Size does matter – Susceptibility of apple for gray mold is affected by cell size

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Size does matter – susceptibility of apple for grey mould is affected by cell size

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Apple is a seasonal product that is stored for long periods of time, during which fungal-caused decay can occur. Previous infection experiments of intact Jonagored apple with *Botrytis cinerea* displayed apparent differences in lesion expansion rate with respect to inoculation position on the fruit (on, above or below the equator). The goal of this study was to investigate whether these differences are consistent or not and if so, to relate them to fruit characteristics. The study involved measuring the hue angle of the intended inoculation spots prior to inoculation, and firmness and total soluble solids content of fruit from the same batch. Results showed that firmness correlated somewhat (-0.55 and -0.72 for shadow and sun side, respectively) with lesion diameter expansion rate. In a subsequent step, X-ray imaging was carried out for samples from each position. Analysis of 3D reconstructions by micro computed tomography of these

diffraction images showed that cell size was strongly correlated (0.996) to lesion diameter expansion rate. Finally, it was investigated if cell size could also be used to rank different apple cultivars for their susceptibility to *B. cinerea*. The result shows that there is a clear distinction between Jonagold and Golden Delicious (non-blushing variety), which have a smaller cell size, and Braeburn and Kanzi, which have a larger cell size (overall correlation of 0.87). This indicates that cell size may also be an important factor in determining susceptibility across cultivars.

Keywords: *Botrytis cinerea*, grey mould, *Malus × domestica*

Introduction

Apple (*Malus × domestica*) is a seasonal fruit harvested in autumn. To provide consumers with high quality fruit year-round, the fruit is stored at lowered temperatures and controlled atmosphere conditions in order to slow down its metabolism (Watkins, 2003). During storage, decay caused by fungal pathogens may occur, next to other postharvest deterioration processes. A study into postharvest decay of apple and pear conducted in the Netherlands and Belgium in 2013–2014 found that in extreme cases, losses could even surmount 60% for certain batches (Wenneker *et al.*, 2015). Major pathogens causing postharvest rot in apple are *Penicillium* species (blue mould) and *Botrytis cinerea* (grey mould) (Watkins, 2003; Mari *et al.*, 2009). Both primarily infect apples through wounds, although this is not necessarily the case (Sugar, 2002; Watkins, 2003). As the fruit ripens it gets easier for these pathogens to cause decay, because of multiple metabolic and physiological changes in the fruit (Alkan & Fortes, 2015). Ripening is associated with fruit softening due to cell wall disassembly. There is evidence to show that this

may facilitate pathogen entry into the fruit. Non-softening tomato mutants *Never ripe (Nr)* and *non-ripening (nor)* have been shown to be less susceptible to necrotrophic pathogens, and simultaneous suppression of *LePG* and *LeExpI* during ripening lead to a reduced susceptibility to *B. cinerea* (Cantu *et al.*, 2008). Another factor that changes during ripening is fruit colour. The concentrations and relative contributions of anthocyanins, carotenoids and chlorophyll pigments make up the colour (Dong *et al.*, 1995; Reay, 1998; Vázquez-Padín *et al.*, 2009). Anthocyanins and carotenoids accumulate in the peel during ripening. They function as antioxidants and can therefore have an enhancing effect on disease resistance (Zhang *et al.*, 2013). Chlorophyll levels decrease during ripening. More recently, research has focused on the role of chloroplasts in plant defence (Stael *et al.*, 2015; de Torres Zabala *et al.*, 2015). The organelle appears to have its own branch of calcium-ROS signalling, biosynthesis of several defence hormones occurs in it, and it has a link with the metabolic state of the cell. Ripening fruit exhibit a rise in sugar content. This could be of significance, because sugar availability could be a trigger for pathogen activation (Prusky, 1996). Furthermore, nutrient availability could affect gene expression and pH modulation by the pathogen, thus altering its pathogenicity (Prusky & Wilson, 2018). Fruit will also undergo a pH change during ripening. The pH affects enzyme activity and gene expression in the pathogen. Pathogens will even modulate their ambient pH by either secreting ammonia or oxalic acid to cause alkalization or acidification, respectively (Prusky *et al.*, 2013). Lastly, levels of preformed antifungal compounds decrease in ripening fruit and induction of wound and defence responses upon pathogen recognition declines (Vilanova *et al.*, 2012; Alkan & Fortes, 2015).

Building on this knowledge, research efforts have been carried out to predict susceptibility based on quality measures such as firmness and colour. Attempts were made by

Chávez *et al.* (2014) who found no correlation for total soluble solids (TSS), titratable acidity, pH and colour, and by Ahmadi-Afzadi *et al.* (2013) who found very weak correlations ($R^2 < 0.4$) for firmness, softening rate and harvest time with disease severity measured as lesion diameter. One reason for this could be that the biological variability inherent to apple fruit obscures any correlation. Apple is grown under non-standardizable field conditions, which can impact susceptibility from year to year, from field to field, and even the location in a field or of the fruit on the tree may have an effect. Another reason may be that many factors contribute towards susceptibility, and that looking at one or a few of them does not necessarily give a clear image.

While developing the infection assay protocol for subsequent research purposes, it was considered whether the position where the fruit is infected makes a difference. If this is actually the case, it is a very interesting path to pursue, because it allows investigation of factor(s) affecting susceptibility, while not having to deal with the inherent large biological variability of apple as much as when using different apple fruit. Preliminary experiments suggested that there was at least an apparent difference in susceptibility between positions on the fruit. Therefore, the goal of this research was to investigate whether these differences are consistent or not and if so, to relate it to fruit characteristics.

Materials and methods

Plant and fungal material

The details of the fruit material used in the experiments above is described in Table 1. Apples were not disinfected prior to the experiments, as plant physiological effects were noticed in earlier research (Naets *et al.*, 2018). The *Botrytis cinerea* isolate B05.10 was kindly provided by

Julia Schumacher (Institut für Biologie & Biotechnologie der Pflanzen, University of Münster). *Botrytis cinerea* was cultured on potato dextrose agar. Sporulation was induced by exposing the plates to near UV light overnight. Harvested spores were suspended in 12 g L⁻¹ potato dextrose broth at a concentration of 10⁶ conidia mL⁻¹.

Pathogenicity assay

For each inoculation spot, a wound was made of about 5.1 mm depth and 0.8 mm diameter in experiment 1, and 10.1 mm depth and 1.7 mm diameter in experiments 2 and 3 by using a needle or nail, respectively, mounted on a piece of cork. Each wound was inoculated with 2 µL of the spore suspension which was pipetted directly into the wound. Disease severity was followed up by measuring the maximum horizontal lesion diameter using a digital caliper. For each assay a total of 30 biological replicates was used.

Colour

For the second experiment, a circle was drawn around each inoculation spot prior to inoculation and the colour was measured as the hue angle using a hand-held spectrophotometer (CM-2600D; Minolta) in accordance with Gwanpua *et al.* (2014). The measurement was repeated five times for each spot.

Firmness and total soluble solids content

Magnus Taylor firmness was measured as in Gwanpua *et al.* (2014). Briefly, a Texture Analyzer (TA-XT2i; Stable Microsystems) was used with a self-cutting probe 11 cm in diameter that penetrated the fruit to a depth of 8 mm at a speed of 8 mm s⁻¹. This was carried out for the sun and shadow side of each fruit. Juice from the wound was captured using a Pasteur pipette and the total soluble solids (TSS) content of it was measured using a digital refractometer (PR-101α; Atago).

X-ray microcomputed tomography

Apple pieces were excised from Jonagored (experiment 2), and Kanzi, Braeburn, Jonagold and Golden Delicious (experiment 3) cultivars for microCT examination. The 5 × 5 × 10 mm pieces were placed into a freshly prepared 10% (w/v) caesium iodide contrast agent solution (Acros Organics) for approximately 2 h with periodic agitation. All samples were subsequently prepared according to previously described procedures (Wang *et al.*, 2017) and subjected to microCT scans at a voxel resolution of 2.5 μm. For experiment 2, volumes of interest (VOIs) of 2500 × 2500 × 2500 μm were examined which started at a depth of about 3 mm, while experiment 3 examined VOIs 3000 × 3000 × 875 μm that started at a depth of about 5 mm. In either case, the VOIs well exceeds the representative elementary volumes (REV) for apple hypanthium tissue of 1.3 mm³ (Mendoza *et al.*, 2007). Regardless of the size of the VOIs, the image processing workflow from previous work was applied to extract cell volumes of the different cultivars and used for further analysis in the current study (Wang *et al.*, 2017).

Statistics

For the determination of lesion diameter expansion rate, colour, firmness, TSS and cell volume, repeated measurements on the same biological replication were carried out, which consequently gave correlated data which did not allow for standard ANOVA. Therefore, linear mixed effect (LME) models were used. These were implemented in R (R Development Core Team, 2011) making use of the LME4 package (Bates *et al.*, 2015). Least square means comparisons of model parameters were performed using the LSMEANS package (Lenth, 2016).

Results

Experiment 1

In the first experiment, apples of cultivar Jonagored were inoculated at three different positions, i.e. on the equator, above the equator (superior), and below the equator (inferior) (Fig. 1). This was done for both the shadow and sun exposed side of the fruit. Each of the positions was replicated twice within the same fruit, and 30 biological replicates were used. Infection progress was followed up by measuring the lesion diameter (Fig. 2). An LME model was constructed predicting lesion diameter by position on the fruit, side (sun/shadow) of the fruit, time (days post-inoculation, dpi), and all possible level 1 interactions. Replicates on the same subject were added as a random structure in the model.

$$\begin{aligned} \text{lesion diameter}_{ij} &= \alpha_1 + \alpha_2 \cdot \text{position}_i + \alpha_3 \cdot \text{side}_j + \alpha_4 \cdot \text{time} + \alpha_5(\text{position}_i \times \text{time}) + \alpha_6 \\ &\quad (\text{position}_i \times \text{side}_j) + \alpha_7(\text{side}_j \times \text{time}) + \epsilon_{ij} \end{aligned}$$

In this model, $\alpha_1 - 7$ are the estimated parameters and ϵ_{ij} is the error. Backwards elimination of the model terms resulted in the interaction side $\times$ time being removed from the

model ($P = 0.16$; Table 2). There was a significantly different increase in lesion diameter over time between the different positions (position $\times$ time, $P < 0.001$), and differences between positions were significantly different between sides (position $\times$ side, $P = 0.047$). Estimates for the lesion expansion rates were 1.35 ± 0.20 , 4.20 ± 0.20 and 3.47 ± 0.20 mm per day for inferior, equator and superior, respectively, and these estimates were all significantly different from one another ($P < 0.05$).

Experiment 2

Here, the effect of fruit colour on lesion expansion was examined by measuring the colour of each spot prior to inoculation. Because this was the first time that these within subject differences in susceptibility had been investigated, it was necessary to make sure that results from the previous experiment were not caused due to even the slightest (invisible) inoculum runoff. Therefore, half of the fruit of this experiment had been turned upside down.

The result of the second experiment was in line with that of the first one (Fig. 3). The more exponential trend for lesion expansion is probably a result of the fact that the fruit of experiment 2 had already been in storage for a longer time (1 month more) in comparison with that of experiment 1, as fruit tend to get more susceptible when they ripen (Alkan & Fortes, 2015). Furthermore, measurements in experiment 1 started only from 2 dpi which is the more linear part of the curve. Lastly, experiment 2 was carried out with an updated infection protocol in which wound size was larger, because more consistently successful infections were observed when using larger wounds. An identical LME as in experiment 1 was constructed (Table 3), but lesion diameter was log transformed to make the profile linear and more homoscedastic (Fig.

S1). In agreement with the first experiment, the interaction of side and time (dpi) was not significant ($P = 0.09$) in the second experiment, whereas that of position with time ($P < 0.001$) and position with side ($P < 0.001$) were significant. There was no significant offset for position ($P = 0.07$). This can be explained by the fact that the first measurements of lesion size the same day of the inoculation will be the same for all positions, and this part was not present in experiment 1. Expansion rates (of log transformed lesion diameters) were 0.46 ± 0.0059 , 0.53 ± 0.0059 and 0.51 ± 0.0059 for inferior, equator and superior position, respectively, and these estimates were all significantly different from one another ($P < 0.05$). Again, the lesion expansion rate was the lowest at the inferior position and the highest at the equator. The only exception was the sun side in the case of apples that were put upright.

The colour of each inoculated spot had been measured prior to inoculation. There was a clear difference between sides, and between positions (Fig. 4a), but there was no correlation with lesion diameter (Fig. 4b). The hue angle of each spot was measured five times. Spots that had more mixed red and green colour therefore display a larger error.

Total soluble solids (TSS) content and firmness, both destructive measurements, were measured on a different set of fruit from the same batch as experiment 2 (Table 4). Both firmness and TSS exhibited significant differences between positions. TSS content also differed significantly between sides of the fruit. The equator position had a significantly lower firmness than either the inferior or superior position, and it was also the position with the largest lesion diameter. The correlation of firmness with lesion diameter expansion rate was -0.55 and -0.72 for shadow and sun side of the fruit, respectively.

Firmer fruit often have smaller cells with less interspace (Musacchi & Serra, 2018). It was hypothesized that firmness differences within the fruit could also be related to cell size. Therefore, X-ray microcomputed tomography (micro-CT) was used to investigate if cell size varied between the positions studied. Because making and analysing these scans takes a long time, only samples of the shadow side of the fruit (for which differences in lesion expansion rate were the highest) were analysed. Three biological replicates were used and for each sample over 1500 cells were measured (Figs 5 & 6). Different positions differed significantly in average cell size and there was a strong correlation with log lesion diameter expansion rate (correlation of 0.996; Fig. 6), suggesting a link between cell size (or factors correlating with cell size) and susceptibility.

Experiment 3

A final experiment was carried out in which four apple cultivars were ranked according to their susceptibility for *B. cinerea* based on their average cell volume at their equator. In-house micro-CT scans available for Jonagold, Golden Delicious, Braeburn and Nicoter (Kanzi®) were analysed for cell volume. For each of these, samples were collected from the equator of the fruit, one from each quadrant. Additionally, susceptibility to *B. cinerea* was tested for each cultivar (Fig. 7). Fruit were harvested at each cultivar's respective optimal harvest date and subsequently stored for 4 weeks at 1 °C in order to have sufficient successful infections to draw conclusions from, as susceptibility to *B. cinerea* increases during fruit ripening (Alkan & Fortes, 2015). It can be seen that Golden Delicious and Jonagold were clearly less susceptible than Braeburn and Nicoter.

Discussion

The aim of experiment 1 was to investigate whether differences in susceptibility between inoculation positions on the fruit were consistent or not. The result clearly demonstrated that the lesion expansion rate differed significantly between the different positions tested. There was a significant interaction of position and side, and a significant effect of side. This is because the measurements started from 2 dpi with an immediate offset between sides of the fruit which persisted throughout the experiment, and the magnitude of this difference was also position dependent with 0.001 ± 0.44 ($P = 0.998$), 1.36 ± 0.63 ($P = 0.03$) and 1.33 ± 0.63 ($P = 0.03$) mm for inferior, equator and superior position, respectively. The red skin colour on the sun side is a result of anthocyanin pigmentation which occurs in response to environmental stimuli such as light and temperature (Saure, 1990). It is an adaptive mechanism to abiotic stress and may contribute to cross-tolerance to biotic stress. Anthocyanins have been directly implicated in enhanced resistance to *B. cinerea* in tomato fruit (Zhang *et al.*, 2013). Therefore, a correlation between colour of the inoculation spot and susceptibility was investigated. Additionally, light exposure has previously been linked to firmness (Sams, 1999; Johnston *et al.*, 2002;) and sugar levels (Saure, 1990) in the fruit and, therefore, differences in firmness and sugar concentration between the positions was also investigated.

A second experiment was carried out to investigate these consistent position-based differences. Although there are distinct differences in colour between positions, these colour differences are not linked to lesion diameter. This could be because the fruit colour is a result of copigmentation of anthocyanins, carotenoids and chlorophyll (Dong *et al.*, 1995; Reay, 1998;

Vázquez-Padín *et al.*, 2009), as well as pH of the vacuoles (Dong *et al.*, 1995). Furthermore, pigmentation occurs mostly in the fruit peel, while the inoculation occurred with wounds that penetrate into the flesh below the skin.

TSS and firmness were determined on a different set of fruit from the same batch as those used in experiment 2. Differences in TSS could be a result of different light and temperature exposure on the tree (Saure, 1990). TSS content did not seem to show a clear link with susceptibility. Nutrient availability can affect pathogenicity (Prusky & Wilson, 2018), but the maximum difference between positions amounted to 0.9 °Brix and there was already 12 g L⁻¹ potato dextrose broth in the inoculation suspension.

Between sun and shadow side of the fruit, there were no significant differences in firmness, even though the sun side of the fruit often has a higher firmness than the shadow side (data not shown). It has been described previously that higher sun exposure, if the level stays below the damaging limits, can lead to firmer fruit (Sams, 1999). Within a side of the fruit, the equator position had the lowest firmness and this is also the most susceptible position. Because firmer fruit tend to have smaller cells with less interspace (Johnston *et al.*, 2002), it was investigated whether within a fruit these firmness differences were also linked to differences in cell size, and if they correlated with susceptibility.

Micro-CT imaging was performed on samples from the shadow side of the fruit where differences in lesion expansion rate were highest. There seems to be a linear relationship between cell volume and the log lesion expansion rate (correlation of 0.996). It is hypothesized that this could be due to (i) smaller cells leading to a higher cell density with more cell wall material per unit volume for the fungus to digest; (ii) a higher cell density being related to a

stronger defence response, because more DNA copies and transcriptional machinery is present per unit volume; and/or (iii) smaller cells leading to smaller spaces between the cells, making the cell wall less accessible. A greater biosynthetic capacity per tissue volume, resulting from a higher cell density, could correlate with a more rapid accumulation of defensive metabolites. It is then likely that the link with cell size would extend to different types of fruit and even to other tissue types of plants. In the case of *Pseudomonas syringae* infecting *Arabidopsis*, the quantitative nature of the plant defence response is an important factor in determining if an interaction is compatible (Tao *et al.*, 2003).

To further investigate this link with cell size, four cultivars were ranked according to their susceptibility to *B. cinerea* based on their average cell size at the equator. There is an indication that factors correlating with cell volume play an important role in determining susceptibility, but that more factors varying between cultivars affect susceptibility. Furthermore, it is important to notice that apples get more susceptible to *B. cinerea* as they mature and that the rate at which this happens can vary across cultivars. This work should therefore be extended to a larger set of cultivars and multiple time points throughout storage.

This work investigated if differences in susceptibility for *B. cinerea* observed within apple fruit were consistent and could be linked to characteristics of the inoculation position. The results demonstrate that within fruit there is a strong correlation of lesion expansion rate with cell volume. The study investigated whether a relationship of susceptibility with cell volume exists across cultivars and found an indication that this may be the case. Future research should focus on expanding this work to more cultivars and different storage durations to confirm or reject observations made here.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Figure S1 Standard deviation of the lesion diameter measured in experiment 2 at different time points, for each tested position, and for each treatment displayed for non-transformed (circles) and log transformed (triangles) data.

Figure legends

Figure 1 Picture of the shadow side of a Jonagored apple inoculated below (inferior), on (equator) or above (superior) its equator with *Botrytis cinerea*.

Figure 2 Lesion diameter as a function of time for apple fruit inoculated with *Botrytis cinerea* at inferior, equator or superior position on the shadow or sun exposed side in experiment 1. Lines show the fitted linear mixed effect model. Error bars denote standard error of the mean of 30 biological replicates.

Figure 3 Lesion diameter as a function of time for apple fruit inoculated with *Botrytis cinerea* at inferior, equator or superior position on the shadow or sun exposed side in experiment 2. Lines show the bias-corrected prediction by the linear mixed effect model fitted on the log transformed lesion diameters. Bias-correction was carried out according to McMeekin *et al.* (1993). Error bars denote the standard error of the mean of 15 biological replicates.

Figure 4 (a) Box plots of hue angles of the inferior, equator and superior position, and (b) the relation with lesion diameter at 5 days post-inoculation. Error bars denote standard errors of the mean of five replicate measurements.

Figure 5 Segmented cell volumes in 3D with different colours attributed to cells in the same neighborhood. Images are of (a) inferior, (b) equator and (c) superior position. For each position the average cell volume with standard error, resulting from error propagation of three biological replicates, is displayed.

Figure 6 Cell volume of the shadow side of apple for inferior, equator and superior position versus expansion rate of the log transformed lesion diameter in experiment 2. Error bars for cell volume are the result of error propagation of the standard errors for the three individual

biological replicates. Error bars for expansion rate of the log(lesion diameter) represent the standard error of the mean of 30 biological replicates.

Figure 7 Lesion diameter on the equator of Golden Delicious, Jonagold, Braeburn and Nicoter at 4 days post-inoculation versus the cell volume of these cultivars measured at their equator.

Error bars for cell volume denote standard errors with $n = 4$ for Jonagold and Kanzi and $n = 5$ for Braeburn and Golden Delicious. Error bars for lesion diameter represent the standard error of the mean of 30 biological replicates.

For Peer Review

Shadow Plant Pathology

Sun

Lesion diameter [mm]

Position on the fruit

- inferior
- equator
- superior

Upright

Turned

Time post infection [days]

(a)
 $(3.27 \pm 0.02) \times 10^6 \mu\text{m}^3$

(b)
 $(3.89 \pm 0.02) \times 10^6 \mu\text{m}^3$

(c)
 $(3.66 \pm 0.02) \times 10^6 \mu\text{m}^3$

Plant Pathology

Braeburn

Nicoter

Golden Delicious

Jonagold

plantpath@bspp.org.uk

Cell volume [μm^3]

Table 1 Background information of the apples used in each experiment in Belgium

Cultivar	Experiment	Harvest date	Field location	Planting year	1 °C storage until
Jonagored	1	1, 14, 23 Oct 2015	Rillaar	2014	20 May 2016
Jonagored	2	1, 14, 23 Oct 2015	Rillaar	2014	21 Jun 2016
Golden Delicious	3	13 Sep 2018	Sint-Truiden	2005	11 Oct 2018
Jonagold	3	18 Sep 2018	Sint-Truiden	2010	17 Oct 2018
Kanzi	3	27 Sep 2018	Sint-Truiden	2005	25 Oct 2018
Braeburn	3	22 Oct 2018	Sint-Truiden	2005	19 Nov 2018

All cultivars were managed by integrated pest management (IPM), on M9 rootstocks.

Table 2 ANOVA table for the fixed effects from the model predicting lesion diameter in experiment 1

	Eliminated	SS	MS	d.f. _{num}	d.f. _{den}	<i>F</i>	<i>P</i>
Position	0	770.2	385.1	2	1394.0	16.4807	8.4 × 10 ⁻⁸ ***
Time (dpi)	0	16224.6	16224.6	1	1394.1	694.3351	<2.2 × 10 ⁻¹⁶ ***
Side	0	287.4	287.4	1	1394.1	12.3011	4.6 × 10 ⁻⁴ ***
Position × time	0	2616.3	1308.2	2	1394.0	55.9834	<2.2 × 10 ⁻¹⁶ ***
Position × side	0	143.3	71.6	2	1394.0	3.0657	0.047*
Side × time	1	45.69	45.69	1	1393.1	1.9566	0.162

SS, sum of squared errors; MS, mean sum of squared errors; d.f._{num}, numerator degrees of freedom; d.f._{den}, denominator degrees of freedom; *, *P* < 0.05; ***, *P* < 0.001.

Table 3 ANOVA table for the fixed effects from the model predicting lesion diameter in experiment 2

	Eliminated	SS	MS	d.f. _{num}	d.f. _{den}	<i>F</i>	<i>P</i>
Position	0	0.40	0.20	2	2445.9	2.6862	0.068
Time (dpi)	0	1623.39	1623.39	1	2448.0	21555.1533	<2.2 × 10 ^{-16***}
Side	0	2.16	2.16	1	2445.9	28.6558	9.4 × 10 ^{-8***}
Position × time	0	5.82	2.91	2	2445.9	38.6090	<2.2 × 10 ^{-16***}
Position × side	0	2.84	1.42	2	2445.9	18.8476	7.5 × 10 ^{-9***}
Side × time	1	0.22	0.22	1	2444.9	2.8760	0.090

SS, sum of squared errors; MS, mean sum of squared errors; d.f._{num}, numerator degrees of freedom; d.f._{den}, denominator degrees of freedom; ***, $P < 0.001$.

Table 4 Firmness and total soluble solids (TSS) measurements for each investigated position on the fruit

Position	Side	Firmness ^a		TSS ^a	
		N	SE	%	SE
Inferior	Shadow	56.6 a	2.3	13.1 bc	0.3
Equator	Shadow	52.3 b	1.3	12.6 ab	0.3
Superior	Shadow	57.4 a	1.5	12.2 a	0.2
Inferior	Sun	57.7 a	1.3	13.3 c	0.2
Equator	Sun	51.0 b	1.9	13.2 c	0.3
Superior	Sun	57.0 a	1.7	13.0 bc	0.3

Lowercase letters denote significance for a 95% confidence level.

^a*n* = 10.