

**Effect of fruit canopy position, harvest maturity
and storage duration
on post-harvest mealiness development
of 'Forelle' pears (*Pyrus communis* L.)**

by

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Declaration

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SUMMARY

Preliminary studies indicated a link between fruit canopy position and higher total soluble solids (TSS), respectively, and mealiness development during ripening of 'Forelle' pear. In this study this link is further explored to establish whether different parts of the canopy result in differences in maturity and ripening rates which affect mealiness incidence after ripening post-harvest. This study also investigates whether mealiness incidence is related to the micro-climatic differences within the canopy.

Mealy textured pears were in general bigger sized fruit associated with higher TSS, lower titratable acid (TA), a redder blush colour, yellower background colour, and lower firmness after a period of ripening. Mealy fruit were also associated with a lower juice area and juice mass that were measured using a confined compression method. Mealiness incidence was the highest for red blushed outer canopy 'Forelle' pears associated with the highest exposure to sunlight, coupled with the highest fruit surface temperatures and vapour pressure deficit. The shading of outer canopy pears reduced mealiness incidence significantly, compared to that of sun-exposed outer canopy pears, which could be an indication that direct exposure to full sunlight coupled with high fruit temperatures for most part of the day could be one of the determining factors in 'Forelle' mealiness development. However, not all outer canopy fruit developed a mealy texture and therefore another unidentified tree factor might also play a role.

The ripening rate developed earlier for outer canopy pears (earlier loss of firmness and an earlier transition to a more yellow ground colour) compared to intermediate and shaded inner canopy pears for both seasons, irrespective of harvest maturity. This is an indication that outer canopy fruit are in a more advanced stage of maturity than the other fruit positions. Fruit harvested at post-commercial maturity seems to be more susceptible to mealiness development. Highest mealiness incidence was observed after 8 weeks of cold storage at -0.5 °C with 4, 7 and 11 days of ripening at 20 °C (8w RA + 4, 7 and 11d SL), while mealiness decreased with prolonged cold storage. Mealiness does however, not seem to be directly linked to ethylene production rate.

OPSOMMING

Voorlopige studies dui op 'n ooreenkoms tussen die effek van boomposisie van 'Forelle' pere, hoër totale opgeloste vastestowwe (TOV) en die ontwikkeling van melerigheid gedurende die proses van rypwording. In hierdie studie is die verband verder getoets om vas te stel of vrugte van verskillende boomposisies tot verskillende rypheide met gevolglike verskille in melerigheid tydens die na-oes periode lei. Verder het die studie gepoog om vas te stel of hierdie verskille moontlik gekoppel kan word aan mikro-klimaat verskille van vrugte op verskillende boomposisies.

Pere met 'n melerige tekstuur was oor die algemeen groter, tesame met hoër totale opgeloste vastestowwe (TOV), laer titreerbare suur (TS), rooier bloskleur, geler agtergrond kleur en laer fermheid na 'n periode van na-oes rypwording. Melerigheid was ook geassosieer met 'n laer sap area en sap gewig wat verkry was deur die begrensde kompressie metode. Die voorkoms van melerigheid was die hoogste vir die rooier bloskleur 'Forelle' pere wat geassosieer is met die hoogste persentasie blootstelling aan maksimum sonlig tesame met die hoogste vrugoppervlaktemperatuur- en dampdruk verskille. Wanneer buitevrugte beskadu was, het die voorkoms van melerigheid betekenisvol afgeneem in vergelyking met dié van sonblootgestelde buitevrugte. Dit kan daarop dui dat direkte blootstelling aan vol sonlig tesame met hoë vrugtemperatuur vir die grootste gedeelte van die dag, een van die deurslaggewende faktore kan wees in die ontwikkeling van 'Forelle' melerigheid. Nie alle buitevrugte het egter 'n melerige tekstuur ontwikkel nie, wat kan dui op 'n onbekende boomfaktor wat ook moontlik 'n invloed kan uitoefen.

Die buitevrugte ontwikkel vroeër rypheid ontwikkel (vroeër afname in fermheid en oorgang na 'n geler agtergrondkleur) as die intermediêre- en binneste vrugposisies vir beide seisoene, ongeag die oesrypheid. Dit is 'n aanduiding dat buite vrugte in 'n meer gevorde rypheid stadium is as vrugte afkomstig van ander boomposisies. Vrugte wat na die optimale-kommersiële rypheid gepluk is, blyk om meer vatbaar te wees vir die ontwikkeling van melerigheid. Die hoogste voorkoms van melerigheid is waargeneem 8 weke na koelopberging by $-0.5\text{ }^{\circ}\text{C}$, opgevolg deur 4, 7 en 11 dae van rypwording by $20\text{ }^{\circ}\text{C}$ (8w RA + 4, 7 en 11d RL) terwyl melerigheid in meeste gevalle afgeneem het met 'n verlengde periode van koelopberging. Die ontwikkeling van 'Forelle' melerigheid blyk ook nie direk gekoppel te wees aan die vlak van etileen produksie nie.

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GENERAL INTRODUCTION AND OBJECTIVES

In South Africa, Forelle (*Pyrus communis* L.) is considered the most valuable bicolour pear cultivar, with 26% of South Africa's total area of pear production comprising of 'Forelle'. Its bicolour rivals, 'Rosemarie', 'Flamingo' and 'Cape Rose' contribute only 4%, 1% and 4%, respectively to total pear production (HORTGRO, 2018).

'Forelle' pears' ability to develop an exceptional red blush colour under South African conditions sets them apart from 'Rosemarie', which are more heat sensitive (Steyn et al., 2005) and the other bicolour pear cultivars are smaller fruiting cultivars (Human, 2002). 'Flamingo' pears have a tendency to undergo internal breakdown (Crouch, 2011), and 'Cape Rose' is the latest released cultivar of which plantings are gradually increasing.

The red blush of 'Forelle' pear is extremely important, since without a lack of red blush development, fruit are marketed under the 'Vermont Beauty' label which are sold at a lower premium. Consumers prefer red blush pears that demand higher prices than green or full red fruit (Steyn et al., 2004). The characteristic red blush of 'Forelle' pears which mainly determines their success (Manning, 2009), is inclined to develop a mealy texture after ripening to a firmness below 4 kg (Crouch et al., 2005). Mealiness is the most important internal physiological disorder of South African 'Forelle' pears (Martin, 2002; Crouch, 2011; Cronjé et al., 2015; Muziri et al., 2015). The term mealiness is ascribed to fruit flesh with a soft, floury and dry texture in association with a lack of crispness and juiciness (Barreiro et al., 1998; Crouch, 2011). Pears with a juicy, buttery melting flesh texture combined with a characteristic pear flavour are considered as good eating quality pears (Eccher-Zerbini, 2002).

Forelle is a European pear cultivar with a high post-harvest cold requirement for inducing normal and uniform ripening (Villalobos-Acuña and Mitcham, 2008). Susceptibility of 'Forelle' pears for mealiness development increases when their exposure to cold storage is inadequate, and pome fruit harvested at a post-optimum maturity are more inclined to a mealy texture with a poor storage potential (Mellenthin and Wang, 1976 (pear); Peirs et al., 2001 (apple); Martin, 2002 ('Forelle' pear); Carmichael, 2011 ('Forelle' pear)). Therefore, a mandatory 12-week cold storage period at -0.5 °C is needed for South African 'Forelle' pears to experience minimum mealiness incidence (de Vries and Hurndall, 1993). The mandatory period has an adverse effect, since it causes a loss of South African bicolour pear continuity on European markets and this might lead to a permanent shift of buyers switching to fruit

from offshore competitors (Crouch and Bergman, 2013). This period also prevents South African 'Forelle' pears from reaching the earlier European markets that offer premium prices (Crouch and Bergman, 2013). This resulted in previous research focusing on the mandatory 12-week cold storage period, but no treatment could ensure constant low levels of mealiness. The studies consisted of: evaluating the effect of controlled atmosphere (CA) storage in combination with regular atmosphere (RA) storage intervals (De Vries and Hurndall, 1993; De Vries and Hurndall, 1994; De Vries and Moelich, 1995), various intermittent warming treatments (de Vries and Humdall, 1993), and ethylene treatments (Du Toit et al., 2001). Crouch and Bergman (2013) developed a program called 'Forelle' early market access (FEMA) that supplies crunchy 'Forelle' pears to the European markets. Despite the great success of the FEMA program, the mealiness problem was not solved, since the consumers, particularly from European origin, still preferred the characteristic soft, sweet buttery flesh of 'Forelle' pears (Crouch and Bergman, 2013; Manning, 2009).

Production of South African 'Forelle' pears mainly occurs in the Western Cape and Eastern Cape (Langkloof) production regions with contrasting climatic conditions (HORTGRO, 2018). Different fruit positions within the tree canopy experience different levels of irradiance and ambient temperature, as well as differences in the supply of water, mineral nutrients and endogenous hormones (Kingston, 1994; Tomala, 1999). Time of flowering also tends to be different for different canopy positions. Thus, harvest maturity and ripening potential of fruit could be influenced (Carmichael, 2011), and modifications of post-harvest fruit characteristics could appear, which could have an influence on eating quality and visual appearance which play a major role in consumer preference for the fruit (Bramlage, 1993; Fouche et al., 2010). The duration that pears can be stored before a decline of fruit quality arises is directly linked to the fruit maturity at the time of harvest (Kader, 1999).

There have been several studies conducted on the role of different factors on mealiness development, which mainly focused on pre-harvest factors which include: growing seasons with high total heat units of pears (Hansen, 1961); high temperatures 6 weeks prior to harvest of 'd' Anjou' pears (Mellenthin and Wang, 1976); 'La France' pears in the orchard exposed to cool temperatures (Murayama et al., 1999); pre-harvest temperatures above 40 °C and overhead cooling of 'Forelle' pears (Crouch et al., 2005); harvest maturity of 'Forelle' and 'La France' (Murayama et al., 1998; Carmichael, 2011) and a preliminary study of fruit canopy

position of 'Forelle' pears (Cronjé, 2014). A few studies focused on post-harvest factors, such as: post-harvest storage duration of 'Forelle' pears (Martin, 2002; Carmichael, 2011; Crouch, 2011) and climatic and ripening models of 'Forelle' (Lötze and Bergh, 2004).

However, it is not clear why some 'Forelle' fruit on a given tree, are predisposed to a mealy texture after storage and ripening, and others not. A closer understanding of the association between fruit position in the canopy, microclimate and a susceptibility to develop a mealy texture once harvested, will shed light on the subject. Pollination, the type of flower in a cluster, the number of fruit in a cluster, carbon assimilation due to sink strength, fruit position, and the varied rate of ripening, are a few of the factors which may affect fruit anatomy and physiology, which affect the fruits' susceptibility to have a mealy texture after storage and ripening. The finding of 'Forelle' pears with higher total soluble solids (TSS) developing a mealy texture, and an independent preliminary trial finding that outer canopy 'Forelle' pears may be more prone to mealiness, suggest a link between fruit position and the development of a mealy texture after storage and ripening (Cronjé, 2014; Muziri et al., 2016; Muziri, 2016). This could be explained by the fact that outer canopy fruit possibly have higher TSS concentrations and are possibly slightly riper and more inclined to have a mealy texture than inner canopy fruit, considering the fruit were harvested at the same time. Alternatively, outer canopy flowers and fruit are exposed to higher irradiance and temperature; consequently, possessing a higher sink strength and therefore higher carbon assimilation, resulting in higher TSS, larger cells and more intercellular airspaces (less dense). Currently, it is not yet known whether fruit position influences either the ripening rate or the fruit tissue density. This knowledge could not only lead to customised harvesting and storage protocols, reducing the risk of the development of a mealy texture, but could also improve the fruit quality of 'Forelle' pears after storage and ripening.

In order to obtain knowledge on 'Forelle' pear fruit development and the factors associated with 'Forelle' mealiness a literature review was carried out. The effect of different environmental conditions/factors and internal tree factors, such as hormones and nutrients was focussed on, as well as its effect on fruit development and final fruit quality.

Fruit ripening is also considered. The review was followed by three experimental studies which were carried out in the Elgin region of the Western Cape, South Africa in 2016 and 2017. The objective of our first study was to establish whether different fruit positions within

the tree canopy differ in susceptibility to mealiness development and which environmental factors, such as sunlight, temperature and relative humidity influence mealiness, as well as whether a link exists between maturity indices and mealiness (Chapter 2). The aim of the second study (Chapter 3) was to determine if fruit canopy position is linked to mealiness development through external environmental factors, such as light and temperature by applying shading treatments on outer canopy fruit. The purpose of the third study (chapter 4) was to establish whether mealiness incidence is related to storage potential and ripening rate differences within the canopy, as well as harvest maturity for these canopy positions.

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CHAPTER 1: LITERATURE REVIEW

THE EFFECT OF DIFFERENT ENVIRONMENTAL CONDITIONS/FACTORS AND INTERNAL TREE FACTORS ON FRUIT DEVELOPMENT AND FINAL FRUIT QUALITY, FOCUSING ON 'Forelle' PEAR MEALINESS.

1.1 INTRODUCTION

'Forelle' (*Pyrus communis* L.), a late season blushed pear cultivar in South Africa, is mainly produced in four climatically diverse areas in the Western and Eastern Cape provinces, viz., The Warm Bokkeveld [Wolseley, EGVV (Elgin, Grabouw, Vyeboom, Villiersdorp)], the Koue Bokkeveld and the Langkloof region (HORTGRO, 2018). The success of 'Forelle' is mainly attributed to their exceptional blush, which is favoured by consumers (Manning, 2009). The fact that 'Forelle' pear has the ability to develop an exceptional blush under South African conditions has set the cultivar apart from other bicolour pear cultivars, such as Cheeky, Rosemarie and Flamingo, with the latter two being heat sensitive, leading to a lack of pigmentation (Steyn et al., 2005). This is evident in that 'Forelle' takes up 26% of South Africa's total pear production area, whereas 'Flamingo', 'Rosemarie' and the new cultivar, Cheeky contribute a mere 1%, 4% and 4%, respectively (HORTGRO, 2018). However, 'Forelle' pear fruit is susceptible to develop a mealy texture after ripening to a firmness lower than 4 kg (Crouch et al., 2005).

Mealiness is a dry textural disorder (Crouch, 2011) accompanied by a floury sensation in the mouth, as well as a lack of juiciness, crispness and firmness (Barreiro et al., 1998). As a result, South African 'Forelle' pears have a mandatory cold storage period of at least 12 weeks at -0.5 °C, to achieve uniform ripening and to minimize mealiness incidence (de Vries and Hurndall, 1993). The phenomenon of reduced mealiness with extended cold storage is unique to 'Forelle' pears, considering other European pear cultivars, such as d' Anjou, Marguerite Marillat and La France show an increase in mealiness with extended cold storage (Chen et al., 1983; Murayama et al., 2002). It is known that 'Forelle' has a resistance to normal ripening, if the cold storage duration is insufficient (Martin, 2002; Crouch et al., 2005). The mandatory cold storage period of 'Forelle' has a negative impact on South Africa's exports, considering a gap is created in the supplying of bicolour pears to the European market. With a lack of

continuity in the supply of bicolour pears, it is possible that consumers may migrate to other cultivars as well as other offshore competitors' fruit, which could possibly become a permanent arrangement. To date, 'Forelle' pears from South America reach the European market several weeks earlier than South African 'Forelle', mainly due to fruit from South America being packaged and exported directly after harvesting (Crouch and Bergman, 2013). A programme, namely FEMA ('Forelle' Early Market Access), was recently developed in South Africa, which shortened the mandatory 12-week cold storage period at $-0.5\text{ }^{\circ}\text{C}$ to packaging and shipping periods which typically consist of 4 to 6 weeks of cold storage at $-0.5\text{ }^{\circ}\text{C}$. For the FEMA programme, fruit are left on the tree for a longer period to reach a certain TSS (above 14%). Harvested fruit are subjected to a 1-MCP (1-methylcyclopropene; SmartFreshSM; ©AgroFresh Inc., USA) application to prevent ripening, thereby enabling earlier marketing of non-mealy, crisp, sweet and juicy 'Forelle' pears. Although FEMA reduces the risk of mealiness of 'Forelle' pears, there are certain countries, such as the United Kingdom, which prefer the traditional soft, buttery and juicy 'Forelle' pears (Crouch and Bergman, 2013). The majority of South African pears are, however, destined for the European market which makes up 32% of the total export, with the United Kingdom making up 6%, and the Far East and Asia 20% (HORTGRO, 2018).

South Africa is the second largest pear producer in the Southern Hemisphere, with Argentina being the largest. In South Africa, Packham's Triumph is the most popular pear cultivar, followed by 'Forelle', Williams Bon Chretien and Abate Fetel (HORTGRO, 2018). South Africa's pear industry represents 16% of the total area of deciduous fruit production in South Africa.

The review was conducted with the goal of gaining knowledge on 'Forelle' pear fruit development and aspects associated with 'Forelle' mealiness. The focus is on the influence of various environmental conditions and internal tree factors, such as seed producing hormones and nutrients, on fruit development and final fruit quality. Fruit ripening and aspects associated with mealiness are also considered.

1.2 Process of fruit development

Fruit growth is defined as an irreversible change in mass and size (Robinson and Nel, 1986). The irreversible change is brought about via anatomical and physiological changes, which are controlled by exogenous and endogenous factors. Light levels, nutrients, water and temperature are regarded as the main environmental factors, which influence plant growth

and subsequently fruit growth. Endogenous factors include the genetics of the tree (including cultivar/rootstock combinations), crop load, and plant hormones and nutrients (Corelli-Grappadelli and Lakso, 2002).

Pear fruit have, as a rule, five carpels with each carpel potentially having two seeds. In contrast, parthenocarpic fruit may develop without seeds or fruit may develop with flat, empty seeds where the embryo aborted (Nyéki and Soltész, 1997). Parthenocarpy is the set of fruit without fertilization of the ovules (Gillaspy et al., 1993).

Fruit development can be categorized according to the following phases: pre-pollination; pollination; fertilization and fruit set; post-fruit set; ripening and senescence (Srivastava and Handa, 2005).

Pre-pollination development includes the initiation of the floral and fruit primordia (ovary and ovule) which undergo development up to the commencement of pollination and fertilization. For normal development of fruit of seeded plants, the successful initiation of fruit formation is required, which is dependent on the completion of pollination and fertilization of the ovule. Fertilization starts cell division and triggers the development of the ovary to form a fruit, with further fruit growth aided by plant hormones, principally gibberellins, auxins and cytokinins (Hedden and Hoad, 1985; Gillaspy et al., 1993). Most cell division takes place in the first few weeks following the pollination/fertilization of the flowers and is most likely influenced by the relative sink strength of the fruit and the effectiveness with which the available resources are supplied. The sink strength is more than likely determined by the quality of the flowers, the size of the vascular connection, the number of seeds present and the movement of natural hormones to and from the flower (Webster, 2002). After cell division, further growth occurs through cell enlargement until harvest (Dreyer, 2013). Fruit maturation is followed by ripening with a later transition to senescence (Crane, 1969).

The first step of sexual reproduction, namely flowering, is not triggered by a single factor, but rather by several factors, such as nutrition, plant hormones and different environmental factors (Crabbé, 1984). In pears, floral induction is the process where the meristem becomes committed to the formation of flower buds. The tendency of buds to develop as floral buds is determined by a multitude of factors and varies with morphological aspects of bud development and bud position. Buds on spurs have a higher tendency to develop into floral

buds in comparison to terminal and/or lateral buds on long shoots, which vary with the cultivar, age and vigour of the tree (Bubán and Faust, 1982).

Floral induction requires that the meristem has a strong sink activity during the period of induction. Floral induction occurs approximately at the start of the preceding bloom period and lasts for several weeks after full bloom. For optimal induction the inhibiting effect of gibberellins, originating from fruit seeds, must be limited (Bubán and Faust, 1982).

Floral initiation follows floral induction and commences approximately sixty days after full bloom, at the time of shoot growth cessation (Pratt, 1988; Van Zyl, 1979). Floral initiation takes place several weeks earlier in terminal spur buds compared to buds on longer shoots (Walters, 1968). Floral differentiation refers to the morphological transformation of the bud apex, after the completion of floral initiation, which leads to the formation of the inflorescence (Verheij, 1996). The morphological transformation is characterised by an increase in mitotic activity and cell division (Bubán and Faust, 1982). Shortly before bud opening and during bud opening in spring, the final development processes take place, viz. development of pollen sacs and ovules (Tromp, 2000). The number of flowers per inflorescence is determined largely by tree genetics and less so by the prevailing environmental conditions (Verheij, 1996).

During anthesis, which takes place in spring of the year following floral induction, stamens release pollen and the pistil is receptive to pollination and fertilisation. On completion of pollination, flowers can set fruit or abscise (Gillaspy et al., 1993).

During parthenocarpic fruit development the ovary grows into a seedless fruit without pollination and/or fertilization (Gorguet et al., 2005). Parthenocarpic fruit can occur naturally or be artificially induced with the application of various hormones, such as gibberellin (Gillaspy et al., 1993).

Pear fruit set parthenocarpically more regularly than apples, although some cultivars achieve this more than others, eg. Conference; Abbe-Fétel (Nyéki and Soltész, 2003); Williams Bon Chretien under certain growing conditions (Weinbaum et al., 2001) and 'Forelle' (Theron, 2010). The ability to undergo parthenocarpic fruit set on a regular basis has a great advantage for producers in areas with adverse conditions; such as late spring frosts, rain, cold temperatures or wind during bloom, which prevents the efficient pollination by insects,

mainly bees (Nyéki et al., 1998). Adverse conditions during pollination generate less viable seed (Nyéki and Soltész, 1997). Under conditions favourable for pollination, parthenocarpic fruit set is on average lower than when cross-pollination occurs (Pauwels et al., 1996).

Although parthenocarpic fruiting has advantages, there are a few disadvantages associated with parthenocarpic fruit. Fruit shape is strongly influenced by the number of full, viable seeds which are present (Gillaspy et al., 1993; Wertheim, 2000c; Buccheri and Di Vaio, 2004) and Pauwels et al. (1996) found that parthenocarpic 'Summerred' apple fruit have a greater length, while the diameter of the fruit was smaller than in pollinated fruit. Misshapen fruit and smaller fruit (Varoquaux et al., 2000), as well as a predisposition to post-harvest disorders (Sharifani and Jackson, 2001) are often associated with parthenocarpic fruit. Parthenocarpic fruit more often have calcium deficiency symptoms (Pauwels et al., 1996).

Miranda et al. (2005) found that the parthenocarpic 'Blanquilla' pear trees had a lower total yield than pollinated trees. The lower yield can be attributed to decreased sink strength of the fruitlets due to the absence of viable seeds, and therefore lower fruit set (Weinbaum et al., 2001). Considering that fruit drop is influenced by the number of seed, parthenocarpic fruit are more susceptible to fruit drop, although parthenocarpic fruit set can be increased with the application of GA₃ during bloom. However, one must keep in mind that different cultivars react differently to these applications (Pauwels et al., 1996).

Sink strength influences the amount of assimilates which can be utilised by the fruit which ultimately determines final fruit size. The sink strength of fruit can be conceptualised as the product of two components, namely: sink activity, which is measured as the potential flux or assimilate accumulation; and sink size, which is measured as a potential volume for biomass gain (Patrick, 1988). Both components are subject to hormonal regulation (Reynolds, 2004). Phloem unloading and/or metabolism of carbon assimilates in pear fruit is promoted by GA₃ and GA₄ which results in increased sink demand (Zhang et al., 2005; 2007b).

1.2.1 Role of plant hormones during fruit development and growth

It is known that plant hormones regulate the development and ripening of fruit (Crane, 1969). There are five classical hormones, namely: cytokinins, gibberellins, auxins, abscisic acid and ethylene, which are involved in the modulation of growth and development during different

stages of the developing fruit. Fruit act as mobilisation centres for mineral nutrients, during which time the hormones possibly modulate the process (Ozga and Reinecke, 2003).

Endogenous plant growth hormones, especially cytokinins, influence the early cell divisions of fruit development (Looney, 1993). Cytokinins are associated with the stage of rapid cell division (Gillaspy et al., 1993). Cytokinins are primarily synthesized in the root tips and transported via the xylem (transpiration stream) to different plant organs, with the highest concentration in young organs, such as fruit, seed and leaves (Went, 1992). The study of Bohner and Bangerth (1988b) found a positive correlation between cytokinin levels in developing seeds and cell division activity in nearby tissue. Insufficient endogenous cytokinin levels are considered one of the main factors which limit fruit growth and subsequently final fruit size (Flaishman et al., 2001; Shargal et al., 2006). The application of synthetic cytokinin to pear fruit resulted in parenchyma, which forms fruit flesh between the epidermis and the seed layers to have significantly smaller cells, but a larger number of cells in comparison to control fruit. The increase in the number of parenchyma cells was associated with an extended period of the cell division phase, therefore resulting in an increase in the number of cell divisions (Shargal et al., 2006).

Gibberellic acid (GA) is the hormone most frequently negatively associated with reproductive bud formation of pear. The two main sources of endogenous GA originate from the terminal regions of rapidly elongated shoots, particularly the young, rapidly expanding leaves and from developing seeds during the period of rapid embryo growth. GA is non-polar and may be transported throughout the plant via the xylem and phloem (Reynolds, 2004). Commercially, parthenocarpic fruit development is induced solely through the exogenous application of GA or in combination with other plant growth regulators (Westwood and Bjornstad, 1974). GA is also involved during seed germination, trichome development, stem and leaf elongation, flower induction, anther development, as well as fruit and seed development (Hedden and Phillips, 2000). GA inhibits floral induction of perennial fruit trees (Bangerth, 2006), but seed-produced GA's (GA₃ and GA₇) enhance fruit growth and development (Groot et al., 1987), as well as facilitate uptake of mineral elements (Buccheri and Di Vaio, 2004).

The study by Zhang et al. (2007a) reported that an application of GA during the early period of pear fruit development leads to a greater final fruit size, which is an indication that GA plays a role in cell division of pear fruit, as well as the maintaining of cell expansion (Ozga and

Reinecke, 2003). The cell enlargement phase after cell division has stopped, is primarily responsible for fruit growth that is dependent on carbohydrate accumulation and water uptake (Atkinson et al., 1998).

Pollen produced GA may play a role in the induction of auxin production in the ovary, which then possibly acts as a signal for fruit set and additional cell division (Gillaspy et al., 1993). GA and auxin produced by viable seeds enhance fruit growth and facilitate uptake of mineral elements (Buccheri and Di Vaio, 2004). It is suggested that auxin is involved in the initiation of the cell expansion phase and in the final embryo development phase (Mapelli et al., 1978; Gillaspy et al., 1993). The effect of auxin on reproductive development is unclear, considering that an early application of auxin is inclined to inhibit flower induction. However, later applications may well encourage the development of reproductive buds (Reynolds, 2004).

The dominance that certain fruit exercise over others is not necessarily due to morphological differences, but rather primigenic dominance, which means that earlier developed fruit dominates the fruit which develop later (Bangerth, 1989; Maguylo et al., 2014). There is some evidence that suggests that indole-3-acetic acid (IAA) is possibly involved in the transfer of the dominance signal (Reynolds, 2004). The export rate of auxin from a plant organ is an important factor in determining dominance and therefore indicates the importance of seeds in dominant fruit (García-Martínez and Carbonell, 1980). As mentioned previously, parthenocarpic fruit set may be induced by phytohormones, however, subsequent development may be restricted or prevented by the simultaneous presence of competing seeded fruit (Retamales and Bukovac, 1986).

The hormone balance of a tree may have a marked influence on the final fruit size and quality. Climatic variables (discussed at a later stage), as well as internal tree factors can exhibit an influence on the synthesis and distribution of endogenous hormones. The hormone ethylene plays an important role in climacteric fruit ripening and is discussed in the following section.

1.3 Ripening of climacteric fruit

Ripening of most fruit is associated with textural changes which are collectively referred to as softening (Brummell and Harpster, 2001), but which reflect multiple sensory attributes (Szczesniak, 2002). Ripening includes the processes which take place during the latter stages of fruit growth and the early stages of senescence (Kader, 1999). Fruit ripening is important

for the development of flavour, texture, aroma and the loss of astringency, which are important for obtaining optimum eating quality (Carmichael, 2011). Although factors such as cellular turgor and morphology (Lin and Pitt, 1986; Shackel et al., 1991; Harker et al., 1997) contribute to the overall fruit texture, the loss of fruit firmness is principally attributed to cell wall disassembly (Wakabayashi, 2000) and a decline in cell-to-cell adhesion, due to the dissolution of the pectinaceous middle lamella (Ben-Arie et al., 1979; Hallett et al., 1992). Therefore, fruit softening is typically accompanied by the depolymerization and solubilization of various classes of cell wall polysaccharides, such as pectin and hemicellulose, as well as by an increase in the expression of genes, proteins and enzyme activity (Wakabayashi, 2000; Giovannoni, 2001). In pear fruit, an increase in the amount of water-soluble polyuronides is usually found during normal ripening (Yoshioka et al., 1992; Murayama et al., 1998; Crouch, 2011).

A period of cold storage is required for autocatalytic ethylene synthesis to be induced (Knee, 1987; El-Sharkaway et al., 2004). The period of cold storage, however, varies according to the growth conditions of the fruit (El-Sharkaway et al., 2004). 'Forelle' pears have a high cold requirement for the induction of ethylene synthesis (Crouch et al., 2005). The ethylene climacteric is required for ripening and the development of the characteristic soft, buttery texture of 'Forelle' pear fruit (Crouch, 2011). Ethylene production by climacteric fruit, such as pears (Hiwasa et al., 2003) during the ripening process is regulated by two systems. The first system, called System I, produces low ethylene levels during the preclimacteric stage which increases the readiness of fruit to enter the climacteric stage via the possible deactivation of a "ripening inhibitor" (Yang and Oetiker, 1994). The path of ethylene biosynthesis begins with methionine, proceeds through S-adenosylmethionine (SAM) and 1-aminocyclopropane-1-carboxylic acid (ACC) and ultimately to ethylene. For ethylene biosynthesis, two main enzymes are involved, namely ACC synthase (ACS) and ACC oxidase (Yang and Hoffman, 1984). Sufficient ACC needs to build up for ripening which is initiated by ACS and cold temperatures. The enzyme, ACC oxidase is responsible for the final step to produce ethylene (Martin, 2002; Crouch, 2011).

Cell wall modifying enzymes are classified as pectolytic or non-pectolytic, depending on the class of polysaccharide, which is used as a substrate. Pectolytic enzymes function by cleaving or modifying the nature of the polysaccharide backbone or removing neutral sugars from the

branched side chains. Endo- and exopolygalacturonases (PG), pectate lyases, pectin methyl-esterases (PME), pectin acetyl-esterases, β -galactosidases en α -L-arabinofuranosidases are classified as pectolytic enzymes (Goulao and Oliveira, 2008). The fruit ripening enzyme which has been studied the most is polygalacturonase (PG) (DellaPenna et al., 1986); this is a hydrolase enzyme, which, to a large extent, is responsible for pectin depolymerisation (Wakabayashi et al., 2003). For pectin depolymerisation to take place, it is required that pectin is first de-methyl-esterified by PME (Brummell and Harpster, 2001).

Non-pectolytic enzymes bring about a modification of hemicellulose and include enzymes such as endo-1,4- β -glucanases (EGase), endo-1,4- β -xylanases, β -xylanases, xyloglucan endotransglycosylase/hydrolases and expansins (Goulao and Oliveira, 2008). The protein expansin has a direct, as well as a regulatory effect on fruit ripening enzymes (Payasi et al., 2009). Payasi et al. (2009) and Rose et al. (1997) reported that the role of expansins during the ripening process is to increase access for other cell wall modifying enzymes to cell wall polymers in a pH-dependent manner. The suppression or increased levels of a specific fruit ripening expansin results in altered rates of fruit softening and depolymerization of different classes of cell wall polysaccharides (Brummell et al., 1999).

Ripening pear fruit exhibit high expansin activity, as well as an accumulation of expansins during the ripening process, and potentially contribute to cell wall metabolism associated with ripening (Rose et al., 2000). The cooperative action of expansins with other enzymes, such as polygalacturonase, may possibly be an important factor in the softening process of pear fruit (Hiwasa et al., 2003). According to Hiwasa et al. (2003), there are at least ten expansin genes present in pear fruit and the expression of expansin genes does not take place simultaneously; the specific stage of fruit development determines the expression of a specific expansin gene. Certain expansin proteins are upregulated during ripening when softening commences; a decreased expression is observed in the over-ripe stage (Hiwasa et al., 2003). The presence of ethylene and other endogenous signals is important for certain expansins to have an effect, as well as after the onset of ripening in order to bring about fruit softening (Hiwasa et al., 2003).

1.4 Reported factors influencing mealiness development of pear fruit

Numerous studies have been done previously on factors influencing mealiness development of pear fruit, which included growing seasons with high total heat units (Hansen, 1961),

maximum temperatures six weeks prior to harvest on 'd' Anjou pears (Mellenthin and Wang, 1976), intermittent warming on 'Forelle' pears (de Vries and Hurndall, 1993), exposure to cool temperatures in the orchard on 'La France' pears (Murayama et al., 1999), climatic and ripening models of 'Forelle' (Lötze and Bergh, 2004), pre-harvest temperatures above 40 °C and overhead cooling on 'Forelle' pears (Crouch et al., 2005); storage duration after harvest of 'Forelle' (Carmichael, 2011; Crouch, 2011; Martin, 2002), harvest maturity on 'Forelle' and 'La France' (Carmichael, 2011; Murayama *et al.*, 1998), and canopy position (Cronjé, 2014).

The two factors which are mainly associated with increased susceptibility of 'Forelle' pears to develop a mealy texture after the ripening period is the insufficient cold storage duration at -0.5 °C (Martin, 2002; Crouch et al., 2005; Carmichael, 2011) and the harvesting of pears at a post-optimum maturity (Carmichael, 2011). Cold storage duration is an important aspect of 'Forelle' pear mealiness, considering that prolonged cold storage resulted in a decrease in mealiness of 'Forelle' pear fruit (Crouch et al., 2005). A higher 'Forelle' mealiness incidence was also associated with red blushed outer canopy fruit in a preliminary study by Cronjé (2014), as well as with bigger sized fruit and fruit with high TSS (Muziri, 2016).

1.5 Mechanism of mealiness development

In general, there are two mechanisms associated with mealiness development: firstly, the forming of relatively high molecular mass non-soluble methoxy pectic substances (Ben-Arie and Lavee, 1971; Dawson et al., 1992; Zhou et al., 2000a), and, secondly, the loss of cell-to-cell adhesion (Ben-Arie et al., 1979; Harker and Hallett, 1992; Crouch, 2011; Muziri et al., 2016).

In stone fruit, mealiness or soft dry textural disorders can be accompanied by internal gel breakdown (Brummell et al., 2004). The combination of mealiness with internal gel breakdown is attributed to the abnormal chilling-induced destruction of cell wall pectin (Ben-Arie and Lavee, 1971; Dawson et al., 1992), which is attributed to the imbalance between polygalacturonase (PG) and pectin methyl-esterase (PME) (Ben-Arie and Sonogo, 1980; Zhou et al., 2000a, b, c) and the cell membrane (Jooste, 2012). Chilling-injured fruit contain relatively high PME and low PG activity, with the result that the pectin matrix is de-esterified without the succession of depolymerisation (Manganaris et al., 2005). This leads to the accumulation of relatively high molecular mass of non-soluble methoxy pectic substances, which have the capacity to form gel structures, possibly aided by cell wall calcium and binds

free moisture (juice) (Dawson et al., 1992; Zhou et al., 2000a). As a result, a dry, mealy texture emerges (Obenland and Carrol, 2000). The chilling injury is related to low ethylene levels (Zhou et al., 2001), with the result that ethylene regulated cell wall modifying enzymes are influenced (Brummell et al., 2004).

The second mechanism of mealiness development is the reduction in cell-to-cell adhesion, as was proven by Harker and Hallett (1992) in apple. Mealiness is associated with high levels of intercellular air spaces, which is possibly related to the degradation of the middle lamella (Harker and Hallett, 1992), as well as the limited breakdown of cellulose (cell wall). A decrease in cell-to-cell adhesion can result in cell-to-cell sliding, which, in turn, prevents the breakage of cells and prevents the release of juice (Brummell et al., 2004). If the cell wall is stronger than the middle lamella, the parenchyma tissue gives way and as a result, the content of the cell is prevented from being released during mastication (Ben-Arie et al., 1979). Mealiness development of 'Forelle' pear fruit is also due to a more broken-down middle lamella with a loss of cell-to-adhesion, resulting in cell sliding during mastication as no high molecular mass pectins were found after ripening in mealy tissues (Crouch, 2011; Muziri, 2016).

With the ripening of fruit, a reduction in fruit turgor pressure occurs (Shackel et al., 1991; Harker and Sutherland, 1993). According to Brummell (2006), the associated reduction in turgor pressure is possibly due to the accumulation of osmotic solutes inside the apoplast, resulting in water loss. The expansionary pressure exerted on the cell wall decreases with the reduction of fruit turgor pressure, contributing to altered textural characteristics of fruit (Brummell, 2006). By using tensile tests, it has been reported that the cells of fresh, firm fruit break in a different manner compared to those of stored soft fruit, in that cells in fresh firm fruit predominantly break over the fruit equator (cell fracture), in contrast to soft fruit where the cells separate at the middle lamella without damage (cell-to-cell debonding) (Harker et al., 2002). With reference to the above results, it could possibly be said that a decrease in turgor pressure gives rise to a reduction in fruit firmness.

1.6 Synthesis and function of the primary cell wall

The cell wall is the strongest mechanical component of the cell and acts as an exoskeleton, which gives form to the plant cell, as well as enabling it to manage high turgor pressure. The cell wall participates in cell-to-cell adhesion, cell-to-cell signalling, defence and various other growth and differentiation processes (Cosgrove, 1997).

The cell wall of plant cells consists of approximately 25% cellulose, 20% hemicellulose, 40% pectin and possibly 5% structural protein on a dry mass basis (Taiz et al., 2015). Biosynthesis of cellulose takes place via dynamic complexes, which move within the plasma membrane, while the synthesized cellulose is added directly to the cell wall (Lerouxel et al., 2006). On the contrary, matrix polysaccharides such as hemicellulose and pectins, are synthesized inside the Golgi apparatus (Lerouxel et al., 2006), whereafter they migrate via vesicles and fuse to the plasma membrane. As a result, the matrix polysaccharides are released inside the extracellular space and are deposited in the cell wall (Baluška et al., 2005).

Cellulose plays the main role in determining the strength and the structural basis of cell walls. Hemicelluloses, like xyloglucan, bind to cellulose surfaces, which most likely form tethers, which bind cellulose microfibrils together or act as a lubricating coating, which prevents direct contact between microfibrils. Pectins form a gel phase in which the cellulose-hemicellulose network is embedded and possess the ability to act as a hydrophilic filler to prevent aggregation and the collapse of the cellulose network (Jarvis, 1992), as well as to modulate the porosity of cell walls (Baron-Epel et al., 1988). Pectins also provide charged surfaces, which modulate wall pH and regulate cell-to-cell adhesion at the middle lamella and junction zones (Jarvis et al., 2003). The hydrolase enzyme, α -L-arabinofuranosidase (α -AFase) combined with xylanases is responsible for the degrading of hemicelluloses to component sugars. The enzyme, α -AFase is considered to be one of the most important enzymes associated with mealiness (Saha, 2000), on account of apples (Pena and Capita, 2004) and peaches (Yoshioka et al., 2010) associated with mealiness, containing elevated levels of this enzyme.

Pectins are characterised by their high galacturonic acid content (Carpita and Gibeaut, 1993; Toivonen and Brummell, 2008). Pectin-containing polysaccharides can be differentiated into five types, namely: homogalacturonan (HGA), xylogalacturonan (XGA), rhamnogalacturonan I and II (RG-I and RG-II) (Toivonen and Brummell, 2008) and apiogalacturonan (AP) (Longland et al., 1989). HGA and RG-I are the two pectin types, which are mostly involved in dry textural disorders (Crouch, 2011).

The total cell wall of 'Forelle' pear fruit contains high amounts of arabinose and xylose; intermediate amounts of galactose; small amounts of rhamnose and glucose; and very small amounts of fructose and mannose (Crouch, 2011). It has previously been reported that

arabinose is the most abundant sugar in the cell walls of apples and pears (Dick and Labavitch, 1989; Gross and Sams, 1984). The possible function of arabinans in the cell wall is the modulating of homogalacturonan (Jones et al., 2003; Vincken et al., 2003), affecting water binding and cell wall characteristics (Brummell et al., 2004).

The cell wall is a continuously modified going through the plant development stages and adapting to environmental conditions. The middle lamella and primary cell wall are laid down by the plant cell during initial growth and expansion of the cell (Caffall and Mohnen, 2009).

1.7 Differences between mealy- and non-mealy cell wall compositions

Differences in the cell wall composition of mealy and non-mealy fruit has been reported in various previous studies (Brummell et al., 2004; Crouch, 2011; Hobbs et al., 1991; Zhou et al., 2000b). Mealiness is generally associated with a few common cell wall characteristics, of which two are most prevalent, namely: greater cell separation (De Smedt et al., 1998; King et al., 1989) and limited solubilization of pectins (Brummell et al., 2004; Hiwasa et al., 2004; Manganaris et al., 2008).

In the end stages of ripening, the porosity of mealy 'Forelle' is significantly greater than that of non-mealy fruit. The cells are also larger and oval-shaped, whereas the cells of non-mealy fruit are more rounded (Muziri, 2016). A study by Crouch (2011) found that mealy 'Forelle' pear fruit have less galacturonic acids in their middle lamella and that water-soluble pectin is depolymerised at an earlier stage of ripening. During normal ripening, fruit cells are typically released individually, whereas the cells of mealy fruit are released in small clumps (Brovelli et al., 1998). Non-mealy fruit are associated with a relatively high cell-to-cell adhesion, while the cell wall strength declines so that the cells can rupture easily (Crouch, 2011).

1.7.1 Parameters which influence fruit textural characteristics

Firmness is mostly determined by the physical anatomy of the tissue, particularly cell size, shape and packing; cell wall thickness and strength, the extent of cell-to-cell adhesion, combined with turgor status (Toivonen and Brummel, 2008). Many of the factors are inter-related, for example tissue with small cells are inclined to have a greater content of cell walls, a relatively lower content of cytoplasm and vacuole (cell sap), a greater area of cell-to-cell

contact and low amounts of intercellular air spaces, with the result that the tissue is firmer (Toivonen and Brummell, 2008).

On the contrary, the cells of mealy fruit are normally larger compared to the cells of non-mealy fruit. The larger cells, as well as larger intercellular spaces result in smaller areas of cell adhesion, increasing the susceptibility for the development of mealiness [De Smedt et al., 1998 (apples); Muziri, 2016 ('Forelle' pears)]. Fruit juiciness is possibly influenced by the ratio between cell walls, cytoplasm and vacuole. Juice is only released freely from vacuoles, in relation to other cell compartments which require relatively stronger force. Thus, a large vacuole surrounded by a thin layer of cytoplasm and cell wall is associated with the highest perceived juiciness. A decrease in the ratio between the vacuole and other organelles leads to a reduction in juice that mixes with the cytoplasm and cell wall. As a result, a dry sensation can develop even if there is an equal amount of cell moisture content (Crouch, 2011).

1.8 Factors influencing fruit development and fruit quality

Numerous studies have been done previously on the role environmental factors play in the development of mealiness, but relatively little attention has been given to mealiness in 'Forelle' pear fruit. The link between environmental factors and mealiness is to date not fully understood, possibly due to climatic variables across different seasons. Environmental factors also have an effect on endogenous factors, such as the carbon balance of the tree (Corelli-Grappadelli and Lakso, 2002). Fruit is considered a living system, consisting of various biochemical pathways, which may possibly be affected by different environmental factors (Wills et al., 2007). The optimising of pre-harvest factors is essential for obtaining high quality fruit, considering that fruit quality, in general, cannot be improved during the post-harvest period, but only be maintained (Bramlage, 1993).

1.8.1 Environmental factors and fruit canopy position

Climatic variables, specifically prevailing light (Bramlage, 1993) and temperature during periods of fruit growth have a fundamental effect on post-harvest quality and ripening behaviour of pome fruit (Villalobos-Acuña and Mitcham, 2008). Fruit is produced throughout the tree canopy, with the result that fruit is exposed to different irradiance levels, ambient temperature, water and nutrient flow, as well as the supply of endogenous hormones (Kingston, 1994; Tomala, 1999), which could possibly serve as an explanation for the

variability which occurs in the post-harvest life of pome fruit (Woolf and Ferguson, 2000). Because of predisposition to different environmental conditions, fruit also develop at different timings because of a difference in the flowering habit within a tree canopy.

A study by Fouchè et al. (2010) using 'Granny Smith' apple, showed that inner canopy fruit can receive as little as 2% ($33 \mu\text{mol.m}^{-2}.\text{s}^{-1}$) full sunlight, in comparison to outer canopy fruit which can be exposed to 54% ($962 \mu\text{mol.m}^{-2}.\text{s}^{-1}$) or more. The primary role of solar radiation as the source of energy, which is needed for the biological production of dry matter, ultimately determines the fruit yield (Dreyer, 2013). Consequently, the interception of light by the tree canopy is important and interception is determined by the number and arrangement of leaves, fruit and branches within the tree crown, tree shape and size, tree spacing, row orientation and the angular distribution of light from the sun and sky (Palmer, 1981). The timing of light penetration is important, considering that young developing fruit are poor sinks, with fruit set and/or fruit size which may be reduced in the presence of early competition by vegetative shoots (Avery et al., 1979; Ferree and Palmer, 1982), or by low irradiance, which, for example, inhibits floral initiation of spurs (Cain, 1971).

Light penetration into tree canopies can be improved by means of pruning, which leads to an increase in net photosynthesis of interior spurs (Rom, 1991). Maximum shoot expansion of most cropping pears takes place between 40 and 60 days after full bloom and the control of the expansion, at this time, is important to ensure sufficient light penetration and fruit set (Garriz et al., 1998). Temperature during the growing season can influence floral initiation, in that moderate to high temperature, which is necessary for inducing high vegetative vigour, can possibly lead to a decrease in floral initiation (Tromp, 1976). Excessive shoot growth has an inhibitory effect on flower bud formation, which is mostly attributed to GA originating from terminal regions of rapidly elongating shoots. In addition, young leaves and the upper internodes are a main source of GA (Verheij, 1996). The early cessation of shoot growth (vegetative growth) may possibly be conducive to flowering, in that flower initiation and differentiation can take place at a time when cytokinin levels are sufficiently high (Verheij, 1996).

The flesh of outer canopy fruit, which are exposed to direct sunlight, can reach a temperature of 15 °C higher than the ambient temperature. Consequently, fruit require an impressive homeostatic control of cell metabolism (Woolf et al., 1999a). A thermal gradient as great as

10 – 15 °C could develop across the fruit, which stretches from the sun exposed side to the shaded side. The fruit temperature of water stressed trees can increase dramatically, resulting in the movement of water from the warmer side to the cooler side, possibly leading to wilting on the warmer side (Woolf and Ferguson, 2000). The thermal gradient across the fruit can possibly lead to a dispersion of minerals and uneven ripening (Woolf et al., 1999b). Considering that outer canopy 'Forelle' pear fruit are more inclined to be mealy (Cronjé, 2014) and to have a lower free Ca²⁺ concentration than non-mealy pears (Muziri, 2016), the thermal gradient can possibly influence calcium distribution in the fruit with the result that less free calcium will be present. The function of calcium will be discussed later.

Fruit colour is influenced by the concentration and distribution of anthocyanins, carotenoids and chlorophylls (Steyn, 2012). The anthocyanin concentration of fully red and blushed pears is normally a maximum midway between anthesis and harvest (Steyn et al., 2004a), thereafter a gradual decrease in anthocyanin concentration is observed, associated with a loss of red colour due to a combination of decreasing synthesis, natural turnover, degradation at high temperatures and dilution (Steyn et al., 2004b). Although sunlight is required for anthocyanin synthesis, Steyn et al. (2005) reported that light has two opposite effects in pears, because light is required for anthocyanin synthesis, but also contributing to the loss of red color through increased anthocyanin degradation.

The surfaces of darker pigmented fruit (sun fruit) can be 15 to 20 °C warmer than shaded fruit (Raffo et al., 2011); larger fruit is also warmer, as the radiation absorbed varies with fruit radius (Smart and Sinclair, 1976). Harvest maturity can differ between fruit, depending on the different canopy positions (Crisosto et al., 1995), as reported by Cronjé (2014) where inner canopy 'Forelle' pear fruit were less ripe than outer canopy fruit, as well as being significant less mealy. The study of Carmichael (2011) reported that 'Forelle' pears harvested at a post-optimum maturity are more prone to mealiness development.

With reference to the importance and consequences of irradiation on fruit quality as mentioned earlier, tree canopies are indeed regularly subjected to shading, which can be a main stress factor in various crop species, having an influence on the final fruit quality (Garriz et al., 1997). Cultural practices such as type of tree training system, winter and summer pruning and fruit culling, result in changes in the irradiance levels within tree canopies (Corelli-Grappadelli and Lakso, 2002). With the development of leaves during late spring there

is an increase in the interception of photosynthetically active radiation (PAR), which is important in providing photo assimilates to fruit. The microclimate has a variety of effects on fruit development and appears to be related to the variations in irradiance experienced by the tree canopy (Garriz et al., 1997). A positive correlation exists between spur leaf area index (LAI) and apple crop yield, while the LAI of extension shoots does not show the same correlation regarding yield (Wünsche and Lakso, 2000). Similar results were found by Barritt et al. (1991), Palmer (1988) and Wünsche et al. (1996). Although there is a strong correlation between spur LAI and fruit yield, it is however important to note that the same spur leaf area on a tree with heavy shading by the exterior extension shoots will not produce the same fruit yield as a tree with less extension shoots. Consequently, spur LAI can be a controlling factor in very open canopies found in young orchards, but not necessarily in denser canopies with many exterior extension shoots (Wünsche and Lakso, 2000). It is therefore important to thin dense canopies by pruning to increase the yield and fruit quality; the total light interception is reduced, but improved light distribution patterns and improved exposure of the spur canopy is obtained. The importance of extension shoots for canopy development in young orchards and its supporting of fruit growth late in the season, especially in heavily cropped trees, must not be ignored (Wünsche and Lakso, 2000).

1.8.2 Carbon balance

Carbohydrates influence the quality and yield, as well as the sweetness of fleshy fruit (Feng et al., 2014). All plant organs of perennial woody plants can assume the role of storage organs, but root tissue normally contains the highest carbohydrate concentrations. Reserves vary greatly through the course of the year. During spring, when budding occurs, early vegetative and reproductive development takes place and the reserves diminish rapidly. Once the tissue has reached its minimum in resources, there is an immediate accumulation of reserves. In some instances, accumulation is interrupted during the period of fruit maturation (Roper et al., 1988).

The period when reserves accumulate is highly sensitive to late season stresses and to cultural practices. A decline in reserve accumulation can greatly affect the tree's performance in a negative manner the following year, considering reserves serve as substrate for shoot respiration and growth and are also required for flowering and initial fruit development during the period when the leaf canopy is not yet fully developed (Loescher, 1990). Nutrient

accumulation by spur leaves mainly occurs during the early part of the season. Due to the importance of spur leaves in the development of fruit, any limitation in nutrient supply at the beginning of the season resulting in reduced leaf growth, will have serious repercussions on subsequent fruit development (Buwalda and Meekings, 1990). Most of the cell division energy requirement is supplied with photo assimilate from spur and/or extension leaves (Garriz et al., 1997, 1998).

The photosynthetic activity of leaves for provision of photo assimilates for the fruit, is important for final fruit size, as reported by Garriz et al. (1997), with shaded 'Bartlett' pear fruit which had significantly lower fresh fruit mass, as well as a smaller diameter than fruit bearing branches exposed to sunlight. Lakso and Corelli-Grappadelli (1992) obtained similar results, where fruit bearing branches that received 65% less irradiance had a negative fruit growth rate four weeks after full bloom and shortly before harvest. It must however be kept in mind that high irradiance and high temperatures can have negative effects and moderate shade, under these circumstances, could lead to a higher photosynthetic activity and stomatal conductance (Garriz et al., 1997). The combination of high irradiation and stress conditions limits the conversion of photosynthetic energy by reducing the supply of CO₂, which may promote photoinhibition (Herppich, 1999). Photosystem II is the component of the photosynthetic apparatus, which is most sensitive to heat and high light stress (Havaux et al., 1996).

In cases where fruit set is extremely abundant, there is a reduction in the partitioning of photosynthetic assimilates and nutrients which are important for the support of floral primordia development. The flower quality of the subsequent season will therefor also be poor (Webster, 2002). "Poor quality" flowers, in general, produce smaller size fruit or misshapen fruit, and also abscise more frequently (Gillaspy et al., 1993; Webster, 2002).

According to the carbon balance model for 'Empire' apple fruit (Lakso et al., 2001), there is an indication that crop load has an influence on the amount of resource supply to fruit. When the sink (fruit) demand is higher than the maximal supply, the fruit experience a limitation to their growth (Corelli-Grappadelli and Lakso, 2002). Fruit from a normal crop load can be subject to a limited supply of resources early in the season (Lakso et al., 1997), when the canopy is still busy developing, the leaves have not yet reached their full photosynthetic potential and vegetative growth competes for the same resources (Corelli-Grappadelli and

Lakso, 2002). During the first four to five weeks after full bloom, the growing extension shoot tips act as a high priority sink with the ability to draw the fixed carbon from the leaves on fruiting spurs to themselves (Corelli-Grappadelli et al., 1994). The fruit does, however, receive the most fixed carbon after this period and as a result, fruit become the prevailing sink (Corelli-Grappadelli and Lakso, 2002). The carbon shortage in fruit shortly after full bloom explains the thinning effect of shade and photosynthetic inhibitors, as reported by various previous studies (Byers et al., 1985). The fruit bearing site influences sink strength, as reported by Reynolds et al. (2005); fruit on thick bearing units exceed the sink strength of the fruit present on thin bearing units, in that fruit on thick and short bearing units are possibly better provided of metabolites via the xylem (transpiration stream).

Carbohydrates originating from photosynthesising leaves are needed for the growth of the plant, as well as for fruit growth (Loescher et al., 1990; Lebon et al., 2008). Therefore, vegetative growth is essential for the maintenance of leaves and the provision of new bearing sites for the following year (Lauri et al., 2004). The balance between vegetative growth and fruit load is, however, important considering vegetative growth competes with fruit for photosynthetic products from the leaves (Tomala, 1999). The early growth period of pear fruit has a great demand for nitrogen; however, excessive nitrogen levels lead to excessive vegetative growth, which disrupts the balance of the tree (Deckers and Schoofs, 2002). The successful coexistence of vegetative growth and reproductive growth (fruiting) is assured by a temporal and spatial separation of the two processes (du Plooy et al, 2002). For example, pear fruit development occurs preferentially on spurs and brindles (du Plooy et al, 2002; Sansavini, 2002).

1.8.3 Nutrition, water content versus tree and fruit growth

Turgor pressure of fruit cells plays an important role in fruit growth and flesh texture. The maintenance of turgor pressure is dependent on the semi-permeable cell membrane and the physical strength of the cell wall. The loss in selective permeability, which may occur during ripening or fruit damage, results in textural changes (Harker and Sutherland, 1993; Shackel et al., 1991). Plant nutrition has a great influence on final fruit quality, with nitrogen, phosphorus, potassium and calcium, in particular, having pronounced effects on fruit texture (Blanpied et al., 1978).

The plant nutrient mostly associated with fruit quality, especially with flesh firmness, is calcium (Shear, 1975). Calcium forms cross-links with negatively charged pectins, which provide structural rigidity to the cell wall (Hepler and Winship, 2010).

The role of Ca^{2+} in fruit mealiness development has already been investigated, however very little has been found on the relationship between mealiness and fruit Ca^{2+} content (Mignani et al., 1995; Saftner et al., 1998). Muziri (2016) found a lower free Ca^{2+} concentration with mealy 'Forelle' pears compared to non-mealy pears suggesting a possible link between fruit calcium levels and mealiness.

The calcium content of fruit influences fruit metabolism by bringing about changes in intracellular and extracellular processes. Fruit firmness, rate of softening, fruit quality and the appearance of various physiological disorders is dependent on fruit calcium content (Poovaiah, 1988). Calcium is also important in the stabilization of the structure of respiratory enzymes in fruit (Faust and Shear, 1972); there is a negative correlation between fruit calcium content and fruit respiration (Cooper and Bangerth, 1976; Al-Ami and Richardson, 1987). The physiological role of calcium entails the binding of neighbouring cell walls, as well as the maintenance of the integration and semi-permeable properties of the membrane (Tomala, 1997; Poovaiah, 1988). The lower concentration of free Ca^{2+} in mealy textured 'Forelle' pears (Muziri, 2016) may possibly play a role in the increase of the permeability of the membrane to enzymes, resulting in a more broken-down middle lamella. The mechanism of mealiness development of 'Forelle' pears entails a more broken-down middle lamella, leading to the loss of cell adhesion (Crouch, 2011; Muziri, 2016), as calcium-pectin is the principal material contributing to intercellular adhesion (Van Buren, 1991).

Fruit calcium shortage can occur because of environmental factors such as drought, salinity, low relative humidity, shoot and root temperatures, light irradiation, as well as mineral imbalance conditions (Ho and White, 2005). Other factors such as vegetative growth, crop load, fruit position, plant growth regulators, functional xylem vessels, period of calcium availability to the fruit and number of seed present in fruit, can cause differences in fruit calcium content (Sauer, 2005; De Freitas and Mitcham, 2012). In addition, parthenocarpic fruit are generally associated with calcium deficient symptoms (Pauwels et al., 1996).

Calcium deficiency disorders can occur when the rapidly expanding fruit tissue's demand for Ca^{2+} exceeds the immediate xylem supply (Ho et al., 1993; Ho and White, 2005). Thus, fruit

position in the canopy and the origin of buds may influence the calcium levels of fruit, as reported by Tomala (1997) and Ferguson et al. (1993), where fruit closer to the top of the tree contained lower calcium concentrations, as well as fruit of lateral bud origin, in comparison to fruit from terminal buds. Various other studies have also reported the effect of fruit position on fruit calcium content (Bertin et al., 2000; Ho et al., 1993; Ferguson and Triggs, 1990). It was found that higher light intensity and air temperature during fruit growth and development increased Ca^{2+} deficiency disorders; possibly because of fruit calcium content being diluted due to accelerated fruit expansion (Saure, 2005). Thus, outer canopy fruit, which is generally larger, can possibly be more susceptible to a calcium shortage.

The presence of high fruit nitrogen levels results in a reduction in fruit calcium, resulting in turn in a decline of the calcium/potassium ratio (Tahir et al., 2007). Therefore, the excessive nitrogen causes a reduction in fruit firmness combined with accelerated post-harvest softening rates (Tahir et al., 2007). Adequate nitrogen levels are, however, important for fruit development, fruit size, fruit colour and flavour (Tahir et al., 2007). Excessive fruit potassium content, relative to fruit calcium, increases the occurrence of fruit disorders associated with an undesirable texture (Sharples, 1984). Fruit with low fruit phosphorus content results in a loss in flesh firmness, especially in fruit with low fruit calcium levels (Sharples, 1980). The effect of a shortage of fruit potassium can reduce fruit acidity and there could be a reduction in anthocyanin synthesis (Neilsen et al., 2008).

Tree water content has a direct influence on cell turgor and as a result, the degree of cellular hydration results in a marked change in texture (Shackel et al., 1991). Prolonged periods of water stress can cause wilting and a significant loss in textural quality (Iritani, 1981). Water stressed 'Nijisseki' pear trees exhibit a significantly lower rate of photosynthesis and stomatal conductance in relation to well-watered trees (Behboudian et al., 1994). It is important that the water content of trees is at its optimal throughout the season, as the rewatering of water stressed trees does not immediately revive them, resulting in the continuation of a lowered photosynthesis rate and stomatal conductance (Behboudian et al., 1994). The decrease in photosynthesis during the water stress period during the phase of rapid increase in fruit diameter is due to the reduction in stomatal conductance, as well as to an impaired photosynthetic system (Behboudian et al., 1994). Water availability to fruit during the cell enlargement phase is an important factor for fruit growth (Tukey, 1974; Webster, 2002).

1.9 CONCLUSION

Mealiness development is the most serious internal textural disorder of South African 'Forelle' pears (Cronjé et al., 2015; Crouch, 2011; Martin, 2002; Muziri et al., 2016) and has the ability to damage the image of 'Forelle' pears worldwide. Numerous studies have been done on factors influencing mealiness development of pear fruit. The studies included the evaluation of the effect of growing seasons with high total heat units (Hansen, 1961), maximum temperatures six weeks prior to harvest on 'd' Anjou pears (Mellenthin and Wang, 1976), intermittent warming on 'Forelle' pears (de Vries and Hurndall, 1993), exposure to cool temperatures in the orchard on 'La France' pears (Murayama et al., 1999), climatic and ripening models of 'Forelle' (Lötze and Bergh, 2004), pre-harvest temperatures above 40 °C and overhead cooling on 'Forelle' pears (Crouch et al., 2005); storage duration after harvest of 'Forelle' (Carmichael, 2011; Crouch, 2011; Martin, 2002), harvest maturity on 'Forelle' and 'La France' (Carmichael, 2011; Murayama et al., 1998), and canopy position (Cronjé, 2014). Mealiness development of 'Forelle' pears are mainly associated with insufficient cold storage at -0.5 °C (Martin, 2002; Crouch, 2011; Carmichael, 2011) and with post-optimum harvest fruit (Carmichael, 2011). Little research has focused on the effect of canopy position and pollination on 'Forelle' pear mealiness development.

In conclusion, it is relatively evident that pre-harvest factors and endogenous tree factors play an important role in fruit development and final fruit quality. Fruit texture is influenced by traits such as cellular organelles and biochemical constituents, water content or turgor, and cell wall composition. As a result, any external factor or internal tree factor has the ability to influence fruit quality by influencing the aforementioned traits and may alter ripening behaviour, resulting in modification of flesh texture (Sams, 1999). Considering 'Forelle' pear mealiness only develops after the period of ripening, the studying of 'Forelle' mealiness development remains a great challenge. Thus, clear knowledge of environmental factors, such as light irradiation and temperature, and endogenous plant factors related to mealiness development is necessary to minimize mealiness, as well as to implement correct post-harvest practices to minimize further mealiness development. Knowledge of fruit maturity variables, such as flesh firmness, ground colour, total soluble solids, titratable acidity, ethylene production and carbon dioxide production, which are possibly associated with 'Forelle' mealiness is also required in order to identify potential mealy fruit at an early

ripening stage. The use of X-ray computed tomography could be a promising technique for the non-destructive determination of mealiness at an early stage of ripening (Muziri, 2016).

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CHAPTER 2:

THE EFFECT OF COLOUR, CANOPY POSITION ON 'Forelle' PEAR MEALINESS DEVELOPMENT

Abstract

Forelle (*Pyrus communis* L.) is the most valuable bicolour pear in South Africa. 'Forelle' pears tend to develop a soft, dry textural disorder associated with a lack of juiciness, called mealiness, after ripening. The objective of this study was to determine if fruit from different positions within the tree canopy differ in their susceptibility to develop mealiness. In addition, the link between mealiness and fruit maturity indices as well as environmental factors such as irradiance, temperature and vapour pressure deficit, were evaluated over the 2016 and 2017 seasons. Irradiance and fruit surface temperature (FST) were measured for five fruit canopy positions in the 6 weeks prior to harvest. Canopy temperature and relative humidity were measured continuously during the 6 weeks. The five fruit canopy positions included: outer-canopy, highly red blushed pears on the eastern and western sides of north to south orientated tree rows, slightly blushed pears in the intermediate/middle canopy on the eastern and western sides and shaded inner-canopy pears with no blush. Fruit were harvested at commercial maturity (± 6.2 kg firmness) and maturity indices, as well as mealiness incidence, ethylene production and respiration rate were determined after 8 weeks of cold storage at -0.5 °C and 7 days or 11 days of ripening at 20 °C [8w RA (regular atmosphere) + 7d or 11d SL (shelf life)]. In both seasons, mealiness was significantly higher for red blushed outer canopy fruit, which was also associated with a significantly higher irradiance (sunlight exposure), average fruit surface temperature and vapour pressure deficit (VPD). Mealy fruit seemed to be in a more advanced stage of maturity and to be bigger in size. Interestingly, outer canopy fruit with a non-mealy texture released a significantly lower amount of juice than non-mealy textured fruit from the intermediate and inner canopy. The large temperature fluctuations experienced by outer canopy fruit may affect fruit metabolism, which eventually might lead to the fruit being more susceptible to mealiness development or seeing that outside canopy fruit are larger, their cell-to-cell connections may not be able to keep up with the expansion rate/ sink strength. It seems as if the exposure of fruit to high irradiance, in conjunction with high fruit surface temperatures coupled with high VPD, is one of the determining factors in 'Forelle' pear mealiness development.

Keywords: canopy position, fruit surface temperature, irradiance, mealiness, *Pyrus communis* L., vapour pressure deficit.

2.1 INTRODUCTION

In South Africa the pear industry contributes 16% to the total area of deciduous fruit production (HORTGRO, 2018). The pear production takes place mainly in the Western Cape Province with also some production in the Eastern Cape Province (HORTGRO, 2018). These areas vary in climatic conditions, which has the potential to influence harvest maturity and the ripening potential of fruit (Carmichael, 2011). Forelle (*Pyrus communis* L.) is the most valuable bicolour pear cultivar in South Africa and contributes 26% to South Africa's total pear production area, whereas other bicolour cultivars, Rosemarie, Flamingo and Cape Rose contribute only 4, 1 and 4%, respectively (HORTGRO, 2018). The dominance of 'Forelle' pears is attributed to their ability to develop a better red blush than 'Rosemarie' under South African conditions (Steyn et al., 2005), whilst 'Flamingo' tend to undergo internal breakdown (Crouch, 2011). Cape Rose is a recently released cultivar of which plantings are gradually increasing.

'Forelle' is a "winter pear" with a high cold requirement for the induction of ethylene synthesis (Crouch et al., 2005). The ethylene climacteric is required for ripening and development of the characteristic soft, buttery flesh of 'Forelle' pears (Crouch, 2011). Two of the most essential factors that determine consumer acceptance are eating quality and fruit appearance (Eccher-Zerbini, 2002). However, the development of the characteristic red blush of 'Forelle' pears, which is favoured by consumers (Human, 2002), are more prone to develop a dry, mealy texture after ripening to a firmness below 4 kg (with a 7.9 mm penetrometer tip) (Crouch et al., 2005). Mealiness is classified as a textural disorder associated with a dry, floury mouthfeel during eating and a lack of juiciness, crispness and firmness (Barreiro et al., 1998). Mealiness was first reported in the 1980s (Hurndall, 2011) and is the most serious internal quality defect of South African 'Forelle' pears (Martin, 2002; Crouch, 2011; Cronjé, 2014; Muziri et al., 2015).

Mealiness development of 'Forelle' pear fruit is associated with post-optimum harvest maturity (Carmichael, 2011) and insufficient cold storage (Martin, 2002; Carmichael, 2011).

This resulted in a mandatory cold storage period of at least 12 weeks at $-0.5\text{ }^{\circ}\text{C}$ for South African 'Forelle' pears for the purpose of reducing the incidence of mealiness (de Vries and Hurndall, 1993). This mandatory cold storage period causes a loss in South African bicolour pear continuity in the European market, with the risk that consumers could make a permanent move to offshore fruit, even if South African 'Forelle' pears are available.

Considering that South American, especially Chilean export volumes of 'Forelle' show a sharp yearly increase, it is a high priority to reduce the mandatory cold storage period. This will ensure the availability of South African 'Forelle' pears in Europe from week 15 already, when premium prices can be achieved (Crouch and Bergman, 2013).

Numerous studies have been done on pre-harvest and post-harvest factors affecting mealiness development of pear fruit. Studies focused on pre-harvest factors such as growing seasons with high total heat units on pears (Hansen, 1961), high temperatures six weeks prior to harvest on 'd' Anjou' pears (Mellenthin and Wang, 1976), the exposure to cool temperatures in the orchard on 'La France' pears (Murayama et al., 1999), pre-harvest temperatures above $40\text{ }^{\circ}\text{C}$ and overhead cooling on 'Forelle' pears (Crouch et al., 2005), and a preliminary study on fruit canopy position on 'Forelle' pear mealiness (Cronjé, 2014). The effect of harvest maturity on 'La France' and 'Forelle' was also studied (Murayama et al., 1998; Carmichael, 2011). Studies on post-harvest factors investigated the effect of intermittent warming on 'Forelle' pears during storage (de Vries and Hurndall, 1993), storage duration after harvest of 'Forelle' (Martin, 2002; Carmichael, 2011; Crouch, 2011), and climatic and ripening models of 'Forelle' (Lötze and Bergh, 2004).

Fruit located near well-illuminated leaves ~~near them~~, have the best chance to achieve their growth potential (Jackson, 1980). Numerous studies associated an increase in light exposure with improved fruit growth and increased final fruit size (Tustin et al., 1988; Khemira et al., 1993 Kappel and Neilsen, 1994). However, the preliminary study of Cronjé (2014) found outer canopy 'Forelle' pears to have a higher mealiness incidence than fruit in the shaded inner parts of the canopy. In addition, Muziri (2016) associated 'Forelle' mealiness with higher total soluble solids (TSS) and bigger fruit, which is generally associated with outer canopy fruit. There is currently very little research done on the role canopy position plays in mealiness development of 'Forelle' pear fruit.

Temperature influences fruit metabolism, which results in cellular structures and other components being affected indirectly (Sams, 1999). Fruit textural changes during ripening are dependent on various metabolic events, which involve the loss in turgor pressure, physiological changes in membrane composition, starch degradation, and modifications in the cell wall structure and dynamics (Hadfield and Bennett, 1998). The most important factors influencing the textural changes of fruit are variations in the cell wall mechanical strength and cell-to-cell adhesion (Fischer and Bennett, 1991; Hadfield and Bennett, 1998). The mechanism of 'Forelle' pear mealiness development entails a loss of cell-to-cell adhesion due to a weaker middle lamella, which results in cell sliding during mastication (Crouch, 2011; Muziri, 2016). As mentioned previously, Cronjé (2014) and Muziri (2016) associated outer canopy 'Forelle' pears with a higher mealiness incidence. Thus, post-harvest textural differences between fruit of different canopy positions can possibly be expected. Fruit development, fruit growth rates and quality properties such as carbohydrate concentrations of fruit are mainly determined by temperature (Woolf and Ferguson, 2000). The different fruit positions within the canopy experience different levels of irradiance and ambient temperature, as well as differences in the supply of water, mineral nutrients and endogenous hormones (Tomala, 1999). Consequently, fruit mineral concentrations may differ greatly within a single pear tree (Sanchez et al., 1991). Muziri (2016) associated mealy 'Forelle' pears with lower concentrations of free Ca^{2+} than non-mealy pears, thus fruit position within the canopy may possibly alter the partitioning of mineral nutrients, as well as the partitioning within the fruit. The cell enlargement phase, which takes place after cell division, is primarily responsible for fruit growth and is dependent on carbohydrate accumulation and water uptake (Atkinson et al., 1998). The rate and duration of the cell division phase is a determining factor for the final number of cells within the fruit (Denne, 1960). The mechanical properties of the fruit cortical tissue are influenced by the number and size of the fruit cells. Recently Muziri et al. (2015) reported a positive linear relationship between cell volume and mealiness in 'Forelle' pears. Thus, differences in textural quality and susceptibility of fruit to post-harvest physiological disorders could be expected (Atkinson et al., 1998).

It is not clear why some 'Forelle' fruit on the same tree are predisposed to a mealy texture after storage and ripening, whilst others are not. A closer understanding of the relation between fruit position on the tree, microclimate and susceptibility to develop a mealy texture

once harvested, will shed light on the subject. An understanding of the possible pre-harvest factors that increase the incidence of 'Forelle' pear mealiness development, could possibly lead to the modification of cultural practices to produce fruit that are less prone to mealiness. The objective of this study was therefore to confirm the link between mealiness development and fruit position within the canopy, to understand the differences between mealy and non-mealy fruit within the canopy, as well as the possible drivers for mealiness development within the canopy.

2.2 MATERIALS AND METHODS

2.2.1 2016 season

2.2.1.1 Fruit material

Ten 'Forelle' pear (*Pyrus communis* L.) trees were selected on 12 January 2016 at Glen Fruin farm in Elgin, Western Cape, South Africa, based on similar vigour and crop load to ensure uniformity. The trees were in a single row. The experimental design was a completely randomised block design, with the trees as blocks.

The orchard was planted on BP3 rootstock in 1991 at a spacing of 4.5 x 1.5 m in a north-south row orientation and trained to a central leader training system. On each of the ten trees, five fruit positions were tagged (between 1m and 3.5m from the ground): the outer canopy red blushed pears on the eastern and western sides, the slightly blushed pears in the intermediate/middle canopy on the eastern and western sides and fruit with no blush in the completely shaded parts inside the canopy. For each of the five positions, eight fruit per tree were tagged.

2.2.1.2 Irradiation, fruit surface temperature and vapour pressure deficit

Irradiance levels and fruit surface temperatures were measured from mid-January until 2 March 2016 on cloudless days between 07:00 and 17:00, four times a day (three times a week). Irradiance levels of fruit of different canopy positions were measured using a light meter (LI-250, LI-COR, Lincoln, NEB, USA) with a quantum sensor attached to it. The light meter was held next to each fruit with the sensor perpendicular with the position of the sun.

Maximum irradiance levels were measured in the open next to the treatment trees prior to the onset of light irradiation measurements.

Fruit surface temperature was measured at the position of the fruit facing the current position of the sun, using a high-performance infrared thermometer (Rayner MX4, Raytek Corporation, Santa Cruz, CA, USA).

During the period from mid-January until the time of harvest on 2 March 2016, canopy temperature and relative humidity were continuously measured by means of Tinytag Plus 2 data loggers (Model TGP-4500, Gemini Data Loggers, United Kingdom). In total, 10 Tinytag loggers were used, where each Tinytag logger was placed within a bollard top. Five Tinytags were placed in the outer canopy of five different trees, approximately 1.5m from the ground (two on the eastern side and three on the western side). The other five Tinytags loggers were placed in the shaded inner canopy of five different trees, approximately 1.5m from the ground. The hourly vapour pressure deficit (VPD) of the five different fruit canopy positions was calculated by using the canopy temperature and relative humidity data in combination with the fruit surface temperatures of the sun exposed side of the fruit if the fruit position was in the sun.

2.2.1.3 Maturity and quality indices

Tagged pear fruit were harvested at commercial harvest maturity (± 6.2 kg firmness) on 2 March 2016. The trial entailed the harvest of a total of 400 fruit, comprised out of 80 fruit from each of the five canopy positions. After harvest, pears were transported and stored directly in polyethylene bag (37.5 μm) lined commercial cartons for 8 weeks of cold storage at -0.5 °C with 7 and 11 days of ripening at 20 °C (8w RA + 7 and 11d SL) at the Department of Horticultural Science, Stellenbosch University, South Africa.

Maturity indexing (MI) was conducted at harvest and after 8w RA at -0.5 °C + 7 and 11d SL. Fruit that were ripened for 7 days did not ripen sufficiently in order to evaluate mealiness and were hence ripened further for 11 days for the MI evaluation. On each of the evaluation days, maturity and quality indices were measured 12 h later. For each evaluation, two fruit per canopy position, per tree were measured.

2.2.1.3.1 Fruit background colour and blush colour

Fruit background colour represents the change in colour from a green to a more yellowish ground colour. The background colour of the pears was determined according to the colour chart developed by Unifruco Research Services (URS) [Pty] Ltd., South Africa for apples and pears, on a scale ranging from 0.5 to 5 (0.5=dark green and 5=deep yellow). The blush chart P. 16 developed specifically for 'Forelle' pears by URS was used for the determination of the fruit blush coverage on a 1 to 12 scale (1=dark red and 12= green).

2.2.1.3.2 Ethylene production and respiration rate

For fruit from each of the ten trees, the ethylene production ($\mu\text{L}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$) and respiration ($\text{mg CO}_2\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$) rates were measured on one fruit from selected from each of the five respective canopy positions. Single fruit were enclosed in a 5 L airtight plastic container (jar) for 30 min at room temperature. With the completion of the 60 min, airtight 10 mL syringes were used for taking gas samples, which were then injected into an Agilent N6980 Gas Chromatography system (Agilent Technologies, Wilmington, Delaware, USA) with PorapakQ and Molsieve packed columns and flame ionization and thermal conductivity detectors. The total fruit mass and volume of free space in the container (jar) was measured in order to calculate ethylene production and respiration rates.

2.2.1.3.3 Diameter, mass and length

A Cranston gauge and an electronic balance were used to determine fruit diameter and mass, respectively. Both apparatus were connected to a fruit Texture Analyser (model TA. xTPlus, Stable Micro Systems, Inc., Surrey, UK), which measured the flesh firmness. A digital calliper (Mitutoyo Corp., Japan) with a range of 0 to 150 mm, was used to measure fruit length.

2.2.1.3.4 Seed count (normal and aborted)

Fruit were cut equatorially and the seeds were removed for counting. Flattened seeds were classified as aborted seeds and full, round seeds as normal, viable seeds.

2.2.1.3.5 Firmness

A penetrometer (Fruit Texture Analyser, GÜSS Manufacturing (Pty) Ltd., Strand, South Africa) fitted with a 7.9 mm probe was used to measure flesh firmness on both the sun exposed and shaded side of the fruit, after removing the peel (± 1 mm thick) with a potato peeler. In the case of fully shaded pears, firmness was measured on opposite sides of the fruit.

2.2.1.3.6 TSS and TA

Equatorial pear fruit slices (without seeds), representing all the different sides of the fruit were juiced with an electric juicer (AEG Electrolux, Type JE- 107 no. 91100085/ PNC 950075206, P.R.C., city?, Country?) for determination of total soluble solids (TSS) using a digital refractometer (TSS 0-32%, Atago, Tokyo, Japan). Fruit titratable acidity (TA), expressed as percentage malic acid equivalents, was measured by using an automated titrator (Metrohm Titrando with an 815 Robotic USB Sample Processor and Tiamo software, Metrohm, Switzerland).

2.2.1.3.7 Mealy texture and juiciness evaluation

Sensory analysis was used for the determination of mealy texture. The analysis consisted of three trained evaluators evaluating the texture of each fruit. The panel had a minimum of 8 years' experience on texture determination. The fruit were in cold storage at -0.5 °C for a period of 8w RA + 11d SL at 20 °C before sensory analysis was performed. To determine the presence or lack of free juice, the slices of pear tissue were organoleptically evaluated and hand squeezed, which was then rated for texture. A scale of 0 to 2 (0=non-mealy; 1=partly mealy and 2=mealy) was used to classify the mealiness score per fruit.

For the validation of the differences in fruit perceived as mealy, partly mealy and non-mealy by the trained panel, a confined compression juiciness test as described by Barreiro et al. (1998) was used on the same fruit. The confined compression test entails the measuring of the expressible juice (mg) obtained from each fruit upon compression of a 1 cm high and 1 cm diameter tissue wedge (radial bar of tissue). The source of the tissue wedge was from the equator region of each fruit and the Texture Analyser compressed the tissue wedge. The texture analyser was set to move at a speed of 1 mm·s⁻¹ and return back at 10 mm·s⁻¹. The tissue was compressed to a distance of 2 mm from the platform of the instrument. A 10 kg steel block was used to calibrate the instrument. In order to collect the juice released upon compression, Benchkote protector filter paper (Whatman No. 2300 916, GE Healthcare, Buckinghamshire, UK) served as sample holding paper. At the end of complete deformation, the probe moved back to its original position. After weighing the filter paper in order to determine the juice mass (mg), the paper was air-dried for 48 h followed by a 24 h period of oven drying at 40 °C for the purpose of developing colour on the juice covered area. After the

completion of the scanning of the filter paper, ImageJ (Wayne Rasband, National Institute of Health, City?, USA) was used for the measuring of the total area covered by the released juice. At the end, each fruit was associated with a mealy texture score, a juice area and juice mass as described by Muziri et al. (2016).

2.2.1.3.8 Data analysis

The repeated measures procedure was used for the separation of temperature and irradiance data. In order to establish differences between the five different fruit canopy positions, with regard to the maturity and quality indices, one-way analysis of variance (ANOVA) was performed on these data. The Kruskal-Wallis test was used for confirmation in cases where the residuals were not normally distributed. A Levene's test for homogeneity of variances was also performed. If the hypothesis was rejected, Games-Howell multiple comparisons were done to incorporate heteroscedasticity. The Fisher's least significant difference (LSD<0.05) at a 95% confidence level was applied to calculate the mean separation. Analysis was performed using Statistica 13.2 (StatSoft, Tulsa, OK, USA).

2.2.2. 2017 season

2.2.2.1 Fruit material

In the same orchard used in 2016, twenty 'Forelle' pear (*Pyrus communis* L.) trees in a single row, with similar vigour and crop load, for purposes of uniformity, were selected on 10 January 2017. The experimental design was a completely randomised block design, with the trees as blocks. On each of the 20 trees, fruit from three canopy positions were tagged: western outer canopy red blushed pears, slightly blushed pears in the intermediate canopy on the western side, and shaded green fruit with no blush in the inner parts of the canopy. For each of the three positions, eight fruit per tree were tagged.

2.2.2.2 Irradiation, fruit surface temperature and vapour pressure deficit

Irradiation and fruit surface temperature were measured seven times in the six weeks prior to harvest (from 12-January until 21 February), twice a day, on cloudless days from 10:00am until 17:00pm. The same procedure as for 2016 season was followed for the continuous measurement of the canopy temperature and relative humidity (mid-January until 21 February). The hourly vapour pressure deficit of the five different fruit canopy positions was calculated in the same manner as for the 2016 season.

2.2.2.3 Maturity and quality indices

Tagged pear fruit were harvested at commercial harvest maturity (± 6.2 kg firmness) on 21 February 2017. A total of 480 fruit were harvested, consisting of 160 fruit from each of the three canopy positions. After harvest, the same procedure as for season 2016 was followed. The only differences for the 2017 season were 1) that the maturity indexing was conducted after 8w RA + 7d SL as fruit ripened sufficiently for mealiness determination and 2) six fruit per position, per tree were measured.

A chromameter (Model CR-400; Minolta Co., Ltd., Tokyo, Japan) was used to record the hue angle (H) of the outer surface of the pear fruit. The hue angle was taken on the darkest (reddest) area and on the green side of each fruit. 0° = red/purple, 90° = yellow and 180° = blue/green as described by McGuire (1992).

The same procedures as reported for the 2016 season were used to assess fruit background colour, blush coverage, firmness, fruit size and mass, TSS and TA.

2.2.2.3.1 Ethylene production and respiration rate

For each tree, six fruit from each of the three canopy positions were used for the calculation of ethylene production ($\mu\text{L}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$) and respiration rate ($\text{mg CO}_2\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$). Each of the fruit samples were sealed in a 5 L airtight container (jar) at room temperature for 30 min; where after the same procedure was followed as for season 2016.

2.2.2.3.2 Seed count (normal and aborted)

The same procedure was followed as for season 2016, except that normal seeds from the sun and shaded side of fruit were also counted separately.

2.2.2.3.3 Mealy texture and juiciness evaluation

The same procedure was followed as in 2016.

2.2.2.3.4 Data analysis

The same procedure was followed as in 2016, except that a one-way ANOVA was also performed to determine differences between mealy and non-mealy fruit. Ethylene levels, respiration rates, TSS and TA levels could not be analysed according to mealiness since data were collected on pooled samples per replicate. Comparison of pears with a mealy texture to non-mealy pears could only be done for the western outer canopy fruit of 2017. This was due

to fruit from the other canopy positions (in both seasons) having very little or no mealy fruit for analysis. Analysis was conducted on 45 mealy outside-west fruit and 46 non-mealy outside-west fruit, using Statistica 13.2 (StatSoft, Tulsa, OK, USA).

2.3 RESULTS

2.3.1 2016 season

Only the results of 8w RA + 11d SL will be reported on, as very little ripening occurred and therefore mealiness incidence could not be measured after 8w + 7d (data not shown).

2.3.1.1 Irradiance and fruit surface temperature (FST)

Outer canopy red blushed fruit received a significantly higher average irradiance percentage of full sunlight, although outside-east fruit received on average 10% more sunlight than outside-west fruit (Table 1). Slightly blushed middle-east canopy fruit received a significantly higher irradiance percentage of full sunlight compared to slightly blushed mid-west canopy fruit (Table 1). Shaded no blushed inner canopy fruit was exposed to a significantly lower average irradiance percentage (*ca.* 2% of full sunlight) throughout the season. The difference between the average maximum and minimum irradiance percentage of no blushed inside fruit was only 2.5% (Table 1). The percentage intercepted irradiance varied the most for red blushed outer canopy fruit, where the difference between the average maximum and minimum irradiance percentage for outside-west and outside-east fruit was 65.9 and 63.7%, respectively.

Red blushed outside-east pears exhibited on average a significantly higher FST, while no blushed inner canopy pears had a significantly lower surface temperature (Table 2). Western red blushed outer canopy and slightly blushed middle-east canopy fruit exhibited the second highest average FST (26.8 °C and 26.9 °C, respectively), with slightly blushed middle-west canopy fruit being 1 °C cooler at 25.9 °C. The average ambient air temperature during the same time (07:00 – 17:00) was 23.4 °C.

However, the average maximum FST of red blushed outside-west pears was significantly higher compared to the slightly blushed middle-east pears (35.2 °C and 30.7 °C, respectively; Table 2). The average maximum FST of red blushed outside-west fruit was 1.2 °C higher than

the outside-east fruit, although non-significant (Table 2). Slightly blushed middle-west canopy fruit exhibited a significantly higher average maximum FST than middle-east fruit and did not differ statistically from the red blushed outside-east fruit. No blushed inner canopy and slightly blushed middle-east fruit exhibited a significantly lower average maximum FST (Table 2).

The average minimum FST of the outside-east and middle-east pears was significantly higher than the other three canopy positions. On average the minimum FST of outside-east and middle-east fruit was approximately 5.3 °C and 4.1 °C higher, respectively, than the other canopy positions. Thus, outside-west and middle-west fruit experienced larger fluctuations in their FST (Table 2). For a single point measurement, the maximum FST of a single fruit from the outside-west canopy was 51.6 °C, middle-west canopy 49 °C, inner canopy 34.8 °C, middle-east canopy 43.3 °C and outside-east canopy 46.5 °C. The minimum temperature of a single fruit from the western outer canopy was 8.1 °C, middle-west canopy 8.4 °C, inner canopy 9.0 °C, middle-east canopy 8.4 °C and outer east canopy 10.6 °C.

2.3.1.2 Vapour pressure deficit (VPD)

The average VPD (07:00-17:00) of outside-east fruit was significantly higher, followed by outside-west fruit (Table 3). Inner canopy fruit had a significantly lower average VPD, whilst the average VPD of the middle (west and east) canopy fruit was significantly higher than that of the inner canopy fruit and significantly lower than the outside-west and east fruit (Table 3).

Throughout the day (07:00 - 17:00) the VPD for middle-east fruit was similar to that of the outside-east fruit (Fig. 1), but the average VPD was significantly lower (Table 3). The VPD peak for middle-west fruit was approximately 1000Pa higher than that of the outside-east fruit, but outside-east fruit was exposed to a higher VDP for a longer period. The VPD for outside-west, middle-west and inside fruit was similar up to 12:00, after which the VPD for outside-west and middle-west fruit increased sharply. At 15:00 and 16:00, respectively it was about double the inner canopy VPD (Fig 1). The diurnal VPD pattern for middle-west fruit was similar to the outside-west fruit, but the average VPD was significantly lower. The VPD peak for outside-west fruit was at 17:00.

2.3.1.3 Fruit background colour and blush colour

At the time of harvest and after 8w RA + 11d SL the sun-exposed side of outside-west fruit had a significantly redder blush colour than fruit from the other canopy positions (Table 4 and 5). The red blush colour of outside-east and middle-west fruit did not differ statistically but, was significantly redder than the middle-east fruit (Table 5). Inner canopy fruit did not have a red blush (Table 5). Fruit background colour at the time of harvest and after 8w RA + 11d SL did not differ between canopy positions (Table 4 and 5).

2.3.1.4 Ethylene production and respiration rate

Ethylene production after 8w RA + 11d SL was significantly higher for red blushed inner canopy fruit ($23.3 \mu\text{L}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$). The red blushed outer canopy fruit from both sides of the tree produced significantly lower levels of ethylene, with ethylene levels being significantly lower for red blushed outside-east fruit. There was no significant difference between the slightly blushed intermediate canopy fruit on the western and eastern sides (Table 6).

The respiration rate was significantly higher for red blushed outer canopy and no blushed inner canopy fruit, although red blushed outside-east fruit did not differ statistically from the slightly blushed middle-west fruit ($P_{>F} = 0.0798$). The slightly blushed middle-east canopy fruit exhibited a significantly lower respiration rate compared to all other treatments (Table 6).

2.3.1.5 Diameter, mass and length

Red blushed fruit from the Western outer canopy exhibited the biggest diameter and highest mass of all the fruit from the different canopy positions after 8w RA + 11d SL (Table 7). Red blushed fruit from the outside-west was 24 g heavier and 3 mm bigger in diameter than outside-east fruit, although statistically non-significant ($P_{>F} = 0.1453$ and 0.1416 , respectively). Fruit diameter and mass did not differ statistically for red blushed fruit from the outside-east and slightly blushed fruit from the mid-west canopy ($P_{>F} = 0.5628$ and 0.5851 , respectively), although red blushed fruit from the outside-east was 1.1 mm bigger in diameter and 8.9 g heavier than the slightly blushed fruit from the middle-west. No blushed fruit from the inner canopy and slightly blushed fruit from the middle-east canopy exhibited a significantly smaller diameter and lower mass than fruit from the other canopy positions (Table 7). The red blushed fruit from the outside-west canopy exhibited the longest length, although only significantly longer than slightly blushed fruit from the middle-west and no

blushed fruit from the inner canopy (Table 7). The latter had significantly the shortest fruit length compared to the other positions (Table 7).

The diameter to length ratio of the eastern canopy pears did not differ statistically from the outside-west, middle-west and inside fruit, although only statistically significant from the middle-east fruit (Table 7).

2.3.1.6 Seed count (normal and aborted)

The average number of viable seeds did not differ significantly between the five different fruit canopy positions ($P_{>F} = 0.1985$). Only the red blushed fruit from the outside-west canopy had one normal seed per fruit (Table 8).

Slightly blushed fruit from the middle-east canopy exhibited the highest number of aborted seeds, whilst the red blushed fruit on the outer-west canopy west fruit had the lowest number. The red blushed fruit on the outside-east, slightly blushed fruit on the middle-west and no blushed fruit on the inside canopy did not differ significantly in the number of aborted seeds compared to the middle-east (highest) and outside-west fruit (lowest) (Table 8).

2.3.1.7 Firmness

Fruit colour and canopy position had no effect on flesh firmness at harvest (p -value; Table 9) and after 8w RA + 11d SL ($P_{>F} = 0.3600$; Table 10).

2.3.1.8 TSS and TA

At the time of harvest the red blushed fruit on the outer canopy exhibited significantly the highest TSS and lowest TA compared to the no blushed fruit inside and slightly blushed fruit in the middle canopy. The slightly blushed fruit in middle canopy had similar TSS- and TA levels compared to the no blushed fruit in the inner canopy at harvest time (Table 9).

The TSS after 8w RA + 11d SL was significantly higher for both red blushed fruit on the outer canopy than no blushed fruit in the inner canopy (13.2%) (Table 10). Red blushed fruit on the Western outer canopy exhibited the highest TSS (15.0%), but did not differ significantly from the outer canopy east fruit, as well as fruit from the slightly blushed middle canopy on the eastern side. The TSS of slightly blushed fruit from the middle-west (14.1%) did not differ significantly from the red blushed fruit on the outside-east canopy (14.2%) and slightly

blushed fruit on the middle-east canopy (14.1%), but were significantly different from the red blushed fruit on the outside-west canopy (Table 10).

No blushed inner canopy fruit exhibited a significantly higher TA (0.15%) after 11d SL whilst the TA of red blushed outside-west fruit was significantly lower (0.09%; Table 10). TA of slightly blushed middle canopy fruit on the eastern and western sides, as well as red blushed fruit on the outer east did not differ significantly (Table 10).

2.3.1.9 Mealy texture and juiciness evaluation

Red blushed outer canopy fruit on the eastern side exhibited a significantly higher average mealiness score, followed by red blushed outer canopy west fruit (Table 10). Fruit from the intermediate canopy on the eastern and western sides, as well as the inner canopy fruit did not differ significantly in mealiness incidence (Table 10).

The three different mealiness classes showed a clear separation in mean juice mass and juice area ($P_{>F} = 0.0001$ and 0.0001 , respectively), which confirmed the mealiness classes used by the trained sensory panel (Table 11). Non-mealy textured fruit had significantly the highest juice mass and juice area, whilst juice mass and juice area were significantly the lowest for mealy textured fruit (Table 11).

Inner canopy fruit, associated with a non-mealy texture, released a significantly higher total amount of juice and had a larger juice area, whilst the outer canopy fruit from both sides released a significantly lower amount of juice and had a smaller juice stained area (Table 11). The mean juice mass of all five different fruit positions associated with a partly mealy texture did not differ significantly from one another, although the mean juice area of partly mealy inner canopy fruit was significantly larger than that of the outer canopy fruit (Table 11). The middle canopy pears of partly mealy texture exhibited a significantly bigger juice area than that of outside-east fruit (Table 11).

2.3.2 2017 season

2.3.2.1 Irradiance and fruit surface temperature

Red blushed Western outer canopy fruit received the highest irradiance throughout the season, followed by slightly blushed western intermediate canopy fruit and no blushed shaded inner canopy fruit (Table 12). No blushed inner canopy fruit received very little irradiance

throughout the day, as their average maximum and minimum irradiance percentage was 2.5% and 0.9% of full sunlight, respectively (Table 12). The difference between the maximum and minimum irradiance percentage was significantly the biggest for outside-west fruit (71.2% difference) compared to the other two positions. Inner canopy fruit exhibited significantly the smallest difference (1.6%) (Table 12).

The average FST of red blushed outside-west fruit was significantly higher than fruit from the other canopy positions, being 1.7 and 4.5 °C warmer than slightly blushed middle-west and no blushed inside fruit, respectively (Table 13). The average maximum and minimum FST was also significantly higher for red blushed outside-west fruit (Table 13). The average FST, average maximum and minimum FST of no blushed inner canopy fruit was between 25 °C and 28 °C. The FST of red blushed outside-west and slightly blushed middle-west fruit exhibited relatively large fluctuations in their fruit surface temperatures, compared to no blushed inner canopy fruit (Table 13). For a single point measurement, the maximum FST recorded for a single red blushed outside-west, slightly blushed middle-west and no blushed inside fruit was 48.5, 47.2 and 33.8 °C, respectively. The minimum FST for a single red blushed fruit from the western outer canopy, slightly blushed fruit from the mid-west canopy and no blushed fruit from the inner canopy was 20.5, 20.8 and 21.0 °C, respectively. The average ambient air temperature during the same time (10:00 – 17:00) was 25.3 °C.

Non-mealy red-blushed outside-west fruit was on average exposed to 6.6% higher irradiance than the mealy red blushed outside-west fruit, although non-significant ($P_{r>F} = 0.0911$; Table 14). The average maximum irradiance percentage was significantly higher for red blushed non-mealy outside-west fruit ($P_{r>F} = 0.0386$). The average minimum irradiance percentage also did not differ significantly ($P_{r>F} = 0.3998$). The mealy and non-mealy red blushed outside-west fruit did not differ significantly regarding their average, maximum and minimum FST (Table 15).

2.3.2.2 *Vapour pressure deficit*

The VPD pattern throughout the day (10:00 – 17:00) was the same for all three canopy positions, peaking at 15:00 although at different rates (Fig. 2). Red blushed outside-west fruit experienced on average a significantly higher VPD, followed by slightly blushed middle-west fruit and no blushed inside canopy fruit, with the VPD being significantly lower for the latter (Table 16). The maximum VPD for no blushed inside fruit was approximately 50% of the

maximum VPD for red blushed outside-west and slightly blushed middle-west fruit (Table 16). The only difference was between 12:00 and 13:00, where the VPD for no blushed inner canopy fruit decreased, while the VPD for slightly blushed middle-west fruit stayed reasonably constant. The VPD for red blushed outside-west fruit increased during the day. The average VPD of mealy and non-mealy outside-west fruit did not differ statistically ($Pr > F = 0.1102$; Table 17).

2.3.2.3 Hue angle

At the time of harvest and after 8w RA + 7d SL the hue angle of the blush on fruit from western outer canopy was significantly lower than for middle-west fruit and inner canopy fruit (Table 18 and 19). As expected, the shaded inner canopy fruit had a significantly higher hue angle, which is typical of green fruit. The mealy and non-mealy outside-west fruit did not differ in the degree of red blush colour ($Pr > F = 0.1504$; Table 20).

2.3.2.4 Fruit background colour and blush colour

At the time of harvest and after 8w RA + 7d SL the outside-west fruit had a significantly redder blush colour on their sun-exposed side compared to middle-west and inner canopy fruit (Table 18 and 19). Blush colour was significantly redder for middle-west fruit than for inside canopy fruit ($Pr > F = 0.0001$). The peel of inner canopy fruit did not develop any red colouration (Table 18 and 19). The mealy outside-west fruit and non-mealy outside-west fruit exhibited a similar degree of red blush after 8w RA + 7d SL (Table 20). Background colour did not differ significantly for the canopy positions (Table 19). However, the background colour was significantly more yellow ($Pr > F = 0.0001$) for the mealy outside-west fruit compared to the non-mealy outside-west fruit (Table 20).

2.3.2.5 Ethylene production and respiration rate

Slightly blushed pears from the middle-west canopy produced significantly higher ethylene production rates after 8w CA + 7d SL compared to the red blushed fruit from the outside-west and no blushed fruit from the inside (Table 21). Ethylene production rates did not differ statistically between red blushed from from the outside-west and no blushed fruit from the inside canopy ($Pr > F = 0.5648$), where ethylene production rates were 12.0% and 16.3% lower, respectively, compared to slightly blushed middle-west fruit (Table 21).

The respiration rate of no blushed inner canopy pears was significantly higher (12.1%) compared to red blushed fruit from outside-west but did not differ significantly from slightly blushed mid-west canopy fruit (Table 21).

2.3.2.6 Diameter, mass and length

The red blushed fruit from the western outer canopy were significantly larger in diameter and higher in mass compared to the other two canopy positions (Table 22). The red blushed outside-west fruit was 4.6 mm larger and 24.1 g heavier than slightly blushed middle-west fruit and 8.7 mm bigger and 45.6 g heavier than no blushed inner canopy fruit (Table 22). The diameter and mass of slightly blushed middle-west canopy pears were significantly higher than the shaded no blushed inner canopy fruit (Table 22). Red blushed outer canopy west fruit exhibited a significantly larger diameter to length ratio (0.80) than that of slightly blushed middle-west (0.76) and no blushed inside fruit (0.76; Table 22).

The red blushed mealy outside-west fruit was significantly bigger and heavier than the red blushed non-mealy fruit (Table 23). The diameter to length ratio was 0.02 larger for mealy fruit compared to non-mealy fruit, although this difference was non-significant (Table 23). The diameter of mealy outside-west fruit was 6.0 mm bigger, while the mass was 40.0 g heavier and fruit were 5.6 mm longer in length.

2.3.2.7 Seed count (normal and aborted)

Red blushed, outer canopy west fruit had significantly more viable seeds than slightly blushed middle-west and no blushed inner canopy fruit (Table 24). The sun-exposed side of red blushed western-outer canopy fruit had significantly more viable seeds than the sun-exposed side of slightly blushed middle-west fruit, although on average less than one viable seed was present in the outside-west fruit. The number of viable seeds on the shaded side of the fruit did not differ statistically between the canopy positions (Table 24).

The number of aborted seeds was significantly lower in the outside-west pears, whilst middle-west and inside fruit did not differ statistically (Table 24). Middle-west canopy and inner canopy fruit had on average two more aborted seeds than outside-west fruit.

The number of viable seeds did not differ statistically between the mealy outside-west fruit and non-mealy outside-west fruit ($Pr > F = 0.9539$; Table 25). The sun-exposed and shaded side

of the fruit did not differ with regards to the seed count (Table 25). Mealy and non-mealy outside-west fruit also had a similar number of aborted seeds ($P_{>F} = 0.3795$; Table 25).

2.3.2.8 Firmness

At time of the harvest the firmness of all three fruit positions was the same (Table 26). Canopy position had no significant effect ($P_{>F} = 0.1800$) on flesh firmness after 8w CA + 7d SL (Table 27). However, the firmness of mealy outside-west fruit (1.7 kg) was significantly lower ($P_{>F} = 0.0006$) than the firmness of non-mealy outside-west fruit (2.0 kg) (Table 28).

2.3.2.9 TSS and TA

At the time of commercial harvest, the TSS of outside-west fruit was significantly higher than the other two fruit positions, whilst inner canopy fruit exhibited significantly the lowest TSS (Table 26). However, the inner canopy had a significantly higher TA at the time of harvest in comparison to the outside-west and middle-west pears, whereas the latter two exhibited the same TA level (Table 26).

Western outer canopy pears were significantly higher in TSS and significantly lower in TA than middle-west and inside canopy fruit after 8w RA + 7d SL (Table 27). The TSS of mid-west canopy fruit was significantly higher than inner canopy pears. TA was significantly higher in inner canopy fruit (Table 27). Mealy outside-west fruit was associated with a significantly higher TSS compared to the non-mealy outside-west fruit (15.4 and 14.9%, respectively; Table 28). The TA was 0.14% for the mealy and non-mealy outside-west fruit (Table 28).

2.3.2.10 Mealy texture and juiciness evaluation

Mealiness incidence was significantly the highest for outside-west fruit compared to middle-west and inside canopy fruit after 8w RA + 7d SL (Table 27). The average mealiness score of middle-west fruit (0.24) was slightly lower than inner canopy fruit (0.28), although non-significant (Table 27). The three mealiness classes differed significantly in the total amount of juice released, with non-mealy fruit releasing the highest amount of juice mass and juice area, followed by partly mealy and mealy fruit, respectively (Table 29). These results confirmed the mealiness classes classified by the trained panel.

Non-mealy outside-west fruit had significantly lower juice mass and area than the non-mealy middle-west and inner canopy fruit (Table 29). Non-mealy inner canopy fruit exhibited

significantly the highest juice mass and juice area upon compression (Table 29). The three different fruit canopy positions associated with a partly mealy texture had a similar juice mass, however, the juice area of partly mealy inner canopy fruit was significantly higher compared to that of the middle-west and outside-west fruit (Table 29). The mean juice area of middle-west fruit was not significantly higher than that of outside-west fruit (Table 29).

2.4 DISCUSSION

In both seasons, the mealiness score was significantly higher for red blushed outer canopy fruit, which showed a significantly higher exposure to sunlight, average FST and average VPD. A preliminary study by Cronjé (2014) also found outer canopy 'Forelle' pears to have a higher mealiness incidence than inner canopy pears. Interestingly, in 2016 and 2017, red blushed non-mealy outer canopy fruit exhibited significantly lower juice mass and juice area than slightly blushed non-mealy intermediate and no blushed inner canopy fruit (Tables 11 and 28).

The characteristic pear ripening process entails a loss of flesh firmness, background colour transition from green to yellow, decrease in TA and an increase in TSS and ethylene production. This causes an increase in protein and water-soluble polyuronides, which eventually leads to a juicy fruit (Eccher-Zerbini, 2002).

Cronjé (2014) associated red blushed outer canopy 'Forelle' pears with a more advanced maturity with a higher mealiness incidence, similar to the study by Carmichael (2011). According to the results obtained in 2016 and 2017, the maturity stage of the different canopy positions did not differ. However, in 2017, mealy red blushed outside-west pears were associated with a significantly more yellow background colour and lower firmness than non-mealy red blushed outside-west fruit (Tables 20 and 28). This possibly indicates that mealy outside-west fruit exhibited a more advanced stage of maturity.

In 2016 and 2017, the mealiness incidence of the slightly blushed intermediate canopy fruit and no blushed inside canopy fruit did not differ significantly. However, the average percentage irradiance exposure and average FST were significantly higher for the intermediate canopy fruit (Tables 1, 2, 12 and 13). This result was unexpected as fruit from different canopy positions are exposed to different levels of irradiance and ambient

temperature, as well as differences in the supply of nutrients, water and endogenous hormones (Kingston, 1994; Tomala, 1999). It seems as if there is a threshold temperature/irradiance level for initiating mealiness development. Cronjé (2014) suggested that canopy microclimate possibly influences the tendency of 'Forelle' pears to develop a mealy texture, whilst Woolf and Ferguson (2000) attributed the differences in post-harvest life of pears to varying pre-harvest environmental factors experienced by the fruit. The similar mealiness scores obtained for slightly blushed intermediate canopy fruit and no blushed inside fruit could be attributed to the general trend of mealiness development, where mealiness increases, peaks and decreases again (Martin, 2002). Mealiness development of slightly blushed middle-west fruit could already have occurred or could possibly develop at a later stage of ripening. This hypothesis is further evaluated in Chapter 4, where maturity differences, as well as mealiness differences within the canopy are explored at more storage and ripening times.

The higher irradiance levels received by outer canopy fruit gave rise to a higher degree of red blush (Tables 5 and 19), because sunlight is required for the synthesis of the red pigment, anthocyanin (Steyn et al., 2005). However, high intercepted levels of irradiance are associated with radiant heating (Curry, 1997; Reay, 1999). In pears, light has two opposing effects on anthocyanin synthesis. Anthocyanin synthesis is light dependent, whilst a specific irradiance level can induce anthocyanin synthesis or degradation; environmental and endogenous factors determine the gain or loss in anthocyanin (Steyn et al., 2004). In our study, the red blush colour of the sun-exposed sides of outside-east and middle-west 'Forelle' pears in 2016, did not differ (Table 5), although the outside-east fruit were exposed to higher levels of irradiance and fruit surface temperatures (Tables 1 and 2). It is important to note that if irradiance and fruit surface temperature were logged continuously from sunrise to sunset, the results might have looked slightly different.

Kappel and Nielsen (1994) found a negative correlation between fruit background colour and light exposure in 'Bartlett' and 'Anjou' pears. In agreement, Tustin et al. (1988) reported 'Granny Smith' apples from the lower, inner parts of the canopy to exhibit the greenest background colour. However, the results obtained in this study did not agree, since the background colour throughout the canopy did not differ. In 2017, a more yellow background colour was observed for mealy outside-west fruit, which received on average 7% less

irradiance than non-mealy outside-west fruit. It is important to note that a colour chart was used, which is not always very precise. Consequently, chromameter readings (especially L values) might have coincided with the referenced authors.

The association of high irradiance exposure in conjunction with a high FST, reported by several previous studies (Smart and Sinclair, 1976; Raffo et al., 2011), appears not to agree with the results obtained in this study, for both seasons. It is important to note these studies looked at differences between ambient temperature and exposed fruit while they were exposed to sunlight. In our study, the combined average FST for both seasons for the outer canopy fruit was a mere 1.6 °C higher than the intermediate canopy fruit. The differences in the FST might have been larger if measured more regularly, as well fruit are not exposed to the sun the entire day, so there are times when outside fruit are shaded and should not differ too much from the ambient temperature. In 2016 the maximum temperature difference between outside and inside fruit was nearly 6 °C and nearly 9 °C in 2017 (Table 2 and 13). The FST may possibly have a cumulative effect on the incidence of mealiness development, since outer canopy fruit have a higher susceptibility to become mealy after cold storage and ripening (Table 10 and 27). Since fruit temperature is a function of radiation and air circulation (Bergh et al., 1980), the slight differences in FST between the fruit from various canopy positions, might be explained by outer and intermediate canopy fruit being exposed to higher wind speeds compared to inside fruit, with the result that convective heat loss takes place. FST could decrease by approximately 5 °C with an increase in wind velocity of 0.5 – 3.5 m.s⁻¹ (Schrader et al., 2003). Furthermore, the FST measurements during the 2016 season started early (07:00) in the morning and continued until late (17:00) in the afternoon. The FST early in the morning were similar amongst all five-canopy positions (data not shown). According to Schrader et al. (2003) maximum fruit temperature occurred prior to 17:00, whilst air temperature was at least 30 °C. The low FST values have a greater impact on the average temperature of outer and middle canopy fruit, compared to the inside fruit. Thus, the FST of outer canopy fruit varies greatly during the course of the day, to such an extent that early in the morning the outer canopy fruit have similar low FSTs as inside fruit, whilst later in the day outer canopy fruit temperature can be up to 15 °C warmer than inner canopy fruit (data not shown). The large temperature fluctuations experienced by outer canopy fruit might result in negative changes in fruit metabolism, such as the enhancing of membrane permeability which

leads to the loss of electrolytes, inactivation of enzymes, loss of membrane integrity and alterations in the partitioning of photo-assimilates which are just a couple of several changes that could occur during heat stress (Wahid et al., 2007). This eventually leads to deterioration of cellular components during ripening, and that might contribute to a more advanced ripening stage and make the fruit more prone to mealiness development. In several fruit species, a shift takes place during the final stage of development from accumulation of organic acids to sugar synthesis (Etienne et al., 2013). Titratable acidity in fruit is known to decrease with increased temperature during fruit growth or storage (Gautier et al., 2005). Lobit et al. (2006) reported a reduced ability of malate accumulation in fruit with increasing temperature. Enzymes involved during respiration are temperature dependent, causing modification of the reaction rates of glycolysis and the Krebs cycle, which may result in changes in the fruit organic metabolism. It is proposed that increased temperature might bring about changes in malate transport at the tonoplast, resulting in a drop in malate content of the vacuole, coupled with an increase in cytosol malate content, which is then available to use in fruit metabolism (Etienne et al., 2013). This is in agreement with Cronjé (2014) and Muziri (2016) who associated higher TSS with more mature 'Forelle' pears. Similar results were obtained in our study.

Larger differences could be expected in fruit flesh temperatures, as reported by Woolf et al. (1999), where the flesh temperature of outer canopy fruit, exposed to direct sunlight, could be up to 15 °C higher than the ambient temperature. Therefore, fruit require a homeostatic control of cell metabolism. A temperature difference can be found between the sun-exposed and the shaded side of fruit, as reported by Thorpe (1974) and Woolf et al. (1999) with apple and avocado fruit, respectively, where the sun-exposed side was up to 10 °C warmer than the shaded side. However, in our study mealiness incidence did not differ between the exposed and shaded sides of outer canopy pears (data not shown).

The higher mealiness incidence of outer canopy fruit was also associated with higher TSS and lower TA, unlike fruit from the intermediate and inner parts of the canopy (Tables 10 and 27). This phenomenon agrees with Muziri (2016), who associated mealy 'Forelle' pears with higher concentrations of TSS. A number of studies reported a positive relationship between fruit TSS and irradiance levels, coupled with high FSTs (Jackson et al., 1977 (apples); Tustin et al., 1988 (apples); Kappel and Neilsen, 1994 (pears); Nilsson and Gustavsson, 2007 (apples)). This may

be because of a higher photosynthetic rate of the outside leaves and outer canopy fruit that possess stronger sink strength for carbohydrate assimilation (Garriz et al., 1997).

In general, larger fruit are associated with larger cells, reduced cell wall material per unit fruit volume (which contributes to lower fruit tissue strength) and lower flesh firmness than smaller fruit (Harker et al., 1997). The lower flesh firmness of large fruit is related to a higher proportion of intercellular spaces (Volz et al., 2004). According to the results obtained in this study, the relationship between firmness and fruit size was not as clear.

Fruit that grow in the presence of a high sugar supply, due to a high source: sink ratio, are bigger and have a higher respiration rate (Etienne et al., 2013). Ferguson et al. (1998) found that the synthesis of heat shock proteins (HSPs) is promoted by high FST while on the tree. Consequently, Woolf et al. (1999) proposed that pre-harvest temperatures could possibly influence fruit post-harvest tolerance to high and/or low temperature. Exposure of fruit to frequent high temperatures over the long term could possibly lead to morphological and physiological adaptations (Woolf et al., 1999). The physiological maturity of apples is achieved sooner, with higher ambient temperatures early in the growth phase (Warrington et al., 1999). In 2017, the higher mealiness incidence of western outer canopy pears in association with the lower respiration rate and ethylene levels similar to inner canopy fruit, suggests that outside fruit might already have reached a respiration peak before the evaluation period. Alternatively, outside-west fruit's ability to produce normal ethylene levels was altered. As mentioned earlier, Cronjé (2014) associated outer canopy 'Forelle' pear fruit with a higher mealiness incidence and with an advanced stage of maturity. Unfortunately, fruit of the 2016 season showed an unexplained resistance to ripening, since the average firmness was more than double that of the 2017 fruit during the evaluation time (Tables 8 and 27). At the time of harvest, the average firmness of the 2016 fruit was approximately 0.4 kg lower than that of the 2017 fruit (Table 9 and 26). Thus, respiration rates and ethylene levels of 2016 fruit will not be discussed, seeing that no clear pattern was obtained and fruit did not ripen normally.

The final size of pears is influenced by a combination of the number of cells at fruit set, the number of subsequent cell divisions and cell expansion after completion of cell division (Shargal et al., 2006). Any limitations early in the season which lead to reduced spur leaf growth, has major repercussions on subsequent fruit growth (Buwalda and Meekings, 1990), since most of the cell division phase is dependent on photo-assimilates from spur and/or

extension leaves (Garriz et al., 1998). Photo-assimilates are also important in sustaining further fruit growth through cell expansion. Mealiness in apples is generally associated with bigger fruit, as well as larger cell sizes and intercellular airspaces, with the result that smaller areas of cell adhesion are present (De Smedt et al., 1998). This leads to increased cell sensitivity for mealiness development in apples (De Smedt et al., 1998). Muziri (2016) also found that larger fruit were more prone to mealiness, but this correlation was not always present for all locations. However, decreased cell adhesion or large intercellular airspaces were directly linked to fruit that would become mealy. Smaller fruit are normally associated with a smaller number of cells, as well as smaller cells (Warrington et al., 1999). Muziri (2016) reported that cell size was positively correlated to 'Forelle' pear mealiness development.

The larger fruit diameter and mass of outside fruit (Table 7 and 22) might have been influenced by the higher photosynthetic activity of their leaves, as a result of a higher percentage of sunlight being intercepted by the outside leaves, or due to a higher sink strength, as well as originating from a bud of higher quality. Outer canopy fruit could possibly, however very unlikely, have primigenic dominance over the intermediate canopy and inner canopy fruit. This means that earlier developed fruit dominates later developed fruit, with the result that more photo-assimilates and nutrients are supplied to earlier developed fruit, which influence final fruit size (Madail et al., 2012). There can be speculation that primigenic dominance could probably be the reason for the bigger diameter and greater mass of the mealy outside-west fruit compared to the non-mealy outside-west fruit, which received a similar percentage of irradiance (Table 14). As mentioned above, this is only a speculation and other factors, such as bud quality may play a role.

Garriz et al. (1997) reported that shaded 'Bartlett' pears exhibited a significantly lower fruit mass, as well as a smaller fruit diameter, compared to fruit bearing branches exposed to sunlight. This agrees with Jackson et al. (1997, apple) and Ramos et al. (1994, pear). In this study fruit that were mealy, were also larger in size, whereas inside fruit were the smallest and had low levels of mealiness. However, fruit size of intermediate canopy pears on the western side was significantly bigger than middle-east canopy and inner canopy fruit in both seasons (Tables 7 and 22). Yet, their mealiness class did not differ. Since fruit size may play a role, more factors are possibly involved in fruit size as well as mealiness development.

Warrington et al. (1999) reported a negative relationship between the duration of apple fruit cell division and mean temperature. It might be speculated that the inner canopy and possibly intermediate canopy pears experience a slightly more controlled cell division period, with the result that better-quality cells with better cell connections develop. Accordingly, Bergh (1990) found increased apple fruit growth rates during early season warm temperatures and associated the increased growth rates with increased cell division rates in the cortical region of fruit.

Cell expansion rates of fruit increase with temperature (Warrington et al., 1999). Bigger fruit size is associated with increased rates of cell expansion (Etienne et al., 2013), resulting in fruit cells requiring more energy/carbohydrates and good water relations. In 2017 and 2016, the outer canopy fruit associated with a non-mealy texture had a significantly lower juice mass and juice area than non-mealy fruit from the intermediate and inner canopy (Table 28). This can be an indication that particular cells in the neck tissue of outer canopy fruit, may be less pliable for higher enlargement rates. This may lead to the development of large intercellular airspaces in the neck tissue of 'Forelle' pears, due to cellular breakage and cell separation, as reported by Muziri et al. (2016). The neck tissue of 'Forelle' pears is the area where mealiness development initiates, seeing the neck is where ripening starts, after which it spreads downwards and throughout the fruit flesh (Crouch, 2011; Muziri, 2016).

At physiological temperatures, membranes are in a fluid phase, but membrane fluidity can be lost under high temperature conditions due to membrane-phospholipids changing from a liquid crystalline phase to a gel phase (Crowe et al., 1998). Membrane leakage increases when the liquid phase and gel phase coexist, resulting in the semi-permeability of the membrane being lost (Murray et al., 1989). Thus, there may be a possibility that the greater VPD experienced by outer canopy fruit during their early to mid-development (when pears are permeable to water) may have influenced internal tissue properties and the higher FST fruit may alter the distribution of mineral nutrients within the fruit and cause uneven ripening (Woolf et al., 1999), resulting in a higher susceptibility to mealiness development.

The outside-east and middle-west fruit have a similar diameter, mass, diameter: length ratio and TSS, but differ significantly in mealiness incidence. This difference can be ascribed to the differences in percent porosity, while cell size did not differ between fruit positions. The pore size of outside fruit is significantly larger (higher percentage porosity) than that of the middle-

canopy fruit with the pores more connected in outside fruit, although non-significant compared to middle fruit. A higher percentage porosity is coupled to weaker cell-to-cell connections within fruit (Crouch et al., 2018). The mechanism described for 'Forelle' pear mealiness development entails a loss in cell-to-cell adhesion, resulting in cell sliding due to a weaker middle lamella compared to the cell wall (Crouch, 2011; Muziri, 2016).

2.5 CONCLUSION

According to the results obtained in this study, it seems as if the exposure of fruit to high irradiance levels coupled with high FST, is one of the determining factors in 'Forelle' pear mealiness development. However, an unidentified tree factor may also be involved in mealiness development, since the mealy outside-west fruit received on average 7% less irradiance than the non-mealy outside-west fruit, while their FST was similar.

Outer canopy 'Forelle' pears, which are exposed to high levels of irradiance coupled with high FST and possibly high internal fruit temperatures, are more susceptible to mealiness development. A higher mealiness incidence was associated with a significantly redder blush colour, higher VPD, higher TSS and lower TA, which can be attributed to the higher irradiance levels and FSTs experienced by the fruit.

The higher mealiness incidence of outer canopy fruit was mainly associated with a larger fruit size, which infers a higher growth rate. The control of fruit homeostasis and the regulating of cell division and -enlargement during fruit growth are important in obtaining optimum quality fruit. The outside canopy fruits' cell-to-cell connection may not have the ability to keep up with a high expansion rate/sink strength, which decreases the fruits' ability to regulate the growth rate in a controlled manner, as well as changes the distribution of mineral nutrients. Therefore, changes could appear in the way fruit cells divide and enlarge, as well as in their capacity/ability to divide and expand. This may cause fruit to be more susceptible to mealiness development.

The characteristic red blush colour of 'Forelle' is of great importance in the success of 'Forelle' pear production, since blushed pears are desired by consumers. The fact that red blushed outer canopy pears are associated with higher exposure to irradiance, higher FST and higher VPD, is an indication that shade netting could possibly be used to develop outer canopy fruit,

which are less prone to mealiness development. However, further research is needed to determine the effect of shade netting on 'Forelle' pear fruit size, TSS and red blush colour development. Further research can also focus on the effect of warmer vs colder winters, knowing that the number of cells of buds are determined by the type of winter (temperature) experienced. Effect of different crop loads on mealiness can be researched, a higher crop load and resultant smaller fruit size may possibly decrease mealiness development.

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2.7 TABLES AND FIGURES

Table 1: Effect of 'Forelle' pear fruit canopy position on the average-, maximum- and minimum intercepted irradiance percentage in the 6 weeks prior to harvest (07:00 – 17:00). Fruit were harvested in 2016 at commercial maturity on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

Fruit canopy position	Irradiance average (%)	Maximum irradiance average (%)	Minimum irradiance average (%)	Maximum – minimum irradiance average (%)
Outside-west	34.5 b ²	74.3 a	8.4 b	65.9 a
Middle-west	16.1 d	38.3 c	2.3 c	36.0 c
Inside	1.8 e	3.4 d	0.9 c	2.5 d
Middle-east	23.3 c	52.9 b	6.5 b	46.4 b
Outside-east	44.3 a	78.8 a	15.1 a	63.7 a
Source of variation:	Pr>F			
Position	0.0001	0.0001	0.0001	0.0001

²Means in the same column followed by the same letter are not significantly different at 5% level (LSD).

Table 2: Effect of 'Forelle' pear fruit canopy position on the average-, maximum- and minimum fruit surface temperature (°C) (FST) in the 6 weeks prior to harvest. Fruit were harvested in 2016 at commercial maturity on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

Fruit canopy position	FST average (°C)	Maximum FST average (°C)	Minimum FST average (°C)	Maximum – minimum FST average (°C)
Outside-west	26.8 b ²	35.2 a	19.0 b	16.2 a
Middle-west	25.9 c	33.4 b	18.9 b	14.5 a
Inside	24.8 d	29.4 c	19.6 b	9.8 b
Middle-east	26.9 b	30.7 c	23.3 a	7.4 c
Outside-east	29.0 a	34.0 ab	24.5 a	9.5 b
Source of variation:		Pr>F		
Position	0.0001	0.0001	0.0001	0.0001

²Mean values in the same column followed by the same letter are not significantly different at 5% level (LSD).

Fruit surface temperature (FST) was measured three times a week, approximately four times a day, on cloudless days between 07:00 and 17:00.

Table 3: Average vapour pressure deficit (VPD) (kPa) in the 6 weeks prior to harvest (07:00–17:00) of five different ‘Forelle’ pear fruit canopy positions in 2016, as measured on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

Fruit canopy position	Average VPD (kPa)
Outside-west	2.1 b ^z
Middle-west	1.8 c
Inside	1.4 d
Middle-east	1.8 c
Outside-east	2.4 a
Source of variation	Pr>F
Position	0.0001

^zMeans followed by the same letter are not significantly different at 5% level (LSD).

Table 4: Effect of fruit canopy position on 'Forelle' pear average blush colour and ground colour at the time of commercial harvest maturity. Fruit were harvested in 2016 on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

Fruit canopy position	Blush colour (chart index) ^x	Ground colour (chart index) ^y
Outside-west	1.4 d ^z	1.9 ^{*NS}
Middle-west	8.5 b	1.8
Inside	12.0 a	2.1
Middle-east	9.0 b	2.0
Outside-east	3.1 c	2.1
Source of variation:	Pr>F	
Position	0.0001	0.4031

*NS = Non-significant

^zMean values in the same column followed by the same letter are not significantly different at 5% level (LSD)

^xChart values 1-12: where 1=red; 12=green

^yChart values 0.5-5: where 0.5= green; 5= pale green/ yellow

Table 5: Effect of fruit canopy position on 'Forelle' pear blush colour and ground colour after 8w RA storage at -0.5°C + 11d shelf-life at 20°C. Fruit were harvested in 2016 at commercial maturity on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

Fruit canopy position	Blush colour (chart index)^x	Ground colour (chart index)^y
Outside-west	1.7 d ^z	3.3 *NS
Middle-west	4.5 c	3.3
Inside	11.7 a	3.2
Middle-east	8.5 b	3.5
Outside-east	3.6 c	3.5
Source of variation:	Pr>F	
Position	0.0001	0.3300

*NS = Non-significant

^zMean values in the same column followed by the same letter are not significantly different at 5% level (LSD)

^xChart values 1-12: where 1=red; 12=green

^yChart values 0.5-5: where 0.5= green; 5= pale green/ yellow

Table 6: Effect of fruit canopy position on 'Forelle' pear ethylene production and respiration rate after 8w RA storage at -0.5°C + 11d shelf-life at 20°C. Fruit were harvested in 2016 at commercial maturity on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

Fruit canopy position	Ethylene production rate ($\mu\text{L.kg}^{-1}.\text{h}^{-1}$)	Respiration rate ($\text{mg CO}_2.\text{kg}^{-1}.\text{h}^{-1}$)
Outside-west	12.0 c ²	176.7 a
Middle-west	17.4 b	104.5 b
Inside	23.3 a	149.2 a
Middle-east	17.6 b	46.2 c
Outside-east	8.5 d	142.8 ab
Source of variation:	Pr>F	
Position	0.0001	0.0001

²Mean values in the same column followed by the same letter are not significantly different at 5% level (LSD)

Table 7: Effect of fruit canopy position on 'Forelle' pear average diameter, mass, length, diameter: length, TSS and TA after 8w RA storage at -0.5°C + 11d shelf-life at 20°C. Fruit were harvested in 2016 at commercial maturity on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

Fruit canopy position	Diameter (mm)	Mass (g)	Length (mm)	Diameter:Length
Outside-west	69.9 a ^z	213.0 a	91.6 a	0.77 a
Middle-west	65.8 b	180.1 b	88.5 ab	0.75 ab
Inside	56.9 c	126.9 c	75.7 c	0.76 a
Middle-east	57.8 c	127.7 c	83.5 b	0.70 b
Outside-east	66.9 ab	189.0 ab	85.7 ab	0.78 a
Source of variation:		Pr>F		
Position	0.0001	0.0001	0.0001	0.0146

^zMean values in the same column followed by the same letter are not significantly different at 5% level (LSD)

Table 8: Effect of 'Forelle' pear canopy position on the average number of normal (viable) and aborted seeds after 8w storage at -0.5°C + 11d shelf-life at 20°C. Fruit were harvested in 2016 at commercial maturity on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

Fruit canopy position	Number of normal seeds	Number of aborted seeds
Outside-west	1.2 *NS	7.0 b ^z
Middle-west	0.2	8.6 ab
Inside	0.4	8.2 ab
Middle-east	0.3	9.3 a
Outside-east	0.8	8.9 ab
Source of variation	Pr>F	
Position	0.1985	0.0009

*NS = Non-significant

^zMeans in the same column followed by the same letter are not significantly different at 5% level (LSD).

Table 9: Effect of fruit canopy position on 'Forelle' pear average firmness, TSS and TA at the time of commercial harvest maturity. Fruit were harvested in 2016 on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

Fruit canopy position	Firmness (kg)	TSS (%)	TA (%)
Outside-west	5.9 *NS	13.6 a ^z	0.12 b
Middle-west	5.9	12.1 b	0.15 a
Inside	5.7	11.5 b	0.16 a
Middle-east	5.8	11.6 b	0.15 a
Outside-east	6.0	13.5 a	0.12 b
Source of variation:		Pr>F	
Position	0.0716	0.0001	0.0001

*NS = Non-significant

^zMean values in the same column followed by the same letter are not significantly different at 5% level (LSD)

Table 10: Effect of fruit canopy position on 'Forelle' pear average mealiness score, firmness, TSS and TA after 8w 8w storage at -0.5°C + 11d shelf-life at 20°C. Fruit were harvested in 2016 at commercial maturity on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

Fruit canopy position	Mealiness Class score average ^x	Firmness (kg)	TSS (%)	TA (% malic acid)
Outside-west	0.70 b ^z	3.7 ^{*NS}	15.0 a	0.09 c
Middle-west	0.30 c	3.9	14.1 bc	0.12 b
Inside	0.30 c	4.1	13.2 c	0.15 a
Middle-east	0.40 c	3.5	14.1 abc	0.12 b
Outside-east	1.10 a	4.3	14.2 ab	0.11 b
Source of variation:		Pr>F		
Position	0.0001	0.3600	0.0071	0.0001

*NS = Non-significant

^xMealiness classes: where 0=non-mealy, 1=partly mealy and 2=mealy

^zMean values in the same column followed by the same letter are not significantly different at 5% level (LSD)

Table 11: Average juice mass and area of different mealiness classes and fruit positions of 'Forelle' pear after 8w storage at -0.5°C + 11d shelf-life at 20°C. Fruit were harvested in 2016 at commercial maturity on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

Factor:	Mean juice mass (mg)		Mean juice area (cm²)	
Mealiness class				
Non-mealy (0)	0.12 a ^z		12.5 a	
Partly mealy (1)	0.09 b		10.6 b	
Mealy (2)	0.05 c		3.5 c	
Source of variation:	Pr>F			
Mealiness class	0.0001		0.0001	
Factor:	Non-mealy		Partly mealy	
Fruit position	Juice mass (mg)	Juice area (cm²)	Juice mass (mg)	Juice area (cm²)
Outside-west	0.10 c ^z	11.6 c	0.09 *NS	10.1 bc
Middle-west	0.13 b	12.6 b	0.10	11.0 ab
Inside	0.16 a	13.7 a	0.11	11.3 a
Middle-east	0.14 b	12.7 b	0.09	10.8 ab
Outside-east	0.10 c	11.7 c	0.06	9.9 c
Source of variation:	Pr>F			
Position	0.0001	0.0001	0.0912	0.0084

*NS = Non-significant

^zMealiness class means in the same column followed by the same letter are not significantly different at 5% level (LSD)

^xAnalysis of mealy textured fruit for the different positions were not done due to low mealiness incidence for certain canopy positions

Table 12: Effect of 'Forelle' pear fruit canopy position on the average-, maximum- and minimum intercepted irradiance percentage in the 6 weeks prior to harvest. Fruit were harvested in 2017 at commercial maturity on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa. Light irradiation was measured seven times in the last six weeks prior to harvest, twice a day on cloudless days between 10:00 and 17:00.

Fruit canopy position	Irradiance average (%)	Maximum irradiance average (%)	Minimum irradiance average (%)	Maximum – minimum irradiance average (%)
Outside-west	52.4 a ²	86.5 a	15.3 a	71.2 a
Middle-west	29.6 b	60.3 b	4.8 b	55.5 b
Inside	1.6 c	2.5 c	0.9 c	1.6 c
Source of variation:	Pr>F			
Position	0.0001	0.0001	0.0001	0.0001

²Means in the same column followed by the same letter are not significantly different at 5% level (LSD).

Light irradiation was measured seven times in the last six weeks prior to harvest, twice a day on cloudless days between 10:00 and 17:00.

Table 13: Effect of 'Forelle' pear fruit canopy position on the average-, maximum- and minimum fruit surface temperature in the 6 weeks prior to harvest. Fruit were harvested in 2017 at commercial maturity on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

Fruit canopy position	FST average (°C)	Maximum FST average (°C)	Minimum FST average (°C)	Maximum – minimum FST average (°C)
Outside-west	31.1 a ²	36.4 a	27.0 a	9.4 a
Middle-west	29.4 b	33.7 b	26.5 a	7.2 b
Inside	26.6 c	27.5 c	25.7 b	1.8 c
Source of variation:	Pr>F			
Position	0.0001	0.0001	0.0001	0.0001

²Means in the same column followed by the same letter are not significantly different at 5% level (LSD).

Fruit surface temperature was measured seven times in the last six weeks prior to harvest, twice a day on cloudless days between 10:00 and 17:00

Table 14: Average-, maximum- and minimum intercepted irradiance percentage in the 6 weeks prior to harvest (10:00 – 17:00) of mealy and non-mealy outside-west 'Forelle' pears in 2017 as measured on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

Factor:		Outside-west Canopy fruit		
Mealiness class	Irradiance average (%)	Maximum irradiance average (%)	Minimum irradiance average (%)	
Mealy	48.9 ^{*NS}	73.2 b ^z	23.5 ^{NS}	
Non-mealy	55.5	82.4 a	28.6	
Source of variation:		Pr>F		
Mealiness class	0.0911	0.0386	0.3998	

*NS= Non-significant

^zMeans in the same column followed by the same letter are not significantly different at 5% level (LSD).

Light irradiation was measured seven times in the last six weeks prior to harvest, twice a day on cloudless days between 10:00 and 17:00

Table 15: Average-, maximum- and minimum fruit surface temperature (°C) (FST) in the 6 weeks prior to harvest (10:00 – 17:00) of mealy and non-mealy outside-west 'Forelle' pears as measured in 2017 on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

Factor:		Outside-west Canopy fruit		
Mealiness class	FST average (°C)	Maximum FST average (°C)	Minimum FST average (°C)	
Mealy	31.1 ^{*NS}	34.8 ^{NS}	27.5 ^{NS}	
Non-mealy	30.8	34.7	26.9	
Source of variation:		Pr>F		
Mealiness class	0.6276	0.9726	0.3981	

*NS= Non-significant

Fruit surface temperature was measured seven times in the last six weeks prior to harvest, twice a day on cloudless days between 10:00 and 17:00.

Table 16: Average vapour pressure deficit in the 6 weeks prior to harvest (10:00-17:00) of three different 'Forelle' pear fruit canopy positions in 2017 as measured on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

Fruit canopy position	Average VPD (kPa)
Outside-west	2.9 a ^z
Middle-west	2.4 b
Inside	1.6 c
Source of variation	Pr>F
Position	0.0001

^zMeans in the same column followed by the same letter are not significantly different at 5% level (LSD).

Table 17: Average vapour pressure deficit (kPa) in the 6 weeks prior to harvest of mealy and non-mealy outside-west 'Forelle' pears as measured in 2017 on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

Mealiness class	Average VPD (kPa) of outside-west fruit
Mealy	2.9 ^{*NS}
Non-mealy	2.8
Source of variation	Pr>F
Mealiness class	0.1102

*NS= Non-significant

Table 18: Effect of fruit canopy position on 'Forelle' pear average Hue angle, blush colour and ground colour at the time of commercial harvest maturity (\pm 6.2 kg). Fruit were harvested in 2017 on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

Fruit canopy position	Hue (°) blush side ^x	Blush colour (chart index) ^y	Ground colour (chart index) ^z
Outside-west	41.6 c ^v	1.0 c	2.2 *NS
Middle-west	72.8 b	6.0 b	2.2
Inside	112.4 a	12.0 a	2.1
Source of variation:		Pr>F	
Position	0.0001	0.0001	0.6217

*NS = Non-significant

^vMean values in the same column followed by the same letter are not significantly different at 5% level (LSD).

^xHue was measured at the reddest position.

^yBlush colour chart values 1-12: where 1=red; 12=green.

^zGround colour chart values 0.5-5: where 0.5= green; 5= pale green/ yellow.

Table 19: Effect of fruit canopy position on 'Forelle' pear average hue angle, blush colour, ground colour after 8w storage at -0.5°C + 7d shelf-life at 20°C. Fruit were harvested in 2017 at commercial maturity on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

Fruit canopy position	Hue (°) blush side^x	Blush colour (chart index)^y	Ground colour (chart index)^z
Outside-west	49.4 a ^v	1.0 a	3.2 ^{*NS}
Middle-west	71.5 b	7.3 b	3.2
Inside	103.5 c	12.0 c	3.1
Position	0.0001	0.0001	0.3300

*NS = Non-significant

^vMean values in the same column followed by the same letter are not significantly different at 5% level (LSD).

^xHue was measured at the reddest position.

^yBlush colour chart values 1-12: where 1=red; 12=green.

^zGround colour chart values 0.5-5: where 0.5= green; 5= pale green/ yellow.

Table 20: Average hue angle, blush colour and ground colour of mealy and non-mealy outside-west 'Forelle' pears after 8w storage at -0.5°C + 7d shelf-life at 20°C. Fruit were harvested in 2017 at commercial maturity on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

Factor:		Outside-west canopy fruit		
Mealiness class	Hue (°) blush side	Blush colour (chart index)^x	Ground colour (chart index)^y	
Mealy	47.2 ^{*NS}	1.0 ^{*NS}	3.4 a ^z	
Non-mealy	51.7	1.0	2.9 b	
Source of variation:		Pr>F		
Mealiness class	0.1504	1.0000	0.0001	

*NS = Non-significant

^zMeans in the same column followed by the same letter are not significantly different at 5% level (LSD).

^xBlush colour chart values 1-12: where 1=red; 12=green.

^yGround colour chart values 0.5-5: where 0.5= green; 5= pale green/ yellow.

Table 21: Effect of fruit canopy position on 'Forelle' pear ethylene production and respiration rate after 8w storage at -0.5°C + 7d shelf-life at 20°C. Fruit were harvested in 2017 at commercial maturity on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

Fruit canopy position	Ethylene production rate ($\mu\text{L}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$)	Respiration rate ($\text{mg CO}_2\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$)
Outside-west	106.4 b ²	269.1 b
Middle-west	120.9 a	286.3 a
Inside	101.2 b	306.0 a
Source of variation:	Pr>F	
Position	0.0021	0.0001

²Means in the same column followed by the same letter are not significantly different at 5% level (LSD).

Table 22: Effect of fruit canopy position on 'Forelle' pear average diameter, mass, length and diameter: length after 8w storage at -0.5°C + 7d shelf-life at 20°C. Fruit were harvested in 2017 at commercial maturity on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

Fruit canopy position	Diameter (mm)	Mass (g)	Length (mm)	Diameter:Length
Outside-west	63.6 a ²	155.4 a	80.2 a	0.80 a
Middle-west	59.0 b	131.3 b	77.8 a	0.76 b
Inside	54.9 c	109.8 c	72.7 b	0.76 b
Source of variation:		Pr>F		
Position	0.0001	0.0001	0.0001	0.0002

²Mean values in the same column followed by the same letter are not significantly different at 5% level (LSD).

Table 23: Average diameter, mass, length and diameter: length ratio of mealy and non-mealy outside-west 'Forelle' pears after 8w storage at -0.5°C + 7d shelf-life at 20°C. Fruit were harvested in 2017 at commercial maturity on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

Factor:		Outside-west canopy fruit			
Mealiness class	Diameter (mm)	Mass (g)	Length (mm)	Diameter:Length	
Mealy	66.8 a ²	177.4 a	83.4 a	0.81 ^{NS}	
Non-mealy	60.8 b	137.4 b	77.8 b	0.79	
Source of variation:		Pr>F			
Mealiness class	0.0001	0.0001	0.0121	0.2420	

*NS= Non-significant

²Mean values in the same column followed by the same letter are not significantly different at 5% level (LSD).

Table 24: Effect of 'Forelle' canopy position on the average number of normal (viable) seeds and aborted seeds after 8w storage at -0.5°C + 7d shelf-life at 20°C. Fruit were harvested in 2017 at commercial maturity on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

Fruit canopy position	Number of normal seeds	Number of normal seeds (sun side)	Number of normal seeds (shade side)	Number of aborted seeds
Outside-west	0.70 a ^z	0.36 a	0.31 *NS	7.23 b
Middle-west	0.18 b	0.03 b	0.13	9.63 a
Inside	0.21 b	0.00 c	0.21	9.43 a
Source of variation:			Pr>F	
Position	0.0001	0.0001	0.1136	0.0001

*NS = Non-significant

^zMean values in the same column followed by the same letter are not significantly different at 5% level (LSD).

Table 25: Average number of normal (viable) seeds and aborted seeds of mealy and non-mealy outside-west 'Forelle' pears after 8w storage at -0.5°C + 7d shelf-life at 20°C. Fruit were harvested in 2017 at commercial maturity on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

Factor:		Outside-west canopy fruit		
Mealiness class	Number of normal seeds	Number of normal seeds (sun side)	Number of normal seeds (shade side)	Number of aborted seeds
Mealy	0.69 ^{*NS}	0.36 ^{NS}	0.33 ^{NS}	6.95 ^{NS}
Non-mealy	0.67	0.43	0.24	7.45
Source of variation:	Pr>F			
Mealiness class	0.9539	0.7099	0.5495	0.3795

*NS= Non-significant

Table 26: Effect of fruit canopy position on 'Forelle' pear average firmness, TSS and TA at the time of commercial harvest maturity (\pm 6.2 kg). Fruit were harvested in 2017 on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

Fruit canopy position	Firmness (kg)	TSS (%)	TA (% malic acid)
Outside-west	6.3 *NS	14.4 a ^z	0.17 b
Middle-west	6.3	13.0 b	0.17 b
Inside	6.3	12.7 b	0.21 a
Source of variation:		Pr>F	
Position	0.9907	0.0001	0.0018

*NS = Non-significant

^zMean values in the same column followed by the same letter are not significantly different at 5% level (LSD).

Table 27: Effect of fruit canopy position on 'Forelle' pear average mealiness score, firmness, TSS and TA after 8w storage at -0.5°C + 7d shelf-life at 20°C. Fruit were harvested in 2017 at commercial maturity on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

Fruit canopy position	Mealiness Class score averagex	Firmness (kg)	TSS (%)	TA (% malic acid)
Outside-west	0.99 a ^z	1.9 *NS	15.1 a	0.14 c
Middle-west	0.24 b	1.8	14.0 b	0.15 b
Inside	0.28 b	1.8	13.6 c	0.19 a
Position	0.0001	0.0001	0.0001	0.0001

*NS = Non-significant

^zMean values in the same column followed by the same letter are not significantly different at 5% level (LSD).

*Mealiness classes: 0=non-mealy, 1=partly mealy and 2=mealy.

Table 28: Average firmness, TSS and TA of mealy and non-mealy outside-west 'Forelle' pears after 8w storage at -0.5°C + 7d shelf-life at 20°C. Fruit were harvested in 2017 at commercial maturity on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

Factor:		Outside-west canopy fruit		
Mealiness class	Firmness (kg)	TSS (%)	TA (% malic acid)	
Mealy	1.7 b ^z	15.4 a	0.14 *NS	
Non-mealy	2.0 b	14.9 b	0.14	
Source of variation:		Pr>F		
Mealiness class	0.0006	0.0109	0.9268	

*NS = Non-significant

^zMeans in the same column followed by the same letter are not significantly different at 5% level (LSD).

Table 29: Average juice mass and area of different mealiness classes and fruit positions of 'Forelle' after 8w storage at -0.5°C + 7d shelf-life at 20°C. Fruit were harvested in 2017 at commercial maturity on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

Factor:	Mean juice mass (mg)		Mean juice area (cm²)	
Mealiness class:				
Non-mealy (0)	0.23 a ^z		14.5 a	
Partly mealy (1)	0.11 b		9.9 b	
Mealy (2)	0.04 c		3.6 c	
Source of variation:	Pr>F			
Mealiness class	0.0001		0.0001	
Factor:	Non-mealy		Partly mealy	
Fruit position:	Juice mass (mg)	Juice area (cm²)	Juice mass (mg)	Juice area (cm²)
Outside-west	0.18 c ^z	13.3 c	0.11 *NS	8.9 b
Middle-west	0.23 b	14.5 b	0.11	9.7 b
Inside	0.26 a	16.0 a	0.12	11.5 a
Source of variation:	Pr>F			
Position	0.0001	0.0001	0.9384	0.0001

*NS = Non-significant

^zMeans followed by the same letter are not significantly different at 5% level (LSD).^xAnalysis of mealy textured fruit for the different positions were not done due to low mealiness incidence for certain canopy positions.

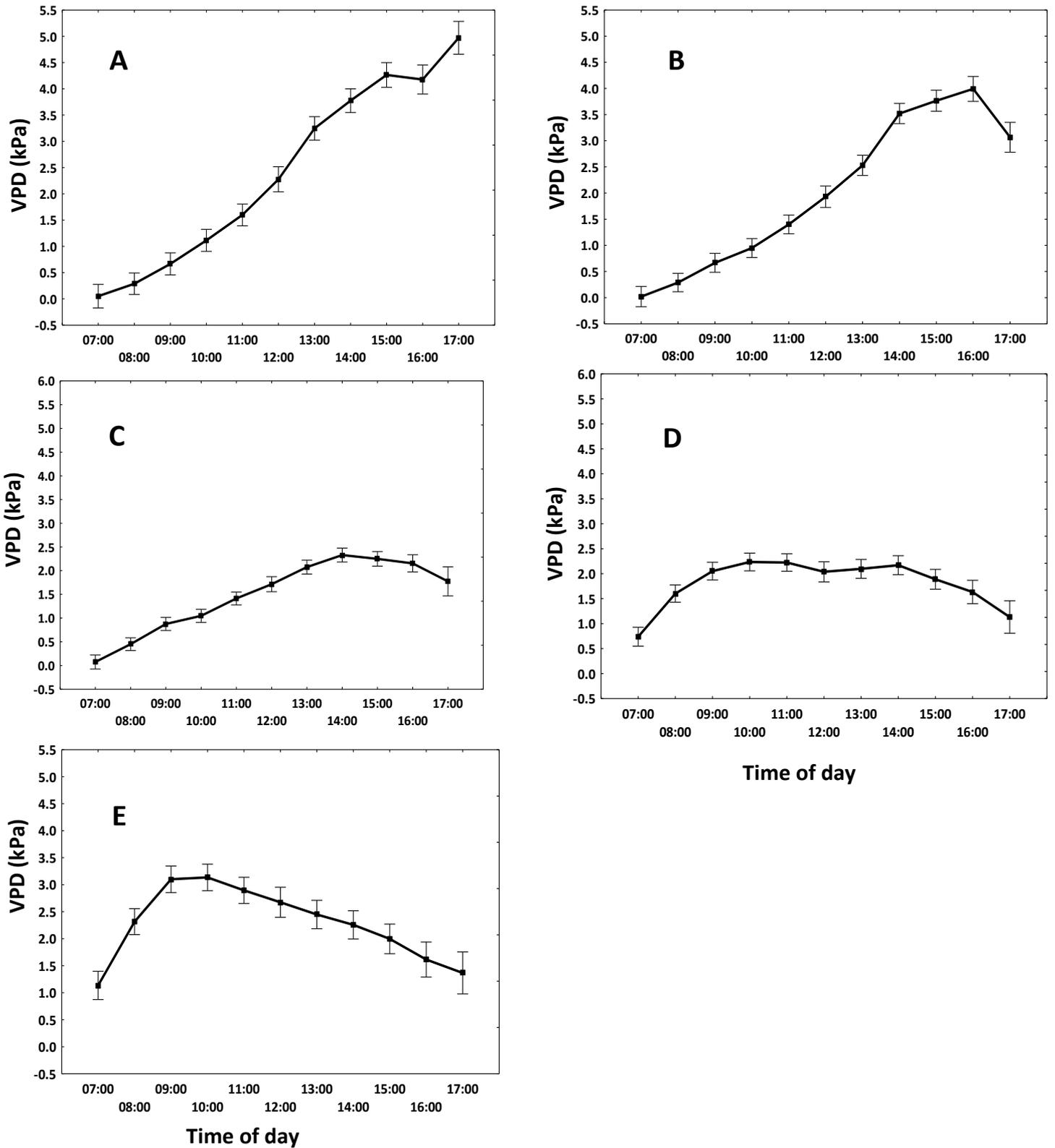


Figure 1: The diurnal vapour pressure deficit (VPD), in the 6 weeks prior to harvest for A) outside-west, B) middle-west, C) inside, D) middle-east and E) outside-east ‘Forelle’ pear fruit as measured in 2016 on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

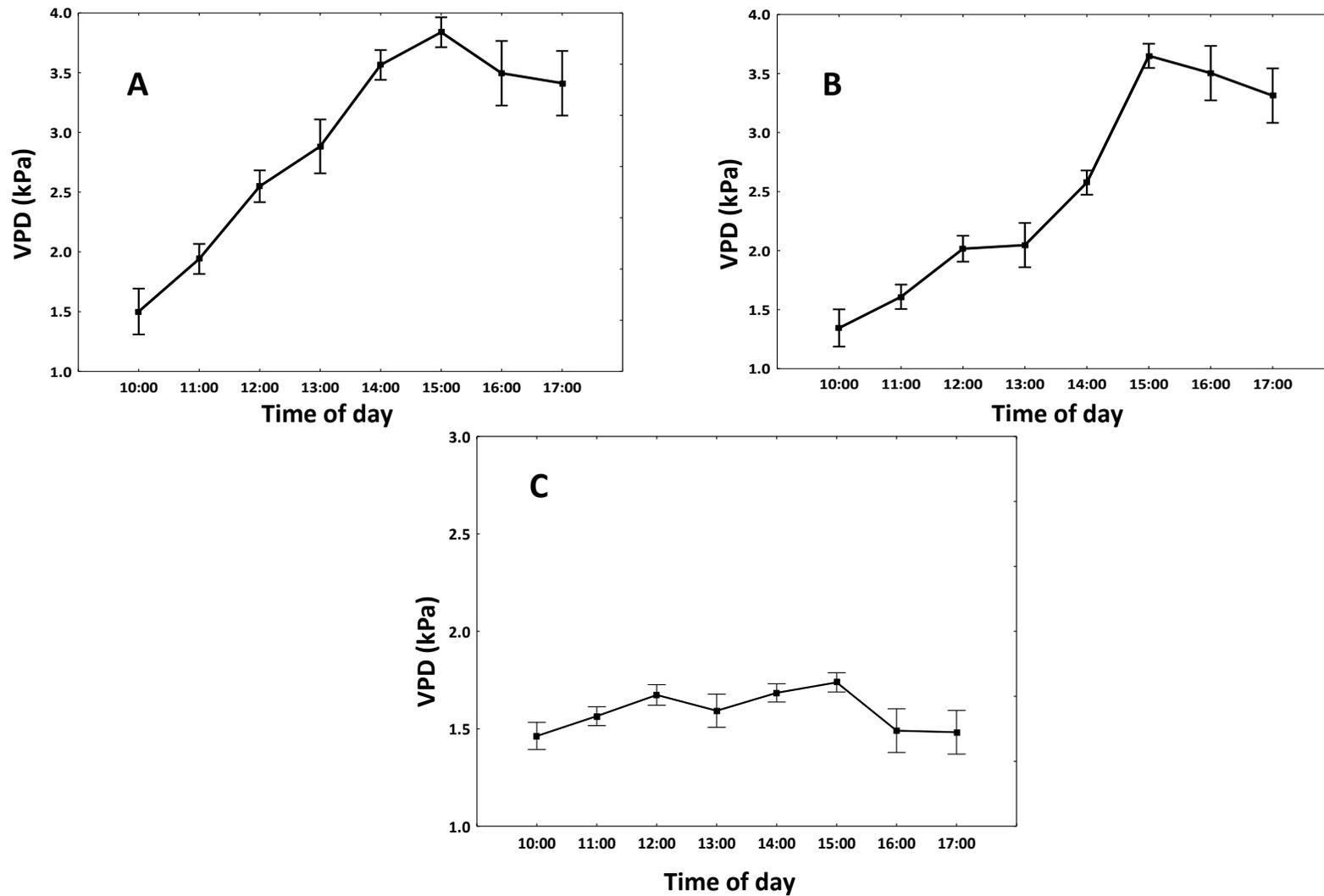


Figure 2: The diurnal vapour pressure deficit (VPD), in the 6 weeks prior to harvest for A) outside-west, B) middle-west and C) inside 'Forelle' pear fruit as measured in 2017 on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

CHAPTER 3:

POST-HARVEST 'FORELLE' MEALINESS INFLUENCED BY SHADING THE OUTSIDE CANOPY FRUIT

Abstract

Forelle (*Pyrus communis* L.) is the most widely grown bicolour pear cultivar in South Africa. Fruit of this cultivar have a propensity to develop mealiness, a soft, dry textural disorder of the flesh. Susceptibility to mealiness is linked to canopy position with outer canopy fruit more prone to develop the disorder. The aim of this study was to determine if mealiness susceptibility can be modified by complete shading of the outer canopy fruit. Three shading treatments were randomly applied to outer canopy pears on the western side of the tree row on 13 December 2016: (1) fully exposed control; (2) shading of fruit and their surrounding leaves; (3) shading of fruit, but not the surrounding leaves. Fruit surface temperature and irradiance were measured on the day of harvest during the hottest part of the day. Fruit were harvested at optimum maturity (6.2 kg firmness) and maturity indices, as well as mealiness incidence were determined after 8 weeks of regular atmosphere (RA) storage at -0.5 °C and 7 days of shelf-life at 20 °C (8w RA + 7d SL). The fruit surface temperature of fully exposed outer canopy fruit was 8 °C higher compared to the shaded fruit. After cold storage, fully exposed outer canopy fruit were significantly mealier compared to the shaded fruit even though fruit size did not differ significantly. The higher mealiness incidence was associated with a yellower background colour, higher total soluble solids, lower titratable acids and lower firmness. The greater susceptibility of exposed fruit to mealiness development suggests that light and temperature might be determining factors involved in 'Forelle' pear mealiness development.

Keywords: *Pyrus communis* L., mealiness, outer canopy, shading, irradiance, fruit surface temperature.

3.1 INTRODUCTION

Forelle pear (*Pyrus communis* L.), a late season blushed cultivar produced in South Africa, makes up 26% of South Africa's total pear production area, whereas other bicolour cultivars, viz. Rosemarie, Cape Rose and Flamingo, contribute 4, 3 and 1%, respectively (HORTGRO,

2018). Despite the success of 'Forelle' pears, mainly attributable to their red blush that is favoured by consumers (Manning, 2009), the fruit are prone to develop mealiness, a textural disorder characterised by a floury mouthfeel, combined with a lack of juiciness, crispness and firmness (Barreiro et al., 1998; Crouch, 2011). As a result, South African 'Forelle' pears have a mandatory cold storage period of at least 12 weeks at $-0.5\text{ }^{\circ}\text{C}$ to minimise the development of a mealy texture (de Vries and Hurndall, 1993; Martin, 2002). There is relatively little research available on mealiness development in pear fruit, although a number of studies have been done on different factors influencing mealiness incidence in a variety of fruit. Several past studies focused on pre-harvest factors affecting mealiness incidence, such as high temperatures 6 weeks prior to harvest on 'd'Anjou' pears (Mellenthin and Wang, 1976), exposure of 'La France' pears to cool temperatures in the orchard (Murayama et al., 1999), pre-harvest temperatures above $40\text{ }^{\circ}\text{C}$ and overhead cooling (Crouch, et al., 2005) on 'Forelle' pears, harvest maturity on 'Forelle' and 'La France' (Carmichael, 2011; Murayama et al., 1998) and fruit canopy position on 'Forelle' pear (Cronjé et al., 2015). Previous research also focused on the post-harvest factors affecting 'Forelle' mealiness incidence, which included intermittent warming (de Vries and Hurndall, 1993) and storage duration after harvest (Martin, 2002; Carmichael, 2011; Crouch, 2011).

Fruit is borne throughout the tree canopy, with the result that fruit from the same tree are exposed to considerable differences in irradiance levels, resulting in differences in fruit temperature, water and nutrient flow, as well as the supply of endogenous hormones (Kingston, 1994; Tomala, 1999). This could possibly serve as an explanation for the variability in the post-harvest life of pome fruit (Woolf and Ferguson, 2000). A preliminary study by Cronjé et al. (2015) found that outer canopy red blush 'Forelle' pears exhibited a higher mealiness incidence compared to inner canopy fruit. In addition, Muziri (2016) associated higher total soluble solids (TSS) with 'Forelle' pear mealiness, which could possibly mean that outer canopy fruit are slightly riper and mealiness development commences sooner. Murayama et al. (1998) linked mealiness development in 'La France' pear with a post-optimum harvest maturity. The link between environmental factors and mealiness in pears is, however, not fully understood. It is not clear whether or how high irradiance and high temperatures experienced by outside canopy fruit affect fruit development, resulting in fruit being more susceptible to mealiness development.

Environmental factors, such as low relative humidity and irradiation, as well as mineral imbalances can lead to mineral deficiencies in various fruit types. One such mineral is calcium, which plays an important role in cellular integrity within the fruit (Ho and White, 2005). High irradiance coupled with high temperatures accelerates the rate of fruit expansion (De Kock et al., 1982). Muziri et al. (2015) also reported that cell volume and cell diameter display a positive linear relationship with 'Forelle' pear mealiness percentage. Therefore, the fruit canopy position may affect cell division/expansion, which eventually has an effect on the anatomy and tissue structure.

The objective of the study was to determine if 'Forelle' pear mealiness susceptibility can be modified by the complete shading of the sun-exposed outer canopy fruit. If true, it would show that the lower incidence of mealiness of inner canopy fruit is not necessarily due to their position on the tree structure *per se* but might rather be ascribed to the lower irradiance/temperature to which they are exposed. The purpose of the shading treatment was to manipulate outer canopy fruit to represent inner canopy fruit with regard to light exposure and temperature. This paper explores the shading of sun exposed outer canopy fruit and its effects on texture after storage and ripening.

3.2 MATERIAL AND METHODS

3.2.1 Fruit material

Three treatments were randomly applied to the western outer canopy of two rows of 'Forelle' pears (*Pyrus communis* L.) on 13 December 2016. Trees of similar vigour and crop load were used for purposes of uniformity. The orchard was planted in 1991 on BP3 rootstock at a spacing of 4.5 m x 1.5 m in a north-south row orientation and trained to a central leader training system. A completely randomized was used.

Treatment one consisted of shading 100 western outer canopy 'Forelle' pear fruit and their surrounding leaves (spur and bourse shoot) by using Tetrapak juice carton canopies (Tetra Prisma®Aseptic, The Republic of South Africa), with the aluminium side to the sun (Fig. 1). Treatment two was the same as treatment one, except that the leaves directly next to the fruit were not shaded. Treatment three consisted of unshaded, outer canopy red blush pears on the western side of the tree row, which served as the control. In total, 300 fruit were used

(100 fruit per treatment) allowing for fruit that might drop prior to harvest thereby ensuring that enough fruit would be available for assessments.

Fruit surface temperature at a position perpendicular to the current position of the sun was measured at 16:00 on the day of harvest, 23 February 2017 (a warm, cloudless day) using a high-performance infrared thermometer (Rayner MX4, Raytek Corporation, Santa Cruz, CA, USA). This time of the day has been previously established to result in fruit surface temperatures being the highest (Chapter 2). For treatments one and two, fruit surface temperature was measured immediately upon removal of the Tetrapak cover.

3.2.2 Maturity and quality indices

A total of 210 fruit (90 of the 300-fruit dropped during the season) were harvested at commercial harvest maturity (± 6.2 kg firmness) consisting of 54 shaded pears from treatment one, 78 shaded pears from treatment two, and 78 unshaded outer canopy red blush pears on the western side of the tree canopy (treatment 3). After harvest the same procedure as for chapter 2 was followed. Maturity indexing was conducted after 8w RA + 7d SL.

For the evaluation, treatment one consisted of six replicates of nine fruit each, and treatment two and three, each consisted of six replicates of thirteen fruit each.

3.2.2.1 Hue angle and peel colour

The same procedure was followed as for chapter 2. The blush chart P. 16 was used (Fig 2).

3.2.2.2 Fruit background colour, firmness, TSS and TA, mealiness, juiciness, diameter, mass and length.

The same procedure was followed as for chapter 2.

3.2.2.3 Data analysis

One-way analysis of variance (ANOVA) was done and confirmed with Kruskal-Wallis in the cases where residuals were not normally distributed. A Levine's test for homogeneity of variances was performed. If this hypothesis was rejected, a Games-Howell multiple comparison was done to incorporate the heteroscedasticity. Mean separation was done using Fisher's least significant difference (LSD 0.05) at a 95% confidence level. The analysis was performed using Statistica 13.2 (StatSoft, Tulsa, OK, USA). A one-way ANOVA was used to

determine the differences between mealy textured and non-mealy textured fruit regarding the standard maturity parameters.

The TSS and TA levels could only be analyzed for differences between treatments and not between mealiness classes as fruit were pooled per replicate and not evaluated for individual fruit.

3.3 RESULTS AND DISCUSSION

This study showed that the higher mealiness incidence of the outside unshaded fruit (Fig. 3A) was associated with a significantly redder blush, yellower background colour, higher TSS, lower TA and lower flesh firmness compared to shaded fruit with uncovered leaves after 8w RA + 7d SL (Table 1). This possibly indicates that the unshaded red blush pear fruit had a higher ripening rate during cold storage and shelf life since at the time of harvest, firmness did not differ between treatments ($P > F = 0.1879$). The review of Musacchi and Serra (2018) reported that fruit that developed in the shade have a lower TSS level and are delayed in maturity compared to sun-exposed fruit. In our study, the unshaded control fruit and shaded fruit with their surrounding leaves exhibited a similar stage of maturity. Therefore, since there was no difference in mealiness incidence between the two covered treatments, the difference in mealiness incidence between covered and control fruit cannot simply be ascribed to differences in maturity and ripening.

Fruit ripening is also associated with a transition of background colour from green to yellow (Maga, 1974). This means that shaded fruit with exposed surrounding leaves could possibly have a better storage life, as these fruits were greener, had a higher firmness and TA than the unshaded outer canopy fruit and the shaded fruit with their surrounding leaves after 8w RA + 7d SL (Table 1).

Control fruit had a significantly higher mealiness incidence (51%) compared to shaded treatments (5% on average) (Fig. 3A). It is not clear why fruit that were shaded with their surrounding leaves exhibited a similar firmness and background colour to unshaded red blush control fruit (Table 1). It is possible that shading the leaves and fruit resulted in lower quality fruit that ripened sooner than shaded fruit with their surrounding leaves exposed to sunlight.

The lower quality may be ascribed to the lower supply of photo-assimilates from the surrounding leaves, seeing that photosynthesis is a light-dependent process.

In general, mealy textured fruit was associated with a significantly redder blush colour, yellower ground colour and lower firmness compared to non-mealy pear fruit (Table 2). Mealy and partially mealy pears were also bigger in diameter (63.3 mm and 63.6 mm, respectively compared to 60.9 mm). Partly mealy fruit were heavier and longer in length than non-mealy fruit, but mealy (153.0 g; 79.4 mm) and non-mealy (139.6 g; 77.8 mm) fruit did not differ significantly in mass or length (Table 2).

Sensorial classifications of mealiness and the amount of juice released and measured differed significantly between fruit of the three different mealiness classes ($P > F = 0.0001$) (Table 3). Non-mealy pears had significantly higher mean juice mass and juice area than partly mealy (0.097 mg; 9.61 cm²) and mealy textured fruit (0.042 mg; 4.05 cm²), with mealy pears exhibiting significantly the lowest juice mass and juice area (Table 3). Unshaded outer canopy pears that were non-mealy released a lower amount of juice in terms of juice mass and juice area than non-mealy fruit that were shaded with their surrounding leaves (Table 4).

Fruit canopy position may influence fruit maturity, carbohydrate utilization, biosynthesis of pigments, and the metabolism of amino acids as well as the capacity of fruit to undergo ripening, which may cause metabolic profiles to differ between different fruit canopy positions (Rudell et al., 2008; Hamadziripi, 2012; Rudell et al., 2017). Cronjé et al. (2015) found that outer canopy red blush 'Forelle' pears exhibited a higher mealiness incidence than inner canopy fruit, and Muziri (2016) associated a higher TSS with 'Forelle' pear mealiness. In agreement with these studies, the unshaded red blushed outer canopy fruit were significantly mealier (Fig. 3A) and had a significantly higher percentage of TSS compared to the two shaded treatments (Table 1). The 15.3% TSS of unshaded outer canopy fruit was, however, only 0.5% higher. There are various studies which associated high irradiance and high fruit surface temperature with higher fruit TSS in apples (Jackson et al., 1977; Nilsson and Gustavsson, 2007), and pears (Kappel and Neilsen, 1994). This may be as a result of higher carbon assimilation due to higher photosynthesis and sink strength, but also due to faster conversion of starch to soluble carbohydrates. The difference in TSS between shaded and exposed outer canopy fruit was less than found between outer and inner canopy fruit (chapter 2). This

suggests that shaded outer canopy fruit are better supplied with photo-assimilates compared to inner canopy fruit.

These differences in maturity indices and mealiness incidence between the three treatments may be due to the micro-climate created as all the fruit originated from the outer canopy. Woolf and Ferguson (2000) suggested that differences in ethylene production, protein synthesis/breakdown, cell wall breakdown and membrane permeability can be expected upon ripening if fruit reached high pre-harvest temperatures and/or if fruit were exposed to high temperatures during development. Increasing temperature during fruit growth decreases fruit TA in apples (Robinson et al., 1983). This agrees with our results, as unshaded control fruit were typically 8 °C warmer on the day of measurement (Fig. 3B) compared to shaded fruit and had significantly lower TA (Table 1). Modifications in organic acid metabolism in response to temperature are most likely the effect of glycolysis and the tricarboxylic acid (TCA) cycle, through modifying enzyme activities (Etienne et al., 2013), as well as the effect on mitochondrial systems which are involved (Halestrap, 1975). This causes the higher rate of fruit organic acids usage as substrates with an increased respiration rate at higher temperatures.

Pear fruit growth involves an initial cell division period followed by a period of cell expansion (Gillaspy et al., 1993). Higher cell division rates in apple are associated with an increase in temperatures (Bergh, 1990; Bergh and Cloete, 1992; Lakso et al., 1995). The outer canopy fruit is exposed to excessive high irradiance coupled with high fruit surface temperatures and high vapour pressure deficits (VPD) (as reported in chapter 2). This could possibly lead to hardening of walls of cortex cells in particular in the most exposed neck tissue, which then becomes less pliable during further cell enlargement as they are unable to expand. This may result in big cavities in the neck tissue due to cellular breakage and cell separation as reported by Muziri et al. (2016), resulting in fruit that may be more susceptible to mealiness development. It is known that porosity affects flesh texture and thus mealiness development (Muziri et al., 2016). Schoeman found sun-exposed control fruit to have higher porosity which corresponds to lower connectivity between the cells (Crouch et al., 2017). This agrees with Muziri et al. (2016) who found mealy 'Forelle' pears to have larger cells, with respect to cell volume and cell area. However, even though differences in mealiness exist between the sun-exposed fruit and shaded fruit, the fruit size did not differ significantly (Table 1). The higher

carbon assimilation of the sun-exposed control fruit may cause a rapid increase in fruit volume and the cell wall perhaps is not plastic enough to maintain cellular adhesion, resulting in a higher porosity. Pores are formed by separated tissue and breakage (Muziri et al., 2016). Consequently, the higher porosity and mealiness incidence of sun-exposed control fruit compared to the shaded fruit can possibly be attributed to the difference in irradiance levels and temperature.

The temperature difference between the sun exposed side of fruit and the shaded side can be up to 15 °C in apples (Thorpe, 1974) and 8 °C in red tomatoes (Woolf and Ferguson, 2000). Thus, a thermal gradient can exist over unshaded red blush pear fruit due to their high FST (Fig. 2B), which may lead to the dispersion of mineral nutrients in the fruit and uneven ripening (Woolf et al., 1999). The significantly higher mealiness incidence of the sun exposed side compared to the shaded side of red blush control fruit (Fig. 4), can possibly be attributed to the higher irradiance levels coupled with higher fruit temperatures on the exposed side. In shaded fruit where such an irradiance and temperature differential between the sides of the fruit was absent, mealiness levels did not differ between the sides of the fruit.

Lakso et al. (1989) also correlated the growth rate of apple fruit during the first five weeks after bloom with the exposure of spurs to sunlight. They proposed that the effect of canopy shade on final fruit size occurs during the first five weeks after bloom. Young fruit are not strong sinks and with the early competition with vegetative shoots or lowered irradiance, fruit size can be greatly decreased (Avery et al., 1979; Ferree and Palmer, 1982). The importance of leaves closest to the fruit can be seen by the significantly higher mass of shaded outer canopy fruit excluding their leaves compared to shaded leaves and fruit (157.2 g and 138.9 g, respectively), although fruit diameter did not differ significantly (63.2 mm and 60.9 mm, respectively). In general, the shaded fruit with their leaves exposed seem to be of a higher quality, regarding to texture. This is ascribed to sufficient supplying of photo-assimilates to the fruit in association with fruit temperatures suitable for normal fruit development to occur.

These results therefore show the importance of the leaves nearest to the fruit in supplying photo-assimilates to the fruit in order for fruit development to occur at full potential. In our study, fruit drop was high where fruits' surrounding leaves were covered. However, temperatures exceeding 33 °C may have a detrimental effect on fruit growth (Calderón-Zavala et al., 2004), resulting in fruit susceptible to mealiness development.

3.4 CONCLUSION

It appears that fruit and the sides of fruit that were directly exposed to high irradiance in association with high fruit temperatures are more susceptible to mealiness development. This indicates the possibility of fruit temperature being one of the determining factors involved in 'Forelle' pear mealiness development and not so much fruit position within the tree canopy. It seems that the higher irradiance and fruit temperatures may result in higher porosity of the sun-exposed outer canopy fruit compared to the shaded outer canopy fruit, although this needs to be verified. However, since not all unshaded outer canopy fruit developed a mealy texture, an unidentified tree factor may also be involved in 'Forelle' mealiness development.

The higher mealiness incidence was associated with a significantly redder blush colour, yellower background colour, higher fruit surface temperature, higher TSS, lower TA and lower firmness after 8w RA + 7d SL compared to the shaded fruit excluding their surrounding leaves. The parameters indicate that the unshaded outer canopy red blush pear fruit may possibly have a higher ripening rate. This may be ascribed to their higher fruit temperatures which may increase the rate of several fruit reactions/processes such as an increased rate of starch breakdown (SB) that increases fruit TSS and lowers TA levels due to potentially higher respiration rates. Fruit development and the manner of fruit ripening of the shaded outer canopy fruit may occur in a more normal manner, seeing that their FST was 8 °C lower.

According to the results obtained and as reported in numerous publications, the leaves closest to the fruit have an important role in supplying the fruit with photo-assimilates to ensure fruit develop to their full potential (size, TSS, TA, firmness, flesh texture). However, fruit exposure to high irradiance coupled with high fruit temperatures play an important role in determining final fruit quality and fruits' susceptibility to develop mealiness. This can be seen with the fruit shaded excluding their surrounding leaves, which were significantly firmer, had a greener ground colour, a higher TA content and were significantly heavier in comparison to the fruit shaded with their surrounding leaves. Of these three treatments, it appears that the shading of outer canopy fruit excluding their surrounding leaves may be a solution for a reduction of 'Forelle' pear mealiness development but this practice may not be practical since red colour development is reduced. Insufficient red colour development of red blush pear fruit in South Africa results in the downgrading of the fruit from 'Forelle' to 'Vermont Beaut'.

The results could mean that shade netting could possibly be used to reduce 'Forelle' mealiness development. In contrast to the current trial, this would only provide partial shading which could in turn deliver sufficient blush but leaves will be shaded to a degree.

Further studies are needed to determine during which stage of development the fruit are most susceptible to high fruit temperatures so that shading might be limited to a certain period to ensure the development of a sufficient red blush colour and for fruit to obtain the highest possible quality.

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3.6 TABLES AND FIGURES

Table 1: Effect of shading of outer canopy 'Forelle' pear fruit including or excluding their surrounding leaves on the average firmness at the time of harvest, as well as the effect on the average hue angle on the blushed side and green background colour, background colour, TSS, TA, firmness, diameter, mass and length after 8w storage at -0.5°C + 7d shelf-life at 20°C compared to uncovered control fruit. Hue angle was measured at the reddest position of control fruit. Fruit were harvested in 2017 at commercial harvest maturity on the western side of the tree on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

Treatment	Firmness at harvest (kg)	Hue angle (°) blush side	Hue angle (°) background	Ground colour (chart value ^x)	TSS (%)	TA (% malic acid)	Firmness (kg)	Diameter (mm)	Mass (g)	Length (mm)
Control	6.1 ^{*NS}	43.3 b	104.4 b	3.4 a	15.3 a	0.13 c	1.9 b	62.7 ^{NS}	150.0 ab	78.7 ab
Fruit covered	6.2 ^{NS}	98.1 a	106.9 a	3.0 b	14.7 b	0.18 a	2.8 a	63.2 ^{NS}	157.2 a	81.8 a
Fruit + leaves covered	6.2 ^{NS}	95.4 a	105.2 b	3.4 a	14.8 b	0.15 b	2.0 b	60.9 ^{NS}	138.9 b	77.1 b
Source variation:					Pr>F					
Treatment	0.1879	0.0001	0.0001	0.0002	0.0001	0.0001	0.0001	0.1470	0.0372	0.0311

*NS = Non-significant

^zTreatment means in the same column followed by the same letter are not significantly different at 5% level (LSD).

^xChart values 0.5-5: where 0.5= green; 5= pale green/ yellow.

Table 2: Average hue angle on the blushed side and non-blushed side, background colour chart index, TSS, TA, firmness, diameter, mass, length of mealy, partly mealy and non-mealy 'Forelle' pears after 8w storage at -0.5°C + 7d shelf-life at 20°C. Fruit were harvested in 2017 at commercial maturity on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

Mealiness class	Hue (°) blushed side	Hue (°) background	Ground colour (chart index)*	Firmness (kg)	Diameter (mm)	Mass (g)	Length (mm)
Mealy	49.9 c ^z	104.1 c	3.5 a	1.9 c	63.3 a	153.0 ab	79.4 ab
Partly mealy	80.4 b	105.5 b	3.3 b	2.2 b	63.6 a	159.3 a	81.4 a
Non-mealy	88.7 a	106.4 a	3.1 c	2.6 a	60.9 b	139.6 b	77.8 b
Source of variation:			Pr>F				
Mealiness class	0.0001	0.0001	0.0001	0.0001	0.0200	0.0200	0.0900

^zMealiness class means in the same column followed by the same letter are not significantly different at 5% level (LSD).

*Chart values 0.5-5: where 0.5= green; 5= pale green/ yellow.

Table 3: Average juice mass and juice area of different mealiness classes of 'Forelle' after 8w storage at -0.5°C + 7d shelf-life at 20°C. Fruit were harvested in 2017 at commercial maturity on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

Mealiness class	Mean juice mass (mg)	Mean juice area (cm ²)
Non-mealy (0)	0.195 a ^z	13.930 a
Partly mealy (1)	0.097 b	9.609 b
Mealy (2)	0.042 c	4.051 c
Source of variation:	Pr>F	
Mealiness class	0.0001	0.0001

^zMealiness class mean followed by the same letter are not significantly different at 5% level (LSD).

Table 4: Effect of unshading and shading of outer canopy 'Forelle' pear fruit including or excluding their surrounding leaves on average juice mass and juice area of different mealiness classes after 8w storage at -0.5°C + 7d shelf-life at 20°C. Fruit were harvested in 2017 at commercial maturity on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa. Analysis of treatment with mealy texture was not possible owing to low numbers of mealy texture fruit.

Treatment	Mealiness class			
	Non-mealy (0)		Partly mealy (1)	
	Juice mass (mg)	Juice area (cm ²)	Juice mass (mg)	Juice area (cm ²)
Control	0.175 b ^z	13.238 b	0.090 *NS	9.376 NS
Fruit covered	0.191 ab	13.576 b	0.086 NS	9.710 NS
Fruit + leaves covered	0.225 a	15.206 a	0.115 NS	9.826 NS
Source of variation:	Pr>F			
Treatment	0.0208	0.0016	0.3059	0.5526

*NS= Non-significant

^zTreatment means in the same column followed by the same letter are not significantly different at 5% level (LSD).



Figure 1: Tetrapak juice cartons used as a canopy for the western outside 'Forelle' fruit with or without the surrounding leaves. The aluminium side of the Tetrapak™ juice carton canopy was facing the sun.

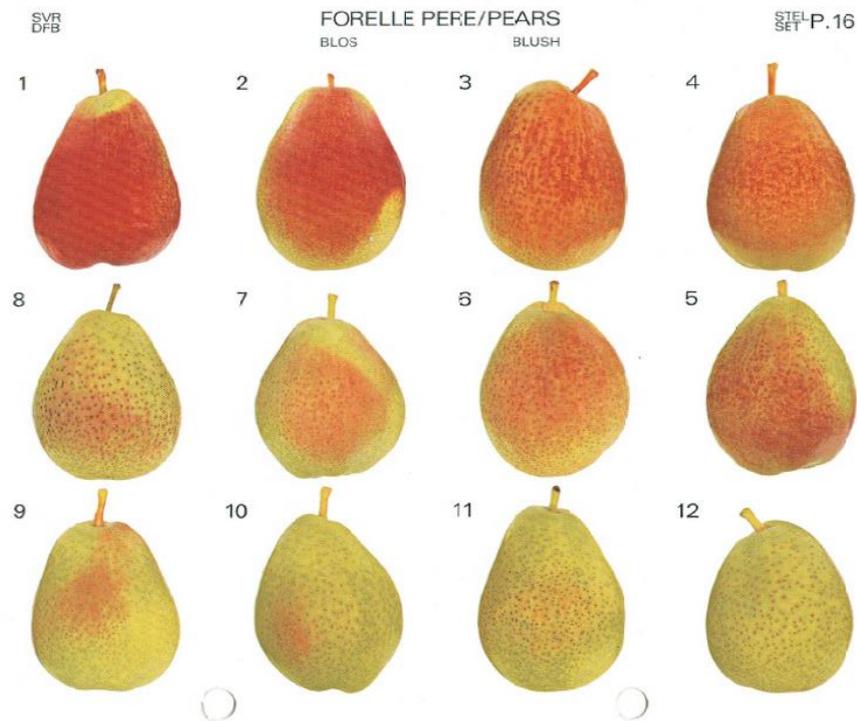


Figure 2: Peel blush colour chart P.16 developed for 'Forelle' pears by Unifruco Research Services (URS) [Pty] Ltd., South Africa, on a scale of 1 to 12 (1=fully covered on one side with a bright red blush and 12= green with no blush)

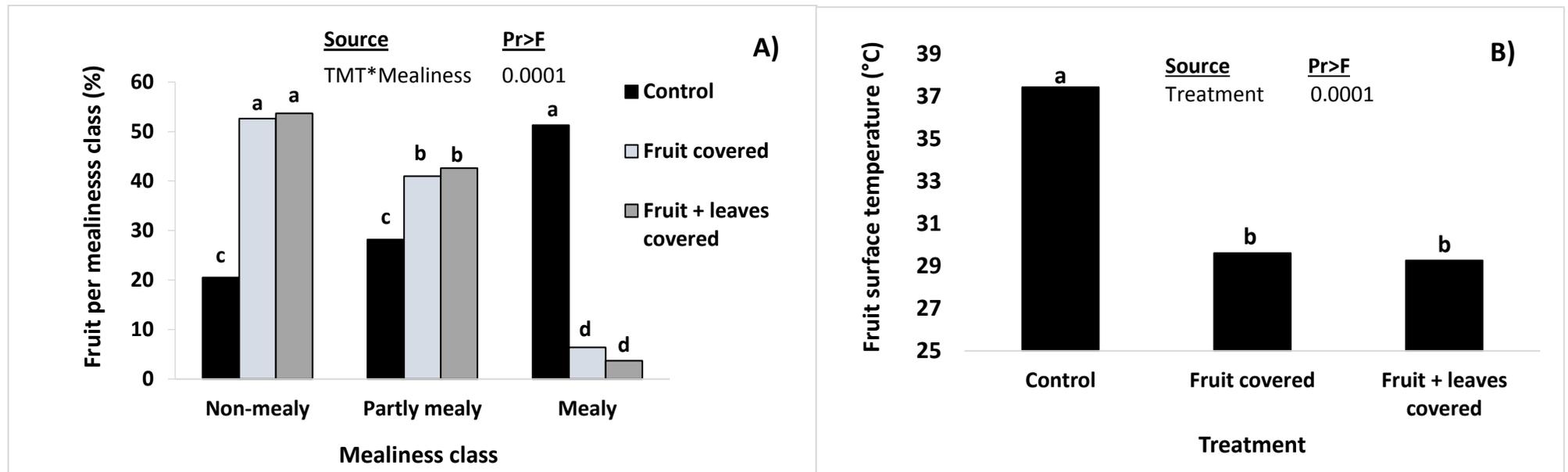


Figure 3: A) Percentage of the total 'Forelle' pear fruit per mealiness class after 8w storage at $-0.5\text{ }^{\circ}\text{C}$ + 7d shelf-life at $20\text{ }^{\circ}\text{C}$ and B) Average fruit surface temperature ($^{\circ}\text{C}$) for sun exposed red blush outer canopy 'Forelle' pear fruit (control) and shading of outer canopy fruit including/excluding their surrounding leaves (Average air temperature was $32.9\text{ }^{\circ}\text{C}$ at the time of measurement). Fruit were harvested in 2017 at commercial harvest maturity on the western side of the tree on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa. Different letters show significant differences between treatments at $p \leq 0.05$. The three mealiness classes consisted of non-mealy; partly mealy and mealy texture.

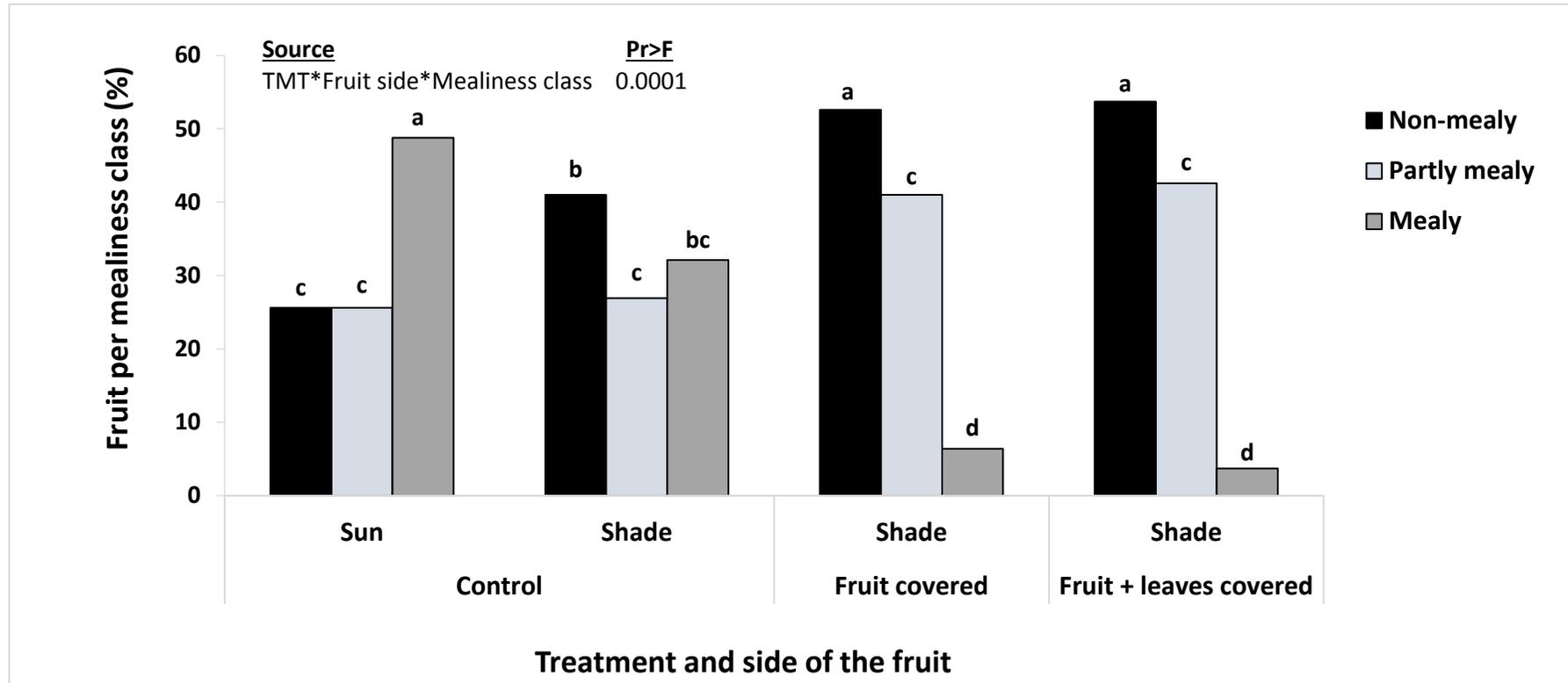


Figure 4: Effect of sun exposed side of fruit and shaded side of fruit on the percentage of the total 'Forelle' pear fruit per mealiness class for unshaded red blush outer canopy 'Forelle' pear fruit (control) and the effect of opposite cheek of the shaded outer canopy fruit with/without their surrounding leaves after 8w RA storage at $-0.5\text{ }^{\circ}\text{C}$ + 7d shelf-life at $20\text{ }^{\circ}\text{C}$. The shaded outer canopy treatments did not have a sun exposed side. Fruit were harvested in 2017 at commercial harvest maturity ($\pm 6.2\text{ kg}$) on the western side of the tree on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa. Different letters show significant differences between treatments at $p \leq 0.05$. The three mealiness classes consisted of non-mealy, partly mealy and mealy texture.

CHAPTER 4:

POST-HARVEST 'Forelle' (*Pyrus communis* L.) MEALINESS INFLUENCED BY CANOPY POSITION, HARVEST MATURITY AND STORAGE DURATION

Abstract

'Forelle' pears have the tendency to develop a mealy texture after ripening. Therefore, South African 'Forelle' pears have a mandatory 12-week cold storage period at -0.5 °C for even ripening to occur and to obtain a good eating quality. The aim of this study was to explore whether differences in ripening potential of outer, middle and inner canopy fruit, harvested at different harvest maturities, may influence mealiness expression, as well as to identify which maturity indices are linked to mealiness development.

'Forelle' pears from five different canopy positions were harvested at two maturities (commercial and post-commercial maturity) and evaluated immediately and after cold storage (0, 8, 12, 16 weeks at -0.5 °C) with subsequent ripening (0, 4, 7, 11 days at 20 °C). The five canopy positions consisted of the outer canopy red blush pears on the eastern and western sides, as well as slightly blushed pears in the middle canopy on the eastern and western sides, and non-blushed pears in the inner shaded parts of the canopy.

Post-commercially harvested 'Forelle' pears seem to be more susceptible to mealiness development and red blushed outer canopy pears were significantly more inclined to a mealy texture than middle and inner canopy fruit. Inner canopy fruit mealiness remained low for both harvest maturities, regardless of cold storage and ripening duration. The higher mealiness incidence of outer canopy pears was generally associated with a redder blush colour, higher total soluble solids (TSS), lower titratable acidity (TA), and bigger fruit size, compared to that of inner canopy fruit. In general, fruit associated with a mealy texture exhibited a redder blush colour, yellower ground colour, lower firmness, and bigger fruit size. In 2016 and 2017, post-commercial harvested fruit generally had lower ethylene production rates post storage than fruit harvested at commercial maturity. Fruit canopy position and harvest maturity did not influence the fruits' ability to achieve their full ripening potential. However, the ripening rate developed earlier (earlier loss of firmness and earlier transition to a yellower ground colour) for outer canopy fruit than for the inner canopy fruit. In both seasons, independent of harvest maturity, mealiness incidence of fruit started to decrease

with prolonged cold storage at $-0.5\text{ }^{\circ}\text{C}$. The non-mealy outer canopy pears of both seasons and harvest maturities released significantly less juice on average than non-mealy textured inside and middle canopy pears. In conclusion, 'Forelle' pears harvested at post-commercial maturity and red blushed outer canopy pears are more prone to mealiness development. 'Forelle' mealiness susceptibility seems to be a combination of fruit maturity and fruit size, seeing that larger fruit size is associated with bigger pores and the progressing of harvest maturity may cause an increase in the size of the pores, resulting in mealiness incidence.

Keywords: *Pyrus communis L.*, mealiness, canopy position, irradiance, temperature, ethylene

4.1 INTRODUCTION

Mealiness is the most important physiological disorder of South African 'Forelle' pears (Martin, 2002; Crouch, 2011; Cronjé et al., 2015; Muziri et al., 2015). Mealiness is a textural disorder that is associated with a floury, soft and dry sensation in the mouth with a low amount of extractable juice (Barreiro et al., 1998; Martin, 2002). Various other fruit, such as peaches (Obenland and Carroll, 2000; Brummell et al., 2004), nectarines (Lurie and Crisosto, 2005), apples (De Smedt et al., 1998; Barreiro et al., 2000), Japanese plums (Taylor et al., 1994), tomatoes (Jackmann et al., 1992) and other pear cultivars (Chen et al., 1983; Murayama et al., 2002) are also susceptible to a dry and soft texture.

Forelle is the second most planted pear cultivar in South Africa and the most important bicolour pear (HORTGRO, 2018). Total area planted with pears in South Africa is 12 319 hectares, with 'Forelle' contributing 26% of the total area (HORTGRO, 2018).

European pear cultivars, which including Forelle, requires cold storage after harvesting, for fruit to ripen normally and uniformly (Villalobos-Acuña and Mitcham, 2008; Crouch and Bergman 2013b). Increased susceptibility of 'Forelle' pears to develop mealiness, is mainly associated with fruit that are not exposed to a sufficient period of cold storage at $-0.5\text{ }^{\circ}\text{C}$, or with pears that are harvested at a post-optimum maturity (Martin, 2002; Crouch et al., 2005; Carmichael, 2011). The decrease in mealiness incidence with prolonged cold storage is unique to 'Forelle' pears, since other European pear cultivars, such as Marguerite Marillat, La France (Murayama et al., 2002) and d' Anjou (Chen et al., 1983) exhibit an increase in mealiness incidence after prolonged periods of cold storage.

Consequently, the protocol for marketing of South African 'Forelle' pears entails a mandatory 12 week cold storage period at $-0.5\text{ }^{\circ}\text{C}$ for fruit to ripen evenly to an acceptable eating quality and to minimize mealiness incidence (de Vries and Hurndall, 1993), as well as to achieve good colour, texture and flavour (Lelièvre *et al.*, 1997; Agar *et al.*, 1999). The mandatory cold storage period causes a loss of South African bicolour pear continuity on European markets which could result in a consumer shift to fruit from offshore competitors (Crouch and Bergman, 2013a). Higher market prices of more than 50% per box could be obtained for 'Forelle' pears if they could be available earlier (from week 15 in Europe) (Martin, 2002). Consequently, past research focused on reducing the mandatory 12 week cold storage period, but no other treatment could ensure a consistent low level of mealiness. The studies included: intermittent warming treatments (de Vries and Hurndall, 1993), controlled atmosphere (CA) storage in combination with regular atmosphere (RA) storage intervals (de Vries and Hurndall, 1993; de Vries and Hurndall, 1994; de Vries and Moelich, 1995), and ethylene treatments (Du Toit *et al.*, 2001). However, a program called 'Forelle' Early Market Access (FEMA) was initiated which enables the earlier marketing of 'Forelle' as crisp, but sweet and juicy pears. However, despite the great success of the FEMA program, the characteristic soft, sweet buttery flesh of 'Forelle' pear fruit is still preferred by many consumers, particularly those from European origin (Manning, 2009; Crouch and Bergman, 2013b).

Pears are climacteric fruit which means that fruit must be harvested at physiological maturity, allowing fruit to ripen normally after a critical period of cold storage (Hansen, 1961; de Vries, 2001; Martin, 2002). The cold-dependent accumulation of the precursor of ethylene, 1-aminocyclopropane-1-carboxylic acid (ACC), to a certain threshold is necessary to overcome ripening resistance (Wang *et al.*, 1985; Martin, 2002). At room temperature ACC is oxidized by the enzyme ACC oxidase, to ethylene. Thus, autocatalytic ethylene production occurs that leads to normal and uniform ripening of fruit (Oetiker and Yang, 1995). This is an important aspect for the development of the traditional soft, buttery flesh of the 'Forelle' pear (Crouch, 2011). The correct harvest maturity is also important for optimum ripening potential to ensure normal ripening of fruit (Hansen and Mellenthin, 1979; Carmichael, 2011). The loss of ripening potential is associated with abnormal fruit softening patterns (Predieri and Gatti, 2009). Fruit harvested at a post-optimum maturity is more inclined to a mealy texture, also associated with a poor storage life (Mellenthin and Wang, 1976 (pear); Hansen and

Mellenthin, 1979 (pear); Peirs et al., 2001 (apple); Martin, 2002; Carmichael, 2011 ('Forelle' pear)], and pear fruit harvested at an immature maturity do not have the ability to reach a climacteric, and also fail to ripen, or ripen unevenly (Ben-Arie et al., 1979).

Differences in climatic conditions amongst different cropping seasons has the ability to affect the harvest maturity and ripening potential of climacteric fruit (Matthee, 1988; Frick, 1995), causing variations in climacteric respiration and ethylene production of fruit (Nordey et al., 2016). Mellenthin and Wang, (1976) reported that high total heat units 6 weeks prior to harvest caused 'd' Anjou' pears not to ripen fully, and to be of a lower quality. Hansen (1961) associated high seasonal heat units with pear mealiness development. Carmichael (2011) also found a higher mealiness incidence of 'Forelle' pears growing in warmer areas, and Cronjé (2014) found outer canopy 'Forelle' pears to be more susceptible to mealiness development.

The proposed mechanism of 'Forelle' mealiness development is attributed to a more broken-down middle lamella in association with a stronger cell wall, resulting in reduced cell-to-cell adhesion which prevents the release of cell fluids during mastication, due to cell sliding (Crouch, 2011; Muziri, 2016). As mentioned earlier, the preliminary study by Cronjé (2014) associated a higher mealiness incidence with outer canopy 'Forelle' pear fruit, and bigger sized fruit seemed to be mealier ((De Smedt et al., 1998 (apples); Muziri, 2016 ('Forelle' pears)). Consequently, a difference among different fruit positions within the tree canopy could be expected in terms of physiological maturity at the time of harvest, as well as the degree of changes in maturity indices, which may affect the ripening potential and the way in which cellular changes occur during ripening.

The objective of this study was to determine whether mealiness differences found within the canopy are related to storage potential and ripening potential differences for fruit from different canopy positions, and harvest maturity.

4.2 MATERIAL AND METHODS

4.2.1 2016 season

4.2.1.1 Fruit material

Storage and ripening potential of five different 'Forelle' pear (*Pyrus communis* L) fruit positions, namely the outer canopy red blush pears on the eastern and western sides, as well

as slightly blushed pears in the intermediate/middle canopy on the eastern and western sides, and non-blushed pears in the inner, shaded parts of the canopy, were determined. The experimental design used was a completely randomized design.

The same orchard was used as in chapter 2 on Glen Fruin Farm, in the Elgin region of the Western Cape Province, South Africa. Fruit were harvested from two rows with similar vigour and crop load, for purposes of uniformity. The fruit were harvested on two different dates - 2 March (commercial maturity) and 9 March (post-commercial maturity).

4.2.1.2 Maturity and quality indices

In total, 1950 fruit (390 per position) were harvested, on each of the two harvest dates. Directly after harvesting the same procedure as for chapter 2 was followed. Maturity indexing was conducted at harvesting and again after 8, 12 and 16 weeks of cold storage at $-0.5\text{ }^{\circ}\text{C}$ under regular atmosphere (RA), plus 0, 4, 7 and 11 days of ripening at $20\text{ }^{\circ}\text{C}$. On each of the evaluation days, fruit from cold storage were allowed to reach room temperature and maturity and quality indices were measured. Individual fruit from a random sample of 30 fruit (six replicates of five fruit each) for each position, per evaluation day, were numbered to maintain identity of quality attributes per fruit. For each evaluation, 30 fruit (six replicates of five fruit each) per position were measured.

The standard MI parameters measured were fruit background colour and blush chart index, flesh firmness, diameter, mass, length, total soluble solids (TSS) and titratable acidity (TA), seed number (normal and aborted), and an average mealiness score and juiciness evaluation, as well as an ethylene production and respiration rate following the same procedure as for chapter 2. The average mealiness score was determined on a scale of 0 to 2 (0 = non-mealy; 1 = partly mealy and 2 = mealy).

4.2.1.2.1 Ethylene production and respiration rate

Ethylene production rate ($\mu\text{L}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$) and respiration rate ($\text{mg CO}_2\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$) were measured on five fruit from each of the five different canopy positions, per replicate. Each sample was placed in a 5L airtight plastic jar and left at room temperature for 60 min. The same procedure as for chapter 2 was used to calculate the ethylene production rate and respiration rates of the fruit.

4.2.1.2.2 *Data analysis*

Factorial analysis of variance (ANOVA) was performed on maturity and quality indices data. A Levene's test for homogeneity of variances was performed. If this hypothesis was rejected, then a Games-Howell multiple comparison was done to incorporate the heteroscedasticity. Mean separation was done using Fisher's least significant difference (LSD 0.05) at a 95% confidence level. A one-way ANOVA was used to determine the differences between mealy textured and non-mealy textured fruit regarding the standard MI parameters. Ethylene production rates and respiration rates, TSS and TA could not be analyzed for mealiness classes due to a pooled replicate sample. The analysis was performed using Statistica 13.2 (StatSoft, Tulsa, OK, USA).

4.2.2 *2017 season*

4.2.1.1 *Fruit material*

Storage and ripening potential of three different 'Forelle' pear (*Pyrus communis* L.) fruit positions namely, the outer canopy red blush pears and the slightly blush pears in the intermediate/middle canopy on the western side, as well as non-blushed pears in the inner shaded parts of the canopy, were determined. Fruit on the west side were used instead of those from the east side, since results from 2016 showed that fruit on the west side were more exposed to the sun during the hottest time of day. Thus, bigger differences in canopy position are obtained between fruit from the western side and fruit from the shaded inner parts of the canopy. Fruit were harvested from the same orchard and two rows as in 2016.

The fruit were harvested on two dates – 21 February (commercial maturity) and 6 March (post-commercial maturity). Harvest dates were further apart (compared to the 2016 season) to obtain clearer differences in fruit maturity and quality indices between the two harvest maturities. The same post-harvest procedure was followed as for 2016.

4.2.1.2 *Maturity and quality indices*

In total, 1170 fruit (390 per position) were harvested, for each harvest date. After harvest, the same procedure as for season 2016 was followed. The same standard MI and quality parameters were measured and mealiness assessed as for 2016. The only addition for the 2017 season was that the hue angle was also measured following the same procedure as for chapter 2.

4.2.1.2.1 *Ethylene production and respiration rate*

Ethylene production rate ($\mu\text{L}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$) and respiration rate ($\text{mg CO}_2\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$) were measured on five fruit from each of the three positions, per tree. Each sample was placed in a 5 L airtight plastic jar and left at room temperature for 30 min, where after the same procedure was followed as in 2016.

4.2.1.2.2 *Data analysis*

The same procedure as for season 2016 was followed.

4.3 RESULTS

4.3.1 2016 season

4.3.1.1 *Mealy texture and juiciness evaluation*

The eastern outer canopy pears of the first harvest showed a different mealiness incidence development over storage duration and ripening time compared to outside-west fruit (Fig. 1A, B, C). The outside-west fruit of harvest one already reached a mealiness peak after 8w RA + 7d and 11d SL, whereas mealiness development only started after 12 weeks at $-0.5\text{ }^\circ\text{C}$, with subsequent ripening for the pears from the eastern outer canopy (Fig. 1A). In fact, for harvest one the outside-east fruit had a similar low mealiness incidence compared to the inner canopy fruit after 8 weeks of cold storage plus subsequent ripening. However, after 12w RA + 4, 7 and 11d SL, outside-east fruit were substantially mealier compared to the fruit from the outer west fruit canopy (Fig. 1B).

The mealiness incidence over storage and ripening duration for harvest two outer canopy fruit from both sides of the tree exhibited a similar pattern that peaked already after 8 weeks of cold storage plus subsequent ripening (Fig 1D). The outside-east fruit of harvest two developed mealiness after 8w RA + 4d SL, which is slightly earlier than 8w RA + 7d SL of outside-west fruit (Fig. 1D). In contrast, the western outer canopy of harvest two exhibited a higher mealiness incidence earlier than outside-east fruit (Fig. 1E).

The maximum mealiness incidence was, however, higher for the second harvest of outside-east fruit after 8w RA +11d SL but did not differ significantly for outside-west fruit between harvest times at 8w RA +11d SL (Fig. 1E). Outside-west pears of the second harvest showed

significantly higher mealiness incidence after 16w RA + 11d SL compared to harvest two, outside-east fruit (Fig. 1F).

For both harvest dates, the maximum mealiness incidence for middle-east fruit was after 8w RA + 11d SL, as well after 16w RA + 11d SL for harvest two (Fig. 1A, D, F). The maximum mealiness incidence of middle-east fruit from harvest one (8w RA + 11d SL) and harvest two (8w and 16w RA + 11d SL) did not differ significantly (Fig. 1D, F). Middle-west fruit, just like middle-east fruit from both harvests, reached a mealiness peak after 8w RA + 11d SL (Fig. 1A, D). However, harvest one middle-west fruit reached similar maximum mealiness incidence after 12w RA + 11d SL (*ca.* 0.8) and 16w RA + 11d SL (*ca.* 1.0) (Fig. 1B, C) compared to after 8w RA + 11d SL. For both harvests, the maximum mealiness incidence of middle-east fruit was significantly higher than that of the middle-west fruit (Fig 1A-F).

Mealiness incidence of inner canopy pears of the first harvest was negligibly small, whereas for harvest two there was a significantly higher mealiness incidence after 8 weeks of cold storage with 11 days of ripening (Fig. 1A, D). However, in comparison with the other fruit positions the mealiness incidence was considerably lower.

The mealiness incidence of all five fruit positions of both harvests started to decrease with prolonged cold storage at $-0.5\text{ }^{\circ}\text{C}$, although mealiness incidence still appeared for all fruit from different canopy positions and was quite high for harvest two after 11 days of ripening (Fig. 1C, F). For harvest one the mealiness incidence of middle-east and middle-west fruit was similar after 12 and 16w RA + 11d SL (Fig. 1B, C) whereas the outer canopy fruit of the first harvest exhibited a lower mealiness incidence after 16w RA + 11d SL than after 12w RA + 11d SL (Fig. 1B, C). For the second harvest the mealiness incidence after 12w RA + 11d SL and 16w RA + 11d SL was similar for four of the fruit canopy positions, except for the middle-east canopy fruit which had a significantly higher mealiness incidence after 16w RA + 11d SL than after 12w RA + 11d. (Fig. 1E, F).

The three different mealiness classes differed significantly ($Pr > 0.0001$) from one another in terms of juice mass and juice area (Table 1). Non-mealy flesh textured fruit released on average 0.092 and 0.120 mg more juice than partly mealy and mealy textured fruit, respectively. This was significantly more juice mass in non-mealy fruit than both mealy textures (Table 1). Like juice mass, the juice area (cm^2) of non-mealy textured fruit was significantly larger than that of partly mealy and mealy fruit (Table 1). Juice mass and juice

area were significantly the lowest for mealy textured fruit (Table 1). There was no significant interaction between fruit canopy position and harvest date on total mean juice mass and juice area for the three different mealiness classes (Table 2). Harvest date also did not have a significant effect on juice mass and juice area (Table 2). There were no statistical differences in juice mass and juice area of mealy fruit between the five respective fruit positions (Table 2). However, the non-mealy outer canopy pears of both sides of the tree exhibited significantly lower average juice mass and juice area than non-mealy middle canopy and inside fruit (Table 2). Partly mealy outer canopy fruit had a lower juice mass than middle-east and inner canopy fruit.

4.3.1.2 Ethylene production and respiration rate

The ethylene production rate for the fruit harvested at the first date was almost similar for all five fruit positions after 8 weeks of storage at -0.5 °C before ripening (Fig. 2A). After 8w RA + 4d SL, ethylene production was still very low for the five fruit positions. The middle canopy fruit ethylene production was significantly higher after 8w RA + 7d SL than that of the outer canopy and inner canopy fruit (Fig. 2A). Outside-east fruit ethylene production was also significantly lower after 8w RA + 11d SL compared to other fruit positions. Ethylene production of inner canopy fruit at 8w RA + 7d SL was also low but increased significantly from 8w RA + 7d SL to 8w RA + 11d SL even though it was lower compared to middle-east, middle-west and outside-west fruit (Fig. 2A). Ethylene production increased substantially after 12 weeks of cold storage with subsequent ripening (Fig. 2B). The highest ethylene maxima of harvest one fruit were achieved by outside-east fruit (12w RA + 4d SL), followed by middle-west (12w RA + 0d SL), outside-west (12w RA + 4d SL), middle-west (12w RA + 4d SL) and middle-east fruit (12w RA + 4d SL; Fig. 2B). Inner canopy fruit had the lowest ethylene maxima (12w RA + 7d SL; Fig. 2B). After 16 weeks of cold storage plus 11 days of ripening ethylene levels of all the fruit positions were similar to levels obtained after 8 weeks of storage plus ripening (Fig. 2C and 2A).

For harvest two, the ethylene production of inside fruit gradually increased as the ripening period increased after each of the three respective cold storage durations, except from 12w RA + 7d SL to 12w RA + 11d SL that showed a slight decline in ethylene production (Fig. 2D, E, F). Maximum ethylene production was already reached after 8w RA + 11d SL for inside fruit (Fig. 2D). The middle-west and middle-east fruit also exhibited a gradual increase in ethylene

production with ripening and the maxima production occurring after 12w RA + 7 and 11d SL, respectively (Fig. 2E). For harvest two, ethylene production decreased substantially from 12 weeks of storage with subsequent ripening to 16 weeks plus ripening (Fig. 2F). The inner canopy and middle-west fruit produced significantly higher levels after 16w RA + 11d SL than harvest one fruit at the same evaluation time (Fig. 2F).

Middle-west fruit, however, did reach a higher ethylene production maximum than the middle-east fruit, although it was not significantly different. Ethylene production of both outer canopy positions was unpredictable (Fig. 2D, E, F). The highest level of ethylene production was associated with outside-east fruit of the first harvest after 12w RA + 4d SL (Fig. 2B).

The interaction between fruit position x storage duration and ripening x harvest date did not have a significant influence on the average respiration rate (Fig. 3). However, a brief summary will follow.

The respiration rate of inner canopy fruit varied and consisted of two major peaks for both harvest maturities (Fig. 3B, D). Harvest two inside fruit respiration rate maxima were reached after 8w RA + 11d SL, which is earlier than the first harvest after 12w RA + 4d SL. However, the maxima of harvest one inside fruit were higher than that of harvest two (Fig. 3B, D). There was no clear respiration pattern obtained for the outside-east fruit gradually decreasing after 12 weeks of cold storage with ripening period. However, the outside-west fruit from harvest two exhibited a respiration peak after 8 weeks of cold storage plus 4 days of ripening, which is earlier than harvest one after 8w RA + 7d SL (Fig. 3A, D). For harvest one and harvest two, the respiration maxima of outside-west fruit was obtained at 12w RA + 0d SL and decreased with further cold storage and ripening (Fig. 3E, F).

The middle-west fruit achieved a respiration maximum after 12 weeks of cold storage for both harvest maturities (Fig. 3B, E). The respiration rate maxima were, however, higher for the second harvest date (12w RA + 7d SL; Fig. 3E).

The respiration pattern of middle-east fruit differed between the two harvest maturities. Harvest one had maxima already after 8 weeks of cold storage with 7 days of ripening, where after respiration decreased with further cold storage (Fig. 3A). In contrast, the respiration rate

of harvest two middle-east fruit increased with prolonged cold storage with maxima after 16 weeks of cold storage plus subsequent ripening (Fig. 3F).

The main effects did not have a significant effect on the respiration rate of the different fruit positions and different harvest dates (data not shown).

4.3.1.3 Blush colour and Fruit background colour

Blush colour did not differ between harvest dates (Table 3). As expected for both harvest dates, the outer canopy pears from both sides of the canopy had a significantly redder blush than the middle canopy and inside fruit. The blush colour did not differ significantly between middle-west and middle-east fruit at the time of the both harvest dates (Table 3).

Fruit associated with a mealy flesh texture had a significantly redder blush colour compared to that of partly mealy and non-mealy fruit at the time of fruit harvest. Non-mealy pears were significantly less red than partly mealy fruit (Table 4).

Outside-east fruit from the first harvest showed a significantly more yellow background colour compared to the other fruit positions from the same harvest (Table 5). Shaded inner canopy fruit exhibited a greener ground colour at harvest one, compared to outside-west, middle-east and outside-east fruit from the same harvest. The ground colour for the second harvest was similar for all five fruit positions (Table 5). The ground colour was significantly yellower for the outside-west, middle-west and inside fruit from harvest two compared to fruit from the first harvest, except for outside-east fruit. Outside-east fruit exhibited a significantly greener ground colour at the time of harvest two than at harvest one. No statistical difference was found between the two harvest dates for the middle-east fruit ($P > F = 0.1188$).

Background colour of fruit that were harvested after commercial maturity changed faster from green to a yellow colour during storage and shelf life compared to the fruit of the first harvest (Fig. 4). Fruit of the first harvest only developed the same yellow ground colour after 12 weeks of cold storage with 11 days of ripening (12w RA + 11d SL) compared to harvest two fruit after 8w RA + 11d SL (Fig. 4B, D). However, after 12w RA + 0d SL and 12w RA + 4d SL, harvest one fruit was more yellow compared to harvest two fruit at 12w RA + 4d SL (Fig. 4B, E). For harvest one, canopy position played a role after 12 weeks of cold storage and ripening, with inside canopy fruit being greener compared to the other fruit positions (Fig. 4B). For

harvest two, fruit yellowing for fruit from different positions did not seem to be as different after 12 weeks of cold storage with subsequent ripening, compared to harvest one (Fig. 4E). After 11 days of ripening, the yellowing of both harvest maturities and canopy positions did not differ, as all fruit exhibited a deep yellow ground colour (colour chart index of 4.0 to 4.5). After 16 weeks of storage the five fruit positions of harvest one exhibited a yellower background colour compared to harvest two fruit, although only middle-west and outside-east fruit was significantly yellower than the harvest two fruit (Fig.4C, F).

The background colour of non-mealy fruit was significantly greener than the ground colour of partly mealy and mealy fruit ($P_{r>F} = 0.0312$; Table 4). The latter two mealiness classes exhibited a similar background colour (Table 4).

4.3.1.4 Firmness

The firmness did not differ between harvest dates or canopy positions (Table 5). For the first harvest, after 8 weeks of cold storage with subsequent ripening, the firmness of outside-west and middle canopy fruit declined to under 3.5 kg, whereas inside and outer canopy east fruit firmness only declined after 12 weeks plus ripening, to below 3.5 kg (Fig. 5A, B). The harvest one outside-west fruit had a drastic drop in firmness from 8w RA + 7d SL to 8w RA + 11d SL (Fig. 5A). This was noted for middle-east and middle-west fruit as well, although to a lesser extent (Fig. 5A). The firmness of harvest two fruit already declined after 8w RA + 7d SL to below 3.5 kg (Fig. 5D). Unlike for harvest one fruit after 12 weeks of cold storage with subsequent ripening for 4 and 7d, the firmness of the different fruit positions of harvest two did not differ (Fig. 5B, E). All the fruit positions of harvest one experienced a drastic decrease in firmness from 12w RA + 0d SL to 12w RA + 4d SL, except for inside canopy fruit that remained firm (Fig. 5B).

Flesh firmness of harvest two fruit, except for inner canopy fruit, was softer after 4 days of shelf-life after 8 weeks of cold storage, compared to harvest one at the same time (Fig. 5A, D). Inner canopy harvest two fruit was softer after 8 weeks of cold storage and 7 days of ripening compared to harvest one inside fruit (Fig. 5A, D).

For harvest two, the eastern canopy fruit exhibited a softer flesh after 8w RA + 4d SL than the western canopy and inner canopy fruit (Fig. 5D). At 8w RA + 7d SL the firmness of the outer canopy fruit was the lowest, however only significant from the middle-west fruit (Fig. 5D). The

firmness of the outside-west and middle-west fruit of harvest one was significantly higher after 8w RA + 7d SL than the middle-east fruit. The outside-west fruit were significantly firmer ~~was~~ compared to outside-east fruit. However, after 8w RA + 11d SL the firmness of the different fruit positions changed drastically (Fig. 5A). Western outer canopy fruit had the lowest firmness, whilst outside-east and inside fruit exhibited the highest firmness at this time (Fig. 5A). The longer the storage (12 weeks compared to 8 weeks), the shorter the shelf-life period required to reach 2 kg (Fig. 5). Inside fruit from harvest one only reached 2 kg after 12w RA + 11d SL, which is unusual when harvested at an optimum firmness. For the second harvest the outside-west, middle-west and outside-east fruit had a significantly higher firmness after 16 weeks of cold storage than during the same time for harvest one fruit (Fig. 5C, F). At 16w RA + 11d SL, irrespective of harvest date, firmness decreased to below 2 kg (Fig. 5C, F).

Non-mealy pears (3.1 kg) were significantly firmer than partly mealy and mealy fruit (1.9 and 1.7 kg, respectively; Table 4). The mealy fruit were associated with a significantly lower firmness than partly mealy fruit. This indicates that partly mealy and mealy fruit undergo an advanced breakdown of internal cellular structures, which enhances mealiness incidence.

4.3.1.5 TSS and TA

At the time of harvest one, the outer and middle canopy fruit from the western side had significantly the highest TSS (Table 5). TSS of outside-east fruit of the first harvest was significantly higher than that of the middle-east fruit, while inner canopy fruit had the lowest TSS. The TSS of outside-west and outside-east fruit was significantly the highest at the time of harvest two, although the latter being non-significantly higher than middle-west fruit. At harvest two, middle-west fruit had a higher TSS than inside and middle-east fruit, however, these differences were not statistically significant between middle-west and inside fruit but middle-west and middle-east fruit did differ significantly (Table 5).

For harvest one, the TSS of only outside-west fruit increased with prolonged cold storage with subsequent ripening, whereas a decrease in TSS was observed for middle-east, middle-west and inside fruit (Fig. 6A-C). The outside-east fruit of harvest one exhibited a similar maximum TSS after 8 and 16 weeks of cold storage with subsequent ripening (Fig. 6A,C). The TSS of outer canopy pears from both sides of the tree from harvest two, showed a slight decrease from 8 to 16 weeks at $-0.5\text{ }^{\circ}\text{C}$ with subsequent ripening (Fig. 6D-F). The mid-east canopy fruit of

harvest two exhibited a slight increase in their TSS with prolonged cold storage, plus subsequent ripening, however, TSS was still lower than the outer canopy fruit (Fig. 6D-F). The TSS of harvest two middle-west fruit, like harvest one, also decreased with prolonged cold storage with subsequent ripening, whilst the TSS of the inner canopy fruit from the second harvest remained fairly unchanged after the different cold storage durations and ripening periods (Fig. 6D-F). For all five-fruit canopy positions the TSS in most cases were higher for harvest one fruit compared to harvest two fruit (Fig. 6A-F).

The TA at the time of harvest one was significantly the lowest for outside-east fruit (0.14%), while the other four positions had almost a similar TA (Table 5). At the time of harvest two, the TA of inner canopy fruit (0.25%) was significantly higher, compared to the outer canopy fruit and middle canopy fruit (Table 5). Outer canopy fruit exhibited significantly the lowest TA at the time of harvest two compared to the middle- and inner canopy fruit, except for outside-west fruit which did not differ significantly from the middle-east fruit (Table 5). The TA of inside fruit was significantly higher for harvest two than for harvest one ($P_{>F} = 0.0011$). The TA of the other fruit positions did not differ statistically between the harvest dates (Table 5).

For the first harvest the TA of all five fruit positions decreased with prolonged cold storage and ripening (Fig. 7A-F). In most cases the inner canopy pears exhibited higher TA levels than the outer canopy fruit, whilst middle canopy fruit, especially middle-west fruit, showed similar TA levels as inside fruit (Fig. 7A-C). A similar pattern was found for harvest two fruit, although greater differences occurred between the inside and outside fruit, with inner canopy fruit showing higher TA levels most of the time (Fig. 7D-F). The TA of outer canopy fruit from harvest two, already dropped to minimum levels after 8 weeks of cold storage with subsequent ripening, where after the TA remained unchanged with further cold storage and ripening (Fig. 7D-F).

4.3.1.6 Diameter, mass and length

Later harvested fruit were significantly larger, heavier and longer than harvest one fruit at each of the five respective fruit positions (Table 6). The outside-west fruit of harvest one were significantly the biggest, whilst the inner canopy fruit were significantly the smallest (Table 6). Diameter, mass and length did not differ statistically for outside-east and middle canopy fruit

of harvest one. The middle-east and middle-west fruit did not differ in size between harvest dates (Table 6).

For the second harvest, the average diameter and mass of outer canopy fruit were significantly the highest (Table 6). As with harvest one, harvest two inside fruit had significantly the smallest fruit size (diameter and mass). ~~An interesting finding was that~~ The diameter of inside fruit of harvest two did not differ statistically from harvest one outside-east and middle canopy fruit, while inner canopy fruit from harvest two were significantly heavier and longer compared to outside-east and middle canopy fruit from harvest one. The outer canopy fruit from both sides of the tree, as well as the middle-east fruit were significantly longer than the inside fruit, for the second harvest date. The middle-west fruit did not differ statistically in length from the inside fruit, as well as from the outer canopy and middle-east fruit (Table 6).

The average diameter, mass and fruit length of partly mealy and mealy textured fruit did not differ statistically ($Pr > F = 0.8688, 0.9974$ and 0.5654 , respectively). However, non-mealy fruit were significantly smaller in both length and diameter than partly mealy and mealy fruit and also significantly lighter (Table 4).

4.3.1.7 Seed count (normal and aborted)

The number of normal seeds present in fruit did not differ for the two harvest maturities in each of the five respective fruit positions ($Pr > F = 0.3163$; Table 7). The outer canopy fruit from both sides of the tree contained on average significantly more normal (viable) seeds than the other fruit positions (Table 7). The number of normal seeds present in middle-west fruit were significantly more than inner canopy fruit. The outside fruit had a less than average number of normal seeds e.g. an outer canopy fruit contained only one normal seed on average, whilst in 100 fruit only 57 middle-west-, 35 inside- and 41 middle-east fruit had one normal seed (Table 7).

The number of aborted seeds present in fruit did not differ statistically for the two harvest maturities for each of the five respective fruit positions ($Pr > F = 0.2447$; Table 7). The western and eastern outer canopy fruit had significantly less aborted seeds on average, compared to the other positions (Table 7).

Non-mealy, partly mealy and mealy textured fruit did not differ statistically from one another with regard to the average number of normal and aborted seeds present in the fruit ($P_{r>F} = 0.0732$ and 0.0841 ; Texture Main effect, respectively) (Table 4).

4.3.2 2017 season

4.3.2.1 Mealy texture and juiciness evaluation

For both harvest maturities, mealiness incidence of all three fruit positions decreased with prolonged cold storage (from 8 to 16 weeks of cold storage plus ripening). However, mealiness incidence was still quite high after 12 weeks of cold storage with subsequent ripening (Fig. 8B, E). For harvest two, the mealiness incidence of outside-west fruit was higher after 16 weeks of cold storage plus 11 days of ripening than the highest mealiness incidence obtained for the outside-west fruit of the first harvest (12w RA + 4d SL; Fig. 8B, F). The outside-west and middle-west fruit of harvest one exhibited a higher mealiness incidence after 16w RA + 11d SL than the inside fruit at the same time, although non-significant (Fig. 8C).

The mealiness incidence of harvest two outside-west fruit developed slightly earlier (after 8w RA + 4d SL) compared to harvest one fruit (after 8w RA + 7d SL). However, mealiness incidence of harvest two outside-west fruit increased rapidly after 8 weeks of cold storage with subsequent ripening and peaked at 8w RA + 11d SL (Fig. 8D). The maximum mealiness incidence of harvest two outside-west fruit was significantly higher than that of harvest one outside-west fruit at the respective cold storage durations with subsequent ripening.

Mealiness developed earlier for harvest two middle-west fruit than for harvest one middle-west fruit, seeing as the mealiness incidence of harvest two, middle-west fruit was higher after 8w RA + 4d SL and 8w RA + 7d SL compared to harvest one mid-west fruit. However, a similar incidence was obtained after 8w RA + 11d SL. For the second harvest, mealiness incidence decreased with further cold storage and ripening, whereas middle-west fruit from the first harvest reached a similar mealiness incidence after 12w RA + 4d SL compared to 8w RA + 11d SL (Fig. 8B). The middle-west and outside-west fruit of the second harvest exhibited a similar mealiness incidence pattern, although the mealiness maxima of outside-west fruit was significantly higher (Fig. 8D, E, F).

For both harvests, mealiness incidence of inside fruit increased slightly after 8 weeks of cold storage plus subsequent ripening, where after mealiness almost disappeared completely with

further cold storage for harvest one inside fruit (Fig. 8A-F). In contrast, harvest two inside pears were at their most susceptible for mealiness development after 12 weeks of cold storage plus subsequent ripening, with a maximum incidence after 12w RA + 7d SL, which is similar to that of the western outer canopy pears (Fig. 8E). After 16 weeks of cold storage plus subsequent ripening the harvest two inside fruit, as found for harvest one, showed virtually no mealiness incidence (Fig. 8C, F).

The three different mealiness classes evaluated by the trained panel showed a significant difference regarding juice mass and juice area (Table 8). The average juice mass and juice area of non-mealy fruit was significantly higher than partly mealy and mealy fruit (Table 8). Partly mealy fruit had a significantly higher juice mass and juice area than mealy fruit (Table 8). The interaction between fruit canopy position and harvest date was not significant for average juice mass (Table 9A). However, the average juice area of the three different mealiness classes differed significantly between canopy positions (Table 9A). Non-mealy and partly mealy outside-west fruit had a significantly lower juice area than middle-west and shaded inner canopy fruit whilst mealy textured outside-west fruit exhibited a significantly lower juice area than mealy inside fruit (Table 9A).

Harvest date had a significant effect on the average juice mass and juice area of non-mealy and partly mealy textured fruit (Table 9B). Non-mealy fruit of the first harvest date had a significantly higher juice mass and juice area compared to non-mealy harvest two fruit (Table 9B). The partly mealy fruit of the first harvest had a significantly higher juice mass and juice area than partly mealy fruit of the second harvest (Table 9B). Harvest date did not affect the juice mass or juice area of mealy fruit (Table 9B).

4.3.2.2 Ethylene production and respiration rate

A minimum of 12 weeks at $-0.5\text{ }^{\circ}\text{C}$ under RA was needed for maximum ethylene production for all three fruit positions of harvest one (Fig. 9A, B, C). Ethylene production of all three fruit positions was similar after 8 weeks of cold storage, and subsequently only after 11 days of ripening was ethylene production substantially higher for the inside fruit compared to that of the outside-west and middle-west fruit (Fig. 9A). Whereas, after 12 weeks of cold storage, the ethylene production was significantly higher for inner canopy fruit than for the outside-west fruit after 12w RA + 4d SL until 12w RA + 7d SL, and the same was found after 16 weeks of cold storage plus 4, 7 and 11 days of ripening (Fig. 9B, C). For harvest one, in most cases,

middle canopy west fruit exhibited higher ethylene production than the outside-west fruit, although not always significantly higher.

The ethylene production of harvest two fruit after 8, 12 and 16 weeks of cold storage with 11 days of ripening, was higher for the inner canopy pears compared to the outside-west fruit (Fig. 9D, E, F).

Fruit harvested at post-commercial maturity, needed 16 weeks at $-0.5\text{ }^{\circ}\text{C}$ to produce maximum ethylene levels, except the inner canopy fruit that already produced their maximum level after 12w RA + 11d SL that was significantly lower than the ethylene maximums reached of harvest one fruit after only 12 weeks of cold storage with subsequent ripening (Fig. 9B, F). Ethylene production of harvest two outside-west, middle-west and inner canopy fruit increased after 8w RA + 7d SL, which is slightly earlier than that of harvest one after 8w RA + 11d SL. (Fig. 9A, D).

For harvest one, the respiration rate of all three fruit positions fluctuated more between the different cold storage durations and ripening periods compared to that of the fruit of the second harvest (Fig. 10A-F). The respiration rate for outside-west fruit from both harvest maturities was the highest after 8 weeks of cold storage plus subsequent ripening, however, harvest two fruit achieved a lower rate (Fig. 10B, E). With further cold storage and subsequent ripening, the respiration rate of harvest two outside-west fruit stayed constant. The same pattern was found for middle-west and inside fruit of harvest two, although at a higher rate. In fact, the respiration rate was, in most cases, substantially higher for inner canopy and middle canopy fruit. For harvest two the respiration rate was only significantly higher after 8w RA + 0d SL, 16w RA + 0d SL and 16w RA + 11d SL for inner canopy and only after 8w RA + 11d SL and 16w RA + 11d SL for middle-west fruit compared to the outside-west fruit (Fig. 10D-F). Middle-west canopy and inner canopy pears harvested at commercial maturity showed a different respiration pattern to the outside-west fruit (Fig. 10A-C). Maximum respiration rate was achieved after 12w RA + 7d SL and 12w RA + 11d SL for harvest one middle-west and inside fruit, respectively, which levels harvest one outside-west fruit never reached (Fig. 10B). However, as seen in harvest two, the respiration rate of harvest one was in most cases higher for middle-west (after 8w RA + 7d SL, 12w RA + 4d SL, 12w RA + 7d SL, 16w RA + 0d SL and 16w RA + 7d SL) and inside fruit (after 12w RA + 4d SL, 12w RA + 11d SL, 16w RA + 0d SL and 16w RA + 7d SL) (Fig. 10D-F).

4.3.2.3 Hue angle and blush colour

The interaction between fruit position and harvest date did not have a significant effect on the hue angle and colour chart values at the time of the two harvest dates (Table 10A). The outside west fruit exhibited significantly the reddest blush, followed by middle west fruit. The inner canopy fruit did not develop any red blush (Table 10A). Harvest date did not affect blush colour.

Hue angle and blush colour chart (Pl. 16) value of the blushed side of mealy textured fruit was in general, significantly lower, indicating that these fruits were redder compared to partly mealy and non-mealy fruit (Table 11). Non-mealy fruit were significantly less red than partly mealy fruit (Table 11).

4.3.2.4 Fruit background colour

Except for outside west and inner canopy harvest two fruit which were significantly more yellow, background colour of other positions at both harvests did not differ significantly (Table 10B). In contrast to the above, the interaction between fruit position and harvest date was not significant for the average hue angle of the green background colour. The outside west fruit exhibited a significantly yellower ground colour (lower hue value) than the inside fruit (Table 10B) but did not differ significantly from the middle west fruit.

According to background colour chart values, harvest two fruit of all three positions had a slightly earlier transition from green to a more yellowish ground colour (after 8w RA + 4d SL), whilst harvest one fruit started to change colour only after 8w RA + 7d SL (Fig. 11A. A, D). This was less evident when considering changes in hue angle (Fig. 11B. A, D). The outside west fruit from both harvest maturities seemed to change slightly earlier from green to a more yellow ground colour compared to the middle west and inside fruit.

For both harvest maturities outside west fruit developed a significantly deeper yellow ground colour after 8+11 and 12+11, compared to middle west canopy fruit (Fig. 11A. A-D). However, no significant differences were found with the hue angle (Fig 11B. A-D). The outside west and inside fruit of the first harvest exhibited a similar deep yellow colour after 8+11, 12+11 and 16+11 (Fig 11A. A-D and 11B. A-D), whereas for harvest two the outside west fruit exhibited a significantly yellower ground colour after 8+11 and 16+11, compared to that of the inner canopy pears (Fig. 11A. D-F). The latter is not in agreement with the hue angle results (Fig.

11B. D-F). The degree of yellowing, measured by the colour chart index, of middle west and inside fruit of harvest two was similar after 11 days of ripening after each of the three respective cold storage durations (8, 12 and 16 weeks of storage) (Fig. 11A. D, E, F). The same was found with the hue angle; however, harvest two inside fruit were significantly yellower after 12+11 than middle west fruit according to the hue angle (Fig 11B. E).

Non-mealy pears exhibited a significantly greener background colour (according to both colour chart and hue angle) compared to fruit with a mealy or partly mealy texture (Table 11) whilst the mealy textured fruit had a significantly yellower ground colour than the partly mealy fruit (Table 11).

4.3.2.5 Firmness

The average firmness at the time of harvest was not significantly influenced by the interaction between fruit canopy position and harvest date (Table 12A). However, harvest two fruit were significantly less firm than harvest one fruit at the time of harvest (Table 12A). Firmness did not differ between canopy positions.

For both harvest maturities, the firmness of canopy positions varied most after 8, 12 and 16 weeks of cold storage with 4 days of ripening, (Fig 12 A-F) with the firmness of outside west fruit significantly lower compared to inside fruit except for harvest 1, 8+4 (Fig. 12A, B, C). The firmness of outside west fruit from harvest two was significantly lower after 4 days of ripening after each cold storage duration than the middle west and inside fruit (Fig. 12D, E, F). Middle west fruit exhibited a lower firmness than the inner canopy fruit after 4 days of ripening, but the difference was only significant for 12w + 4d (Fig. 12D, E, F). The firmness of all three positions of harvest two declined slightly earlier to below 3.5 kg (after 8+4) compared to harvest one fruit (after 8+7) (Fig. 12A, D). For both harvest maturities the firmness of all three fruit positions declined to below 2 kg after 11 days of ripening following 8, 12 and 16 weeks of cold storage (Fig. 12A-F).

The average flesh firmness of 3.6 kg of non-mealy fruit was significantly higher than that of partly mealy and mealy fruit (2.1 and 1.6 kg, respectively; Table 11), while the firmness of partly mealy fruit was significantly firmer than mealy textured fruit (Table 11).

4.3.2.6 TSS and TA

The interaction between fruit canopy positions and at the time of harvest for average TSS was not significant (Table 12A). Harvest date and fruit position did, however, have a significant effect on the average TSS at the time of harvest. The TSS of fruit at the time of the second harvest date was significantly higher compared to the first harvest (Table 12A). The outer west canopy fruit exhibited a significantly higher TSS at the time of harvest than middle west and inside fruit and the latter had significantly the lowest TSS (Table 12B).

The TSS of harvest two outside west, middle west and inside fruit increased with prolonged cold storage plus subsequent ripening, whereas not the same was found for harvest one fruit (Fig. 13A-F). The TSS of harvest one outside west fruit was already at 15.0 (%) after 8+4, whereas outside west fruit of harvest two only reached similar levels at 12 weeks at -0.5 °C plus subsequent ripening (Fig. 13A, E).

The harvest one inside fruit TSS levels did not differ after 8 and 12 weeks of cold storage plus subsequent ripening, from the inside fruit of the second harvest (Fig. 13A, B, D, E). However, after 16 weeks with subsequent ripening the TSS of harvest two inside fruit was much higher than that of the harvest one inside fruit (Fig. 13C, F).

The TSS of all three fruit positions increased in most cases with more days at 20 °C, peaking mostly after 7 and/or 11d days of ripening, although there was not a clear pattern for all the positions (Fig. 13A-F). The TSS of outside west fruit of both harvest dates after 12+0 was similar to the TSS levels obtained with 4, 7 and 11 ripening (Fig. 13B, E). For both harvest maturities outside west fruit reached significantly higher TSS peaks than middle west and inner canopy fruit, whilst the middle west fruit exhibited significantly higher TSS peaks than the inside fruit (Fig. 13A-F).

There was no statistically significant interaction between fruit canopy position and time of harvest on the average TA (Table 12A). The TA of fruit at the time of the second harvest was, however, significantly lower compared to at the time of the first harvest date (Table 12A). At the time of harvest the average TA was significantly the lowest for outside-west fruit, with TA being significantly the highest for shaded inner canopy fruit (Table 12B).

The TA of harvest one outside west fruit decreased gradually with longer cold storage (Fig. 14A-C). In contrast, harvest two outside west fruit showed a drastic decrease in TA levels

already after 8 weeks of cold storage plus subsequent ripening, after which the TA remained fairly constant with further cold storage and ripening (Fig. 14D, E, F). The TA of harvest two outside west fruit was generally lower than that of harvest one outside west fruit (Fig. 14A-F). The inner canopy pears of harvest two had significantly higher TA levels than outside west fruit during each of the evaluation times (Fig. 14D, E, F). The TA of the middle west fruit corresponded at times with that of outside west fruit and other times again with inner canopy fruit (Fig. 14D, E, F).

4.3.2.7 Diameter, mass and length

For both harvest maturities, western outer canopy pears were significantly bigger in diameter and greater in mass than the middle west and inside fruit (Table 13). The average diameter and mass of outside west fruit of harvest one and harvest two did not differ significantly. In contrast, the middle west and inner canopy fruit were significantly bigger in diameter and heavier for harvest two compared to harvest one fruit.

The diameter and mass of middle west fruit were significantly higher compared to inside fruit, for each of the respective harvests, however, the inner canopy fruit from harvest two exhibited a similar diameter and mass than for harvest one middle west fruit (Table 13).

The outside west fruit from the first harvest was significantly the longest compared to the other two positions from either harvest (Table 13). The middle west and inside fruit exhibited a similar length for the respective harvests. Fruit harvested after the commercial harvest date had a similar length (Table 13). The average length of middle west and inside fruit from harvest two was significantly longer than for harvest one fruit, for the respective fruit positions (Table 13). In contrast, harvest one outside west fruit were significantly longer than harvest two outside west fruit ($Pr > F = 0.0126$).

Mealy textured fruit were significantly bigger and heavier compared to non-mealy and partly mealy fruit, but fruit length did not differ between the mealiness categories (Table 11). Partly mealy fruit were bigger and heavier than non-mealy fruit.

4.3.2.8 Seed count (normal and aborted)

The number of normal seeds (viable seeds) did not differ between the two harvest dates (Table 14). The average number of normal seeds was significantly higher for outside west fruit compared to middle west and inside fruit.

The inner canopy fruit had significantly the lowest number of normal seeds (Table 14). The number of aborted seeds present in fruit did not differ statistically between the two harvest dates (Table 14). The outer canopy west fruit contained on average significantly fewer aborted seeds compared to the other two positions (Table 14).

The average number of viable and aborted seeds present in non-mealy, partly mealy or mealy fruit did not differ significantly (Table 11).

4.4 DISCUSSION

In 2016 and 2017, post-commercial maturity harvested 'Forelle' pear fruit seemed to be more inclined to mealiness development following cold storage and ripening (Fig. 1 and 8). Fruit position within the tree canopy did have a noticeable effect on 'Forelle' pear mealiness development, with red blushed outer canopy fruit being more susceptible to the development of a mealy texture, compared to middle canopy fruit and shaded inner canopy fruit (Fig. 1 and 8).

For the 2017 season, outer canopy fruit harvested at post-commercial maturity were in a more advanced stage of maturity than shaded inner canopy fruit. These outside canopy fruit exhibited in general a more yellow background colour, lower flesh firmness (only after ripening), higher TSS, lower TA, as well as a lower level of ethylene production and respiration rate compared to that of post-commercial harvested inside fruit (Fig. 11A, B; 12; 13; 14; 9 and 10, respectively). Ripening of pears is associated with a decrease in firmness, a change from green to a more yellow ground colour, and a climacteric rise in ethylene production (Chen and Mellenthin, 1981; Chen et al., 1983; Martin, 2002).

This could be an indication that outer canopy fruit were already at commercial harvest maturity in a more advanced stage of maturity, compared to the inner canopy fruit. The change in background colour, firmness, TSS and TA happened faster for the outer canopy fruit. The difference in maturity indices is just smaller between fruit harvested at commercial maturity, but there could probably already be a difference in internal cellular structures and fruit metabolism between outside and inside fruit. However, similar differences did not occur in 2016 between post-optimum harvested outside and inside fruit. This could be ascribed to the two harvest dates that were too close together and to flesh firmness that was slightly

higher at the time of harvest two than at the time of the first harvest date in each of the respective fruit positions (Table 6 and Fig. 5). It is known that the winter pears harvested at an advanced stage of maturity are more susceptible to develop a coarse texture, and they have a shorter post-harvest life (Hansen and Mellenthin, 1979).

Some maturity indices of middle canopy fruit were similar to outside fruit, whilst others were similar to inside fruit. This indicates that the stage of maturity was less advanced for middle canopy fruit compared to outside fruit, but that they were in a more advanced stage of maturity than the inside fruit. Further discussion will mainly focus on the differences in fruit parameters between outside fruit and inner canopy fruit.

The stage of fruit maturity at the time of harvest could influence the rate at which fruit softening takes place (Chen and Mellenthin, 1981). According to studies by Murayama et al. (1998) and Peirs et al. (2001), faster softening of fruit causes faster development of a mealy texture. According to the results obtained in this study, it seems as if ripening rate of outer canopy fruit developed earlier than for inside fruit, irrespective of harvest maturity with regard to firmness and change in background colour. For the 2016 season the firmness of inside fruit harvested at either maturity took longer to decline to below 3.5 kg ('edible firmness for soft pears') than the outer canopy fruit (Fig. 5). The same pattern did not occur for the 2017 season, although the firmness of the outer canopy fruit was, in most cases, lower at each of the respective evaluation days, irrespective of harvest maturity (Fig. 12). The difference in ripening rate of fruit can be further seen with outside-east fruit of the 2016 season, which exhibited a different pattern of mealiness development during ripening compared to outside-west fruit of the first harvest date (Fig. 1). Mealiness of harvest one outside-east fruit, increased significantly after 8 to 12 weeks of cold storage with subsequent ripening. This could possibly be associated with heightened ethylene production observed after 12 weeks of cold storage for the outside-east fruit (Fig. 2). Ethylene is needed for the initiating of the softening process of fruit (Hiwasa et al., 2003). The result possibly indicates that outside-east fruit were riper during harvest two than for harvest one, due to mealiness that already occurred after 8 weeks of cold storage plus subsequent ripening. Although this may be true for outside-east fruit, ethylene production for outside-west fruit during both seasons, also increased after 12 weeks of cold storage plus further ripening. However, mealiness levels were already high after 8 weeks of ripening (Fig. 1). Low levels of ethylene

must have been sufficient for fruit to soften and mealiness to be measured. In addition, mealiness may not be directly linked to ethylene production, as ethylene levels of inside fruit in many cases were higher during parts of the evaluation time for both seasons (Fig. 2 and 9), and yet they never got as mealy as the outside fruit (for both seasons). This occurrence indicates that the sensitivity of fruit to ethylene from different canopy positions may differ depending on whether the fruit have the ability to ripen. This agrees with several other studies, where fruit softening is already induced before the climacteric rises of ethylene and respiration (Du Toit et al., 2001; Wang et al., 1972 (pear)). Variations in climacteric ethylene production and respiration rate can be induced by several factors, including environmental factors during fruit development and the physiological age of fruit at the time of harvest (Nordey et al., 2016). The study by Mellenthin and Wang (1976) reported that 'd' Anjou' pears which received higher daily hourly average (DHA) temperatures six weeks prior to harvest, showed a much lower ethylene production and respiration rate than fruit with lower DHA temperatures and which gave no indication of a climacteric rise. They found that pears which experienced higher DHA, failed to ripen properly and they were of a lower quality. It agrees with our results obtained in the 2017 season that, in general, the shaded inner canopy fruit exhibited higher ethylene production and respiration rates than outside fruit (Fig. 9 and 10), and they possessed a better internal quality in terms of mealiness incidence and juiciness (Fig. 8 and Table 10A). However, the fruit from different canopy positions in this study did ripen to the same extent.

The manner whereby fruit from different canopy positions ripen can differ. This is confirmed seeing that the flesh firmness of the different fruit canopy positions was the same in most cases, but at the same time showed significant differences in mealiness. This is possibly an indication that prior to ripening, fruit already have a predisposition to undergo normal ripening, and that only slight changes in fruit metabolism are required for the development of mealiness. This can be seen with outer canopy pears of both seasons associated with a non-mealy texture having a lower juice mass and juice area than non-mealy textured inside and middle canopy fruit (Table 2 and 10A). These results agree with Delaire et al. (2015) who reported that apple fruit may have similar fruit size and fresh mass but may differ in their growth pattern during the cell division and cell elongation phases, which may potentially influence the textural fruit quality during ripening. Fruit ripening is associated with processes

that are dependent on hormones and are catalysed in a controlled manner by various endogenous disassembly enzymes (Cantu et al., 2008). Consequently, high internal flesh temperatures and large temperature fluctuations during the growing season that outer canopy fruit experience (as reported in chapter two), could possibly cause alterations in the activity of several pre- and post-harvest enzymes resulting in changed cell wall degradation, such as advanced breakdown of the middle lamella. This may relate to the higher susceptibility of outer canopy fruit to develop a dry, mealy texture. The mechanism of 'Forelle' mealiness development entails the collapsing of cells in addition to extensive cell-to-cell debonding (Muziri et al., 2016; Crouch, 2011). This is due to the weaker strength of the middle lamella compared to the cell wall ((Yamaki et al., 1983 (pear); Harker and Hallett, 1992 (pear); Crouch, 2011; Muziri, 2016)), resulting in limited release of juice because of a lack of cell rupture (Harker and Hallett, 1992).

Several factors have an influence on fruit flesh texture, of which size and shape of cells, strength and thickness of the cell wall, size of intercellular spaces (Harker et al., 1997; Muziri, 2016), as well as cell-to-cell adhesion (Fischer and Bennett, 1991) and cell turgor (Harker and Sutherland, 1993; Ilker and Szczesniak, 1990) are the most important. All these different components evolve/change during fruit growth and post-harvest storage and thereby influence fruit textural quality (Delaire et al., 2015). De Smedt et al. (1998) associated mealiness in apples with bigger sized fruit coupled with larger sized cells and intercellular spaces (larger pore size). Muziri et al. (2016) found the same with 'Forelle' pear fruit. Fruit size seems to play a role in 'Forelle' mealiness development in both seasons (Table 6 and 13). However, harvest two inside fruit were significantly bigger than harvest one outside east fruit of the 2016 season (Table 6) but exhibited less mealiness (Fig. 1). This indicates that internal fruit factors/cellular structures related to fruit canopy position, prior to ripening, play a meaningful role in mealiness development.

For 2016 and 2017 season, independent of harvest maturity, mealiness incidence of fruit started to decrease with prolonged cold storage at $-0.5\text{ }^{\circ}\text{C}$ (Fig. 1 and 8). Crouch (2011) reported similar results by showing that mealiness development of 'Forelle' pears decreased with an extended cold storage period prior to ripening. Muziri (2016), Carmichael (2011) and Crouch (2011) suggested a decrease in 'Forelle' mealiness with a longer storage at $-0.5\text{ }^{\circ}\text{C}$, could be attributed to cell wall degrading enzymes that are less active during ripening,

resulting in cell walls that break during mastication which causes the release of cellular components (juice). Surprisingly, outer canopy and middle canopy fruit in our study showed an increase in mealiness incidence after 16w RA + 11d SL (Fig. 1 and 8). This is an indication that perhaps a longer cold storage was needed in this instance but that in other fruit positions this prolonged cold storage had the potential to reduce the potential of Mealiness development during ripening.

4.5 CONCLUSION

According to the results obtained in this study the harvest of 'Forelle' pears at their commercial maturity, is an important protocol to follow since later harvesting increases the susceptibility of fruit for mealiness development. The mandatory 12-week cold storage period to minimize 'Forelle' mealiness development agrees with our results. Although eight weeks at -0.5 °C was sufficient to induce 'Forelle' ripening, it was associated with the highest mealiness incidence. Interestingly, however, 16 weeks at low temperature was not sufficient in reducing mealiness incidence of outer canopy and middle canopy fruit during ripening. Mealy textured pears were generally associated with a redder blush colour, yellower ground colour, bigger size and lower firmness.

Mealiness may not be directly linked to ethylene production as ethylene production rates of inside fruit were in many cases higher during periods of the evaluation time, and yet they never got as mealy as the outside fruit (in both seasons). To determine whether mealiness development is linked to ethylene production, it is suggested that ethylene production should already be measured at the time of harvest and thereafter at one-week intervals of cold storage and ripening. Internal ethylene could be measured per individual fruit and linked to mealiness. However, this procedure is a destructive analysis replacing air spaces with water making the measurement of juice mass and area as well as sensory evaluation impossible.

Fruit canopy position and harvest maturity did not influence ripening potential, since firmness declined to the same extent and a similar deep yellow fruit background colour developed, irrespective of fruit position and harvest maturity. However, the ripening rate developed earlier for outer canopy fruit than for the inner canopy fruit. This possibly indicates that outside fruit may be in a more advanced stage of maturity, as well as that the sensitivity may differ depending on whether the fruit have the ability to undergo ripening. The outer canopy

red blushed pears could already be in an advanced stage of maturity at the commercial harvest date, causing outside fruit to be harvested possibly a week or two earlier. The significantly higher mealiness incidence of outer canopy fruit compared to that of inside fruit with the same ripening potential, possibly indicates that the way that the cell wall develops and degrades during ripening, might differ amongst the different fruit positions. The varying environmental conditions, particularly irradiation coupled with fruit temperature that the different fruit positions experience, could affect various fruit development processes. Consequently, these changes could influence internal cellular structures that may affect the texture of the fruit. Mealy fruit were larger possibly indicating that the same number of cells in a fruit grow larger but affecting the cell-to cell bonding and hence developing large cellular pores as fruit size increases. This could later possibly result in cell sliding whilst ripening if fruit were not stored long enough. In conclusion, outside canopy fruit are possibly more prone to mealiness due to a combination of structural canopy differences and harvest maturity advancing these structural characteristics while on the tree.

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4.7 TABLES AND FIGURES

Table 1: Combined average juice mass (mg) of different mealiness classes of 'Forelle' after 8, 12 and 16 weeks of cold storage in regular atmosphere (RA) at -0.5 °C with 0, 4, 7 and 11 days of ripening at 20 °C (8w, 12w and 16w RA + 0d, 4d, 7d and 11d SL). Fruit were harvested in 2016 at commercial maturity and a week after the commercial harvest date on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

Mealiness class	Mean juice mass (mg)	Mean juice area (cm²)
Non-mealy (0)	0.153 a ²	11.375 a
Partly mealy (1)	0.061 b	8.413 b
Mealy (2)	0.033 c	3.121 c
Source of variation:	Pr>F	
Mealiness class	0.0001	0.0001

²Mealiness class mean followed by the same letter are not significantly different at 5% level (LSD)

Table 2: Effect of fruit canopy position on 'Forelle' pear average juice mass and juice area of different mealiness classes after 8w, 12w and 16w storage at -0.5 °C RA + 4d, 7d and 11d shelf-life at 20 °C. Fruit were harvested in 2016 at commercial maturity and a week after the commercial harvest date on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

Fruit position	Mealiness class					
	Non-mealy (0)		Partly mealy (1)		Mealy (2)	
	Juice mass (mg)	Juice area (cm ²)	Juice mass (mg)	Juice area (cm ²)	Juice mass (mg)	Juice area (cm ²)
Outside-west	0.142 b ^z	10.748 b	0.056 b	8.239 ^{NS}	0.033 ^{NS}	3.016 ^{NS}
Middle-west	0.161 a	11.730 a	0.061 ab	8.516 ^{NS}	0.034 ^{NS}	3.504 ^{NS}
Inside	0.161 a	11.617 a	0.071 a	8.378 ^{NS}	0.038 ^{NS}	3.308 ^{NS}
Middle-east	0.156 a	11.787 a	0.066 a	8.574 ^{NS}	0.035 ^{NS}	3.224 ^{NS}
Outside-east	0.143 b	10.834 b	0.055 b	8.369 ^{NS}	0.030 ^{NS}	2.878 ^{NS}
Source of variation:	Pr>F					
Position	0.0001	0.0001	0.0143	0.0790	0.5312	0.0620
Harvest	0.0838	0.0909	0.3077	0.1539	0.0502	0.0725
Position*Harvest	0.9730	0.3974	0.1646	0.7273	0.4634	0.7046

*NS = Non-significant

^zFruit canopy position means in the same column followed by the same letter are not significantly different at 5% level (LSD).

Table 3: Effect of fruit canopy position on 'Forelle' pear average blush colour at the time of the first harvest (commercial maturity) and second harvest date (post-commercial maturity). Fruit were harvested in 2016 at commercial maturity and a week later (post-optimum) on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

Fruit canopy position	Blush colour (chart index)^x
Outside-west	1.6 c ^z
Middle-west	7.7 b
Inside	12.0 a
Middle-east	7.8 b
Outside-east	1.6 c
Source of variation:	Pr>F
Position	0.0001
Harvest	0.7909
Position*Harvest	0.4816

^zFruit canopy position mean followed by the same letter are not significantly different at 5% level (LSD).

^xChart values 1-12: where 1=red; 12=green.

Table 4: Combined average (harvest one and two) blush colour, background colour, firmness, diameter, mass, length, number of normal (viable) seeds and aborted seeds of mealy, partly mealy and non-mealy 'Forelle' pears after 8, 12 and 16 weeks of cold storage at -0.5 °C plus 0, 4, 7 and 11 days of ripening at 20 °C. Fruit were harvested in 2016 at commercial- and post-commercial maturity on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa. The combined average firmness of the three different mealiness classes were determined after 8w RA + 11d SL (harvest one); 8w RA + 4d, 7d, 11d SL (harvest two); 12w RA + 4d, 7d, 11d SL (both harvests) and 16w RA + 11d SL (both harvests). The evaluation times were left out in cases where the firmness was above 4 kg for all five fruit positions, because fruit are more prone to mealiness development after ripening below 4 kg (Crouch et al., 2005).

Mealiness class	Blush colour (chart index) ^x	Ground colour (chart index) ^y	Firmness (kg)	Diameter (mm)	Mass (g)	Length (mm)	Normal seeds	Aborted seeds
Mealy	3.9 c ^z	4.2 a	1.7 c	66.2 a	182.3 a	89.0 a	0.73 ^{*NS}	9.24 ^{NS}
Partly mealy	4.6 b	4.1 a	1.9 b	65.9 a	182.3 a	89.5 a	0.82 ^{NS}	9.13 ^{NS}
Non-mealy	6.9 a	3.2 b	3.1 a	63.8 b	165.3 b	87.1 b	0.65 ^{NS}	9.33 ^{NS}
Source of variation	Pr>F							
Mealiness class	0.0001	0.0312	0.0001	0.0001	0.0001	0.0001	0.0732	0.0841

*NS = Non-significant

^zMealiness class means in the same column followed by the same letter are not significantly different at 5% level (LSD)

^xChart values 1-12: where 1=red; 12=green.

^yChart values 0.5-5: where 0.5= green; 5= pale green/ yellow

Table 5: Effect of fruit canopy position and harvest maturity on 'Forelle' pear average ground colour, firmness, TSS and TA at the time of the first harvest (commercial maturity) and second harvest date (post-commercial maturity). Fruit were harvested in 2016 at commercial maturity and a week later (post-optimum) on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

Fruit canopy position	Harvest	Ground colour (chart index) ^x	Firmness (kg)	TSS (%)	TA (% malic acid)
Outside-west	1	2.3 cd ^z	5.9 ^{*NS}	15.0 a ^z	0.19 cde
Outside-west	2	2.9 ab	6.3 ^{NS}	14.9 ab	0.17 de
Middle-west	1	2.2 de	5.9 ^{NS}	14.9 a	0.21 bc
Middle-west	2	2.6 bc	6.1 ^{NS}	14.0 cd	0.22 b
Inside	1	1.9 e	5.7 ^{NS}	11.4 f	0.20 bcd
Inside	2	2.7 b	6.1 ^{NS}	13.5 de	0.25 a
Middle-east	1	2.3 cd	5.8 ^{NS}	13.4 de	0.20 bc
Middle-east	2	2.6 bc	5.8 ^{NS}	13.2 e	0.19 cd
Outside-east	1	3.1 a	6.0 ^{NS}	14.3 bc	0.14 f
Outside-east	2	2.8 b	6.1 ^{NS}	14.7 ab	0.16 ef
Source of variation			Pr>F		
Position		0.0004	0.1913	0.0001	0.0001
Harvest		0.0002	0.2471	0.0690	0.0025
Position*Harvest		0.0006	0.4542	0.0001	0.0017

*NS= Non-significant

^zFruit canopy position means in the same column followed by the same letter are not significantly different at 5% level (LSD).

^xChart values 0.5-5: where 0.5= green; 5= pale green/ yellow

Table 6: Effect of fruit canopy position and harvest maturity on 'Forelle' pear average diameter, mass and length after 8w, 12w and 16w storage at -0.5 °C RA + 0d, 4d, 7d and 11d shelf-life at 20 °C. Fruit were harvested in 2016 at commercial maturity and a week later (post-optimum) on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

Fruit canopy position	Harvest	Diameter (mm)	Mass (g)	Length (mm)
Outside-west	1	66.8 b ^z	181.9 b	88.8 cd
Outside-west	2	69.3 a	206.4 a	90.8 ab
Middle-west	1	63.1 d	155.3 d	85.7 e
Middle-west	2	65.5 c	181.0 b	89.6 bcd
Inside	1	56.6 e	117.8 e	79.1 f
Inside	2	63.1 d	166.6 c	88.5 d
Middle-east	1	62.3 d	151.2 d	85.6 e
Middle-east	2	65.4 c	180.2 b	90.6 abc
Outside-east	1	62.6 d	150.6 d	84.9 e
Outside-east	2	68.5 a	201.1 a	91.8 a
Source of variation		Pr>F		
Position		0.0001	0.0001	0.0026
Harvest		0.0001	0.0001	0.0002
Position*Harvest		0.0003	0.0010	0.0001

^zFruit canopy position means in the same column followed by the same letter are not significantly different at 5% level (LSD).

Table 7: Effect of 'Forelle' canopy position on the average number of normal (viable) seeds and aborted seeds after 8w, 12w and 16w storage at -0.5 °C RA + 0d, 4d, 7d and 11d shelf-life at 20 °C. Fruit were harvested in 2016 at commercial maturity and a week after the first harvest date on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

Fruit position	Number of normal seeds	Number of aborted seeds
Outside-west	1.19 a ^z	8.79 c
Middle-west	0.57 b	9.41 b
Inside	0.35 c	9.62 a
Middle-east	0.41 bc	9.53 ab
Outside-east	0.91 a	8.98 c
Source of variation	Pr>F	
Position	0.0001	0.0001
Harvest	0.6355	0.5903
Position*Harvest	0.3163	0.2447

^zFruit canopy position means in the same column followed by the same letter are not significantly different at 5% level (LSD)

Table 8: Combined average juice mass and juice area of different mealiness classes of 'Forelle' after 8w, 12w and 16w storage at -0.5 °C RA + 4d, 7d and 11d shelf-life at 20 °C. Fruit were harvested in 2017 at commercial maturity and two weeks after the commercial harvest date on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

Mealiness class	Mean juice mass (mg)	Mean juice area (cm²)
Non-mealy (0)	0.215 a ^z	12.478 a
Partly mealy (1)	0.075 b	8.566 b
Mealy (2)	0.042 c	2.991 c
Source of variation:	Pr>F	
Mealiness class	0.0001	0.0001

^zMealiness class mean followed by the same letter are not significantly different at 5% level (LSD).

Table 9A: Effect of fruit canopy position on 'Forelle' pear average juice mass and juice area of different mealiness classes after 8w, 12w and 16w storage at -0.5 °C RA + 4d, 7d and 11d shelf-life at 20 °C. Fruit were harvested in 2017 at commercial maturity and two weeks after the commercial harvest date on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

Fruit canopy position	Mealiness class					
	Non-mealy (0)		Partly mealy (1)		Mealy (2)	
	Juice mass (mg)	Juice area (cm ²)	Juice mass (mg)	Juice area (cm ²)	Juice mass (mg)	Juice area (cm ²)
Outside-west	0.210 ^{*NS}	12.211 b	0.076 ^{NS}	8.418 b	0.040 ^{NS}	2.868 b
Middle-west	0.220 ^{NS}	12.583 a	0.072 ^{NS}	8.708 a	0.044 ^{NS}	3.093 ab
Inside	0.216 ^{NS}	12.718 a	0.079 ^{NS}	8.712 a	0.053 ^{NS}	3.488 a
Source of variation:	Pr>F					
Position	0.2470	0.0028	0.4711	0.0022	0.4753	0.0187
Harvest	0.0029	0.0001	0.0318	0.0012	0.1861	0.9143
Position*Harvest	0.9988	0.8808	0.4588	0.5971	0.3027	0.4983

*NS = Non-significant

²Means in the same column followed by the same letter are not significantly different at 5% level (LSD).

Table 9B: Effect of harvest maturity on 'Forelle' pear average juice mass (mg) of different mealiness classes after 8w, 12w and 16w storage at -0.5 °C RA + 4d, 7d and 11d shelf-life at 20 °C. Fruit were harvested in 2017 at commercial maturity and two weeks after the first harvest date on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

Harvest	Mealiness class					
	Non-mealy (0)		Partly mealy (1)		Mealy (2)	
	Juice mass (mg)	Juice area (cm ²)	Juice mass (mg)	Juice area (cm ²)	Juice mass (mg)	Juice area (cm ²)
1	0.222 a ²	12.709 a	0.082 a	8.781 a	0.050 *NS	3.096 ^{NS}
2	0.206 b	12.184 b	0.069 b	8.396 b	0.037 ^{NS}	2.930 ^{NS}
Source of variation:			Pr>F			
Position	0.2470	0.0028	0.4711	0.0022	0.4753	0.1859
Harvest	0.0029	0.0001	0.0318	0.0012	0.1861	0.9143
Position*Harvest	0.9988	0.8808	0.4588	0.5971	0.3027	0.4983

*NS = Non-significant

²Means in the same column followed by the same letter are not significantly different at 5% level (LSD)

Table 10A: Effect of fruit canopy position on 'Forelle' pear average hue angle, blush colour and hue angle on the green background colour at the time of the first harvest (commercial maturity) and second harvest date (post-commercial maturity). Fruit were harvested in 2017 at commercial maturity and a week later (post-optimum) on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

Fruit canopy position	Hue (°) blush side	Blush colour (chart index P. 16) ^x	Hue angle (°) background
Outside-west	40.7 c ^z	1.0 a	110.9 b
Middle-west	72.6 b	6.2 b	111.7 ab
Inside	111.9 a	12.0 c	112.0 a
Source of variation	Pr>F		
Position	0.0001	0.0001	0.0301
Harvest	0.5591	0.3999	0.5271
Position*Harvest	0.8143	0.9245	0.2304

^zFruit canopy position means in the same column followed by the same letter are not significantly different at 5% level (LSD).

^xChart values 1-12: where 1=red; 12=green.

Table 10B: Effect of fruit canopy position and harvest maturity on 'Forelle' pear average ground colour and hue angle on the green background colour at the time of the first harvest (commercial maturity) and second harvest date (post-commercial maturity). Fruit were harvested in 2017 at commercial maturity and a week later (post-optimum) on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

Fruit canopy position	Harvest	Ground colour (chart index) ^x	Hue angle (°) background
Outside-west	1	2.2 b ^z	110.9 ^{*NS}
Outside-west	2	2.7 a	110.9 ^{NS}
Middle-west	1	2.2 b	111.5 ^{NS}
Middle-west	2	2.3 b	111.9 ^{NS}
Inside	1	2.1 b	112.5 ^{NS}
Inside	2	2.5 a	111.5 ^{NS}
Source of variation:		Pr>F	
Position		0.0123	0.0301
Harvest		0.0413	0.5271
Position*Harvest		0.0191	0.2304

*NS = Non-significant

^zFruit canopy position means in the same column followed by the same letter are not significantly different at 5% level (LSD).

^xChart values 0.5-5: where 0.5= green; 5= pale green/ yellow.

Table 11: Combined average (harvest one and two) hue angle on the blushed side and on the green background colour, blush colour, background colour chart index, firmness, diameter, mass, length, number of normal (viable) seeds and aborted seeds of mealy, partly mealy and non-mealy 'Forelle' pears after 8w, 12w and 16w storage at -0.5 °C RA + 4d, 7d and 11d shelf-life at 20 °C. Fruit were harvested in 2017 at commercial- and post-commercial maturity on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

Mealiness class	Hue (°) blush side	Blush colour (chart index) ^x	Ground colour (chart index) ^y	Hue angle (°) background	Firmness (kg)	Diameter (mm)	Mass (g)	Length (mm)	Normal seeds	Aborted seeds
Mealy	49.2 c ^z	3.6 c	4.5 a	97.1 c	1.6 c	65.0 a	170.9 a	84.6 *NS	0.45 NS	9.22 NS
Partly mealy	57.6 b	4.9 b	3.9 b	100.6 b	2.1 b	63.5 b	160.9 b	82.8 NS	0.80 NS	9.14 NS
Non-mealy	73.2 a	7.2 a	3.3 c	103.7 a	3.6 a	61.5 c	148.9 c	82.0 NS	0.59 NS	9.22 NS
Source of variation:				Pr>F						
Mealiness class	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0815	0.0653	0.2904

*NS = Non-significant

^zMealiness class means in the same column followed by the same letter are not significantly different at 5% level (LSD).

^xChart values 1-12: where 1=red; 12=green.

^yChart values 0.5-5: where 0.5= green; 5= pale green/ yellow.

Table 12A: Effect of harvest maturity on 'Forelle' pear average firmness, TSS and TA at the time of the first harvest (commercial maturity) and second harvest date (post-commercial maturity). Fruit were harvested in 2017 at commercial maturity and a week later (post-optimum) on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

Harvest	Firmness (kg)	TSS (%)	TA (% malic acid)
1	6.3 a	13.4 b	0.19 a
2	5.8 b	14.0 a	0.17 b
Source of variation:		Pr>F	
Position	0.4831	0.0001	0.0001
Harvest	0.0001	0.0003	0.0027
Position*Harvest	0.4770	0.8054	0.0889

²Harvest means in the same column followed by the same letter are not significantly different at 5% level (LSD).

Table 12B: Effect of fruit canopy position on 'Forelle' pear average TSS and TA at the time of the first harvest (commercial maturity) and second harvest date (post-commercial maturity). Fruit were harvested in 2017 at commercial maturity and a week later (post-optimum) on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

Fruit canopy position	TSS (%)	TA (% malic acid)
Outside-west	14.7 a ²	0.15 c
Middle-west	13.4 b	0.17 b
Inside	12.9 c	0.21 a
Source of variation:		Pr>F
Position	0.0001	0.0001
Harvest	0.0003	0.0027
Position*Harvest	0.8054	0.0889

²Fruit canopy position means in the same column followed by the same letter are not significantly different at 5% level (LSD).

Table 13: Effect of fruit canopy position and harvest maturity on 'Forelle' pear average diameter, mass and length after 8w, 12w and 16w storage at -0.5 °C RA + 0d, 4d, 7d and 11d shelf-life at 20 °C. Fruit were harvested in 2017 at commercial maturity and two weeks after the commercial harvest date on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

Fruit canopy position	Harvest	Diameter (mm)	Mass (g)	Length (mm)
Outside-west	1	65.1 a ^z	172.4 a ^z	85.4 a
Outside-west	2	65.4 a	170.3 a	83.4 b
Middle-west	1	60.4 c	142.2 c	80.7 cd
Middle-west	2	62.1 b	152.2 b	82.3 b
Inside	1	58.3 d	130.3 d	79.4 d
Inside	2	60.2 c	143.2 c	82.2 bc
Source of variation:			Pr>F	
Position		0.0001	0.0001	0.0001
Harvest		0.0002	0.0005	0.0773
Position*Harvest		0.0434	0.0045	0.0001

^zFruit canopy means in the same column followed by the same letter are not significantly different at 5% level (LSD).

Table 14: Effect of 'Forelle' canopy position on the average number of normal (viable) seeds and aborted seeds after 8w, 12w and 16w storage at -0.5 °C RA + 0d, 4d, 7d and 11d shelf-life at 20 °C. Fruit were harvested in 2017 at commercial maturity and two weeks after the commercial harvest date on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa.

Fruit canopy position	Number of normal seeds	Number of aborted seeds
Outside-west	0.92 a ²	8.94 b
Middle-west	0.56 b	9.29 a
Inside	0.38 c	9.42 a
Source of variation	Pr>F	
Position	0.0001	0.0001
Harvest	0.0872	0.4704
Position*Harvest	0.7080	0.7310

²Fruit canopy position means in the same column followed by the same letter are not significantly different at 5% level (LSD).

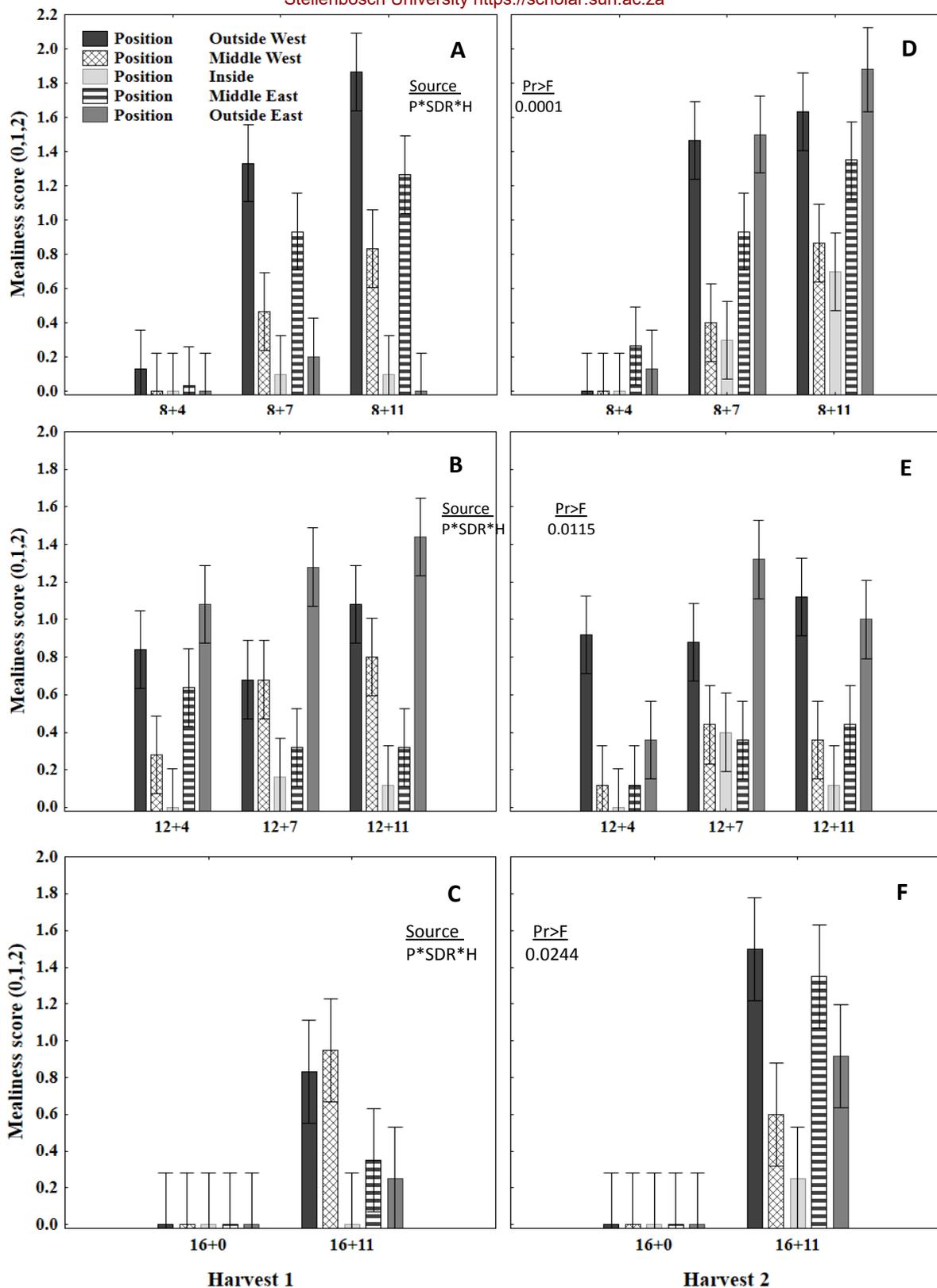


Figure 1: Average 'Forelle' mealiness class score (0=non-mealy; 1=partly; 2= mealy) after 8w, 12w and 16w storage at -0.5 °C RA + 0d, 4d, 7d and 11d shelf-life at 20 °C (8+4, 8+7, 8+11; 12+4, 12+7, 12+11; 16+0, 16+11, respectively) for different fruit canopy position of harvest one (commercial maturity) and harvest two (one week after harvest one) fruits. Different letters show significant differences between fruit position at $p \leq 0.05$. Fruit were harvested in 2016 on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa. P = Fruit position; SDR = Storage duration and ripening; H = Harvest.

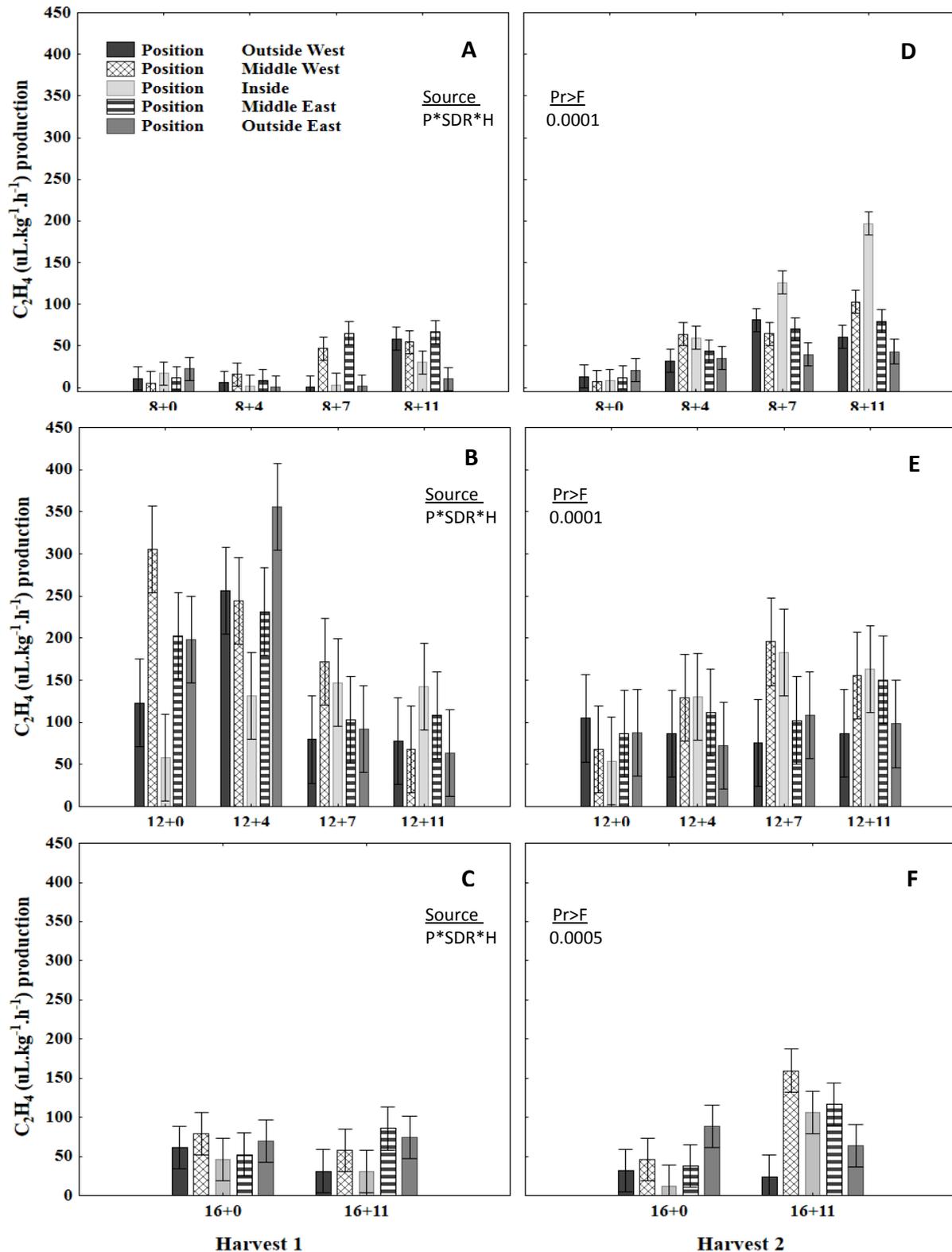


Figure 2: Average 'Forelle' ethylene production rates after 8w, 12w and 16w storage at -0.5 °C RA + 0d, 4d, 7d and 11d shelf-life at 20 °C (8+0, 8+4, 8+7, 8+11; 12+0, 12+4, 12+7, 12+11; 16+0, 16+11, respectively) for different fruit canopy position of harvest one (commercial maturity) and harvest two (one week after harvest one) fruits. Fruit were harvested in 2016 on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa. P = Fruit position; SDR = Storage duration and ripening; H = Harvest.

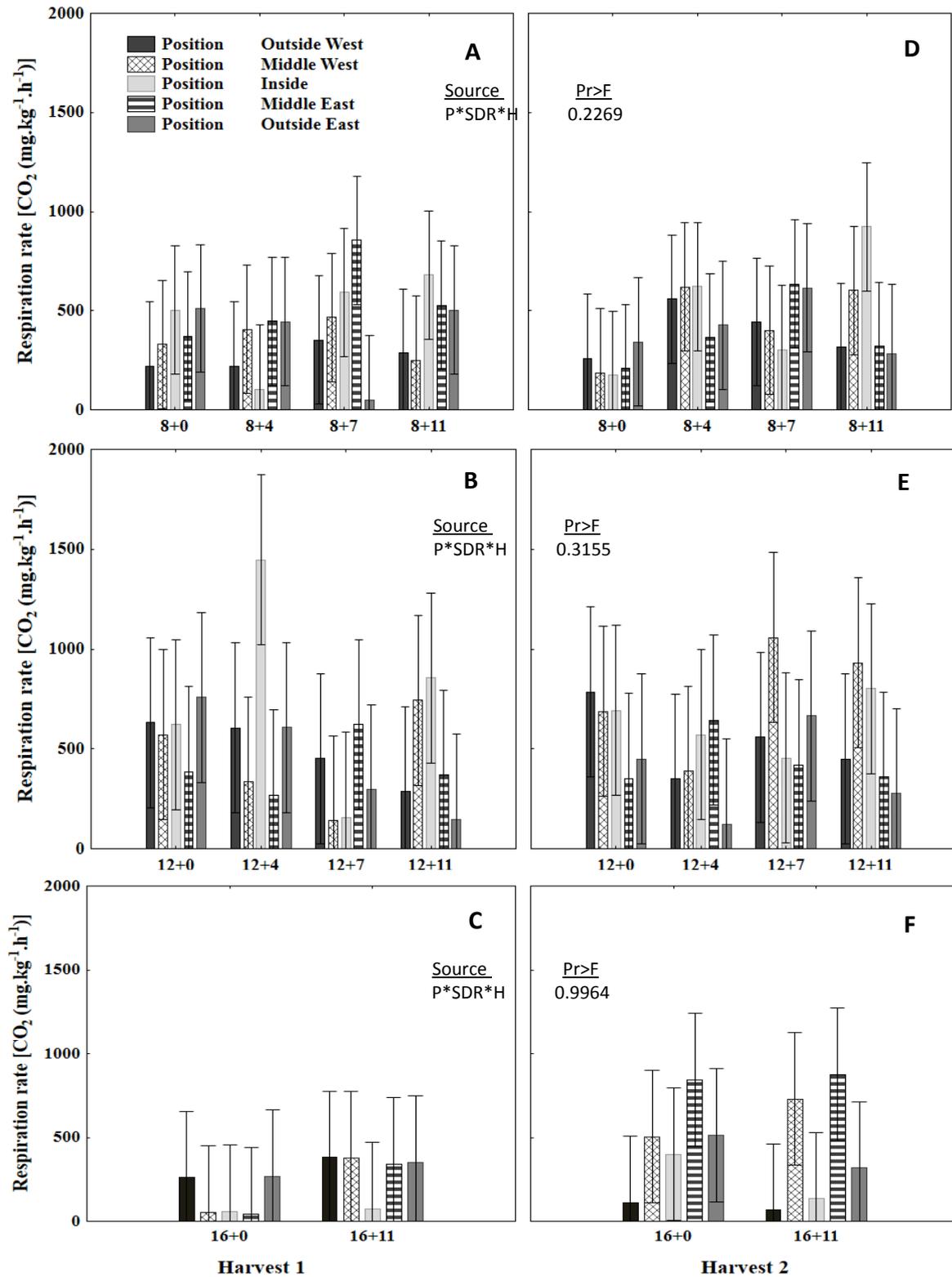


Figure 3: Average 'Forelle' respiration rate 8w, 12w and 16w storage at -0.5 °C RA + 0d, 4d, 7d and 11d shelf-life at 20 °C (8+0, 8+4, 8+7, 8+11; 12+0, 12+4, 12+7, 12+11; 16+0, 16+11, respectively) for different fruit canopy positions of harvest one (commercial maturity) and harvest two (two weeks after harvest one) fruits. Fruit were harvested in 2016 on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa. P = Fruit position; SDR = Storage duration and ripening; H = Harvest.

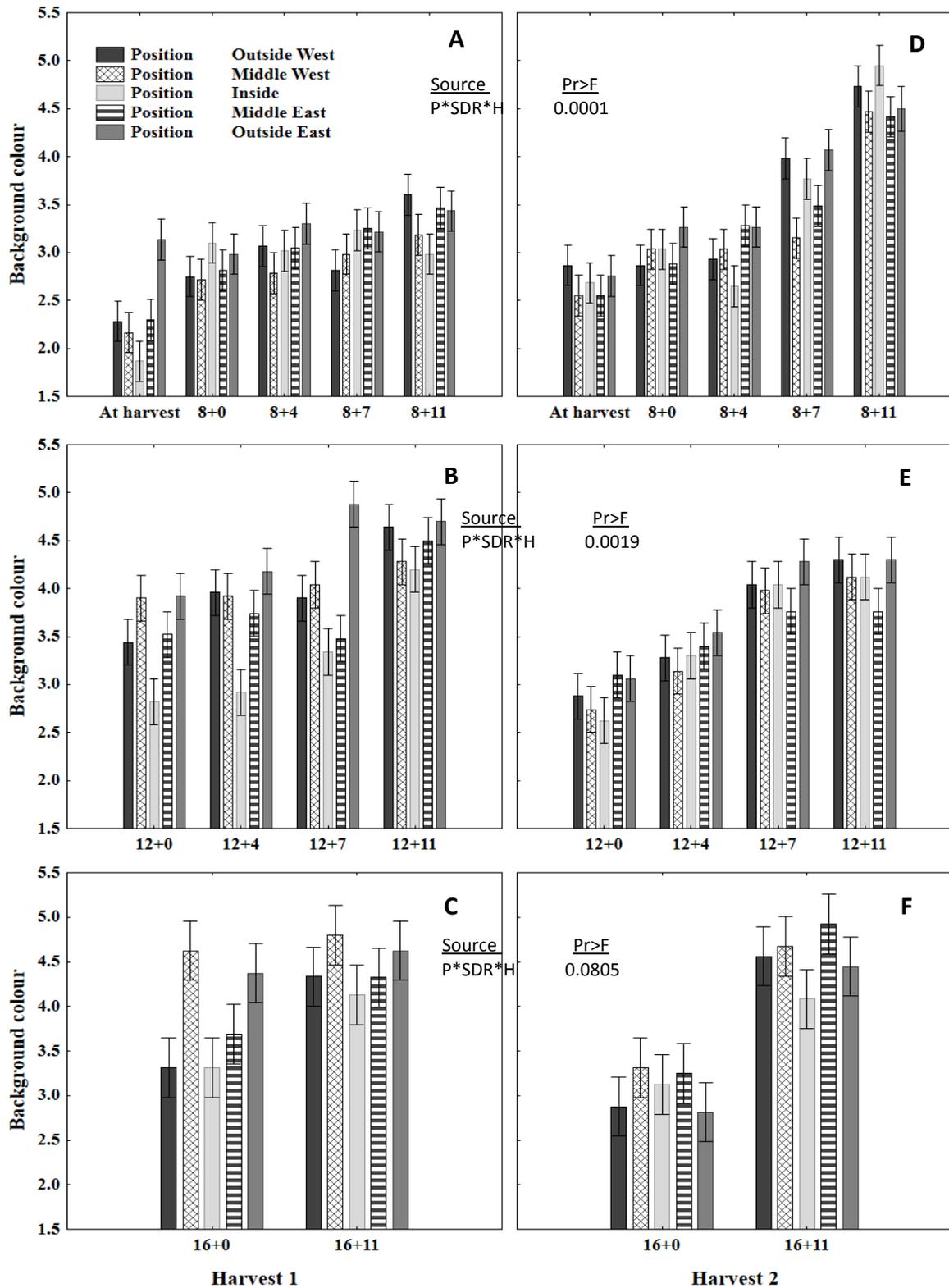


Figure 4: Average 'Forelle' background colour (0.5 = dark green; 5 = deep yellow) after 8w, 12w and 16w storage at -0.5 °C RA + 0d, 4d, 7d and 11d shelf-life at 20 °C (8+0, 8+4, 8+7, 8+11; 12+0, 12+4, 12+7, 12+11; 16+0, 16+11, respectively) for different fruit canopy position of harvest one (commercial maturity) and harvest two (one week after harvest one) fruits. Fruit were harvested in 2016 on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa. P = Fruit position; SDR = Storage duration and ripening; H = Harvest.

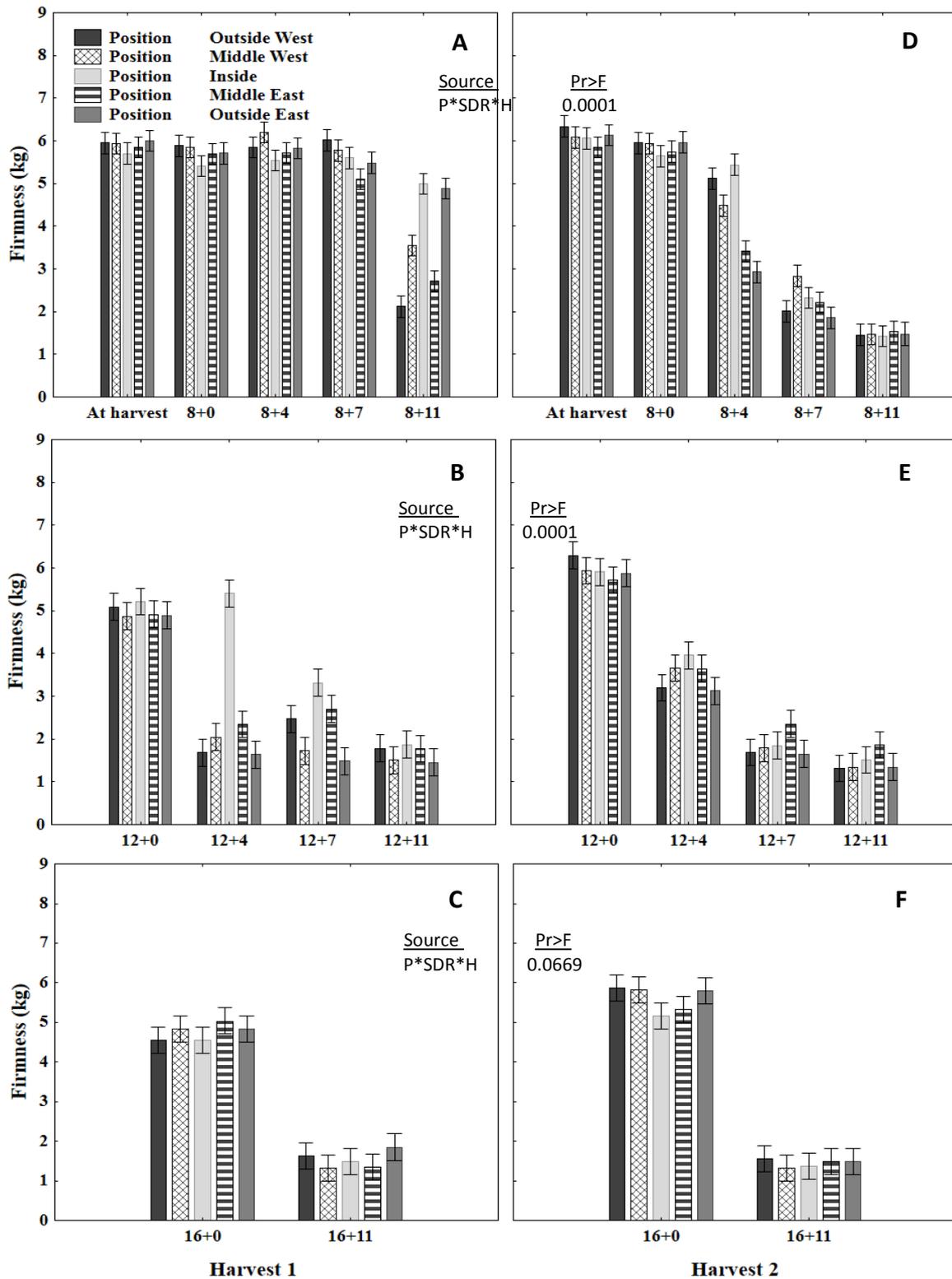


Figure 5: Average 'Forelle' firmness after 8w, 12w and 16w storage at -0.5 °C RA + 0d, 4d, 7d and 11d shelf-life at 20 °C (8+0, 8+4, 8+7, 8+11; 12+0, 12+4, 12+7, 12+11; 16+0, 16+11, respectively) for different fruit canopy position of harvest one (commercial maturity) and harvest two (one week after harvest one) fruits. Fruit were harvested in 2016 on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa. P = Fruit position; SDR = Storage duration and ripening; H = Harvest.

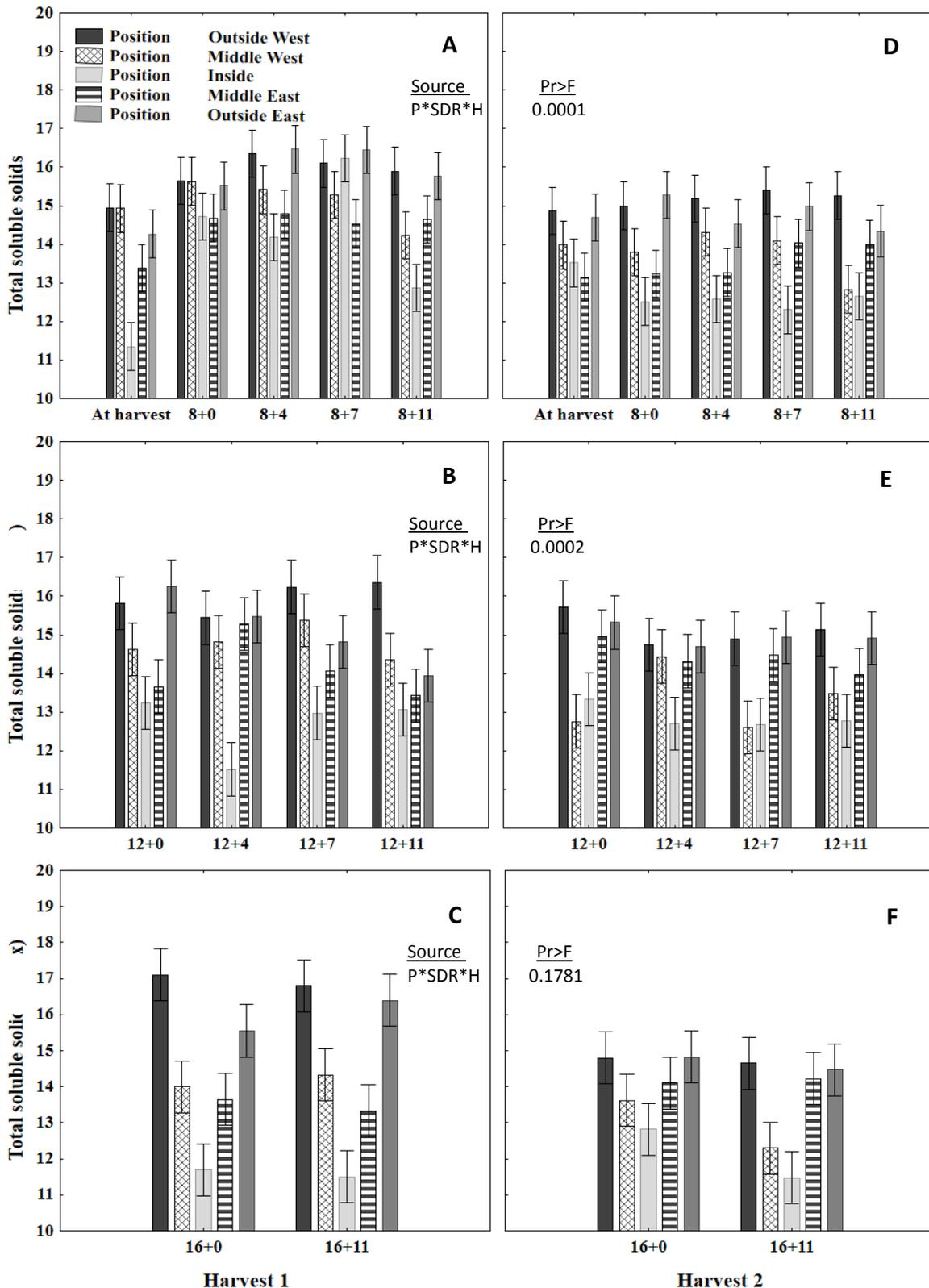


Figure 6: Average 'Forelle' total soluble solids (TSS) after 8w, 12w and 16w storage at -0.5 °C RA + 0d, 4d, 7d and 11d shelf-life at 20 °C (8+0, 8+4, 8+7, 8+11; 12+0, 12+4, 12+7, 12+11; 16+0, 16+11, respectively) for different fruit canopy position of harvest one (commercial maturity) and harvest two (one week after harvest one) fruits. Fruit were harvested in 2016 on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa. P = Fruit position; SDR = Storage duration and ripening; H = Harvest.

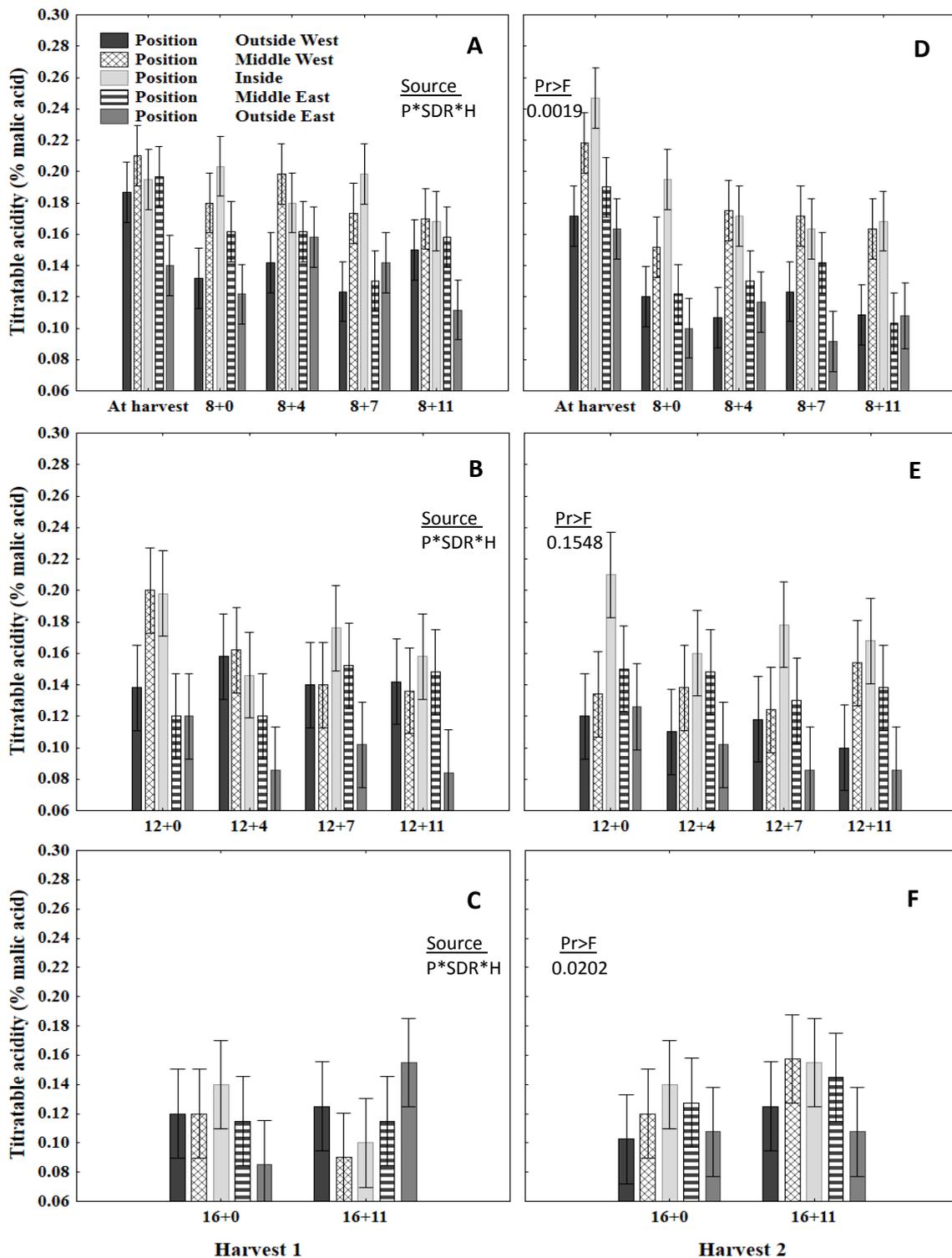


Figure 7: Average 'Forelle' titratable acidity (TA) after 8w, 12w and 16w storage at -0.5 °C RA + 0d, 4d, 7d and 11d shelf-life at 20 °C (8+0, 8+4, 8+7, 8+11; 12+0, 12+4, 12+7, 12+11; 16+0, 16+11, respectively) for different fruit canopy position of harvest one (commercial maturity) and harvest two (one week after harvest one) fruits. Fruit were harvested in 2016 on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa. P = Fruit position; SDR = Storage duration and ripening; H = Harvest.

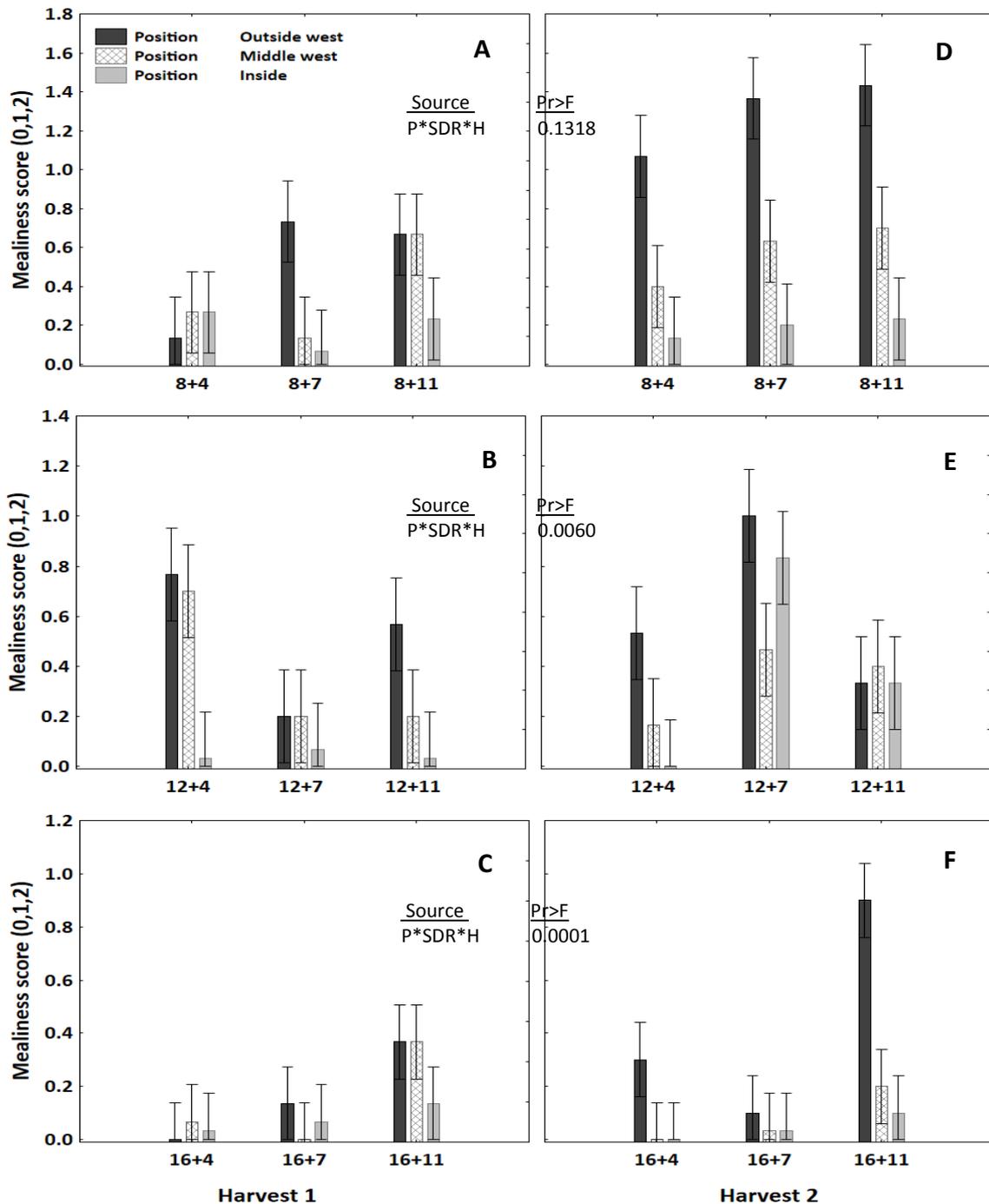


Figure 8: Average 'Forelle' mealiness class score (0=non-mealy; 1=partly; 2= mealy) after 8w, 12w and 16w storage at -0.5 °C RA + 4d, 7d and 11d shelf-life at 20 °C (8+4, 8+7, 8+11; 12+4, 12+7, 12+11; 16+4, 16+7, 16+11, respectively) for different fruit canopy position of harvest one (commercial maturity) and harvest two (two weeks after harvest one) fruits. Different letters show significant differences between fruit position at $p \leq 0.05$. Fruit were harvested in 2017 on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa. P = Fruit position; SDR = Storage duration and ripening; H = Harvest.

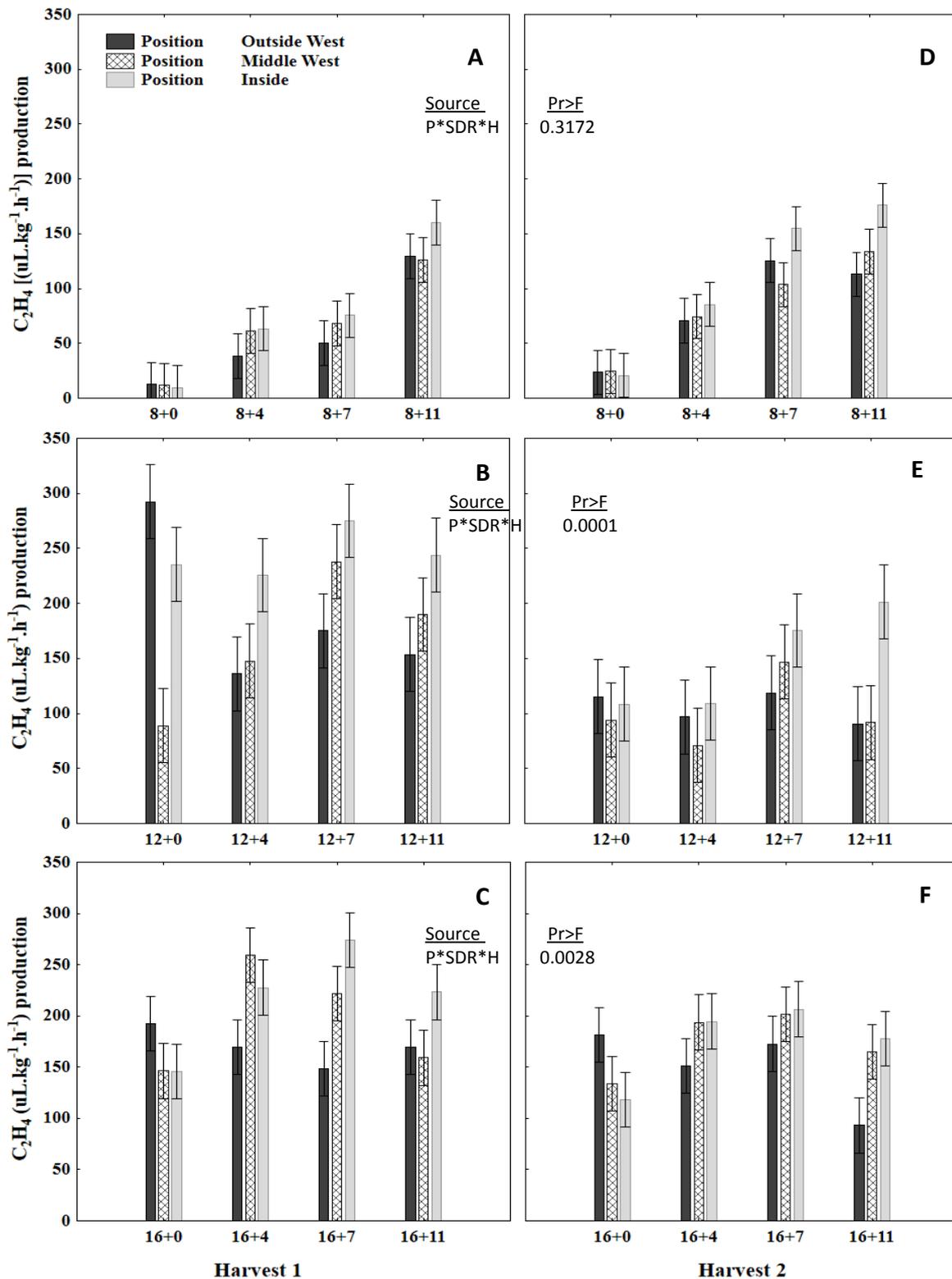


Figure 9: Average 'Forelle' ethylene production rates after 8w, 12w and 16w storage at -0.5 °C RA + 0d, 4d, 7d and 11d shelf-life at 20 °C (8+0, 8+4, 8+7, 8+11; 12+0, 12+4, 12+7, 12+11; 16+0, 16+4, 16+7, 16+11, respectively) for different fruit canopy position of harvest one (commercial maturity) and harvest two (two weeks after harvest one) fruits. Different letters show significant differences between fruit position at $p \leq 0.05$. Fruit were harvested in 2017 on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa. P = Fruit position; SDR = Storage duration and ripening; H = Harvest.

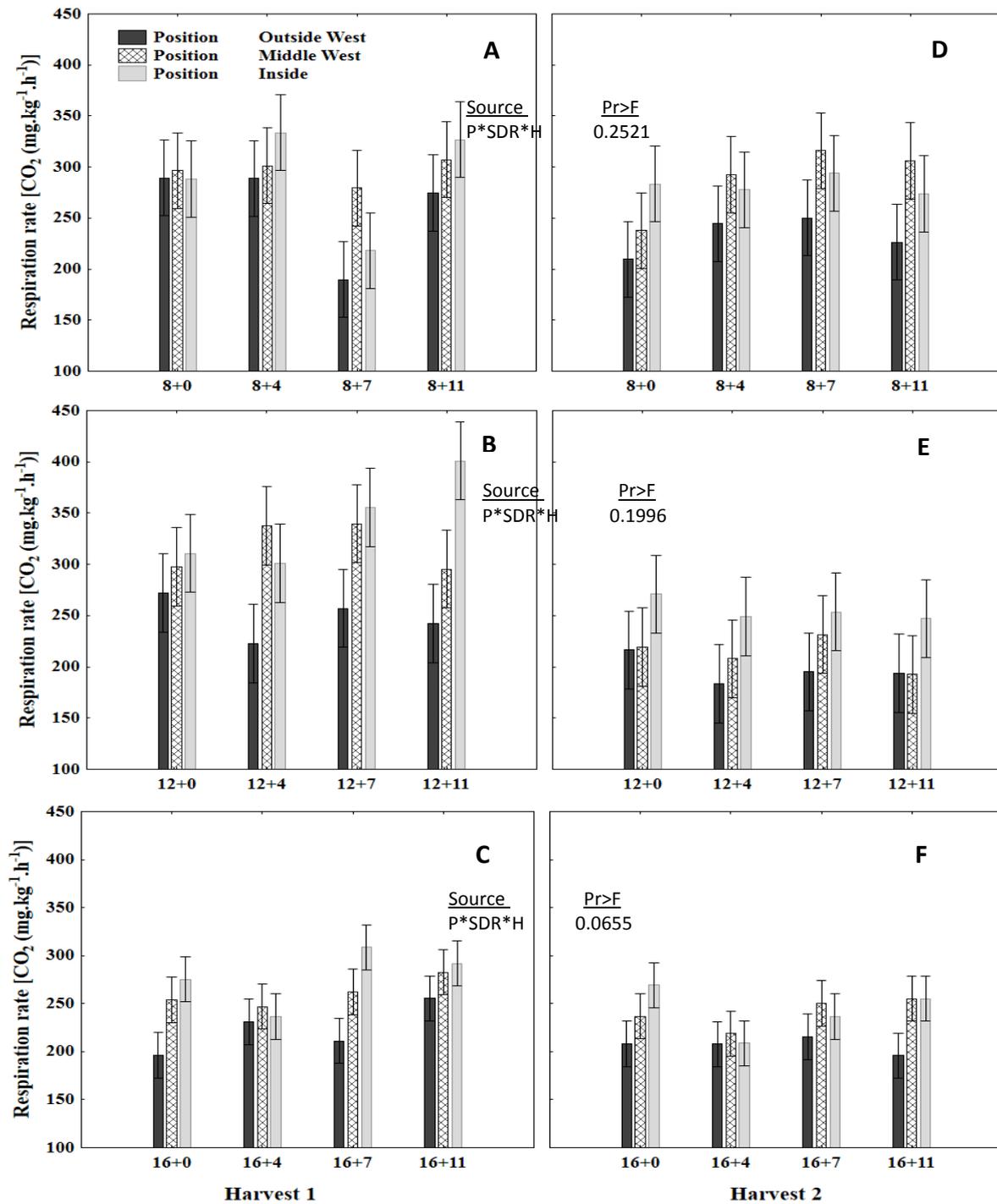


Figure 10: Average 'Forelle' respiration rate after 8w, 12w and 16w RA storage at $-0.5\text{ }^{\circ}\text{C}$ RA + 0d, 4d, 7d and 11d shelf-life at $20\text{ }^{\circ}\text{C}$ (8+0, 8+4, 8+7, 8+11; 12+0, 12+4, 12+7, 12+11; 16+0, 16+4, 16+7, 16+11, respectively) for different fruit canopy position of harvest one (commercial maturity) and harvest two (two weeks after harvest one) fruits. Fruit were harvested in 2017 on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa. P = Fruit position; SDR = Storage duration and ripening; H = Harvest.

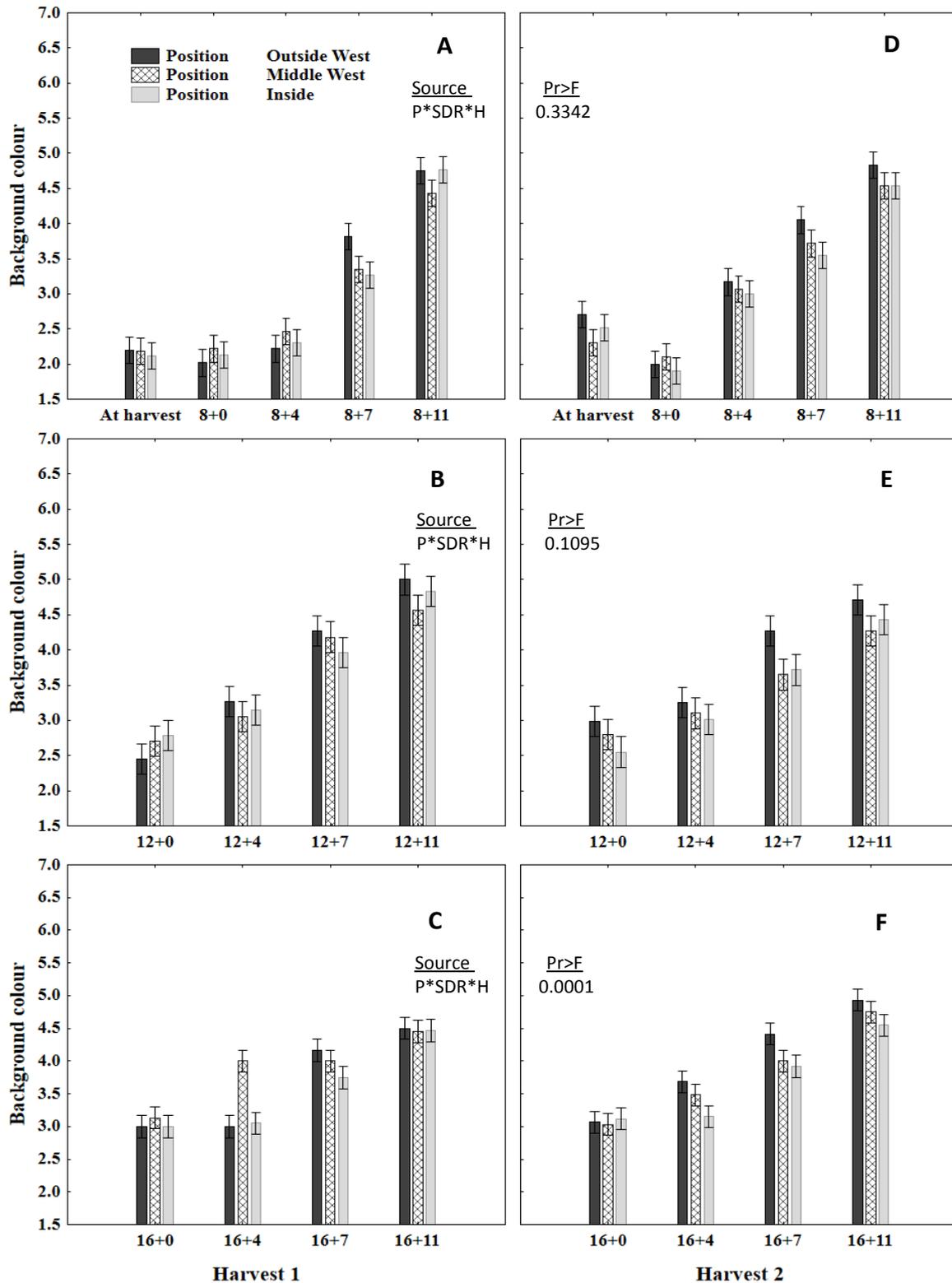


Figure 11A: Average ‘Forelle’ background colour chart index (Unifruco apple and pear chart) (0.5 = dark green; 5 = deep yellow) after 8w, 12w and 16w storage at -0.5 °C RA + 4d, 7d and 11d shelf-life at 20 °C (8+0, 8+4, 8+7, 8+11; 12+0, 12+4, 12+7, 12+11; 16+0, 16+4, 16+7, 16+11, respectively) for different fruit canopy position of harvest one (commercial maturity) and harvest two (two weeks after harvest one) fruits. Fruit were harvested in 2017 on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa. P = Fruit position; SDR = Storage duration and ripening; H = Harvest.

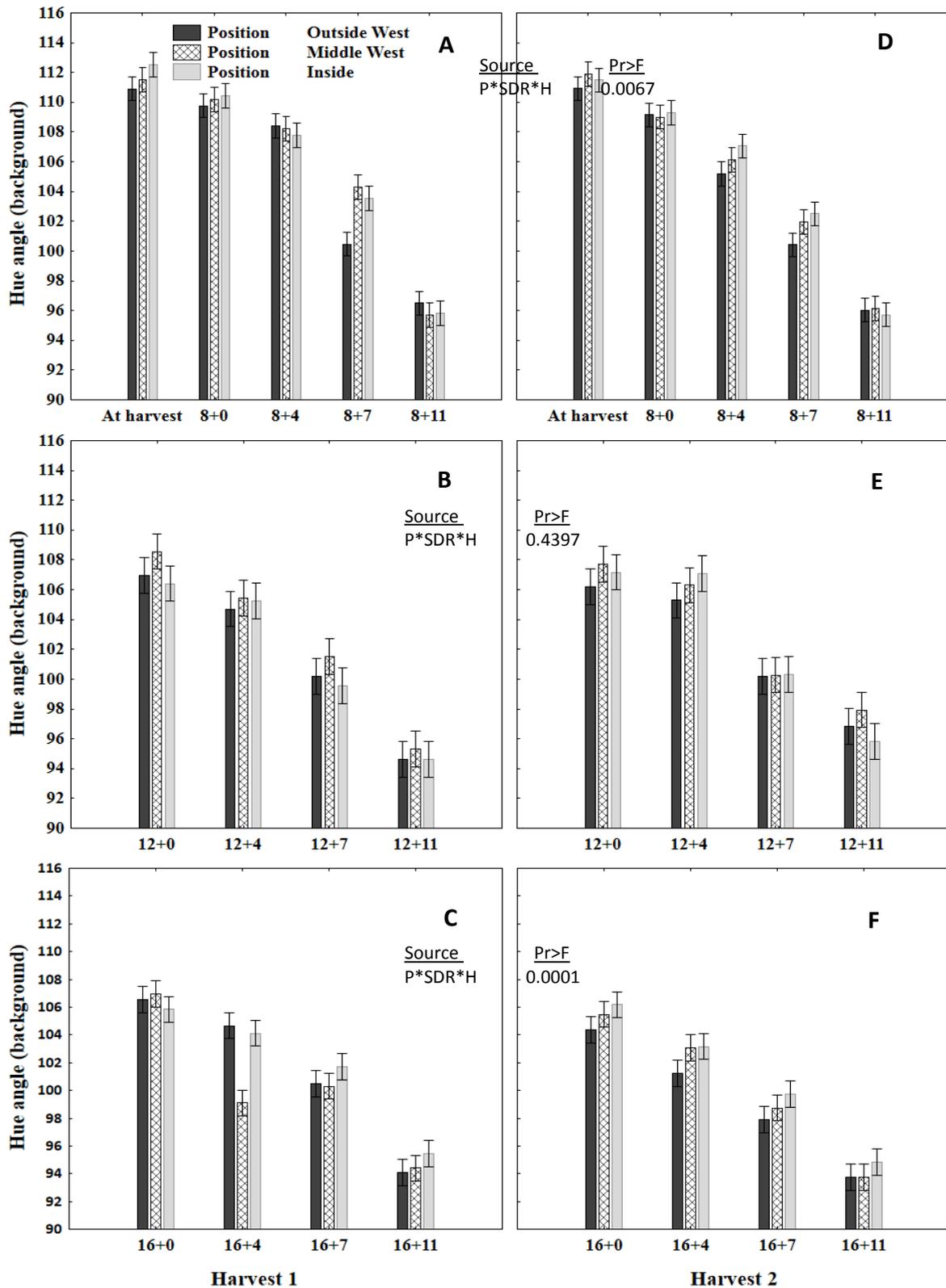


Figure 11B: Average 'Forelle' hue angle of background colour after 8w, 12w and 16w RA storage at $-0.5\text{ }^{\circ}\text{C}$ RA + 0d, 4d, 7d and 11d shelf-life at $20\text{ }^{\circ}\text{C}$ (8+0, 8+4, 8+7, 8+11; 12+0, 12+4, 12+7, 12+11; 16+0, 16+4, 16+7, 16+11, respectively) for different fruit canopy position of harvest one (commercial maturity) and harvest two (two weeks after harvest one) fruits. Fruit were harvested in 2017 on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa. P = Fruit position; SDR = Storage duration and ripening; H = Harvest.

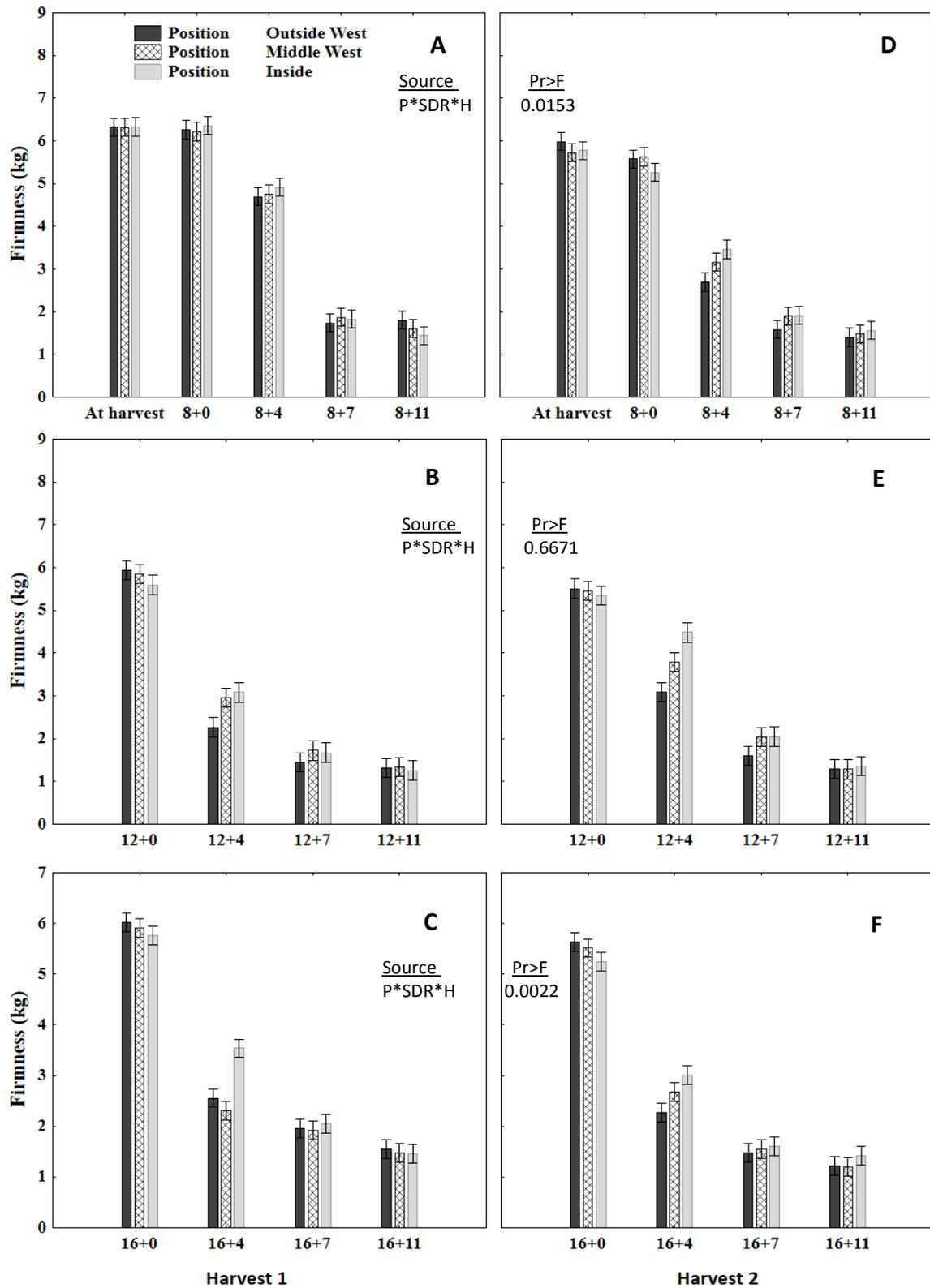


Figure 12: Average 'Forelle' firmness after 8w, 12w and 16w storage at -0.5 °C RA + 0d, 4d, 7d and 11d shelf-life at 20 °C (8+0, 8+4, 8+7, 8+11; 12+0, 12+4, 12+7, 12+11; 16+0, 16+4, 16+7, 16+11, respectively) for different fruit canopy position of harvest one (commercial maturity) and harvest two (two weeks after harvest one) fruits. Fruit were harvested in 2017 on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa. . P = Fruit position; SDR = Storage duration and ripening; H = Harvest.

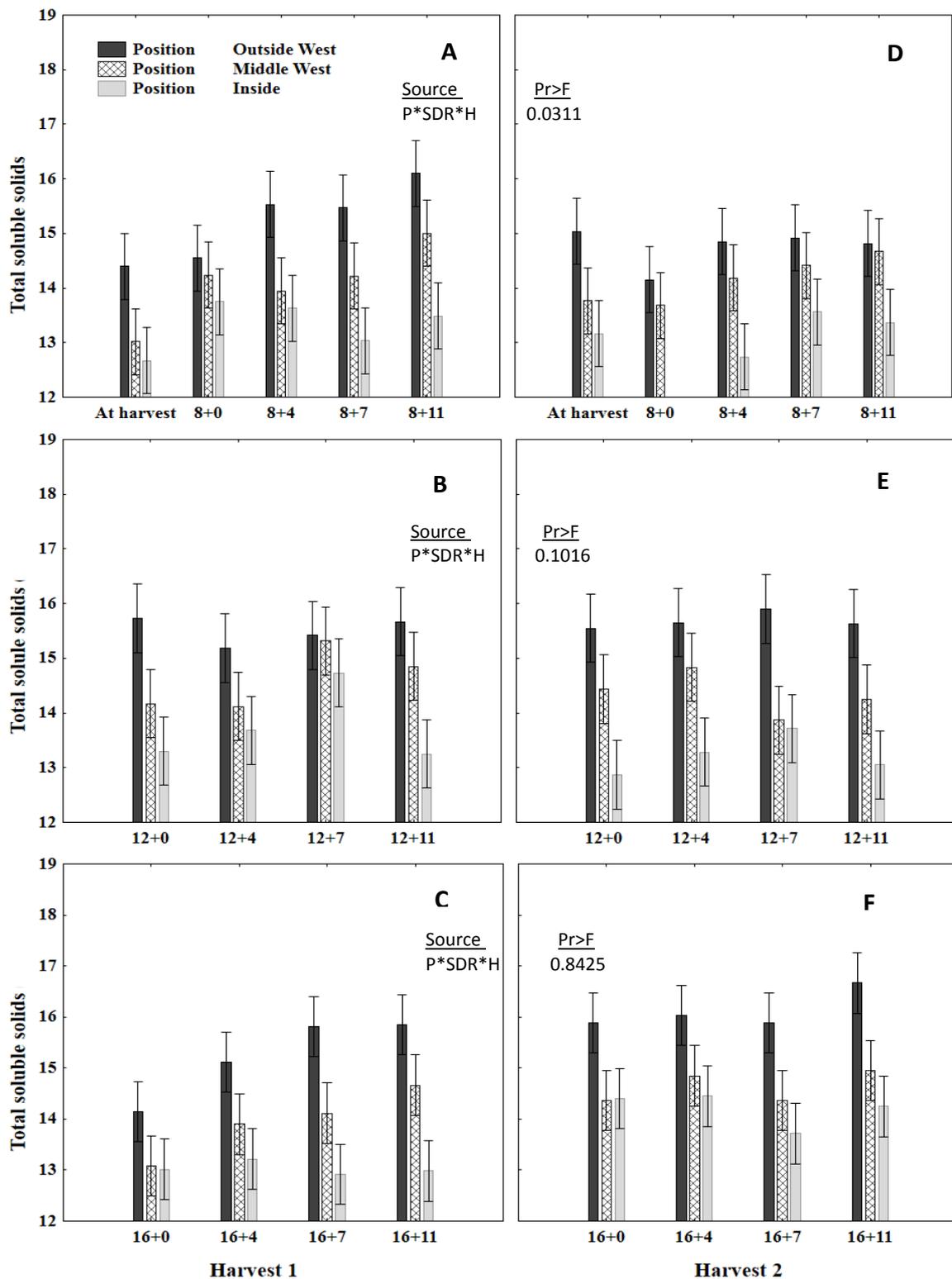


Figure 13: Average 'Forelle' total soluble solids (TSS) after 8w, 12w and 16w storage at -0.5 °C RA + 0d, 4d, 7d and 11d shelf-life at 20 °C (8+0, 8+4, 8+7, 8+11; 12+0, 12+4, 12+7, 12+11; 16+0, 16+4, 16+7, 16+11, respectively for different fruit canopy position) of harvest one (commercial maturity) and harvest two (two weeks after harvest one) fruits. Fruit were harvested in 2017 on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa. . P = Fruit position; SDR = Storage duration and ripening; H = Harvest.

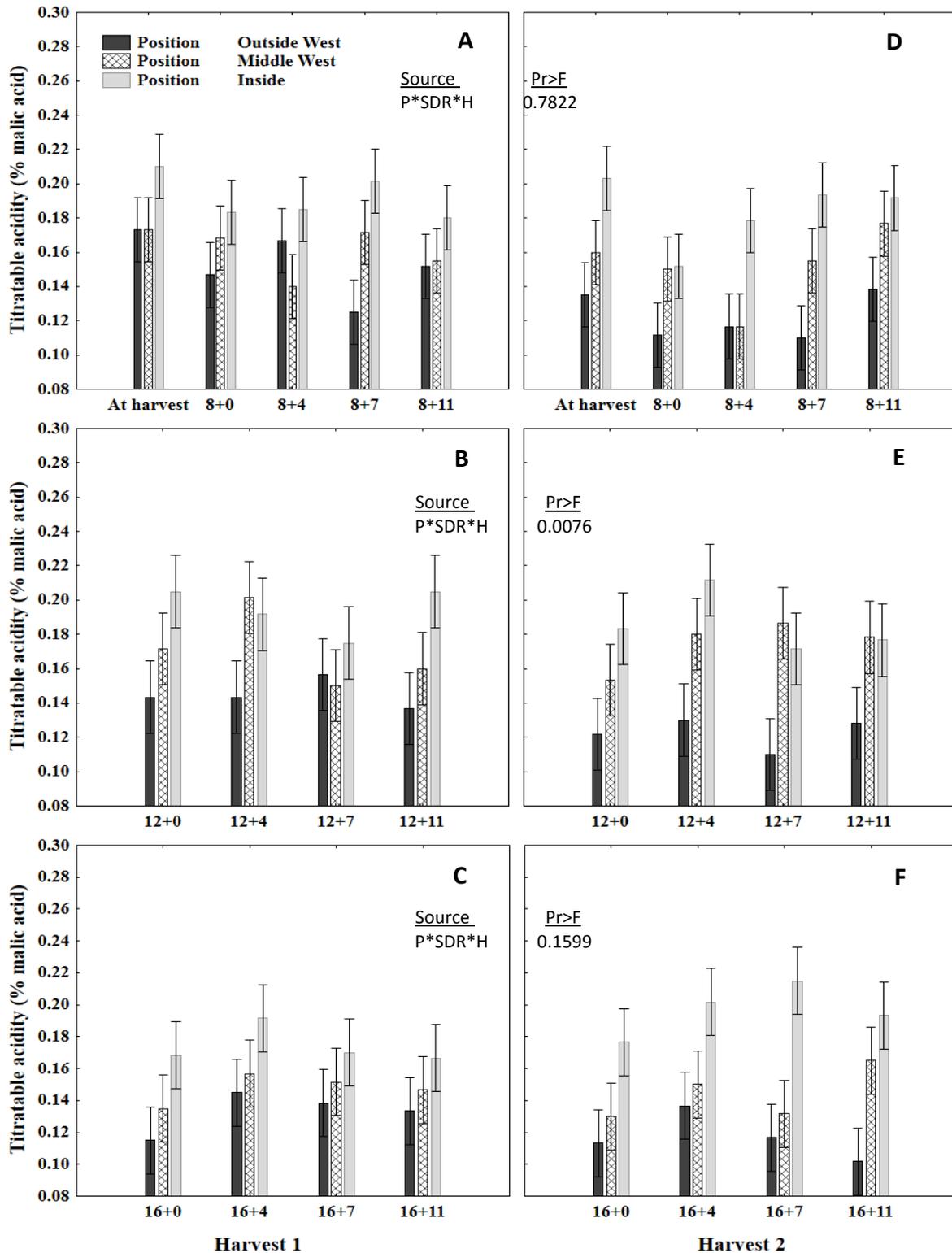


Figure 14: Average 'Forelle' titratable acidity (TA) after harvest, 8w, 12w and 16w storage at -0.5 °C RA + 0d, 4d, 7d and 11d shelf-life at 20 °C (8+0, 8+4, 8+7, 8+11; 12+0, 12+4, 12+7, 12+11; 16+0, 16+4, 16+7, 16+11, respectively) for different fruit canopy position of harvest one (commercial maturity) and harvest two (two weeks after harvest one) fruits. Fruit were harvested in 2017 on the Glen Fruin farm in the Elgin region of the Western Cape, South Africa. P = Fruit position; SDR = Storage duration and ripening; H = Harvest.

GENERAL DISCUSSION AND CONCLUSION

In South Africa, Forelle pear is the most valuable bicolour cultivar and second most planted pear cultivar in South Africa. Their total hectares contribute 26% of South Africa's total area of pear production (HORTGRO, 2018). The ability to obtain the characteristic red blush under South African conditions is of great importance to 'Forelle' pears' success (Steyn et al., 2005). Post-harvest disorders are an everyday problem that influences all types of fruit of which 'Forelle' pears are no exception. 'Forelle' pears have a tendency to develop mealiness after ripening to a firmness lower than 4 kg (Crouch et al., 2005). Mealiness is a dry textural disorder coupled with a floury sensation in the mouth that is associated with a lack of juiciness (Barreiro et al., 1998; Martin, 2002). There is a mandatory 12-week cold storage period at -0.5 °C for South African 'Forelle' pears to ripen uniformly and to minimize mealiness incidence (de Vries and Hurndall, 1993). The mandatory period prevents the availability of 'Forelle' pears for earlier European markets providing premium prices (Crouch and Bergman, 2013). Currently, the mandatory cold storage period is the only practice available to minimise 'Forelle' mealiness.

Several studies have been conducted in the past regarding the roles that environmental factors play in mealiness development of fruit. Mealiness development of 'Forelle' pear fruit, specifically, however, has been given relatively little attention. Different fruit positions within the tree canopy experience differences in irradiance levels, ambient temperature, water and mineral-nutrients flow, as well as the provision of endogenous hormones (Kingston, 1994; Tomala, 1999). The post-harvest fruit quality, harvest maturity, and ripening potential are mainly influenced by environmental factors, such as sunlight (irradiance) and temperature during the period of fruit growth (Matthee, 1988; Villalobos-Acuña and Mitcham, 2008). Currently, the effects of environmental factors on mealiness development are not fully understood, which could be mainly because of the difference in environmental conditions between seasons. Fruit must be harvested at optimum maturity for optimum ripening potential and for the fruit to ripen normally (Carmichael, 2011). Consequently, differences among different fruit positions within the tree canopy could be expected in terms of physiological maturity at the time of harvest, as well as the degree of changes in maturity indices, which may affect the ripening rate and the way in which cellular changes occur during ripening.

The objective of our first study (Chapter 2) was to determine whether different fruit positions within the tree canopy differ in susceptibility to mealiness development, and whether environmental factors such as sunlight, temperature and relative humidity, as well as maturity indices, are linked to mealiness. In both seasons, the mealiness class score was significantly the highest for red blushed outer canopy fruit, associated with significantly the highest percentage exposure to sunlight (irradiance), that linked with the highest average fruit surface temperature (FST) and highest vapour pressure deficit (VPD). Several other studies also reported the role of temperature on mealiness (Hansen, 1961; Mellenthin and Wang, 1976; Carmichael, 2011; Cronjé, 2014). The outer canopy fruit received on average 44% more sunlight than shaded inner canopy pears, together with an average FST of 4 °C higher and an average maximum FST of 7 °C higher.

Growth of pear fruits entails a period of cell division and cell expansion, where the rate of these process increases with temperature (Gillaspy et al., 1993; Warrington et al., 1999) and fruit size (Etienne et al., 2013), with fruit cells developing a greater need for energy/carbohydrates, as well as for water availability. This could possibly lead to hardening off of cell walls, in particular the neck tissue that is exposed most, which then become less pliable during further cell enlargement, as they are unable to expand. This may result in cellular breakage and cell separation that leads to big cavities in the fruit tissue as reported by Muziri et al. (2016), resulting in fruit which may be more susceptible to mealiness development. The mechanism of 'Forelle' mealiness development entails the advanced degradation of the middle lamella with a loss of cell-to-cell adhesion, resulting in cell sliding during mastication, with limited release of cellular contents (juice) (Harker and Hallet, 1992; Crouch, 2011; Muziri, 2016). The study by Muziri et al. (2016) and Crouch et al. (2017) found mealy 'Forelle' pears to have larger cells and higher porosity which corresponds with lower cell-to-cell connectivity.

In this study, the higher mealiness incidence of the outer canopy fruit was associated with bigger sized fruit, as well as with higher total soluble solids (TSS) levels and lower titratable acidity (TA) levels, compared to that of the inner canopy fruit. This agrees with De Smedt et al. (1998) that associated apple mealiness development with bigger sized fruit together with larger sized cells and larger intercellular spaces. Muziri et al. (2016) reported the same about 'Forelle' pears. The bigger size and higher TSS levels of the outer canopy, might be because of

higher photosynthesis rates of the leaves that are exposed to the sun and outer canopy pears that are a stronger sink for mineral nutrients/carbohydrates and that are linked to a higher growth rate. Since not all fruit with high TSS levels and red blushed outer canopy fruit developed a mealy texture, it is an indication that an unidentified tree factor might also be involved in 'Forelle' mealiness development.

In light of the above, the aim of our second trial (Chapter 3) was to determine whether a link exists between fruit canopy position and mealiness development through external environmental factors, such as light and temperature, or possibly through the provision of mineral nutrients, photo-assimilates, endogenous hormones and water. Three shading treatments were randomly applied on the western outer canopy, red blush pears: (1) totally exposed control; (2) shading of fruit and their surrounding leaves (3) shading of fruit, but not the fruits' surrounding leaves. After 8w RA storage at $-0.5\text{ }^{\circ}\text{C}$ + 7d shelf-life at $20\text{ }^{\circ}\text{C}$ the shaded outer canopy pears without their surrounding leaves exhibited a significantly higher firmness and greener background colour than the unshaded outer canopy fruit. The unshaded outside fruit had a similar diameter than the shaded outer canopy pears without their surrounding leaves, while the latter were 7.2 g heavier. Since the sun-exposed outside fruit were significantly mealier, it was an indication that the exposure of the fruit to a high percentage of sunlight (irradiance) for most part of the day, coupled with high FST and VPD (as mentioned earlier), played a significant part in 'Forelle' mealiness development. As mentioned earlier, the high temperatures that unshaded outer canopy fruit experienced, could have a negative influence on fruit metabolism, resulting in the formation of internal cellular structures during cell division and cell enlargement happening differently, causing the pre-harvest unshaded outer canopy fruit to be predisposed in developing a mealy texture during the ripening period.

The objective of our third study (Chapter 4) was to determine whether mealiness differences within the canopy are related to storage potential and ripening potential differences for fruit from different canopy positions, as well as the effect of harvest maturity on 'Forelle' mealiness. For both harvest maturities, maturity indexing was conducted at harvest and again after 8, 12 and 16 weeks of cold storage at $-0.5\text{ }^{\circ}\text{C}$ under regular atmosphere (RA), plus 0, 4, 7 and 11 days of shelf-life storage at $20\text{ }^{\circ}\text{C}$.

According to the results it seems that 'Forelle' pears harvested at a post-optimum maturity, are more inclined to a mealy texture. This agrees with the studies of Mellenthin and Wang,

(1976; pear); Peirs et al. (2001; apple); Martin, (2002; 'Forelle' pear) and Carmichael, (2011; 'Forelle' pear). As in the previous two trials, the red blushed outer canopy pears were generally more susceptible to mealiness development, compared to the intermediate and inner canopy fruit.

The rate of fruit softening could be influenced by the degree of maturity at the time of harvest (Chen and Mellenthin, 1981). The characteristic pear ripening process is associated with a loss of flesh firmness, a colour transition from green to yellow, decrease of TA, increase of TSS and an increase in ethylene production (Eccher Zerbini, 2002). In this study, ripening potential was not influenced by fruit canopy position and harvest maturity. However, the ripening rate of red blushed outer canopy pears was more advanced than for inner canopy fruit, irrespective of harvest maturity. Outer canopy pears were in a more advanced stage of maturity, considering the earlier decrease in flesh firmness of outside fruit as well as the earlier transition from a green to a more yellow fruit background colour. In general, the outer canopy fruit had higher TSS levels and lower TA levels than inner canopy fruit. The results are similar to those in the study done by Carmichael (2011) and Cronjé (2014), who associated mealy 'Forelle' pears with a more advanced stage of maturity.

Eight weeks of cold storage at $-0.5\text{ }^{\circ}\text{C}$ was enough for inducing 'Forelle' pear ripening, however, it was associated with the highest mealiness incidence. Carmichael (2011) reported the same result with 'Forelle' pears. Our results agree with the mandatory 12-week cold storage period to minimize 'Forelle' mealiness development. For both seasons, independent of harvest maturity, mealiness incidence of all the different fruit positions decreased after 16 weeks at $-0.5\text{ }^{\circ}\text{C}$. This agrees with Martin (2002) and Carmichael (2011) who perceived a decrease in 'Forelle' pear mealiness with prolonged cold storage duration. It is noteworthy that mealiness incidence of outer canopy and mid-canopy pears decreased after 16 weeks of cold storage plus 11 days of ripening.

The firmness of outside west fruit for both harvest maturities showed a sharp decrease from 8w RA storage at $-0.5\text{ }^{\circ}\text{C}$ + 7d shelf-life at $20\text{ }^{\circ}\text{C}$ to 8w RA storage at $-0.5\text{ }^{\circ}\text{C}$ + 11d shelf-life at $20\text{ }^{\circ}\text{C}$ in 2016 and from 8w storage at $-0.5\text{ }^{\circ}\text{C}$ + 4 to 7d shelf-life at $20\text{ }^{\circ}\text{C}$ in 2017. This decrease occurred before the climacteric rise of ethylene. This agrees with several other studies where fruit softening inducing happened before the climacteric rise (Wang and Hansen, 1970 (pear) [Cited by Du Toit et al., 2001]; Wang et al., 1972 (pear); Chen and Mellenthin, 1981 (pear).

This indicates that a very small amount of ethylene is needed for fruit softening, and that the sensitivity of fruit for ethylene might differ depending on their ability to ripen. According to the results of both seasons (2016 and 2017), no direct link was found between 'Forelle' mealiness and ethylene production, seeing that ethylene levels of the inner canopy fruit were higher during most periods of the evaluation time, and yet their mealiness incidence was never as high as the outside pear fruit.

Since the firmness of red blushed outer, slightly blushed intermediate and no blush inner canopy fruit during the evaluation times was mostly similar, but in mealiness incidence differed significantly, it indicates that internal fruit factors/cellular structures related to colour and fruit canopy position prior to ripening, played a meaningful part in the way post-harvest fruit softening (cell wall degradation) occurred and could cause outer canopy fruit to be more susceptible to mealiness development. However, according to the previous two trials, as well as this trial, an unidentified tree factor plays an important part in 'Forelle' mealiness. This is while bearing in mind that not all sun-exposed red blushed outer canopy pears developed mealiness and not all shaded no blush inner canopy fruit have a non-mealy texture.

In conclusion, according to the results obtained in our study, it seems as if pre-harvest factors, such as the exposure of fruit to high irradiance levels coupled with high FST are some of the determining factors in 'Forelle' pear mealiness development. However, an unidentified tree factor may also be involved in mealiness development, since not all red blushed outer canopy fruit, as well as the no blushed shaded inner canopy fruit (that were associated with a mealiness), developed a mealy texture. It seems the susceptibility of fruit for mealiness development is already determined whilst on the canopy.

The higher mealiness incidence of red blushed outer canopy pears, irrespective of harvest maturity was generally associated with a more advanced stage of maturity, resulting in the outer canopy fruit that are at commercial harvest maturity, are probably already in a more advanced stage of maturity. The red blushed outer canopy pears could probably be harvested a week or two before the commercial harvest date, since it could advance their post-harvest behaviour and even influence their mealiness incidence.

This study paved the way for further research regarding the part that pre-harvest factors play in 'Forelle' mealiness development, with the emphasis on external and internal tree factors that influence cell division and enlargement. Further studies are needed to determine during

which stage of development the fruit are most susceptible to high FST and according to that, it can be determined when fruit should be shaded (by using drape nets) and exposed to sunlight to ensure the development of a sufficient red blush colour. Insufficient red colour development of red blush pear fruit in South Africa results in the downgrading of the fruit (Huysamer, 1998). Outer canopy fruit could be shaded at 'pea' size and computed tomography (CT)-scanning could be done weekly to visualise the internal structure of the fruit to establish at what stage of development the fruit are predisposed for a mealy texture, since mealy 'Forelle' pears are associated with large intercellular airspaces (Muziri et al., 2015).

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