acid ($\mu g.g^{-1}$ FW)	Oxidized glutathione ($\mu g.g^{-1}$ FW)	Reduced ascorbic acid ($\mu g.g^{-1}$ FW)	Reduced glutathione ($\mu g.g^{-1}$ FW)
10 DAS						
1. Control	1070.3 ns	65.3 ns	325.2 ns	15.9 ns	745.1 a[z]	49.4 ns
2. 400 ppm S-ABA, 40 DAFB	841.0	58.6	288.3	15.5	552.7 b	43.1
Pr>F	0.2044	0.1404	0.7814	0.7673	0.0095	0.0999
20 DAS						
1. Control	912.8 ns	75.7 ns	207.1 ns	12.4 ns	705.6 ns	63.3 ns
2. 400 ppm S-ABA, 40 DAFB	947.7	74.0	230.8	13.3	716.9	60.7
Pr>F	0.4716	0.9368	0.3618	0.2725	0.6120	0.8003

[z] Means with a different letter differ significantly at the 5% level (LSD).

ns - Not significant.

Table 5. Effect of S-ABA application at 80 DAFB on the concentration of total ascorbic acid, total glutathione, oxidized ascorbic acid, oxidized glutathione, reduced ascorbic acid and reduced glutathione in the peel of 'Granny Smith' apples at Disseldraai, Grabouw, during the 2012-2013 season.

Treatment	Total ascorbic acid (μg.g^{-1} FW)	Total glutathione (μg.g^{-1} FW)	Oxidized ascorbic acid (μg.g^{-1} FW)	Oxidided glutathione (μg.g^{-1} FW)	Reduced ascorbic acid (μg.g^{-1} FW)	Reduced glutathione (μg.g^{-1} FW)
10 DAS						
1. Control	750.9 b^{z}	92.1 ns	189.3 b	17.6 ns	561.6 ns	74.5 ns
2. 400 ppm S-ABA, 80 DAFB	842.9 a	90.8	316.4 a	24.4	526.5	66.4
Pr>F	0.0491	0.8974	0.0499	0.2334	0.6001	0.3137
20 DAS						
1. Control	717.4 ns	83.2 ns	194.4 ns	15.7 ns	523.0 ns	67.5 ns
2. 400 ppm S-ABA, 80 DAFB	675.8	83.4	162.4	15.7	513.4	67.7
Pr>F	0.7006	0.7669	0.9631	0.5330	0.6996	0.8173

^{z}Means with a different letter differ significantly at the 5% level (LSD).

ns - Not significant.

Table 6. The effect of S-ABA application at 40 DAFB on the conentration of total antioxidants and total phenolics in the peel of 'Granny Smith' apples at Disseldraai, Grabouw, during the 2012-2013 season.

Treatment	40 DAFB		80 DAFB	
	Total antioxidants ($mg.g^{-1}$ FW)	Total phenolics ($mg.g^{-1}$ FW)	Total antioxidants ($mg.g^{-1}$ FW)	Total phenolics ($mg.g^{-1}$ FW)
10 DAS				
1. Control	16.3 a[z]	385.8 a	19.0 ns	306.5 ns
2. 400 ppm S-ABA	13.6 b	342.5 b	16.0	313.1
Pr>F	0.0131	0.0032	0.1449	0.8066
20 DAS				
1. Control	15.5 ns	309.5 ns	14.6 ns	228.8 ns
2. 400 ppm S-ABA	11.8	312.4	12.8	172.2
Pr>F	0.0995	0.8629	0.4396	0.0846

[z] Means with a different letter differ significantly at the 5% level (LSD).

ns - Not significant.

Table 7. The effect of S-ABA application at 123 DAFB on net carbon assimlation rate (*A*), stomatal conductance(g_s), transpiration rate (*E*), fruit surface temperature and stem water potential of 'Granny Smith' apples at Disseldraai, Grabouw, during the 2010-2011 season.

Treatment	*A* ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	g_s ($mol \cdot m^{-2} \cdot s^{-1}$)	*E* ($mmol \cdot m^{-2} \cdot s^{-1}$)	FST (°C)	SWP (MPa)
1. Control	16.7 a^z	0.38 a	6.3 a	36.8 ns	-1.59 b
2. 250 ppm S-ABA	13.8 b	0.23 b	4.4 b	37.3	-1.61 b
3. 500 ppm S-ABA	12.4 b	0.19 b	3.7 b	35.6	-1.28 a
4. 1000 ppm S-ABA	13.7 b	0.19 b	4.2 b	37.2	-1.42 ab
Pr>F	0.0188	0.0001	0.0002	0.1127	0.0470
S-ABA vs Control	0.0033	0.0001	0.0001	0.1902	0.1752
S-ABA linear	0.8955	0.2813	0.7673	0.8554	0.1299

^{z}Means with a different letter differ significantly at the 5% level (LSD).

ns-Not significant.

Table 8. The effect of S-ABA application 10 and 20 days after spraying (DAS) at 40 and 80 days after full bloom (DAFB) on net carbon assimilation rate (*A*), stomatal conductance (g_s), transpiration rate (*E*), fruit surface temperature (FST) and stem water potential (SWP) of 'Granny Smith' apples at Disseldraai, Grabouw, during the 2012-2013 season.

Treatment	*A* (µmol·m^{-2}·s^{-1})	g_s (mol·m^{-2}·s^{-1})	*E* (mmol·m^{-2}·s^{-1})	FST (°C)	SWP (MPa)
40 DAFB, 10 DAS					
1.Control	16.2 ns	0.14 ns	3.47 ns	31.8 ns	-1.74 b[z]
2. 400 ppm S-ABA	12.9	0.14	3.96	31.4	-1.28 a
Pr>F	0.1624	0.8346	0.3607	0.4704	0.0182
40 DAFB, 20 DAS					
1.Control	14.5 ns	0.38 ns	4.77 ns	35.3 ns	-0.78 ns
2. 400 ppm S-ABA	11.1	0.20	3.59	35.0	-0.77
Pr>F	0.1666	0.0940	0.0948	0.9579	0.7804
80 DAFB, 10 DAS					
1.Control	19.1 a[z]	0.41 a	4.97 a	39.8 ns	-1.88 b
2. 400 ppm S-ABA	15.2 b	0.25 b	3.82 b	39.3	-1.50 a
Pr>F	0.002	0.0136	0.0069	0.7123	0.0001
80 DAFB, 20 DAS					
1.Control	16.8 ns	0.35 ns	4.81 ns	40.2 ns	-1.79 ns
2. 400 ppm S-ABA	14.6	0.24	3.90	39.6	-1.64
Pr>F	0.1440	0.0619	0.1793	0.4555	0.1068

[z] Means with a different letter differ significantly at the 5% level (LSD).

ns-Not significant.

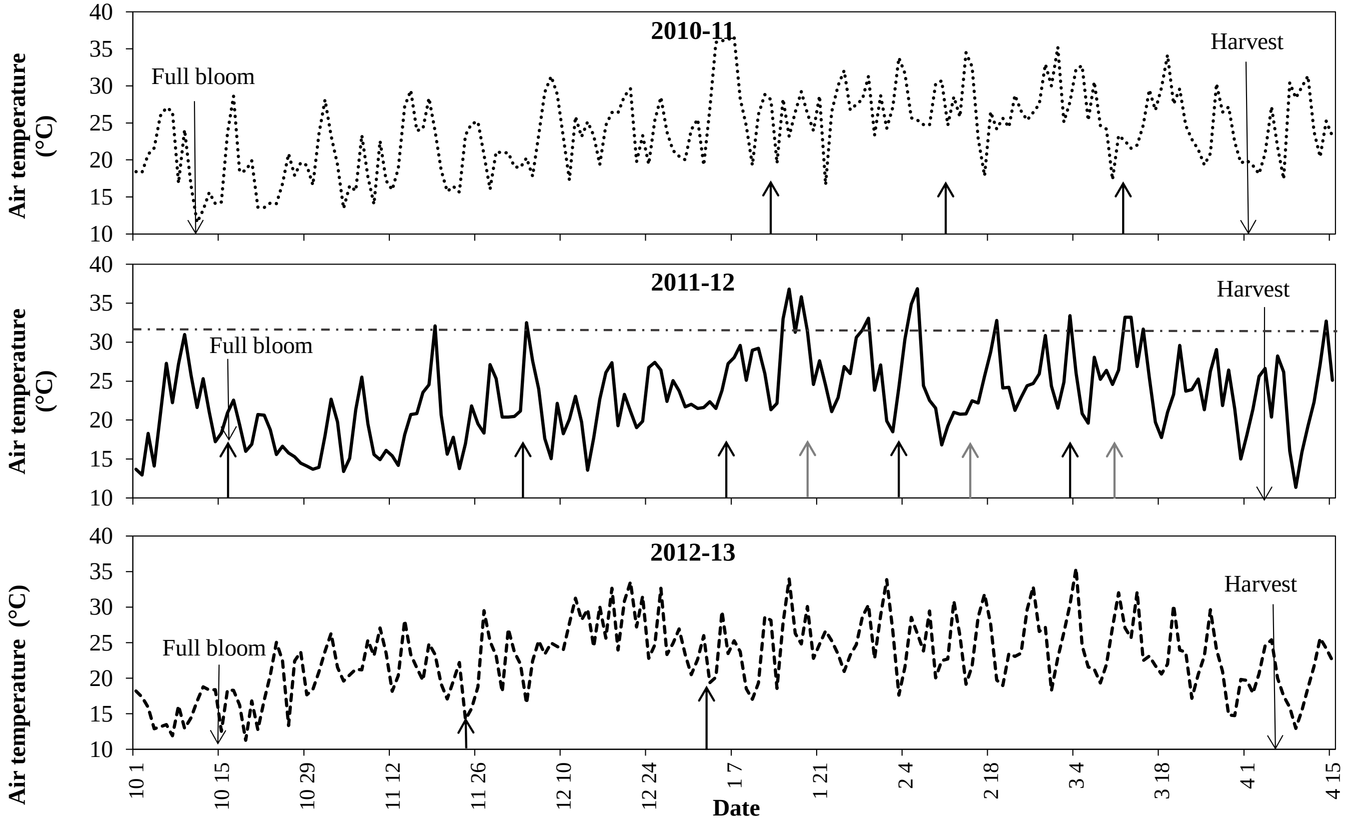

Figure 1. Daily maximum air temperatures recorded at Oak Valley Estate in Grabouw, South Africa (Latitude: 34°15’S; Longitude: 19°07’E, Alt 375 m) in close proximity to Disseldraai, Grabouw during the 2010-11, 2011-12 and 2012-13 seasons. Grey arrows indicate treatments prior to the arbitrary 32ºC sunburn threshold for treatment prior to heat waves in 2011-12. Other treatments are indicated by black arrows.

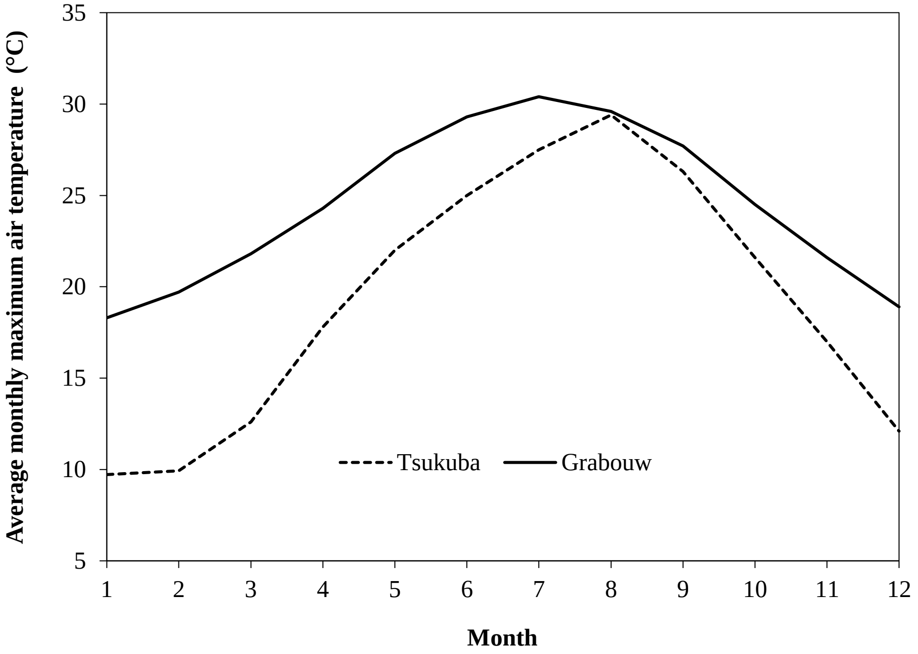

Figure 2. Historical average monthly maximum air temperature (Jan 1983-Dec 2004) for Tsukuba Japan, where Iamsub et al. (2009) conducted their study on the effect of S-ABA application on sunburn in apple at the University of Tsukuba (36°10’ N, 140°10’ E) and Disseldraai, Grabouw, South Africa (34°15’S; 19°07’E) used in the current study (https://eosweb.larc.nasa.gov/cgi-bin/sse/grid.cgi?email=skip@larc.nasa.gov). The data from Grabouw has been shifted by six months so that summer months for both hemispheres overlap.

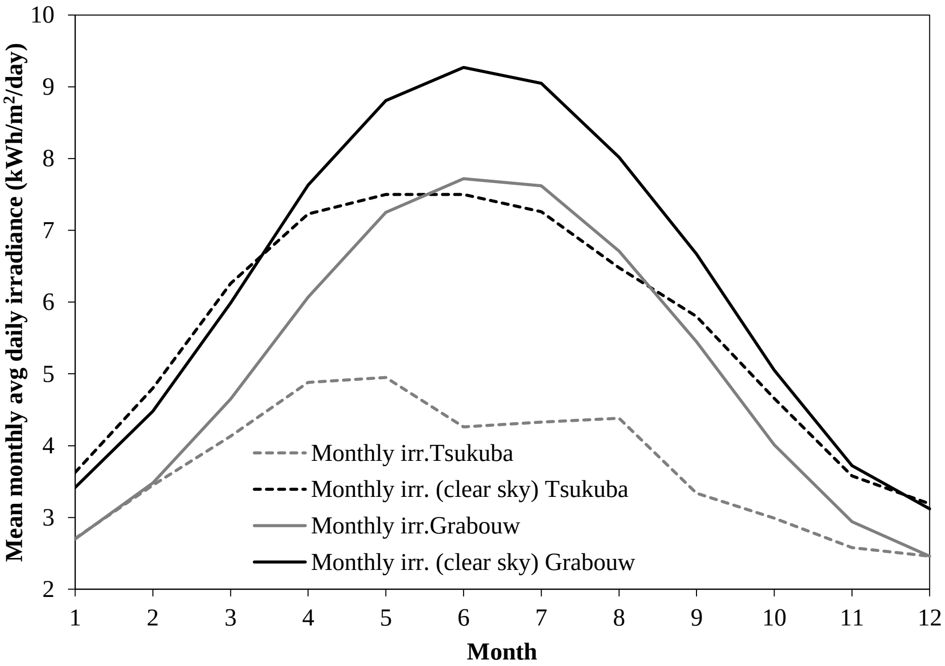

Figure 3. Historical monthly average amount of the total solar radiation (Jul 1983-Jun 2005) for all days and for clear days (<10% cloud cover) for Tsukuba Japan, where Iamsub et al. (2009) conducted their study on the effect of S-ABA application on sunburn in apple at the University of Tsukuba (36°10’ N, 140°10’ E) and Disseldraai, Grabouw, South Africa (34°15’S; 19°07’E) used in the current study (https://eosweb.larc.nasa.gov/cgi-bin/sse/grid.cgi?email=skip@larc.nasa.gov). The data from Grabouw has been shifted by six months so that summer months for both hemispheres overlap.

Commented [S13]: The usual units for daily radiation are MJ/m2/d. Use the conversion that 1 kWh = 3.6 MJ